CKJ REVIEW

The anaemia treatment journey of CKD patients: from epoetins to hypoxia-inducible factor–prolyl hydroxylase inhibitors

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ABSTRACT

The discovery and development of erythropoiesis-stimulating agents was a journey lasting more than a century, leading to the cloning and approval of recombinant human erythropoietin (rHuEpo). This was an impressive clinical advance, providing the possibility of correcting the symptoms associated with anaemia in chronic kidney disease. Associated iron use was needed to produce new haemoglobin-containing blood red cells. Partial anaemia correction became the standard of care since trials aiming for near-normal haemoglobin levels showed a higher risk of adverse cardiovascular events. Hoping to reduce the cardiovascular risks, a new category of drugs was developed and tested. Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are small molecules than can be formulated into orally active pills. They simulate reduced tissue oxygen pressure, thus stimulating the production of endogenous erythropoietin (Epo) by the kidneys and liver. Clinical trials with these compounds demonstrated that HIF-PHIs are at least as effective as rHuEpo in treating or correcting anaemia in non-dialysis and dialysis patients. Trials with HIF-PHIs did not demonstrate superiority in safety outcomes and in some trials, outcomes were worse. There was also a focus on oral delivery, a possible beneficial iron-sparing effect and the ability to overcome Epo resistance in inflamed patients. A negative effect is possible iron depletion, which may explain adverse outcomes.

LAY SUMMARY

Erythropoiesis-stimulating agents (ESAs) have been used for >30 years to treat anaemia in CKD. They have become the standard of care, as haemoglobin (Hb) levels can be raised and maintained within target ranges, with an acceptable safety profile. The typical Hb target range (9–12 g/dl) is lower than the normal range (13–15 g/dl) because of safety concerns, including increased strokes, and cost when targeting higher Hb levels. A new class of orally active agents that simulate low oxygen tension, hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs), have been discovered and developed. They have the possible added advantage of promoting iron mobilisation to support Hb synthesis. HIF-PHIs were not superior to ESAs in treating or correcting anaemia and did not demonstrate superiority in safety outcomes in phase 3 clinical trials. In some trials, strokes and other thrombotic events were increased beyond that of originator ESAs. The mechanism is not understood, but one possibility is that iron depletion due to mobilization of iron from stores is the cause.

INTRODUCTION

Understanding the cause of anaemia in chronic kidney disease (CKD) patients and the associated clinical symptoms, including fatigue, worsening of quality of life and cardiovascular complications, was a multidecade effort. Patients with CKD complained of extreme fatigue, due to severe anaemia, associated with progressive plasma retention of urea (as a marker of uraemic toxins). In addition to depurative or dietetic strategies, the possibility of reverting or at least reducing the symptoms associated with CKD anaemia was an important focus. However, effective methods to treat anaemia were elusive.

Discovery of erythropoietin (Epo) and the development of erythropoiesis-stimulating agents (ESAs) for treatment of anaemia

In 1863, Jourdanet first reported that blood was more viscous at high altitude than at sea level [1]. This was later found to be due to increased numbers of red blood cells (RBCs). RBCs carry oxygen from the lungs to tissues and working muscles (Fig. 1). The lifespan of RBCs is ≈4 months, so changes in RBC numbers are controlled by regulating their rate of production. Epo is a circulating hormone and is the primary regulator of erythropoiesis. Its level increases rapidly in response to low oxygen tension, explaining the increase in RBC numbers at high altitude. In 1977, Epo was purified [2]; the encoding gene was cloned in 1983 [3]. This allowed for the manufacture and testing of the first recombinant human erythropoietin (rHuEpo; epoetin alfa).

Prior to the advent of rHuEpo, repeated transfusions were used to correct anaemia, although quality of life remained poor, with negative consequences due to iron accumulation and the risk of infection. We can therefore imagine the enormous enthusiasm of doctors, nurses and patients regarding the possibility of using rHuEpo for the treatment of anaemia due to CKD. Patients who barely survived, with unspeakable fatigue and innumerable symptoms, were revived. An injection of epoetin alfa into the dialysis line three times per week was enough to restore an acceptable well-being and reduce the need for transfusions. Seminal publications of the first clinical experiences using rHuEpo in anaemic CKD dialysis-dependent (DD) patients were published late in the 1980s [4–6]. Administration of rHuEpo has subsequently been expanded to patients with non-dialysis (ND) CKD, peritoneal dialysis and kidney transplant patients who had a deterioration in their kidney function. Over the years, ESA molecules obtained treatment indications for other diseases, including anaemia during chemotherapy. Indications other than CKD anaemia are molecule specific.

With the marketing of rHuEpo came attempts to optimise its use. Costs were high and efforts to reduce doses were attempted. Subcutaneous (SC) administration turned out to provide some convenience (self-administration) and an approximate 30% dose savings on average, probably due to a lower concentration of rHuEpo in the blood, but with longer drug blood persistence, allowing a reduced frequency of administration to twice and then once a week. SC self-administration is of importance particularly in CKD patients not receiving haemodialysis (HD). Preservation of peripheral veins of the arms is also very important for
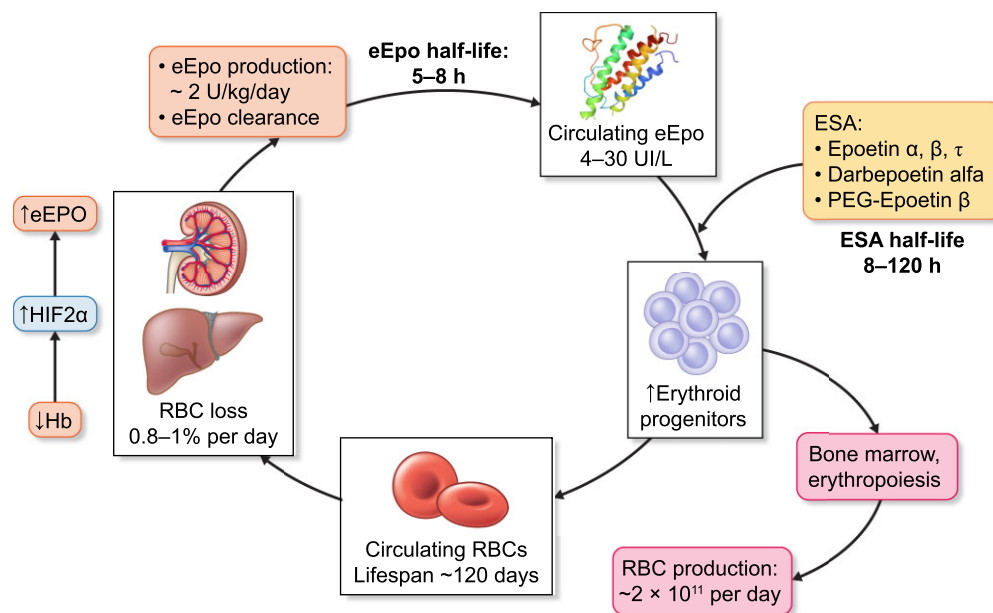

Figure 1: RBC homeostasis and Epo-stimulated erythropoiesis. Following stimulation of the HIF system, eEpo production from the kidney (and to a lesser extent from the liver) is increased. HIF-2α is the main subunit regulating eEpo synthesis. Under physiological conditions, 2 IU/kg of eEpo are produced daily with a half-life of 5–8 hours, resulting in a circulating concentration of 4–30 UI/L. ESA administration alters this balance by markedly increasing ESA levels. ESA half-life depends on molecular characteristics, ranging from 8 (epoetins) to 120 hours (methoxy polyethylene glycol-epoetin β) and administration route (for epoetins). eEpo stimulates erythroid progenitor development, leading to their maturation into reticulocytes and RBCs. Nearly 2×10^{11} RBCs are produced daily. Their life span is ≈120 days (shorter in patients with CKD). Senescent RBCs are cleared from circulation via macrophage phagocytosis (0.8–1% per day). The consequent haemoglobin decrease is a stimulus for HIF-2α increase and stimulation of new erythropoiesis.

future insertion of a fistula for performing HD. Unfortunately, SC administration also resulted in a rare but increased risk of antibody-mediated pure red cell aplasia (PRCA), a serious and life-threatening condition where antibodies to the recombinant protein cross-reacted with endogenous Epo (eEpo), thereby inactivating it [7, 8]. Consequently, for safety reasons and convenience, intravenous (IV) administration became the preferred route in HD patients.

Dose frequency of ESAs is determined by a combination of convenience and pharmacokinetics and pharmacodynamics of the molecules. The first ESAs were administered two to three times a week to match the dialysis interval. Further extension, to once a month in selected patients, was limited because of fast clearance and the need of higher doses and thus higher peak Epo plasma levels required to keep ESA levels above the threshold of erythropoiesis for extended time intervals. An ESA with a longer half-life, 'long-acting Epo', was made by increasing the sugar content of rHuEpo, resulting in a 3-fold increase in serum half-life [9]. This first long-acting agent (darbepoetin alfa) offered the possibility of administration initially once a week and then up to once every 4 weeks, thereby reducing needle sticks and increasing convenience. Later, a second long-acting molecule was constructed with an even longer half-life by 'pegylating' rHuEpo (epoetin beta, methoxy peg); it can be administered every 2–4 weeks [10, 11].

As patents expired internationally for the first two ESAs, epoetin alfa and beta, short-acting biosimilars were introduced in Europe. A biosimilar of darbepoetin alfa is also under clinical development in the USA. Biosimilars are similar but not equal to the originators and inevitably present the same limitations.

Other agents followed, including peginesatide, a long-acting Epo mimetic protein that bound to the Epo receptor, similar to rHuEpo, thereby activating it and stimulating erythropoiesis. It was pegylated to increase its serum half-life and thereby fell into the category of a long-acting ESA [12], but differed from previous ESAs because it is a synthetic small peptide not requiring recombinant DNA technology. Initial clinical results with peginesatide were positive, increasing or maintaining Hb levels in CKD patients [13]. Quite unexpectedly, in ND patients an increased hazard ratio was found for the cardiovascular safety endpoint, with higher incidences of death, unstable angina and arrhythmia compared with darbepoetin alfa [14]. While approved for use in DD patients, it was later withdrawn from the market because of serious hypersensitivity reactions, including anaphylaxis, possibly due to the stabilizer in the multidose formulation. At present, a modified version of peginesatide is undergoing phase 3 clinical development (https://clinicaltrials.gov/ct2/show/NCT03902691).

Another important issue with ESAs is target haemoglobin (Hb). Initially doses were adjusted to keep patients in a Hb range below that of normal subjects (10–12 g/dl) but higher compared with untreated patients <10 g/dl). There was a hypothesis that disease progression, cardiovascular problems, survival and wellbeing would be improved if patients had a normal haematocrit. This led to the Normal Hematocrit Cardiac Trial (NHCT), a large randomized controlled trial of patients targeted to a normal haematocrit of 42 ± 3% versus maintaining partial correction of 30 ± 3% [15, 16]. The trial was halted early for futility. While there was a clinically meaningful increase of 7.2 points in the physical function scale, there was an increased death rate at the 1- and 2-year intervals in the higher haematocrit arm together with an increase in the rate of vascular access thrombosis. Similar negative or neutral results were obtained in other ESA trials designed to see if outcomes were improved with complete anaemia correction in ND patients [17–20]. In meta-analyses of

these and other trials, there was an increased risk of thrombosis with higher doses [21] and higher target Hb [22]. In some trials there was also an increase in all-cause and cardiovascular mortality. However, in meta-analyses, worsening of mortality endpoints when comparing ESA to placebo [23–25] or low to high Hb targets [26] did not reach statistical significance.

In those trial designs, the doses were progressively increased in patients unable to reach the Hb target, indicating the presence of ESA hyporesponse. The side effects of ESAs were greater in the patients unable to reach a Hb target, especially if receiving high-dose ESA [27]. The patients reaching Hb levels >12 g/dl with small doses of ESAs showed the best prognosis [28]. Similar results were seen in observational studies [29].

Overall, these observations resulted in restrictions on ESA use [30, 31]. In this respect, targeting Hb levels >13 g/dl should be avoided, especially when high ESA doses are needed. This is particularly true in hyporesponsive patients, because of possible increased risk of venous or arterial thrombotic events.

Another possibility that was explored was that ESAs might reduce renal disease progression through direct or indirect effects. Multiple small and moderate-sized trials were initiated to test the hypothesis, but no consistent benefits in CKD outcomes were observed [32].

Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs): a different way to treat anaemia

Pushed by the possibility that ESAs with a different mechanism of action might have a better safety profile, and to possibly improve the treatment of patients who responded poorly to rHuEpo, a new category of drugs was developed, HIF-PHIs. Multiple orally active compounds with various structures have been discovered and developed and some of them have completed clinical trials. Currently, six different drugs—roxadustat, vadadustat, daprodustat, molidustat, desidustat and enarodustat—have received marketing authorization in China, Japan and other countries. Roxadustat has been approved by the European Medicines Agency (EMA), while the US Food and Drug Administration (FDA) denied approval of roxadustat (https://www.drugs.com/nda/roxadustat_210811.html) and vadadustat (https://www.drugs.com/nda/vadadustat_220404.html) because of safety concerns, including evidence of increased thrombotic events compared with epoetin alfa [33] (https://www.fda.gov/media/150728/download). Daprodustat use has recently been approved by the FDA for prevalent dialysis patients; it is under evaluation in Europe.

To underline the importance of the developed knowledge in this area and the discovery of HIF and HIF-PHIs [34, 35] not only for the treatment of anaemia, but also in other areas of medicine, three doctors—William Kaelin, Sir Peter Ratcliffe and Gregg Semenza—received the Nobel Prize in Medicine. Many articles describe in detail the mechanism of action of HIF-PHIs [36–38]. They mimic the body's exposure to hypoxia by inhibiting prolyl hydroxylase activity, thus increasing HIF-α levels (Fig. 2). HIF, a dimer comprised of HIF-α and HIF-β, stimulates transcription of the Epo gene by the kidneys and to a lesser extent by the liver. Among the three HIF-α isoforms, HIF-2α has the major role in eEpo synthesis. HIF also increases iron absorption from the gut and reduces hepcidin levels, mainly through indirect mechanisms, allowing mobilisation of iron from stores. The final result is increased iron availability to match the demand for iron to make Hb. It also adjusts cellular metabolism according to the availability of oxygen. Furthermore, many other genes are stimulated that activate pathways involved in protection
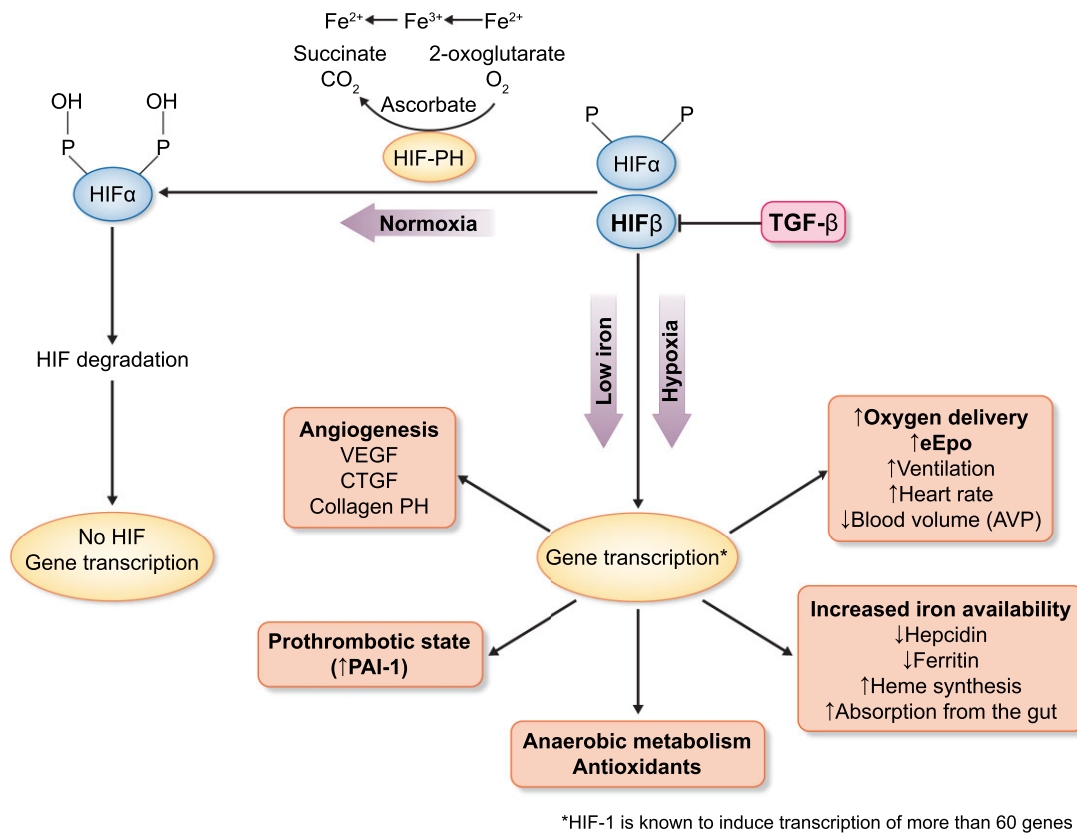
Figure 2: Physiology of the HIF system. HIF-PH activity is affected by oxygen, inflammation and iron. In conditions of normal oxygen tension, HIF-α is hydroxylated by the enzyme HIF-PH and undergoes von Hippel–Lindau protein-dependent ubiquitination and rapid proteasomal degradation, with a half-life of only 3 minutes. HIF-PH activity is regulated by oxygen levels and iron; both factors are necessary for its function. With hypoxia or low iron, HIF-PH is inactive and cannot hydroxylate HIF-α, which then accumulates and forms heterodimers with the HIF-β subunit in the nucleus, resulting in an active HIF complex (HIF). HIF promotes transcription of multiple genes, whose function is aimed at restoring oxygen balance and reducing the consequences of tissue hypoxia. Among these functions is the stimulation of eEpo and regulators of iron metabolism.

from hypoxia in cells and tissues. HIF can also influence cell metabolism, angiogenesis, inflammation and immune responses.

One early question was whether HIF-PHIs could be used to treat anaemia in CKD patients since eEpo production is primarily in the kidney and is already compromised in kidney disease. However, these compounds can stimulate production of eEpo in the liver as well, even in anephric CKD patients, at levels sufficient to increase and maintain Hb within target ranges. The mechanisms of eEpo synthesis following HIF-PHIs have been investigated by several experimental studies with conflicting findings. Some suggested the possibility that following stimulation, myofibroblasts can restore their ability to produce eEpo. Others demonstrated that eEpo synthesis can occur only in non-fibrotic areas [39, 40].

HIF-PHIs induce synthesis of eEpo, raising it to levels that can stimulate erythropoiesis, but at a lower serum concentration compared with SC or IV injected rHuEpo. The differences in the pharmacokinetics could partially explain this. HIF-PHIs are administered daily, or at most three times a week, depending on the half-life of HIF-PHI molecules; rHuEpo is typically administered less frequently than HIF-PHIs. More frequent ESA administration is known to be more efficient, albeit less convenient, especially from the patient perspective. The fact that responses with 'Epo-resistant' inflamed patients are possibly better argues against increased efficiency of more frequent administration as

the sole explanation. Instead, HIF-PHIs may make erythropoiesis more 'sensitive' to Epo via increased iron availability to make Hb or by its effects on pathways regulating inflammation and immune responses.

It has been proposed that negative outcomes observed in some ESA clinical trials may be attributed to higher doses of rHuEpo when targeting near-normal Hb levels. Thus HIF-PHIs, by stimulating eEpo production at much lower plasma concentrations and improving iron absorption and utilization, may translate into less risk of cardiovascular complications. Unfortunately, the results of phase 3 trials did not support this hypothesis. They showed overall non-inferiority compared with ESAs [41–44] or placebo [45], with relative increases in major adverse cardiac events (MACEs) and thrombotic events in some trials [43].

There is a general agreement that all the HIF-PHIs are at least as effective as ESAs in increasing and maintaining Hb levels within target ranges in both ND and DD CKD patients [46]. However, the trials were generally designed to demonstrate non-inferiority to ESAs and superiority with respect to placebo. There is evidence that target Hb can be achieved faster with HIF-PHIs, possibly because of a relative higher 'effective' dose of HIF-PHI chosen for the investigation. Accordingly, there were also increases in Hb overshoots due to the rapid rates of increase. Lower doses are recommended in Asia for this reason; also considering that this population in general is of smaller size and much less
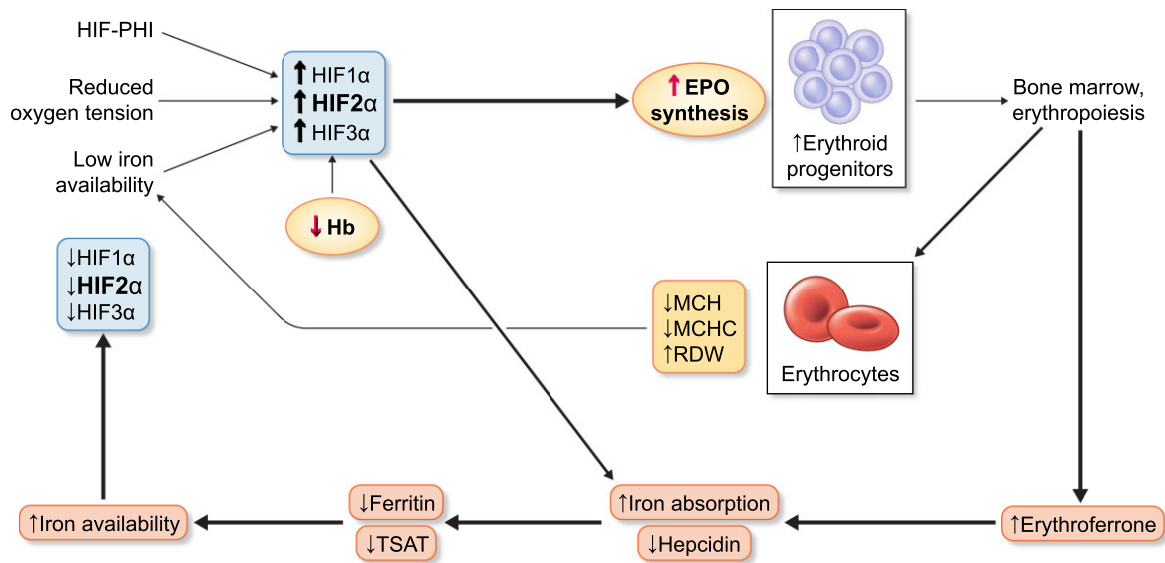
Figure 3: Iron metabolism changes following increased erythropoiesis. There are three HIF-α subunits: HIF-1α, HIF-2α and HIF-3α. The HIF-2α subunit is the one mostly involved in the promotion of increased eEpo synthesis and erythropoiesis. In the bone marrow, this process leads to the production of erythroferrone in erythroblasts. In turn, this substance decreases hepcidin expression and increases iron mobilization. HIF activation also regulates iron metabolism by increasing iron absorption from the gut and exporting it to the circulation via increased expression of the iron exporter ferroportin 1. The direct effect of HIF on hepcidin is still a matter of debate.

inflamed. While earlier data suggested a possible benefit of HIF-PHIs in reducing blood pressure, the results of phase 3 trials did not confirm these favourable results [33].

A very interesting effect of some HIF-PHIs is a reduction in total and low-density lipoprotein (LDL) cholesterol along with a smaller decrease in high-density lipoprotein (HDL). The overall effect may translate into a benefit, at least in ND patients. However, in this population the benefit of a reduction in LDL is likely to be inversely associated with the level of renal function deterioration. Considering that anaemia treatment is started when renal function is already heavily deteriorated, only a partial benefit would be expected. In DD patients, cardiovascular complications are more due to vascular calcifications than to LDL deposition, so the benefit would be negligeable. It is unknown whether HIF-PHIs have the same favourable cardiovascular effects as statins when reaching the same LDL target.

Iron deficiency, heart failure and thrombosis

Total body iron in humans is ≈3.5 g and iron in Hb-containing red blood cells (RBCs) represents ≈40% of the total. Iron uptake from the gut is relatively slow, so increased demand is met by mobilisation from stores into the circulation and then to the bone marrow where erythropoiesis occurs. A substantial number of RBCs, 2×10^{11}, are generated per day (Fig. 1) and they constitute 90% of circulating cells. Hb represents ≈96% of the protein in an RBC, thus large quantities of iron are required to maintain this level of Hb.

An imbalance between need and availability of iron can cause iron deficiency (ID), resulting in decreased mean corpuscular Hb (MCH) and mean corpuscular Hb concentration and increased red cell distribution width (RDW), and this can have negative consequences. In heart failure patients, ID is associated with worse all-cause mortality and increased hospitalisations [47–52], and increased RDW is negatively correlated with left ventricular ejection fraction and New York Heart Association class [53]. ID is also a predictor of thrombotic events. Multiple

studies suggest a relationship between ID and the risk of stroke in infants and pre-menopausal women [54–56] and in patients with pulmonary arteriovenous malformations [57]. In a nationwide case–control study in Taiwan, ID was associated with an increased risk of stroke [58]. Moreover, chronic ID in animal studies has been shown to increase fibroblast growth factor 23 (FGF23) transcription by activating HIF signalling [59]; the negative cardiac effects of FGF23 are well recognised. There also may be an interplay between ID, thrombosis and increased mortality in patients with acute pulmonary embolism [60]. In contrast, in studies with high-dose IV iron administration [61], there was evidence that the risk of cardiovascular events was reduced.

Iron use and safety with HIF-PHIs: a novel hypothesis

In ESA trials there was an increased incidence of cardiovascular events, thromboembolic events and strokes [21, 22]. However, no conclusive mechanism to explain this has emerged. It was assumed ESA dose and high Hb were contributors, so lower doses and lower target Hb were recommended to reduce risk [31]. Another possibility is that iron depletion plays a role.

Anaemia correction with ESAs can significantly deplete iron stores and create functional ID. Assuming a total blood volume of 5 L in a 70-kg man, an increase in Hb of 1 g/dl requires 170 mg of iron. Therefore, to increase Hb from 9 to 12 g/dl, as in some anaemia correction studies, would require ≈500 mg of iron. Assuming stores contain 1–2 g of iron, the active patient arm in anaemia correction studies would be depleted by 25–50%.

In the absence of iron administration, stimulating erythropoiesis with both ESAs and HIF-PHIs can mobilise iron to support enhanced erythropoiesis, resulting in increases in serum iron and decreases in iron stores. In support of this, transferrin saturation (TSAT) and serum iron increased in CKD studies, but the iron storage markers ferritin and hepcidin decreased [62, 63] (Fig. 3). In animal studies with roxadustat, serum iron and TSAT also went up and hepcidin went down [64]. In clinical ND-CKD studies with roxadustat and vadadustat, serum iron

was unchanged or went up and ferritin and hepcidin went down [63, 65–71]. Similarly in DD patients, ferritin and hepcidin were decreased with vadadustat [63]. In addition, there is evidence of iron-restricted erythropoiesis, because iron flux may not increase sufficiently to meet demand, resulting in decreases in Hb per cell. This may explain why, in healthy humans treated with ESAs, MCH went down and RDW went up [72].

ID is a frequent complication of CKD, being detected in more than half of the ND [73] and 20–25% of the DD populations [74]. Despite increased need, IV iron is rarely administered to ND-CKD patients, in part because of the so-called therapeutic inertia, but also because of organisational issues. Thus, if they do get iron, it is usually oral iron, with less benefit.

Table 1 summarises iron needs and management during the larger controlled ESA trials testing different Hb targets. In DD patients, iron levels, RBC survival and iron turnover increased demand because of chronic blood loss from retention in the HD dialyzers and blood lines and limited iron availability from stores because of chronic inflammation, but with increased iron administration. Consequently, DD patients can have higher iron stores [75]. Because there are different treatment methods and iron demands, ESA and iron administration should be carefully considered according to the CKD stage, target Hb and iron status.

Clearly the need for iron by absorption from the gut or by parenteral administration becomes more essential as stores are depleted, especially as demand increases, as occurs with increasing ESA dose or with a faster rate of Hb increase. If patients are given IV iron, total iron should go up, thereby relieving stress on stores, allowing for more iron for other essential processes.

In most trials targeting higher Hb with ESAs, the active treatment groups received similar or even lower amounts of iron compared with control groups, despite a greater need in the former. While RBC transfusions could provide an additional source of iron, the intervention groups typically also had lower transfusion rates. Note that in the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT), IV iron was rarely administered in the active treatment arm [19].

The same applies to HIF-PHIs. Indeed, no IV iron was permitted in an early clinical trial with roxadustat [76]. In phase 3 clinical trials, iron treatment was often left to the investigators and was suboptimal compared with what is recommended by international guidelines (Tables 2 and 3). In the case of roxadustat, iron treatment policies differed between the experimental and control group. Moreover, in most HIF-PHI studies, iron use went down in the treatment arm over time. It appears that in most clinical trials, even though the treatment arms needed more iron, they got less than the comparator group (Tables 4 and 5). This may be because TSAT was the same or went up, and any decrease of TSAT was interpreted as due to the increase in transferrin production and not related to ID. Thus it was very likely assumed that iron was not needed.

We could suppose that the idea of the developers of HIF-PHIs was to produce a 'global erythropoietin' drug able to both stimulate eEpo production and to provide iron by favouring its absorption and mobilisation. Consequently, they possibly hypothesised that parenteral iron was unnecessary. Different anaemia correction methods can have different impacts on iron and RBC levels and different advantages and disadvantages with different demands for iron (Table 6). Further, the strategy used to treat anaemia will require adjustments to ensure that iron demand and iron availability are properly coordinated. In our opinion,

the reduction of stores was the possible trigger for the failure to show superiority in reducing the risk of MACEs with increased thrombotic events and this may be correctable with appropriate iron management.

Induced ID may also explain, at least partially, why targeting but not achieving a higher Hb level and why non-responders did worse in ESA trials [77]. Note that the primary cause of non-response to an ESA is ID [78], although inflammation causing functional ID is of increasing relevance.

We may need to think differently about what constitutes ID. Is ID in patients presenting with anaemia in the hospital similar to ID induced by treatment with ESAs? In this regard, ID is a stronger predictor of mortality than anaemia in patients with heart failure [48]. In TREAT, baseline ferritin and TSAT versus outcomes show no relationship [79]. Thus it is possible that the issue is the depletion of storage iron and failure to compensate for the induced ID by administering IV iron, and not baseline levels *per se*. Note that in TREAT [19], IV iron was administered to only 15% of patients and the ESA arm had iron depletion and increased strokes.

With HIF-PHIs, iron was mobilised to a greater extent and rates of Hb increase were greater than with ESAs, so likely the stores of patients treated with HIF-PHIs were exhausted to a greater degree than with ESAs. In this scenario of increased need, it is unlikely that iron absorption would provide enough iron for supporting more erythropoiesis. This is consistent with the decrease in ferritin and hepcidin in many of the HIF-PHI studies.

There is an interaction between iron and oxygen, where a change in either results in modulation of the same pathways [37, 38, 80]. Since both oxygen and iron are cofactors for HIF-PH, depletion of either can increase HIF levels. HIF activation is associated with promoting a prothrombotic state [81–84] as well as pulmonary hypertension [85, 86]. Consistent with a role of oxygen and iron in pulmonary hypertension, hypoxia due to high altitude or hypoxia due to venosection resulted in pulmonary hypertension, and this was reversed with IV iron [87, 88].

The results of the Proactive IV Iron Therapy in Haemodialysis Patients (PIVOTAL) trial are informative [89]. There was a significantly lower incidence of cardiovascular events and deaths in DD patients randomised to receive proactive (400 mg/month) iron administration (the limit was no more than a ferritin level of 700 ng/ml and TSAT of 40%) compared with patients randomised to a reactive iron administration. Other support comes from the trials demonstrating a reduction in cardiovascular events in patients with heart failure treated with iron [90], while no positive results were shown in anaemic patients with heart failure treated with ESA alone [91].

Because of safety concerns, in approving the clinical use of roxadustat, the EMA recommended to not shift stable DD patients treated with ESA to roxadustat without valid reasons (https://www.ema.europa.eu/en/medicines/human/EPAR/evrenzo). If the above interpretation is correct, administration of more IV iron to avoid induced ID may be a simple treatment to improve patient outcome. We suspect the negative consequence of iron depletion would be worse for patients already having low iron stores since the greater the depletion, the worse may be the outcome. Recommendations by the EMA (https://www.ema.europa.eu/en/medicines/human/EPAR/evrenzo) and from the Asian Pacific Society of Nephrology [92] to start HIF-PHI in iron-replete patients goes in this direction. Whether treatment with HIF-PHIs provides any advantage in patients with functional ID compared with ESA is still an open question.

Table 1: Iron management in ESA CKD anaemia correction studies.

Study	Baseline Hb (g/dl)	Target Hb: high versus low (g/dl)	Iron needed to reach target Hb (mg)	Baseline ferritin: control versus high Hb group (μg/L)	Iron (IV) administered: control versus high Hb group		Transfusions: control versus high Hb group
					Patients, %	Dose (mg)	Patients, %
HD							
Normal haematocrit [15, 16]	10.2	14 versus 10 (target) 13.3 versus 10.4 (achieved)	0 versus 646	403 versus 334	75 versus 85 (iron dextran)	119 ± 133 versus 145 ± 179; survivors (152 ± 150) versus died (214 ± 190)	31 versus 21
ND-CKD							
Foley 2000 [20]	9–11	13.5 versus 10	0 versus 595	NA	45 versus 66 (iron dextran)	44–46 versus 64–68 mg/week	NA
CHOIR [18]	10.1	13.5 versus 11.3	204 versus 578	167.8 ± 157.2 versus 179.0 ± 171.5	IV 2.6 versus 1.6, oral 26.5 versus 26.7	NA	NA
CREATE [17]	11.6	13–15 versus 10.5–11.5	0 versus 323	189 versus 174	IV 42 versus 52, at least one dose; oral 62 versus 60, at least one dose	At discretion of investigators	10.9 versus 8.6
TREAT [19]	10.5	<11 versus 13	0 versus 425	137 versus 131	20.4 versus 14.8		25 versus 15

Table 2: Strategies for iron supplementation in the main phase 3 randomised clinical trials with HIF-PHI in ND patients.

Trial	Study characteristics	Choice of the route of administration	Oral	IV
ANDES [95]	Roxadustat versus placebo RR 2:1 N = 922	Oral iron encouraged in both groups	NA	NA
ALPS [96]	Roxadustat versus placebo RR 2:1 N = 594	The same for both study drugs	At baseline, 39.9% on oral iron in both groups To support erythropoiesis and as the first line for prevention and treatment of iron deficiency unless the patient was intolerant 200 mg/day of elemental iron	Hb level not increased after two or more dose increases of study drug while taking oral iron (unless not tolerated) Hb <8.5 g/dl and ferritin <100 ng/ml, or TSAT <20% Single iron dose ≤250 mg 8 weeks after single dose IV iron administered if Hb <9.0 g/dl and ferritin <100 ng/ml or TSAT <20% After this 8-week period, full IV iron rescue criteria to be met again for a second course of IV iron at a later point
OLYMPUS [97]	Roxadustat versus placebo RR 2:1 N = 2781	According to the study protocol		Permitted in patients intolerant or unresponsive to oral iron and with Hb <8.5 g/dl and ferritin <100 mg/L or TSAT <20%
DOLOMITES [41]	Roxadustat versus darbepoetin alfa RR 1.1 N = 616	R: per protocol DA: left to the investigator's discretion	R: first-line treatment (ferritin <100 ng/ml or TSAT <20% DA: ferritin <100 ng/ml or TSAT <20%	R: inadequate Hb response after at least two dose increases or the maximum dose limit was reached and iron deficiency or intolerance to oral iron DA: ferritin <100 ng/ml or TSAT <20%
PRO₂TECT [43] (correction)	Vadadustat versus placebo RR 1:1 N = 1751	Left to the investigator's discretion	Encouraged if iron depletion	Encouraged if iron depletion
PRO₂TECT [43] (conversion)	Vadadustat versus placebo RR 1:1 N = 1725	Left to the investigator's discretion	Encouraged if iron depletion	Encouraged if iron depletion
ASCEND ND [44]	Daprodustat versus placebo RR 1:1 N = 1751	Route of administration and dose of iron based on the patient's iron status and local clinical practice	Iron therapy was administered if ferritin ≤100 ng/ml and/or TSAT ≤20% All iron (excluding multivitamins) stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%	Iron therapy was administered if ferritin ≤100 ng/ml and/or TSAT ≤20% All iron (excluding multivitamins) stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%

Table 3: Strategies for iron supplementation in the main phase 3 randomised clinical trials with HIF-PHI in dialysis patients.

Trial	Study characteristics	Choice of the route of administration	Oral	IV
HYMALAYAS [98]	Roxadustat versus epoetin alfa RR 1:1 N = 1043 (incident to dialysis)	The same for both study drugs	Oral iron as the first-line iron supplementation in both groups	IV iron if, in the opinion of the investigator, Hb had not responded adequately, iron deficiency (ferritin <100 ng/ml and TSAT <20%) IV iron discontinued once iron repletion (ferritin ≥100 ng/ml and TSAT ≥20%)
SIERRAS [99]	Roxadustat versus epoetin alfa RR 1:1 N = 731	The same for both study drugs	No restriction	≤250 mg per dosing cycle; no limit under protocol amendment 2 Inadequate response or intolerant to oral iron or deficient (ferritin <100 ng/ml or TSAT <20%). Iron was discontinued after iron repletion (ferritin ≥100 ng/ml and TSAT ≥20%)
PYRENEES [98]	Roxadustat versus ESA RR 1:1 N = 836	According to the study protocol	Oral iron permitted	R: IV iron allowed if Hb had not increased adequately to roxadustat after two consecutive dose increases or if the maximum dose limit had been reached and ferritin <100 ng/ml or TSAT <20% or was intolerant to oral iron ESA: according to local standard of care
ROCKIES [99]	Roxadustat versus epoetin alfa RR 1.1 N = 2133	According to the study protocol	Permitted in both groups without restriction	R: if Hb did not increase sufficiently after two or more dose increases, ferritin <100 ng/ml or TSAT <20% Epoetin alfa: according to standard of care
INNO₂VATE (incident) [42]	Vadadustat versus placebo RR 1:1 N = 3923	Left to the investigator's discretion	Encouraged to avoid iron depletion, ferritin <100 ng/ml or TSAT <20%	Encouraged to avoid iron depletion, ferritin <100 ng/ml or TSAT <20%
INNO₂VATE (prevalent) [42]	Vadadustat versus placebo RR 1:1 N = 3554	Left to the investigator's discretion	Encouraged to avoid iron depletion, ferritin <100ng/ml or TSAT <20%	Encouraged to avoid iron depletion, ferritin <100 ng/ml or TSAT <20%
ASCEND D [102]	Daprodustat versus injectable ESA RR 1:1 N = 2964	Route of administration and dose of iron based on the patient's iron status and local clinical practice	Iron therapy administered if ferritin ≤100 ng/ml and/or TSAT ≤20% All iron (excluding multivitamins) stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%	Iron therapy administered if ferritin ≤100 ng/ml and/or TSAT ≤20% All iron (excluding multivitamins) stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%

Table 3: Continued.

Trial	Study characteristics	Choice of the route of administration	Oral	IV
ASCEND TD [103]	Daprodustat versus conventional epoetin RR 2:1 N = 407	Iron dose and route of administration were determined by the investigator	Administered if ferritin ≤100 ng/ml and/or TSAT was ≤20%; stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%	Administered if ferritin ≤100 ng/ml and/or TSAT was ≤20%; stopped if ferritin >800 ng/ml and TSAT >20% or TSAT >40%
ASCEND-ID [104]	Daprodustat versus darbepoetin alfa RR 1:1 N = 312 incident to dialysis	Not specified	Required if ferritin ≤100 ng/ml and/or TSAT ≤20% Halted if ferritin >800 ng/ml and TSAT >20% or TSAT >40% (regardless of ferritin concentration). Investigators could stop administration of iron at a lower ferritin or transferrin saturation level according to local guidelines as long as patients remained iron replete	Required if ferritin ≤100 ng/ml and/or TSAT ≤20% Halted if ferritin >800 ng/ml and TSAT >20% or TSAT >40% (regardless of ferritin concentration). Investigators could stop administration of iron at a lower ferritin or transferrin saturation level according to local guidelines as long as patients remained iron replete

Table 4: Hb increases, iron use and transfusion needs in the main phase 3 randomised clinical trials with HIF-PHI in ND patients.

Study	HIF-PHI molecule	Comparator	Baseline Hb (g/dl)	Hb increase (g/dl)	Iron needed to achieve Hb (mg)[a]	Baseline ferritin (ng/ml)	Iron replete, %	Iron administered, %		Patients with transfusions, %
								IV	Oral	
ANDES [95]	Roxadustat	Placebo	9.1	2.02 versus 0.18	R 839; P 530	R 306 ± 388; P 308 ± 352	R 39.1; P 43.8	R 2.5; P 4.9	NA	R 5.6; P 15.4
ALPS [96]	Roxadustat	Placebo	≈9.1	R 1.98; P 0.4	R 832; P 506	R ≈550; P ≈600	R 52.2; P 53.7	R 5.4; P 5.9	NA	R 8; P 16.7
OLYMPUS [97]	Roxadustat	Epoetin alfa	9.1	R 1.75; P +04	R 794; P 567	R 248.32; P 241,35	R 58.5; P 58	R 4.26; P 7.85	64.4 of the whole cohort	R 12,72; EA 23.26
DOLOMITES [41]	Roxadustat	Darbepoetin alfa	≈9.5	R 2.5; DA 2.3	R 920; P 886	R 525 ± 519; DA 505 ± 466	56.3 versus 51.9	NA. R superior in time to first use of IV iron during weeks 1–36 (HR 0.45 (95% CI 0.26–0.78); P = .004	NA	NA
PRO₂TECT [43] (correction)	Vadadustat	Darbepoetin alfa	9.1 ± 0.8	V 1.43; VA 1.38	V 740; DA 731	V 368 ± 294; DA 360 ± 287	100	At baseline: V 2.5, DA 2.3	At baseline: V 42.7, DA 44.1	V: 5.1; DA: 4.4
PRO₂TECT [43] (conversion)	Vadadustat	Darbepoetin alfa	9.1 ± 0.8	V 0.41; VA 0.42	V 561; DA 570	V 369 ± 285; DA 383 ± 319	100	At baseline: V 5.0, DA 5.7	At baseline: V 46.5, DA 41.1	V 4.0; DA 3.1
ASCEND-ND [44]	Daprodustat	Darbepoetin alfa	9.9 ± 0.9	D 0.74, DA 0.66	D 624, DA 610	[b]D 267 (64–456); DA 275 (171–449)	100	At baseline: D 11.7, DA 11.8	At baseline: D 49.9, DA 49	D 2.8; DA 13.5

[a]Calculated with the Ganzoni's formula: total iron needed (mg) = body weight (kg) × [target Hb − actual Hb (g/dl) × 2.4] + iron stores (mg) with a fixed body weight of 70 kg and fixed estimated iron stores of 500 mg.

[b]Median and interquartile range values.

R: roxadustat; P: placebo; V: vadadustat; D: daprodustat; DA: darbepoetin alfa; HR: hazard ratio; NA: not available.

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Table 5: Hb increases, iron use and transfusion needs in the main phase 3 randomised clinical trials with HIF-PHI in ND patients.

Study	HIF-PHI molecule	Comparator	Baseline Hb (g/dl)	Hb increase (g/dl)	Iron needed to achieve Hb (mg)[a]	Baseline mean ferritin (ng/ml)	Iron replete, %	Iron administered		Patients with transfusions, %
								IV (mean monthly dose in mg and/or % of patients treated)	Oral (% of patients)	
HIMALAYAS [98]	Roxadustat	Epoetin alfa	R 8.4 ± 1.1; EPO 8.5 ± 1.0	R 2.57 ± 1.27; EPO 2.36 ± 1.21	R 931; EPO 896	R 441 ± 337; EPO 437 ± 311	R 77.8; EPO 77.9	R 58.14 ± 110.58; EPO 88.67 ± 122.49	R 83.7; EPO 85.4	R 7.3; EPO 6.4
SIERRAS [99]	Roxadustat	Epoetin alfa	R 10.30 ± 0.66; EPO 10.31 ± 0.66	R 0.39 ± 0.93; EPO 0.09 ± 0.84	R 565; EPO 515	R 1002; EPO 959	R 97.3; EPO 97.8	R 17.1 ± 53.4; EPO 37.0 ± 106.8	NA	R 12.5; EPO 21.1
PYRENEES [100]	Roxadustat	ESA	R 10.75 ± 0.62; ESA 10.77 ± 0.62	[c]R 0.512; ESA 0.286	R 586; ESA 548	R ≈1420; ESA ≈1640	R 86; ESA 87.1	R 25.2; ESA 56.0 (from baseline to EOT) [c]R 12 ± 47.6; ESA 44.8 ± 88.6	NA	R 9.2; ESA 12.9
ROCKIES [101]	Roxadustat	ESA	[b]R 10.2; [b]ESA 10.3	R 0.77; ESA 0.68	R 629; ESA 614	R 542.96; ESA 555.78	NA	R 58.71 mg; ESA 91.37 mg; $P < .001$ for superiority	R 20.7; ESA 18.0	R 9.8; ESA 13.2
INNO₂VATE (incident) [42]	Vadadustat	Darbepoetin alfa	V 9.4 ± 1.1; DA 9.2 ± 1.1	V 1.26 ± 0.11; DA 1.58 ± 0.1	V 740; DA 731	V 469 ± 316; DA 527 ± 401	100	At baseline: V 10.5%, DA 4.8% V 9.9% and DA 6.9% receiving both IV and oral	At baseline: V 50.8, DA 58.5	V 5.1; DA 4.4
INNO₂VATE (prevalent) [42]	Vadadustat	Darbepoetin alfa	V 10.6 ± 0.9; DA 10.2 ± 0.8	V 0.19 ± 0.03; DA 0.36 ± 0.03	V 561; DA 570	V 846 ± 562; DA 840 ± 538	100	At baseline: V 6.9%, DA 6.6%; 4.7% and 4.8[a] receiving both IV and oral	At baseline: V 51.3, DA 48	V 4.0; DA 3.1
ASCEND-D [102]	Daprodustat	Epoetin alfa for HD, darbepoetin alfa for PD	10.4 ± 1.0	D 0.28 ± 0.02; ESA 0.10 ± 0.02	D 547; ESA 516	[b]D 589 (344–976); [b]ESA 604 (341–948)	100	NA	At baseline: D 64.3, ESA 63.8	D 15.7; ESA 18.3
ASCEND-TD [103]	Daprodustat	Epoetin alfa	D 10.44 ± 0.83; EPO 10.59 ± 0.93	D −0.04; EPO 0.02	D 493; EPO 503	[b]D 589 (334–933); [b]EPO 553 (364–918)	100	At baseline: D 64%, EPO 73%	At baseline: D 6, EPO 7	D 2; EPO 2
ASCEND-ID [104]	Daprodustat	Darbepoetin alfa	D 9.46 ± 1.00; DA 9.49 ± 0.97	D 1.02 ± 0.09; DA 1.12 ± 0.09	D 671; DA: 688	[b]D 365 (221–518); DA 373 (239–649)	100	D 142 ± 161; DA 128 ± 137	At baseline: D 16; DA 14	D 12; DA 14

[a] Calculated with the Ganzoni's formula: total iron needed (mg) = body weight (kg) × [target Hb (g/dl) − actual Hb (g/dl) × 2.4] + iron stores (mg) with a fixed body weight of 70 kg and fixed estimated iron stores of 500 mg.
[b] Median and interquartile range values.
[c] Week 28–36 and weeks 1–28.
R: roxadustat; P: placebo; V: vadadustat; D: daprodustat; DA: darbepoetin alfa.

Table 6: Anemia treatment options.

	Short- and long-acting Epos	HIF-PHIs	IV iron	Oral iron
Benefits and advantages	Effective increase in Hb Avoidance of transfusions Improvement in fatigue Modest mobilization of iron Specific targeting to Epo receptors IV administration convenient in HD patients Long-term experience	Effective increase in HB Avoidance of transfusions Improvement in fatigue Mobilization of iron Oral administration Stimulation of 'global erythropoiesis' Possibly more effective in inflamed patients No need for a refrigerator Oral administration possibly convenient in ND-CKD patients or PD	Rapid change in serum iron Reduces Epo resistance?? Improvement in cardiovascular complications Decrease in ESA dose Later start of ESA (especially in ND-CKD)	Oral administration Modest increase in Hb levels Inexpensive
Risks and disadvantages	Can deplete iron stores Thrombotic events Decreased survival/tumour progression? Need for refrigeration (cold chain preservation) Patients with agoraphobia	Can deplete iron stores Thrombotic events Decreased survival/tumor progression? Possible worsening of diabetic retinopathy or maculopathies Possible worsening of pulmonary hypertension Possible worsening of autosomal dominant polycystic kidney disease Central hypothyroidism? Possible off-target effects due to inhibition of other 2-oxoglutarate–dependent enzymes Adherence (pill burden) Oral drug interaction Limited long-term experience	Requires IV infusion Potential of rare but severe hypersensitivity reactions Potential for iron toxicity and accumulation at high doses Potential for increase in bacterial infections Peripheral vein damage in ND-CKD patients in the perspective of an arteriovenous fistula Logistical issues for ND-CKD patients	Poor absorption Nausea or intestinal side effects (diarrhoea or constipation) Oral pill burden Oral drug interaction Less effective than IV administration in increasing Hb

CONCLUSIONS

HIF-PHIs are effective drugs in correcting anaemia in CKD patients and have moved from a hopeful clinical superiority to clinical non-inferiority compared with the present ESAs, with lively debate around relative safety [93].

In assessing which ESA or HIF-PHI would be better for treating anaemia of CKD patients, we should consider patient characteristics, including comorbidities, CKD stage (ND, peritoneal dialysis, home HD or in-centre HD), and the expected adherence to oral or parenteral therapy, including phobia about needles. The fact that HIF-PHIs do not need a refrigerator for their preservation is a favourable aspect, especially in countries where it is difficult to guarantee a strict cold chain and with long travel distances to cover for drug delivery or for holidays. In considering the economic constraints, we anticipate that the cost of HIF-PHIs will impact their marketing penetration and clinical use.

The balance between positive and negative effects of these new drugs (to be further evaluated in the long term) should determine how to treat patients and with which molecule (Table 6). HIF-PHIs may be a better choice in ND patients treated with iron, in those who are intolerant or allergic to iron therapy and in inflamed patients. Conversely, they should be used with cau-

tion in patients with polycystic kidneys, proliferative retinopathy or macular degeneration, pulmonary hypertension, low thyroid-stimulating hormone or severe vascular calcification. Caution is warranted also in patients at risk of thrombosis or suffering from cancer, especially if cure is the anticipated outcome. However, this holds true for all anti-anaemic drugs, including iron products, HIF-PHIs and ESAs. Given their different mechanism of action, HIF-PHIs could be an effective alternative in the rare case of PRCA with ESA therapy [94].

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All the authors contributed equally to manuscript conception, writing and revision.

DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

CONFLICT OF INTEREST STATEMENT

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