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Roxadustat in the treatment of anaemia in chronic kidney disease

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ABSTRACT

Introduction: Anaemia is one of the hallmarks of advanced chronic kidney disease (CKD); it correlates with a lower quality of life and increased cardiovascular risk. Currently its management is based on iron and erythropoiesis-stimulating agents (ESAs) therapy. Given safety issues on ESA therapy and excessive iron use, anaemia management is still suboptimal.

Areas covered: The inhibitors of the prolyl-hydroxylases domain (PHD) are oral drugs which activate the hypoxia-inducible factors (HIF) and stimulate the production of endogenous erythropoietin. Roxadustat (FG-4592) is a second-generation PHD inhibitor; it is undergoing now phase-III clinical development.

Expert opinion: Phase-II clinical trials have shown that roxadustat is effective and save in the short term in either non-dialysis or dialysis CKD patients. Roxadustat is a chemical drug and thus has the potential of being cheaper than traditional ESAs. Given that the peaks of endogenous EPO are much lower than those observed with traditional ESA, it is possible to speculate the roxadustat (and more in general PHD inhibitors) will be safer than ESA on cardiovascular safety end-points. Considering that HIFs are involved in different pathways, with possible promotion of relevant side effects, their safety must be proven in long-term studies.

1. Introduction

Anaemia is a hallmark of advanced chronic kidney disease (CKD) [1]. It is mainly caused by a relative or absolute decrease in the production of erythropoietin (EPO) by the failing kidneys. Other factors, such as absolute or functional iron deficiency, occult blood loss, inflammation, infection, oxidative stress, hyperparathyroidism, and inadequate dialysis, may contribute to its development; they also influence the response to anaemia therapies. Several observational studies have highlighted a clear association between severe chronic anaemia and an increased risk for negative outcome, namely death, cardiovascular comorbidities, and hospitalization [2,3]. Anaemia also negatively influences patient’s quality of life [4].

Since the early 1990s, the management of anaemia has been based on iron and erythropoiesis-stimulating agents (ESAs) therapies in CKD patients. This approach usually reaches target haemoglobin (Hb) levels in a significant percentage of patients and reduces the need for blood transfusions. After more than 20 years of clinical use, there is still no clear evidence that anaemia correction with ESA therapy can significantly reduce the risk for hard end points (namely mortality or cardiovascular events) or simply improve quality of life. Actually, safety issues have been raised in patients who had been treated with ESA aiming at high Hb levels [5]. Moreover, ESAs are expensive drugs and many patients are hyporesponsive to their action, mainly because of the coexistence of inflammation and functional iron deficiency.

The research on drugs stimulating erythropoiesis has received renewed interest following the discovery of the hypoxia inducible factors (HIFs) [6,7] and the development of drugs stabilizing these labile transcription factors. Among many other functions, HIFs regulate the production of endogenous EPO by stimulating its expression in the kidney and liver under hypoxic conditions (Figure 1)[8,9].

2. Overview of the market

The ESA market is huge worldwide. It accounts for nearly $7.2 billion in 2015. In the last 15 years, it has been progressively growing, thanks to the increasing prevalence of CKD throughout the world. However, the market is starting to see a decline for several reasons. First, the entrance of biosimilars, which are cheaper compared to patented drug, has decreased the prices also of the originators. Second, because of safety issues around ESA therapy, Hb values at which start ESA treatments and ESA maintenance doses have decreased worldwide. New drugs entering the market have to face stringent criteria for registration by regulatory bodies, which ask for large, phase-III clinical program to test cardiovascular safety. This implies huge and high-risk economic investments. Despite an expensive clinical development, new drugs need to be more effective and/or safer than ESA or being cheaper to enter this mature and complicate market.

3. Inhibitors of the prolyl hydroxylases domain – mechanism of action

The inhibitors of the prolyl-hydroxylases domain (PHD-i) are a class of small molecules, which mimics the effect of hypoxia.
HIFs are heterodimers consisting of an O₂-sensitive α subunit and a constitutively expressed β-subunit. They are transcription factors that produce a physiological and orchestrated response to reduced tissue oxygen in the body. To obtain this, they activate the expression of target genes that are involved in several functions, spanning from erythropoiesis to angiogenesis, lipid and glucose metabolism, glycolysis, mitochondrial function, cell growth and survival, vasodilation, and cell migration. Many of the HIF-activated pathways are still to be identified. In mammals, there are three HIF-α subunits [10]. HIF-1-α is ubiquitous and together with HIF2-α facilitates O₂ delivery and cellular adaptation to hypoxia by stimulating a number of biological processes [11]. HIF-2-α is expressed in a cell-restricted manner; it is the key regulator of EPO synthesis and iron metabolism [12–14]. The functions of HIF-3α are still partially unknown [15]. Some short HIF-3α variants act as dominant-negative regulators of HIF-1/2α actions, other variants can inhibit HIF-1/2α actions by competing for HIF-β [16]. Two paralogs of the HIF-β subunit (aryl hydrocarbon receptor nuclear translocator [ARNT], ARNT2) have been recognized [16]. Either HIF-1α or HIF-2α can heterodimerize with any of the HIF-β subunits to form functional HIF transcription factor complexes.

Interestingly, HIF-1α is widely expressed and is detected in virtually all innate and adaptive immune populations including macrophages, neutrophils, dendritic cells, and lymphocytes [17]. Recent work has shown that the HIF transcription factors are key elements in the control of immune cell metabolism and function.

PHDs belong to a family of dioxygenase enzymes that require oxygen, iron, and 2-oxoglutarate (2-OG) for their catalytic activity. They split O₂ and couple oxidation (hydroxylation) of HIF-α to oxidative decarboxylation of 2-OG to succinate and CO₂. Both iron and oxygen promote the binding of HIFα to the von Hippel-Lindau protein–elongin B/C complex and then signaling for proteosomal degradation of HIFα [18]. Under normal oxygen conditions, HIF-α half-life is of approximately 5 min as a consequence of PHD degradation. By contrast, under hypoxia, PHD activity decreases and HIF-α accumulates. HIF-α can then bind to the HIF-β subunit (also known as ARNT, resulting in the activation of a large array of target hypoxia-responsive genes [19].

There are three PHD proteins, namely PHD1, PHD2, and PHD3 according to their distinctive prolyl-4-hydroxylase domains [20]. PHD2 plays a dominant role in oxygen sensing and is considered critical in regulating the HIF pathway. In mice with conditional knockout of PHD2, enhanced angiogenesis and increased levels of vascular endothelial growth factor (VEGF)-A and EPO were observed [21]. Interestingly, PHD2 gene mutations have been identified in patients with congenital polycythemia [22]. PHD1 controls the expression level of nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) by hydroxylation-mediated activation of the NF-κB pathway.

**Figure 1.** The mechanism of action of roxadustat compared to ESA.  
HIF: hypoxia-inducible transcription factors; PHD: prolyl hydroxylase domain; ESA: erythropoiesis stimulating agents; EPO: erythropoietin.
inactivation of the inhibitor of NF-κB kinase β during inflammation [23]. In addition, PHD1 is involved in cell proliferation [24]. PHD3 plays major roles in neural development, immune system function, cell migration, and apoptosis [25].

The majority of PHD-i have been developed through structure-based drug discovery programs [26]. They reversibly inhibit PHD catalytic activity by binding to the ferrous-iron-containing active site, thereby blocking the entry of the cosubstrate 2OG. The final result is mimicking the effect of hypoxia on the HIF system. In particular, this process results in an increased endogenous production of EPO by the kidneys. Recently, experimental data have shown that in damaged kidneys, the selective inactivation of PHD1, PHD2, and PHD3 can restore EPO production in myofibroblast-transformed renal EPO-producing cells [27]. Moreover, following liver-specific deletions of genes encoding PHD isofoms, EPO production can occur also in the liver (EPO production by hepatocytes physiologically occurs only during embryonic life) [28]. This explains why PHD-i are effective also in anephric subjects [9].

The positive effect of PHD-i on anemia is not only due to the stimulation of endogenous EPO, but also to a better utilization of iron stores and to an increase in the absorption of iron from the gut to meet increased iron demand for erythropoiesis in the bone marrow. Indeed, the HIF system acts on several steps of iron metabolism [29] (Table 1).

Hepcidin is the key regulator of iron metabolism; its synthesis is stimulated by inflammation and systemic iron excess. Conversely, anemia, hypoxia, and increased erythropoiesis inhibit its expression, thereby increasing the amount of iron available for erythropoiesis. The effect of HIF on hepcidin has been a matter of debate. Following HIF stimulation, hepcidin levels significantly decrease [30]. Some years ago, Peyssonnaux et al. [31] suggested that hepcidin is a direct transcriptional target of HIF-1. However, subsequent studies provided support that the suppression of hepcidin under conditions of HIF activation occurs indirectly via the induction of erythropoiesis [32–34].

This indirect mechanism is likely mediated by the production of erythroferrone by the erythroblasts [35]. In addition to regulating iron metabolism, the HIF–PHD pathway has direct effects on the bone marrow. In hypoxic conditions, it stimulates the expression of the EPO receptor, regulates components of the Hb synthesis pathway, and modulates stem-cell maintenance, lineage differentiation, and maturation [30].

### 4. Roxadustat

Roxadustat (FG-4592) is the second-generation, orally active PHD-i. It has been developed by Fibrogen, Astellas, and AstraZeneca (Box 1). Its precursor, FG-2216, was the first, promising molecule of the PHD-i class that underwent clinical development more than one decade ago. In phase-II clinical studies, it was found effective in increasing Hb levels in healthy volunteers and HD patients [36]. Its clinical development was halted following a case of acute fatal hepatitis (although the death was then found to be not caused by the drug). Of note, the effect of this precursor on plasma EPO levels was higher in HD patients than in healthy volunteers [29].

Roxadustat was developed by the addition of a phenoxy group in the quinolone core of FG-2216.

#### 4.1. Phase-I clinical studies

Roxadustat is orally bioavailable and is predominantly eliminated by phase-I oxidation and phase-II conjugation (glucuronidation and glucosidation). It is highly bound to proteins in human plasma, predominantly albumin.

A single-center, single-dose, open-label study compared the pharmacokinetics and pharmacodynamics of roxadustat in eight subjects with moderate hepatic impairment and eight control subjects with normal hepatic function [37]. After the administration of a single 100 mg dose of roxadustat, exposure to roxadustat (AUC∞) was higher in those with moderate hepatic impairment, while $C_{\text{max}}$ was lower compared with subjects with normal hepatic function. Roxadustat was absorbed rapidly in both groups, with a median $t_{\text{max}}$ of 1.5–2 h; it was eliminated more slowly in subjects with moderate hepatic impairment (17.72 vs. 12.79 h, respectively). In these subjects, the average percentage unbound was only slightly higher; this was not considered to be clinically significant. Altogether, despite these slight differences, no different dosing strategy is needed for subjects with moderate hepatic impairment.

An open-label, single-sequence crossover study was conducted in healthy volunteers to assess possible interference of warfarin therapy on roxadustat parameters [38]. The volunteers received oral doses of 200 mg of roxadustat thrice a week for 2 weeks with or without concomitant warfarin therapy. No clinically significant pharmacokinetic interactions and limited influence on warfarin pharmacodynamic parameters were found.

#### 4.2. Phase-II clinical studies

Table 2 summarizes the phase-II clinical studies that have been published so far.

A phase-II, randomized, placebo-controlled dose-ranging and pharmacodynamics study (NCT00761657) evaluated the efficacy and safety of roxadustat in patients with stage 3 or 4 CKD and with Hb ≤11.0 g/dl [39]. Initially, there were pharmacokinetic/pharmacodynamic cohorts and treatment cohorts. Following a stop of 2 years in 2007 and a protocol amendment, the study was resumed only with treatment cohorts and with lower roxadustat doses to avoid excessive

### Table 1. PHD inhibitors and iron metabolism [30].

<table>
<thead>
<tr>
<th>PHD Inhibitors</th>
<th>Function</th>
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<tbody>
<tr>
<td>Divalent metal transporter 1 (Dmt1)</td>
<td>↑ Duodenal iron uptake</td>
</tr>
<tr>
<td>Duodenal cytochrome b reductase 1 (Dcytb)</td>
<td>↑ Reduction of Fe$^{2+}$ to Fe$^{3+}$</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>↓ (Indirect effect) Iron regulator</td>
</tr>
<tr>
<td>Transferrin</td>
<td>↑ Transport of Fe$^{3+}$</td>
</tr>
<tr>
<td>Transferrin receptor 1</td>
<td>↑ Cellular iron uptake</td>
</tr>
<tr>
<td>Ferroportin</td>
<td>↑ Iron exporter</td>
</tr>
<tr>
<td>Heme-oxygenase-1</td>
<td>↑ Iron recycling</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>↑ Oxidation of Fe$^{2+}$ to Fe$^{3+}$</td>
</tr>
</tbody>
</table>
Hb increases. One hundred and seventeen subjects were randomized to roxadustat (four dose cohorts of 0.7, 1.0, 1.5, and 2.0 mg/kg) or placebo two times weekly (BIW) or three times weekly (TIW) for 4 weeks, with a 3:1 ratio. The patients had been then followed for 12 weeks. ESA, IV iron, androgens, and blood transfusions were prohibited during the 4 weeks of treatment period but allowed during the 12 weeks of follow-up period. Oral iron was always permitted. Nearly half of the patient population was already treated with oral iron at randomization and continued it throughout the study; only a minority started iron therapy during the trial. Roxadustat increased Hb levels in a dose-dependent manner, with a mean change from baseline of 0.8 ± 0.9 g/dl to 2.2 ± 0.8 g/dl. The effect was dose dependent. Similarly, the percentage of patients achieving an Hb response (ΔHb of ≥1 g/dl) was higher at higher doses (30% and 58% in the roxadustat 0.7 mg/kg BIW and TIW groups, and 100% in both 2.0 mg/kg groups). Peak endogenous EPO increased with dose but not frequency. At 2 mg/kg dose, the median peak level was 397 IU/l with a maximal observed value of 705 IU/l. EPO levels returned to baseline within 48 h. Serum hepcidin levels decreased significantly during treatment with roxadustat compared with placebo.

Another phase-II clinical study tested roxadustat in 145 non-dialysis (ND) CKD patients with Hb levels ≤10.5 g/dl [40]. Six randomization cohorts of varying roxadustat starting doses (tiered weight and fixed amounts) and frequencies (BIW and TIW) were foreseen. Hb maintenance was then obtained with roxadustat one to TIW. Treatment duration was 16 or 24 weeks. The placebo control group was not foreseen by the study protocol. One-third of the patients were receiving oral iron at randomization, which was continued throughout the study. At randomization, nearly half of the patients were not iron replete. Similarly to the study mentioned above, a high percentage of patients had an Hb response following roxadustat therapy (cumulative rate of 91.6%). The cohorts with higher weekly starting dose had a shorter median Hb response time. During the maintenance phase, 84% and 96% of initial responders of the two TIW tiered weight dose cohorts (those achieving the best correction rate) were able to maintain Hb levels, despite 20–30% dose reductions in mean weekly maintenance dose. Among those not iron replete at baseline, 92.8% achieved Hb response; mean ΔHb at the end of treatment was similar to that of iron-replete patients. Interestingly, Hb correction was independent of baseline C-reactive protein (CRP). One unexpected finding of this trial was a significant decrease in total serum cholesterol (together with similar reductions in low-density lipoproteins (LDL) cholesterol and increases in high-density lipoproteins (HDL)-to-LDL ratio). Following treatment discontinuation, total cholesterol returned to baseline levels.

Roxadustat was also tested in 90 HD patients treated with epoetin alfa who were randomized to 6 treatment cohorts with various starting doses and adjustment rules (1.0–2.0 mg/kg or tiered weight based) over 25 weeks [41]. The study consisted of two parts, the first to define optimal roxadustat starting dose and the second to compare the drugs. A period of 6 weeks of treatment was observed for the first part of the study, with 8 weeks of follow up, while the second part provided 19 weeks of therapy and 4 weeks of follow up. Intravenous iron was prohibited. Compared to the epoetin alfa group, a significant higher percentage of patients in pooled roxadustat 1.5–2.0 mg/kg groups had ΔHb of +0.5 g/dl or greater from baseline (33% and 79%, respectively). The average roxadustat dose requirement for Hb level maintenance was ~1.7 mg/kg. A positive impact of roxadustat on iron availability was observed. Indeed, in the patients who discontinued iron therapy, a higher decline in serum Hb, transferrin saturation, serum iron, and reticulocyte count was observed during epoetin alfa therapy compared to roxadustat treatment. As for the ND-CKD populations, also in HD patients, roxadustat significantly decreased hepcidin levels; the magnitude of the effect was higher compared to epoetin alfa therapy. Moreover, average weekly roxadustat maintenance dose was not associated
with CRP levels. As expected, the patients treated with epoetin alfa had significantly higher mean peak EPO levels (median dose of 90 U/kg/week, EPO of ~700 mIU/ml) compared to those treated with roxadustat (mean dose of 1.3 mg/kg, EPO of ~130 mIU/ml).

Another open-label, randomized, clinical trial tested the role of iron therapy during roxadustat treatment [42]. Sixty incident dialysis patients were all started to roxadustat therapy for 12 weeks and randomized to no iron, oral iron, or IV iron. Starting from a quite low mean baseline Hb (8.3 ± 1.0 g/dl), roxadustat at titrated doses significantly increased mean Hb within 7 weeks regardless of baseline iron repletion status, CRP level, and iron regimen. Of note, in patients receiving no iron, hepcidin decrease was higher and ΔHb was lower than in iron-treated patients, possibly suggesting that a certain amount of iron therapy is necessary to guarantee erythropoiesis when using PHD-i. Considering that HD patients have different iron requirements from patients on peritoneal dialysis, the enrollment of a mixed dialysis populations is a limitation of this study.

Recently, Chen et al. [43] reported the findings of a phase-II clinical trial, which was conducted in Chinese CKD patients. It included a double-blinded study of 91 ND-CKD patients and an open-label study of 47 HD patients. ND-CKD patients were randomized with a 2:1 ratio to either placebo or roxudastat (low and high dose regimens, 1.1–1.75 and 1.50–2.25 mg/kg, respectively). Eighty percent of the subjects in the low-dose cohort and 87.1% in the high-dose cohort had an Hb increase ≥1 g/dl from baseline versus 23.3% in the placebo arm. HD patients were randomized 3:1 either to roxadustat (sequentially to 1.1–1.8 mg/kg [low], 1.5–2.3 mg/kg [medium], and 1.7–2.3 mg/kg [high]) per dose TIW on dialysis days, using weight-based dosing) or to continue epoetin alfa. Subjects were stratified by baseline epoetin alfa dose. In this cohort, the primary end point was the percentage of subjects with an Hb level maintained at no less than ~0.5 g/dl below mean baseline value. A total of 59.1%, 88.9%, and 100% of subjects randomized to the low-, mid-, and high-roxadustat dose, respectively, met the primary end point as compared with only 50% of the subjects who continued on epoetin alfa. These findings may suggest that Chinese patients have similar dose requirements than Western populations.

Similarly to what observed in the other clinical trials, roxadustat treatment caused a decrease in hepcidin levels and serum cholesterol. Moreover, subjects with the highest platelet counts experienced modest decreases in platelet numbers.

Finally, in a phase-IIb study of ND-CKD and HD patients, the 36-Item Short Form Health Survey and the Functional Assessment of Cancer Therapy-Anemia scores were reported to be significantly improved from baseline after treatment with roxadustat, particularly in patients presenting with low baseline scores [44].

### 4.3. Phase-III clinical studies

Roxadustat has an advanced phase-III development program, with a total of 15 phase-III studies and a target enrolment of about 10,000 patients worldwide. The aim of these studies is to support independent regulatory approvals of roxadustat in both NDD-CKD and DD-CKD patients in the United States, Europe, Japan, and China. Table 3 summarizes the phase-III clinical development of roxadustat. Some of these trials will enroll thousands of patients and will follow them for years to obtain data on hard end points. Notably, two phase-III clinical studies have recently been completed in China [45].

### 5. Safety and tolerability

Altogether, roxadustat was found to be well tolerated in the short term [40–44]. The rate of adverse events was similar or slightly higher than that of placebo or comparator therapy and the majority of reported events were expected for the CKD population. No deaths were reported to be related to roxadustat treatment. From 6% to 20% of the patients treated with roxadustat complained of diarrhea.

Hypertension is a possible side effect occurring with ESA therapy; only a minority of the patients treated with roxadustat reported a worsening in blood pressure control. Given the case of fatal hepatitis that occurred with FG-2216, liver function was monitored closely in phase-II, clinical studies. No evidence of liver toxicity or sustained increases of liver enzymes or serum bilirubin was reported.

One episode of acute pancreatitis was found to be possibly related to roxadustat treatment accordingly to study investigators [42]. One patient developed a first-degree arteriovenous block during roxadustat therapy that reverted to normal at end of treatment [43].

So far published studies of roxadustat did not report data on VEGF changes during roxadustat therapy.

Long-term data of roxadustat safety will be available at the end of the phase-III clinical development. None of the ongoing, phase-III clinical trials have been interrupted so far for safety issues.

### 6. Regulatory affairs

AstraZeneca and FibroGen are collaborating for the development and commercialization of roxadustat in the United States, China, and other markets. In China, FibroGen China is conducting all clinical trials and will hold all roxadustat regulatory licenses and permits to be issued by China regulatory authorities. After market approval, FibroGen China will manage manufacturing and medical affairs and AstraZeneca will manage launch and commercialization activities in China. Very recently, after the conclusion of two phase-III clinical trials [46], the China Food and Drug Administration has accepted the New Drug Application for registration of roxadustat in this country [47].

Astellas and FibroGen are collaborating for the development and commercialization of roxadustat in Europe, Japan, the Commonwealth of Independent States, the Middle East, and Africa. The registration of roxadustat is expected no early than 2019 for the United States and Europe.
<table>
<thead>
<tr>
<th>Clinicaltrials.gov identifier</th>
<th>Estimated patients</th>
<th>Study design</th>
<th>Intervention</th>
<th>Primary end point</th>
<th>Country</th>
<th>Follow-up</th>
<th>Status</th>
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<td>NCT02174731 2100 dialysis patients</td>
<td>Multicenter, randomized, open-label, active-controlled</td>
<td>Roxadustat Epoetin alfa</td>
<td>Mean change in Hb from baseline to week 52 Major adverse CV events (MACE)</td>
<td>North, Central and South America, Europe, Australia, India, Philippines</td>
<td>Event-driven, anticipate 1–2 years</td>
<td>Recruiting</td>
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<td>NCT02273726 820 dialysis patients</td>
<td>Multicenter, randomized, open-label, active-controlled</td>
<td>Roxadustat Epoetin alfa</td>
<td>Hb change from baseline to week 52 in the United States to week 36 extra United States</td>
<td>North, Central and South America, Europe, Asia, Australia, Philippines</td>
<td>52 weeks</td>
<td>Recruiting</td>
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<td>Multicenter, randomized, double-blind, placebo-controlled study</td>
<td>Roxadustat Placebo</td>
<td>Major adverse CV events (MACE)</td>
<td>North, Central and South America, Europe, Asia, Australia</td>
<td>Event driven</td>
<td>Active, not recruiting</td>
<td></td>
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<td>NCT02021318 DOLOMITES 570 ND-CKD patients</td>
<td>Randomized, open-label, active-controlled</td>
<td>Roxadustat Darbepoetin alfa</td>
<td>Hb response without the use of rescue therapy (time frame: up to week 24)</td>
<td>Europe</td>
<td>108 weeks</td>
<td>Recruiting</td>
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<td>Randomized, double-blind, placebo-controlled study</td>
<td>Roxadustat Placebo</td>
<td>Hb response without the use of rescue therapy (time frame: up to week 24) Change in Hb from baseline to week 52</td>
<td>Europe</td>
<td>108 week</td>
<td>Active, not recruiting</td>
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<td>NCT02052310 HIMALAYAS 900 incident dialysis patients</td>
<td>Multicenter, randomized, open-label, active-controlled</td>
<td>Roxadustat Epoetin alfa</td>
<td>United States: Mean Hb change from baseline to week 52 Extra United States: Proportion who achieve an Hb response during the first 24 weeks of treatment</td>
<td>North and South America, Europe, Asia</td>
<td>Approximately 3 years</td>
<td>Recruiting</td>
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<tr>
<td>NCT02278341 PYRENEES 838 dialysis patients</td>
<td>Randomized, open-label, active-controlled</td>
<td>Roxadustat Epoetin alfa Darbepoetin alfa</td>
<td>EU: Hb change from baseline to week 36 without rescue therapy United States: Hb change from baseline to S2 regardless of rescue therapy</td>
<td>Europe</td>
<td>A minimum of 52 weeks up to a maximum of 104 weeks</td>
<td>Active, not recruiting</td>
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<td>Efficacy in anemia correction and maintenance</td>
<td>North, Central, and South America, Asia, Australia, New Zealand</td>
<td>A minimum of 52 weeks and maximum of 52 weeks after the last subject is randomized</td>
<td>Recruiting</td>
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<td>Long-term study</td>
<td>Roxadustat</td>
<td>Hb response rate from week 18 to 24</td>
<td>Japan</td>
<td>52 weeks</td>
<td>Active, not recruiting</td>
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<td>NCT02780141 75 ESA-naives HD patients</td>
<td>A multicenter, randomized, 2-arm, open-label study</td>
<td>Roxadustat</td>
<td>Hb response rate</td>
<td>Japan</td>
<td>24 weeks</td>
<td>Active, not recruiting</td>
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<td>Multicenter, open-label, parallel group</td>
<td>Roxadustat, low dose, high dose, and previously treated with ESA</td>
<td>Hb response rate from weeks 18 to 24</td>
<td>Japan</td>
<td>24 weeks</td>
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<td>Multicenter, randomized, 2-arm parallel, double-blind, active-comparator</td>
<td>Roxadustat Darbepoetin alfa</td>
<td>Hb change from baseline to week 24</td>
<td>Japan</td>
<td>24 weeks</td>
<td>Active, not recruiting</td>
<td></td>
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<td>Multicenter, randomized, 2-arm, open-label</td>
<td>Roxadustat</td>
<td>Hb change from baseline to week 24</td>
<td>Japan</td>
<td>24 weeks</td>
<td>Recruiting</td>
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<td>Multicenter, randomized, open-label, active-comparator conversion study</td>
<td>Roxadustat, darbepoetin alfa</td>
<td>Hb change from baseline to week 24</td>
<td>Japan</td>
<td>52 weeks</td>
<td>Recruiting</td>
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Table 3. (Continued).

<table>
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<th>Intervention</th>
<th>Primary end point</th>
<th>Country</th>
<th>Follow-up</th>
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<td>NCT01630889</td>
<td>50 ND-CKD (phase-II) and dialysis patients (phase-III)</td>
<td>Open-label extension study</td>
<td>Roxadustat</td>
<td>Efficacy and safety</td>
<td>The United States and Puerto Rico</td>
<td>Up to 5 years</td>
<td>Enrolling</td>
</tr>
<tr>
<td>NA</td>
<td>151 ND-CKD patients</td>
<td>Double-blind, placebo-controlled randomized, open-label</td>
<td>Roxadustat Placebo (2:1)</td>
<td>Hb increase</td>
<td>China</td>
<td>8 weeks</td>
<td>Completed</td>
</tr>
<tr>
<td>NA</td>
<td>340 dialysis patients</td>
<td>Randomized, placebo-controlled double-blind, open-label</td>
<td>Roxadustat Epoetin alfa (2:1)</td>
<td>Hb increase</td>
<td>China</td>
<td>26 weeks, 52 weeks safety assessment</td>
<td>Completed</td>
</tr>
</tbody>
</table>

CV: Cardiovascular; ND-CKD: non-dialysis chronic kidney disease; ESA: erythropoiesis-stimulating agent.

7. Conclusions

In the last decades, ESA and iron have been the main therapeutic agents for the management of anemia in CKD patients. Over the last decade, anemia correction and maintenance has become suboptimal due to economic constraints and safety issues surrounding ESA therapy when used at excessive doses or when aiming at too high Hb levels.

Following the discovery of the HIF, several drugs inhibiting the PHD domain have been developed. They simulate the effect of hypoxia on the HIF system, causing a stimulation of endogenous EPO production and an increase in iron availability. Differing from ESA, they can be administered orally. Roxadustat is one agent of this promising class. Accordingly to the data of phase-II clinical studies, roxadustat is effective and safe in both ND and dialysis patients. Interestingly, it seems to be equally effective in inflamed and non-inflamed CKD patients. As an ancillary effect, it can also decrease serum cholesterol. Differing from traditional ESA, roxadustat does not cause significant rises in blood pressure.

Phase-III clinical studies of roxadustat are ongoing in many continents to assess long-term safety.

8. Expert opinion

PHD-i are a new and promising class of oral agents stimulating erythropoiesis.

Differing from ESA, PHD-i stimulate the production of endogenous EPO, thus avoiding or limiting nonphysiological plasma levels of EPO. This could be particularly useful for inflamed patients, who are more likely to be exposed to excessive ESA peaks and intravenous iron doses, and possibly develop complications related to excessive ESA doses. In this particular subset of patients, who are often hyporesponsive to traditional ESA therapy, PHD-i can become a new and effective therapeutic strategy. PHD-i can increase the utilization of functionally blocked iron stores (thanks to a significant reduction of hepcidin levels) and increase iron absorption from the gut. Notably, data from phase-II studies seem to suggest that treatment with roxadustat require less iron therapy compared to traditional ESAs. Given the concerns around excessive iron therapy, especially when given intravenously to patients with functional iron deficiency [6], this represents a potential advantage of PHD-i.

Cardiovascular mortality is the leading cause of death in CKD patients [46]. Roxadustat does not increase blood pressure significantly, possibly decreasing the cardiovascular risk in comparison to ESA-treated patients. Roxadustat also significantly reduces serum cholesterol. Even if the observed decrease affects not only total serum cholesterol and LDL cholesterol but also HDL cholesterol, it increases the HDL-to-LDL ratio. The potential of this ancillary effect of roxadustat is still to be investigated, also considering that hypercholesterolemia not necessarily contribute to the pathogenesis of cardiovascular disease in advanced CKD [48].

HIF stabilizers are the new agents that more likely will modify the anemia management in the next 5–10 years. A huge phase-III clinical development is ongoing with roxadustat and other PHD-i globally, testifying the interest of the developing companies.

Many factors will influence the clinical use of roxadustat; different scenarios can be then imagined. The final selling price of roxadustat will condition its likeliness to deeply penetrate or not the ESA market. Given that roxadustat is a chemical compound with a cheaper manufacturing process compared to ESA, it has the chance to enter the market at a lower price than all traditional ESAs, including biosimilar. However, the final selling price will be influenced not only by the manufacturing process, but also by the costs of its clinical development, which will be huge.

According to phase-II clinical studies, roxadustat is effective as traditional ESAs on anemia correction and maintenance. So, it is likely that, at the end of the phase-III clinical program, it will be confirmed equally effective than ESA (in inflamed patients it may be more effective). Interestingly, its efficacy is similar in dialysis and ND patients; long-term data will prove the capability of the failing kidneys to produce endogenous EPO over long treatment periods.

In addition to the selling price, the other key factor that will influence the future of roxadustat is long-term safety. At the current stage of clinical development, roxadustat has been proven safe. However, the suboptimal safety profile of ESAs on cardiovascular, thromboembolic, and cancer risk could apply also to PHD-i. During ESA therapy, the possible occurrence of
cardiovascular or thrombotic events may be due either to ESA (more likely when given at high doses or in inflamed patients) or to anemia correction (high Hb levels). If the suboptimal cardiovascular safety profile of ESA is caused by high EPO peaks, then roxadustat (and more in general PHD-i) has the potential of having a better cardiovascular safety profile compared to ESA (lower EPO peaks). Differing from ESAs, they seem to have a neutral effect on blood pressure values, possibly further reducing the risk of cardiovascular events.

The HIF system is involved in different pathways other than EPO synthesis. In particular, as a response to hypoxia, it can stimulate angiogenesis, glucose, and fat metabolism. The main mediator of angiogenesis is VEGF. Following PHD-i therapy, VEGF may increase, with a possible promotion of tumor growth and a worsening of diabetic retinopathy. Till now, no abnormalities and no changes in systemic VEGF levels have been reported in patients treated with PHD-i. This aspect is particularly relevant when considering the oncogenic risk related to VEGF synthesis. On the other side around, the activation of the HIF system on vessels and neoangiogenesis may have a protective effect on ischemic tissues.

Given that roxadustat (more in general PHD-i) causes the stimulation of complex and widespread pathways, there is also the potential for the development of unexpected side effects (but also unexpected additional advantages or therapeutic indications other than anemia). The long-term consequences of a chronic ‘hypoxic’ state in patients who are often affected by several comorbidities are also to be elucidated. For instance, some patients with activating mutations of HIF-2 alpha, who have a persistent activation of the HIF system, can develop severe pulmonary hypertension [49].

The oral administration of roxadustat may be helpful in ND-CKD and PD patients. However, it may be not necessarily an advantage in HD patients (who receive ESA intravenously) or if compared to long-acting ESAs with monthly administration. Nevertheless, the thrice weekly administration of roxadustat could remain convenient for HD patients, who could receive the drug at the end of the dialysis session. Conversely, this regimen may become confusing in ND patients, who receive their drug treatment home.

The PHD-i molecules display a different degree of selectivity on the HIF system. While all the molecules act on HIF-2 alpha, many of them also influence the HIF-1 alpha and HIF-3 alpha pathways. These differences in drug selectivity will possibly influence the efficacy and safety profile of the single molecules. According to a molecular comparison of different PHD-i [50], they have similar effects on the upregulation of HIF target genes but differ in the kinetics of their effects and in extent of inhibition of hydroxylation of the N- (NODD) and C-terminal-dependent degradation domains (CODD). In particular, in comparison to the other PHD-i, roxadustat has an intermediate potency in inhibiting HIF-2alpha and it is the only molecule that inhibits both the N- and C-terminal-dependent degradation domains (the other PHD-i inhibits mainly the N-terminal-dependent degradation domain). This different NODD/CODD activity may be of biological relevance [51]. At the present stage of clinical development and in the lack of direct comparisons, it is premature to conclude that any PHD-i have any advantage/disadvantage over another molecule of the class.

Declaration of interest
L. Del Vecchio has served on advisory boards for Astellas and received speakers fees from Roche. F. Locatelli is or was a member of advisory board for Akebia, Amgen and Astellas. He has also been a speaker at symposia supported by Amgen, Astra Zeneca, Fibrogen, Roche, Vifor Fresenius Pharma. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

One peer reviewer declares that they have served on an advisory board for Akebia (producer of vadadustat) and received editorial assistance funded by AstraZeneca (sponsor of roxadustat) in the development of a manuscript they co-authored on HIF-PHIs (published in June 2017).

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.
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• The development of first PHD inhibitors.
• A comprehensive review about the HIF system.
• A comprehensive review about the physiology of the HIF system.
A review covering the link between iron homeostasis and the HIF system.


**A phase-II clinical study of roxadustat in non-dialysis CKD patients.**


**A phase-II clinical study of roxadustat in non-dialysis CKD patients.**


**A phase-II clinical study of roxadustat in hemodialysis patients.**


**A phase-II clinical study of roxadustat in dialysis patients randomised to different iron regimens.**


**A phase-II clinical study of roxadustat in non-dialysis and dialysis Chinese CKD patients.**


