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REVIEW ARTICLE

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Erythropoiesis and chronic kidney disease–related anemia: From physiology to new therapeutic advancements

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Abstract

Erythropoiesis is triggered by hypoxia and is strictly regulated by hormones, growth factors, cytokines, and vitamins to ensure an adequate oxygen delivery to all body cells. Abnormalities in one or more of these factors may induce different kinds of anemia requiring different treatments. A key player in red blood cell production is erythropoietin. It is a glycoprotein hormone, mainly produced by the kidneys, that promotes erythroid progenitor cell survival and differentiation in the bone marrow and regulates iron metabolism. A deficit in erythropoietin synthesis is the main cause of the normochromic normocytic anemia frequently observed in patients with progressive chronic kidney disease. The present review summarizes the most recent findings about each step of the erythropoietic process, going from the renal oxygen sensing system to the cascade of events induced by erythropoietin through its own receptor in the bone marrow. The paper also describes the new class of drugs designed to stabilize the hypoxia-inducible factor by inhibiting prolyl hydroxylase, with a discussion about their metabolism, disposition, efficacy, and safety. According to many trials, these drugs seem able to simulate tissue

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hypoxia and then stimulate erythropoiesis in patients affected by renal impairment. In conclusion, the in-depth investigation of all events involved in erythropoiesis is crucial to understand anemia pathophysiology and to identify new therapeutic strategies, in an attempt to overcome the potential side effects of the commonly used erythropoiesis-stimulating agents.

KEYWORDS
erythropoiesis-stimulating agents (ESA), erythropoietin, hypoxia-inducible factor (HIF), HIF stabilizers, pleiotropic effects

1 | INTRODUCTION

Oxygen is the most common chemical element of the earth crust (47% of the mass), while it is present in the percentage of 21% of the volume and 23% of the mass in the atmosphere. It is essential for life. As known, aerobic organisms including humans require oxygen to produce energy through the oxidation of different substrates (for example, carbohydrates and fatty acids). This is the reason why many efforts are physiologically made to ensure an adequate oxygen delivery to all body cells.

In the blood, molecular oxygen (O_2) is transported by hemoglobin within erythrocytes.

The maintenance of normal blood oxygen levels is the result of complex metabolic pathways involving the kidneys, the bone marrow and many molecules (hormones, growth factors, cytokines, vitamins, etc), which act according to a very rigorous "timetable" to regulate erythropoiesis as needed.[1] Abnormalities in one or more of these factors may induce different kinds of anemia that require different treatments.

Normochromic normocytic anemia is particularly frequent among patients with progressive chronic kidney disease (CKD). The causes are many and include: inadequate production of erythropoietin, erythropoiesis inhibition due to the accumulation of uremic toxins, reduced red blood cell survival, iron deficiency, malnutrition, inflammation, folate and/or vitamin B12 deficiency, dysregulated iron metabolism, oxidative stress, chronic gastrointestinal blood loss, secondary renal hyperparathyroidism, and blood losses during hemodialysis sessions in patients with end-stage renal disease requiring replacement therapy.[2–5]

The severity of anemia has been associated with poor outcomes in patients with CKD. Foley and colleagues demonstrated that mean hemoglobin was an independent risk factor for left ventricular dilatation, development of cardiac failure, and mortality in a cohort of 432 patients on dialysis (hemodialysis or peritoneal dialysis) followed prospectively for about 41 months.[6] Moreover, anemia seems to accelerate the progression of renal damage through different mechanisms. Firstly, low hemoglobin levels reduce oxygen delivery to the kidneys with resulting medullary hypoxia that favors interstitial fibrosis. Anemia may also stimulate renal sympathetic nerve activity and then induce an increase in glomerular pressure and proteinuria, which is another factor contributing to CKD progression.[7]

A complete understanding of the pathophysiology of anemia as well as the correct management of this disorder makes necessary a thorough knowledge of the physiological cascade of events triggered by hypoxia, from the renal oxygen sensor system to the formation of the red blood cell in the bone marrow.

The aim of the present review has been therefore to summarize the most recent findings about each step of this process, also describing all drugs, already available or still experimental, able to promote erythropoiesis in CKD, with a discussion about their metabolism and disposition.

2 | THE OXYGEN SENSING SYSTEM AND THE ROLE OF HIF IN THE KIDNEY

Erythropoietin is a glycoprotein hormone playing a key role in the production of erythrocytes.

The starting point of the erythropoietic process is an oxygen sensing system that is located in the kidney at the level of cells known as renal erythropoietin-producing (REP) cells.

The discovery and identification of REP cells are the result of many experimental studies. At first, it was thought that cells producing erythropoietin were endothelial cells of the renal cortex and external medulla.[8] Later studies showed that erythropoietin is produced by interstitial fibroblasts localized between tubules and capillaries in the kidney mid-cortical region.[9–11] These spaces are characterized by low oxygen delivery and high oxygen consumption; it follows that REP cells detect hypoxia with great sensitivity.[12]

Interestingly, REP cells have shown remarkable plasticity that makes them capable of changing into myofibroblasts under experimental conditions of kidney injury. In such circumstances, REP cells lose their ability to produce erythropoietin and switch to a fibrogenic cellular phenotype, through a mechanism probably involving nuclear factor κB signaling as observed by Souma et al[13] in a mouse model of unilateral ureteral obstruction. These authors also demonstrated that the reversion of inflammation induced by unilateral ureteral obstruction resulted in the recovery of REP cell physiologic phenotype and erythropoietin-producing potential. Then, they concluded that phenotypic transition

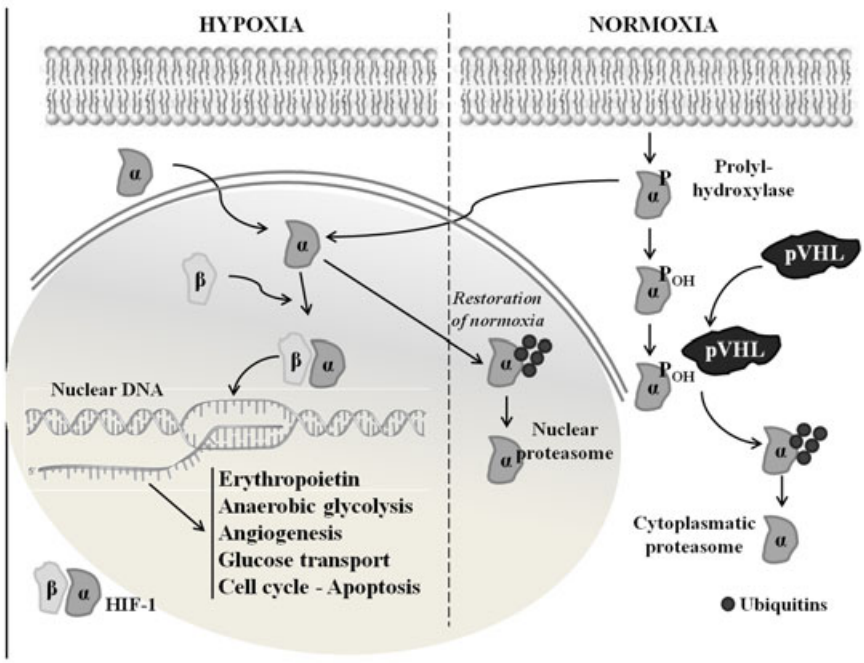

FIGURE 1 Regulation of HIF-1 activity by oxygen. HIF-1 beta subunit is constitutively synthesized whereas alpha subunit expression is regulated by oxygen levels. Under normoxia conditions, HIF-1 alpha is rather unstable and is rapidly degraded (within 5 minutes) in the cytoplasmic proteasomes through a process triggered by prolyl hydroxylase domain (PHD)-containing proteins (activated by O_2 and other factors) and involving the von Hippel-Lindau protein (VHL). When blood oxygen concentration is low, prolyl hydroxylase activity is reduced. Consequently, HIF-1 alpha is not degraded and moves into the nucleus where it binds to the beta subunit to form HIF-1, which in turn stimulates the expression of genes responsible for the adaptation of cells to hypoxia, including the gene codifying for erythropoietin. After oxygen concentration normalization, HIF-1 alpha is degraded at the level of nuclear proteasomes. HIF-1, hypoxia-inducible factor

of REP cells to myofibroblasts could represent the link between anemia and tubulointerstitial fibrosis, which is the pathophysiological event responsible for the histological and functional changes leading to progressive CKD.[14]

Hypoxia induces a complex adaptive response that aims at facilitating cellular metabolism and restoring normal oxygen blood levels through a process involving the transcription factor known as hypoxia-inducible factor-1 (HIF-1). The compensatory mechanisms stimulated by hypoxia include erythropoiesis through increased erythropoietin production, angiogenesis, anaerobic glycolysis, glucose transport, and regulation of cell cycle and apoptosis.[15]

HIF-1 is a heterodimeric transcription factor consisting of two subunits known as alpha and beta. While HIF-1 beta is constitutively synthesized, HIF-1 alpha expression is tightly regulated by oxygen concentrations. When blood oxygen is normal, HIF-1 alpha is highly unstable since it is continually produced and degraded. The process of degradation is started by prolyl hydroxylase domain–containing proteins that are activated by iron, O_2, α-ketoglutarate and reactive oxygen species, and hydroxylate specific HIF-1 alpha proline residues.[16] These modified proline residues are recognized by the von Hippel-Lindau protein, which in turn mediates the recruitment of some proteins (Elongin C and B, CUL2, RBX1, and E2 ubiquitin-conjugating enzyme) with the consequent formation of the E3 ubiquitin ligase complex. Ubiquitination ultimately results in the degradation of HIF-1 alpha in the cytoplasmic proteasomes.[1]

Under hypoxic conditions, prolyl hydroxylase activity is reduced because of oxygen deficiency; it follows that HIF-1 alpha does not undergo degradation and moves into the nucleus where it binds to HIF-1 beta. The formed HIF-1 stimulates the transcription of genes, which allows cell adaptation to hypoxia, including the gene codifying for erythropoietin.[17] In particular, HIF-1 interacts with a hypoxia response element present within an enhancer located at the 3′-terminal of the erythropoietin gene.[18] The following increase in oxygen tension will lead to HIF-1 alpha degradation at the level of nuclear proteasomes (Figure 1).[17]

An important role in the regulation of the erythropoietin gene transcription is played by a GATA factor-binding sequence, known as GATA box, which has been identified in the promoter region of the erythropoietin gene. When blood oxygen is normal, binding of GATA transcription factors to the GATA element inhibits the expression of the erythropoietin gene; conversely, hypoxia reduces such interaction, hence inducing gene transcription.[19]

The adaptive response to hypoxia occurs in all body cells, but erythropoietin gene expression is physiologically limited to the kidney and liver. The cell type–specific erythropoietin synthesis seems to be precisely due to a mechanism involving GATA box. Indeed, it has been reported that GATA factors constitutively repress ectopic erythropoietin gene expression in distal tubular epithelial cells also under hypoxic conditions.[18,20]

3 | ERYTHROPOIETIN: SYNTHESIS AND BIOLOGICAL ACTIVITIES

Erythropoietin is a glycoprotein hormone of approximately 30 kDa mainly produced by the kidneys and only minimally by the liver in adult life, whereas the liver represents the main source during embryological development. The gene codifying for human erythropoietin has been detected on chromosome 7 in the region q11-q22.[21,22]

Erythropoietin synthesis is a key example of oxygen-dependent regulation of gene expression. Hypoxia can increase erythropoietin serum levels up to a thousand times from baseline through the already described mechanism involving the transcription factor HIF-1, with the ultimate purpose of improving oxygen delivery to the tissues.[23] The main function of the hormone is indeed to stimulate erythropoiesis by preventing apoptosis and promoting late maturation of erythroid progenitors in the bone marrow.

Erythropoiesis is also regulated by angiotensin II, the main effector of the renin-angiotensin system, through a mechanism mediated by the angiotensin II type 1 receptor (AT1R). In particular, angiotensin II acts both as a growth factor on erythroid progenitor cells and as an erythropoietin secretagogue, favoring the maintenance of high levels of this hormone despite the increase in hematocrit.[24] This may explain the reduction in hematocrit observed in patients receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.[25]

A recent study suggests that the effects of the renin-angiotensin system on erythropoietin concentrations depend on more complex signaling pathways involving not only angiotensin II and AT1R but also the angiotensin II

type 2 receptor, nitric oxide levels, NADPH oxidase 4 (which produces specific reactive oxidant species products), and heme oxygenase-1 (which protects against oxidative stress and exerts anti-inflammatory actions).[26–28]

The biological activities of erythropoietin are mediated by the interaction with its receptor (erythropoietin receptor [EpoR]), which belongs to the class I cytokine receptor superfamily[29] and is located on the surface of erythrocyte precursor cells.[30]

The human EpoR gene has been detected on chromosome 19 (19p13.3-p13.2) and codifies for a protein made up of 508 amino acids, which is later transported to the cell surface.[31,32] Maximal EpoR expression takes place at the colony-forming unit-erythroid (CFU-E) and proerythroblast stages of erythrocyte differentiation. Almost 1000 receptors for the cell are expressed during this phase while the expression declines as cells mature.[33] EpoR is modulated by other factors, for example, under conditions of iron deficiency transferrin receptor (trf-rec) 2 acts to balance erythrocyte production with the available iron.[34,35]

EpoR expression at the plasma membrane enables the interaction with erythropoietin. Before being inserted in the cell membrane as a transmembrane receptor, this protein undergoes the cleavage of a signal peptide at the N-terminal end as well as modifications because of glycosylation, phosphorylation, and ubiquitination processes.[36,37] A single erythropoietin molecule binds to two EpoRs. The cross linking of the two receptors is followed by the phosphorylation of EpoR and Janus kinase 2 (JAK2), with consequent activation of a molecular signaling cascade responsible for the effects of erythropoietin on the red blood cell formation.[29]

Besides stimulating erythropoiesis to restore tissue oxygen homeostasis, erythropoietin has several pleiotropic actions. These nonerythropoietic effects occur at the level of many organs and tissues including brain, blood vessels, liver, and heart, where the receptor for the hormone is present. Actually, in these cases, erythropoietin seems to interact with a heteroreceptor complex formed by EpoR and a β-common receptor, which is a common subunit of the receptor specific for interleukin-5, interleukin-3, and granulocyte-macrophage colony-stimulating factor.[37]

Through this mechanism erythropoietin is, for example, able to induce vasoconstriction, in such a way determining an increase in arterial blood pressure. The hormone also stimulates angiogenesis and acts as a growth factor also for several other cells in addition to endothelial cells.[38] Thereby, it is able to promote tissue repair and regeneration under experimental conditions of kidney,[39,40] heart[41,42] and central nervous system injury.[43,44]

However, because of the ability to favor angiogenesis and cell regeneration, erythropoietin may not only provide tissue protection but also stimulate cancer development and progression as demonstrated in experimental models[45–47] and observed in human studies.[48,49] Therefore, the pleiotropic properties of this molecule are considered to be responsible for the side effects of the administration of high doses of recombinant human erythropoietin reported in large clinical trials.[50,51]

The difference between the protective effects of erythropoietin in experimental models of tissue damage and the potentially negative results observed in patients receiving ESAs may be explained by the fact that in human studies such effects are observed in vivo and the observation time is generally longer than that of experimental studies. Another important consideration derives from the different affinity for erythropoietin of the two kinds of receptors. In particular, the receptor mediating the pleiotropic effects of erythropoietin has a lower affinity for its ligand compared with the erythropoietic receptor; it follows that higher ESA doses are required to stimulate the nonerythropoietic actions and that doses generally used to treat CKD-related anemia should not be associated with a significantly increased risk of side effects.[52]

4 | RECOMBINANT HUMAN ERYTHROPOIETIN: PHARMACEUTICAL FORMULATIONS, DOSAGES, AND MODALITIES OF ADMINISTRATION

Until the marketing of recombinant human erythropoietin in the 1980s, anemia caused by progressive chronic renal failure required frequent blood transfusions. This implied a high risk of infectious complications, sensitization,

transfusion-induced iron overload, and other adverse effects, in addition to the difficulty of finding enough blood units.[52] The chance to use recombinant human erythropoietin has meant a real revolution for renal patients, inducing a marked improvement of anemia correction and quality of life.

A number of pharmaceutical formulations are currently available, which differ in terms of molecular structure, half-life, and time intervals of administration.

Epoetin alpha and *epoetin beta* have been obtained by the recombinant DNA technology from Chinese hamster ovary cells. They consist of 165 amino acids with a carbohydrate portion that is slightly different between the two forms.[53] Many dosages are available (from 1000 to 40 000 IU for epoetin alpha, and from 500 to 50 000 IU for epoetin beta) and the recommended administration is twice or three times a week according to hemoglobin levels and body weight.

Darbepoetin alpha is derived from recombinant human erythropoietin through the addition of sialic acid residues to the glucidic region. This modification is responsible for a reduced affinity to the erythropoietin receptor, a three-fold longer serum half-life, and an increased biological activity, and allows administering the drug every week or every two weeks.[54,55] The dosing interval may be greater in CKD patients on conservative therapy with stable values of hemoglobin. One micrograms of darbepoetin alpha is approximately equal to 200 IU of epoetin alpha or beta, and the dosages range from 10 to 500 mcg.

A further addition of glucidic residues and sialic acid molecules has led to the development of *methoxy polyethylene glycol-epoetin beta*, which has an even longer half-life (135-139 hours) and can be administered every two weeks or once a month.[56] The dosages vary from 30 to 360 mcg and 15 mcg of methoxy polyethylene glycol-epoetin beta correspond to about 1000 IU of epoetin alpha or beta.

Over the last years, some biosimilars including epoetin zeta (SB309) and epoetin alfa (HX-575) have been marketed for the management of anemia in patients with renal impairment. By definition, biosimilars are not identical to the reference product due to their structural complexity and differences in the production methods. Nevertheless, they are expected to be as safe and effective as the originator drugs.[57]

All these medications, known as erythropoiesis-stimulating agents (ESAs), can be administered both subcutaneously and intravenously. The first way is preferred in patients with CKD not on dialysis, while the second one is basically used in hemodialysis patients.

As mentioned before, erythropoietin not only stimulates red blood cell production but also has potentially harmful pleiotropic actions. In the last decades, several studies have been performed identifying pros and cons of ESA therapy, and the hemoglobin range to be reached in CKD-related anemia has been often resized according to the progressive discovery of the potential side effects of these drugs, primarily represented by the greater risk of cancer progression and cardio- and cerebrovascular events observed in patients treated with higher dose ESAs.[50–52] For example, neoplastic cells were demonstrated to express erythropoietin receptors[58] as well as endothelial cells, and erythropoietin plays a role in neoangiogenesis and progression from progenitor endothelial cells into vessels.[28,59,60] In patients with neoplastic disease, therapy with ESAs promotes tumor cell growth.[61] Recombinant human erythropoietin also induces an increase in platelet production, megakaryocytic mean diameter, and serotonin content, leading to an increased risk of thromboembolic events in parallel with hematocrit increase.[62,63] The most recent KDIGO guidelines[64] recommend initiating ESA therapy when hemoglobin values are between 9 and 10 g/dL in dialysis adult patients (Guideline 3.4.3), whereas in CKD patients not on dialysis ESAs should not be started if hemoglobin is greater than or equal to 10 g/dL (Guideline 3.4.1), unless this could improve the quality of life (Guideline 3.4.4); in any case, hemoglobin concentration should not intentionally rise above 13 g/dL in treated patients (Guideline 3.6). Moreover, great attention must be paid to CKD patients with a history of stroke or malignancy or with active cancer (Guideline 3.3), since they are at higher risk of ESA-related adverse events.

KDIGO guidelines also suggest adjusting ESA dose based on hemoglobin levels, rate of change in hemoglobin concentration, and clinical conditions (Guideline 3.8.2). The optimal increase rate of hemoglobin values in patients receiving initial ESA therapy should be 1.0 to 2.0 g/dL per month, avoiding rises greater than

2.0 g/dL over the same period of time. Indeed, it is advisable to be cautious in correcting hemoglobin too rapidly especially in patients with left ventricular hypertrophy, decreased arterial compliance, and poorly controlled arterial blood pressure.[65]

The increase rate in hemoglobin levels strictly depends on individual ESA responsiveness. Factors inducing resistance to ESA administration include: female (sex), history of cardiovascular disease, overweight, iron deficiency, and inflammation.[66] In particular, CKD patients are characterized by a systemic inflammation that contributes, together with iron status and hypoxia, to regulate the production of the peptidic hepatic hormone hepcidin. Under physiological conditions, high levels of iron stores and transferrin-bound serum iron increase the production of hepcidin, which stimulates the internalization and degradation of the iron transporter ferroportin with consequent reduction in iron uptake from enterocytes and from iron stores to maintain normal iron levels.[67] Inflammation alters these homeostatic mechanisms since inflammatory cytokines such as interleukin-6 induce an increase in serum levels of hepcidin. This event contributes to the pathogenesis of anemia in patients with impaired renal function because hepcidin halts duodenal iron absorption and iron release from the liver and the splenic macrophages involved in the recycling of iron deriving from senescent erythrocytes, reducing iron availability for erythropoiesis.[68,69]

4.1 | Use of ESAs beyond chronic kidney disease

ESAs are also administered in clinical situations other than CKD-related anemia.

Myelodysplastic syndromes (MDSs) include a group of clonal hematopoietic stem cell disorders characterized by cytopenias consequent to ineffective hematopoiesis, and morphological alterations of blood and bone marrow cells. According to the International Prognostic Score System, MDSs can be classified in lower risk and higher risk. Higher risk MDS may potentially evolve toward acute myeloid leukemia, whereas the major issue in the management of patients with lower risk disease is represented by symptomatic anemia resulting in fatigue and transfusion dependency. ESA therapy, with or without the administration of granulocyte colony-stimulating factor, is effective in 40% to 50% of lower risk MDS patients with anemia.[70] Patients who do not respond to ESAs are treated with packed red blood cell transfusions.[71] According to some studies, other potential therapeutic strategies may include lenalidomide, immunosuppressive agents or transforming growth factor-beta inhibitors, with the aim to reduce transfusion needs.[72,73]

ESAs, in addition to transfusions and iron therapy, are also usually used in clinical practice to improve anemia in patients with solid tumors and hematological malignancies receiving chemotherapy.[74] ESAs have been approved for the treatment of chemotherapy-induced anemia since 1993.[75] These drugs have been demonstrated to ameliorate hemoglobin levels and anemia-related symptoms[76] and to reduce the need for red blood cell transfusions. However, there are concerns about the reduction in overall survival and the increased risk of serious adverse events that have been associated with ESA therapy in some studies because of the known nonerythropoietic effects of erythropoietin, especially as regards thrombosis-related complications and cancer progression.[77–79] These results suggest to use ESAs cautiously and not to reach high hemoglobin values in patients with cancer. The last ESMO (European Society of Medical Oncology) Clinical Practice Guidelines[80] recommend considering ESAs in the management of patients with cancer on chemotherapy after the correction of iron deficiency and other underlying causes of anemia other than the malignancy or its treatment [I, A]. More specifically, ESAs should be used in patients with symptomatic anemia under chemotherapy alone [I, A] or combined with radiotherapy [II, B] with hemoglobin <10 g/dL, as well as in patients treated with chemotherapy with asymptomatic anemia and hemoglobin <8 g/dL. According to the same guidelines, the hemoglobin target range to consider is a stable value of about 12 g/dL without red blood cell transfusions [I, A]; moreover, ESA dose increases and changes from one ESA to another in subjects who do not respond within 4 to 8 weeks are not recommended and, in the absence of at least an initial hemoglobin response at this time, ESA therapy should be stopped.

5 | NEW THERAPEUTIC PERSPECTIVES FROM HIF STABILIZERS

Because of the potential harmful effects of ESA administration, research is looking for alternative therapeutic strategies for the treatment of renal anemia. Among them are drugs that seem able to stabilize HIF, with following increase in the transcription of the erythropoietin gene.

These molecules, known as HIF stabilizers, are 2-oxoglutarate analogs that reduce the degradation of the alpha subunit of HIF-1, mediated by prolyl hydroxylation, through a mechanism that simulates tissue hypoxia. The result of this process is the increased expression of erythropoietin in the kidney.[81]

Various studies have been completed and others are currently ongoing to evaluate safety and efficacy of HIF stabilizers in patients with CKD-related anemia (Table 1). The research on clinicaltrials.gov website[82] has been conducted on February 2018.

The first molecule of this class to be studied was FG-2216, which was administered to 12 hemodialysis patients and six healthy subjects achieving a considerable increase in erythropoietin levels. Unfortunately, the use of this compound was withdrawn due to a case of fatal hepatitis.[83] Then, the same company produced a new molecule called roxadustat (FG-4592), and Besarab et al[84] performed a phase 2a study (clinicaltrials.gov *identifier*: NCT00761657) to test its efficacy and safety in anemic CKD patients. The results showed an increase in erythropoietin and hemoglobin levels as well as a reduction in hepcidin concentrations in 116 CKD patients treated with roxadustat (0.7-2.0 mg/kg administered 2 or 3 times per week) compared with the controls within 4 weeks of treatment in a dose-dependent manner. There were no differences in adverse events between the two groups. These authors performed another phase 2 clinical trial[85] including 60 anemic hemodialysis and peritoneal dialysis patients treated with roxadustat for 12 weeks. The drug was administered three times a week and the weekly dose was 4.3 mg/kg. The study showed an average increase in hemoglobin concentration of 2.0 g/dL within 7 weeks of treatment independently from iron status. In addition, the authors found a reduction in hepcidin levels and reported no adverse events. The limitations of this study are the small sample size and the lack of a control group. Another randomized phase 2 study,[86] evaluating the efficacy and safety of the drug, showed that three times-weekly oral roxadustat produced an improvement of hemoglobin levels greater than that achieved with Epoetin alpha in hemodialysis patients, without adverse events. Furthermore, hepcidin levels were lower in the group treated with Roxadustat. Efficacy and safety of Roxadustat (three times or twice a week at varying doses) were also assessed in 145 CKD patients in a phase 2b study (NCT01244763).[87] The results revealed that 92% of patients achieved a hemoglobin increase ≥1.0 g/dL with reduced serum hepcidin levels, independently from baseline C-reactive protein values and iron repletion status. In addition, Roxadustat was well tolerated in all patients. Currently, two phase 3 clinical trials are ongoing to evaluate the safety and efficacy of roxadustat compared to epoetin alpha (NCT02174731) and to epoetin alpha or darbepoetin alpha (NCT02278341) for the treatment of anemia in CKD patients on dialysis.

Another prolyl hydroxylase inhibitor, Vadadustat (AKB-6548), has been studied in a 20-week placebo-controlled phase 2b study (NCT01906489)[88] to assess its efficacy and safety in patients with anemia and stage 3a to 5 CKD compared with controls receiving placebo. The primary endpoint was to achieve a hemoglobin level of 11.0 g/dL or more or a mean increase in hemoglobin of 1.2 g/dL. The study showed that 54.9% of patients treated with vadadustat (450 mg once daily) met the primary outcome compared to 10.3% of controls. The incidence of adverse events was similar in both groups. The same molecule, orally administered for 16 weeks, has been tested in two phase 2 studies to assess its efficacy, safety, and tolerability in nondialysis (NCT03054337) and in hemodialysis patients (NCT02260193); however, the results have not yet been published. Two phase 3 studies are currently recruiting participants to evaluate the effects of Vadadustat compared to Darbepoetin alpha in CKD patients who do not require (NCT02680574) or receive (NCT02892149) hemodialysis treatment. Other trials on Vadadustat are ongoing (NCT03054350; NCT02648347; NCT03140722; NCT02865850) whereas one study (NCT03242967) has been withdrawn because of the revision of study design.

TABLE 1 Ongoing and completed trials on HIF stabilizing compounds

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
FG-2216	[51]	Phase 1 open-label, single-dose study	n = 12 long-term HD patients (of which n = 6 with native kidneys and n = 6 anephric) • n = 6 healthy control subjects	Long-term HD patients and healthy volunteers	Single dose of 20 mg/kg body weight	No control group	7 d	• Biological activity • Pharmacokinetics • Safety	Completed	• EPO raised 30.8-fold in HD patients with kidneys, 14.5-fold in anephric HD patients, 12.7-fold in controls • Reticulocytes increased in nephric HD patients and did not decrease further in the anephric HD group • No significant changes in Hb • HD patients had similar T_{max} and C_{max}, longer half-life, lower apparent clearance, and higher AUC vs controls • No change of FG-2216 levels during the dialysis period • Dialysis removed only a small fraction of the drug dose

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
										• No significant change in laboratory and clinical findings • 17 AEs (two in controls, six in nephric HD and nine in anephric HD patients) recorded (diarrhea, nausea, abdominal discomfort, rash, headache, dizziness, lesion of a radial nerve branch due to venipuncture, hyperkalemia, sweating, arterial hypotension, an accidental scalp wound) • two not drug-related SAEs
Roxadustat (FG-4592)	00761657[52]	Phase 2a multicenter,	• n = 88 patients receiving	Anemia and stage 3 or	0.7, 1.0, 1.5, and 2.0 mg/kg	Placebo	4-wk treatment	• ΔHb from baseline	Completed	• Hb raised in a dose-related

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
		single-blind (subjects) RCT with sequential dose	roxadustat • n = 29 patients receiving placebo	4 CKD	BIW or TIW		period and up to a 12-wk follow-up period	• Proportion of Hb responders (ΔHb ≥ 1.0 g/dL) • Pharmacodynamic evaluation • AE frequency and severity		manner • Maximum ΔHb within the first 6 wk was higher in the 1.5 and 2.0 mg/kg groups • Hb responder rates were dose-dependent and ranged from 30% in the 0.7 mg/kg BIW group to 100% in the 2.0 mg/kg BIW and TIW groups vs 13% in placebo • AEs were similar in all groups
	[53]	Phase 2b open-label study	n = 60 patients on Roxadustat randomly assigned to: no iron (n = 24 HD patients) oral iron (n = 12 HD and n = 12 PD patients) IV iron (n = 12 HD patients)	Anemia in HD and PD	Mean weekly roxadustat dose administered TIW: starting dose: 4.0 mg/kg during the first week 4.3 mg/kg during week 12	No control group	• 12-wk treatment period • 4-wk follow-up period	• Maximum ΔHb • Percentage of patients with ΔHb of ≥1.0 g/dL and median time to Hb response • Changes in iron parameters of TSAT, ferritin, hepcidin,	Completed	• Mean Hb increased by ≥ 2.0 g/dL within 7 wk regardless of baseline iron status, CRP level, iron therapy, or dialysis technique • Maximal ΔHb was 3.1 ± 0.2 g/dL over 12 wk • In groups receiving oral or

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
								soluble transferrin receptor, and reticulocyte Hb content		IV iron, maximal ΔHb was similar and larger than in the no-iron group • Hb response was achieved in 96% of patients • Mean serum hepcidin decreased significantly
	[54]	Phase 2, open-label, active-comparator, safety and efficacy RCT	Part 1: • n = 54 patients randomly assigned 3:1 roxadustat to epoetin alpha Part 2: • n = 67 patients on roxadustat • n = 23 patients on epoetin alpha	HD patients previously treated with epoetin alpha	Part 1: • oral roxadustat doses fixed at 1.0, 1.5, 1.8, or 2.0 mg/kg TIW Part 2: various starting doses	Epoetin alpha	Part 1: 6-wk Part 2: 19-wk	• Hb level response, defined as end-of-treatment ΔHb of −0.5 g/dL or greater from baseline (part 1) and as mean Hb level ≥ 11.0 g/dL during the last 4 treatment weeks (part 2)	Completed	Part 1: • Hb level responder rates were 79% in pooled roxadustat 1.5 to 2.0 mg/kg vs 33% in epoetin alpha groups • Hepcidin decrease was greater at roxadustat 2.0 mg/kg vs epoetin alpha Part 2: • The average roxadustat dose need for Hb level maintenance was ~1.7 mg/kg. The least-squares-

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
										mean ΔHb in roxadustat cohorts was similar to that in epoetin alpha group • Roxadustat significantly reduced mean total cholesterol • No safety concerns were raised
	01244763[55]	Phase 2b multicenter, open label RCT	145 patients randomized in six cohorts each with varying roxadustat starting doses (tiered weight and fixed amounts) and frequencies (BIW and TIW)	Nondialysis CKD patients with Hb ≤ 10.5 g/dL	• Cohorts A and B: starting doses of 1.0-1.7 mg/kg TIW (doses tiered by body weight) • Cohort E: tiered weight starting doses BIW • Cohorts C, D, and F:	No control group	• Screening period of up to 4 wk • Treatment period of 16 or 24 wk • Follow-up period of 4-wk	• Cumulative proportion of patients in each cohort achieving Hb response by the end of week 16 • Effect on Hb levels • Safety • Assessment of hepcidin, serum iron, transferrin, TSAT, and ferritin	Completed	• 92% of patients achieved Hb response • Higher starting doses led to earlier achievement of Hb response than lower ones • Hb increases were independent of baseline CRP levels and iron repletion status • Hepcidin values decreased • Reticulocyte Hb content was

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
					50, 100, and 70 mg TIW respectively (fixed starting doses)do			• Lipid profiles		maintained, and Hb increased • Total cholesterol decreased • No SAEs were recorded
	02174731	Phase 3 multicenter, open-label, active-controlled RCT	2070 patients (estimated)	Anemia in HD or PD	Dose not specified administered TIW to achieve an Hb level of 11 g/dL and maintain a Hb level of 11 ± 1 g/dL	Epoetin alpha	52 wk	• ΔHb averaged over week 28 to week 52 • Major adverse CV events • Time to first occurrence of any-cause death, stroke, MI, unstable angina or HF, vascular access thrombosis, deep vein thrombosis, pulmonary embolism, hypertensive emergency • Time to first rescue	Recruiting (estimated study completion date: April 2, 2018)	–

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
								therapy • Changes in self-reported health status		
	02278341	Phase 3 open-label, active-controlled RCT	838 patients	Anemia in ESRD on stable dialysis	• Doses not specified • Dose adjustments allowed	Epoetin alpha and darbepoetin alpha	• Screening period: up to 6 wk • Treatment period: from 52 up to 104 wk • Follow-up period: 4 wk	• ΔHb from baseline to the average Hb of weeks 28 to 36 (primary endpoint)	Active, not recruiting (estimated study completion date: July 2018)	–
Vadadustat (AKB-6548)	01906489[56]	Phase 2b multicenter double-blind, placebo-controlled RCT	• n = 138 vadadustat • n = 72 placebo	Anemia in stage 3A to 5 nondialysis-dependent CKD	• Once-daily vadadustat started at 450 mg, titrated (minimum	Placebo	20 wk	• Percentage of patients who in the last 2 wk achieved or maintained a mean Hb of ≥11.0 g/dL or an increase in	Completed	• The primary endpoint was met in 54.9% of patients on vadadustat and 10.3% of patients on placebo • Increases in

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
					of 150 mg, maximum of 600 mg) based on Hb response • Dose adjustment allowed • Drug suspended if Hb ≥ 13.0 g/dL			Hb of ≥1.2 g/dL over the predose average (primary endpoint) • Hb levels • ESA and transfusion rescue • Absolute reticulocyte count • Iron parameters • Dosage, compliance • AEs and SAEs		reticulocytes and TIBC and decreases in serum hepcidin and ferritin levels were observed in patients on vadadustat • The overall incidence of AEs was similar • SAEs occurred in 23.9% and 15.3% of the vadadustat- and placebo-treated patients, respectively • Three deaths occurred in the vadadustat arm
	03054337	Phase 2 double-blind, placebo-controlled, dose-finding RCT	51 patients	Anemia in nondialysis-dependent CKD	Doses not specified • Daily dose 1 • Daily dose 2 • Daily dose 3	Placebo	16 wk	• ΔHb • Time to reach target Hb from baseline • Need for RBC transfusion or rescue with an ESA • AEs and SAEs • Pharmacokinetics • Pharmacodynamics	Completed	No results posted

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
	02260193	Phase 2 open-label non-RCT	94 patients	Anemia in chronic HD patients	• Doses not specified • Dose adjustment based on Hb • Three groups: starting dose 1, once daily; starting dose 2, once daily; starting dose 3, TIW	No control group	16 wk	• Hb response • Number of subjects requiring transfusion and/or ESA rescue • AEs, vital signs, ECGs, laboratory results) • Concentration of drug and its metabolites pre- and post-dialysis • Iron metabolism • IV iron utilization	Completed	No results posted
	02680574	Phase 3 multicenter, open-label, active-controlled RCT	2100 patients (estimated)	Anemia in nondialysis-dependent CKD	Doses not specified	Darbepoetin alpha	Event-driven end of study, minimum 1 y	• ΔHb • Major adverse CV events • Proportion of subjects with mean Hb within target range • AEs and SAEs	Recruiting (estimated study completion date: September 2019)	–
	02892149	Phase 3 multicenter, open-label,	2200 patients (estimated)	Anemia in dialysis-dependent	Doses not specified	Darbepoetin alpha	Event-driven end of	• ΔHb • Major adverse CV	Recruiting (estimated study	–

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
		active-controlled RCT		CKD			study, minimum 1 y	events • Proportion of patients with mean Hb within target range • AEs and SAEs	completion date: September 2019)	
	03054350	Phase 2 double-blind, placebo-controlled, dose-finding RCT	48 patients (estimated)	Anemia in dialysis-dependent CKD	Three experimental arms receiving three different not specified doses	Placebo	16 wk	• ΔHb • Time to reach target Hb level • ΔHb between pre-treatment and end of dose adjustment and maintenance period • Need for RBC transfusion or rescue with an ESA • AEs and SAEs	Active, not recruiting (estimated study completion date: December 2017)	–
	03242967	Phase 3 open label, active-controlled RCT	0 patient	Anemia in dialysis-dependent CKD	Doses not specified TIW	Darbepoetin alpha	52 wk	• ΔHb • AEs and SAEs	Withdrawn (revised study design)	–
	02648347	Phase 3 multicenter, open-label, active-	1000 patients (estimated)	Anemia in nondialysis-dependent CKD	Doses not specified	Darbepoetin alpha	Event-driven end of study,	• ΔHb • Major adverse CV events	Recruiting (estimated study comple-	–

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
		controlled RCT					minimum 1 y	• AEs and SAEs	tion date: September 2019)	
	03140722	Phase 2 open-label RCT	50 patients (estimated)	Anemia in dialysis-dependent CKD patients hyporesponsive to ESAs	Doses not specified, adjustable based on Hb level	Epoetin alpha	Treatment period: 20 wk	• ΔHb • Proportion of subjects receiving epoetin alpha rescue or RBC transfusion • AEs and SAEs	Active, not recruiting (estimated study completion date: December 2018)	–
	02865850	Phase 3 multicenter, open-label, active-controlled RCT	400 patients (estimated)	Anemia in CKD patients who have recently initiated dialysis	Not specified	Darbepoetin alpha	52 wk	• ΔHb • Major adverse CV events • AEs and SAEs	Recruiting (estimated study completion date: September 2019)	–
Daprodustat (GS-K127886-3)	01047397[57]	Phase 2a, multicenter, single-blind, placebo-controlled, parallel-group RCT	• n = 70 CKD patients • n = 37 HD patients	• Anemia in stages 3-5 CKD • Anemia in HD	• CKD patients received GS-K1278863 (10, 25, 50, or 100 mg) once daily • HD patients received GS-K1278863	Placebo	28 d	• Rate of response in achieving target Hb • Rate of Hb increase • Absolute Hb value and maximum change from baseline • Rate of Hb decrease after stopping	Completed	• Both groups showed a dose-dependent increase in EPO, reticulocytes and Hb levels • The main cause for withdrawal (CKD patients, 30%; HD patients, 22%) was a high rate of Hb increase or a high absolute Hb

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
					(10 or 25 mg) once daily			therapy • AEs, vital signs, safety laboratory tests, ECGs, clinical monitoring • Pharmacokinetics • Pharmacodynamics (change in endogenous EPO, Ht, reticulocyte and total RBC count, VEGF, TIBC, TSAT, iron, hepcidin, fetal Hb, ferritin)		concentration • The experimental group showed a dose-dependent decrease in hepcidin and an increase in total and unsaturated iron binding capacity
	01587898 [58]	Phase 2a, double-blind placebo-controlled, parallel-group, multicenter RCT	n = 73	Anemia in stages 3-5 CKD patients not taking rhEPO and not on dialysis	0.5, 2, or 5 mg once daily, oral dose	Placebo	• Screening phase: up to 2 wk • Treatment phase: 4 wk • Follow-up phase:	• Hb response • Change from baseline in hepcidin, ferritin, transferrin, TSAT, TIBC, total iron, hsCRP, Ht, reticulocytes, EPO, RBC count, VEGF	Completed	• Hb increased in a dose-dependent manner; the highest dose induced a mean increase of 1 g/dL at week 4 • VEGF did not increase significantly. • The drug was generally safe and

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
							2 wk	and other parameters of routine laboratory • AEs and SAEs • Effects on BP, heart rate, ECG parameters • Pharmacokinetics		well tolerated.
	01587924 [58]	Phase 2a, active-controlled, parallel-group, multicenter RCT	n = 83	Anemia in CKD patients on HD being treated with stable doses of rhEPO	0.5, 2, or 5 mg once-daily, oral dose	Continuing on rhEPO	• Screening phase: 2 wk • Treatment phase: 4 wk • Follow-up phase: 2 wk	• Hb response • Change from baseline in hepcidin, hsCRP, EPO, VEGF, Ht, transferrin, TSAT, ferritin, total iron, TIBC, RBC count, reticulocytes, and other parameters of routine laboratory • AEs and SAEs • Pharmacokinetics • Effects on	Completed	• Mean Hb levels were maintained after the switch from rhEPO in the 5-mg arm; conversely, mean Hb decreased in the lower-dose arms. • The increase in endogenous EPO levels was lower than that observed in the rhEPO control group. • VEGF did not increase significantly. • The drug was

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
								BP, heart rate, ECG parameters		generally safe and well tolerated.
	01977573	Phase 2b multicenter, controlled, parallel group RCT	252 patients • Group 1: rhEPO naïve randomized to receive either GSK1278863 once daily or rhEPO in a 3:1 fashion • Group 2: rhEPO users randomized in a 1:1 fashion to GSK1278863 once daily or to the control arm	Anemia in CKD not on dialysis	Film-coated tablets containing 0.5, 1, 2, 5 mg	Locally sourced rhEPO as necessary per standard of care	• Screening phase of at least 4 wk • 24-wk treatment phase • follow-up visit approximately 4 wk after completing treatment	• Summary of Hb level at week 24 • Change or percent change from baseline in hepcidin, serum EPO, VEGF, ferritin, total iron, transferrin, TSAT, TIBC, reticulocyte Hb, Ht, RBC count, reticulocyte count • Pharmacokinetics • Characteristics of dose adjustments • Number of patients receiving blood transfusions,	Completed	No published results

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
								IV iron or rhEPO • Safety		
	02876835	Phase 3 multicenter open-label (sponsor-blind), active-controlled, parallel-group, event-driven RCT	4500 patients (estimated)	Anemia in CKD not on HD	• The initial dose of oral daprodustat for ESA naïve patients is based on Hb and for ESA users on prior ESA dose. • Dose is adjusted to achieve the target range.	Darbepoetin alpha • The initial dose for ESA naïve patients is based on Hb and weight, and for ESA users on converting the prior ESA dose to the nearest available study darbepoetin alpha dose • Dose is adjusted to achieve	Event-driven end of study, up to 4.1 y	• Time to the first occurrence of adjudicated major adverse CV events • ΔHb and percentage of responders (mean Hb within range) • Time to progression of CKD • All-cause and CV mortality • Time to first occurrence of hospitalization • Effects on BP • Time to stopping randomized treatment due to meeting rescue criteria	Recruiting (estimated study completion date: August 17, 2020)	–

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
						the target range		• Mean change in SF-36 Health Related Quality of Life (HRQOL) scores		
Molidustat (BAY 85-3934)	02064426	Phase 2 controlled, parallel group, open-label, multicenter extension RCT	88 patients	Anemia in CKD on dialysis	• Titrated dose treatment (15, 25, 50, 75, 100, and 150 mg once daily) • Same treatment received in the parent study (16208)	Epoetin alpha/beta • Same treatment received in the parent study (16208)	36 mo	• Change in Hb level • SAEs • Maintenance in Hb target range • Duration of treatment exposure • Number of subjects requiring dose titration • Change of RBC and reticulocyte count, Ht, Hb from baseline of this study and from baseline of study 16208	Completed	No results posted
JTZ-951	01971164	Phase 1 single-blind (patient),	29 patients	Anemia in ESRD on HD	Sequential ascending doses once	Placebo	15 d	• AEs • Vital signs and ECG	Completed	No results posted

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
		placebo-controlled, RCT			daily			• Pharmacokinetics • Pharmacodynamics • RBC count, Hb, TSAT, ferritin		
	JapicCTI-152881[60]	Phase 2 multicenter study Part 1: (initial treatment period): double-blind study Part 2: (extended treatment period): open study	160 patients	Anemia in CKD not requiring dialysis • Group 1, ESA-naive: Hb ≥ 8.0 and ≤10.5 g/dL • Group 2, ESA-treated: Hb ≥ 9.5 and ≤12.0 g/dL	Doses not specified	No control group	Not specified	• Hb level • Dose-response and safety • Maintenance dose and safety in extended exposure	Completed	No results posted
DS-1093a	02299661	Phase 1 study Part A: open-label,	31 patients	Part A: Anemia and stage 3b or 4	Part A: Three single doses:	No control group	28 d post-dose	• Drug plasma concentrations • Change in serum EPO	Completed	No results posted

(Continues)

TABLE 1 (Continued)

Drug	NCT Identifier and/or [ref.]	Study design	No. of patients	Study population	Dose of study drug	Control	Vintage	Outcome(s)	Status	Main results
		noncontrolled, parallel group pilot RCT Part B: open, noncontrolled study		CKD Part B: CKD subjects (n = 6) receiving HD	• 7.5 mg • 25 mg • 50 mg Part B: Single dose determined based on data from Part A			• Change in Hb, Ht, reticulocyte and RBC count • Change in VEGF and iron metabolism parameters (iron, transferrin, TSAT, hepcidin-25) • AEs		

AE, adverse event; AUC, area under the curve; BIW, twice a week; BP, blood pressure; CKD, chronic kidney disease; CRP, C-reactive protein; CV, cardiovascular; ECG, electrocardiogram; EPO, erythropoietin; ESA, erythropoiesis-stimulating agent; ESRD, end-stage renal disease; Hb, hemoglobin; HD, hemodialysis; HF, heart failure; hsCRP, high sensitivity C-reactive protein; Ht, hematocrit; IV, intravenous; MI, myocardial infarction; PD, peritoneal dialysis; RBC, red blood cell; RCT, randomized clinical trial; rhEPO, recombinant human erythropoietin; SAE, serious adverse event; TIBC, total iron binding capacity; TIW, three times a week; TSAT, transferrin saturation; VEGF, vascular endothelial growth factor; vs, versus; ΔHb, mean Hb change.

Daprodustat (GSK1278863), another orally administered HIF-inhibitor, has been evaluated in a phase 2a study to estimate its efficacy and safety in anemic CKD patients and anemic hemodialysis patients (NCT01047397).[89] Both groups were randomized to Daprodustat (10, 25, 50, or 100 mg once a day) or placebo for 28 days. The results showed that this molecule is able to increase erythropoietin concentrations, reticulocyte, and hemoglobin levels and to decrease hepcidin values in both groups in a dose-dependent manner compared with placebo. Holdstock et al[90] conducted two phase 2a studies to evaluate the hemoglobin response to Daprodustat within 4 weeks of treatment with an oral single dose (0.5, 2, or 5 mg) in anemic CKD (NCT01587898) and anemic hemodialysis (NCT01587924) patients. Participants were randomized to Daprodustat or control therapy (placebo for CKD patients and recombinant human erythropoietin for hemodialysis patients). Both studies demonstrated that this drug was effective in increasing the level of hemoglobin compared to controls and was generally safe and well tolerated at the doses and duration evaluated. A phase 2b randomized clinical trial has been performed on 252 CKD patients not on dialysis but results have not yet been published (NCT01977573). Currently, a phase 3 study is recruiting participants to examine the safety and effects of daprodustat compared to darbepoetin alpha in CKD patients not on hemodialysis (ASCEND-ND study; NCT02876835).

A novel HIF stabilizer is Molidustat (BAY 85-3934). Experimental studies showed that the oral administration of Molidustat resulted in the increase of hemoglobin levels compared with controls, and it was also effective in rats with renal anemia.[91] A phase 2b study in hemodialysis patients is currently in progress (NCT02064426).

Other HIF stabilizers such as JTZ-951 (NCT01971164; JapicCTI-152881)[92] and DS-1093a (NCT02299661) were tested in clinical trials but results are not available.

In conclusion, the use of HIF stabilizing compounds stimulates the physiological erythropoietin synthesis, avoiding those circulating erythropoietin peaks associated with the administration of ESA that may be responsible for negative and dangerous effects. Furthermore, the oral administration would facilitate their use in particular in patients with CKD on conservative therapy.

6 | CASCADE EVENTS TRIGGERED BY ERYTHROPOIETIN IN THE BONE MARROW ERYTHROID PROGENITOR CELLS

Erythropoietin released by the kidneys reaches the bone marrow through the blood circulation. Here, it acts on erythroid progenitor cells favoring cell survival and differentiation and regulating iron metabolism. The binding of erythropoietin to its receptor generates a cascade of events leading in the end to an increase in erythrocyte production (Figure 2).

After binding, the erythropoietin-EpoR complex is activated, internalized, and some is degraded in lysosomes, with the remaining recycled to the cell surface.

The increase in the intracellular concentration of calcium ions (Ca^{2+}) is one of the starting events happening in erythroid progenitor cells under erythropoietin stimulation. Such enhanced Ca^{2+} influx depends on erythropoietin dose and occurs through the calcium-permeable transient receptor potential channels of canonical type (transient receptor potential canonical [TRPC] channels). Calcium is a universal intracellular second messenger triggering many cell functions; in erythroid cells, it has a fundamental role by inducing colony's growth and acting during terminal stages of differentiation. Particularly, Ca^{2+} uptake contributes to the differentiation and proliferation of erythroid precursors at the stages of burst-forming unit-erythroid (BFU-E) and CFU-E. This has been demonstrated by the inhibition of erythroid precursor's differentiation in Ca^{2+}-free medium. Novel signaling pathways of erythropoietin may be identified by studying the mechanisms regulating TRPC channels activation, membrane expression, and physiological function in erythroid cells. This may further lead to new approaches for therapeutic intervention in diseases involving abnormal erythropoiesis.[93–95] Moreover, a recent analysis in red blood cells confirmed a direct correlation between the intracellular concentration of free Ca^{2+} and the hemoglobin oxygen saturation.[96]

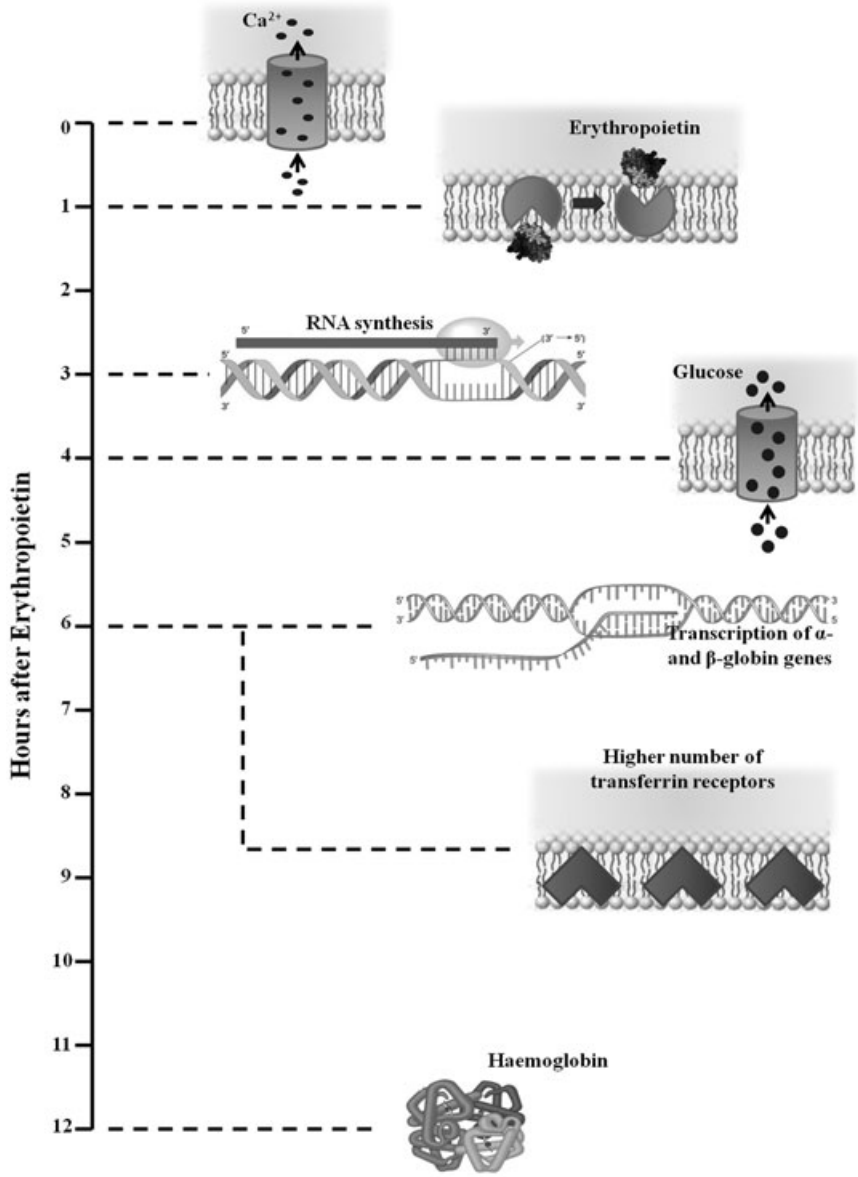

FIGURE 2 Cascade of events triggered by the binding of erythropoietin to its receptor. One of the starting events is represented by an increase in the intracellular concentration of calcium ions (Ca^{2+}) through the calcium-permeable transient receptor potential channels of canonical type (TRPC channels). Afterward, the activation of several signaling pathways leads to an increase in RNA synthesis. The changes in the expression of specific genes induced by erythropoietin allow enhancing hemoglobin and heme synthesis and alpha and beta globin assembly. At this stage of red blood cell production, erythropoietin also stimulates the cell surface expression of transferrin receptors to provide an adequate iron uptake. TRPC, transient receptor potential canonical

The second step after erythropoietin signal activation is an increase in RNA synthesis in bone marrow cells. In vitro, it happens within 15 minutes after the addition of an erythropoietin extract. RNA synthesis is triggered by a series of redundant stimuli. Several signaling cascades are involved in mediating the erythropoietin-induced changes in gene expression and subsequent effects on cell differentiation, proliferation, and survival. At first, erythropoietin induces a quick and transient EpoR tyrosine phosphorylation that returns to the basal level within

15 to 30 minutes. JAK2 tyrosine kinase is constitutively associated with the EpoR and its activation by dephosphorylation is the first step of erythropoietin intracellular signaling. JAK2 activation leads to the phosphorylation of several proteins including the EpoR itself, which undergoes phosphorylation of eight tyrosines located within the intracellular domain, and other proteins (SHP1, SHP2, phosphatidylinositol 3-kinase [PI3K], SHIP, Shc, STAT5, and CIS), whose phosphorylation triggers their respective signaling pathways. Among them, erythropoietin activates the PI3K signaling cascade directly by recruiting the p85 regulatory subunit to EpoR Y479, and indirectly through binding to Casitas B lymphoma (Cbl), Gab1, Gab2, and insulin-receptor substrate 2.[97]

The PI3K-mitogen–activated protein (MAP) kinase pathway is the principal CFU-E's differentiation way, and it is sufficient for normal erythroid differentiation induction. More in detail, PI3K activates the ERK2 (extracellular-signal-regulated)-MAP kinase through a Ras-independent pathway. Downstream protein kinase B (PKB)/Akt is activated by PI3K, and afterward many downstream substrates of PKB/Akt have been investigated, including the forkhead box O3A (FoxO3a) transcription factor, which is essential in erythropoiesis.

Moreover, erythropoietin activates two isoforms of the signal transducer and activator of transcription pathway (STAT5A and STAT5B). Especially, the phosphorylated STAT5 dissociates from the receptor, dimerizes, moves to the nucleus and turns on gene expression. Finally, STAT5 activates transcription of the Bcl2-related protein long isoform (BclXL) gene promoting cell proliferation. Indeed, BclXL has a leading role in preventing the apoptosis of primitive and definitive erythrocytes at the end of maturation. However, the precise role of STAT5 in erythropoiesis became the protagonist of many scientific controversies. In some trials it resulted to be responsible for differentiation ad proliferation of erythroid cells.[98] On the other hand, mice defective in both STAT5 isoforms A and B did not show any evident abnormality in erythrocyte production, thus STAT5 seems not to have an essential role in erythroid development. Probably, STAT5 activation alone is not sufficient to support normal erythroid differentiation.[99]

Other actors in red blood cell growth are CIS and the tyrosine phosphatase SHP, which exhibit a negative regulator effect on CFU-E differentiation. In particular, SHIP-1 null mice showed elevated formation of BFU-E and CFU-E in bone marrow.[100]

Erythropoietin/EpoR signals therefore control the expression of selected cell-cycle regulatory genes that are proposed to modulate stage-specific decisions for erythroblast cell-cycle progression.

Erythropoietin has a well-known effect enhancing hemoglobin and heme synthesis and alpha and beta globin assembly. CFU-E showed a decreased hemoglobin synthesis in the absence of erythropoietin, while it was significantly augmented after about 5 hours of incubation with erythropoietin.[101]

Finally, a sufficient iron reserve is a fundamental prerequisite of red blood cell production. Erythropoietin is an important regulator of iron homeostasis as well, for example an association between the increase in trf-rec messenger RNA levels and erythropoietin levels has been demonstrated starting from the 1997. In particular, erythropoietin regulates the cell surface expression of trf-recs, thus increasing iron uptake.[102] This is the reason why the concomitant administration of erythropoietin and iron improves the response to ESA therapy in CKD anemia.

7 | CONCLUSIONS

Anemia is commonly observed in patients with progressive CKD. The marketing of recombinant human erythropoietin in the 1980s has revolutionized the treatment of renal anemia, which before required frequent blood transfusions. However, due to the potential harmful effects of ESAs administration, research is looking for alternative therapies.

The in-depth investigation of all pathophysiological events involved in erythropoiesis has suggested the idea to build molecules able to stabilize HIF to simulate tissue hypoxia and then amplify the transcription of the erythropoietin gene. According to many trials, these drugs effectively increase hemoglobin levels and are

generally safe and well tolerated. However, longer studies performed on larger samples are needed to confirm these results and guarantee about the safety of such therapeutic strategies in the long term, considering that HIF has a ubiquitous localization in human tissues and intervenes in a lot of physiological and pathophysiological processes.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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