

# Physiology and Pharmacology of Erythropoietin

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## Keywords

Anemia · Chronic kidney disease ·  
Erythrocytic progenitors · Hypoxia ·  
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## Summary

Human erythropoietin (Epo) is a 30.4 kDa glycoprotein hormone composed of a single 165 amino acid residues chain to which four glycans are attached. The kidneys are the primary sources of Epo, its synthesis is controlled by hypoxia-inducible transcription factors (HIFs). Epo is an essential factor for the viability and proliferation of erythrocytic progenitors. Whether Epo exerts cytoprotection outside the bone marrow still needs to be clarified. Epo deficiency is the primary cause of the anemia in chronic kidney disease (CKD). Treatment with recombinant human Epo (rhEpo, epoetin) can be beneficial not only in CKD but also for other indications, primarily anemia in cancer patients receiving chemotherapy. Considering unwanted events, the administration of rhEpo or its analogs may increase the incidence of thromboembolism. The expiry of the patents for the original epoetins has initiated the production of similar biological medicinal products ('biosimilars'). Furthermore, analogs (darbepoetin alfa, methoxy PEG-epoetin beta) with prolonged survival in circulation have been developed ('biobetter'). New erythropoiesis-stimulating agents are in clinical trials. These include compounds that augment erythropoiesis directly (e.g. Epo mimetic peptides or activin A binding protein) and chemicals that act indirectly by stimulating endogenous Epo synthesis (HIF stabilizers).

## Introduction

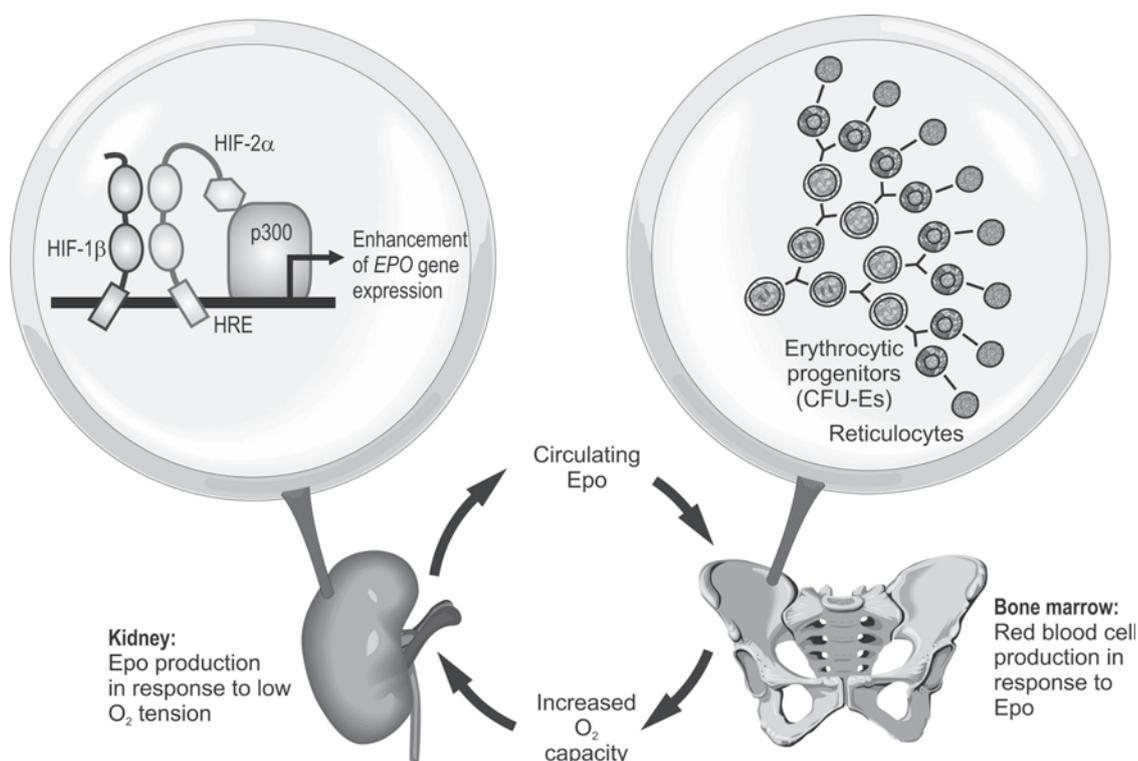
The viability, proliferation, and differentiation of hematopoietic stem and progenitor cells are controlled by pleiotropic and blood cell lineage-specific factors. The production of red blood cells (RBCs) is regulated by the hormone erythropoietin (Epo), which maintains the blood hemoglobin (Hb) concentration constant under normal conditions. RBCs circulate for 100–120 days. Then, they are engulfed by macrophages in the bone marrow, and possibly also in the spleen and the liver. Balancing this loss, the bone marrow produces about 2.5 million reticulocytes, every second. Epo deficiency results in anemia.

About 25% of patients with chronic kidney disease (CKD) needed regular blood transfusions before recombinant human Epo (rhEpo) became available. Transfusion therapy is an important option, and RBC transfusions are often life-saving. However, blood products also bear risks, such as the transmission of infectious diseases, acute and chronic hemolytic transfusion reactions, and transfusion-related lung injury. This was especially true for the 1980s (HIV transmission by blood and plasma derivatives) in which rhEpo became clinically available. The launch of rhEpo and analogous erythropoiesis-stimulating agents (ESAs) has by now been advantageous to millions of patients compromised by chronic anemia [reviewed in 1]. Of note, in this period the safety of blood transfusions has also further been improved as exemplified by the German Haemovigilance Report 2010 of the Paul-Ehrlich-Institut ([www.pei.de](http://www.pei.de)).

## Sites and Control of Epo Production

Peritubular fibroblasts in the renal cortex are the main site of Epo synthesis. Epo mRNA is also detectable in liver, spleen, bone marrow, lung and brain, and Epo may be translated in small amounts in these organs. Actually, the liver is the main site of Epo production in the fetal stage. The concentration of circulating Epo increases exponentially with de-

**Fig. 1.** Scheme of erythropoiesis. The kidneys are the main site of the production of erythropoietin (Epo). The *EPO* enhancer is activated by the hypoxia-inducible transcription factor complex HIF-1 $\beta$ /HIF-2 $\alpha$ . Supported by co-factors such as p300 the HIF complex binds to the hypoxia-response element (HRE) activating *EPO* expression. In bone marrow, Epo promotes the survival, proliferation and differentiation of erythrocytic progenitors, particularly the colony-forming units-erythroid (CFU-Es). Starting about four days after an increase in Epo levels, more reticulocytes enter the blood stream.



creasing Hb concentration in uncomplicated anemia (absence of renal disease or inflammation). The regulated variable is the tissue  $pO_2$ , which depends on Hb concentration, arterial  $pO_2$ , and Hb- $O_2$  affinity. Compared to other organs, the  $pO_2$  in the renal cortex is little affected by blood flow changes, as the renal  $O_2$  consumption decreases in proportion with the blood flow. Hence, the kidneys are most appropriate for producing Epo in a blood  $O_2$  content-regulated way (fig. 1).

Epo production depends on the rate of the transcription of the Epo gene (*EPO*; in chromosome 7). Several transcription factors are involved. The *EPO* promoter is inhibited by GATA-2 and by nuclear factor  $\kappa B$  (NF- $\kappa B$ ), which are likely responsible for the impaired *EPO* expression in inflammatory diseases [2]. The *EPO* enhancer possesses a hypoxia-response element (HRE) that is activated by heterodimeric ( $\alpha/\beta$ , 100–120 kDa each), hypoxia-inducible transcription factors (HIFs). Apart from *EPO*, hundreds of other HIF-responsive genes have been identified. The HIF- $\alpha$  subunits present with isoforms, the main activator of *EPO* is HIF-2, which comprises HIF-1 $\beta$  and HIF-2 $\alpha$  [3].

Importantly, the HIF- $\alpha$  subunits are  $O_2$  labile. Their C-terminus contains two proline residues (Pro<sup>405</sup> and Pro<sup>531</sup> in HIF-2 $\alpha$ ) that are hydroxylated by specific,  $\alpha$ -ketoglutarate requiring, dioxygenases in the presence of  $O_2$  [reviewed in 3–5]. HIF- $\alpha$  undergoes immediate proteasomal degradation on prolyl hydroxylation, as it is tagged by the von Hippel-Lindau tumor suppressor protein (pVHL) in association with an E3 ubiquitin ligase. Further, in the presence of  $O_2$  a HIF- $\alpha$  asparagine residue (Asn<sup>847</sup> in HIF-2 $\alpha$ ) is hydroxylated by the so-called factor inhibiting HIF (FIH). Thereupon, the binding of

the transcriptional co-activator p300/CBP (CREB-binding protein) is prevented. Drugs are under development for manipulation of these processes and, thus, HIF-dependent gene expression [4–6].

### Structure of Epo

Human Epo is an acidic glycoprotein with a molecular mass of 30.4 kDa. Its 165 amino acid residues chain forms four antiparallel  $\alpha$ -helices, two  $\beta$ -sheets and two intra-chain disulfide bridges (Cys<sup>7</sup>-Cys<sup>161</sup>, Cys<sup>29</sup>-Cys<sup>33</sup>). The carbohydrate portion (40% of the molecule) comprises three N-glycans (at Asn<sup>24</sup>, Asn<sup>38</sup>, and Asn<sup>83</sup>) and one O-glycan (at Ser<sup>126</sup>). The N-glycans serve a variety of functions, including the protection of Epo from proteases and the modulation of its receptor binding affinity [7].

The glycosylation isoforms of Epo and its analogs can be distinguished by isoelectric focusing and immunoblotting. These methods are also used for proof of ESA doping in sports [8].

### Action of Epo on Erythrocytic Progenitors

RBCs are the offspring of CD34+ hematopoietic stem cells [9], the ‘CFU-GEMMs’ (colony-forming units generating granulocytes, erythrocytes, monocytes, and megakaryocytes). The ‘BFU-Es’ (burst-forming units-erythroid) are the earliest descendants in the erythrocytic compartment. Their progeny goes through about 12 divisions, giving rise to several hundred

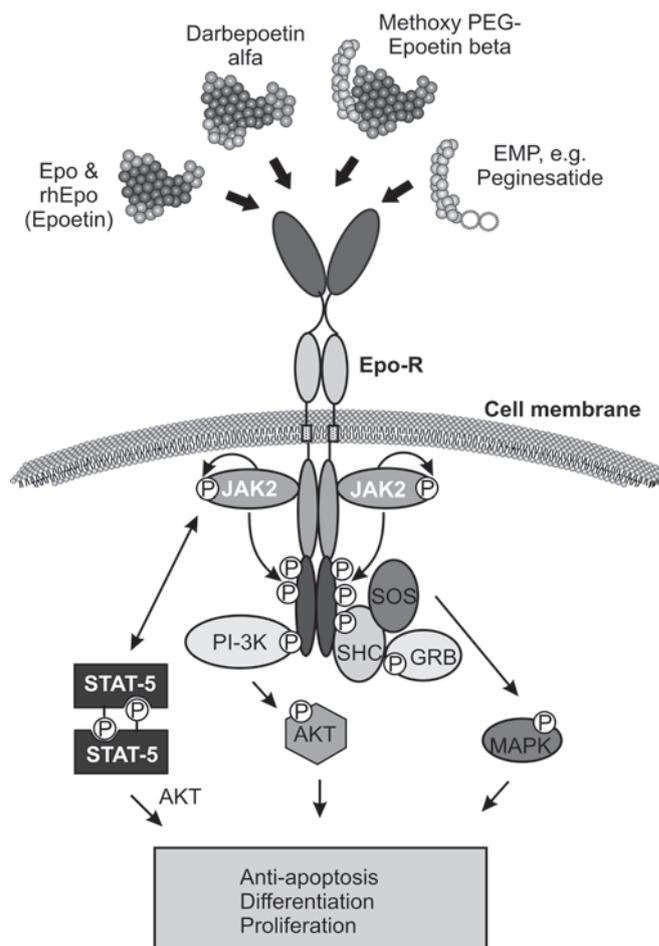
erythroblasts within 10–20 days. Next to the BFU-Es are the ‘CFU-Es’ (colony forming units-erythroid) which express abundant Epo receptor molecules (EpoR) and undergo apoptosis in the absence of Epo. In the presence of Epo, CFU-Es and their progeny divide 3–5 times generating 8–64 erythroblasts within 7–8 days (fig. 1). Once the level of ‘orthochromatic erythroblasts’ (syn. ‘normoblasts’) is reached, the cells extrude their nuclei and then become reticulocytes [10]. Reticulocytes and mature erythrocytes lack EpoR.

As shown in figure 2, the human EpoR is a membrane-spanning approximately 59 kDa glycoprotein (484 amino acid residues, one N-glycan), that acts as homodimer [11]. Epo binding causes the intracellular activation of EpoR-associated Janus kinase 2 (JAK-2). In turn, JAK-2, EpoR and other signaling proteins are tyrosine-phosphorylated [12]. Further, JAK-2 is an essential chaperone for transferring EpoR to the cell surface [13]. The phosphorylated EpoR provides docking sites for proteins containing SRC homology 2 (SH2) domains. EpoR signaling involves signal transducers and activators of transcription such as STAT-5, phosphatidylinositol 3-kinase (PI-3K)/AKT (protein kinase B), and SHC/mitogen-activated protein kinase (MAPK).

The action of Epo is terminated when the EpoR is dephosphorylated by the tyrosine phosphatase SHP-1 (Src homology phosphatase-1) and the Epo/EpoR-complex is internalized. In vitro studies suggest that about 60% of Epo is resecreted, and 40% undergoes proteasomal degradation after internalization [14]. EpoR mediated Epo uptake by its target cells is considered a major mechanism of the degradation of circulating Epo [15].

### Alleged Actions of Epo Outside the Bone Marrow

The human EpoR gene (EPOR; in chromosome 19) is expressed not only in erythrocytic progenitors but in most tissues [16, 17], because the EPOR promoter lacks a TATA box [18]. Human endothelial, cardiac, renal and neuronal cells contain 10- to 100fold lower EpoR mRNA levels than cells highly responsive to Epo [16, 17]. Preclinical studies assigned ESAs pleiotropic cytoprotective effects against hypoxia, ischemia/reperfusion, cytotoxic agents, and inflammation. However, the role of the Epo/EpoR-system outside the bone marrow clearly needs approval [19]. Studies of transgenic mice have shown that the Epo/EpoR-system is dispensable in non-hematopoietic tissues [20]. On the other hand, there are observations of chemical binding of antibodies raised against the EpoR (mostly the polyclonal C-20 antibody; Santa Cruz) to various cells and tissues [reviewed in 21]. Still these observations may have been misinterpreted, because the antibodies (including C-20) employed in earlier studies proved to be not specific and to cross-react with various other proteins such as heat-shock proteins [22, 23]. Recently, a monoclonal antibody specific for EpoR (A-82; Amgen) has been developed, and only this has enabled valid analyses of EpoR protein expres-



**Fig. 2.** Scheme of Epo receptor signaling. Endogenous Epo, its recombinant analogs and Epo-mimetic peptides can bind to the homodimeric EpoR. The EpoR then recruits JAK-2 that catalyzes EpoR/JAK-2 transphosphorylation. The phosphorylated EpoR induces activation of the signal transducer and activator of transcription 5 (STAT-5), the phosphatidylinositol 3-kinase (PI-3K)/AKT, and the SH2 adaptor protein C (SHC)/mitogen-activated protein kinase (MAPK) pathways. STAT-5 induces the expression of anti-apoptotic proteins (for example Bcl-x<sub>L</sub>). PI-3K and MAPK prevent pro-apoptotic proteins (Bax, Bad) from activating caspases, specific cysteine proteases otherwise executing apoptosis. In addition, MAPK activates genes promoting cell proliferation.

sion [24]. Investigations with A-82 have shown that only erythroid cells have significant levels of EpoR [16, 17]. In addition, the studies using A-82 have revealed that tumor-cell lines generally express only low (to undetectable) levels of EpoR and that their EpoR is not functional [25].

Some reports have proposed in vitro angiogenic effects of Epo on human bone marrow-derived endothelial progenitor cells (EPCs) [26]. In one clinical study, ESAs increased the number of EPCs in circulation [27]. However, in other trials ESA treatment failed to affect the number of EPCs in donors for allogeneic peripheral blood stem cell transplantation [28] or in patients with acute myocardial infarction [29]. ESAs also failed to exert effects in an in vivo angiogenesis assay [16]. Taken together, convincing evidence is still missing to assume that ESAs stimulate angiogenesis in the clinical setting [19].

Epo may exert a local function in the brain besides its role in erythropoiesis [30, 31]. It is speculated that Epo acts as a neurotrophic and neuroprotective factor. The therapeutic value of the administration of rhEpo to humans with hypoxic brain injury is a focus of present research [32].

It has been hypothesized that Epo may exert non-hematopoietic effects via heteromeric receptors involving EpoR and CD131, the common  $\beta$ -subunit of cytokine receptors such as for interleukin-3 (IL-3), IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [33]. In preclinical studies asialo-Epo (Epo lacking terminal sialic acid) and carbamoylated Epo (CEPO, Epo treated with cyanate) were assigned cytoprotection without stimulating erythropoiesis. However, other investigators found no evidence for an interaction between EpoR and CD131 [34].

### Assay of Epo

In vivo bioassays for Epo are commonly performed in rodents by measurements of reticulocytes or incorporation of radioactive iron ( $^{59}\text{Fe}$ ) in blood. One international Epo unit (IU) produces the same erythropoietic response in the animals as 5  $\mu\text{mol}$  cobalt ( $\text{Co}^{2+}$ ) chloride. The specific in vivo activity of epoetin (about 200,000 IU/mg peptide) is higher than that of purified human urinary Epo (70,000 IU/mg peptide). In vitro bioassays for Epo can be performed in Epo-responsive cell cultures by measurements of enzyme activities, or of heme or DNA synthesis. In clinical routine, enzyme-linked immuno-sorbent assays (ELISAs) for Epo are commonly used to measure Epo immunoreactivity units (U). The normal concentration of Epo in human plasma amounts to about 15 U/l (~5 pmol/l). The in vivo bioassay is required to calibrate rhEpo for therapeutic purposes because immunoassays provide no clear information on ESA activity in the organism.

### Recombinant Epo Preparations

Transfected Chinese hamster ovary (CHO) cells are generally used for the large-scale manufacture of ESAs. The drug substances are formulated as dry powders or as isotonic sodium chloride/sodium citrate buffered solutions for intravenous (IV) or subcutaneous (SC) administration. They are stabilized by polysorbate (Tween-20 or -80), or human albumin (2.5 mg/ml) and benzyl alcohol (1%).

'Epoetin' is the international non-proprietary drug name (INN) for eucaryotic cell-derived rhEpo, whose amino acid sequence is identical with that of endogenous human Epo. Differences in the amino acid residues chain are indicated by a random prefix (e.g. 'darbepoetin'). The glycosylation pattern is indicated by a Greek letter (alfa, beta, etc.). Two brands of innovator CHO cell-derived rhEpo, namely epoetin alfa and epoetin beta, were launched as anti-anemic agents

about 25 years ago. Epoetin alfa has been marketed in the USA as Epogen<sup>®</sup> (Amgen) for the treatment of CKD patients on hemodialysis and as Procrit<sup>®</sup> (Johnson and Johnson) for other indications through an agreement with Amgen, and outside the USA mainly as Eprex<sup>®</sup> or Erypo<sup>®</sup> (Johnson and Johnson subsidiary Ortho Biotech), and Espo<sup>®</sup> (Kirin). Epoetin beta has been mainly marketed as NeoRecormon<sup>®</sup> (F. Hoffmann-LaRoche) and Epogin<sup>®</sup> (Chugai/F. Hoffmann-LaRoche). The originator epoetins alfa and beta are used for the same major indications (anemias associated with CKD or myelosuppressive chemotherapy treated cancer). In 2009, epoetin theta has been launched as another stand-alone CHO cell-derived rhEpo (Eporatio<sup>®</sup>, Ratiopharm; Biopoin<sup>®</sup>, CT Arzneimittel) in the European Union (EU). In some parts of the world, CKD patients have been treated with epoetin omega, which is expressed in EPO cDNA-transfected baby hamster kidney (BHK, from Syrian hamster) cells, but apparently this product is not widely used.

After the patents for the innovator epoetins expired, other manufacturers have developed similar biological medicinal products ('biosimilars') [35]. The primary rationale for the use of biosimilars is cost saving. Because biopharmaceuticals cannot be exactly copied, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) has issued guidelines regards the approval of biosimilars and product-specific requirements for epoetins ([www.ema.europa.eu/ema/](http://www.ema.europa.eu/ema/)). Similar regulatory pathways exist in many other regions of the world (USA, Canada, Australia, Japan, etc.). The two biosimilar epoetins available in the EU are used at the same dose(s) and dosing regimen(s) for the indications of the reference product, Eprex/Erypo. One of the biosimilars has received the INN epoetin alfa (Binocrit<sup>®</sup>, Sandoz; Epoetin alfa Hexal<sup>®</sup>, Hexal Biotech; Abseamed<sup>®</sup>, Medice Arzneimittel Putter) and the other epoetin zeta (Silapo<sup>®</sup>, Stada; Retacrit<sup>®</sup>, Hospira). The several brand names are accounted for by co-marketing of the same drug substances by different companies. Overall, the naming (INNs) of the epoetins is confusing for physicians in the EU [reviewed in 35].

Physicochemical and functional investigations of purported epoetin alfa copies manufactured and used in a good many Asian and Latin American countries revealed major isoform differences, batch-to-batch variations in potencies as well as endotoxin contamination of some of the products [36].

RhEpo, administered IV in clinically relevant single doses of 50 IU/kg body weight (b.w.) is eliminated at a first-order kinetic rate following the rapid distribution phase (volume of distribution 0.03–0.09 l/kg b.w.). Hence, peak plasma rhEpo concentrations (IU/l) following IV administration can be roughly estimated by multiplying the dose (IU/kg) with the factor 20 [37]. Following SC administration, peak plasma concentrations of rhEpo are achieved after 12–18 h, with bioavailability amounting to about 30%. On SC administration, peak plasma concentrations are approximately one twentieth of the initial values measured after IV administration. How-

ever, the slow absorption allows for about 30% lower drug requirements with SC versus IV application [38].

There are recombinant ESAs with prolonged survival in circulation ('biobetter'). First darbepoetin alfa (Aranesp<sup>®</sup>; Amgen) has come, a hyperglycosylated analog (37.1 kDa) of rhEpo, which contains two additional N-glycans in association with an exchange of five amino acids [7]. Compared with the terminal half-life of IV administered epoetin (6–9 h), the half-life of darbepoetin alfa is three- to fourfold longer (25 h), which allows for less frequent application [39]. Another biobetter is methoxy polyethylene glycol-epoetin beta (methoxy PEG-epoetin beta; Mircer<sup>®</sup>, F. Hoffmann-LaRoche). The half-life of methoxy PEG-epoetin beta (60 kDa) amounts to 130–140 h on IV injection. The prolonged in vivo survival of darbepoetin alfa and methoxy PEG-epoetin beta is in part due to a reduced EpoR binding affinity. 1 µg of darbepoetin alfa or of methoxy PEG-epoetin beta peptide corresponds biophysically to 200 IU rhEpo peptide. Clinically, however, the long-acting products may allow for dose reductions below the predicted 1:200 ratio [39].

### Clinical Applications of ESAs

ESAs can be indicated for the treatment of chronic forms of anemia, but the drugs are not an alternative to RBC transfusions for patients with severe trauma-induced hemorrhage, major blood loss during surgery such as in cardiothoracic or liver surgery, and severe or life-threatening anemia. Depending on individual country regulations ESAs have been approved for i) anemia due to CKD, both predialysis and dialysis, ii) anemia in patients with cancer receiving chemotherapy, iii) anemia associated with zidovudine treatment in HIV infection, iv) support of autologous blood collection, v) elective surgery, and vi) anemia in preterm infants.

Indeed, lack of Epo is the primary factor causing the anemia in CKD. The renal fibroblasts lose their Epo-producing capacity after injury and trans-differentiate to scar-producing myofibroblasts [40]. Other factors implicated in renal anemia are blood loss, shortening of RBC life-span, malnutrition, reduced iron availability, and inhibition of the growth of erythrocytic progenitors by inflammatory cytokines and uremia toxins. ESA therapy can reduce the need for RBC transfusions and the hyperdynamic cardiac state. Physical performance and brain function may also improve on anemia alleviation. The target hematocrit (Hct) in ESA treated patients was generally set in the range 0.30–0.36, and the target Hb concentration 100–120 g/l [41]. However, the present ESA labels in the USA warn that CKD patients in controlled clinical trials experienced greater risks for death, serious adverse cardiovascular reactions, and stroke, when ESAs were used to target Hb concentration > 110 g/l. The US Food and Drug Administration has recommended allowing Hb concentration to decline to 100 g/l in patients with non-dialysis-dependent CKD

and to 100–110 g/l in patients on dialysis ([www.fda.gov/drugs/drugsafety/ucm259639.htm](http://www.fda.gov/drugs/drugsafety/ucm259639.htm)).

In patients with CKD the major cause of resistance towards ESAs is reduced iron availability, due to the action of hepcidin. This is indicated by a low serum ferritin concentration (<100 µg/l), a low transferrin saturation (<20%), and a high proportion of hypochromic RBCs (>10%) [42, 43]. The administration of IV iron can decrease the ESA dosage requirements [44]. However, physicians should be aware that iron overload may cause tissue damage [45].

In cancer patients receiving chemotherapy, the treatment with ESAs (epoetins or darbepoetin alfa) can be initiated when Hb concentration falls <100 g/l. The primary goal of the therapy is to maintain the Hb concentration above the transfusion trigger, yet RBC transfusions are also an option. Clinicians should outweigh potential harms (e.g. thromboembolism) and benefits (e.g. reduced transfusions) of ESAs and compare these with potential harms (e.g. very rare transmissible infections and a variety of immune-associated transfusion reactions) and benefits (e.g. rapid Hb concentration improvement) of RBC transfusions [46]. ESAs should be administered at the lowest dose possible, and the treatment should increase Hb concentration to the lowest level possible if it aims at avoiding RBC transfusions.

In addition, ESA therapy can be indicated for AIDS patients treated with zidovudine. In the surgical setting, rhEpo may be administered preoperatively in order to stimulate erythropoiesis in phlebotomy programs for autologous RBC re-donation or correction of a pre-existing anemia, and postoperatively for recovery of RBC mass in certain interventions. Another approved indication for ESAs can be the anemia of prematurity to reduce the number of RBC transfusions in newborns.

### Safety

Cases of acute toxic effects of approved rhEpo formulations or its analogs have never been reported. ESAs are contraindicated in patients with hypersensitivity to non-human cell-derived products. Patients with a hypersensitivity to human albumin should not be treated with formulations stabilized with this protein. In pregnancy, rhEpo should be administered very cautiously, because the risks for the fetus have not been evaluated in humans.

In ESA-treated CKD patients, the most common unwanted effect is an increase in arterial blood pressure and possibly hypertension (affects 1–10 users in 100). Thus, ESAs are contraindicated in patients with uncontrolled hypertension. The increase in blood pressure can be partly explained by the elevated blood viscosity and the reversal of hypoxia-induced vasodilatation in association with the increase in Hb concentration. Patients with non-renal anemia do not usually develop hypertension on ESA therapy. The use of ESAs may increase the incidence of thromboembolism and the risk of

cardiovascular events, including death. It seems likely that the occurrence of cardiovascular events is partly related to the elevation in Hb concentration and Hct.

Similarly, in cancer patients thromboembolic events are considered a critical risk factor associated with the use of ESAs [47]. The 2012 update of a Cochrane Database review, which included 91 randomized controlled trials (with 20,102 participants) on managing anemia in cancer patients receiving or not receiving anticancer therapy that compared the use of ESAs (plus transfusion if needed) has not only confirmed that use of ESAs reduces the relative risk of RBC transfusions but also provided evidence that ESAs increase the risk for thromboembolic complications and deaths [48]. Possibly, ESA therapy is associated with an increase in platelet numbers. ESAs could stimulate thrombopoiesis in an indirect way, as iron depletion due to increased erythropoiesis can result in thrombocytosis [49]. A recent report proposes to reduce the ESA-associated risk of thrombosis by anti-thrombotic therapy [50].

Another critical issue is whether ESAs could stimulate tumor growth. Laboratory studies applying appropriate biochemical techniques have shown that cancer cells lack functional EpoR protein, although they express low levels of EPOR mRNA [16, 17, 25]. The question of whether Epo and its analogs can promote tumor growth by stimulating angiogenesis is a focus of present research, with some authors answering in the affirmative [51] and others in the negative [19]. Several meta-analyses have examined ESA use and safety outcomes in cancer patients. Bennett et al. [47] and Bohlius et al. [52] reported a negative ESA impact risk on mortality, but not on how ESAs affect disease progression. Meta-analyses have shown that ESA use does not generally impact disease progression [53, 54]. However, safety data from some individual, controlled trials suggest ESAs could affect disease progression and/or mortality in certain cancer patient populations (e.g. head-and-neck cancer patients receiving radiotherapy only) [54]. These statements are in line with the results of the 2012 Cochrane review update, to quote verbatim: 'Whether and how ESAs affects tumour control remains uncertain. The increased risk of death and thromboembolic events should be balanced against the potential benefits of ESA treatment taking into account each patient's clinical circumstances and preferences' [48].

Finally, drug purity and immunogenicity are issues with biopharmaceuticals. The production of ESAs confers to extremely high biotechnological standards in most regions of the world, including Northern America, Europe and Japan. In these regions, ESA containing medicines are rarely immunogenic, even on SC administration. The exposure-adjusted incidence of neutralizing anti-Epo antibodies (anti-Epo Abs)-induced pure red cell aplasia (PRCA) in rhEpo treated CKD patients is at present <0.03 per 10,000 patient years [55]. However, changes in the manufacturing process or the formulation of an ESA may cause an immune reaction. When neutralizing anti-Epo Abs occurred on SC administration of a biosimilar epoetin, investigations carried out by the manufacturer re-

vealed that abnormally high tungsten levels in the pre-filled syringes caused the epoetin to unfold and to aggregate [56]. Hence, a change in the type of syringes could overcome the transient problem.

## Perspectives

Anemia treatment with recombinant ESAs is cost-intensive. Thus, the question arose whether there are alternative therapeutic options [57, 58].

First, there are small, orally active, chemicals that prevent HIF- $\alpha$  from degradation ('HIF stabilizers') and stimulate endogenous Epo production. A phase I clinical trial with the  $\alpha$ -ketoglutarate competitor FG-2216 (FibroGen) in CKD patients has been published [59]. Actually, many different HIF stabilizers have been identified [5, 6, 60]. However, HIF stabilizers induce the expression of numerous genes apart from EPO [5, 60], which may result in adverse events.

Another strategy has been to replace recombinant ESAs by Epo mimetic peptides (EMPs), synthetic cyclic peptides of about 20 amino acids. EMPs show no sequence homology to Epo but signal through the EpoR (fig. 2). As the first EMP, peginesatide (Omontys<sup>®</sup>; Affymax/Takeda) was approved in the USA in 2012 for SC or IV treatment of CKD patients on dialysis. However, the drug was recalled early in 2013, since 0.2% of patients experienced serious and 0.02% of patients experienced fatal hypersensitivity reactions. In an alternative approach, EMPs have been constructed onto human IgG-based scaffolds by recombinant DNA technology. The seminal compound, CNTO 528 (Centocor), increased Hb concentration on IV administration in a phase I clinical trial. The follow-on product CNTO 530, a dimeric EMP fused to a human IgG4 Fc scaffold, was shown to expand the pool of erythrocytic progenitors in vitro and in vivo [61]. However, doubt may be raised as to the advantages of this type of biopharmaceutical over the established ESAs.

The most novel approach has been the administration of sotatercept (ACE-011; Acceleron Pharma/Celgene), a recombinant, chimeric protein consisting of the extracellular domain of the human activin receptor type 2A and the Fc domain of human IgG1. Sotatercept binds and inactivates activin A. In a phase I clinical trial sotatercept produced dose-dependent increases in reticulocytes, RBCs, Hb concentration, and Hct in healthy postmenopausal women [39]. However, in contrast to HIF-stabilizers, which can be taken orally, sotatercept must be injected. In addition, the immunogenic potential of the drug should be studied in more detail.

## Disclosure Statement

The author has had a compensated consultant/advisory role and received honoraria and research funding from pharmaceutical companies producing and/or marketing ESAs.

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