REVIEW ARTICLE

Port J Nephrol Hypert 2010; 24(2): 137-145
Advance Access publication 1 February 2010

Different modalities of erythropoiesis stimulating agents

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Received for publication: 19/11/2009
Accepted: 05/01/2010

ABSTRACT

Erythropoiesis stimulating agents (ESAs) have been used in clinical practice for over 20 years and have benefited a large number of anemia patients. The initial epoetins approved for clinical use, Epoetin alfa and beta, have identical amino acid sequences to endogenous human erythropoietin (Epo). Newer ESAs have different pharmacokinetic (PK) and pharmacodynamic (PD) properties than recombinant human Epo (rHuEpo) and are indicated for less frequent dosing. Darbepoetin alfa is a novel ESA that was engineered through the introduction of new glycosylation sites on the epoetin alfa molecule. Methoxy polyethylene glycol-epoetin beta (PEG-epoetin beta) was developed by attaching a PEG moiety to epoetin beta. Several other approaches are now being used to develop novel ESAs. These molecules include peptides or antibodies that are agonists of the Epo receptor, or small molecules such as HIF-PH inhibitors, which modulate pathways that up-regulate the expression of endogenous Epo. They explore new drug and administration modalities (oral versus parenteral) and new manufacturing approaches (chemical synthesis versus cell culture). Finally, the ESA landscape has recently been changed by the introduction in Europe of biosimilar epoetins, which are similar but not identical to the original epoetins. Because biologics have very complex structures and their properties depend not only on the amino acid composition, but also on post-translational modifications and manufacturing conditions, the equivalence of biological products cannot be established like small molecule generics. Any changes that might not be detectable by current analytical methods might affect the efficacy and immunogenicity of the product, which could have significant safety implications. As such post-approval pharmacovigilance programs are in place to monitor the use of biosimilars. This article does not attempt to be a comprehensive review of the literature on ESAs, but focuses on examples of various approaches that have been used to develop new ESAs.

Key-Words:
Anemia; biologics; biosimilars; erythropoietin.

INTRODUCTION

The production of red blood cells is a highly controlled and dynamic process. Erythrocytes are produced in the bone marrow from hematopoietic progenitor cells and typically last 100 to 120 days in humans, therefore requiring replacement of 0.8 to 1% of the erythrocyte pool every day. Considering that the red cell mass ranges between 23 and 32 ml/kg body weight[1], the magnitude of this process is impressive even under normal homeostatic conditions. The main growth factor controlling erythropoiesis is erythropoietin (Epo), a 165 amino acid protein, produced mostly by the peritubular interstitial cells in the kidney. The levels of Epo production are adjusted in response to changes in oxygen tension, which is detected by a sensor mechanism in the kidney controlled by the transcription factor HIF[2]. Under normal oxygen conditions, HIF is produced, but is rapidly degraded. When oxygen tension decreases, the degradation of HIF is suppressed and

it accumulates in the kidney cells where it activates transcription of hypoxic responsive proteins, including Epo.

The existence of Epo was first postulated by Carnot and Deflandre more than 100 years ago[3,4]. They observed an increase in erythrocytes when serum from anemic rabbits was administered to normal rabbits. The investigators attributed this increase to the presence of a soluble substance in the serum, which was named hemopoietin. Several years later in 1957, Jacobson *et al.*[5] used nephrectomized rats to demonstrate that Epo was produced by the kidney in response to oxygen demand by the rest of the body. After this observation, major efforts were launched by several groups to isolate Epo, but they were not successful. Finally, in 1977 and following a significant endeavor, Miyake, Kung and Goldwasser[6] were able to purify milligram amounts of Epo from 1500L of urine from patients with aplastic anemia. They used a 7-step purification process that included ion exchange chromatography, ethanol precipitation, gel filtration, and adsorption chromatography to purify human Epo. With purification of Epo, the Goldwasser group was able to get the amino acid sequence from the first 26 amino acids of the N-terminus sequence, but this sequence had two errors. Finally, in 1985 two different groups working independently reported the cloning of the human erythropoietin gene[7,8]. The cloning of the human Epo gene led to major advances in understanding of erythropoiesis, which was made possible by the production of large amounts of recombinant protein. Recombinant human Epo produced in CHO cells was shown to have a biologic activity similar to that of endogenous human Epo both *in vitro* and *in vivo*[9]. The mature recombinant human Epo protein produced by CHO cells has a molecular weight (MW) of ~30 kDa and is composed of ~60% amino acids and ~40% carbohydrates[10].

Epo binds as a monomer to a dimer of the Epo receptor (EpoR). A high and a low affinity binding site on the Epo molecule are involved in the interactions with the two receptors subunits[11]. Upon binding of the ligand, the EpoR changes conformation and initiates a signaling cascade involving several signal transduction proteins. The tyrosine kinase JAK2 plays a critical role in initiating this signaling cascade; it not only phosphorylates downstream signaling molecules, but also phosphorylates multiple tyrosine residues on the EpoR, which creates binding sites for several SH2-domain containing proteins such as STA-5, PLC , PI3K and SHP-1, SHP-2, and SHIP[12]. Signaling by EpoR in hematopoietic progenitor cells promotes cell growth, differentiation, and survival. A review of the expression of the EpoR is outside the scope of this manuscript, but this is an area that remains controversial due conflicting data in the literature, poor specificity of antibodies used to detect EpoR expression, and poor agreement between *in vitro* data and *in vivo* animal data (for more on this topic, see Osterborg *et al.*[13]).

EPOETINS AND ENGINEERED ESAS

Epoetin alfa and beta are used for the treatment of anemia in dialysis and non-dialysis patients with chronic renal failure and cancer patients with metastatic, non-myeloid malignancies receiving chemotherapy. Epoetins are commonly administrated 1 to 3 times a week. Darbepoetin alfa is a novel protein that was genetically engineered by altering the amino acid composition of epoetin alfa to generate a molecule with two new glycosylation sites. The additional carbohydrate chains increased the molecular weight of the glycoprotein from approximately 30 to 37 kDa resulting in a molecule that has increased half-life and erythropoietic activity in patients[14]. PEG-epoetin beta was generated by coupling a PEG moiety to the epoetin beta molecule to produce a bulkier molecule that has decreased clearance and increased PD activity *in vivo*[15].

The development of darbepoetin alfa was a complex process that resulted from observations made during the characterization of epoetin alfa. Initial studies by Egrie and Browne[16] demonstrated that glycoforms of Epo with increased sialic acid content had decreased clearance and increased *in vivo* activity. Epo is a glycoprotein that contains three N-linked and one O-linked glycosylation sites. The structure of the carbohydrate chains attached to Epo and other glycoproteins is variable. In mammalian cells, N-linked carbohydrates can have up to four branches, whereas O-linked carbohydrates can only have up to two branches. These branches are capped by negatively charged sialic acid residues, which play a significant role in the clearance of Epo. Epo can have up to 14 sialic acid residues per molecule. When

different glycoforms of Epo with different sialic acid content were separated and tested individually *in vivo*, it was observed that glycoforms with greater sialic acid content had increased erythropoietic activity *in vivo*. This increase in activity was associated with a decreased clearance and increased terminal half-life. These observations led to the hypothesis that increasing the carbohydrate content of Epo beyond that found in endogenous human Epo could increase *in vivo* activity. Elliott *et al.*[17] tested this hypothesis by engineering new N-linked glycosylation sites on the Epo cDNA. After testing a larger number of new glycosylation analogs, they settled on a new construct that contained two additional N-linked glycosylation sites. This molecule differs from the original Epo molecule in that five amino acids were mutated to create two new N-linked glycosylation sites that increased the carbohydrate content from 40% in Epo to about 51% in darbepoetin alfa. The new carbohydrate chains were added to positions on the Epo molecule that are distal from the Epo receptor binding sites (Fig. 1). Initial experiments in mice demonstrated that a single injection of darbepoetin alfa at 100µg/kg elicited a greater hemoglobin response in mice than a much higher dose of rHuEPO (1800 µg/kg). This increase in activity is due to the decreased clearance and increased half-life of darbepoetin alfa. In humans and rodents, the half-life of darbepoetin alfa is approximately threefold higher than rHuEPO. The extended serum half-life of darbepoetin alfa supports extended dosing schedules of up to a single injection every 2 to 4 weeks depending on the patient population[18-20], in contrast with rHuEPO, which frequently requires more than an injection per week[21]. The development of darbepoetin alfa illustrates how research and understanding of the PK and PD relationships of the initial epoetin alfa led to the development of a novel molecule that offers expanded dose schedule options for patients while maintaining clinical activity. The approach used to develop PEG-epoetin beta was different from the darbepoetin alfa. PEG-epoetin beta was engineered through the conjugation of PEG to the epoetin beta molecule to create a molecule that has a molecular weight of about 60 kDa, which nearly doubles the molecular weight of epoetin beta. The pegylation of macromolecules is a pharmaceutical technology that has been used effectively in the laboratory to increase the size of different therapeutics, such as interferons, G-CSF and others, to produce molecules that have reduced *in vivo* clearance through diminished kidney filtration and protection against enzymatic digestion[22,23]. The half-life of PEG-epoetin beta is approximately 130 hours[24] for IV administration compared to a range of 4 to 12 hours for epoetin beta. Recent clinical studies have shown, that in patients with CKD who are in dialysis, a dosing regimen of once-monthly

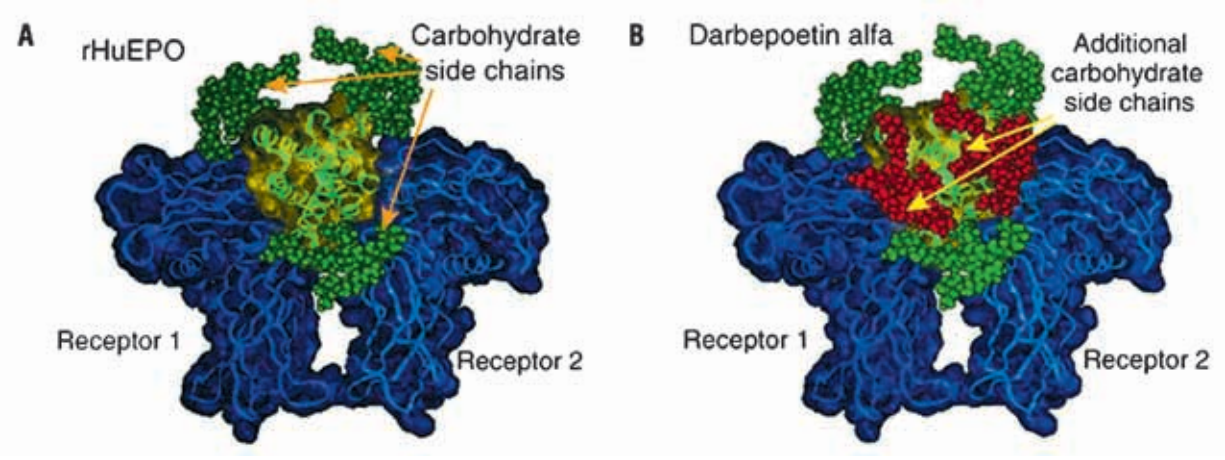

Figure 1

Molecular comparison of rHuEpo and darbepoetin alfa bound to a dimer of the Epo receptor. The original carbohydrate side chains are shown in green and the new carbohydrate side chains of darbepoetin alfa are shown in red. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology[17], copyright 2003.

PEG-epoetin beta can correct anemia and maintain hemoglobin levels[25,26].

OTHER PROTEIN-BASED ESAS

Several approaches have been used to develop new erythropoietic molecules. Broadly, they fit into two basic categories; large protein molecules that bind and activate EpoR and smaller, synthetic molecules that either activate EpoR or activate other cellular targets that modulate erythropoiesis *in vivo*. The second set of molecules will be discussed further in the next section.

Various protein modalities have been utilized to develop ESAs. Most of these candidate molecules are still at the pre-clinical stage and it is not clear how many will proceed to clinical development. Nevertheless, they highlight the efforts that have been put into developing new ESAs, some of which have no sequence or structural relationship to Epo. One approach that is used to increase the half-life of proteins is to fuse the Fc region of human IgG to the protein of interest. This strategy increases the size of the protein and promotes recycling *in vivo* through FcRn similarly to what happens to antibody molecules. Fc-fusion proteins are expressed as dimers due to the natural propensity of Fc regions to associate with each other. This approach has been used to generated a wide variety of clinical candidate molecules, including approved drugs such as etanercept, an Fc fusion of human IgG1 Fc and the TNF receptor used for the treatment of inflammatory diseases and romiplostin, a fusion protein of human IgG1 Fc, and a peptide that activates the thrombopoietin receptor, which is used to treat patients with chronic idiopathic thrombocytopenic purpura. Bitonti *et al.*[27] used the same principle to create an Epo-Fc fusion protein. Their initial construct contained two Epo molecules fused to two chains of the Fc domain of human IgG1, but they reported that a chimeric molecule containing only a single Epo attached to the Fc dimer had better PK and PD properties in cynomolgus monkeys. In another study, Sytkowski *et al.*[28] used a chemical process to cross-link two Epo monomers and create a dimeric Epo molecule. Even though Epo is naturally active as a monomer, the work by Sytkowski and colleagues showed that a dimeric Epo molecule had increased activity *in vivo*, possibly due to longer residence times in the serum. The plasma half-life of the Epo dimers was greater than 24 hr in rabbits, in contrast with monomeric Epo, which had a half-life of 4 hours.

The EpoR can also be activated by molecules that are not structurally homologous to Epo such as peptides and antibodies. In general, antibodies that bind to growth factor receptors can activate or inhibit receptor signaling. Most of the antibodies in the clinic, especially in oncology settings, have been designed to block binding of the natural ligand and inhibit receptor signaling. However, antibodies can also be designed to mimic the ligand and stimulate receptor signaling. Various groups have shown that antibodies against the EpoR can induce intracellular signaling and can activate bone marrow progenitor cells to promote erythropoiesis both *in vitro* and *in vivo*. Elliott *et al.*[29] raised antibodies against human EpoR and discovered that some clones had agonistic activity. Monovalent Fab fragments of the agonistic antibodies failed to activate the receptor, but the dimeric IgGs stimulated proliferation of the UT-7/EPO cell line suggesting that the EpoR needs to be in a dimeric conformation to signal. The antibodies also promoted the formation of erythroid colonies from $CD34^+$ hematopoietic progenitor cells *in vitro*. Similar results, using a different antibody, MoAb 34, were reported at about the same time by Schneider *et al.*[30] demonstrating that it is possible to use protein modalities other than Epo to activate human EpoR. Liu *et al.*[31] took advantage of newer antibody technologies to generate a fully human agonistic antibody against the human EpoR (ABT007), which has erythropoietic activity in transgenic mouse expressing human EpoR. Interestingly, this antibody binds to a site on EpoR that is different from the Epo binding sites, thus demonstrating that EpoR can be activated by molecules that do not interact with the same binding pocket as the ligand. More recently, a mimetic antibody fusion protein (CNTO 528) was tested in adult healthy volunteers and was effective at increasing and maintaining hemoglobin levels in a phase I clinical trial[32]. This molecule is a fusion protein of an Epo mimetic peptide (EMP1) and the Fc region of human IgG1. The peptide portion activates the EpoR and the Fc region provides protein stability, solubility and improved PK properties *in vivo*. After a single IV administration of CNTO 528, the serum half-life ranged from 1.55 to 7.6 days

at doses between 0.03 and 0.9 mg/kg. The literature indicates that all these molecules have no homology to Epo and as such, they may not induce pure red cell aplasia (PRCA) even if they trigger an antibody response by the patient. The antibodies could alter PK and PD properties of the therapeutic agent and even neutralize it, but they are not likely to cross-react with endogenous Epo and induce PRCA, a situation that although rare, can occur with epoetin ESAs. However, the clinical safety of these novel molecules remains to be determined.

CHEMICALLY SYNTHESIZED ESAS

Protein therapeutics are complex molecules made by living cells and require elaborate purification and formulation procedures. Switching from a cell-based system to a synthetic manufacturing process could simplify production significantly and lower manufacturing costs. In addition, small molecules and peptide based drugs can be stable at room temperature, which facilitates storage and transport. Finally, small molecule-based drugs may be given orally in contrast to protein-based therapeutics, which are typically administered parentally. These and other factors have led several groups to investigate the feasibility of generating erythropoietic agents based on modalities other than protein therapeutics.

One of the first approaches described in the literature was in 1999 when Qureshi *et al.*[33] identified a chemical compound that activates the human EpoR. This group screened a chemical compound library to identify molecules that blocked binding of Epo to the EpoR and isolated compound #1, which was capable of binding to a single sub-unit of the EpoR. Interestingly, the authors postulated that a multimeric version of this compound could potentially work as an Epo mimetic if it could induce dimerization of the receptor. To test this hypothesis, they built a multimeric molecule, compound #5, which contained eight copies of compound #1 held together by a central core. As hypothesized, compound #5 induced dimerization of EpoR and transduced a signal that promoted growth and differentiation of erythroid precursor cells *in vitro*. More potent chemical agonists of EpoR were identified by Goldberg and colleagues[34] through the screen of combinatorial libraries of dimeric iminodiacetic acid diamides. The compounds activated EpoR, but were just partial agonists and much less potent than Epo. Even though these compounds (MW >1000) and compound #5 (MW=6400) are far from being therapeutic candidates and are significantly larger than typical small molecule drugs (MW <500), their research demonstrates that chemically synthesized compounds can activate human EpoR and generate an erythropoietic signal.

Peptides are another class of molecules that can be used to activate the EpoR. Several groups have identified various peptides with distinct sequences that bind different regions of the EpoR and stimulate erythropoiesis. At least one of these peptides has been shown to bind to a site in the EpoR distinct from the ligand. Naranda *et al.*[35] reported on a sequence in the extracellular domain of EpoR, which is involved in the receptor dimerization and synthesized a peptide (ERP) with the identical sequence. This peptide was reported to activate the receptor in the absence of ligand and in combination with Epo had synergistic activity. In another study, Wrighton *et al.* [36] screened a peptide phage display library and isolated several peptides (EMPs) that activated EpoR. In contrast with the ERP peptide, the EMP peptides were selected based on the ability to inhibit binding of Epo to EpoR and as a result they were likely to bind close or to the same binding pocket as Epo. The research by Wrighton's group showed that these peptides acted as full agonists of the EpoR and were able to activate EpoR both *in vitro* and *in vivo*. Analysis of the signal transduction pathways induced by Epo and the peptides showed no differences, even though the potency of the mimetic peptides (EC50s between 200 and 400 nM) was significantly lowered than that of Epo (EC50 ~20 pm). The potency of these peptides could be increased if they were synthesized as dimers, possibly due to an improved efficacy at stabilizing the dimeric conformation of the EpoR[36].

Hematide is a peptidic ESA derived from the original EMP peptides[37]. In contrast to protein therapeutics, Hematide is synthesized chemically without the need for a production cell line. One of the major shortcomings of peptides as therapeutic agents is their poor PK properties. Because of their small size, they are cleared from circulation very rapidly and they need to be administered frequently and at high doses in order to have efficacy *in*

vivo. To overcome this issue, two peptide units in Hematide were coupled to PEG to improve stability and reduce renal clearance *in vivo*. In rats, the terminal half-life of Hematide ranges between 21.5 and 30.7 hours after a single IV injection[37]. Since Hematide does not have any sequence homology with Epo[37], any antibodies that patients might develop against Hematide are unlikely to cross-react with endogenous Epo and induce PRCA. Theoretically, Hematide could even be used to overcome PRCA-induced anemia. This hypothesis was tested recently in a small phase 2 clinical trial in CKD patients with PRCA and it was observed that treatment with Hematide raised the hemoglobin levels from 9.7 g/dl to 11.6 g/dl, which was enough to eliminate blood transfusions[38]. Hematide is currently being tested in phase 3 clinical trials for the treatment of anemia in dialysis and non-dialysis patients with chronic kidney disease. The ultimate clinical value and safety of this molecule awaits the results of these clinical trials. Hemomer is another Epo mimetic peptide agent that is currently in pre-clinical development (AplaGen, Baesweiler, Germany). It combines an EpoR binding peptide with hydroxyethylstarch as carrier instead of PEG as in Hematide. The manufacturers of Hemomer suggest that the conjugation to hydroxyethylstarch not only improves the PK properties of the peptides, but also their activity.

In the body, the production of endogenous Epo is regulated by oxygen tension. Under hypoxic conditions, the concentration of the transcription factor HIF is increased and the expression of hypoxic-responsive genes, including Epo, is rapidly up-regulated. Under normal oxygen conditions, HIF prolyl-hydroxylases (HIF-PH) hydroxylate proline residues in HIF, targeting the protein for rapid degradation by the proteosome. When oxygen tension goes down, the activity of the HIF-PH enzymes is inhibited and HIF is stabilized. HIF then moves to the nucleus where it activates transcription of a large number of genes including Epo, vascular endothelial growth factor, and glycolytic enzymes[2].

The ability of HIF to up-regulate endogenous Epo and promote erythropoiesis has been explored as a possible mechanism to develop new erythropoietic agents. FG-4592/ASP1517 is a small molecule inhibitor of HIF-PH enzymes, which is currently being investigated in clinical trials as an oral treatment for anemia associated with chronic kidney disease (FibroGen Inc., San Francisco, CA, USA). If effective and safe, this agent would provide a new treatment modality for anemia that could potentially eliminate the need for injections. However, HIF-PH inhibitors are likely to have pleiotropic effects because HIF regulates transcription of a large number of genes besides Epo[2].

Another general approach that has been proposed to stimulate erythropoiesis is to use an inhibitor of the SHP-1 phosphatase as a therapeutic agent. SHP-1 binds to a phosphorylated site on the activated EpoR and down-regulates the JAK-STAT signaling pathway through the dephosphorylation of the JAK2 kinase. Akagi *et al.*[39] reported that antisense oligonucleotides which down-regulated SHP-1 expression in hematopoietic progenitor cells from patients that were hyporesponsive to Epo, enhanced erythroid colony formation *in vitro*, suggesting that SHP-1 could be another target to promote erythropoiesis. In addition, mutations on the EpoR that eliminate the binding site for SHP-1 have been linked to polycythemia in humans further implicating SHP-1 as a down-modulator of EpoR signaling[40]. However, this phosphatase is expressed by several hematopoietic cells and is used in the signal transduction pathways of various hematopoietic receptors. Even though inhibitors of this enzyme could theoretically work as erythropoietic agents, these molecules like HIF-PH inhibitors need to be carefully assessed due to their potential to modulate a wide range of biological pathways, which could trigger significant side effects.

BIOSIMILARS

Biosimilars are biological products that claim to be similar to an innovator product, but are not equivalent to the innovator product unlike generics of small molecule drugs[41,42]. These biosimilar products are now being introduced in the market as a result of the patent expirations of some biologics. Several biosimilars have now been approved in the EU, but are not yet available in the US due to later patent expiration and the absence of a legal pathway for approval. Guidelines for the approval and marketing authorization of biosimilars were first established by the EMEA in 2005 and are still being developed as new classes of biopharmaceuticals reach the end of their patents. In the US, legislation for the devel-

opment of biosimilars (also known as follow-on biologics) is currently being discussed in Congress.

Unlike small molecule drugs where direct analytical comparison between the active ingredient of an innovator drug and generics can be established, the complexity of biological products and the current analytical methods make it impossible to establish equivalence between the innovator product and biosimilar versions. The properties and characteristics of biologics are dependent not only on the sequence of the gene encoding the protein product, but also on a wide variety of other factors including the cell lines used for production of the proteins, the growth conditions of the cells, the purification processes, formulation, packaging and storing parameters. State of the art analytical methods are not sensitive enough to fully characterize a biological product and cannot detect all the changes that occur when the same protein is made by two different manufacturers. In addition, the manufacturing processes used for the production of off-patent biologics are proprietary and biosimilar manufacturers do not have access to the specific conditions used by the innovator to produce a particular biological product. The EMEA recognized these limitations and therefore created new legislation to regulate the development and approval of biosimilars ensuring that the impact of these likely manufacturing differences to patients is appropriately assessed.

Of particular concern to the regulatory agencies and manufacturers is the issue of patient safety, in particular the potential for immunogenicity of the biosimilar product. In contrast to small molecules, biological products have a much greater propensity to induce an immune response. This immune response can lead to the generation of neutralizing antibodies, which can act not only on the drug product, but also on the homologous endogenous proteins. In the case of ESAs, there is potential for the generation of auto-antibodies and the consequences can be severe and result in the development of PRCA. Starting around 1998, the incidence of epoetin-associated PRCA increased significantly in the EU, Canada, and Australia. After several investigations it was concluded that it was triggered by changes in manufacturing and formulation of a single epoetin alfa[43]. This case made the medical community, regulators, and manufacturers aware that even small changes in manufacturing, formulation, and cold-chain management can have significant impact on the safety of biological products. Currently, it is impossible to predict how differences in manufacturing and formulation of biosimilar products will affect their safety and efficacy in the clinic. As a result, the biosimilar guidelines issued by the EMEA require that clinical studies be conducted to compare not only PK as in the case of generic molecules, but also the efficacy and safety of the biosimilar and the reference innovator product. The guidelines go further and require that a pharmacovigilance program plan be implemented post-approval to monitor safety and track any possible adverse events as larger numbers of patients are treated with the biosimilar product. To ensure that these adverse events are appropriately ascribed to the biosimilar product, regulators request that adverse events are reported by the brand name, which is unique to each product. Ongoing discussions in Europe specific to biosimilar epoetins suggest that if a different safety profile is seen through adverse event reporting once the product is commercialized, this may question the previous conclusion of biosimilarity.

Biosimilars are expected to reduce the cost of biopharmaceuticals, but the impact on pricing has been significantly less dramatic than in the case of small molecule generics because of the high costs associated with the manufacturing of complex biological molecules and the need for clinical trials prior to marketing authorization. Currently, five biosimilar epoetins have been approved in the EU. These products are produced by two different manufacturers and commercialized by five different companies. They include two epoetin zeta products, Retacrit® and Silapo®, and three epoetin alfa products, Abseamed®, Binocrit®, and Hexal®.

CONCLUSIONS

It is clear that different therapeutic modalities can be used to activate the EpoR and stimulate erythropoiesis. Current ESA therapeutics include recombinant Epo molecules, which have an identical amino acid sequence to the endogenous Epo molecule, and engineered variants of Epo with increased half-life and longer dosing intervals. Darbepoetin alfa is a genetic engineered analog of Epo, in which five amino acids were replaced to create two novel

glycosylation sites and is approved for treating CKD patients as well as anemic patients with non-myeloid malignancy receiving myelosuppressive chemotherapy. PEG-epoetin beta is a pegylated form of recombinant epoetin beta that is significantly larger than the original molecule and is approved for treating CKD patients. Both of these ESAs have altered PK and PD properties and are indicated for extended dosing schedules.

Several therapeutic modalities have been tested to generate molecules that can activate the EpoR. They include small molecules, peptides, and antibodies that bind to human EpoR and generate an erythropoietic signal. Even though several of these molecules are not being pursued anymore, others are now in late pre-clinical or clinical development. These include Hematide, a pegylated erythropoietic peptide, CNTO 528, another mimetic peptide fused to the Fc region of human IgG1, and FG-4592/ASP1517, a small molecule inhibitor of HIF-PH enzymes, which up-regulates endogenous Epo expression. To become successful therapeutic agents, these candidate molecules will have to demonstrate significant advantages over current registered ESAs. Engineered changes in efficacy that allow for more convenient dosing schedules, non-parenteral drug delivery, easier manufacturing, storage and handling conditions are important factors that will affect the success of these agents in clinical practice. And the road to success will not only require improved efficacy and more convenient administration and dosing schedules, but will also require that any new agent will have to demonstrate a stringent safety profile as good as or better than currently marketed ESAs. In addition to these novel agents, epoetin biosimilars have now been introduced in the EU; these products are similar, but not identical to the innovator products and their use is being closely monitored through pharmacovigilance programs.

Conflict of interest statement. The author is an employee of Amgen Inc.

Acknowledgement

The author wishes to thank Glenn Begley and Steven Elliott for their valuable comments. In addition, the author would like to thank Mandy Suggitt, on behalf of Amgen, Inc. and Larry Kovalick, Amgen, Inc., for editorial support.

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