New Treatment Approaches in Chronic Kidney Disease-associated Anaemia

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Abstract

Recombinant human erythropoietin (rHuEPO) is an effective agent for the treatment of anaemia in patients with chronic kidney disease. However, given its relatively short half-life, it requires a relatively frequent administration schedule. Moreover, it can be administered only subcutaneously or intravenously and is unstable at room temperature, making a strict cold chain control necessary. Pharmacological research has focused on the development of new agents to circumvent these relative disadvantages. Some long-acting erythropoietin-stimulating agents (ESAs) are already available for clinical use that require a less-frequent administration schedule. Peginesatide (Hematide™), which is a small dimeric peptide with a chemical structure unrelated to EPO, has recently ended phase III clinical trials. Other new molecules undergoing clinical development are CNTO 530 and CNTO 528, ACE-011 and hypoxia-inducible transcription factor stabilisers. The latter have the advantage that they can be administered orally but their clinical development faces a significant hurdle following a case of fatal hepatitis. Newer molecules in this class are undergoing clinical evaluation. Other strategies, such as EPO fusion proteins, agonistic antibodies targeting the EPO receptor and gene therapy have only been tested in animal models or are undergoing pre-clinical evaluations. Before clinical approval, all these new strategies need to address safety concerns raised recently about the use of ESAs regarding possible increased cardiovascular risks following targeting to high haemoglobin levels and/or exposure to excessive doses without reaching a target in both higher and lower haemoglobin groups, and reduced survival and tumour control in the oncology setting. Many of these molecules will also need careful evaluation for possible immunogenicity.

Keywords

Anaemia, erythropoietin, chronic kidney disease, Hematide™, prolyl hydroxylase inhibitor, erythropoiesis

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Anaemia, resulting primarily from insufficient production of erythropoietin to support erythropoiesis, is a common complication of chronic kidney disease (CKD). Approximately 50% of patients with CKD stage III–V have anaemia.[1] This percentage increases greatly in patients receiving dialysis.[2] Since the late 1980s, the availability of recombinant human erythropoietin (rHuEPO) has revolutionised the management of anaemia in patients with CKD. Today, erythropoiesis-stimulating agents (ESAs), together with iron supplementation, are the main tool used to achieve anaemia correction in CKD patients.

Over the past two decades, several attempts have been made to develop new ESAs with theoretically improved characteristics compared with rHuEPO, to develop easier manufacturing processes and to develop other strategies that may indirectly increase erythropoiesis. *Table 1* summarises new ESAs under clinical development in CKD patients.

Erythropoiesis-stimulating Agents – Molecules on the Market

Epoetin alpha and epoetin beta are synthesised in Chinese hamster ovary cells and share the same amino acid sequence as endogenous EPO but slightly differ in their carbohydrate moieties.[3] The patent of both drugs expired some years ago, opening the way to biosimilars. Some of the biosimilars have their own international non-proprietary names (INN) (epoetin zeta), but their molecular structure is close to that of epoetin alpha.

Epoetin theta is similar to epoetin alpha and received marketing approval from the European Medicines Agency (EMA) in 2009 as an originator. Epoetin omega and epoetin delta differ from epoetin alpha and beta because they are synthesised in cells other than those from the Chinese hamster ovary[4,5] and, consequently, they differ slightly in their glycosylation patterns compared with epoetin alpha and beta.[6] Epoetin delta was discontinued in 2009 for commercial reasons.

Darbepoetin alpha (Amgen Inc.) is the first EPO analogue with a prolonged half-life. Compared with EPO, it has a modified amino acid sequence, higher sialic acid content and molecular weight, and increased negative charge.[7] The half-life of darbepoetin alpha is nearly two to three times that of rHuEPO depending on the administration route. This characteristic allows a less-frequent administration schedule. Dose requirements are almost independent of the administration route.[8]

Continuous erythropoietin receptor activator (CERA) (Roche) is another modified EPO molecule containing a large water-soluble

Table 1: New Erythropoiesis-stimulating Agents Under Clinical Development in Patients with Chronic Kidney Disease According to the Development Phase

Drug	Company	Structure	Mechanism of Action	Administration Route	Development Phase
Peginesatide (Hematide™)	Affymax and Takeda	Small dimeric peptide + PEG moiety	EPO receptor activation	SC and IV	III
ACE-011	Acceleron and Celgene Corporation	Dimeric fusion protein targeting the TGF-β superfamily	Blocking of the SMAD pathway	SC and IV	IIa
FG-2216	FibroGen	Prolyl hydroxylase inhibitor	EPO gene transcription	PO	II, not actively recruiting
FG-4592	FibroGen	Prolyl hydroxylase inhibitor	EPO gene transcription	PO	II
GSK1278863	GlaxoSmithKline	Prolyl hydroxylase inhibitor	EPO gene transcription	PO	I
CNTO 530	Ortho Biotech	Two 20-amino acid peptides + human IgG4 Fc	EPO receptor activation	SC and IV	I
CNTO 528	Ortho Biotech	Two 20-amino acid peptides + human IgG4 Fc human IgG4 Fc	EPO receptor activation	SC and IV	I

EPO = erythropoietin; Ig = immunoglobulin; IV = intravenous; PEG = polyethylene glycol; PO = oral; SC = subcutaneous; TGF = transforming growth factor.

polyethylene glycol (PEG) moiety. The molecular weight of CERA is nearly double that of EPO and it has a much longer half-life (~130 hours).[9] Like darbepoetin alpha, the binding affinity of CERA for the erythropoietin receptor is also reduced but to a far greater extent. This peculiarity may contribute to distinct pharmacological characteristics (it has been hypothesised that the same CERA molecule may activate the EPO receptor several times without being internalised). The dose does not need to be modified according to the administration route.

Erythropoietin Mimetic Peptides

In 1996, peptides with no sequence homology to EPO but with EPO receptor specificity were identified.[10,11] However, because of their low molecular mass they were rapidly excreted in urine. A small dimeric peptide was then conjugated to a polyethylene glycol moiety to form a new drug, peginesatide (Hematide™, Affymax and Takeda), which has a highly specific binding to the EPO receptor, with five-times less binding potency than epoetin or darbepoetin alpha.[12] Unlike rHuEPO and its analogues, and similar to the first peptides, peginesatide is partially cleared by the kidney. Accordingly, CKD patients needed half the dose of healthy volunteers to achieve similar efficacy. Like other long-acting ESAs, peginesatide pharmacodynamics and dose requirement are irrespective of the administration route. Owing to the fact that peginesatide has a molecular structure unrelated to that of EPO, it was used to correct anaemia in a rat model of antibody-mediated pure red-cell aplasia (PRCA)[13] and then found to be effective in 13 out of 14 patients with CKD and PRCA.[14] Phase I and II trials showed a good efficacy and safety profile for the drug in healthy volunteers[15] and in patients with CKD[16] or cancer[17] when given once a month either intravenously or subcutaneously. In June 2010, Affymax and Takeda announced preliminary results of phase III clinical trials in CKD patients.[18] The four studies (EMERALD 1, EMERALD 2, PEARL 1 and PEARL 2), which enrolled more than 2,000 patients for a median follow-up of 1.3 years, showed non-inferiority in the mean change in haemoglobin from baseline compared with epoetin and darbepoetin in correcting and/or maintaining haemoglobin in the target range.

Although the combined four studies showed that peginesatide was not inferior to the other ESAs in the risk of cardiovascular composite safety end-points, subgroup analysis of patients not on dialysis found a higher frequency of cardiovascular events in the peginesatide group compared with the other ESAs (21.6 and 17.1%, respectively; hazard ratio [HR] 1.34, 90% confidence interval [CI] 1.03–1.73). The clinical meaning of this finding is unclear because it is unlikely that a single ESA would increase cardiovascular risk independently from the haemoglobin target, particularly in non-dialysis patients where doses are much lower than in those receiving dialysis. The HR in the non-dialysis patients was primarily driven by higher rates of death, unstable angina and arrhythmia events in the peginesatide group, but these comorbidities are usually more frequent in dialysis than in non-dialysis patients.

Two other non-erythropoietin-derived EPO-receptor agonists have been recently developed by Ortho Biotech Inc. and are undergoing clinical evaluation. Two sequences of a 20-amino acid peptide with weak EPO-like bioactivity (EMP1) were coupled with a human immunoglobulin (Ig) G4 Fc. The molecule obtained, CNTO 530, selectively binds the EPO-receptor.[19] In animal studies, it is a more potent stimulator of erythropoiesis than epoetin-alpha or darbepoetin alpha.[20] A similar molecule, CNTO 528, has undergone phase I clinical development.[21] Single intravenous administration of CNTO 528 at ascending doses (from 0.03 to 0.9mg/kg) stimulated the production of reticulocytes, red blood cells and haemoglobin in 24 healthy volunteers. Similar findings were obtained in another single and fractional ascending dose study of 44 healthy adult volunteers.[22] Of the ESAs developed to date, CNTO 528 has the longest half-life (four to seven days).

Agonistic Antibodies Targeting the Erythropoietin Receptor

Mouse monoclonal antibodies against the soluble extracellular domain of the human EPO receptor were developed some years ago.[23,24] These antibodies mimic EPO activity but activate the EPO receptor poorly. More recently, ABT-007 (Abbott Bioresearch Center) has been developed.[25] According to data obtained using a transgenic mouse model expressing the human EPO receptor (ABT-007 does not recognise the rodent EPO receptor), this is a potent *in vivo* stimulator of erythropoiesis requiring less-frequent dosing than darbepoetin alpha. In addition, ABT-007 dosing in transgenic mice resulted in less fluctuation of haematocrit than that observed following darbepoetin dosing. Like other long-acting ESAs, increases in haematocrit were similar following either subcutaneous or intravenous administration of ABT-007.

Modified Erythropoietin Molecules Under Development

Attempts have been made to fuse EPO with unrelated peptides, such as human chorionic gonadotropin beta (beta HCG)[26] or the Fc part of an antibody,[27] which are known to increase the *in vivo* potency and circulatory half-life of the molecule. This approach has been used to create an Fc fusion protein (Syntonix Pharmaceuticals, Inc) that can be administered by aerosol inhalation.[28]

Albumin is another fusion partner of glycoproteins such as insulin and interferon. Three kinds of albumin–EPO fusion proteins (IALE, AD2LE and AD1LE) have been produced with a similar half-life and *in vivo* efficacy to that of darbepoetin.[29]

A non-glycosylated denatured EPO was obtained from *Escherichia coli* and then refolded and pegylated.[30] The final molecule had a lower bioactivity than rHuEPO but enhanced thermal stability and prolonged circulating half-life in rats. The addition of linear PEGs of increasing size and a branched activated PEG (PEG-2, 40kDa) progressively improves pharmacokinetic performances;[31] the two 40k-PEG conjugates demonstrated comparable *in vivo* efficacies to that of rHuEPO. However, even if they are effective in correcting anaemia, all these EPO-modified proteins raise some concerns about immunogenicity.[32]

Sotatercept (ACE-011)

ACE-011 (Acceleron and Celgene Corporation) is a novel drug being developed for the treatment of chemotherapy-induced anaemia. It is a dimeric fusion protein that targets members of the transforming growth factor-β superfamily that signal through the activin receptor type IIA (ActRIIA). The drug, consisting of the extracellular domain of the ActRIIA linked to the Fc portion of human IgG1, binds to activin, preventing activin from binding endogenous receptors and interfering with downstream signalling cascades, in particular the SMAD pathway. This pathway promotes hepcidin transcription in hepatocytes and maintains systemic iron homeostasis.[33] In a phase I clinical trial in post-menopausal females, sotatercept increased haematocrit levels.[34] The drug also seems to have antitumour activity and promotes new bone formation.[35]

ACE-011 is currently being studied in two phase II clinical trials in cancer patients; a phase IIa, study is also ongoing to test anaemia correction in patients with CKD stage V.

Hypoxia-inducible Transcription Factor Stabilisers

The hypoxia-inducible transcription factors (HIFs) mediate the effects of hypoxia on the cell. By regulating the expression of a large array of target genes during hypoxia, these proteins also direct adaptive changes in several systems, including the hematopoietic system. Under normal conditions, the HIFα subunit is prolyl hydroxylated and then degraded. Under hypoxia, HIFα accumulates and together with HIFβ transcriptionally activates genes such as *EPO*. The HIF-stabilisers prevent HIF inactivation through alpha hydroxylation and thus stimulate erythropoiesis. These agents have the great advantage that they can be administered orally. The clinical development of FG-2216 (FibroGen, Inc.), the first promising molecule in this class, was halted some years ago following a case of fatal hepatitis. In 2008, phase II clinical trials in dialysis and non-dialysis patients were started with a new agent (FG-4592, FibroGen, Inc.). The preliminary findings of one of these phase II trials were presented at the 2010 annual meeting of the American Society of Nephrology.[36] One hundred and seventeen patients with CKD stage III–IV were randomised to either FG-4592 (n=88, four doses escalating from 0.7 to 2mg/kg administered two or three times weekly) or placebo (n=28) for four weeks. The haemoglobin responder rate was dose dependent and maximal in those receiving the highest dose three times weekly. For responders, median time to haemoglobin response was 22–43 and 15–22 days for twice and three times weekly administration, respectively. Despite the rapid rate of haemoglobin rise with higher doses compared with that observed with other ESAs, a significant increase in blood pressure was observed in only one patient. No liver toxicity was described. GlaxoSmithKline is developing another prolyl hydroxylase inhibitor (GSK1278863). A single-escalating-dose, phase I clinical trial was concluded in 2009 in healthy individuals. A phase IIa clinical trial is underway to evaluate the safety, pharmacokinetics and efficacy of repeat doses in anaemic patients with CKD (both dialysis and pre-dialysis).

Gene Therapy

Several techniques have been tried to release a small but continuous amount of EPO into the circulation through *EPO* gene therapy.[37–42] Unfortunately, these approaches are not yet used in clinical practice because it appears difficult to exactly tune the amount of EPO needed to correct anaemia, maintaining a level of expression that promotes erythropoiesis in the long term and varies according to clinic needs. Moreover, *ex vivo* transfected implanted cells may give rise to immunological problems.

PBI-1402

PBI-1402 (Prometic) is a low-molecular-weight synthetic drug with erythropoiesis-stimulating activity. This compound is orally active and although it mimics the biological activity of EPO, it has a mechanism of action that is distinct from EPO because it does not bind to the same cell surface receptor. In multiple pre-clinical models it has also shown anticancer activity. Phase I studies showed a good safety profile and an increase of reticulocyte counts in healthy volunteers. A phase II trial of PBI-1402 showed a significant increase in haemoglobin levels in patients with chemotherapy-induced anaemia. Recent experiments based on a 5/6 nephrectomised rat model have demonstrated the ability of PBI-1402 to correct anaemia in this setting. These results suggest that this agent could also have a potential use in the treatment of anaemia in patients with CKD, but to date no clinical trials with this compound are under way.

Vitamin E-coated Dialysers

Oxidative stress may have an independent negative role on anaemia and ESA responsiveness; anecdotal data suggest that oral vitamin E supplementation may improve ESA responsiveness.[43] Given that blood–membrane interaction plays a key role in generating oxidative stress, direct free-radical scavenging at the membrane site has been proposed. Some studies tested the role of vitamin E-coated membranes on ESA responsiveness. One study found that a significantly higher proportion of patients achieved the recommended haemoglobin target while receiving a lower ESA dose after being shifted from their previous dialyser to a vitamin E-coated membrane.[44] However, improved ESA responsiveness may also have been caused by enhanced membrane biocompatibility. More recently, a pilot, open, controlled, randomised study of 20 haemodialysis patients compared a synthetic polysulphone dialyser with and without vitamin E.[45] A clear trend towards a greater decrease in the ESA resistance index (i.e. the ratio between haemoglobin and ESA dose) was found in the vitamin E

treated group compared with the control group (37 versus 20%, p = not significant); this difference became statistically significant when analysed using multivariate analysis. Further larger studies need to demonstrate that these membranes have significant positive clinical effects in order to balance high treatment costs.

Conclusion

RHuEPO has been available to treat anaemia in patients with CKD for the past twenty years. More recently, long-acting rHuEPOs with modified molecular structures have also entered the market offering the advantage of less-frequent administration schedules. All these molecules are effective in correcting anaemia and decreasing transfusion needs. However, the treatment of anaemia with rHuEPO or its analogues is quite expensive, given that their synthesis, like that of other biomolecules, is complex. New ESAs are or will be even more expensive than rHuEPO because of the high costs of the pharmacological research, which has not yet been covered by years of selling. Moreover, safety concerns have been raised recently about the use of ESAs that may be associated with increased cardiovascular risks following targeting to high haemoglobin levels and/or exposure to excessive doses[46] and reduced survival and tumour control in the oncology setting.[47] This will entail additional requirements to be fulfilled by the drugs under development before clinical approval, which will further increase costs. However, the synthesis of simpler molecules not related to ESA structure may lead to the creation of cheaper ESAs that would revolutionise the market. In this respect, peginesatide seems to be the most promising in the near future. CNTO 530 and CNTO 528, two non-erythropoietin derived EPO-receptor agonists, are also under clinical development. Given their long half-life, they are likely to be administered less frequently than peginesatide. In the meantime, biosimilars of rHuEPO have entered the market in many European countries and compete economically with the originator or long-acting ESAs.

New approaches to stimulate erythropoiesis will not only need to be effective and safe, but will also need to be a substantial improvement on rHuEPO or other available ESAs, or be cheaper thanks to the development of simpler manufacturing processes. ■

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