

# *Treatment of Anemia in Chronic Kidney Disease: Beyond EPO*

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## **Introduction**

Anemia develops in early stages of CKD and is nearly universal in patients with end stage kidney disease. Although pathogenesis of renal Anemia is multifactorial, the primary cause is inadequate production of erythropoietin by damaged kidneys. Liver in fetal life and peritubular cell of the kidney produces erythropoietin(EPO) after birth. The EPO is major hormone involved in erythropoiesis.

Anemia is associated with serious and irreversible complications including cardiac disease and has been shown to increase the risk of mortality. Anemia causes decrease exercise capacity, cognitive impairment and poor quality of life. Anemia has also been implicated in the development of left ventricular hypertrophy and congestive heart failure. Correction of anemia in patients with CKD, prolongs survival, reduces morbidity and improves clinical outcome.

Before the mid-1980's anemic patients with CKD were managed primarily by regular blood transfusions and with anabolic steroids. Both methods of treatment had serious limitations. Regular blood transfusions increased patients risk for infections like hepatitis B, C & CMV, iron overload and developing anti HLA antibodies. Moreover the adverse effect associated with anabolic steroid, hepatic dysfunction, hirsutism and virilization far outweighed its limited efficacy. In 1983, the gene

for human erythropoietin was identified, and 1985 clinical trials were initiated to evaluate the efficacy and safety of recombinant human erythropoietin. By 1990, recombinant human erythropoietin was licensed in the United States and Europe for the treatment of anemia associated with chronic renal failure including patients on dialysis and pre-dialysis period.

Recombinant erythropoietin (rhEPO) is produced with the use of cells transfected with either the human EPO gene or EPO cDNA (the coding sequence of the gene) linked to an expression vector (recombinant DNA) which are integrated into the genome of the host cell and stably expressed over time. The present therapeutic rhEPO preparations are manufactured in mammalian host cells, because EPO is a complex glycoprotein of 165 amino-acids to which four glycans are attached. rhEPO is administered by subcutaneous or intravenous injection 1 to 3 times weekly. Indistinguishable from endogenous erythropoietin rhEPO binds the dimerized erythropoietin receptor on the surface of erythroid progenitor cells. Erythropoietin binding triggers a variety of responses by means of several signalling pathways. Activation of these pathways results in cell proliferation and inhibits apoptosis of the erythroid progenitor cells. EPO is highly effective at stimulating erythropoiesis and produces a consistent increase in hemoglobin level.

**Table 1 : New therapies for the anemia of chronic kidney disease**

1. First generation erythropoiesis stimulating agents.
  - a. Human recombinant erythropoietin alfa, beta, omega, delta, Epoietin bio-similar short-life, has to be given 2-3 times a week.
2. **Second generation ESA**
  - a. Darbepoietin alfa Longer half-life, flexibility of extended dosing
3. **Third generation ESA**
  - a. Methoxy Polyethylene Glycol Epoietin (CERA) Continuous Erythropoiesis Receptor Activator Extended half life – 130 hrs. Completed phase 3 trials
4. Erythropoietin mimetic peptides Hematide Undergoing phase 2 trials
5. **Hypoxia – inducible factor stabilizer**
  - a. FG-2216 / first generation
  - b. FG-4592 / second generation Undergoing phase 1 trials
6. **Gene Therapy**

Availability of rhEPO created real revolution in management of anemia of CKD. Even though rHuEPO changed the way anemia was managed in patients with CKD, it does have its limitations including efficacy, duration of activity, route of administration and concomitant iron deficiency and inflammation. There are several innovative agents available beyond EPO including second generation darbepoietin alfa, third generation CERA erythropoietin - mimetic peptide and hypoxia inducible factor stabilizer are being developed and are currently in various stages of clinical testing. Aim of this article is to give brief review of these agents (Table 1).

### Darbepoietin Alfa

Darbepoietin alpha is second generation ESA soon likely to become available in India.

### Chemistry and Pharmacology

Darbepoietin – alfa is an erythropoiesis stimulating protein similar to rHuEPO that has five carbohydrate chains compared with three in rHuEPO (epoietin alfa). It is produced in Chinese hamster ovary cells by recombinant DNA technology. It differs from rHuEPO in the addition of two N linked oligosaccharide chains. These two additional sites from amino acid substitutions in the peptide

backbone do not interfere with receptor binding. The carbohydrate chains increase the molecular weight of the glycoprotein from approximately 30,000 Daltons for rHuEPO to near 38,000 Daltons for Darbepoietin - a

### Pharmacokinetics

In animal studies Darbepoietin -  $\alpha$  had a longer terminal half life and greater in vivo biologic activity than rHuEPO, allowing for less frequent administration. In humans Darbepoietin alfa had an approximately 3-fold longer mean terminal half – life than rHu EPO (25.3 hrs vs. 8.5 hrs.) respectively.

Clinical studies in patients with chronic renal failure either receiving or not receiving dialysis have shown that Darbepoietin alfa is equivalent to rHuEPO in terms of increases in hemoglobin concentration, percentage of patients achieving target hemoglobin concentration and average time to reach target hemoglobin concentration, although Darbepoietin alfa is administered less frequently.

### Adverse Effect

Adverse event profile of Darbepoietin alfa is similar to that of recombinant human erythropoietin. The most common adverse effect due to Darbepoietin -  $\alpha$  are infection, hypertension, myalgia and headache. There are no reports of antibody formation associated with Darbepoietin alfa in chronic renal failure patients. No cases of pure red cell aplasia have been reported.

### Dosing

The recommended starting dose in patient with CKD is 0.45 microg/ kg once a weekly for both intravenous and subcutaneous administration with subsequent titration based on hemoglobin concentration. Some patients may respond to 0-75 microg/kg every 2 week or 1.5 microg/ kg every 4 weeks.

### Dose Adjustment

Inadequate response: Increase dose by 25% for hemoglobin increase less than 1 gm/ dl after 4 weeks

**Table 2 : General principles of Treatment of Anemia of CKD**

- Hb is measurement of choice (not Hct.)
- Anemia workup should be initiated with Hb less than 11gm/dl in premenopausal women and Hb less than 12g/dl in men
- Anemia workup Hb/Hct, RBC indices, reticulocytes, serum Fe, TIBC, Transferrin saturation, serum ferritin, fecal occult blood.
- Target hemoglobin for EPO therapy- 11 to 12 g/dl.
- Iron stores: targets transferrin saturation more than 20% and serum ferritin more than 200 ng/ml for EPO therapy.
- Before starting EPO rule out other causes of anemia
- EPO should be administered subcutaneously as preferred route.
- Initial EPO dose 80 to 120 units/kg/week in 2 or 3 doses (SC)
- Titrate dose according to response
- Monitor Hb every one to two weeks after initiation or changes to EPO therapy. Once target achieved monitor every 2-4 weeks.
- Serum ferritin and transferrin saturation should be monitored every month in patient not receiving IV Fe and 3 monthly in those receiving IV Fe.
- Supplementation of IV Fe is necessary in-patient on EPO therapy.
- IV Fe sucrose is agent of choice because of safety.
- Inadequate response to EPO is most commonly due to Fe deficiency other causes include infection, inflammation, chronic blood loss, hyper parathyroidism, aluminium toxicity, myeloma, malnutrition, hemolysis and ACEI therapy.
- Blood transfusions are indicated in severely anemic patients with signs or symptoms and EPO resistance with chronic blood loss.
- Monitor BP in all patients receiving EPO antihypertensive need to be increased

Excessive response: decrease dose by 25% when hemoglobin increase  $> 1$  g/dl in any 2- week period or hemoglobin increase and approaches 12 g/dl in any 2 week period.

Clinical uses : Anemia of CKD and cancer. The longer half life of Darbepoietin -  $\alpha$  together with a similar efficacy and safety profile confers the clinical advantage with over rHuEPO of allowing less frequently dosing (once weekly or every other week or once a month versus three times weekly in renal patients.) thus reducing health -

care utilization and probably improving patient compliance.

### Continuous Erythropoiesis Receptor Activator (CERA)

CERA is the third generation erythropoiesis Stimulating Agent. Phase - 3 clinical testing of CERA has recently been completed. Awaiting final regulatory authority approval. CERA is composed of a large methoxy polyethylene glycol polymer chain integrated into erythropoietin molecule and linked primarily by amide bonds. The elimination half life of CERA is considerably longer than that of epoietin (130 hours vs. 8.5 hours for epoietin  $\alpha$ ) and Darbepoietin -  $\alpha$  (25.3 hours) CERA has shown to provoke a greater erythropoietic response in mice than that of epoietin  $\alpha$  despite being less tightly bound to the erythropoietin receptors. CERA has also demonstrated a 45 fold lower affinity for the erythropoietin receptor than epoietin  $\beta$ . In clinical trials CERA has been compared with epoietin at a reduced dose of up to once every 4 weeks for the treatment of anemia in patients with CKD. Data from randomized controlled multicenter studies that were presented at 2006 ASN annual meeting demonstrated that CERA provided long - term tight control of hemoglobin levels in 800 hemodialysis patients who were converted from epoietin administered 1 to 3 times weekly to CERA once every 2 weeks or once monthly and that stable hemoglobin levels were achieved irrespective of the patient's age gender or diabetic status. In clinical trials CERA has also been compared with Darbepoietin -  $\alpha$  at a reduced dose of up to once every 4 weeks for treatment of anemia in patients with CKD. The CERA administered once every two to 4 weeks achieved high response rate in ESA naïve patients who were not on dialysis.

### Erythropoietin - mimetic peptide : Hematide

Erythropoietin - mimetic peptides are a family of peptides that were identified by phase display technology and found to have erythropoietin

– mimetic activity. The discovery of a drug that can mimic the action of erythropoietin is another method for eliminating the need for rHuEPO in CKD. These agents have the same mechanism of action as endogenous and rHuEPO although they are structurally unrelated. Hematide, one agent in this class, is in phase 2 of clinical development. Hematide is a type of erythropoietin – mimetic peptide. It is highly potent synthetic pegylated peptide that bind to and activates EPO receptor triggers intracellular signaling and causes cell proliferation and differentiation. In vivo studies have shown that hematide is well tolerated and can stimulate erythropoiesis in multiple species to produce a sustained increase in hemoglobin levels. As a pegylated peptide hematide has a longer duration of action that allows for once monthly dosing. Other possible benefits of hematide include its stability at room temperature, a lack of cross reactivity with anti erythropoietin antibodies suggesting it will not cause antibody mediated pure red cell aplasia.

### **Hypoxia Inducible Factor Stabilizers**

The first oral therapy for the treatment of anemia in CKD is also in phase – 2 of clinical trials. This oral agent is hypoxia-inducible factor (HIF) stabilizers. HIF is now recognized to be a key regulator of erythropoietic gene expression. Besides erythropoiesis HIF also regulates iron absorption, energy metabolism, pH, and angiogenesis. HIF is negatively, regulated by a prolyl hydroxylase enzyme in presence of oxygen. The HIF stabilizer inhibits the prolyl hydroxylase enzyme which reduces the susceptibility of HIF to degradation and increase erythropoietin production in pseudo-hypoxia conditions. Erythropoietic pH inhibitor induce complete erythropoiesis by coordinately regulating induction of EPO and improving bioavailability and utilization of iron. FG-2216 is a first generation pH inhibitor that elevates endogenous EPO and hemoglobin in healthy subjects and patients with CKD. FG-4592 is a second generation oral pH inhibitor being evaluated to treat anemia of chronic

disease . Phase I studies have shown it to be safe with no serious adverse events with FG-2216. The hemoglobin increase observed was consistent with the level obtain with rHuEPO and Darbepoietin alfa.

### **Gene Therapy**

It is possible that gene therapy may be able to supplant the need for exogenous EPO administration in patients with CKD. As an example, the potential efficacy of this approach was demonstrated in a study of uremic mice, in which myoblast transfer of human EPO gene led to persistent secretion of human EPO and correction of the anemia. Osada and Ebihara reported on the results of gene therapy with human erythropoietin gene as a method of treating anemia of renal origin.

They studied mice with polycystic kidney disease, transfected cells with an adenovirus vector and human EPO gene, and inserted these cells intraperitoneally. There was a significant increase in serum EPO level and reticulocyte response. Similar results have been reported with human EPO gene therapy in primates. This mode of therapy is very promising but needs further trials.

In conclusion various agents are in various stages of development. What we should be looking for is less costly, more convenient to administer, safe, efficacious and non-antigenic Erythropoiesis Stimulating Agent.

### **Summary**

Anemia is a constant feature of Chronic Kidney Disease (CKD), occurs early in the course, mainly due to deficient production of erythropoietin (EPO) by kidneys. Anemia is associated with increased morbidity and mortality due to cardiovascular events. Anemia is also associated with poor quality of life, decreased exercise capacity, decreased cognitive and sexual function. The introduction of human recombinant erythropoietin for treatment of anemia in 1989, dramatically transformed life of patients with CKD. It not only improves anemia but also cognitive, sexual function and overall quality

of life. Recombinant human erythropoietin alfa, beta, omega, delta and epoietin biosimilars are first generation erythropoiesis stimulating agent (ESA) having short half-life when given intravenously or subcutaneously. EPO Alfa and beta have become standard care for anemia of CKD.

Now several new treatments beyond EPO are on the clinical horizon. **Darbepoietin alfa**, second generation, novel erythropoietin stimulating agent is a hyperglycosylated analog of human EPO, having three-fold longer half-life, will soon become available in India. A third generation molecule continuous erythropoiesis receptor activator (CERA) is pegylated form of EPO that has ability to repeatedly activate the erythropoiesis receptor. It has got substantially longer half-life than that of epoietin (130 hours vs. 8.5 hours for epoietin alfa) and darbepoietin alfa (25.3 hours). CERA has undergone phase III studies and awaiting final regulatory approval from US FDA.

Also in the development stages for treatment of anemia of CKD are **erythropoietin – mimetic peptide**. One agent of this class – Hematide is in phase 2 of clinical development. In vitro studies have shown that Hematide binds the erythropoietin receptor, triggers intra-cellular signaling and cell proliferation and differentiation. In vivo studies have shown that Hematide is well tolerated and can stimulate erythropoiesis in multiple species to produce a sustained increase in haemoglobin level. The first oral treatment of Anemia, Hypoxia inducible factor (HIF) stabilizer – FG-2216 is also

in phase 2 of clinical trial. It increases endogenous erythropoietin synthesis. It may surpass the effectiveness of recombinant EPO because of its ability to stimulate iron absorption and to suppress the negative effect of proinflammatory cytokines on red blood cell production. It is possible that **gene therapy** in future may be able to supplant the need for exogenous EPO administration. The potential efficacy of this approach was demonstrated in a study of uremic mice in which myoblast transfer of the human EPO gene led to persistent secretion of human EPO and correction of anemia.

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