

## Erythropoietin

Erythropoietin, or its alternative erythropoetin (pronounced or *EPO*), is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow.

Also called *hematopoietin* or *hemopoietin*, it is produced by the peritubular capillary endothelial cells in the kidney, and is the hormone that regulates red blood cell production. Erythropoietin hormone produced by the kidney and promotes the formation of red blood cells in the bone marrow. EPO is a glycoprotein (a protein with a sugar attached to it). The kidney cells that make EPO are specialized and are sensitive to low oxygen levels in the blood. These cells release EPO when the oxygen level is low in the kidney. EPO then stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood.

Erythropoietin is produced by the kidneys (90%) and liver (10%) in response to hypoxia or low blood oxygen levels. Hypoxia stimulates the kidneys to produce erythropoietin. Erythropoietin selectively stimulates early red blood cell (erythroid) in the bone marrow to increase bone marrow activity. The increased number of red cells, in turn, helps deliver more oxygen to the tissues and resolve some of the symptoms of weakness and fatigue.

It also has other known biological functions. For example, erythropoietin plays an important role in the brain's response to neuronal injury. *EPO* is also involved in the wound healing process.

EPO is the prime regulator of red blood cell production. Its major functions are to promote the differentiation and development of red blood cells and to initiate the production of hemoglobin, the molecule within red cells that transports oxygen.

Human EPO has a molecular weight of 34,000. The EPO gene has been found on human chromosome 7 (in band 7q21). EPO is

produced not only in the kidney but also, to a lesser extent, in the liver. Different DNA sequences flanking the EPO gene act to control kidney versus liver production of EPO.

The measurement of EPO in the blood is useful in the study of bone marrow disorders and kidney disease. Normal levels of EPO are 0 to 19 mU/ml (milliunits per milliliter). Elevated levels of EPO can be seen in polycythemia, a disorder in which there is an excess of red blood cells. Lower than normal levels of EPO are seen in chronic renal failure.

Using recombinant DNA technology, EPO has been synthetically produced for use in persons with certain types of anemia -- such as anemia due to kidney failure, anemia secondary to AZT treatment of AIDS, and anemia associated with cancer.

EPO has been much misused as a performance-enhancing drug in endurance athletes including some cyclists (in the Tour de France), long-distance runners, speed skaters, and Nordic (cross-country) skiers. When misused in such situations, EPO is thought to be especially dangerous (perhaps because dehydration can further increase the viscosity of the blood, increasing the risk for heart attacks and strokes. EPO has been banned by the Tour de France, the Olympics, and other sports organizations. When exogenous *EPO* is used as a performance-enhancing drug, it is classified as an erythropoiesis-stimulating agent (ESA). Exogenous *EPO* can often be detected in blood, due to slight difference from the endogenous protein, for example in features of posttranslational modification.

More recently, a novel erythropoiesis-stimulating protein (NESP) has been produced. This glycoprotein demonstrates anti-anemic capabilities and has a longer terminal half-life than erythropoietin. NESP offers chronic renal failure patients a lower dose of hormones to maintain normal hemoglobin levels.

### *Regulation*

It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver. Regulation is believed to rely on a feed-back mechanism measuring blood oxygenation. Constitutively synthesized transcription factors for EPO, known as hypoxia-inducible factors (HIFs), are hydroxylated and proteosomally digested in the presence of oxygen. It binds to the erythropoietin receptor (EpoR) on the red cell surface and activates a JAK2 cascade. This receptor is also found in a large number of tissues such as bone marrow cells and peripheral/central nerve cells, many of which activate intracellular biological pathways upon binding with Epo.

*Primary role in red cell blood line*

Erythropoietin has its primary effect on red blood cells by promoting red blood cell survival through protecting these cells from apoptosis. It also cooperates with various growth factors involved in the development of precursor red cells. Specifically, the colony forming unit-erythroid (CFU-E) is completely dependent on erythropoietin. The burst forming unit-erythroid (BFU-E) is also responsive to erythropoietin.

Under hypoxic conditions, the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E.

It has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers.

*Available forms as biomedicine*

- Epogen which is made by Amgen
- Betapoyetin which is made by CinnaGen and Zahravi
- ReliPoyetin which is made by Reliance Life Sciences Pvt. Ltd
- Epokine which is made by Intas Biopharmaceutica Pvt. Ltd
- Procrit (also known as Eprex),
- NeoRecormon

- Methoxy Polyethylene Glycol-Epoetin Beta (MIRCERA)

### *Uses*

Erythropoietin is available as a therapeutic agent produced by recombinant DNA technology in mammalian cell culture. It is used in treating anemia resulting from chronic kidney disease, from the treatment of cancer (chemotherapy and radiation) and from other critical illnesses (heart failure).

#### *Anemia due to chronic kidney disease*

In patients who require dialysis (have stage 5 chronic kidney disease (CKD)), iron should be given with erythropoietin.

Outside of people on dialysis, erythropoietin is used most commonly to treat anemia in people with chronic kidney disease and not on dialysis (those in stage 3 or 4 CKD and those living with a kidney transplant). There are two types of erythropoietin for people with anemia due to chronic kidney disease (not on dialysis), these are:

- epoetin
- darbepoetin

Erythropoietin has been shown to be beneficial in certain neurological diseases like schizophrenia.

#### *Anemia due to treatment for cancer*

Synthetic Erythropoietin should be used with extreme caution while treating anemia caused due to cancer. A clinical alert for doctors was sent out on February 16, 2007, about the use of erythropoiesis-stimulating agents (ESAs) such as epogen and darbepoetin. The advisory recommended caution in using these agents in cancer patients receiving chemotherapy or off chemotherapy, and indicated a lack of clinical evidence to support improvements in quality of life or transfusion requirements in these settings. In addition, on March

9, 2007, drug manufacturers agreed to new black box warnings about the safety of these drugs.

ESAs should only be used in patients with cancer when treating anemia specifically caused by chemotherapy and not for other causes of anemia. Further, it states that ESAs should be discontinued once the patient's chemotherapy course has been completed.

### *Anemia in critically ill patients*

In a recent randomized controlled trial, erythropoietin was shown to not change the number of blood transfusions required by critically ill patients.

A surprising finding in this study was a small mortality benefit in patients receiving erythropoietin. This result was statistically significant after 29 days but not at 140 days. This mortality difference was most marked in patients admitted to the ICU for trauma.

Several hypotheses of potential etiologies for reduced mortality are speculated, but given the known increase in thrombosis and increase benefit in trauma patients as well as marginal nonsignificant benefit (adjusted hazard ratio of 0.9) in surgery patients, one might conclude that some of the benefit might be secondary to the procoagulant effect of erythropoietin. Further research may be necessary to see which critical care patients, if anyone, might benefit from administration of erythropoietin. Any benefit of erythropoietin must be weighed against the 50% increase in thrombosis, which has been well substantiated by numerous trials.

Erythropoietin is also associated with an increased risk of adverse cardiovascular complications in patients with kidney disease if it is used to increase hemoglobin levels above 13.0 g/dl.

Early treatment with erythropoietin correlated with an increase in the risk of Retinopathy of prematurity in premature infants who had anemia of prematurity, raising concern that the angiogenic actions of

erythropoietin may exacerbate retinopathy. However, since anemia itself increases the risk of retinopathy, the correlation with erythropoietin treatment may simply be incidental and reflect that anemia induced retinopathy.

Erythropoietin has been shown to interact with Erythropoietin receptor.

### *Erythropoietin (Epoetin alfa)*

A weakened bone marrow due to cancer infiltration (marrow replacement), lymphoma or leukemia may lead to low red cell production. These patients may respond to epoetin alfa (the commercial equivalent to erythropoietin) therapy. Patients who cannot produce adequate erythropoietin due to kidney or liver disease may also be helped by injections of Epoetin alfa.

Erythropoietin injections are generally well tolerated. Some of the uncommon side effects include: hypertension (elevated blood pressure), iron deficiency, occasionally minor allergies, edema, and occasional increased diarrhea. It can cause growth of some tumors, especially myeloid leukemias.

### *The Effects of Epoetin Alfa on the Central Nervous System*

In rodents, who received brain infusions of Erythropoietin, it was noted to help alleviate ischemia due to anemia, which caused learning and navigational disabilities. *A decrease in the neuronal (nerve) cell death* in those who received Epoetin Alfa before or during the early phase of brain trauma or stroke was noted.

There are now ongoing studies to determine the effectiveness of Epoetin Alfa in protecting the central nervous system for strokes and chemotherapy-induced problems in cognitive thinking. In anemic dialysis patients, Epoetin Alfa has been found to reduce anemia and improve cognitive function. There was both a prolongation of the attention span and improvement in memory skills.

Many studies have been done on recurring chemotherapy patients. Schagn, reported in Cancer, 1999, along with Van Dam and others, studying cognitive function and impairment of brain function along with increased levels of anxiety, depression and fatigue. There was increased brain dysfunction with more intense doses of chemotherapy, such as bone marrow transplantation or higher-dose chemotherapy (CMF or CEF). The estimation is that at least 19% of these patients had cognitive dysfunction or impairment in memory and concentration, which was also correlated with a decrease in hemoglobin.

When one under goes surgery, receives radiation and chemotherapy, the affects can be more severe.

In summary, there is a direct correlation between cognitive deficits and anemia. In addition, there are beneficial effects seen experimentally in clinical trials, which are ongoing, to study the beneficial affects of Epoetin Alfa and anemia, as well as the neuro-protective properties in acute stroke patients.

Preliminary studies are looking favorable. In patients with cancer, it has been noted that anemia can affect both the young and elderly alike. Cancer treatments can promote anemia with a secondary side effect of cognitive impairment. As the anemia becomes more severe, it can exacerbate cognitive defects.

Epoetin Alfa, thus, can help diminish (at least for six months) cognitive deficits secondary to chemotherapy and can also help reduce the significance of anemia. Multiple studies have shown that it takes somewhere between four and eight weeks for Epoetin Alfa to help raise the hemoglobin between one and two grams. In general, those with hematologic malignancies do better with improved survival versus those with solid tumors. One study showed that those having a greater than 10.5-gm hemoglobin had improved survival compared to those with a less than 10.5-gm hemoglobin.

There are currently two forms of Erythropoietin. One is Epoetin Alfa, which has a shorter half-life, averaging approximately 8.5 hours, versus Darbepoetin Alfa, with a half-life of about 25 hours.

Studies have shown that Epoetin Alfa biologic activity has increased several times compared to Darbepoetin. There is a need for a head-to-head trial to compare Darbepoetin to Epoetin alfa to see which has the best therapeutic advantage. Based on current studies, Darbepoetin has a higher molecular rate because of added sialic branches with a 3.5 x longer T<sub>1/2</sub> half-life but less biologic activity. There is not enough clinical evidence to specify whether one is more effective than the other. The studies on longer- and shorter-acting Erythropoietin agents have yet to be accomplished. Studies are in progress in general, and the head-to-head study has to be set up to compare the 200 mg dose of Darbepoetin versus 40,000 units of Erythropoietin (Procrit®). The consensus is unclear as to which is a superior drug. They are both expensive, and one should find out which is least expensive with an equivalent dose.

Epoetin alfa for chemotherapy- or radiotherapy-induced anemia has been evaluated by many investigators. They all show that with breast cancer, there is a rise in the hemoglobin with Epoetin alfa approximately 1.6 to 2.1 gm with an overall improvement in quality of life between 23 and 26%.

### *Hair Follicles Can Manufacture Blood-Doping and Life-Saving Substance, Erythropoietin*

A stunning discovery by German scientists may make blood doping and the treatment of severe anemia as easy as washing your hair. In the October print issue of The FASEB Journal (<http://www.fasebj.org/>), researchers show that the estimated 100,000 hair follicles on each person's head have the potential to become erythropoietin (EPO) factories. EPO, the hormone primarily responsible for the creation of red blood cells, is used illegally to



enhance athletic performance and is used legally to treat severe anemia associated with kidney failure and chemotherapy.

“The ultimate hope is that we’ll be able to up the production of natural EPO in our hair follicles whenever we need it, safely and at a low cost,” said Ralf Paus, senior author of the study. “Our study also highlights that ancient hormones are engaged in many more activities than conventional medical wisdom has assigned to them.”

Normally, EPO is created and released by the kidneys. When the kidneys fail, or when someone undergoes chemotherapy, this process is disrupted and severe anemia occurs.

Today, most people are treated using synthetic EPO to bring red blood cells back to normal levels, but synthetic versions of this hormone are relatively expensive. Blood-doping athletes use synthetic EPO to help their bodies bring red blood cells to above-normal levels. This increased concentration of red blood cells allows the blood to deliver more oxygen to muscles than normal, significantly improving endurance and performance. The major danger in boosting the number of red blood cells above normal is that as the blood thickens with red blood cells, the possibility of heart attack increases.

“This study opens doors to an entirely new approach for treating EPO-related anemia,” said Gerald Weissmann, MD, Editor-in-Chief of The FASEB Journal. “The study also is important because it suggests that there is still much to learn about ‘well known’ processes in the body.”