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Erythropoietic Agents and the Elderly

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Abstract

Erythropoietin is a peptide hormone that stimulates erythropoiesis. There are several agents in clinical use and in development, which either act as ligands for the cell surface receptors of erythropoietin or promote erythropoietin production that stimulates erythropoiesis. These are known as erythropoietic agents. The agents already in use include epoetin alfa, epoetin beta, and darbepoetin alfa. Newer agents stimulating erythropoiesis (such as continuous erythropoietin receptor activator (CERA) or proline hydroxylase inhibitors that increase HIF-1 thereby stimulating erythropoietin production and iron availability and supply) are under active investigation. Erythropoietic agents have been shown to promote neuronal regeneration and to decrease post-stroke infarct size in mouse models. They have also been reported to shorten survival when used to treat anemia in many cancer patients and to increase thromboembolism. In contrast, rapid decrease of erythropoietin levels as observed in astronauts and high-altitude dwellers upon rapid descent to sea level leads to the decrease of erythroid mass, a phenomenon known as *neocytolysis*. The relative decrease in the serum erythropoietin level is known to occur in some subjects with otherwise unexplained anemia of aging. Anemia by itself is a predictor of poor physical function in the elderly and is a significant economic burden on society. One out of every five persons in the United States will be elderly by 2050. Erythropoietic agents, by preventing and treating otherwise unexplained anemias of the elderly and anemia associated with other disease conditions of the elderly, have the potential to improve the functional capacity and to decrease the morbidity and mortality in the elderly, thereby alleviating the overall burden of medical care in society.

INTRODUCTION

Erythropoietin (Epo), an acidic glycoprotein hormone, is the principal regulator of erythropoiesis and is produced mainly by the renal tubular cells(1) but also by the liver(2) in response to hypoxia. Epo acts a ligand for the cell surface-bound polypeptide receptors (EpoR) (3) and stimulates proliferation, survival, and differentiation of the erythroid progenitor cells (4,5). Epo signaling also takes place in erythroid tissues and some of these non-erythroid effects are beneficial, while others are detrimental. EpoR-null mice and Epo-null mice die *in utero* because of lack of mature red blood cell production(6), while a rapid decrease of Epo level experienced by astronauts and high-altitude dwellers upon rapid descent to sea level leads to decrease of the erythroid mass, a phenomenon known as *neocytolysis*(7). Epoetin alfa, epoetin beta, and darbepoetin alfa are all erythropoiesis-stimulating agents (ESAs) that act as ligands

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for the EpoR and thus stimulate erythropoiesis but have different half-lives. There are other agents in different stages of clinical development.

Non-erythroid effects of erythropoietin

It has become apparent that the pleiotropic effects of Epo in nonerythroid tissues are the result of Epo binding to EpoR, and, as in erythroid cells, the Epo-EpoR interaction initiates a signal transduction process that regulates the survival, growth, and differentiation of the involved tissue. Epo and EpoR have been shown to play a physiological role in many non-erythroid cells, including endothelial cells(8), megakaryocytes(9), cells of the brain (10), heart(11), uterus(12), breast(13), and testes(14), but in some tissues (e.g., brain, heart and kidney), the signaling mechanism can be different since Epo can interact with EpoR and CD131 heterodimers(15). Epo and EpoR exhibit a beneficial role in neural, cardiovascular, and retinal tissues and in immune function and tissue repair. Other effects, including improved athletic performance and improved neurocognition, have been attributed to Epo/EpoR signaling but are not convincingly substantiated. Detrimental effects of Epo include increased cancer mortality(16–18), increased blood pressure, and thromboses. The possible benefits as well as the toxicities in non erythroid tissues have recently been comprehensively reviewed(19).

This review will summarize the mechanism of regulation of erythropoiesis, various erythropoietic agents in clinical use and in development and their mechanism of action, known erythropoietic and non-erythropoietic effects of the ESAs on various physiologic and disease conditions with emphasis on those afflicting the elderly population, the prevalence of anemia in the elderly population, and the potential role of ESAs in the management of anemia in elderly.

Regulation of erythropoietin production by hypoxia

The *EPO* gene is one of many “hypoxia-regulated” genes whose expression is controlled by the transcription factor hypoxia-inducible factor (HIF-1). Hypoxia-inducible factor-1 and -2 (HIF-1, HIF-2) (each composed of dimers of α and β subunits) are master transcription factors that control transcriptional regulation of multiple genes involved in diverse processes, including cell proliferation and survival, glycolytic metabolism, angiogenesis, and iron metabolism(20). Epo, vascular endothelial growth factor (VEGF), genes encoding glycolytic enzymes, transferrin, and transferrin receptor genes are just a few of the genes that are regulated by HIF-1(21,22). Only the HIF α subunits are regulated by hypoxia and their expression is controlled post-transcriptionally. Under normoxic conditions, HIF-1 α is rapidly degraded by the ubiquitin-proteasome pathway(23). This targeting and subsequent polyubiquitination of HIF-1 α requires von Hippel-Lindau protein (pVHL), iron, O² and proline hydroxylase activity; this complex constitutes the oxygen sensor(24,25). In normoxia, the prolyl residues of HIF-1 are hydroxylated by the enzyme prolyl hydroxylase, which allows pVHL to bind to HIF-1 α (24). pVHL is part of a multiprotein complex that acts as a ubiquitin ligase(26,27). Subsequent to the binding of pVHL to HIF-1 α , a polyubiquitin tail is added to HIF-1 α which is rapidly degraded by multiprotein complex known as the proteasome (Figure 1). HIF-1 α and HIF-2 α exhibit high sequence homology but have different mRNA expression patterns: HIF-1 α is expressed ubiquitously, whereas HIF-2 α expression is restricted to certain tissues(28,29). Both HIF-1 α and HIF-2 α are regulated by identical mechanisms by hypoxia and form a heterodimer with the same HIF- β subunit. The kidney is the main site of Epo production and HIF-1 is the principal regulator of *EPO* transcription in the kidney (28). In other tissues, such as brain (30) and liver (31) (that generates ~ 20% of circulating Epo), *EPO* gene transcription is HIF-2-dependent (29). The recent discovery of an iron-responsive element in the 5' untranslated region of *HIF-2 α* reveals a novel regulatory link between iron availability and *HIF-2 α* expression (32) that may also influence control of erythropoiesis. Therefore, when the iron supply is limited, HIF-2 α decreases, and when iron is abundant, liver HIF-2 α increases, thereby

increasing liver-synthesized Epo production and further promoting erythropoiesis. The importance of HIF-2 α in regulation of the *EPO* gene was recently demonstrated by a gain-of-function *HIF-2 α* mutation causing erythrocytosis(33).

Treatment of cells with HSP90 inhibitors induces degradation of HIF-1 α , even under hypoxic conditions. HSP90 competes with RACK1 for binding to spermidine/spermine-N-acetyltransferase-2 (SSAT2) that binds to HIF-1 α and promotes its ubiquitination/degradation by stabilizing the interaction of VHL and elongin C. In addition, SSAT1, which shares a 46% amino acid identity with SSAT2, also binds to HIF-1 α and promotes its ubiquitination/degradation. However, in contrast to SSAT2, SSAT1 acts by stabilizing the interaction of HIF-1 α with RACK1. Thus, SSAT1 and SSAT2 play complementary roles in promoting O₂-independent and O₂-dependent degradation of HIF-1 α . Similarly, elongin-C can be recruited by both oxygen-dependent and -independent binding to HIF-1 α (Figure 1).

Another novel mechanism of HIF-1 α regulation has been recently described: a)RSUME (RWD-containing sumoylation enhancer) is induced by hypoxia and enhances the sumoylation of HIF-1 α , promoting its stabilization and transcriptional activity during hypoxia(34); b) calcineurin, along with calcium and calmodulin-dependent serine/threonine phosphatase, inhibits the ubiquitination and proteasomal degradation of HIF-1 α (35).

Erythropoiesis-Stimulating Agents (ESAs) in Clinical Use: Epoetin alfa, Epoetin beta, and Darbepoetin alfa

Recombinant human erythropoietins (rhEpo) were approved by the FDA for clinical use in 1993. Currently, three different rhEpos are used commonly: epoetin alfa (Procrit, Johnson & Johnson, New Brunswick, NJ; Epogen, Amgen, Thousand Oaks, CA), epoetin beta (Recormon, Roche Diagnostics, Basel, Switzerland), and a second-generation erythropoietic agent, darbepoetin alfa (Aranesp, Amgen, Thousand Oaks, CA). The epoetins currently available are produced using recombinant DNA technology in Chinese hamster ovary cell lines, which leads to a glycosylation structure that differs from endogenous human Epo(36). The native Epo has a half-life of 8.5 hours. After a subcutaneous injection, the half lives of rhEpos in plasma increase in the following manner: epoetin alfa to 20.5 hours, epoetin beta to 24 hours and darbepoetin alfa to 49 hours. Different sialic acid-containing carbohydrate components added to the 165 amino acids of epoetin alfa and epoetin beta confer longer half-lives to these compounds(37). Darbepoetin alfa, a hyperglycosylated rhEpo, contains additional N-linked oligosaccharide chains that are accomplished by substitutions at five positions along the 165-amino-acid backbone without altering the tertiary structure. These additional carbohydrates confer a three-times-longer terminal half-life and a five-times-lower affinity for Epo receptors relative to erythropoietin alfa. Despite different pharmacodynamic and pharmacokinetic properties, all these products are considered to have similar clinical efficacy(38,39). Epogen, Procrit and Aranesp are FDA-approved to treat anemia in patients with chronic kidney failure and anemia caused by chemotherapy in certain patients with cancer. Epogen and Procrit are also approved for use in certain patients with anemia who are scheduled to undergo major surgery to reduce blood transfusions during or shortly after surgery and for the treatment of anemia associated with zidovudine (AZT) therapy in HIV patients(40).

For epoetin alfa and epoetin beta, the recommended dosing is as follows: for anemia associated with chronic renal disease it is given subcutaneously (intravenous route is used for hemodialysis patients because of convenience), 50–100 units/kg three times/week; for anemia associated with chemotherapy it is given subcutaneously, 150 units/kg 3 times/week or 10,000 units 3 times/week or 40,000 units once weekly; to achieve and maintain hemoglobin levels between 10–12 g/dL. Hemoglobin levels should not exceed 12 g/dL(41–43).

For darbepoetin alfa, the recommended dosing is as follows: for patients with anemia of chronic renal disease it is given intravenously or subcutaneously, 0.45 mcg/kg once weekly; for anemia associated with chemotherapy it is given subcutaneously, 2.25 mcg/kg once weekly or 200 mcg every two weeks or 500 mcg once every 3 weeks; to achieve and maintain hemoglobin levels between 10–12 g/dL. Hemoglobin levels should not exceed 12 g/dL(41,44).

Newer Erythropoiesis-Stimulating Agents (ESAs)

Epoetin delta

Epoetin delta (Dynepo, Shire Pharmaceuticals, Wayne, PA, USA) is the first human cell line-derived Epo and therefore has a human-type glycosylation profile. Epoetin delta can be administered intravenously or subcutaneously and given on a weekly basis. In a multicenter, open-label, uncontrolled study on predialysis chronic renal disease patients, epoetin delta was found to be an effective and well-tolerated agent for the management of anemia(45,46). It is approved for clinical use in European countries but not in the United States.

Continuous Erythropoietin Receptor Activator (CERA)

CERA (Mircera, Hoffmann-La Roche Inc. (Roche), Nutley, N.J.), is composed of a large methoxy-polyethylene glycol polymer chain integrated into the Epo molecule and linked primarily by amide bonds(47). This results in a longer half-life of approximately 130 hours after both intravenous and subcutaneous administration. Compared with epoetin alfa, CERA has different binding characteristics at the Epo receptor, mainly because it associates with the receptor more slowly, but better stability gives it a longer half-life (48,49). In multiple, randomized phase III trials, CERA has been found to be as safe as currently-approved and in-use ESAs and has been shown to be efficacious in the management of anemia of chronic renal disease when given intravenously at 2-week or 4-week dosing intervals(50,51). It is FDA-approved but not yet in clinical use.

HIF prolyl hydroxylase inhibitors (PHIs)

HIF PHIs are also known as selective HIF stabilizers. HIF PHIs pharmacologically inhibit HIF-prolyl hydroxylase, thereby preventing HIF- α degradation and leading to augmentation of HIF-dependent transcription. This eventually leads to an increase in serum Epo level. FG-2216, FG-4539, FG-4592 (FibroGen, Inc., South San Francisco, CA) are some of the PHIs under different phases of development. FG-2216 leads to stabilization of HIF-2 α isoform and selective activation of Epo and increases serum Epo levels in normal rhesus macaques when chronically administered by an oral route(52). In a phase 1 trial of FG-2216 in hemodialysis patients, a single oral dose of FG-2216 raised median Epo levels from 7.8 to 240.6 mIU/mL (in patients with partially functioning kidneys), from 4.4 to 57.8 mIU/mL (in anephric patients), and from 6.4 to 81.2 mIU/ml in healthy controls. These data suggest that renal anemia appears to be due to disturbed oxygen sensing in the kidney rather than destruction of Epo-producing cells of the kidney. In addition, increase in Epo in anephric patients suggests that extra-renal sources (liver) were stimulated to release Epo by FG-2216(53). In a phase 2, randomized, single-blind, placebo-controlled, dose-escalation study, FG-4592 was administered orally 2 or 3 times weekly for 4 weeks in patients with chronic kidney disease. Five of eight subjects (62%) treated with doses of FG-4592 ranging from 40 to 120 mg had increases in hemoglobin of 1 g/dL during the 4-week treatment period and maintained that increase for 2–4 wks after discontinuing treatment, compared to the control. No difference in adverse effects was noted in the two groups(54).

Non-erythropoietin-derived Epo-R agonists

1) Erythropoietin mimetic peptide-1 (EMP1)—EMP1 is a 20-amino acid peptide that has no sequence homology with Epo and was discovered by screening combinatorial libraries of random sequence peptides using phage display technology. EMP1 binds to Epo receptors and expresses Epo-like bioactivity. However, the binding of EMP1 to Epo-R is relatively weak, was found to be antigenic, and the peptide has a short *in vivo* half-life, making it unsuitable for clinical use(55,56).

2) Hematide—Hematide (Affymax, Inc, Palo Alto, CA) is an Epo mimetic peptide coupled to polyethylene glycol (PEG) to enhance its stability and prolong its half-life in the circulation (57). Hematide has no sequence homology to Epo. In a phase I trial on 28 healthy human volunteers, a one-time intravenous administration stimulated erythropoiesis in a dose-dependent manner with an increase in the Hb level lasting for more than a month(58). In phase II studies in patients with chronic renal disease, Hematide corrected anemia in predialysis patients and maintained elevated Hb levels in patients on hemodialysis(59,60). In a phase 2, dose-finding study of Hematide in cancer patients receiving chemotherapy, Hematide dosed subcutaneously every three weeks resulted in an increase in baseline Hb of ≥ 1 g/dL in more than 50% of patients and was well-tolerated at all doses studied(61). Advantages of Hematide include its stability at room temperature, simpler manufacturing processes, avoiding the need for complex manipulation, product processing from cell lines and genetic engineering techniques, and no cross-reactivity with anti-Epo antibodies(56,62).

3) CNTO 530—This compound is a 58 kD glycoprotein Fc domain fusion protein and includes two EMP1 sequences as the pharmacophore on a human IgG4 Fc framework. CNTO 530 has been shown to selectively bind Epo-R, to mediate Epo-R signaling, and to support proliferation of Epo-dependent cells. In mice, CNTO 530 was shown to have extended pharmacokinetics and to stimulate erythropoiesis leading to a durable increase in hemoglobin in mice(63).

K-11706 (GATA inhibitor)

EPO gene expression is under the control of hypoxia-inducible factor 1 (HIF-1) and is negatively regulated by GATA, which binds to the GATA site of the *EPO* promoter. Thus, drugs that are GATA inhibitors might increase the production of Epo. K-11706 was shown to inhibit GATA and to enhance HIF-1 binding activity, and a subsequent increase in *EPO* promoter activity and Epo protein production *in vitro* and in an *in vivo* mouse assay. Oral administration of K-11706 reversed the decreases in hemoglobin and serum Epo concentrations, reticulocyte counts, and in numbers of erythroid colony-forming units (CFU-Es) in a mouse model of anemia of chronic disease(64).

CID (chemical inducer of dimerization)

CIDs depend upon engineered hematopoietic cells to express modified receptors which lack extracellular domains. These modified receptors, in the absence of an extracellular domain, are insensitive to endogenous ligand such as Epo and are specifically activated by the small molecule drugs called chemical inducers of dimerization (CID)(65,66). CIDs, in combination with a derivative of the thrombopoietin receptor (F36VMpl), have been shown to stimulate erythropoiesis in mice(67), in dogs(68) and in human CD34+ cells following transplantation into immune deficient mice(69).

Non erythropoietic effects of erythropoietin and ESAs

Functional EpoR has been identified in non-erythroid cells such as endothelial, muscle, and neural cells, and there is increasing evidence that Epo can act to stimulate cell proliferation and cell-specific function, or promote cell survival in these tissues(70). In contrast to

erythropoiesis, which is mediated by an EPOR homodimer, Epo signaling in some non-erythroid tissue employs a heteromeric receptor consisting of EPOR and the β common receptor used by GM-CSF, IL-3, and IL-5(71). EpoR and β common receptor together constitute a tissue-protective heteroreceptor in non-erythroid tissues(15). Activation of neuronal Epo receptors (EpoRs) prevents apoptosis induced by NMDA (*N*-methyl-d-aspartate) or NO by EpoR-mediated activation of Jak2, leading to phosphorylation of the inhibitor of NF- κ B (I κ B), subsequent nuclear translocation of the transcription factor NF- κ B, and NF- κ B-dependent transcription of neuroprotective genes(72).

Neuroprotection

The role of Epo in the protection and repair of the nervous system is an area of active investigation. Epo receptors are expressed in the nervous system and other tissues and neuroprotection mediated by Epo has been demonstrated in cell cultures and animal models of neurological disorders(73). In a mouse model of intracranial hemorrhage, administration of recombinant human Epo ameliorated brain injury by two mechanisms. First, it reduced apoptotic cell death by down-regulating the expression of TNF- α , Fas and Fas-L mRNA and the activity of caspases-8, -9 and -3. Second, it increased expression of endothelial(72) nitric oxide synthase (eNOS) and p-eNOS, pAkt, pSTAT3 and pERK, factors that promote regeneration and survival of neural tissues(74). Epo is a survival factor for retinal photoreceptors and acts as a neurologic protection factor in diabetic neuropathy(75). However, Epo has the potential to worsen the proliferative diabetic retinopathy by potentiating retinal angiogenesis independent of VEGF(76). In a double-blind placebo controlled phase II study on patients with stroke, intravenous supplementation of rhEPO was associated with significantly better clinical recovery, faster normalization in the circulating serum marker of injury, S100 beta, and a more favorable outcome on imaging studies (MRI)(77). In an early phase exploratory study in chronic progressive multiple sclerosis, EPO treatment was found to be safe and well tolerated. Using an intra-individual follow-up design, investigators found significant clinical improvement and a tendency of electrophysiological improvement of motor function in chronic progressive MS upon treatment with high-dose rhEPO (48000 IU weekly) but not with low-dose EPO (8000 IU weekly)(78).

Effect on cancer

Several clinical trials in patients with different cancers have shown worsening of survival on treatment with Epo(79). In most of these trials, the investigators sought to maintain the Hb above 12 gm/dl, a non-FDA-approved indication. The antibody that was used to detect the Epo receptor in most of these clinical studies is nonspecific and cross-reacts with heat-shock protein, which is up-regulated in aggressive cancers(16,80). Many of these patients received intravenous iron supplementation. Iron stimulates tumor growth and iron chelation retards tumor growth and stimulates rapid ubiquitination and proteasomal destruction of cyclin D and other cancer-promoting cell cycle regulators(81,82). Recently, Bennett and colleagues analyzed data from phase 3 clinical trials reported between January 1993 and January 2007 to assess ESA-associated risks of VTE and mortality in anemic patients with cancer. These authors reported that patients treated with ESAs had a 1.55-fold increased risk of VTE and a 1.10-fold increased risk of mortality compared with patients who received placebo or standard care(83).

Effect on the quality of life

In a review of clinical trials published in English between January 1993 and September 2005 that evaluated the impact of epoetin alfa on hemoglobin concentration and health-related quality of life (HRQL) across various populations with different underlying causes of anemia

(including cancer, HIV/AIDS and chronic kidney disease), Kimel and colleagues found a statistically and clinically significant impact on HRQL, particularly fatigue(84).

Cardiovascular effects of Epo and ESAs

Kidney

Epo receptors have been demonstrated in vascular as well as non-vascular kidney tissue, specifically on renal tubular cells(85). Administration of rHuEpo is protective in many experimental settings of acute renal failure (ARF), in ischemia/reperfusion injury, or cisplatin-induced kidney damage. Epo improves renal dysfunction by reducing apoptotic cell death if administered before induction of acute renal failure(86). Bahlmann and colleagues investigated whether low-dose subcutaneous darbepoetin-alfa could protect against renal dysfunction and injury in rats with induced chronic renal failure. Chronic low-dose weekly therapy with darbepoetin alfa resulted in improved renal function and reduced histological evidence of renal injury, greater weight gain than controls, and improved survival without any change in systemic blood pressure or any increase in the packed cell volume. This effect was related to activation of the Akt1/protein kinase B pathway(87).

Heart

Epo can reduce infarct size and attenuate ventricular dysfunction after myocardial infarction without changing hematocrit(88). In rats, treatment with Epo prevented left ventricular dilatation and myocardial functional decline by regenerating the myocardial microvasculature (89). In contrast, in a randomized, prospective, open-label trial of 1233 patients (median age 65 years) with congestive heart failure or ischemic heart disease who were undergoing hemodialysis, Besarab and colleagues found a 1- to 5- fold increased risk of myocardial infarctions and vascular access thrombosis when ESAs were administered with target hemoglobin levels of 14 g/dL(90). However, in the largest multicenter, randomized, double-blind, placebo-controlled trial to date evaluating the effect of treating anemia in heart failure (median age of the study population was 69 years), darbepoetin alfa was well-tolerated and effectively raised hemoglobin but was not associated with significant clinical benefit but a trend of lower risk of morbidity and mortality was observed in the group treated with darbepoetin alfa (91).

Thromboembolic effects

The patients with familial polycythemia who have a gain-of-function EpoR mutation(92) and patients with Chuvash polycythemia(93) who overexpress Epo because of a homozygous germline von Hippel-Lindau mutation (*VHL* 598C→T) are at risk for thromboembolic events that may be due to aberrant EpoR stimulation in nonerythroid tissues. Subsequent to these early reports on the thromboembolic risk with Epo, trials in other populations (use of epoetin alfa in patients with cancer and those with chronic renal failure) with target hemoglobin concentrations above 12 g/dL have reported an increase in the risk of thrombotic complications and mortality(94,95).

Critically ill patients

In 2003, in a multicenter, randomized, double-blind trial on patients hospitalized in medical or surgical intensive care units, use of rHuEpo (40,000 U once weekly, maximum of 4 injections) was associated with significantly decreased need for blood transfusion but no significant differences between groups in 28-day mortality or hospital length of stay(96). In 2007, the same researchers reported another randomized trial with 1460 patients with hemoglobin levels below 12 g/dL who were expected to stay in the ICU for at least several days(97). Patients received epoetin alfa (40,000 U) or placebo weekly for 3 weeks when

hemoglobin concentrations were below 12 g/dL. Epoetin alfa did not decrease the number of transfusions, but four-week mortality was significantly lower with epoetin alfa than with placebo among trauma patients (3.5% vs. 6.6%) but not among other surgical or medical patients. However, epoetin alfa recipients were more likely to have a thrombotic venous or arterial vascular event than placebo recipients (16.5% vs. 11.5%).

Anemia in the elderly: prevalence, functional consequences, economic burden and potential role of erythropoietic agents

Using the World Health Organization definition of anemia (< 13g Hb/dL for men and < 12 g Hb/dL for women), 11.0% of men and 10.2% of women 65 years and older and living in the community (i.e. noninstitutionalized elderly) were anemic according to the Third National Health and Nutrition Examination Study (NHANES III) data set. After age 50 years, prevalence of anemia increased sharply to a rate greater than 20% at age 85 and older. The NHANES III study indicated that close to three million elderly men and women (> 65 year and over) in the United States may be anemic and that even when "mild" anemia is present, it either causes and/or is associated with both significant functional impairment and perhaps increased patient mortality(98,99).

In a longitudinal Swedish study, healthy elderly (70 year old) subjects were followed at 1–5 year intervals for 18 years. Mean blood concentrations of hemoglobin were found to progressively decline with aging(100). In a cross-sectional study of community-dwelling older persons in the Chianti area in Italy (In CHIANTI study), after adjustment for age, sex, body mass index, Mini-Mental State Examination score, creatinine level, and the presence of various comorbid conditions, anemia was significantly associated with disability, poorer physical performance, and lower muscle strength(101,102). In a cross-sectional, observational study (Health and Anemia Study) of community-dwelling elderly subjects in Italy, after adjustment for a large number of demographic and clinical confounders, mild anemia remained significantly and independently associated with impaired selective attention performance and disease-specific quality of life ratings(103). Similarly, in a prospective cohort study of 1,744 men and women, aged 71 years or older, from a random household sample in Durham, North Carolina, anemia was independently associated with increased mortality and functional and cognitive decline(104). Semba and colleagues, in a population based study (Women's Health and Aging Study I) of disabled community-dwelling older women (> 65 years of age), showed that older disabled women with anemia associated with renal disease or anemia of inflammation are significantly more likely to die compared with those who are not anemic; however, no elevated risk of death was observed in those with anemia associated with nutrient deficiency or unexplained/idiopathic anemia(105).

Thirty-four percent of elderly anemic subjects in the NHANES III study were not found to have any cause of anemia and were categorized as having anemia of unexplained etiology.

Ershler and colleagues longitudinally followed (over 8 years) serum Epo levels of participants in the Baltimore Longitudinal Study of Aging (BLSA) in relationship with changes in hemoglobin concentration, age, and the diagnosis of hypertension and diabetes mellitus(106). The authors concluded that: 1) in patients who do not develop anemia, higher levels of Epo are required to sustain normal hemoglobin concentrations as a person ages, and 2) in persons who develop anemia, some processes interfere with the production or secretion of Epo to sustain a normal hemoglobin concentration. The conclusions from the BLSA study are suggestive of defect in the hypoxia/Epo-sensing mechanism, at least in some individuals, with increasing age; however, direct evidence for this assumption is lacking. The use of Epo and Epo-mimicking agents that stimulate endogenous Epo (e.g., FG 2216) are of clear interest in this patient group(99).

In a large longitudinal study, the economic impact of anemia was examined in a managed care population(107). Health-care costs (both direct and indirect) were compared between anemic and non-anemic patients with any one of the following diseases known to have a high prevalence associated with anemia: rheumatoid arthritis, inflammatory bowel disease, chronic obstructive pulmonary disease, chronic renal disease, cancer, and congestive heart failure, all of which are relatively more prevalent in the elderly population. After adjusting for differences in demographics, a portion of condition severity, and comorbidity; anemic patients had substantially higher health costs than non-anemic patients. For a population of 1 million, anemia-associated costs were estimated at \$110 million for these six conditions. This study showed a sizable economic burden associated with anemia, both on an individual and a population level.

CONCLUSION

With the continued increase in the elderly population, anemia will likely continue to be a significant economic burden on society. The erythropoietic agents have the potential to play a therapeutic role in this patient population. Though the use of ESAs have negative consequences (e.g., increased blood pressure and thromboembolism), the risks of Epo therapy should be weighed against the potential beneficial effects of improving anemia, quality of life, neurocognitive performance, and of decreasing the impact of ischemia in brain, heart, and other organs. The molecular basis of anemia in a significant number of elderly remains to be unraveled, and we submit that when that is achieved, rational, targeted therapy of the pathophysiological mechanism(s) of anemia should be more effective and likely safer than a nonspecific stimulation of erythropoiesis.

References

1. Koury ST, Bondurant MC, Koury MJ. Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. *Blood* 1988 Feb;71(2):524–527. [PubMed: 3337914]
2. Koury ST, Bondurant MC, Koury MJ, Semenza GL. Localization of cells producing erythropoietin in murine liver by in situ hybridization. *Blood* 1991 Jun 1;77(11):2497–2503. [PubMed: 2039831]
3. D'Andrea AD, Lodish HF, Wong GG. Expression cloning of the murine erythropoietin receptor. *Cell* 1989 Apr 21;57(2):277–285. [PubMed: 2539263]
4. Koury, MJ.; Bondurant, MC. *Science*. Vol. 248. New York, NY: 1990 Apr 20. Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells; p. 378–381.
5. Kelley LL, Koury MJ, Bondurant MC, Koury ST, Sawyer ST, Wickrema A. Survival or death of individual proerythroblasts results from differing erythropoietin sensitivities: a mechanism for controlled rates of erythrocyte production. *Blood* 1993 Oct 15;82(8):2340–2352. [PubMed: 8400286]
6. Lin CS, Lim SK, D'Agati V, Costantini F. Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. *Genes & development* 1996 Jan 15;10(2):154–164. [PubMed: 8566749]
7. Rice L, Alfrey CP. The negative regulation of red cell mass by neocytolysis: physiologic and pathophysiologic manifestations. *Cell Physiol Biochem* 2005;15(6):245–250. [PubMed: 16037689]
8. Anagnostou A, Lee ES, Kessimian N, Levinson R, Steiner M. Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America* 1990 Aug;87(15):5978–5982. [PubMed: 2165612]
9. Fraser JK, Tan AS, Lin FK, Berridge MV. Expression of specific high-affinity binding sites for erythropoietin on rat and mouse megakaryocytes. *Experimental hematology* 1989 Jan;17(1):10–16. [PubMed: 2535696]
10. Nagai A, Nakagawa E, Choi HB, Hatori K, Kobayashi S, Kim SU. Erythropoietin and erythropoietin receptors in human CNS neurons, astrocytes, microglia, and oligodendrocytes grown in culture. *Journal of neuropathology and experimental neurology* 2001 Apr;60(4):386–392. [PubMed: 11305874]

11. Wu H, Lee SH, Gao J, Liu X, Iruela-Arispe ML. Inactivation of erythropoietin leads to defects in cardiac morphogenesis. *Development (Cambridge, England)* 1999 Aug;126(16):3597–3605.
12. Yasuda Y, Masuda S, Chikuma M, Inoue K, Nagao M, Sasaki R. Estrogen-dependent production of erythropoietin in uterus and its implication in uterine angiogenesis. *The Journal of biological chemistry* 1998 Sep 25;273(39):25381–25387. [PubMed: 9738005]
13. Juul SE, Zhao Y, Dame JB, Du Y, Hutson AD, Christensen RD. Origin and fate of erythropoietin in human milk. *Pediatric research* 2000 Nov;48(5):660–667. [PubMed: 11044488]
14. Magnanti M, Gandini O, Giuliani L, Gazzaniga P, Marti HH, Gradilone A, et al. Erythropoietin expression in primary rat Sertoli and peritubular myoid cells. *Blood* 2001 Nov 1;98(9):2872–2874. [PubMed: 11675366]
15. Brines M, Cerami A. Discovering erythropoietin's extra-hematopoietic functions: biology and clinical promise. *Kidney international* 2006 Jul;70(2):246–250. [PubMed: 16738535]
16. Agarwal N, Gordeuk VR, Prchal JT. Are erythropoietin receptors expressed in tumors? Facts and fiction--more careful studies are needed. *J Clin Oncol* 2007 May 1;25(13):1813–1814. [PubMed: 17470878]author reply 5
17. Yoon D, Agarwal N, Prchal JT. Does erythropoietin promote tumor growth? *Clin Cancer Res* 2008 Mar 15;14(6):1920. [PubMed: 18347197]
18. Hardee ME, Cao Y, Fu P, Jiang X, Zhao Y, Rabbani ZN, et al. Erythropoietin blockade inhibits the induction of tumor angiogenesis and progression. *PLoS ONE* 2007;2(6):e549. [PubMed: 17579721]
19. Arcasoy MO. The non-haematopoietic biological effects of erythropoietin. *British journal of haematology* 2008 Apr;141(1):14–31. [PubMed: 18324962]
20. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proceedings of the National Academy of Sciences of the United States of America* 1995 Jun 6;92(12):5510–5514. [PubMed: 7539918]
21. Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nature genetics* 2002 Dec;32(4):614–621. [PubMed: 12415268]
22. Semenza G. HIF-1 and mechanisms of hypoxia sensing. *Curr Opin Cell Biol* 2001;13:167–171. [PubMed: 11248550]
23. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999 May 20;399(6733):271–275. [PubMed: 10353251]
24. Ivan M, Kondo K, Yang H, et al. HIF-1 targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 2001;292:464–468. [PubMed: 11292862]
25. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-1 to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 2001;292:468–472. [PubMed: 11292861]
26. Gordeuk V, Sergueeva AI, Miasnikova GY, Okhotin D, Voloshin Y, Choyke PL, Butman JA, Jedlickova K, Prchal JT, Polyakova LA. Congenital disorder of oxygen sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors. *Blood* 2004;103:3924–3932. [PubMed: 14726398]
27. Iwai K, Yamanaka K, Kamura T, Minato N, Conaway RC, Conaway JW, et al. Identification of the von Hippel-Lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. *Proceedings of the National Academy of Sciences of the United States of America* 1999 Oct 26;96(22):12436–12441. [PubMed: 10535940]
28. Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor 1. *Crit Rev Oncol Hematol* 2006 Jul;59(1):15–26. [PubMed: 16716598]
29. Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, Simon MC. Acute postnatal ablation of Hif-2alpha results in anemia. *Proc Natl Acad Sci U S A* 2007 Feb 13;104(7):2301–2306. [PubMed: 17284606]
30. Chavez JC, Baranova O, Lin J, Pichiule P. The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes. *J Neurosci* 2006 Sep 13;26(37):9471–9481. [PubMed: 16971531]
31. Rankin EB, Biju MP, Liu Q, Unger TL, Rha J, Johnson RS, et al. Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. *J Clin Invest* 2007 Apr;117(4):1068–1077. [PubMed: 17404621]

32. Sanchez M, Galy B, Muckenthaler MU, Hentze MW. Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. *Nat Struct Mol Biol.* 2007 Apr 8;
33. Percy MJ, Furlow PW, Lucas GS, Li X, Lappin TR, McMullin MF, et al. A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. *The New England journal of medicine* 2008 Jan 10;358(2):162–168. [PubMed: 18184961]
34. Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* 2007 Oct 19;131(2):309–323. [PubMed: 17956732]
35. Liu YV, Hubbi ME, Pan F, McDonald KR, Mansharamani M, Cole RN, et al. Calcineurin promotes hypoxia-inducible factor 1alpha expression by dephosphorylating RACK1 and blocking RACK1 dimerization. *The Journal of biological chemistry* 2007 Dec 21;282(51):37064–37073. [PubMed: 17965024]
36. Skibeli V, Nissen-Lie G, Torjesen P. Sugar profiling proves that human serum erythropoietin differs from recombinant human erythropoietin. *Blood* 2001 Dec 15;98(13):3626–3634. [PubMed: 11739166]
37. Goldwasser E, Kung CK, Eliason J. On the mechanism of erythropoietin-induced differentiation. 13. The role of sialic acid in erythropoietin action. *The Journal of biological chemistry* 1974 Jul 10;249(13):4202–4206. [PubMed: 4368980]
38. Egrie JC, Dwyer E, Browne JK, Hitz A, Lykos MA. Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. *Experimental hematology* 2003 Apr;31(4):290–299. [PubMed: 12691916]
39. Beutel G, Ganser A. Risks and benefits of erythropoiesis-stimulating agents in cancer management. *Seminars in hematology* 2007 Jul;44(3):157–165. [PubMed: 17631180]
40. (FDA). USFaDA. 2007. <http://www.fda.gov/bbs/topics/NEWS/2007/NEW01740.html>
41. (FDA). USFaDA. Drugs and Therapeutic Biological Products (CDER). [Accessed April 6, 2008]. <http://www.fda.gov/medwatch/safety/2007/safety07.htm#ESA>
42. Procrit (epoetin alfa) full prescribing information. [accessed on April 6, 2008]. <http://www.procrit.com/procrit/shared/OBI/PI/ProcritBooklet.pdf#page=1>
43. Reocormon(Epoetin beta)full prescribing information. [accessed on April 6, 2008]. <http://www.medsafe.govt.nz/profs/Datasheet/r/Reocormoninj.htm>
44. Aranesp (darbepoetin alpha) full prescribing information. [accessed on April 6, 2008]. <http://www.aranesp.com/>
45. Kwan JT, Pratt RD. Epoetin delta, erythropoietin produced in a human cell line, in the management of anaemia in predialysis chronic kidney disease patients. *Current medical research and opinion* 2007 Feb;23(2):307–311. [PubMed: 17288685]
46. Martin KJ. The first human cell line-derived erythropoietin, epoetin-delta (Dynepo), in the management of anemia in patients with chronic kidney disease. *Clinical nephrology* 2007 Jul;68(1):26–31. [PubMed: 17703832]
47. Macdougall IC. CERA (Continuous Erythropoietin Receptor Activator): a new erythropoiesis-stimulating agent for the treatment of anemia. *Current hematology reports* 2005 Nov;4(6):436–440. [PubMed: 16232379]
48. Fishbane S, Pannier A, Liogier X, Jordan P, Dougherty FC, Reigner B. Pharmacokinetic and pharmacodynamic properties of methoxy polyethylene glycol-epoetin beta are unaffected by the site of subcutaneous administration. *Journal of clinical pharmacology* 2007 Nov;47(11):1390–1397. [PubMed: 17962427]
49. Hirsh V, Glaspy J, Mainwaring P, Manegold C, Ramlau R, Eid JE. Phase II study of two dose schedules of C.E.R.A. (Continuous Erythropoietin Receptor Activator) in anemic patients with advanced non-small cell lung cancer (NSCLC) receiving chemotherapy. *Trials* 2007;8:8. [PubMed: 17341293]
50. Sulowicz W, Locatelli F, Ryckelynck JP, Balla J, Csiky B, Harris K, et al. Once-monthly subcutaneous C.E.R.A. maintains stable hemoglobin control in patients with chronic kidney disease on dialysis and converted directly from epoetin one to three times weekly. *Clin J Am Soc Nephrol* 2007 Jul;2(4):637–646. [PubMed: 17699476]
51. Levin NW, Fishbane S, Canedo FV, Zeig S, Nassar GM, Moran JE, et al. Intravenous methoxy polyethylene glycol-epoetin beta for haemoglobin control in patients with chronic kidney disease

- who are on dialysis: a randomised non-inferiority trial (MAXIMA). *Lancet* 2007 Oct 20;370(9596):1415–1421. [PubMed: 17950856]
52. Hsieh MM, Linde NS, Wynter A, Metzger M, Wong C, Langsetmo I, et al. HIF prolyl hydroxylase inhibition results in endogenous erythropoietin induction, erythrocytosis, and modest fetal hemoglobin expression in rhesus macaques. *Blood* 2007 Sep 15;110(6):2140–2147. [PubMed: 17557894]
 53. Bernhardt, W.; Wiesener, MS.; Schmieder, RE.; Gunzler, V.; Eckardt, KU. The Prolyl Hydroxylase Inhibitor FG-2216 Stimulates EPO Production in Nephric and Anephric Dialysis Patients - Evidence for an Underutilized Production Capacity in Liver and Kidneys. Vol. 2007. San Francisco: American Society of Nephrology Renal Week; 2007 Nov 2–5. Abstract: SA-PO784
 54. Frohna, P.; Milwee, S.; Pinkett, J.; Lee, T.; Moore-Perry, K.; Chou, J.; Ellison, RH. Results from a Randomized, Single-Blind, Placebo-Controlled Trial of FG-4592, a Novel Hypoxia Inducible Factor Prolyl Hydroxylase Inhibitor, in CKD Anemia. Vol. 2007. San Francisco: American Society of Nephrology Renal Week; 2007 Nov 2–5. Abstract SU-PO806
 55. Johnson DL, Farrell FX, Barbone FP, McMahon FJ, Tullai J, Hoey K, et al. Identification of a 13 amino acid peptide mimetic of erythropoietin and description of amino acids critical for the mimetic activity of EMP1. *Biochemistry* 1998 Mar 17;37(11):3699–3710. [PubMed: 9521688]
 56. Macdougall IC. Novel erythropoiesis-stimulating agents: a new era in anemia management. *Clin J Am Soc Nephrol* 2008 Jan;3(1):200–207. [PubMed: 18077782]
 57. Fan Q, Leuther KK, Holmes CP, Fong KL, Zhang J, Velkovska S, et al. Preclinical evaluation of Hematide, a novel erythropoiesis stimulating agent, for the treatment of anemia. *Experimental hematology* 2006 Oct;34(10):1303–1311. [PubMed: 16982323]
 58. Stead RB, Lambert J, Wessels D, Iwashita JS, Leuther KK, Woodburn KW, et al. Evaluation of the safety and pharmacodynamics of Hematide, a novel erythropoietic agent, in a phase 1, double-blind, placebo-controlled, dose-escalation study in healthy volunteers. *Blood* 2006 Sep 15;108(6):1830–1834. [PubMed: 16720830]
 59. Macdougall, I.; Tucker, B.; Yaqoob, M.; Mikhail, A.; Nowicki, M.; MacPhee, I.; Mysliwiec, M.; Sulowicz, W.; Zamauskaite, A.; Leong, R.; Iwashita, J.; Duliege, AM.; Wiecek, A. Hematide, a synthetic peptide-based erythropoiesis stimulating agent, achieves correction of anemia and maintains hemoglobin in patients with chronic kidney disease not on dialysis; The annual meeting of the American Society of Nephrology; 2006 Nov 14–19. [Abstract F-FC079]
 60. Besarab, A.; Zeig, S.; Geronemus, R.; Pergola, P.; Whittier, F.; Zabaneh, R.; Schiller, B.; Kaplan, M.; Levin, N.; Wright, S.; Swan, S.; Wintz, R.; Wombolt, D.; Leong, R.; Lang, W.; Franco, M.; Duliege, A. National Kidney Foundation Spring Clinical Meeting. Orlando, FL: 2007 Apr 10–14. Hematide™, a synthetic peptide-based erythropoiesis stimulating agent, maintains hemoglobin in hemodialysis patients previously treated epoetin alfa. [Abstract 99]
 61. Pickering L, Cwierka K, Jassem J, Petera J, Pettengell R, Ramlau R, Vorlicek J, Vyzula R, Duliege A, Lang W, Stead RB, Harper PG. Correction of Anemia with Hematide, a Synthetic Peptide-Based Erythropoiesis Stimulating Agent (ESA), in Oncology Patients Receiving Chemotherapy. *Blood* 2007;110(11)Abstract #3666
 62. Woodburn KW, Fan Q, Winslow S, Chen MJ, Mortensen RB, Casadevall N, et al. Hematide is immunologically distinct from erythropoietin and corrects anemia induced by antierythropoietin antibodies in a rat pure red cell aplasia model. *Experimental hematology* 2007 Aug;35(8):1201–1208. [PubMed: 17662888]
 63. Bugelski PJ, Capocasale RJ, Makropoulos D, Marshall D, Fisher PW, Lu J, et al. CNTO 530: Molecular pharmacology in human UT-7(EPO) cells and pharmacokinetics and pharmacodynamics in mice. *Journal of biotechnology* 2008 Mar 20;134(1–2):171–180. [PubMed: 18242752]
 64. Nakano Y, Imagawa S, Matsumoto K, Stockmann C, Obara N, Suzuki N, et al. Oral administration of K-11706 inhibits GATA binding activity, enhances hypoxia-inducible factor 1 binding activity, and restores indicators in an in vivo mouse model of anemia of chronic disease. *Blood* 2004 Dec 15;104(13):4300–4307. [PubMed: 15328158]
 65. Spencer, DM.; Wandless, TJ.; Schreiber, SL.; Crabtree, GR. *Science*. Vol. 262. New York, NY: 1993 Nov 12. Controlling signal transduction with synthetic ligands; p. 1019–1024.
 66. Neff T, Blau CA. Pharmacologically regulated cell therapy. *Blood* 2001 May 1;97(9):2535–2540. [PubMed: 11313238]

67. Weinreich MA, Lintmaer I, Wang L, Liggitt HD, Harkey MA, Blau CA. Growth factor receptors as regulators of hematopoiesis. *Blood* 2006 Dec 1;108(12):3713–3721. [PubMed: 16902155]
68. Neff T, Horn PA, Valli VE, Gown AM, Wardwell S, Wood BL, et al. Pharmacologically regulated in vivo selection in a large animal. *Blood* 2002 Sep 15;100(6):2026–2031. [PubMed: 12200362]
69. Nagasawa Y, Wood BL, Wang L, Lintmaer I, Guo W, Papayannopoulou T, et al. Anatomical compartments modify the response of human hematopoietic cells to a mitogenic signal. *Stem cells* (Dayton, Ohio) 2006 Apr;24(4):908–917.
70. Chen ZY, Asavaritikrai P, Prchal JT, Noguchi CT. Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. *The Journal of biological chemistry* 2007 Aug 31;282(35):25875–25883. [PubMed: 17604282]
71. Brines M, Grasso G, Fiordaliso F, Sfacteria A, Ghezzi P, Fratelli M, et al. Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. *Proceedings of the National Academy of Sciences of the United States of America* 2004 Oct 12;101(41):14907–14912. [PubMed: 15456912]
72. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. *Nature* 2001 Aug 9;412(6847):641–647. [PubMed: 11493922]
73. Genc S, Koroglu TF, Genc K. Erythropoietin and the nervous system. *Brain research* 2004 Mar 12;1000(1–2):19–31. [PubMed: 15053948]
74. Lee ST, Chu K, Sinn DI, Jung KH, Kim EH, Kim SJ, et al. Erythropoietin reduces perihematomal inflammation and cell death with eNOS and STAT3 activations in experimental intracerebral hemorrhage. *Journal of neurochemistry* 2006 Mar;96(6):1728–1739. [PubMed: 16539688]
75. Bianchi R, Buyukakilli B, Brines M, Savino C, Cavaletti G, Oggioni N, et al. Erythropoietin both protects from and reverses experimental diabetic neuropathy. *Proceedings of the National Academy of Sciences of the United States of America* 2004 Jan 20;101(3):823–828. [PubMed: 14718663]
76. Watanabe D, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M, et al. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. *The New England journal of medicine* 2005 Aug 25;353(8):782–792. [PubMed: 16120858]
77. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. *Molecular medicine* (Cambridge, Mass 2002 Aug;8(8):495–505.
78. Ehrenreich H, Fischer B, Norra C, Schellenberger F, Stender N, Stiefel M, et al. Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis. *Brain* 2007 Oct;130(Pt 10):2577–2588. [PubMed: 17728357]
79. Blau CA. Erythropoietin in cancer: presumption of innocence? *Stem cells* (Dayton, Ohio) 2007 Aug;25(8):2094–2097.
80. Elliott S, Busse L, Bass MB, Lu H, Sarosi I, Sinclair AM, et al. Anti-Epo receptor antibodies do not predict Epo receptor expression. *Blood* 2006 Mar 1;107(5):1892–1895. [PubMed: 16249375]
81. Necas E, Prchal JT. The diverse functions of Lipocalin: A recently-recognized mediator of transferrin independent iron transport, innate immunity, and cancer signaling. *Hematologist* 2006;3:3.
82. Whitnall M, Howard J, Ponka P, Richardson DR. A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proceedings of the National Academy of Sciences of the United States of America* 2006 Oct 3;103(40):14901–14906. [PubMed: 17003122]
83. Bennett CL, Silver SM, Djulbegovic B, Samaras AT, Blau CA, Gleason KJ, et al. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. *Jama* 2008 Feb 27;299(8):914–924. [PubMed: 18314434]
84. Kimel M, Leidy NK, Mannix S, Dixon J. Does epoetin alfa improve health-related quality of life in chronically ill patients with anemia? Summary of trials of cancer, HIV/AIDS, and chronic kidney disease. *Value Health* 2008 Jan-Feb;11(1):57–75. [PubMed: 18237361]
85. Westenfelder C, Biddle DL, Baranowski RL. Human, rat, and mouse kidney cells express functional erythropoietin receptors. *Kidney international* 1999 Mar;55(3):808–820. [PubMed: 10027918]

86. Fliser D, Haller H. Erythropoietin and treatment of non-anemic conditions--cardiovascular protection. *Seminars in hematology* 2007 Jul;44(3):212–217. [PubMed: 17631185]
87. Bahlmann FH, Song R, Boehm SM, Mengel M, von Wasielewski R, Lindschau C, et al. Low-dose therapy with the long-acting erythropoietin analogue darbepoetin alpha persistently activates endothelial Akt and attenuates progressive organ failure. *Circulation* 2004 Aug 24;110(8):1006–1012. [PubMed: 15302785]
88. Parsa CJ, Matsumoto A, Kim J, Riel RU, Pascal LS, Walton GB, et al. A novel protective effect of erythropoietin in the infarcted heart. *The Journal of clinical investigation* 2003 Oct;112(7):999–1007. [PubMed: 14523037]
89. Westenbrink BD, Lipsic E, van der Meer P, van der Harst P, Oeseburg H, Du Marchie Sarvaas GJ, et al. Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. *European heart journal* 2007 Aug;28(16):2018–2027. [PubMed: 17576662]
90. Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *The New England journal of medicine* 1998 Aug 27;339(9):584–590. [PubMed: 9718377]
91. Ghali JK, Anand IS, Abraham WT, Fonarow GC, Greenberg B, Krum H, et al. Randomized double-blind trial of darbepoetin alfa in patients with symptomatic heart failure and anemia. *Circulation* 2008 Jan 29;117(4):526–535. [PubMed: 18195176]
92. Prchal JT, Semenza GL, Prchal J, Sokol L. Familial polycythemia. *Science (New York, NY)* 1995 Jun 30;268(5219):1831–1832.
93. Gordeuk VR, Sergueeva AI, Miasnikova GY, Okhotin D, Voloshin Y, Choyke PL, et al. Congenital disorder of oxygen sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors. *Blood* 2004 May 15;103(10):3924–3932. [PubMed: 14726398]
94. Bohlius J, Wilson J, Seidenfeld J, Piper M, Schwarzer G, Sandercock J, et al. Recombinant human erythropoietins and cancer patients: updated meta-analysis of 57 studies including 9353 patients. *Journal of the National Cancer Institute* 2006 May 17;98(10):708–714. [PubMed: 16705125]
95. Singh AK, Szczec L, Tang KL, Barnhart H, Sapp S, Wolfson M, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *The New England journal of medicine* 2006 Nov 16;355(20):2085–2098. [PubMed: 17108343]
96. Corwin HL, Gettinger A, Pearl RG, Fink MP, Levy MM, Shapiro MJ, et al. Efficacy of recombinant human erythropoietin in critically ill patients: a randomized controlled trial. *Jama* 2002 Dec 11;288(22):2827–2835. [PubMed: 12472324]
97. Corwin HL, Gettinger A, Fabian TC, May A, Pearl RG, Heard S, et al. Efficacy and safety of epoetin alfa in critically ill patients. *The New England journal of medicine* 2007 Sep 6;357(10):965–976. [PubMed: 17804841]
98. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood* 2004 Oct 15;104(8):2263–2268. [PubMed: 15238427]
99. Guralnik JM, Ershler WB, Schrier SL, Picozzi VJ. Anemia in the elderly: a public health crisis in hematology. *Hematology / the Education Program of the American Society of Hematology American Society of Hematology* 2005:528–532.
100. Nilsson-Ehle H, Jagenburg R, Landahl S, Svanborg A. Blood haemoglobin declines in the elderly: implications for reference intervals from age 70 to 88. *European journal of haematology* 2000 Nov; 65(5):297–305. [PubMed: 11092459]
101. Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S, et al. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. *Journal of the American Geriatrics Society* 2004 May;52(5):719–724. [PubMed: 15086651]
102. Cesari M, Penninx BW, Lauretani F, Russo CR, Carter C, Bandinelli S, et al. Hemoglobin levels and skeletal muscle: results from the InCHIANTI study. *The journals of gerontology* 2004 Mar;59(3):249–254. [PubMed: 15031309]

103. Lucca U, Tettamanti M, Mosconi P, Apolone G, Gandini F, Nobili A, et al. Association of mild anemia with cognitive, functional, mood and quality of life outcomes in the elderly: the "Health and Anemia" study. PLoS ONE 2008;3(4):e1920. [PubMed: 18382689]
104. Denny SD, Kuchibhatla MN, Cohen HJ. Impact of anemia on mortality, cognition, and function in community-dwelling elderly. The American journal of medicine 2006 Apr;119(4):327–334. [PubMed: 16564775]
105. Semba RD, Ricks MO, Ferrucci L, Xue QL, Chaves P, Fried LP, et al. Types of anemia and mortality among older disabled women living in the community: the Women's Health and Aging Study I. Aging clinical and experimental research 2007 Aug;19(4):259–264. [PubMed: 17726354]
106. Ershler WB, Sheng S, McKelvey J, Artz AS, Denduluri N, Tecson J, et al. Serum erythropoietin and aging: a longitudinal analysis. Journal of the American Geriatrics Society 2005 Aug;53(8):1360–1365. [PubMed: 16078962]
107. Ershler WB, Chen K, Reyes EB, Dubois R. Economic burden of patients with anemia in selected diseases. Value Health 2005 Nov-Dec;8(6):629–638. [PubMed: 16283863]

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