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# Erythropoiesis-stimulating agents and pure red-cell aplasia: you can't fool Mother Nature

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**Subtle alterations in the properties of biopharmaceutical agents may increase their immunogenicity and lead to the production of autoantibodies. Biosimilar agents may not undergo the same quality control in their production, packaging, storage, and distribution as their patented competitors. The extensive use of biosimilar erythropoiesis-stimulating agents led to an epidemic of pure red-cell aplasia in Thailand. The response of Thai regulators may be a model for other countries as the use of biosimilar agents expands.**

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The development of biopharmaceuticals such as erythropoiesis-stimulating agents (ESAs) has been a major therapeutic achievement that has increased our ability to treat many diseases previously thought to be incurable. A biopharmaceutical is defined as 'a medicine whose active substance is made by or derived from a living organism.'[1] There are more than 100 existing biopharmaceutical agents and over 400 new ones under development. A 'biosimilar' (in Europe), or 'follow-on biologic' agent (in the United States), is 'a medicine which is similar to a biological medicine that has already been authorized' and whose patent has typically expired.[1] It is often referred to as a 'generic' biopharmaceutical, but, unlike the generic version of a chemical agent, there is no guarantee that a biosimilar agent is identical to the reference agent, as the structure of the large proteins involved cannot be unequivocally determined. The driving force behind the development of biosimilars is primarily financial, analogous to the cost savings arising from the use of generic or lower-cost 'me too' versions of branded chemical therapeutic agents. In the case of generic chemical competitors, the market usually welcomes the opportunity for cost savings even though data supporting equivalence to the reference agent may be weak. The complexities related to the manufacturing, packaging, storage, and shipping of biopharmaceutical agents make their biosimilar competitors much more vulnerable to efficacy and safety issues, so greater vigilance is required to assure that biosimilars undergo adequate pre- and postmarketing scrutiny to assure their therapeutic value.

Praditpornsilpa *et al.*[2] (this issue) describe the experience in Thailand with biosimilar ESAs. Epoetin-alfa was licensed in Thailand in 1990 and epoetin-beta in 1998. The first biosimilar epoetin-alfa became available in 1997 in Thailand, and, as of 2009, 14 biosimilar ESAs were licensed in Thailand, manufactured in Argentina, China, South Korea, and India. Concomitant with the increased penetration of biosimilar ESAs in the Thai market, the authors noted an alarming increase in the prevalence of pure red-cell aplasia (PRCA) and sought to more systematically diagnose the disorder and characterize the epidemic. They were able to confirm that 23 of 30 patients referred because of loss of ESA efficacy had antibodies to erythropoietin, consistent with PRCA. All of these patients received the biosimilar ESA subcutaneously, confirming previous observations that the interaction between an ill-defined alteration in the erythropoietin molecule and subcutaneous injection of the agent is a prerequisite for the development of PRCA.

PRCA is a relatively rare condition that leads to progressive, severe anemia. It has been associated with a congenital disorder known as Diamond–Blackfan syndrome and has been associated with acquired disorders such as thymoma, systemic lupus, or viral infection.[3] Bone marrow examination in affected patients reveals an almost complete absence of red-cell precursors with normal numbers of white-cell and platelet precursors. The reticulocyte count is extremely low. A number of case reports of an antibody-mediated form of PRCA associated with ESA therapy started to appear in the mid-1990s, but this complication did not receive much attention until an epidemic of ESA-associated PRCA occurred between 1998 and 2002. In 1998, Johnson & Johnson, the manufacturer of Eprex (a brand of epoetin-alfa), changed the stabilizing agent from human serum albumin to polysorbate 80 and glycine. It is hypothesized that the polysorbate 80 interacted with leachates from the uncoated rubber stoppers in the Eprex-prefilled syringes to induce increased immunogenicity of the agent and result in the increased incidence of PRCA, but only when the substance was administered subcutaneously. Once the problem was identified, Johnson & Johnson coated the rubber stoppers in the prefilled syringes with latex, and the PRCA epidemic subsided. The affected patients demonstrated neutralizing antibodies to epoetin that crossreact with endogenous erythropoietin. Since Eprex is not marketed in the United States, patients there were not affected by this PRCA epidemic. In December 2002, health authorities in Europe formally contraindicated subcutaneous administration of Eprex for patients with chronic kidney disease.[4] There have been case reports of PRCA occurring in patients who have received darbepoetin, the Epogen form of epoetin-alfa,

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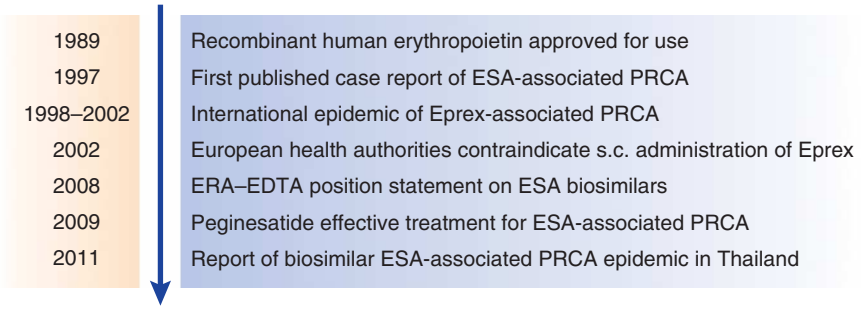


**Figure 1 | Timeline of ESA-associated PRCA.** EDTA, European Dialysis and Transplant Association; ERA, European Renal Association; ESA, erythropoiesis-stimulating agent; PRCA, pure red-cell aplasia; s.c., subcutaneous.

and epoetin-beta, but 91% of 191 antibody-mediated PRCA cases worldwide reported in 2004 were associated with Eprex.[5] A timeline of major events regarding ESA-associated PRCA is shown in Figure 1.

As of 2009, biosimilar, non-patented ESAs were available in many countries of Asia, South and Central America, and Africa.[6] These agents are much less costly than patented ESAs, and their use allows countries with tight health-care budgets to offer ESA therapy to more patients. The subcutaneous administration of short-acting ESAs provides even greater cost savings, as a smaller dose can be used to achieve the same target hemoglobin level as compared with intravenous administration.[7] Praditpornsilpa *et al.* report 23 cases of ESA-related PRCA among an estimated 59,900 patients at risk, for an incidence rate of 1:2068.[2] To put this into perspective, Macdougall[8] noted that the incidence of ESA-related PRCA based on a review of the subject by Rossert *et al.*[9] is in the order of 1:10,000 patients and compares favorably with the 1:5000 risk of developing a life-threatening reaction to the administration of penicillin. 'It seems unlikely that the incidence of biosimilar-induced PRCA would ever approach this level.'[8] Macdougall puts his faith in the fact that 'the very stringent regulations imposed by the EMEA [European Agency for the Evaluation of Medicinal Products, the European Union's counterpart to the medication-review activities conducted by the Food and Drug Administration in the United States] on biosimilar production mean that the chances of inducing a more immunogenic product are probably very small.'[8] This is known as pharmacovigilance, which is a responsibility shared among the pharmaceutical industry, pharmacists, and physicians, with appropriately informed and educated patients. The temptation for hard-pressed health-care economies to use lower-cost biosimilar therapeutic agents must be balanced by a system to ensure that physicians, pharmacists, and patients truly understand the complex arguments and decisions that apply to this new and challenging area. An ESA-associated PRCA incidence rate of 1:2068 in Thailand is unacceptable, and the Food and Drug Administration of Thailand responded appropriately to establish a prospective immunogenicity surveillance registry of ESAs with subcutaneous exposure to estimate the incidence of ESA-associated PRCA and to evaluate the efficacy of the currently available ESA products in that country. Given the magnitude of the safety issues associated with biosimilar ESA administration, Praditpornsilpa *et al.*[2] appropriately propose that these agents not be licensed through the conventional generic paradigm, which mainly focuses on bioequivalence, but that pre- and post-marketing comparisons with the reference product be performed on biosimilar agents to assure their safety and efficacy. The authors note that data from pre-licensing studies are usually too limited to identify all potential adverse effects and recommend that pharmacovigilance and risk management plans be submitted by the manufacturer as part of the evaluation.[2]

The response of Thailand to their epidemic of ESA-associated PRCA should serve as a model for other economies, including in those parts of the world where biosimilars are already used extensively and those, such as the European Union, where increased use of biosimilars is anticipated. In 2005, in the wake of the Eprex-associated PRCA epidemic, Schellekens[10] predicted the recurrence of a PRCA epidemic with biosimilar ESAs and strongly recommended the establishment of stringent country-specific approval and pharmacovigilance protocols by the EMEA, the US Food and Drug Administration, and comparable agencies throughout the world. These would include newly developed or improved methods for analysis of product characteristics, algorithms for predicting immunogenicity (which may have a genetic predisposition), assays for measuring patients' immune response, and improved production methods.

Fortunately, antibody-mediated PRCA, although serious, is not usually fatal and generally resolves after withdrawal of the offending ESA, immunosuppressive therapy (corticosteroids, cyclophosphamide, cyclosporine, mycophenolate, and rituximab have all been used successfully), and multiple transfusions until endogenous red blood cell production resumes. Peginesatide (Hematide), a synthetic, peptide-based erythropoietin receptor agonist with no homology to native erythropoietin, has been shown to be effective in the treatment of 13 out of 14 patients with PRCA due to anti-erythropoietin antibodies.[11] The development of synthetic replacements for biopharmaceutical agents holds promise for decreasing the production of autoantibodies against a biologic agent that crossreact with the native protein. However, given the inevitable possibility of unexpected consequences when therapeutic agents try to 'fool Mother Nature,' a strong pharmacovigilance program for synthetic agents is recommended as well.

## DISCLOSURE

JBW has served as a consultant to Affymax and Sanofi-Aventis; on the scientific advisory boards of Affymax, AMAG, and Sanofi-Aventis; and in speakers bureaus for AMAG and Amgen.

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# SGK3: a novel regulator of renal phosphate transport?

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**Phosphate is a key constituent of several important molecules, and hyperphospatemia has been associated with increased cardiovascular mortality. The kidney plays a crucial role in phosphate metabolism, as it is able to modulate phosphate excretion. Serum- and glucocorticoid-inducible kinase 3 (SGK3) has been shown to regulate a wide variety of transport systems. Bhandaru *et al.* suggest that SGK3 may have a significant role in the regulation of renal tubular phosphate transport.**

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Phosphate is essential in several crucial biological processes, including cellular energy metabolism, nucleic acid synthesis, cell signaling, energy metabolism, and bone mineralization. Alterations in serum phosphate are associated with various pathological effects. Hypophosphatemia is common in rickets, osteomalacia, and other diseases, and hyperphosphatemia in chronic kidney disease has been demonstrated to cause increased risk of cardiovascular morbidity and mortality. Moreover, elevated phosphate concentrations in subjects with normal kidney function are also associated with increased cardiovascular risk and mortality.[1] Therefore it is important to identify the mechanisms involved in phosphate homeostasis. Phosphate balance is coordinated by a complex process involving various organs and tissues, including the intestine, kidney, parathyroid gland, and bone as well as the vitamin D system and phosphatonins. Among the organs, the kidney has a key function in phosphate metabolism due to its ability to excrete phosphate and to control bone and mineral metabolism through the activation of vitamin D.

Phosphate transport in the small intestine and renal tubules is mediated mainly by three type II sodium–phosphate cotransporters: NaPi-2a (SLC34A1), NaPi-2b (SLC34A2), and NaPi-2c (SLC34A3). NaPi-2a is commonly expressed in the apical brush border membrane of the renal proximal tubule (highest in S1 segments and gradually decreasing toward S3 segments), and it is responsible for most of the renal phosphate reabsorption. In contrast, NaPi-2c protein is detected in S1 and S2 and is absent in S3 segments. This transporter has been associated with growth, as its abundance is at its maximum after weaning and decreases in the adult. Both NaPi-2a and NaPi-2c transport divalent phosphate ions ($HPO_4^{2-}$), but with different stoichiometries. Three $Na^+$ ions are transported together with one $HPO_4^{2-}$ ion by NaPi-2a, whereas NaPi-2c exhibits a $2Na^+/HPO_4^{2-}$ stoichiometry. Therefore, transport by NaPi-2a is electrogenic, whereas transport by NaPi-2c is electroneutral (Figure 1).[2] NaPi-2b is mostly localized in the small intestine and is sensitive to vitamin D. NaPi-2b has a low affinity for phosphate, and patients with NaPi-2b-inactivating mutations do not exhibit reduced serum phosphate levels, which would be expected in the case of the lack of a key transporter. However, studies in a conditional NaPi-2b-null mouse model demonstrated that deletion of NaPi-2b resulted in an increase in the expression of NaPi-2a in the renal proximal tubule and serum phosphate concentrations remained normal, most likely owing to compensatory renal reabsorption.[3] Intestinal phosphate absorption may have an effect on renal phosphate handling, perhaps, as recently suggested, by secretion of peptides derived from the intestine.[4] Recently, it was reported that mice knockout for both NaPi-2a and NaPi-2c, while showing severe hypophosphatemia, still had some renal phosphate reabsorption.[5] These results imply the involvement of other secondary active phosphate transporters along the nephron. Recent findings suggest that Pit-2, so far regarded as a ubiquitously expressed housekeeping phosphate transporter, is a new mediator of phosphate reabsorption in the proximal tubule, but with a different adaptive time course than NaPi-2a and NaPi-2c.[6]

The reabsorption of phosphate by the intestine and kidney is controlled by various hormones and peptides, including vitamin D, parathyroid hormone, and the phosphatonins, such as fibroblast growth factor 23, which act to increase intestinal

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