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Table II. Benefits and problems related to the use of biosimilars

Benefits	Problems
Lower pricing than originator medicines	Lack of long-term experience (efficacy, safety, immunogenicity?)
Pressure on innovator companies to reduce prices of originator medicines	Product-specific administration routes (s.c. and/or i.v. authorization?)
Pressure on innovator companies to develop “second-generation products” with improved pharmacodynamic and/or pharmacokinetic properties (e.g. darbepoetin alfa, methoxy-PEG-epoetin beta, pegfilgrastim)	Product-specific indications Product-specific storage and handling → Instruction of medical staff required Confusing naming (some identical INN, some different INN, different brand names for identical substances) → Difficulties in pharmacovigilance recording

Table I. Primary biosimilar recombinant medicines candidates

Active substance (INN)	Main indication	Biosimilar marketing authorization		
		EU	USA	Japan
Epoetin alfa	Anemia in association with CKD, anemia in association with chemotherapy for cancer	+	-	+
Epoetin beta	Anemia in association with CKD, anemia in association with chemotherapy for cancer	-	-	-
Filgrastim (G-CSF)	Neutropenia in association with cancer	+	-	-
Somatropin	Growth hormone deficiency	+	+	+
Insulin	Diabetes mellitus	-	-	-
Interferon alfa	Cancer, hepatitis B/C	-	-	-
Interferon beta	Multiple sclerosis	-	-	-

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Summary

After the patents of biopharmaceuticals have expired, based on specific regulatory approval pathways copied products (“biosimilars” or “follow-on biologics”) have been launched in the EU. The present article summarizes experiences with hematopoietic medicines, namely the epoetins (two biosimilars traded under five different brand names) and the filgrastims (two biosimilars, six brand names). Physicians and pharmacists should be familiar with the legal and pharmacological specialities of biosimilars: The production process can differ from that of the original, clinical indications can be extrapolated, glycoproteins contain varying isoforms, the formulation may differ from the original, and biopharmaceuticals are potentially immunogenic. Only on proof of quality, efficacy and safety, biosimilars are a viable option because of their lower costs.

For Peer Review

Introduction

Biotechnology, *i.e.* the combination of cell culture and genetic engineering, has proved beneficial for the production of diagnostics, vaccines and medicines [1]. Between January 1995 and June 2007, 136 biopharmaceuticals were approved in the United States (US) and 105 in the European Union (EU), with 67 products receiving approval in both regions [2]. With respect to hematology, the primary DNA technology-based products have been recombinant human erythropoietin (rhEPO; epoetin alfa), which received the US orphan drug designation in 1986 and the marketing approval for anemia in chronic kidney disease (CKD) in 1989, and granulocyte-colony stimulating factor (rG-CSF; filgrastim), which obtained the designation in 1990 and the approval in 1994. Recently, the patents of these successful medicines have expired in the EU and elsewhere. Hence, companies other than the innovators have brought up copied products [3-5]. Due to the complex nature of biopharmaceuticals, the EU regulators have introduced the name “biosimilars” (“similar biological medicinal products”; US term: “follow-on biologics”) for the copied medicines, and established specific regulatory pathways for their approval, which differ from those for chemical “generics” [3,6].

Health care authorities and insurance providers expect cost savings from the use of biosimilars. However, there are still concerns with respect to the efficacy and quality of the products [7,8]. Differing from chemically synthesized drugs, biological medicines are engineered in living cells. The active substances exhibit complex three-dimensional structures that cannot be fully characterized by the present analytical tools [1,9]. This problem holds especially true for glycoproteins such as EPO, because these are heterogeneous due to the presence of several isoforms [10,11].

The present article describes first-hand EU experiences with biosimilar recombinant medicines in an attempt to guide follow-on biologics launching plans in the USA and other parts of the world. In particular, core issues will be considered in relation to the clinical use of the two hematopoietic growth factors, primarily the rhEPOs (“epoetins”).

Manufacture of recombinant medicines

Recombinant proteins are produced by cells transfected with a gene or cDNA (the coding sequence of the gene) linked to an expression vector. The recombinant DNA is integrated into the genome of the host cells and stably expressed over time. Transformed bacteria such as *Escherichia (E.) coli* or transfected yeast and filamentous fungi are suited hosts for the production of non-glycosylated recombinant proteins such as growth hormone (GH) or insulin. Only eukaryotic host cells can secrete proteins. Genetically engineered mammalian cells are required for the production of glycoproteins that possess essential *O*-glycans and/or complex *N*-glycans. The integrity of the terminal sugars of the *N*-glycans is of major importance for the pharmacokinetics of glycoproteins.

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3 The main factors influencing the composition of recombinant medicines are: (i) the
4 plasmid (promoter, marker genes), (ii) the host cell (origin, species, clone), (iii) the
5 culturing process (fermenter, culture media), (iv) the purification steps, (v)
6 postranslational modifications (oxidation, deamidation; addition of polymers), and (vi) the
7 formulation and packaging [9,10]. Due to the complex manufacturing processes, it is not
8 possible to exactly copy a biopharmaceutical. All manufacturers use their own cell lines
9 and apply unique fermentation and purification techniques. In addition, biological
10 medicines exhibit batch-to-batch variability. Advanced biophysical, biochemical and
11 immunochemical tests are required to ensure the identity and purity of the active
12 substance in the medicine [9]. The bioactivity of a biopharmaceutical is usually
13 compared with that of an international reference standards of the World Health
14 Organization (WHO), the National Institute for Biological Standards and Control (NIBSC),
15 the European Pharmacopoeia or the US Pharmacopoeia [3,5,9]. In the EU, biosimilars
16 can contain inactive ingredients that differ from those of the reference medicine, which is
17 relevant with respect to the storage and handling requirements. For example, some
18 rhEPO products should be maintained at refrigerator temperature, whereas others may
19 be stored at room temperature for up to three days. Users should be familiar with the
20 product-specific recommendations.
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26 27 **Approval of biosimilars**

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29 **Regulation in the EU.** All biopharmaceuticals must be authorized by the European
30 Agency for the Evaluation of Medicinal Products (EMA; www.ema.europa.eu). In
31 contrast, chemical medicines can be approved by the regulatory authorities of individual
32 EU member countries. According to the EMA “The active substance of a biosimilar
33 medicine is similar to the one of the biological reference medicine” [12]. An EU biosimilar
34 applicant must use a comparator that is filed in the EU. The similarity of the biosimilar
35 with the reference product must extend to the pharmaceutical form, strength and route of
36 administration.
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40 Applications submitted to the EMA are assessed according to the guidelines by its
41 Committee for Human Medicinal Products (CHMP). First, there is an overarching
42 guideline that defines the studies necessary to show “the similar nature, in terms of
43 quality, safety and efficacy” in comparison to the originator’s product [13]. Second, there
44 is a guideline on the quality requirements, which refers to the purity of the product and
45 applies to proteins and peptides, their derivatives and products of which they are
46 components (*e.g.* conjugates). Third, there is a guideline on non-clinical and clinical
47 issues [14]. The non-clinical studies explore pharmacological and toxicological properties
48 *in vitro* (receptor-binding and cell-based assays) and *in vivo* (pharmacodynamic and
49 toxicokinetic studies in animals). The clinical trials must show similarity with respect to
50 the pharmacokinetic and pharmacodynamic properties of the novel drug. The efficacy
51 and safety of most biosimilar products have to be investigated in a few hundred patients
52 [14]. This is in contrast to the approval of generic drugs, which must only show
53 pharmacokinetic similarity in a small number of healthy volunteers. Fourth, product
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3 class-specific guidelines have been issued for the marketing authorization of biosimilar
4 rhEPO [15,16], rG-CSF [17], insulin [18], growth hormone (rhGH, somatropin) [19], and
5 (as a reflection paper) interferon alfa [20], considered the primary candidate substances
6 for the production of biosimilars (Table I). The demands differ, ranging from no need for
7 trials on patients in the case of insulin to two double-blind randomized studies in CKD
8 patients in the case of rhEPO.
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12 When the reference product has more than one therapeutic indication, “the efficacy and
13 safety of the biosimilar medicine may also have to be assessed using specific tests or
14 studies for each disease” [12]. However, the EMEA has regularly admitted the
15 extrapolation to other clinical indications, namely to those for which the reference
16 product was licensed. Upon receipt of a positive opinion from the EMEA, the European
17 Commission issues the marketing authorization. Because only limited clinical data are
18 available at the time of the approval of a biosimilar, the manufacturers have to provide a
19 risk management program (RMP), which comprises risk assessment and safety plans
20 that include clinical trials after marketing authorization (routine pharmacovigilance and
21 post-marketing surveillance) [21].
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26 The stringent regulatory process in the EU has resulted in the application withdrawal
27 respectively rejection of several products [22]. The applications for three human insulins
28 (Marvel Life Sciences, Harrow, UK) were withdrawn in 2008, after the CHMP had
29 expressed doubts regarding the comparability of the copied products with the originator
30 Humulin[®] (Eli Lilly, Indianapolis, IN, USA). In addition, the CHMP was concerned that the
31 applicant had not supplied enough information on how the active substance or the
32 finished products were made [22,23]. Alpheon[®] (Biopartners, Cologne, Germany; with
33 Swiss head-quarters/LG Life Sciences, Korea), an attempted copy of Roferon-A[®]
34 (interferon alfa-2a; Roche; Switzerland, German affiliate), was rejected by the EMEA due
35 to quality and clinical deficiencies compared to the reference product. Roferon-A[®] is
36 used in the treatment of patients with chronic hepatitis C, hairy cell leukemia, and AIDS-
37 related Kaposi's sarcoma. Biferonex[®] (interferon beta-1a; Biopartners) also received a
38 negative opinion from the EMEA, and the application was redrawn in 2009. The CHMP
39 had noted deficiencies in similarity and efficacy [24] compared to the reference products
40 Rebif[®] (Merck Serono, Darmstadt, Germany) and Avonex[®] (Biogen, Zug, Switzerland),
41 which are used to treat patients with relapsing-remitting multiple sclerosis.
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47 Currently, biosimilar rhEPOs (epoetins; for brand names, see below), rG-CSFs
48 (filgrastims) and rhGHs (somatropins) are marketed in the EU. Several of the biosimilars
49 are traded under different brand names by more than one company. There is free online
50 access to Summaries and full length European Public Assessment Reports (EPARs)
51 explaining how the CHMP evaluated each biosimilar medicine (www.ema.europa.eu).
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53 **Regulation in non-EU countries.** The WHO is working out guidelines for biosimilars,
54 which will likely be circulated to interested parties in 2010/11 [25]. The US Food and
55 Drug Administration (FDA) has a similar approach as the EMEA with respect to approval
56 standards for copied biological medicines [26,27]. Previously, the FDA considered only
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3 more simple follow-on protein products. The biological medicines that have been
4 approved under the abbreviated New Drug Application pathway (NDA 505(b)(2)) include
5 recombinant human hyaluronidases, salmon calcitonin, human glucagon and rhGH
6 [25,28]. However, these products are not rated therapeutically equivalent to the
7 innovators' medicines. In Canada, authorities are finalizing the rules for the approval of
8 follow-on-biologics based on the existing legislation for biopharmaceuticals. Under the
9 regulatory term "Subsequent Entry Biologic", a biosimilar somatropin (Omnitrope[®],
10 Sandoz, Holzkirchen, Germany; subsidiary of Novartis, Basel, Switzerland), a copy of
11 Genotropin[®] (Pfizer, New York, NY, USA) has been approved by Health Canada [29].
12 Japan's Ministry of Health, Labor and Welfare (MHLW) has issued "Guidelines for the
13 Quality, Safety and Efficacy Assurance of Follow-on Biologics" in 2008 (updated in
14 2009), regarding the development and marketing approval of biosimilars. A filing in
15 Japan must use a Japanese "Precedent Biotechnology Drug" as the reference.
16 Sandoz' somatropin was already approved as the first Japanese biosimilar in 2009, as its
17 supporting dossier was submitted before the current guidelines were implemented. The
18 first biosimilar epoetin (EPO JR013, epoetin kappa; Japan Chemical Research and
19 Kissei, Tokyo/Matsumoto, Japan) has been ratified according to the novel Japanese
20 guidelines as a follow-on product to epoetin alfa. In Australia, biosimilars have been
21 marketed since the Therapeutic Goods Administration (TGA) adopted the EU ruling. In
22 other parts of the world - f. e. in the Middle East [30] - experts have published
23 recommendations for biosimilars according to the EMEA guidelines. Worldwide, the
24 healthcare systems have to bear high costs, and their reduction would improve the
25 access to biotechnology drugs.
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33 Copied biopharmaceuticals have been used for many years in areas and countries with
34 less strictly controlled markets including Latin America, India, Korea and China.
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37 **Interchangeability and substitution**

38 Interchangeability refers to the clinical practice of switching from one medicine to another
39 that is considered equivalent, in a given clinical setting. The decision for such switch can
40 be made only by the physician choosing an alternative within a certain class of drugs,
41 f.e. angiotensin-converting enzyme inhibitors [31]. In contrast, substitution can be done
42 at the hospital or retail pharmacy level. The FDA lists drugs that are equivalent in
43 "Approved Drug Products With Therapeutic Equivalence Evaluations" (also known as
44 "Orange Book"), which is available both in print and open access online [32]. In Europe,
45 substitution is regulated by the national laws for generic drugs, which differ among the
46 individual EU Member States. Substitution is often based on economic considerations.
47 Less expensive drugs are supplied that have the same quality, safety and efficacy, and
48 usually the same International Nonproprietary Name (INN) as the competitors. In
49 Germany, for example, pharmacists filling prescriptions covered by the statutory health
50 insurance system must, whenever possible, dispense the cheapest product containing
51 the prescribed substance. Substitution is permitted among biosimilars that contain the
52 same active substance but are traded under different brand names. However, the
53 physician can prohibit drug substitution by crossing out "*aut idem*" ("or the like") on the
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3 prescription form. According to the US jurisdiction, the prescriber can state "dispense as
4 written" or "do not substitute".
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6 Biopharmaceuticals are generally physician-administered rather than pharmacy-
7 dispensed. The EMEA does not assess the interchangeability or substitutability of a
8 biosimilar when granting the positive opinion for a marketing authorization application.
9 The EMEA has stated explicitly: "Since biosimilar and biological reference products are
10 not identical, the decision to treat a patient with a reference product or biosimilar
11 medicine should be taken following the opinion of a qualified health professional" [12].
12 Here, the "qualified health professional" is the physician. Countries such as France, Italy,
13 Spain, UK, Netherlands, Sweden and Germany have established legislative rules to
14 prohibit the automatic substitution of biopharmaceuticals. Also, medical societies such as
15 the French [33] and the Portuguese [34] Society of Nephrology have stated that there is
16 no safe interchangeability of biopharmaceuticals. The main concern about switching
17 from one biological medicine to another is the issue of immunogenicity.
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22 **Immunogenicity of recombinant therapeutics**

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24 Due to their structural complexity and potential contaminants, biopharmaceuticals have a
25 greater immunogenic potential than small chemical drugs. Although the occurrence of
26 antibodies (Abs) towards recombinant human proteins is not uncommon, immune
27 reactions have not been a major impediment to their therapeutic use [35]. First, Abs
28 production can be transient. Second, the Abs are mostly non-neutralizing. If occurring,
29 however, neutralizing Abs will not only inhibit the activity of the therapeutic protein, but
30 also that of its endogenous analog. Antibody production can be induced by protein
31 structures that are *a priori* non-self (vaccination). Alternatively, B-cells may lose immune
32 tolerance towards a recombinant human protein, in particular, when it is administered
33 repeatedly. The EMEA has published a draft guideline on immunogenicity assessment of
34 biotechnology-derived therapeutic proteins [36]. The immunogenicity of a
35 biopharmaceutical cannot be deduced from the molecular structure of its active
36 substance or from preclinical laboratory and animal studies [37,38]. Both product-specific
37 (amino acid sequence alteration, posttranslational modification, aggregation, impurity)
38 and patient-specific (application route and frequency, duration of treatment, co-
39 medication, underlying disease) factors impact on the incidence and degree of Abs
40 formation [39-41]. In general, the administration *via* the s.c. route induces a much
41 stronger immunogenic response than i.v. injections. Antibody production often ceases,
42 when the therapeutic protein is no longer administered [38,42].
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49 At the time a biosimilar receives market approval, little is known about its potential to
50 provoke immune reactions, due to the limited number of patients in clinical trials, the
51 limited time of exposure to the medication and, generally, a rather strictly defined patient
52 population.
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55 **Clinical use of rhEPOs**

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3 EPO is essential for red blood cell (RBC) production. It prevents the erythrocytic
4 progenitors from undergoing apoptosis, and it stimulates their proliferation and
5 differentiation. Endogenous EPO is mainly of renal origin. The concentration of the
6 hormone is abnormally low when related to the hemoglobin level ([Hb]) in CKD [43]. The
7 anemia in CKD patients is often aggravated due to accompanying inflammatory
8 processes, reduced iron availability, hemolysis, blood losses, nutritional deficiencies and
9 hyperparathyroidism [44]. Before rhEPO became available, about 25% of renal failure
10 patients on dialysis needed regular RBC transfusions [45]. Epogen[®], an epoetin alfa
11 formulation produced by Amgen (Thousand Oaks, CA, USA), was approved by the FDA
12 in 1989 for the treatment of CKD patients undergoing hemodialysis “to elevate or
13 maintain the red blood cell level and to decrease the need for transfusions” [46]. For
14 other indications epoetin alfa has been marketed by Johnson & Johnson (J&J, New
15 Brunswick, NJ, USA), under the name of Procrit[®], through an agreement with Amgen.
16 Eporex[®] (J&J, subsidiary Ortho Biotech, Bridgewater, NJ, USA), an epoetin alfa
17 formulation marketed outside the USA, was approved in the EU in 1988.
18 NeoRecormon[®], an epoetin beta originally manufactured by Boehringer Mannheim
19 (Germany) and subsequently by Roche (Penzberg, Germany), received EU approval in
20 1990. At present, the most widespread recombinant erythropoiesis stimulating agents
21 (ESAs) include epoetin alfa (Epogen[®], Procrit[®], Eprex[®], Erypo[®], Espo[®]), epoetin beta
22 (NeoRecormon[®], Epogin[®]; outside USA only), biosimilar and copied rhEPOs (outside
23 USA only), and the hyperglycosylated rhEPO analog darbepoetin alfa (Aranesp[®],
24 Amgen) which received regulatory approval in the USA and other countries in 2001/02.
25 ESAs have been of great use to millions of CKD patients, and more recently, cancer
26 patients receiving chemotherapy (for references, see [47,48]). The patients’ benefits
27 include freedom from RBC transfusion and improvements in life quality. However,
28 recombinant ESAs are costly. In the USA, they are among Medicare's top medication
29 expenses (estimated sales \$5 bln per year).

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32 **CKD.** Earlier studies had shown that [Hb] levels <100 g/L were associated with an
33 increased risk of morbidity, hospitalization and mortality in CKD patients [49-52]. Hence,
34 randomized trials investigated whether using ESAs to raise RBC and [Hb] levels into the
35 normal range would further improve clinical outcomes. Unexpectedly, most of the results
36 were negative. Besarab et al. [53] first reported an increased incidence in myocardial
37 infarcts in dialysis patients with congestive heart failure or ischemic heart disease, when
38 RBC concentrations were raised into the normal range (hematocrit 42%). Subsequently,
39 Parfrey et al. [54] showed that the normalization of [Hb] in incident hemodialysis patients
40 had no beneficial effect on cardiac structure, compared with partial anemia correction.
41 The “Correction of Hemoglobin and Outcomes in Renal Insufficiency” (CHOIR) trial was
42 terminated early after an increased risk of death and cardiovascular hospitalization was
43 assessed in predialysis patients treated with epoetin alfa to achieve a target [Hb] of 135
44 g/L instead of 113 g/L. Life quality parameters were not improved in the high-[Hb] group
45 in this study [55]. In contrast, in the “Cardiovascular Risk Reduction by Early Treatment
46 with epoetin beta” (CREATE) study life quality parameters improved in the high-[Hb]
47 (130-150 g/L) group. However, there was also a trend towards more cardiovascular
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3 events [56]. Possibly, iron depletion causing thrombocytosis contributed to the increased
4 mortality in ESA treated CKD patients with normalized [Hb] [57]. The importance of
5 optimal coadministration of iron to reduce the risk for ESA-driven cardiovascular events
6 has been reviewed recently [58]. In the “Trial to Reduce Cardiovascular Endpoints with
7 Aranesp (R) Therapy” (TREAT), 4,038 CKD patients (not requiring dialysis) with type-2
8 diabetes and anemia were randomized in a one-to-one ratio to receive either
9 darbepoetin alfa to a target [Hb] of 130 g/L or placebo, with rescue darbepoetin alfa
10 when the [Hb] was less than 90 g/L [59]. The use of darbepoetin alfa did not produce an
11 adverse effect on all-cause mortality or cardiovascular events. However, the high [Hb]
12 was associated with an increased risk of stroke [59]. Clearly, darbepoetin alfa should not
13 be used in the manner tested in TREAT, which was targeting a [Hb] outside current
14 label. The “Kidney Disease: Improving Global Outcomes” (KDIGO) convention has
15 stated that [Hb] >130 g/L “can be associated with harm” [60]. The “National Kidney
16 Foundation Kidney Disease Outcomes Quality Initiative” (NKF K/DOQI) guidelines
17 recommend a target [Hb] of 110–120 g/L on treatment with recombinant ESAs [61]. The
18 “European Renal Best Practice” (ERBP) guidelines recommend a target [Hb] of >110
19 g/L, with a maximum of 120 g/L for patients with concomitant cardiovascular disease or
20 diabetes [62]. To reduce the mortality risk in renal transplant recipients ESAs should not
21 be administered to subjects with [Hb] >125 g/L [63].
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28 **Chemotherapy associated anemia.** Although ESAs increase [Hb] and reduce the need
29 for RBC transfusions in cancer patients receiving chemotherapy [64, 65], this therapy
30 has been questioned in view of publications indicating an increase in mortality [66-69]. In
31 contrast, the most recent and comprehensive meta-analysis of controlled ESA oncology
32 trials (>15,000 patients) failed to show an effect of ESA therapy on survival or disease
33 progression [70]. However, an increased incidence of venous-thromboembolic events
34 was observed [70]. A high blood viscosity in combination with elevated platelet counts is
35 a risk factor for thrombus formation. Of note, most reports of a detrimental outcome were
36 based on off-label use trials not following current guidelines on the use of ESAs in
37 cancer patients. Both the baseline and the achieved [Hb] often exceeded the
38 recommended values. Whether ESAs directly stimulate tumor growth has remained a
39 controversial issue [71]. Although cancer cells express EPO receptor (EPO-R) mRNA to
40 some extent, functional EPO-R molecules are usually not present on the surface of
41 cancer cells [72-75]. Of note, all immunochemical studies on tumor specimen were
42 hampered by the use of nonspecific anti-EPO-R antibodies that cross-reacted with other
43 proteins, *e.g.* heat-shock proteins [72,73,76,77]. Only very recently, investigators have
44 succeeded in developing a specific antibody for the detection of EPO-R protein on
45 Western blots [78].
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51 Currently, the following rules should generally be respected for the use of ESAs in
52 cancer patients receiving myelosuppressive chemotherapy: (i) the anticipated treatment
53 outcome is not cure, (ii) treatment should be initiated at [Hb] <100 g/L to avoid the need
54 for RBC transfusions, (iii) in cases of less severe anemia treatment should rely on the
55 presence of significant anemia symptoms, and (iv) the [Hb] should not exceed 120 g/L.
56 ESAs are contraindicated for the treatment of non-chemotherapy and non-CKD related
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3 anemias [67]. Dated 02/16/2010, the FDA and Amgen notified healthcare professionals
4 and patients that all ESAs must be used under a Risk Evaluation and Mitigation Strategy
5 (REMS) program. A Medication Guide explaining the risks and benefits of ESAs must be
6 provided to all patients receiving an ESA. Under the ESA APPRISE Oncology program,
7 only those hospitals and healthcare professionals who have enrolled and completed
8 training in the program can prescribe and dispense ESAs to patients with cancer.
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11 Apart from the anemias associated with CKD or myelosuppressive chemotherapy,
12 indications for the administration of ESAs can be prematurity, AIDS and major surgical
13 interventions [79].
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15 **Types of epoetins**

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18 Human EPO is a 30.4 kDa glycoprotein of 165 amino acids, three complex-type *N*-
19 glycans and one small *O*-glycan. The *N*-glycans (at Asn²⁴, Asn³⁸ and Asn⁸³) have a
20 major role in secretion, molecular stability, solubility and elimination of EPO.
21 Therapeutically used rhEPOs are manufactured in mammalian cells transfected with
22 human EPO cDNA. The originators' epoetin alfa and epoetin beta preparations as well
23 as the copies and biosimilars of these (see below) are engineered in Chinese hamster
24 ovary (CHO) cells. The amino acid sequence of all epoetins is identical with that of
25 endogenous EPO, but the glycans of the products exhibit structural differences [11].
26 Epoetin alfa is more homogenous and possesses less basic isoforms than epoetin beta
27 [80,81]. Reportedly, structural differences even exist between the established epoetin
28 alfa formulations, Epogen[®] (Amgen) and Eprex[®] (J&J) [82]. However, this finding has
29 been questioned, since the Eprex[®] bulk substance was isolated from a formulated
30 product considered inappropriate for comparative studies [83]. The originators' epoetin
31 alfa and epoetin beta medicines are used for the same major indications (anemias
32 associated with CKD or myelosuppressive chemotherapy treated cancers). Due to the
33 long medical experience with both products they are considered as interchangeable by
34 healthcare professionals. In contrast to the other epoetins, epoetin omega (Repotin[®],
35 Bioclones, Cape Town, South Africa) is produced in *EPO* cDNA-transfected baby
36 hamster kidney (BHK) cells. Epoetin omega has an *N*-glycan with phosphorylated
37 oligomannoside chains, and it possesses less *O*-glycans than the CHO-cell derived
38 rhEPOs [81,84]. The clinical consequences of these glycosylation differences have not
39 been studied, probably because epoetin omega is not widely used.
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46 **Naming of epoetins**

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48 International Nonproprietary Names (INNs) identify active pharmaceutical substances.
49 INNs are important for pharmacists and physicians to make substitution decisions and to
50 compile postmarketing surveillance reports. Generic drugs use to have the same INN as
51 the originator's product. A pharmacologically active substance having its specific INN
52 may be traded by different companies that use their own registered brand names (or
53 trademarks).
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3 With respect to the naming of the ESAs, the INN Expert Group of the WHO has
4 recommended that an altered amino acid sequence should be denoted by distinct
5 prefixes (such as in “darbepoetin”). Differences in the glycosylation pattern of the
6 epoetins should be indicated by Greek letters added (alfa, beta, omega, *etc.*) [85].
7 Accordingly, the Japanese Accepted Names (JAN) committee has established a precise
8 definition of epoetins that incorporates the cell substrate of origin, the molecular size, the
9 extent of sialylation, and the nature of the *N*- and *O*-linked glycans. In the EU, the
10 applicant for a biosimilar epoetin can apparently at will chose the INN, which may be
11 identical to that of the reference product or not. One epoetin alfa biosimilar (substance
12 HX575) has received EMEA approval under the INN “epoetin alfa” despite its different
13 carbohydrate pattern compared to the reference drug (Eprex[®]/Erypo[®]). HX575 has
14 elevated levels of high-mannose structures and lower levels of *N*-glycolyl-neuraminic
15 acid and diacetylated neuraminic acids [86]. HX575 is with three different brand names
16 on market. It is traded as Binocrit[®] by Sandoz and as Epoetin alfa Hexal[®] by Hexal
17 Biotech (Holzkirchen, Germany), both Novartis subsidiaries. Medice Arzneimittel Putter
18 (Iserlohn, Germany), a Sandoz licensing partner, has the permission to sell the product
19 as Abseamed[®]. Another epoetin alfa biosimilar (substance SB309) has received EMEA
20 approval under the INN “epoetin zeta” (brand names: Silapo[®], Stada, Bad Vilbel,
21 Germany, and Retacrit[®], Hospira, Lake Forest, Ill, USA). SB309 has less *O*-glycans, and
22 lower levels of *N*-glycolyl-neuraminic acid and *O*-acetyl neuraminic acid than the
23 reference product [87]. Clinical consequences of the glycosylation differences have not
24 been reported.
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32 In 2009 the EMEA has granted a marketing authorization for a novel CHO cell-derived
33 rhEPO, epoetin theta (brand names: Biopoin[®], CT Arzneimittel, Berlin, Germany;
34 Eporatio[®] and Ratioepo[®], Ratiopharm, Ulm, Germany), which has been developed by
35 Merckle Biotec (Ulm, Germany) in using epoetin beta as a comparator [88]. However,
36 Epoetin theta is not a biosimilar but has been developed as a stand-alone product. The
37 drug is indicated for the treatment of symptomatic anemia associated with CKD in adult
38 patients and for the treatment of symptomatic anemia in adult cancer patients with non-
39 myeloid malignancies receiving chemotherapy.
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43 In view of the plethora of compounds, it seems mandatory that epoetins are prescribed
44 by brand names to enable pharmacovigilance and to ensure that adverse events are
45 assigned to the correct product.
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48 **Calibration of epoetins**

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51 EPO doses are expressed in International Units (IU) instead of grams or moles, because
52 both endogenous EPO and rhEPOs are mixtures of isoforms that differ in bioactivity. The
53 “EPO unit” was originally defined as the dose eliciting in rodents the same erythropoiesis
54 stimulating response as 5 μmoles of cobaltous chloride. On behalf of the WHO, the
55 NIBSC, UK, has established international human urinary EPO (2nd IRP) [89] and rhEPO
56 (specific activity about 200,000 IU/mg peptide) [90] standard preparations. The
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3 European Directorate for the Quality of Medicines has produced additional biological
4 reference preparations (BRPs, presently batch 3) for the calibration of commercial
5 rhEPOs [91]. According to the European Pharmacopoeia (Ph. Eur. monograph 1316) the
6 activity of clinically used rhEPOs must be assessed by bioassay in mice. Owing to the
7 poor accuracy of this assay, the monograph states: "The estimated potency is not less
8 that 80% and not more than 125% of the stated potency. The fiducial limits of error of the
9 estimated potency are not less than 64% and not more than 156% of the stated potency"
10 [92]. While this regulation allows for significant variations in bioactivity, all EMEA-
11 approved epoetins comply with the requirements [93].
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16 The bioassays are not applicable for measurement of the activities of the second-
17 generation ESAs, darbepoetin alfa (Aranesp[®]; Amgen) and methoxy polyethylene glycol-
18 epoetin beta (methoxy-PEG-epoetin beta; Mircera[®]; Roche). These ESAs have a
19 prolonged survival in circulation. Compared to the epoetins, darbepoetin alfa contains
20 two additional *N*-glycans at novel asparagine residues in positions 30 and 88 as a result
21 of site-directed mutagenesis [94,95]. The terminal half-life of i.v. administered
22 darbepoetin alfa is 3-4-fold longer than that of the epoetins (25 vs. 6-9 h) [96]. Methoxy-
23 PEG-epoetin beta contains a single methoxy-PEG polymer of approximately 30 kDa
24 integrated via amide bonds between the amino groups of either the alanine in position 1
25 or one of the lysines in positions 45 or 52 of EPO [97]. Methoxy-PEG-epoetin beta has
26 an extremely long half-life (130-140 h), which allows for less frequent application [98-
27 101]. Darbepoetin alfa and methoxy-PEG-epoetin beta doses are given in μg instead of
28 in IU. Accordingly, it is almost impossible to compare "Defined daily doses" (DDD) of
29 the first generation ESAs (= epoetins) and the second-generation ESAs (darbepoetin
30 alfa and methoxy-PEG-epoetin beta).
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37 **Potency and marketing authorization of biosimilar epoetins**

38 According to the EPAR, substance HX575 (INN: epoetin alfa; manufacturer: Rentschler
39 Biotechnologie, Laupheim, Germany) showed therapeutic equivalence with
40 Eprex[®]/Erypo[®] on i.v. administration in CKD patients on hemodialysis [86]. A non-
41 comparative efficacy and steady-state pharmacokinetic and pharmacodynamic study for
42 i.v. administered HX575 was performed for approval in cancer patients receiving
43 chemotherapy. In part based on data extrapolation, HX575 has received marketing
44 authorization for i.v. administration in adult CKD patients on peritoneal dialysis or not yet
45 undergoing dialysis, i.v. administration in pediatric CKD patients on hemodialysis, i.v. or
46 s.c. administration in adult patients receiving chemotherapy for malignancies, and
47 patients prior to major elective orthopedic surgery [86].
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52 According to the EPAR, substance SB309 (INN: epoetin zeta; manufacturer: Norbitec,
53 Uetersen, Germany) was about 10% less potent than the reference drug Eprex[®]/Erypo[®],
54 when administered i.v. to CKD patients [87]. The lower potency has been related to
55 differences in the active substance content of the SB309 and Eprex[®]/Erypo[®] batches
56 under study, despite the same nominal dose [102]. The manufacturers use different
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rhEPO standard preparations and bioassays for calibration (SB309: normocytic mouse bioassay preferably used in the EU; Eprex[®]/Erypo[®]: exhypoxic polycythemic mouse bioassay used in the USA). In part based on data extrapolation SB309 has received marketing authorization for i.v. administration in adult CKD patients on peritoneal dialysis or not yet undergoing dialysis, i.v. administration in pediatric CKD patients on hemodialysis, i.v. or s.c. administration in adult patients receiving chemotherapy for malignancies, and patients on an autologous blood donation program [87]. In February 2010, the CHMP has adopted a positive opinion for the s.c. use of SB309 in CKD patients.

Bioactivities of other CHO cell-derived rhEPOs

Copied CHO cell-derived rhEPOs are available from many manufacturers in Asia, Africa, non-Northern America and non-EU Europe [103]. While clinical trials showed equivalence of copied rhEPOs from Cuba [104], China [105] or Korea [106,107], the identity and purity of some of the medicines was found to be less sufficient [108,109]. The purported copies of rhEPOs from Korea, India and China contained more glycoforms and other impurities than the originator's epoetin alfa (Epogen[®], Amgen). Most importantly, covalent aggregates were detected in some of the copied products [108]. Another study identified copied epoetins that were contaminated with endotoxin [109]. There are two reports on the *in vivo* activity of such products. Potency values ranging from 68-119% were assessed by bioassay in normocytic mice in an investigation of 12 purported copies of epoetin alfa from five different manufacturers [109]. *In vivo* activities higher than specification (137-226%) were determined by exhypoxic polycythemic mouse assay in four samples and activities lower than specification (71-75%) in two samples in a study of 11 copies of epoetin alfa from 8 manufacturers [110]. In addition, major batch-to-batch differences in biological activity were assessed.

Anti-EPO antibody-mediated PRCA

Anti-EPO antibody-mediated pure red cell aplasia (PRCA) is characterized by progressive severe normocytic normochromic anemia of sudden onset ([Hb] decrease about 1 g/L blood per day), reticulocytopenia (<10,000/ μ L blood), and the lack of erythroid precursors in the bone marrow [111-114]. The non-erythrocytic cellularity of the bone marrow, and the numbers of leukocytes and thrombocytes in blood are normal. Since iron utilization is reduced, serum ferritin (>1000 mg/L) and transferrin iron saturation (>70%) are increased. The disorder is caused by neutralizing anti-EPO Abs. The Abs are directed against the peptide part of the antigen and not against the glycans. They bind to all recombinant ESAs and to endogenous EPO. For diagnosis of anti-EPO Abs, ligand-binding assays (ELISA, RIP, BIAcore) can be used to screen patients' sera, albeit proof is only provided by *in vitro* bioassay with primary cultures of myeloid erythrocytic progenitors or with EPO-dependent permanent cell lines (TF-1 or UT-7 erythroleukemia cells) [115,116]. The fact that IgG₁ and IgG₄ occur [115] indicates an Ig partial gene switching for the constant region of the heavy chain – a reaction that is

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3 mediated by T_{H2}-cells. There has been no clear case of anti-EPO antibody-mediated
4 PRCA, when ESAs were solely administered *via* the i.v. route.
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6 Almost all patients suffering from anti-EPO antibody-mediated PRCA require regular
7 RBC transfusions. ESA therapy must be discontinued. The administration of
8 immunosuppressive drugs (cyclosporine, glucocorticoids) may accelerate the recovery of
9 erythropoiesis [117]. Recently, an open-label, single-group trial on 14 patients who had
10 anti-EPO antibody-mediated PRCA was performed with a synthetic pegylated EPO
11 mimetic peptide (EMP, Hematide™; Affymax, Palo Alto, CA, USA). The EMP is
12 structurally different from EPO and does not cross-react with anti-EPO Abs. The EMP
13 was administered by s.c. injection at an initial dose of 0.05 mg/kg of body weight every 4
14 weeks [118]. Transfusion requirements diminished in 13 of the 14 patients within 12
15 weeks. Median [Hb] increased from 90 g/L (with transfusion support in the case of 12
16 patients) before treatment to 114 g/L at the time of the last EMP administration. The level
17 of anti-EPO Abs declined over the course of the study and became undetectable in six
18 patients. However, one patient developed Abs against the EMP, and grade 3 or 4
19 adverse events occurred in about 50% of the patients [118].
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25 The incidence of anti-EPO antibody-mediated PRCA in CKD patients increased in the
26 period 1998 - 2003, amounting to over 200 cases worldwide [119]. The majority of cases
27 occurred in patients who received s.c. an epoetin alfa formulation marketed outside the
28 USA (Eprex®/Erypo®; Ortho-Biotech). In 1998, the manufacturer had changed the
29 formulation, in replacing human serum albumin by polysorbate-80 (PS-80) and glycine to
30 avoid any risk of the transmission of prions. At the same time, pre-filled syringes with
31 uncoated rubber stoppers were introduced. After these were replaced by Teflon®-coated
32 stoppers, the incidence of anti-EPO antibody-mediated PRCA decreased to very low
33 rates, again. It has been proposed that the PS-80 released leachates from the rubber
34 stoppers, which acted as adjuvants [120]. An alternative hypothesis suggests that the
35 formation of anti-EPO Abs was induced by micelles loaded with aggregated rhEPO in
36 PS-80 [121]. Note, here, that some ESA formulations contain PS-80 and others PS-20,
37 which may impact on the stability of the drugs [122]. From 2005, exposure-adjusted
38 incidence rates for anti-EPO Abs-mediated PRCA were reportedly as low as 0.02 to 0.03
39 per 10,000 patient-years among patients who received s.c. originator epoetins or
40 darbepoetin alfa [119]. This progress was likely also due to the fact that pharmacists,
41 physicians and their medical staff have become aware that recombinant ESAs are
42 temperature-sensitive products, which need to be maintained in cold chains at 2 - 8 °C
43 from manufacture to administration to avoid structural changes of the drug substance.
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50 The transient increase of anti-EPO antibody-mediated PRCA cases on change of
51 formulation of epoetin alfa has highlighted the relevance of manufacturing processes
52 with respect to the potential immunogenicity of biopharmaceuticals. It has also impacted
53 on the evaluation of biosimilar epoetins. The “Study to Evaluate the Efficacy, Safety and
54 Immunogenicity of Subcutaneous HX575 in the Treatment of Anemia Associated with
55 Chronic Kidney Disease” (SWEEP) was stopped recently. The study included 337 ESA-
56 naïve predialysis patients who were randomly assigned to the biosimilar HX575 or to
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3 Erypo[®]. Two patients in the HX575 arm developed neutralizing anti-EPO Abs [123].
4 PRCA was confirmed by bone marrow biopsy in one patient, but this could not be
5 investigated in the other patient due to his decease following cardiac infarction. HX575
6 contains fewer aggregates than the reference product Erypo[®] [86], which is by itself a
7 parameter of good quality of the biosimilar. In addition, bioburden, endotoxin levels as well
8 as the concentrations of host cell proteins and DNA in the HX575 drug substance met
9 the predefined quality criteria [124]. Thus, it is unclear whether the increased
10 immunogenicity of HX575 was caused by a structural defect of the product or by
11 mishandling during its clinical use.
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16 Cases of anti-EPO Ab-induced PRCA due to the administration of copied rhEPOs from
17 other parts of the world have been reported occasionally [125-127], but information on
18 incidence rates is not available. An investigation of anti-EPO antibody-mediated PRCA
19 cases in Thailand revealed that epoetin prefilled syringes were being smuggled or sold
20 illegally through unauthorized retail pharmacies [128]. These products were stored
21 improperly and contained high levels of aggregates.
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24 **Biosimilar rG-CSFs**

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26 Endogenous human G-CSF is a single polypeptide chain glycoprotein of 174 or 177
27 amino acids with an *O*-glycan at Thr¹³³ (molecular mass about 19 kDa). G-CSF is the
28 most important growth factor for granulocytic progenitors in the bone marrow. In
29 addition, it enhances the effector functions of mature neutrophils, including chemotaxis,
30 phagocytosis and generation of reactive O₂ species.
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34 Both rG-CSFs from *E. coli* (filgrastim; Neupogen[®], Amgen) and from CHO cells
35 (lenograstim; Granocyte[®], Chugai Pharma, Tokyo, Japan) are in clinical use [129]. They
36 are analogs of the 174 amino acid isoform of human G-CSF. Although the *E. coli* protein
37 differs from endogenous human G-CSF and from CHO cell-derived rhG-CSF by an
38 additional *N*-terminal methionine and by the lack of the *O*-glycan, the pharmacological
39 properties of the drugs are apparently very similar. The medicines are indicated for: (i)
40 reduction in the duration of neutropenia and the incidence of febrile neutropenia in
41 patients treated with established cytotoxic chemotherapy for malignancy (with the
42 exception of chronic myeloid leukemia and myelodysplastic syndromes), (ii) reduction in
43 the duration of neutropenia in patients undergoing myeloablative therapy followed by
44 bone marrow transplantation considered to be at increased risk of prolonged severe
45 neutropenia, (iii) mobilization of peripheral blood progenitor cells, (iv) elevation of
46 neutrophil counts and reduction of the incidence and the duration of infection-related
47 events in children or adults with severe congenital, cyclic, or idiopathic neutropenia with
48 an absolute neutrophil count (ANC) of $0.5 \times 10^9/L$, and a history of severe or recurrent
49 infections, and (v) treatment of persistent neutropenia ($ANC \leq 1.0 \times 10^9/L$) in patients with
50 advanced HIV infection in order to reduce the risk of bacterial infections when other
51 options to manage neutropenia are inappropriate.
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3 Two biosimilar rG-CSFs have been launched in the EU in 2008/9, with Neupogen®
4 (Amgen) as the reference product. Both biosimilars are 175 amino acids non-
5 glycosylated methionyl rG-CSFs expressed in *E. coli*. One of the biosimilars
6 (manufacturer: Sandoz, Kundl, Austria) is marketed under two different brand names:
7 Filgrastim Hexal® (Hexal Biotech) and Zarzio® (Sandoz) [130]. The other biosimilar
8 (substance XM02) is marketed by three different companies under four different brand
9 names: Biograstim® (CT Arzneimittel), Filgrastim ratiopharm® and Ratiograstim®
10 (Ratiopharm) and TevaGrastim® (Teva Generics, Radebeul, Germany) [131]. The
11 launching of XM02 has been an instructive example of the complex network of
12 biotechnological and pharmaceutical companies that collaborate, thereby rendering it
13 difficult for the user to apprehend the history of a biopharmaceutical. The manufacturing
14 process for XM02 was established by Sicor Biotech in Vilnius, Lithuania [132]. The drug
15 was developed clinically in Germany by BioGeneriX (Mannheim), a daughter company of
16 Ratiopharm, a subsidiary of the Merckle Group. In March 2010, Teva Pharmaceutical
17 Industries announced that it will acquire Ratiopharm.

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19 The biosimilar rG-CSFs are approved for the same indications as Neupogen®, which
20 include myelosuppressive chemotherapy-induced neutropenia, mobilization of peripheral
21 blood progenitor cells, severe chronic neutropenia (congenital, cyclic or idiopathic) and
22 persistent neutropenia associated with advanced HIV infection. Filgrastims should be
23 prescribed by brand names to enable pharmacovigilance and to ensure that adverse
24 events (AEs) due to the therapy are properly assigned to the correct product.

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26 In February 2010, Teva Pharmaceutical Industries announced that the FDA had
27 accepted for filing Teva's Biologics License Application for XM02 for the reduction in the
28 duration of severe neutropenia and the incidence of febrile neutropenia in patients
29 treated with established myelosuppressive chemotherapy for cancer. The proposed
30 trade name for XM02 in the US is Neutroval.

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32 Finally, it is of note that filgrastim has a short half-life in circulation (about 3 h). Similar to
33 methoxy-PEG-epoetin beta, a long-acting pegylated form of methionyl rG-CSF
34 (pegfilgrastim, Neulasta®; Amgen) has been developed as a second-generation
35 medicine. Compared to filgrastim, pegfilgrastim has a larger molecular mass (40 kDa)
36 and a longer half-life, reducing injection requirements to a single administration *per*
37 chemotherapy cycle [133,134].

38 39 40 41 42 43 44 45 46 47 48 **Conclusions**

49 The pharmacologic properties of biological medicines depend on the production and
50 purification processes. Health care providers encourage the use of biosimilars, because
51 these are usually less costly than the originators' products. However, physicians should
52 not feel obliged to prescribe a certain biopharmaceutical purely for cost reasons. *Primum*
53 *non nocere* ("first, do no harm") has been one of the principal precepts of medical ethics
54 since ancient times. Biosimilars differ from generic drugs. Only if their quality, efficacy
55 and safety are clearly documented biosimilars may be chosen because of their lower
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3 costs. Table II summarizes benefits and problems related to the use of biosimilars. The
4 EMEA has developed specific guidelines for their marketing authorization. The
5 biosimilars must qualify with respect to their authenticity, purity, quality, safety, efficacy
6 and immunogenicity. The term “biosimilar” should only be used for biopharmaceutical
7 follow-on products being approved under a defined regulatory pathway, and not for
8 copied products used in countries with a less controlled market.
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11 Physicians and pharmacists should be familiar with the main legal and pharmacological
12 specialities of biosimilars. (i) The production process of a biosimilar substance can differ
13 from that of the original. (ii) Extrapolation to indications of the original product can be
14 allowed, even when the biosimilar was not tested for these indications. (iii) Recombinant
15 glycoproteins contain isoforms with respect to the glycans. The structure of the glycans
16 varies among products. (iv) The formulation of a biosimilar may differ from the
17 originator’s product. (v) All biological medicines are potentially immunogenic.
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Aggregates are considered the most important risk factor for immunogenicity [42]. It is
important, therefore, that the label and other product information of the biosimilar reflect
the specific characteristics (clinical data, reference product, handling advice, *etc.*). A
comparison of The Summary of Product Characteristics (SPC) approved by the EMEA
and the Package Insert (PI) approved by the FDA for 32 biopharmaceutical products has
revealed that the EU SPCs contain more detailed instructions to the prescriber, including
the positioning of the product with regard to the stage of the disease and to other
therapies. A typical feature of the US PIs is the detailed description of the efficacy and
safety result of the pivotal clinical trials [135].

Several biosimilar rhEPOs have been launched in EU, after the key process patents for
the first-generation epoetins have expired. Presently, the naming of the epoetins is
confusing (identical INN vs. different INN, various brand names for identical drugs). In
some countries, physicians are obliged or encouraged to prescribe by INN. In Germany,
for example, pharmacists filling prescriptions covered by the statutory health insurance
system shall dispense a less expensive medicine that contains the same active
substance, as implicated by identical INNs. Allowing biosimilar products to have the
same INN as the reference product presents safety issues for patients. In case of an
adverse event, it is necessary to identify the responsible drug, by reporting the INN, the
brand name and the relevant batch numbers. Furthermore, the wide price differences
between countries within the EU has resulted in a re-import industry [136], where
suppliers buy drugs at low prices in countries such as Portugal and Greece, and sell
them in countries like Germany and Sweden, where prices are higher.

The primary reason for prescribing a biosimilar is its lower price. Biosimilar epoetins are
around 25-30% less costly than the originators’ products in the EU, depending on the
individual country. The launching of biosimilar epoetins has led some innovator
companies to reduce the prices of their products. The second economic criterion, namely
the equivalence in potency of the drugs, is even more difficult to evaluate. Neither *in vitro*
nor *in vivo* bioassays are precise enough to detect differences with respect to the clinical
efficacy. In addition, with respect to renal anemia the biosimilar epoetin alfa (INN) is
presently only approved for the i.v. administration route. In predialysis patients the s.c.

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3 route is associated with a dose saving (by 24%, according to [137]). These
4 considerations may provide an explanation for the fact that European physicians have
5 not readily embraced the biosimilar epoetins despite their lower price. Here, an
6 exception is Germany, where the epoetin alfa follow-on products have captured about
7 30% of the anemia market. This achievement may be owed to the fact that several
8 generic companies producing biosimilar medicines are based in Germany. Other guiding
9 parameters include the marketing strategies and field staff. Finally, it must be
10 remembered that biosimilars compare with first-generation biopharmaceuticals, for which
11 second-generation products with improved pharmacokinetic properties are already
12 available. Hyperglycosylated (darbepoetin alfa) or pegylated (methoxy-PEG-epoetin
13 beta, pegfilgrastim) recombinant proteins have been established in clinical use.
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21 the manuscript.
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