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Biosimilar therapeutics—what do we need to consider?

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Abstract

Patents for the first generation of approved biopharmaceuticals have either expired or are about to expire. Thus the market is opening for generic versions, referred to as 'biosimilars' (European Union) or 'follow-on protein products' (United States). Healthcare professionals need to understand the critical issues surrounding the use of biosimilars to make informed treatment decisions.

The complex high-molecular-weight three-dimensional structures of biopharmaceuticals, their heterogeneity and dependence on production in living cells makes them different from classical chemical drugs. Current analytical methods cannot characterize these complex molecules sufficiently to confirm structural equivalence with reference molecules. Verification of the similarity of biosimilars to innovator biopharmaceuticals remains a key challenge. Furthermore, a critical safety issue, the immunogenicity of biopharmaceuticals, has been highlighted in recent years, confirming a need for comprehensive immunogenicity testing prior to approval and extended post-marketing surveillance.

Biosimilars present a new set of challenges for regulatory authorities when compared with conventional generics. While the demonstration of a pharmacokinetic similarity is sufficient for conventional, small-molecule generic agents, a number of issues will make the approval of biosimilars more complicated. Documents recently published by the European Medicines Agency (EMEA) outlining requirements for the market approval of biosimilars provide much-needed guidance. The EMEA has approved a number of biosimilar products in a scientifically rigorous and balanced process. Outstanding issues include the interchangeability of biosimilars and innovator products, the possible need for unique naming to differentiate the various biopharmaceutical products, and more comprehensive labelling for biosimilars to include relevant clinical data.

Keywords: bioequivalence; biopharmaceuticals; biosimilars; epoetin alfa; pure red cell aplasia

Introduction

The first-generation biopharmaceuticals are copies of endogenous human proteins, such as erythropoietin (EPO), insulin, growth hormones and cytokines that were developed using recombinant DNA (rDNA) technology or hybridoma techniques. These compounds have revolutionized the treatment of many diseases, including anaemia, diabetes, cancer, hepatitis and multiple sclerosis [1]. Patents for many first-generation biopharmaceuticals have either expired or are about to expire. This has opened the market to non-innovator versions of these products, called 'biosimilars' in the European Union, and 'follow-on protein products' in the United States. The European Union has established legal and regulatory pathways for bringing biosimilars to the market [2–10], while the United States has yet to establish similar pathways [11].

Biosimilars are defined as biological products similar, but not identical, to reference products that are submitted for separate marketing approval following patent expiration of the reference products [3,12–14]. Biosimilars are not generic versions of innovator products. Conventional generics are considered to be therapeutically equivalent to a reference once pharmaceutical equivalence (i.e. identical active substances) and bioequivalence (i.e. comparable pharmacokinetics) have been established and do not require formal clinical efficacy and safety studies. The term 'biogeneric' would imply that the active substance of a biosimilar could be readily characterized and shown to be identical to the active substance of the reference product. This is simply not the case with biosimilars. The active substance of a biopharmaceutical is a collection of large protein isoforms and not a single molecular entity, which is generally the case with conventional small-molecule drugs. Thus, it is highly unlikely that the active substances are identical between two products, and there are currently no analytical techniques to establish biopharmaceutical equivalence [12]. Table 1 shows standard definitions for conventional generic agents, biopharmaceuticals and biosimilars based on terminology used by the European Medicines Agency (EMEA) [3,4,12].

Biosimilars present a new set of challenges compared with conventional generics, and their market approval is more complicated. The complexity of biopharmaceuticals makes it difficult to avoid heterogeneity between batches

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Table 1. Definitions of biological and chemical pharmaceuticals

Generic drug	Chemical and therapeutic equivalent of a low-molecular-weight drug whose patent has expired
Biopharmaceutical	'A medicinal product developed by means of one or more of the following biotechnology practices: rDNA, controlled gene expression, antibody methods'[a]
Biosimilar	'A biological medicinal product referring to an existing one and submitted to regulatory authorities for marketing authorization by an independent application after the time of the protection of the data has expired for the original product'[a]

Data from *Crommelin et al.* [12], reprinted with permission from Pharma Publishing & Media Europe. Copyright 2006. All rights reserved.
[a]European Agency for the Evaluation of Medicinal Products definition.

from the same manufacturing process and between the same proteins from different manufacturers [12]. Moreover, it is difficult to establish therapeutic equivalence of biosimilars with reference products without clinical trials [13,15,16]. Safety of biosimilars is also a critical consideration. An important difference between biopharmaceuticals and conventional drugs with regard to safety is the significant potential to induce an immune response (immunogenicity) [10,17,18]. Documents recently published by the EMEA outlining requirements for market approval of biosimilars provide much-needed guidance [19]. The EMEA also provides public assessment reports for biopharmaceutical products that include the summary of product characteristics and a scientific discussion of the clinical data supporting approval. As of January 2008, the EMEA has advised approval of biosimilar versions of recombinant somatropin [20,21], recombinant human EPO (rHuEPO) [22–26] and filgrastim [27]. One follow-on somatropin product has been approved in the United States, but additional approvals of follow-on protein products will necessitate the establishment of a formal legal and regulatory pathway in the United States [11].

Clinicians need to be aware of the important issues surrounding the use of biosimilars. Greater awareness of the potential differences between products will enable clinicians to make informed prescribing decisions, which are critical for patient safety. This article will highlight some of the main issues with biosimilars and review the current status of regulatory approval procedures and biosimilars that have been approved by the European Union.

Key issues with biosimilars

Manufacture

The complexity of the manufacturing process for biopharmaceuticals is several orders of magnitude higher than that for small-molecule pharmaceuticals [12]. Conventional pharmaceutical agents are small-molecule chemicals with a defined molecular weight typically between 100 and 1000 Da. In contrast, biopharmaceuticals are large, complex and heterogeneous proteins with more variable molecular weights, commonly ranging from 18 000 to 145 000 Da. Compared to the manufacture of small molecular entities, the manufacture of biopharmaceuticals requires a greater number of batch records (>250 versus <10); more product quality tests (>2000 versus <100); more critical process steps (>5000 versus <100) and more process data entries (>60 000 versus <4000) [28]. The molecular size and complexity of biopharmaceuticals and their production in living cells makes the final product very sensitive to changes in production conditions. Changes may occur to the expression systems used for production, culture conditions (e.g. temperature and nutrients), purification and processing, formulation, storage and packaging.

Small changes in, or differences between, manufacturing processes may have a significant impact on the quality, purity, biological characteristics and clinical activity of the final product [12,13,15,18,29]. Even when biosimilars are produced from the same genetic construct, using the same technique, formulation and packaging as the innovator product, there is no guarantee that they will be comparable with the reference product. Structural differences between proteins may arise for a number of reasons, including oligomerization, modification of the protein primary sequence, glycosylation patterns or the conformational state.

In order to maximize comparability between batches, manufacturers of both innovator and biosimilar products must ensure consistency in their production processes and perform rigorous purity and activity profiling. Various analytical tests are available to evaluate the physicochemical properties. However, it is important to recognize the limitations of existing assays [15,16]. For example, subtle differences in the conformational state of a recombinant protein product can be very difficult to detect even with state-of-the-art analytical techniques. Quality assurance assays for biopharmaceuticals are generally less sensitive and precise than tests for small-molecule drugs. There is also a need to standardize assays to enable the comparison of results obtained from different laboratories. Until such standardization is achieved, only data obtained within the same laboratory can be compared. For these reasons, it is difficult to establish biopharmaceutical equivalence (i.e. equivalence for the active substance) between a biosimilar and a reference product. Furthermore, even if molecular characteristics and bioavailability are similar between products, it cannot be assumed that their clinical activity will be the same.

The only way to ascertain the safety and efficacy of a biosimilar will be to conduct pre-clinical tests and clinical trials and implement tailored pharmacovigilance plans [16]. Guidelines published by the EMEA detailing requirements for approval of biosimilars [19] are described later in this article.

Variability

While no biosimilars have been approved yet in the United States and biosimilar products were only recently introduced in the European Union, a number of alternative copies of innovator biopharmaceuticals have been available in South America and the Asia-Pacific region. Analytical studies have revealed the extent of heterogeneity of biopharmaceuticals produced by different manufacturing processes around the world. Variation is illustrated by a

number of studies of innovator and non-innovator versions of rHuEPO. Key differences have been found in the structure, stability, composition, concentration and activity of manufactured epoetins [30–32].

EPO is a 165-amino-acid glycoprotein that stimulates erythropoiesis. Erythropoiesis-stimulating agents (ESAs), based on rHuEPO, have been used successfully for more than 17 years for the treatment of anaemia associated with chronic kidney disease (CKD) or cancer therapy [33,34]. There are a number of first-generation ESAs available in the United States and Europe. Epogen® (epoetin alfa) and Procrit® (epoetin alfa), manufactured by Amgen, USA, are currently available in the United States. Eprex® (epoetin alfa), manufactured by Ortho Biologics LLC, USA, and marketed by Johnson & Johnson, is available outside the United States. NeoRecormon® (epoetin beta), manufactured by Roche, Basel, Switzerland, is available in Europe. In addition, Aranesp® (darbepoetin alfa), manufactured by Amgen, USA, is available in the United States, Europe, Canada and Australia, and MIRCERA® (methoxy polyethylene glycol-epoetin beta), manufactured by Roche, Basel, Switzerland, is available in Europe.

Three non-innovator products for epoetin alfa are now manufactured in Korea (Eporon®, Dong-A Pharmaceutical Company Ltd; Espogen®, LG Life Sciences; Epokine®, CJ Corporation). However, all three brands have been shown to differ from the reference epoetin alfa product manufactured by Amgen (Epogen®), with variations in the activity, concentration and isoforms of the products [30]. For example, iso-electric focusing demonstrated the presence of additional isoforms in all three products from Korea compared with Epogen®, despite claims of their substitutability and bioequivalence. An *in vitro* bioassay showed that both Eporon® and Espogen® had a higher bioactivity than was listed on their respective labels. In addition, an enzyme-linked immunosorbent assay indicated that both of these products had higher concentrations of epoetin alfa than stated on the product labels.

In another study, differences in structure and stability were also found between intravenous formulations of epoetin alfa produced by two different manufacturers. A biophysical comparison of Epogen® (Amgen) with Eprex® (manufactured by Ortho Biologicals LLC and distributed by Johnson & Johnson) showed that the two products were not structurally identical. Small differences were found in the hydrodynamic structure, the degree of alfa helicity and the stability of these products [31].

Combe *et al.* [32] evaluated literature reports of studies conducted with non-innovator epoetin products marketed outside the United States and the European Union. The authors found that analytical studies failed to demonstrate comparability of non-innovator epoetins to the reference product. Products differed in composition, did not consistently meet declared specifications and displayed variation between batches. Additional compounds were detected in 3 of 11 biosimilar products analysed, compared with the reference product, and additional epoetin isoforms were detected in 9 of 11 cases. Furthermore, an *in vivo* bioassay in mice demonstrated that bioactivity was higher than specifications in four samples (137–226%) and below specifications in two samples (71–75%; Figure 1) [32,35]. In addition, few of the clinical evaluations were competitor controlled and studies were not thorough enough to show equivalent safety and efficacy of the biosimilar products to the innovator epoetin alfa.

The variation between products manufactured by different companies underscores the challenge in producing biopharmaceutical proteins to consistent standards. While these non-innovator products are not biosimilars, they illustrate the difficulties facing regulators to ensure the quality and safety of biosimilar products. Biosimilar epoetins and other biosimilar products have been recently approved in Europe and are discussed in detail below.

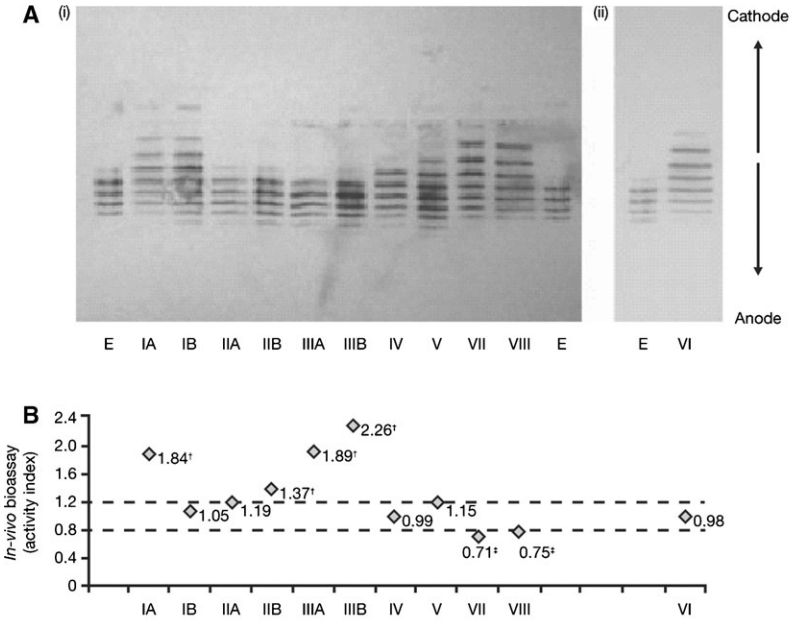
Impact on patient safety

Differences between biological protein products claiming to be similar to approved biopharmaceuticals have been a major concern for the industry and regulatory agencies worldwide. The intrinsic structural and physicochemical heterogeneity of biopharmaceuticals and the complex manufacturing process has the potential to affect their safety and efficacy [13,15,18].

The primary safety concern for biosimilar agents is their potential immunogenicity [17]. The use of biopharmaceuticals to replace endogenous proteins, which may be present at insufficient concentrations (e.g. the use of ESAs in CKD patients with anaemia), carries the serious risk of stimulating the immune system to develop anti-product antibodies (Abs) that may cross-react with endogenous protein [17]. Although these proteins are designed to closely mimic human proteins, they have the potential to induce an immune response, especially when administered as multiple doses over prolonged periods [36,37]. The level of immunogenicity can be markedly different for products considered to be very similar. Computer algorithms can help in the design of less immunogenic proteins [38]. However, no single technique can definitively predict the immunogenicity of a particular protein.

There may be no clinical consequence for developing an immune response to a biopharmaceutical. The patient may develop binding Abs that do not significantly affect the activity of the biopharmaceutical or endogenous protein. On the other hand, anti-product Abs can bind to, and attenuate the activity of, a biopharmaceutical, and general effects include allergy, anaphylaxis or ‘serum sickness’. Major clinical impact can occur if the endogenous protein with essential biological activity is also neutralized. For example, neutralizing naturally occurring EPO can result in a rare condition known as Ab-mediated pure red cell aplasia (PRCA).

One commonly cited example of the impact of variability between biological products on safety is the large increase in the incidence of Ab-mediated PRCA occurring between 1998 and 2003 in CKD patients with anaemia treated with Eprex®, a formulation of epoetin alfa, marketed by Johnson & Johnson (Figure 2) [18,39–42]. The PRCA cases were associated with a breakdown of immune tolerance to treatment with rHuEPO, particularly with subcutaneous administration, resulting in neutralizing Ab formation against both recombinant and endogenous EPO [43]. Previously, PRCA caused by the production of neutralizing anti-EPO



Sample	Concentration (IU/mL)	Country
IA	2000	Korea
IB	4000	Korea
IIA	2000	Korea
IIB	10 000	Korea
IIIA	2000	Korea
IIIB	10 000	Korea
IV	2000	Argentina
V	10 000	Argentina
VI	4000	India
VII	10 000	China
VIII	6000	China

Fig. 1. Physicochemical and biological characteristics of non-innovator epoetin products available outside of the United States and Europe. (**A**) Isoelectric focusing (i)/ western blot (ii) isoform distribution of 11 non-innovator epoetins compared to Eprex® (E). The table shows the location where each sample was obtained. (**B**) Bioactivity, determined by an *in vivo* bioassay in mice, was higher than specifications in four samples (137–226%) and below specifications in two samples (71–75%) [35].

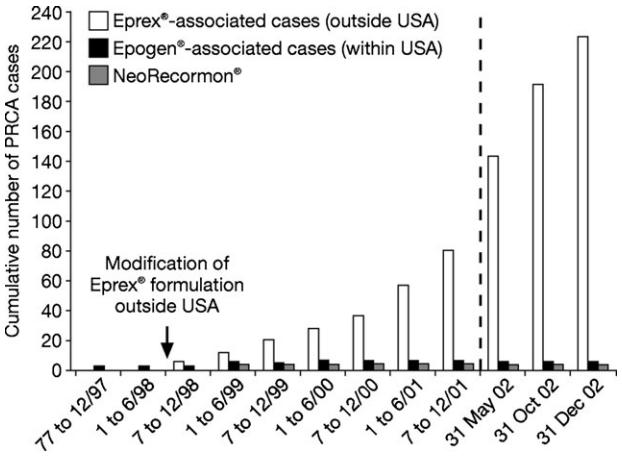


Fig. 2. Antibody-mediated pure red cell aplasia (PCRA) cases, 1997–2002. Data from Schellekens [18], by permission of Oxford University Press.

Abs occurred very rarely with ESA treatment. The apparent increase in immunogenicity coincided with a relatively minor formulation change for Eprex®—replacement of human serum albumin as a stabilizer with glycine and polysorbate 80 in 1998. A contraindication for subcutaneous administration led to a subsequent decrease in the incidence of PRCA.

The Eprex® case highlights concerns regarding the unpredictability and seriousness of immunogenicity of biopharmaceuticals and the potential clinical consequences of their extensive use. Only a small change in the manufacturing process appears to have altered the product's characteristics with a drastic impact on clinical outcome. Many factors have the potential to influence the immunogenicity of proteins. These include variation in amino acid sequence or glycosylation patterns, denaturation or aggregation caused by oxidation due to storage conditions, the presence of contaminants or impurities in the preparation, dose, route of administration, treatment duration and the genetic characteristics of patients [18].

The mechanism by which Eprex® induced PRCA is still not fully understood. A number of possible causes have been proposed [43–45]. These are primarily either micelle formation from polysorbate 80 and epoetin alfa [44,46] or leachates from rubber stoppers breaking B-cell tolerance via an adjuvant effect [45,47,48]. Although many factors are reported to influence the immunogenicity of therapeutic proteins, aggregates play a role in the majority of cases. The Eprex® preparation showed an increase in the levels of aggregates during storage, although the level was never reported to have exceeded specifications. However, product specifications are not defined on the basis of biological effects and aggregates may still be relevant for PRCA induction.

Although cases of Ab-mediated PRCA are rare (ranging from 0.2 to 18 cases per 100 000 patient-years between 2001 and 2003 for the various ESA products [39]), it is a serious complication and one that requires patients to be treated with multiple blood transfusions. The increase in Ab-mediated PRCA cases associated with Eprex® in 1998 highlights how a difference in the manufacturing process can alter product characteristics. This example illustrates just one of the issues to be considered when dealing with these large and complex proteins. With the introduction

of biosimilar epoetins, understanding the cause of Ab-mediated PRCA associated with Eprex® is important for future patient safety [49].

In relation to this issue, it is also important to be aware of the different immunogenic profiles that regulatory approved biosimilars have versus their 'similar biological medicinal products' (SBMPs). For example, a biosimilar epoetin zeta product [SB309 (Retacrit®)] manufactured by Hospira showed a marked difference in immunogenicity in dogs compared with epoetin alfa (its SBMP), although the Abs were non-neutralizing and were not associated with any deterioration in the case of the animals [50]. While there is no hint that SB309 will be more immunogenic than epoetin alfa (at least with respect to clinically relevant neutralizing Abs), only longer term clinical data can fully address the relevance of this difference in immunogenic profile in animals.

A recent case report has documented an instance of PRCA in a haemodialysis patient with CKD ~6 months after treatment with the follow-on epoetin alfa Wepox® (Wockhardt Ltd, Mumbai, India) [51]. The precise mechanism for the development of PRCA in this patient has not been elucidated. However, authors suggest that, as for Eprex®, the increase in immunogenicity could have been due to problems in the manufacturing and storage of Wepox®.

Regulatory approval

General

Limited documentation is required to obtain marketing authorization for a conventional small-molecule generic drug. In general, it is sufficient to show pharmaceutical equivalence and bioequivalence of a generic drug compared with the original product in a small study of volunteers, via an abridged procedure. However, this approach cannot be extrapolated to the majority of biopharmaceuticals because current analytical methods are inadequate to fully characterize these complex proteins. The amount of data required for market approval of biosimilars will be more than for a typical generic drug application but less than for a full new biopharmaceutical application.

EMEA guidelines

The EMEA's Committee for Medicinal Products for Human Use (CHMP) have issued a number of guidelines relevant to biosimilars that detail the requirements for market approval (Table 2). Biosimilar products will be approvable if they have a reference branded market-approved product for which the data protection period has expired. In the United States, the Food and Drug Administration does not have the legal authority to approve most follow-on biologics, and therefore, has not yet issued a specific regulatory pathway. The EMEA guidelines advocate pre-clinical and clinical testing of biosimilars to demonstrate safety and efficacy prior to market authorization, followed by tailored pharmacovigilance plans to monitor potential immunogenicity [10,14,16,19].

The EMEA guidelines cover a range of issues including manufacturing, measurement of comparability, physicochemical and biological analyses and clinical trial requirements. In addition to the pharmaceutical, chemical and biological data normally required for a generic drug application, applications for the market approval of biosimilar products will require additional toxicological and other non-clinical and clinical data. The goal will be to demonstrate that the biosimilar product is similar to the reference product in terms of quality, safety and efficacy. Products will be dealt with on a case-by-case basis, which reflects the complexity and diversity of the products under review. Updates to the guidelines will be published on the EMEA website (www.emea.europa.eu).

The EMEA/CHMP issued an overarching biosimilars guideline [3] to set the scene and guidelines for the development of biosimilars covering quality issues [4], non-clinical and clinical issues [9] and immunogenicity for both biosimilars and innovator products that undergo a manufacturing change [10]. In addition, the EMEA/CHMP released four product class-specific guidelines or concept papers [5–8]. Brief details of these guidelines are outlined below:

- The *Guideline on similar biological medicinal products*, which came into effect in October 2005 had the purpose of 'introducing the concept of similar biological products, outlining the basic principles to be applied and providing applicants with a "user guide" showing where to find relevant scientific information in the various CHMP guidelines, in order to substantiate the claim of similarity' [3].
- The *Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues* came into effect in June 2006. The aim of this document is to 'lay down the quality requirements for a biological medicinal product claiming to be similar to another one already marketed' [4]. Importantly, this guideline addresses the requirements regarding manufacturing processes, analytical methods to assess comparability, factors to consider when choosing a reference product and physicochemical and biological characterization of the SBMP.
- The *Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues* also came into effect in June 2006. This guideline 'lays down the non-clinical and clinical requirements for a biological medicinal product claiming to be similar to another one already marketed' [9]. The non-clinical section addresses the pharmaco-toxicological assessment. The clinical section addresses the requirements for pharmacokinetic, pharmacodynamic, efficacy and safety studies, with emphasis on the evaluation of immunogenicity of the SBMP.
- The *Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins* came into effect in April 2008 [10]. This guideline provides a broad overview of the immunogenic issues that biopharmaceutical companies must adequately address for the approval of a biosimilar product or when a manufacturing change occurs. The guideline discusses factors

Table 2. European Medicines Agency (EMEA) guidelines for biosimilars

Guideline	Focus	Released for consultation	Came into effect
EMEA/CHMP/437/04: Guideline on similar biological medicinal products [3]	General	Nov. 2004	Oct. 2005
EMEA/CHMP//BWP/49348/05: Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues [4]	Quality issues	March 2005	June 2006
EMEA/CHMP/BMWP/42832/05: Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues [9]	Non-clinical and clinical issues	May 2005	June 2006
EMEA/CHMP/BMWP/14327/06: Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins [10]	Immunogenicity assessment	Jan. 2007	April 2008
EMEA/CHMP/94528/05: Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues; guidance on similar medicinal products containing somatropin [6]	Recombinant somatotropin	May 2005	June 2006
EMEA/CHMP/32775/05: Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues; guidance on similar medicinal products containing recombinant human insulin [7]	Recombinant human insulin	May 2005	June 2006
EMEA/CHMP/31329/05: Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues; guidance on similar medicinal products containing recombinant G-CSF [8]	Recombinant G-CSF	June 2005	June 2006
EMEA/CHMP/94526/05: Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues; guidance on similar medicinal products containing recombinant EPOs [5]	Recombinant EPO	May 2005	July 2006

EPO, erythropoietin; G-CSF, granulocyte-colony-stimulating factor.

that might influence immunogenicity and the potential consequences of immunogenicity; the development, design and interpretation of non-clinical and clinical assays to evaluate the immunogenic potency of a product and its comparability to other products; and the implementation of a risk management plan. Many of the concepts discussed in the guideline will likely need to be adapted on a case-by-case basis.

- Four product class-specific guidelines were issued for the development of biosimilars containing recombinant EPO [5], somatotropin [6], human insulin [7] and human granulocyte colony-stimulating factor [8]. These documents outline pre-clinical and clinical data requirements for marketing approval, describing the size of the trials required and the best indication for demonstrating equivalence for each product, in comparison with a reference product.

One example of the product-specific guidelines is the EMEA *Annex to the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues; guidance on similar medicinal products containing recombinant erythropoietins*. This guideline was effective from July 2006 and 'lays down the non-clinical and clinical requirements for EPO-containing medicinal products claiming to be similar to another one already marketed' [5]. Interestingly, the regulatory requirements are more stringent for EPO than for the other recombinant proteins, reflecting its greater molecular complexity and clinical history (i.e. Ab-mediated PRCA). Equivalent therapeutic efficiency with the reference product must be demonstrated in at least two randomized, parallel-group clinical trials, which are preferably double-blind. The document also states that patients with renal anaemia would be the best study population and that after an initial titration phase, the comparative phase should be at least 12 weeks, followed by a maintenance study of at least 3 months. Therapeutic equivalence must be demonstrated for both predialysis and haemodialysis CKD patients, and by both the intravenous and subcutaneous routes of administration. Clinical trials should involve at least 300 patients, and at least 12 months of immunogenicity data should be provided.

Recently, both France and Spain adopted legislation preventing the automatic substitution of a biological medicine for a biosimilar. This decision transposes into French law the European Directive 2004/27/EC [2] governing the definition of generic and biosimilar medicines. Thus, French and Spanish law now forbids the replacement of one biological medicine for another at the pharmacy without the express consent of the prescribing physician.

Post-marketing surveillance

The onset and incidence of immunogenicity is unpredictable; therefore, extended post-marketing surveillance (pharmacovigilance) to monitor potential immunogenicity is very important [14,16]. Biosimilar guidelines from the EMEA state that a pharmacovigilance plan to address immunogenicity and potential rare adverse events should be included in the data package submitted for the product approval [5–9]. The term 'pharmacovigilance' describes the detection, assessment, understanding and prevention of adverse effects after the launch of a product onto the market.

As already discussed previously, there are important lessons to be learned from the experience with Eprex® and Ab-mediated PRCA. Although the innovator product had been in use for years, it was some time before the link between the relatively small modification in the product formulation and the increase in the number of PRCA cases was established [17].

Current biosimilars

Because of the benefits and potential risks associated with biopharmaceuticals and biosimilars, it is important that clinicians familiarize themselves with the relevant literature on the safety and efficacy of these agents in various patient populations. The EMEA provides information on the approval process for human medicines [the European Public Assessment Report EPAR)], including a scientific discussion on the clinical data submitted for approval. Generally, the EPARs for biosimilars have stated that the biosimilar received approval because it was shown to have a comparable quality, safety and efficacy profile to the reference product [52–58]. Despite the comparability of these biosimilars to the reference products, clinicians should be aware of some of the issues that emerged during the development and approval of these products, which highlight the challenges of biosimilars.

Two biosimilar somatropins, Omnitrope® and Valtropin® (marketed by Sandoz and Biopartners, respectively) have been approved by the EMEA. Omnitrope® is a biosimilar version of the reference product, Genotropin® (manufactured by Pfizer). Like Genotropin®, Omnitrope® is a recombinant form of human somatropin that is manufactured with rDNA technology in *E. coli*. The comparability of Omnitrope® to Genotropin® was demonstrated in a randomized controlled trial in 89 children with a lack of growth hormone, with an additional safety study performed in 51 children [52]. During the development of Omnitrope®, an immunogenicity issue emerged with an early version of the product. Up to 60% of patients enrolled in two clinical studies developed anti-growth hormone Abs, which did not appear to affect growth rate. The cause of immunogenicity was linked to excess host cell protein contamination, which was resolved by the manufacturer with additional purification steps [59]. Valtropin®, a biosimilar version of Humatrope® manufactured by BioPartner, was shown to have similar efficacy and safety to the reference product in a 12-month randomized controlled trial involving 149 children lacking growth hormone [53,60]. Clinicians should be aware that while these products have comparable active substances, Humatrope® is synthesized in *E. coli* and Valtropin® is synthesized in the yeast *Saccharomyces cerevisiae*.

Five biosimilar rHuEPOs that are manufactured by two companies have been approved by the EMEA. Abseamed®, Binocrit® and Epoetin alfa HEXAL® are epoetin alfa products and are biosimilar versions of the reference product Eprex®, all produced by Rentschler Biotechnologie GmbH but marketed by three different companies. The approval of these biosimilar epoetin alfa products was based on the demonstration of comparability with Eprex® in quality, safety and efficacy. Comparability exercises demonstrated that although the active substance of the biosimilars was representative of the active substance isolated from Eprex® by immunoaffinity chromatography [61–63], there was a difference in glycosylation levels. The biosimilars contained higher levels of high-mannose-type structures, but this difference was not thought to be clinically significant.

Comparable safety and efficacy between these three biosimilar epoetin alfa products and Eprex® was demonstrated in a randomized controlled trial involving 479 haemodialysis patients with renal anaemia [56–58]. Although the regulatory guidelines for biosimilar rHuEPO developed by the EMEA recommend that comparable efficacy and safety are demonstrated with two randomized trials in the nephrology setting [5], the biosimilar epoetin alfa products were approved based on a single nephrology trial. Data from a study involving 114 cancer patients receiving chemotherapy were also submitted for approval but this study was not adequately powered to demonstrate therapeutic equivalence to the reference product [61–63]. Biosimilar epoetin alfa was approved for indications in cancer patients and patients planning to undergo surgery (for autologous blood transfusions) via data extrapolation—without a full dossier of clinical data for the indication. (For a more detailed review of biosimilar epoetin alfa, see [64].)

Two additional biosimilar versions of Eprex®, Retacrit® and Silapo®, are manufactured by Norbitec GmbH [54,55]. Although this biosimilar manufacturer also used Eprex® as a reference product, the international nonproprietary name (INN) for these products is epoetin zeta rather than epoetin alfa. The active substance of epoetin zeta was shown to be a representative of the active substance found in Eprex®, and the protein structures were comparable. However, differences were noted for the glycosylation profile with respect to glycoforms without an *O*-glycan chain and variants of sialic acid, and a different immunogenicity profile was observed in dogs [65,66]. The comparability of epoetin zeta to Eprex® was demonstrated in two randomized clinical trials, a correction phase study and a maintenance phase study, involving 922 haemodialysis patients with renal anaemia. The correction phase study demonstrated comparability between epoetin zeta and Eprex® for mean haemoglobin levels over the evaluation period. However, comparability was not demonstrated for mean dosage during the evaluation period. Similar results were reported in the maintenance phase study, suggesting a possible difference in the bioactivity of epoetin zeta and Eprex® [65,66]. Data were also presented from a study involving 261 cancer patients receiving chemotherapy, but this study was not designed to demonstrate therapeutic equivalence between products in this patient population. Like biosimilar epoetin alfa, epoetin zeta was approved for indications in renal anaemia, chemotherapy-induced anaemia, and for pre-donation of blood prior to surgery for autologous transfusion [25,26]. Because of its unique INN, epoetin zeta is more readily distinguished from other epoetin products. Unique INNs for biopharmaceuticals may help to facilitate accurate prescribing and dispensing of biopharmaceuticals, as well as pharmacovigilance [67].

The CHMP also issued positive opinions for four biosimilar filgrastim products for the treatment of neutropenia

in February of 2008: Ratiograstim® and Filgrastim ratiopharm® (Ratiopharm GmbH), Biograstim® (CT Arzneimittel GmbH) and Tevagrastim® (Teva Generics GmbH) [68]. These biosimilar versions of filgrastim were shown to be similar to the reference product Neupogen®. These products are awaiting final marketing approval by the EMEA and so the non-clinical and clinical data presented in the EPARs have not yet been made available to the public.

It is important to recognize that the EMEA provides a rigorous and balanced approach to the approval process. Regulators are attempting to meet the demands of the healthcare market while ensuring the quality and safety of biopharmaceutical products. The approval of these biosimilar products does not substantiate interchangeability with reference products [69]. Furthermore, the EMEA has not approved all biosimilar applications. Alpheon®, a biosimilar version of Roferon-A® (interferon alfa-2a), was recently rejected by the EMEA. The manufacturer of Alpheon® had submitted non-clinical data (protein structure, composition and purity) on the biosimilar and conducted a randomized controlled trial in 455 patients with hepatitis C to demonstrate comparable efficacy and safety between the biosimilar and reference product. The reasons for the rejection by the EMEA included quality and clinical differences between Alpheon® and the reference product, inadequate data on the stability of the active substance, inadequate validation of the process for the finished process and insufficient validation of immunogenicity testing [70].

Conclusions

Biosimilar products are very complex molecules and, therefore, cannot be treated the same as conventional generic drugs. There is a need to comprehensively test biosimilars during the production process and always in comparison with an appropriate reference product. Although a variety of assays are available, they may not be adequate to reliably predict the safety and efficacy of a biosimilar product. The validation and standardization of assays will be crucial for future testing and regulation of biosimilars. The regulatory approval of biosimilars will require much more than the demonstration of pharmaceutical equivalence and pharmacokinetic bioequivalence associated with conventional generics. In the post-PRCA era, the immunogenicity of recombinant therapeutic proteins has become a significant safety concern. Ultimately, only clinical studies and post-authorization pharmacovigilance to monitor potential immunogenicity will provide definitive evidence for product comparability to the innovator product with respect to safety and efficacy [14,16].

As manufacturing and clinical experience with the first biosimilar products accumulates, existing EMEA guidelines for the market approval of biosimilars will be revised to include the latest developments and new guidelines will be developed for other biosimilar product classes. Outstanding issues will need to be resolved, including substitution, naming and labelling [67]. Unique naming for all biopharmaceuticals would likely help to differentiate these products, which would facilitate accurate prescribing, dispensing and pharmacovigilance. The labels of the approved biosimilars are nearly identical or are very similar to those of the reference product. A more transparent label that included relevant clinical data for the biosimilar, i.e. the data included in the EPAR, would help clinicians make informed treatment decisions.

Physician awareness of potential differences between biopharmaceuticals and biosimilars and the impact on safety and efficacy is critical for patient safety. Entry of biosimilars onto the market will require transparent, unbiased dissemination of information to prescribers and other healthcare professionals. Clinicians need comprehensive information on biosimilars, and biopharmaceuticals in general, to make informed treatment decisions.

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