Reducing health costs is a hot political issue in many countries, so the introduction and use of generic drugs is stimulated. The generics may be introduced when the patent of an innovative drug expires. With classic drugs, normally produced by chemical synthesis, only limited data for marketing approval application are needed compared with the original drug (1). The generic manufacturer needs to submit data showing the generic to be chemically identical to the innovative drug and pharmaceutically acceptable. In addition, the bioavailability of the generic product must be shown to be equivalent to that of the reference product. This is assessed by pharmacokinetic studies demonstrating an equivalent rate and extent of absorption, typically conducted in healthy volunteers. If the pharmacokinetic parameters such as area under the drug plasma concentration-time curve (AUC) and maximum observed plasma concentration ($C_{\text{max}}$) fall within the range of 0.80 to 1.25 (90% confidence interval), the products are considered bioequivalent. These requirements are based on the assumption that products that demonstrate an identical pharmacokinetic profile will also have identical clinical effects.

The randomized clinical trials required for the approval of new products to demonstrate safety and efficacy are not necessary for the approval of conventional generics. So the clinical data generated with the original product can be extrapolated to the generic. This generic paradigm, made possible by the availability of sophisticated methods for analysis, has resulted in generic products that are safe and effective. Companies marketing a generic product do not need to spend the large investments necessary to develop and launch an innovative drug. They do not need to develop markets and can rely on the extensive experience and expansion of the indications that the innovator may have achieved during the period in which the drug’s patent was protected. So the price of a generic can be relatively low. The introduction of a generic also stimulates innovation because the producer of the original drugs aims to improve their products, which are then protected by new patents. This continuous cycle of price drops and better drugs is good for patients.

This classic generic paradigm cannot be extrapolated to therapeutic proteins for a number of reasons (2). Protein drugs are produced by living cells. They generally are large, complex molecules that mostly also show heterogeneity. This heterogeneity is the result of natural processes in the host cells that modify proteins during their production to protect them during transport through the different cell compartments. Therefore, many therapeutic proteins are mixtures by variations in glycosylation or protein clipping. Modifications of the proteins are also introduced during the extraction and purification from the cells or culture media and during their formulation and storage. Besides these product-related impurities, host cells and culture media may introduce process-related impurities.

Manufacturing therapeutic proteins is a complicated process, and all steps of the production and purification process influence their biologic and clinical properties. The different steps need to be carefully monitored by sophisticated analytical tools and by using many in-house standards. Most of the analytical methods need to be adapted for each specific product. In addition, most production processes, especially those for the first products for which the patents will expire, have undergone important and continuous improvements based on increased experience in manufacturing.

The features of a particular biopharmaceutical are the result of the basic characteristics of the molecule such as amino acid sequence and three-dimensional structure as well as the specific production, purification, formulation, and storage conditions. To produce a biopharmaceutical of constant required quality, a company also needs the experience and the in-house standards to apply the methods used to analyze the structure of a given product. There are various guidelines of the European Medicine Evaluation Agency (EMEA), the United States Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare, and the International Committee for Harmonization (ICH) that require manufacturers to show that they control the production process and are capable of reproducibly manufacturing batches that not only meet product specifications but conform to the definition of the product as established through full characterization. Modifications of the established process are only accepted if the manufacturer can show that the product of the new process is comparable to the initially manufactured product. Comparability studies include revalidation of the process, reevaluation of process- and product-related impurities, recharacterization of the product in side-by-side analyses by all available state of the art methods, and stability studies with the product from the new process in addition to release testing. When considered relevant, these comparability studies also may include clinical studies examining the phar-
macokinetic, pharmacodynamic, and immunogenic properties of the new product and some efficacy and safety studies as well.

Little, if any, of the expertise, analytical methods and in-house standards, specifics of the production process, historical process, and validation data or full characterization data required for comparability assessment of therapeutic proteins are available in the public domain. As a rule, they are proprietary knowledge. It is inconceivable, in the majority of cases, that another manufacturer, on the basis of the patent or published data, is able to manufacture a protein pharmaceutical that can be assumed similar enough to the original innovative product that only a limited documentation of physical chemical characteristics would be sufficient to show equivalence. In most cases only limited data are available in pharmacopeial monographs and scientific reports. Moreover, even the most sophisticated analytical tools are not sensitive enough to fully predict the biologic and clinical characteristics of the product.

Because the generic approach is not applicable to protein drugs, the term biogenerics is considered misleading. Other terms have been used over the years such as multisource products, off-patent biotechnology products, and second entry biologicals (1). The FDA is using the term “follow-on biologics” (3). “Similar biologic medicinal product” is the official terminology in the European Union (EU), but “biosimilars” has become the preferred terminology both in scientific and regulatory discussions and publications and will also be used in this chapter.

Regulatory Implications
In Europe the legislation and regulatory guidelines concerning the marketing authorization is the most advanced. The EU legislation in the Human code stipulates, “Where a biologic medicinal product which is similar to a reference biologic product does not meet the conditions in the definition of generic medicinal products, owing to, in particular, differences relating to raw materials or differences in manufacturing processes of the biologic medicinal product and the reference biologic medicinal product, the results of appropriate preclinical tests or clinical trials relating to these conditions must be provided.” In the annex to this part of the Human code, the issuing of guidelines is left to the EMEA.

The agency has issued a number of general and product-specific guidelines, which give details of what is expected from the companies that apply for a marketing authorization for a biosimilar (4–7). In the EU, two biosimilar growth hormones have been approved, an IFNα-2b has been rejected, and very recently the long-awaited first biosimilar epoetin has been approved.

In the United States the FDA has to wait for legislation enabling the introduction of “follow-on biologics” before it can issue guidelines. Because the recombinant DNA-derived insulins and growth hormones were admitted under the ordinary Drug Act before the specific legislation for biologic products was introduced, follow-on biologics of these products can be approved. It is expected that the US Congress will introduce legislation in 2008 that will enable other follow-on biologics to be marketed. Most experts believe that the there will be much difference in the way the Committee for Medicinal Products for Human Use (CHMP) and the FDA will evaluate biosimilars (3).

The World Health Organization is at present considering the formulation of a basic guideline for biosimilar medicinal products, which can be used by countries with less well developed regulatory systems (8). This worldwide guideline is also expected to advise clinical trials to establish safety and efficacy.

Immunogenicity
The most important safety issue of protein drugs is their potential immunogenicity (9). Except for granulocyte colony stimulating factor, all protein drugs are associated with the induction of antibodies, although the incidence differs. In some cases the immunogenicity is very rare, and in other cases the majority of patients produce antibodies.

The proteins used in medicine before the age of modern biotechnology were either of nonhuman origin or administered to patients with an innate deficiency and no immune tolerance, so their immunogenicity was easy to explain. With the development of recombinant DNA technology, the large-scale production of human homologs like the interferons, growth factors, and hormones became feasible. These products are widely used in patients with a normal immune status and also induce antibodies, which can not be explained by the lack of immune tolerance.

Immunogenicity of therapeutic proteins is based on two different mechanisms. First, the classic reaction to foreign proteins is seen with biopharmaceuticals of bacterial or plant origins such as streptokinase and asparginase. The second mechanism by which antibodies are induced is based on breaking immune tolerance, which normally is directed to self-antigens. This is the mechanism that leads to the antibodies to human homologs like the interferons, IL-2, granulocyte/macrophage colony stimulating factor, and epoetin. The mechanisms by which tolerance is induced or broken are not completely understood. It is becoming clear that aggregation in protein drugs is the most important factor that initiates breaking of B cell tolerance, although many other factors may contribute as well (10,11).

The most potent way to induce high levels of antibodies in experimental systems is to present the self-antigen arrayed on viruses and viral-like particles. The pacing of epitopes with a distance of 50 to 100 Å is unique to viruses and other microbial structures, and the immune system has apparently learned to react vigorously to this type of antigen presentation, be the protein self or nonself. Aggregates of proteins also present epitopes in an array form and explain why they are the main cause of antibody induction (9). Breaking B cell tolerance is a slow process and in general antibodies to products such as IFN and epoetin appear after long-term treatment for more than a year.

In many cases the presence of antibodies is not associated with biological or clinical consequences. The effects that antibodies may induce depend on their level and affinity and can be the result of antigen-antibody reaction in general or of the specific interaction of the antibodies with their target. Severe general immune reactions such as anaphylaxis associated with the use of animal anti-sera have become rare because the purity
of the products increased substantially. Delayed-type infusion-like reactions resembling serum sickness are more common, especially with monoclonal antibodies and other proteins administered in relatively large amounts and the formation of immune complexes. Patients with a slowly but steadily increasing antibody titer are reported to show more infusion-like reactions than patients with a short temporary response.

The consequences of the specific interaction with protein drug is dependent on the affinity of the antibody translating in binding and/or neutralizing capacity. Binding antibodies may influence the pharmacokinetic behavior of the product and both increases and reductions of half-life have been reported, resulting in enhancement or attenuation of activity.

Persisting levels of neutralizing antibodies in general result in loss of activity of the protein drug. Because by definition neutralizing antibodies interact with ligand-receptor interaction, they will inhibit the efficacy of all products in the same class with serious consequences for patients if there is no alternative treatment.

The most dramatic side effects occur if the neutralizing antibodies cross-react with an endogenous factor with an essential biologic function. This had been described for antibodies induced by epoetin alpha, which led to life-threatening pure red cell anemia (PRCA) (12).

Lessons from PRCA
The upsurge of PRCA caused by antibodies induced by epoetin alpha (Eprex™) in countries outside the United States has had a major influence on the regulations regarding biosimilars in Europe. The start of the PRCA epidemic was associated with a formulation change introduced in 1998 when human serum albumin (HSA) as a protein stabilizer was exchanged with polysorbate 80 (13). Several explanations have been offered to explain how this change led to Eprex-associated PRCA. Any explanation should be based on experimental data and have a biological rationale.

Leachates from uncoated rubber stoppers acting as adjuvant are blamed by the manufacturer of Eprex, but the experimental data substantiating this claim are poor. There also is no biological rationale for this because adjuvants are not capable of breaking B cell tolerance as has been shown by many studies during the last 50 years. Leachates as a possible explanation also is inconsistent with the epidemiologic data, because it is unclear why a factor that is present in all syringes should lead to PRCA only in rare instances.

The only explanation that is consistent with all data is a higher tendency for aggregate formation caused by the exchange of HSA by polysorbate 80 as stabilizer. Aggregates offer an undisputed biological rationale, and their appearance is dependent on product storage conditions and handling (13).

The First Biosimilar Epoetin
The first biosimilar epoetins alpha received marketing authorization in August 2007 from the European Commission after positive advice by the EMEA. The public assessment report of Binocrit (Sandoz GmbH, Kundl, Austria), Epoetin alfa Hexal (Hexal Biotech Forschungs GmbH, Holzkirchen, Germany), and Abseamed (Medice Arzneimittel Pütter GmbH, Iserlohn, Germany) was published at the EMEA website in September 2007 (14). Although the biosimilar is marketed by three different companies, it is in fact one product from the same manufacturing plant and the applications for marketing authorizations were identical. The biosimilar was compared in five pharmacology studies investigating pharmacokinetics (PK) and pharmacodynamics (PD) after single-dose as well as multiple-dose intravenous (IV) and subcutaneous (SC) administration performed in healthy male volunteers. Four of these studies were comparative in nature and used either Eprex (epoetin alfa) or NeoRecormon™ (epoetin beta) as comparator. The PK data analysis was considered acceptable by the CHMP, which is the scientific committee of the EMEA, and showed similar PK profiles for IV and SC administration of biosimilar epoetin and Eprex under steady-state conditions.

Although not required in the Guidance on Similar Biologic Medicinal Products Containing Recombinant Epoetins, an acceptance range for PD markers was defined by the manufacturers. The PD profiles showed that the use of same IV or SC dose of the biosimilar and Eprex results in similar increases in hemoglobin in healthy volunteers. The submitted data were accepted by the CHMP to show similar PD effects for IV and SC administration of biosimilar epoetin and Eprex.

In addition, one confirmative, double-blind, randomized, parallel group, multicenter phase III trial was performed to compare efficacy and safety of intravenously administered biosimilar and Eprex in patients with renal anemia on dialysis. The study was designed to evaluate a 1:1 dose conversion from Eprex to biosimilar epoetin with respect to efficacy based on hemoglobin assessment. Of the 314 patients who entered the study in the biosimilar arm, 261 patients completed the study. In the Eprex arm, 142 patients of the 164 patients who entered completed the study. The primary end point of the study was to achieve therapeutic equivalence in mean absolute change in hemoglobin level between the screening baseline period and the evaluation period.

The clinical development program was in line with the guideline on similar biologic medicinal products containing recombinant epoetins and previous scientific advice. However, one exception was noted, i.e., the recommendation to provide results from at least two adequately powered, randomized, parallel group clinical trials demonstrating comparable efficacy and safety for both routes of administration in patients with renal anemia. It was acknowledged that at the time of clinical development Eprex could not be used as comparator in subcutaneous studies in renal anemia patients and therefore no second, randomized, parallel group clinical trial with the subcutaneous route of application could be conducted. This deviation from the guideline was accepted by the CHMP.

The observed difference in hemoglobin change was low between both treatments and within the predefined and acceptable equivalence boundaries of ±0.5 g/dl. All secondary end points, most importantly change in epoetin dose, also supported the conclusion of therapeutic equivalence between Eprex and the biosimilar epoetin. So the CHMP concluded that
comparable efficacy had been established for the intravenous route of administration.

The other exploratory clinical study assessed efficacy and safety of the biosimilar epoetin in the treatment of chemotherapy-associated anemia. In this study, an Eprex group was included as a measure of internal validity. Sixty of the 74 patients in the biosimilar arm completed the study, as did 31 of the 40 patients in the Eprex arm. The primary end point of this study was the absolute increase in hemoglobin value of \( \pm 2.0 \text{ g/dl} \) between the baseline screening period and the evaluation period in the absence of red blood cell transfusions during the preceding 4 wk. However, this study was not designed (i.e., was too small) to establish comparable efficacy for the subcutaneous route of administration. Nevertheless, the CHMP concluded that, on the basis of the demonstration of equivalent efficacy and steady state pharmacokinetics and pharmacodynamics for intravenously administered biosimilar epoetin and Eprex, as well as the finding of similar multiple-dose subcutaneous pharmacokinetic/pharmacodynamic profiles in healthy volunteers, a difference in efficacy for the subcutaneous route of administration appeared highly unlikely.

Overall, there was no significant difference between the treatment groups for the incidence or type of adverse events. The biosimilar epoetin revealed a safety profile similar to that of the innovator comparator Eprex. Although no new safety concerns arose in this clinical trial performed in patients receiving chemotherapy, the size of the population studied was too limited to allow definitive conclusions. The indications allowed by the CHMP are listed in Table 1. The route of administration is restricted to intravenous in patients with renal anemia. In cancer patients and patients undergoing orthopedic surgery, the biosimilar can also be administered by intravenous route.

**But Is It Similar?**

There is no reason to believe that the first biosimilar epoetin admitted to the EU market has a safety or efficacy issue. The companies involved in the development and production of this product have extensive experience in therapeutic proteins. The European regulatory system also is to be commended for taking up the challenge to set a regulatory system into place to evaluate biosimilars. However, questions remain concerning the evaluation of this epoetin. Because two protein drugs can never be considered identical, the concept of similarity has been introduced. On the basis of this concept, manufacturers of biosimilars are allowed to submit restricted documentation for their request for marketing authorization compared with that of the original protein drug. Similarity cannot be defined on a precise quantitative basis, and making the final decision as to how much difference is acceptable, e.g., in the degree of glycosylation and the level of impurities, relies on subjective judgment.

The greatest structural difference between the innovative epoetin alpha and the biosimilar is its high level of mannose. How much more mannose the biosimilar contains is not specified, but it can be assumed to be significant because it is specifically mentioned in the European Public Assessment Report (EPAR). According to the evaluation by the CHMP, this difference is irrelevant because the clinical data showed no difference in parameters like pharmacokinetics and efficacy. However, the margins that were used to define equivalence between the two products were too wide to find relevant pharmacokinetic differences. The limited studies on efficacy and safety also were not discriminatory enough to find possible mannose-related differences.

In the EPAR there also is no discussion whether this difference in structure in itself did not disqualify this epoetin alpha as a biosimilar of the innovator epoetin alpha. Structural similarity is strictly adhered to in the part of the guidelines where selection of the comparator is discussed. In the guideline IFNα-2a and -2b are presented as examples of products considered to be structurally different products and therefore not exchangeable as comparators, although in the many studies done with the two products during the past 25 years not one single biologic or clinical difference between the products was identified.

For the biosimilar epoetins, the CHMP has issued a product-specific guideline that states two phase III clinical trials are necessary. However, in the case of the first biosimilar, the European regulators have accepted the data from a single trial because the subcutaneous route of application of the comparator Eprex was contraindicated at the time of the studies. The CHMP has thus taken the remarkable position to waive the study of the biosimilar in the clinical condition for which the comparator has a safety issue. Therefore, although the safety of the subcutaneous administration of the drug has not yet been tested in patients with chronic renal failure, the only patient group to develop PRCA, the biosimilar can now be given via the subcutaneous route in cancer patients as well. Moreover, extrapolation to the use in children and to orthopedic patients has been allowed without safety and efficacy studies in these specific groups.

The possible immunogenicity of the biosimilar and the potential development of PRCA has apparently been an im-

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**Table 1. Indications of the biosimilar epoetin alpha**

| Treatment of anemia associated with chronic renal failure in pediatric and adult patients on hemodialysis and adult patients on peritoneal dialysis. |
| Treatment of severe anemia of renal origin accompanied by clinical symptoms in adult patients with renal insufficiency not yet undergoing dialysis. |
| Treatment of anemia and reduction of transfusion requirements in adult patients receiving chemotherapy for solid tumors, malignant lymphoma, or multiple myeloma, and at risk of transfusion. |
| The biosimilar can be used to reduce exposure to allogeneic blood transfusions in adult non-iron-deficient patients before major elective orthopedic surgery, if considered at high risk for transfusion complications. |
portant issue in the evaluation. However, the PRCA upsurge in 1998–2002 was associated with a specific change in a specific product (13). This manifestation of immunogenicity had certain typical characteristics that set it apart from what is the antibody response usually seen with therapeutic proteins. An antibody response induced by another epoetin may have a different character and may lead to different clinical consequences. The radioimmunoprecipitation assay used to monitor immunogenicity has been proven useful in the diagnosis of epoetin antibody-induced PRCA (16,17). However, the usefulness of this assay to determine an early immune response in a low-risk population is not clear. With this assay a transient binding antibody response was seen in a number of intravenously treated renal failure patients in the pivotal trial with the innovator and/or biosimilar product. This has never been seen before in clinical trials with epoetins, which questions the specificity of this particular assay.

In the risk management program, a cohort study is described to assess the incidence of PRCA associated with the use of the new biosimilar (14). There is no mention of the number of patients to be involved or the length of the study. The study by Janssen-Cilag to establish safety after the contraindication of subcutaneous use in renal patients was revoked, shows the problems. To power the study to find an increased PRCA incidence, 30,000 patients need to be included. The cost of the study is around $30 million. The recruitment of patients who should be on epoetin treatment for less than a year for the Janssen-Cilag study is far below expectations and it is expected never to attain its goal. This puts in doubt whether the companies marketing the new biosimilar epoetin can realistically be expected to organize such a cohort study, although they are certainly capable of doing these studies.

The naming of the biosimilars has also been an important point for discussion. The innovative industry has lobbied strongly for the biosimilars to have their own specific international nonproprietary name. The CHMP, however, has accepted the international nonproprietary name to be identical to the original epoetin alpha. From a scientific point of view it is logical to accept the same name for proteins that are as similar as possible. To maintain this approach, however, the EMEA should adopt are more restricted approach as to the structural differences they are willing to accept between innovator product and biosimilar.

**Disclosures**

None.

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