

Biologicals and biosimilars

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INTRODUCTION

When a small chemical compound medicine's patent expires, generic drug companies are allowed to produce a chemically identical drug. Market approval is granted by the bioequivalence approach, *i.e.* the pharmacokinetic properties of the generic drug need to fit into certain predefined margins. According to the European Medicines Agency (EMA), "due to the complexity of biological/biotechnology-derived products the generic approach is scientifically not appropriate for these products" (CHMP/437/04). Hence, after patents for biological drugs expire, off-patent biological drugs or biosimilar medicines are produced. These include recombinant human growth hormone, interferon, granulocyte colony-stimulating factor and erythropoietin (EPO). The EMA introduced scientific-based regulatory guidelines in an attempt to secure the quality of the medicines distributed in the European Union and also patient safety. The guidelines mandate that patient safety and the efficacy of the biosimilar as well as the bioequivalence must be shown in a certain number of patients. Importantly, the number of patients is much lower than the numbers that are required nowadays for an originator drug approval, meaning the financial aspect of the second-generation biologicals has also been considered.

The crucial question, however, of if the biosimilars are as good, as potent, or even better than their originator drugs – or if they pose any harm to the

people that are treated with them – remains to be solved. The intention of this review is to discuss some of the critical questions and sketch the background to the manufacture of biological/biosimilar drugs and possible problems and pitfalls.

"THE PROCESS IS THE PRODUCT": THE PRODUCTION OF BIOLOGICAL DRUGS

Small chemical drugs are produced in chemical reactions requiring optimised and exact conditions under which the reaction can be reproduced by every skilled chemist worldwide. Biological drugs, on the contrary, are produced by bioorganisms, meaning all different kinds of cell-based products are summarised: vaccines, blood products, cytokines and hormones as well as monoclonal antibodies. Some 200 years ago, Dr Edward Jenner inoculated material from cowpox blisters into subjects that resulted in vaccination against smallpox. Later years see quite a number of famous names associated with the development of both a worldwide vaccination program of the World Health Organization (WHO) and the specialisation of modern immunology: Pasteur, Koch, Roux, Ehrlich, von Behring, Kitasato and finally Salk and Sabin. Smallpox was extinguished once and for all, proving that drugs derived from biological species clearly exerted positive effects. However, this was the essential starting point for biotechnology and research was paving the way for the production of biopharmaceuticals. The elucidation of the genetic code (aka desoxyribonucleic acid, DNA) was the basis that led to the possibility of forcing cells of different origin and species to produce

foreign protein. This molecular biological approach finally facilitated the large-scale production of therapeutic protein. Further improvements of the production process and the properties of the proteins were easily reached by manipulation of the parent DNA; amino acid side chains could be changed to increase the biological half-life or to enhance the biological potency of the biopharmaceutical. In principle, the vectorial plasmid DNA that contains the genetic information of the therapeutic protein is transfected into appropriate host cells, the process that transports the DNA into the cells. Nowadays, this is achieved by lipofection reagents. In mammalian cells, the protein is produced by ribosomes that firmly attach to the endoplasmic reticulum (ER). The newly synthesised protein intrudes the ER through specialised pores and undergoes a first series of post-translational modifications. Also, the rigid ER quality control checks for correct folding of the protein and glycosylation. In case the protein does not pass, it will be sorted to the ER associated degradation pathway (ERAD); all other proteins will leave for the Golgi apparatus and undergo complex glycosylation or other post-translational modifications. However, biological medicines produced in *E. coli* lack complex glycosylation since the bacteria are not equipped with the necessary machinery. Therefore, biological medicines produced in *E. coli* need to be functional without complex glycosylation steps; recent examples of such biopharmaceuticals include for instance the growth hormones. Glycosylation is the most important post-translational modification because it influences the activity, the folding and stability, and also the pharmacokinetic clearance. Enhanced or reduced glycosylation can be achieved by creating mutants, but also fusion proteins, conjugates with toxins or polymers may be engineered¹. For instance, the attachment of poly-ethylen-glycol repeat sequences (PEGylation) results in altered pharmacokinetic properties: enhanced water solubility and/or a significant increase in size with decreased renal excretion².

However, the production starts with the selection of one clonal derived cell line that will be expanded. One clonal cell does not perfectly resemble its neighbour cell although they were kept under the same conditions and transfected the same plasmid³. Differences in the expression pattern of cellular enzymes and/or the protein machinery render the production of proteins difficult to predict: this is also the reason for batch-to-batch variation – and this

may be seen within one production unit (*i.e.* company) and the same cell line that has been grown over a longer period of time. But the biopharmaceutical companies perform quite a number of different quality control regimens to tightly control the production of biopharmaceuticals³.

Hence, it is safe to conclude that biologicals are produced by unique cell lines and are therefore unique products: differences can occur at a variety of production steps. Therefore, it is not surprising that a recent survey of EPO from several different production units all over the world revealed distinct protein patterns⁴.

■ DIFFERENCES AMONG BIOLOGICALS: ANYTHING NECESSARY TO CONSIDER?

As mentioned above, subtle-to-overt differences of unique biologicals create specific disadvantages: EPO from company A does not resemble EPO from company B; it may not even perfectly match EPO from another cell line within the same company or the EPO from the same cell line after a change in the manufacturing process. In principle, this is an inherent feature of protein production and, also, different proteins released from different tissue in the organism show a distinct pattern. However, it gets more serious if our immune system senses differences in epitopes due to the given structural complexity of proteins which may, in turn, lead to immunogenic problems⁵. The causes that lead to problems include structural properties such as sequence variation, differences in the glycosylation pattern or the downstream processing, also the dose and length of treatment. However, it must always be kept in mind that individual patients' characteristics may also have repercussions on distinct efficacy and safety issues.

A very early example of the importance of single amino acid changes came from the evolution of insulin preparations from porcine source to *E. coli*; porcine and human insulin differ by only one single amino acid. Administration of the porcine insulin preparation resulted in the development of circulating insulin antibodies⁶; these antibodies could potentially contribute to insulin resistance⁷. This

potential threat was completely eliminated after the advent of insulin preparations derived from recombinant bacterial expression systems. However, the advent of molecular biology and the advent of recombinant protein expression did not eliminate all problems instantaneously: despite an identical genetic code being used to express the specific protein molecule, problems with biological drugs have still been reported. One of the most important incidences to occur is the cases of “pure red cell aplasia” (PRCA)⁸. Cases have been reported after subcutaneous administration, but only very rarely after intravenous administration of a specific EPO formulation (Eprex) that resulted in the production of anti-erythropoietin antibodies^{8,9}. While the production of antibodies does not necessarily pose a serious for the patients, these antibodies had an important drawback: they were neutralising in nature and therefore ablated the functional EPO in the body, whether endogenously expressed or exogenously administered. The underlying cause for PRCA is still a matter for intense debate (summarised by Schellekens and Jiskoot¹⁰: considering the “leachate-hypothesis” and the “B-cell tolerance breakdown”, more research is necessary to better define the most likely reason. However, Eprex differed from other EPOs only in the number of sial-glycosylated residues. Apart from the scientific plausibility, this incidence underscores the importance of manufacturing and formulation processes for the activity and safety of biologicals. In a long-term follow-up on the 200 patients hit by erythropoietin-associated PRCA, some 60% received immunosuppressive treatment and some 10% underwent renal transplantation¹¹.

In summary, biological drugs could all potentially result in unpredictable reactions that can ultimately lead to unexpected adverse events; small changes in the molecules can already result in possible immunogenicity and loss in efficacy of the administered drug. Hence, it is of utmost relevance to carry out all necessary measures to ensure patients safety and detect gross group effects by a thorough clinical trials regime.

■ BIOSIMILARS: A REALITY

The EMEA coined the term biosimilars and in the United States they will be named “follow-on

biologics”; these are the new generation biologicals that try to resemble the innovator product as closely as possible. The interest of generics companies in biosimilars stems from (i) an easier market access due to the EU legislative after the expiry of originator patents on several biologicals and (ii) the economic market situation for biologicals that is growing while the traditional small chemical drugs market stagnates¹².

It has been outlined above that biological drugs differ in size. They are of distinct molecular heterogeneity and complexity to classical small drug molecules. Further, their production processes differ vastly: while biologicals are made from living cells, chemicals are synthesised according to standard chemical procedures. Hence, it is easy to reconcile that generic companies synthesise generics; their purity and quality can be tested in standardised procedures (*e.g.* by simply demonstrating bioequivalence): differences between biological drugs render predictions on their physicochemical and biological, thus, clinical effects difficult to impossible. These properties depend on the uniqueness of the cell lines used and the chosen production procedure.

The EMEA issued several stringent guidelines to make sure that the novel biosimilars are manufactured with the greatest care possible to ensure the highest standards in patient safety. Thus, the procedure to approve a biosimilar drug in the European market is much more difficult in comparison to a generic chemical drug. However, during the approval procedure for the first biosimilar EPOs (*i.e.* EPO alfa and EPO zeta), some deviation from the guidelines was tolerated which was difficult to understand. The most striking deviation occurred regarding the reference product which was Eprex in all cases for biosimilar EPO: as Eprex has had a safety issue only for the subcutaneous route of administration during the time the biosimilar manufacturers were in the midst of the clinical trials, scientific advice was granted to omit the comparability study to this administration route. However, this is difficult to understand since Eprex was the compound that caused most if not all of the PRCA-cases, and all by subcutaneous administration.

Furthermore, EPO zeta differed from the reference product in its glycosylation pattern (see EPAR/Silapo/H-760). This was discussed as not relevant

since a comparison of the purity and *in vivo* bioactivity did not reveal any remarkable difference. However, as Delorme¹ showed, a difference in glycosylation can exert an effect on the *in vivo* activity. The need for higher dosage of the test substance (*i.e.* EPO zeta) during the correction study points towards a considerable difference between the original and the biosimilar. Even more strikingly, the maintenance study after a switch from the reference to the test drug showed an increase of the necessary EPO dose by approximately 10-15%. In addition, the haemoglobin values declined by about 5% during the same time frame (see EPAR/Silapo/H-760).

Not only can the activity of a drug be impaired, the biosimilar EPO alfa also had an impact on the adverse events. In study INJ-11 in cancer patients, there were more frequently reported nervous system disorders, as well as neurological signs and symptoms and lymphatic system and cardiovascular system affections (see EPAR/epoetinalfahexal/H-726).

Considering the most important point, the immunogenicity in human subjects, biosimilar EPOs have not been administered by the most problematic route (*i.e.* subcutaneous), only intravenously. The study authors claim that there were no occurrences of novel antibodies. As the most critical part has not been examined, the subcutaneous route of administration has not been granted market authorisation. Moreover, there are extensive post-marketing commitments necessary to examine the safety and quality of the product after market approval.

During the preclinical evaluation of the drug, the company also performed animal tests. There is an antigenicity study performed in dogs intravenously over 13 weeks in which a greater number of dogs that developed large “total positive antibody titres” received the study medication (see EPAR/retacrit/H-872). This was discussed as safe, however, when carefully reading the European public assessment report, there were subcutaneous rat experiments which, in contrast to the dog experiments, indeed led to the production of neutralising antibodies.

To conclude this paragraph, one has to consider that the gross group effects were tested by the originator companies in the early days of the development of EPO as a biopharmaceutical. However, it

will be difficult to pick up any statistically significant effects from the novel biosimilar drugs if i) only a few patients receive biosimilars for treatment and ii) doctors hesitate to use the novel biosimilars. While the EMEA has installed a procedure that allowed for careful assessment of potential problems with biosimilars, it is to be questioned if the number of treated patients is sufficient to clear up all doubts, even after concluding the phase IV trials. This is substantiated even further in the case of a pharmacist checklist for biopharmaceuticals that should provide the best possible overview¹³. In contrast, there is also the opinion that the EMEA approach is overstating the possible threat of biosimilars – and the US government/FDA should use a generic approach also for follow-on biologicals¹⁴.

■ BIOSIMILARS: TO SUBSTITUTE OR NOT SUBSTITUTE...

In case of generic chemical drugs, exchangeability is given between originator drugs and generics; authorities in several countries mandate automatic substitution whenever economically needed. It is more complicated in the case of biosimilars: is the similarity adequate to claim equivalence of the innovator drug and the follow-on drug? Along the lines outlined above, it is safe to conclude that a second-generation biological cannot be a generic drug to the innovator drug; for obvious reasons, bioequivalence may simply not be given. Due to the complex nature of biological drugs, both the biological as well as the biosimilar drug have gone through the same preclinical development process. Therefore, changes from one batch to another even within one brand of biological or biosimilar drug, for instance, can create difficulties, especially when the manufacturing procedure has changed. Hence, automatic substitution from a biological drug to a biosimilar drug seems even less favourable. Consequently, this could also create problems in pharmacovigilance; the traceability of an administered drug might be lost.

However, the first biosimilars entered the European market three years ago¹⁵, two biosimilar somatotropins. Shortly after, the first EPO appeared on the market. Larger differences and, hence, also difficulties may be encountered. The EMEA has put

patient safety as the prime issue in its regulations on biosimilar drugs. It will be of vital importance to extend this also to drug substitution. Therefore, due diligence is mandatory until we ascertain enough data to build up databases as well as true experience of how to deal with biologicals and biosimilars in an appropriate manner.

Conflict of interest statement. None declared.

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