

CMC Issues and Regulatory Requirements for Biosimilars

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Abstract

Chemistry, Manufacturing and Controls (CMC), preclinical and clinical are three critical pieces in biosimilars development. Unlike a small-molecule generic drug, which is approved based on “sameness” to the innovator's drug; a biosimilar is approved based on high similarity to the original approved biologic drug. This is because biologics are large and complex molecules. Many functional-, safety- and efficacy-related characteristics of a biologic depend on its manufacturing process. A biosimilars manufacturer won't be able to exactly replicate the innovator's process. The traditional abbreviated pathway for generic drug approval through the Hatch-Waxman Act of 1984 doesn't apply for biosimilars as drugs and biologics are regulated under different laws. New laws and regulations are needed for biosimilars approval in the US. The EU has issued biosimilars guidelines based on comparative testing against the reference biologic drug (the original approved biologic). A full scale CMC development is required including expression system, culture, purification, formulation, analytics and packaging. The manufacturing process needs to be developed and optimized using state-of-the-art technologies. Minor differences in structure and impurity profiles are acceptable but should be justified. Abbreviated clinical testing is required to evaluate surrogate markers for efficacy and demonstrate no immunogenic response to the product. We anticipate the package for a biosimilars approval in the US will be similar to that in the EU and contain a full quality dossier with a comparability program including detailed product characterization comparison and reduced preclinical and clinical requirements.

Biosimilars Become Inevitable

Biologics developed through biotechnology constitute an essential part of the pipeline for medicines available to patients today. Biologic drugs are quite expensive and many of them are top-selling medicines (see Table 1). Since they come at extremely high prices to consumers, some patients may not be able to afford the use of biologics as the best-available treatments to their conditions. The patent protection on a large number of biologics has expired since 2001. These off-patent biologics include Neupogen, Novolin, Protropin, Activase, Epogen or Procrit, Nutropin, Humatrope, Avonex, Intron A, and Humulin. Traditionally, when a drug patent expires, a generic drug will be quickly developed and marketed. Similarly, generic version of off-patent biologic drugs (also referred to biosimilars or follow-on biologics or biogenerics) represents an extraordinary opportunity to companies that want to seize the potentially great commercial rewards in this unexploited territory. Biosimilars not only benefit the biosimilar manufacturers but also can save patients, and insurance companies, substantial cost and

Table 1: *Estimates of peak sales (in billions) for top selling biologics* ^[1]

Drug	2008 Sales	Year approved	Drug	2008 Sales	Year approved
Avastin	\$9.2	2004	Avonex	\$2.6	1996
Enbrel	\$8.0	1998	Novolin	\$2.5	1991
Remicade	\$7.9	1998	Humalog	\$2.2	1996
Humira	\$7.3	2002	Pegasys	\$2.0	2002
Rituxan	\$7.3	1997	Rebif	\$1.7	2002
Herceptin	\$5.7	1998	Cerezyme	\$1.5	1994
Lantus	\$5.1	2000	Tysabri	\$1.4	2004
Epogen/Procrit	\$5.1	1989	NovoSeven	\$1.4	1999
Neulasta	\$4.2	2002	Synagis	\$1.3	1998
Novolog	\$3.7	2000	Neupogen	\$1.3	1991
Erbix	\$3.6	2004	Betaseron	\$1.2	1993
Aranesp	\$3.2	2001	Humulin	\$1.1	1992
Recombinant	\$2.9	1998	Kogenate FS	\$1.1	1993
Lucentis	\$2.7	2006			

allow patients to gain access to more affordable biologics resulting in market expansion. The government can use biosimilars to reduce healthcare costs. Therefore, development and marketing of biosimilars are supported by both manufacturers and consumers.

Differences between Generic Drugs and Biosimilars

Enacted in 1984, the US Drug Price Competition and Patent Term Restoration Act, informally known as the “Hatch-Waxman Act of 1984” standardized US procedures for an abbreviated pathway for the approval of small-molecule generic drugs. The generic drug approval is based on “sameness”. In comparison to the innovator’s drug, a generic drug is a product that has the same active ingredient, identical in dose, strength, route of administration, safety, efficacy, and intended use. For approval, the generic companies can go through the Abbreviated New Drug Application (ANDA) process with reduced requirement in comparison to approval for a new drug entity. The generic drugs need to show bioequivalence to the innovator drugs typically based on pharmacokinetic parameters such as the rate of absorption or bioavailability in 24 to 36 healthy volunteers. No large

clinical trials for safety and efficacy are required. The generic companies can rely on the FDA’s previous findings of safety and effectiveness of the innovator’s drugs.

However, the abbreviated pathway for generic drugs legally doesn’t apply to biologics as small-molecule drugs and biologics are regulated under different laws and approved through different pathways in the US (Table 2). Small-molecule drugs are regulated under the Food, Drug and Cosmetic Act (FD&C) and require submission of a New Drug Application (NDA) to FDA for drug review and approval. Biologics are regulated under the Public Health Service Act (PHS) and require submission of a Biologic License Application (BLA) to FDA for review and approval. The Hatch-Waxman Act of 1984 doesn’t apply for biosimilars. New laws are needed to establish a pathway for biosimilar approval.

There are some crucial differences between biologics and small-molecule drugs (Table 3). Small-molecule drugs are made from chemical synthesis. They are not sensitive to process changes. The final product of a small-molecule drug can be fully characterized. The development and production of generic drugs are relatively straightforward. Biologics are made from living organ-

Table 2: *Laws and regulatory pathways for drug approval in the US*

Law/Application	Small-molecule drug	Biologics
Law	Food, Drug and Cosmetic Act (FD&C)	Public Health Service Act (PHS)
Drug application	New Drug Application (NDA)	Biologic License Application (BLA)
Generic application	Abbreviated New Drug Application (ANDA)	No pathway yet

isms so that its functional-, efficacy- and safety-related properties depend on its manufacturing and processing conditions. They are sensitive to process changes. Even minor modifications of the manufacturing process can cause variations in important properties of a biological product. Thus it is believed that a biologic product is defined by its manufacturing process. Biologics are 100- or 1,000-fold larger than small-molecule drugs, possess sophisticated three-dimensional structures, and contain mixtures of protein isoforms. A biological product is a heterogeneous mixture and the current analytical methods cannot characterize these complex molecules sufficiently to confirm structural equivalence with the reference biologics.

Immunogenicity Poses a Concern

One of the major complications that biologics can produce is immunogenicity as therapeutic proteins are inherently immunogenic^[2]. Immunogenicity is related to biologics structure and formulation and is dependent on dose, route of administration and frequency of administration. Clinical implications of immunogenicity are not always predictable. Formation of antibodies can result in harmless clinical effect or produce significant adverse events or severe disease. Examples are provided below.

The Eprex (Erythropoietin, EPO) has been marketed by Johnson & Johnson (J&J) in the European Union (EU) countries for 10 years with no noticeable immunogenic-

Table 3: *Differences between small-molecule drugs and biologics*

Comparison property	Small-molecule generics	Biosimilars
Product characteristics	Small, simple molecule (Molecular weight: 100-1,000 Da)	Large, complex molecules, Higher order structures, Post-translational modifications (Molecular weight: 15,000-150,000 Da)
Production	Produced by chemical synthesis	Produced in living organisms
Analytical testing	Well-defined chemical structure, all its various components in the finished drug can be determined	Heterogeneous mixture, difficult to characterize, some of the components of a finished biologic may be unknown
Process dependence	Not sensitive to manufacturing process changes. The finished product can be analyzed to establish the sameness.	Sensitive to minor changes in manufacturing process. The product is defined by the process
Identity and purity	Often meeting pharmacopeia or other standards of identity (e.g., minimums for purity and potency)	Most have no pharmacopeia monographs

ity issues prior to 1998. When J&J made a change in the Eprex formulation by replacing human serum albumin (HAS) with polysorbate 80 and glycine in response to the request from European health authorities, some patients developed pure red-cell aplasia (PRCA), a severe form of anemia. Eprex induced antibodies neutralize all the exogenous rHuEPO and cross-react with endogenous erythropoietic proteins. As a result, serum EPO is undetectable and erythropoiesis becomes ineffective. Upon investigation, J&J found that polysorbate 80 might have caused uncoated rubber stoppers in single-use Eprex syringes to leach plasticizers, which stimulated an immune response that resulted in PRCA. Replacing with Teflon coated stoppers resulted in 90% decrease in PRCA by 2003 [3,4].

The effect of neutralizing antibodies has not always resulted in serious clinical consequences. Three interferon beta products, Betaseron, Rebif and Avonex, are marketed by three different companies. These products induce neutralizing antibodies in multiple sclerosis patients from 5 to 50% after one year treatment. Although these antibodies might be associated with loss of efficacy of treatment resulting in some patients to withdraw from the treatment, it seems no other severe adverse effects were detected [5,6].

Regulatory Landscape

The US, the EU and Japan are the three cornerstone members of the International Conference on Harmonization (ICH), which intends to harmonize the regulatory requirements for drug or biologic approval in these three regions. With the other two members, the EU and Japan, already have established biosimilar approval procedures (see below), the US lags behind in the biosimilar race. There are no formal approval pathways for biosimilars in the US. Congress needs to establish a legal framework in order for FDA to develop guidelines. Legislation has been under discussion in Congress since 2007. The legislative debate is centered on patient safety and preserving incentives to innovate with introduction of biosimilars. Two bills introduced in March 2009 deserve attentions [7,8]. The Waxman bill (H.R. 1427) proposes 5 years of market exclusivity to the innovator companies and requires

no clinical trials for biosimilar development. The Eshoo bill (H.R. 1548) proposes 12 years of market exclusivity to the innovator companies and requires clinical trials for biosimilar development. Obama administration appears to favor a 7-year market exclusivity [9]. Once a legal framework is established for biosimilars, the FDA will likely take a conservative approach using the comparability as an approval principle. Clinical proof of efficacy and safety will be required, probably in reduced scale.

In the EU, the European Medicines Agency (EMA) issued regulatory guidelines for approving biosimilars in 2005 (Figure 1) [10-16]. These include two general guidelines for quality issues [11] and non-clinical and clinical issues [12] and four class-specific annexes for specific data requirements for Granulocyte-Colony Stimulating factor (G-CSF) [13], Insulin [14], Growth hormone [15] and Erythropoietin [16]. In addition, a concept paper on interferon alpha [17] is also available. So far, there are eleven biosimilar products which received market authorization in the EU and they are biosimilar versions of human growth hormone, Epoetin and filgrastim. It is estimated six to eight years on average for a biosimilar to be developed [18].

The EMA treats a biosimilar medicine as a medicine which is similar to a biological medicine that has already been authorized (the “biological reference medicine”) in the EU. The active substance of a biosimilar medicine is similar to the one of the biological reference medicine. A biosimilar and the biological reference medicine are used in general at the same dose to treat the same disease. A biosimilar and the biological reference medicine are not automatically interchangeable because biosimilar

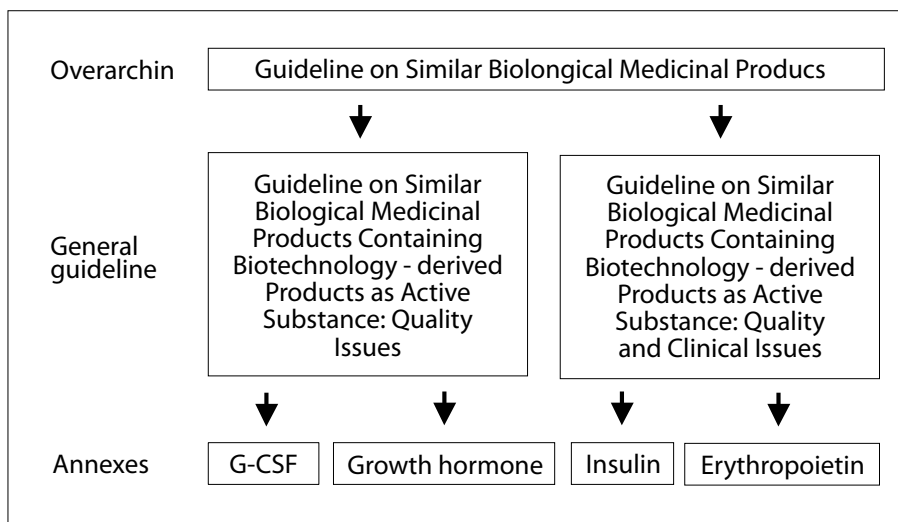


Figure 1: Overview of EMA guidelines for biosimilar

and biological reference medicine are only similar but not identical. A physician or a qualified healthcare professional should make the decision to treat a patient with a reference or a biosimilar medicine. Since the biosimilar may contain different inactive ingredients, the name, appearance and packaging of a biosimilar medicine differ to those of the biological reference medicine. In addition, a pharmacovigilance plan must be in place for post-marketing safety monitoring.

Japan's Ministry of Health, Labor and Welfare (MHLW) issued guidelines for follow-on proteins or biosimilars approval in March 2009. The first biosimilar, Sandoz' growth hormone Somatropin, was approved in June 2009. The MHLW's guidelines consider biosimilars drugs which are equivalent and homogeneous to the original biopharmaceuticals in terms of quality, efficacy and safety. Biosimilars are also requested to be developed with updated technologies and knowledge. Biosimilars need to demonstrate enough similarity to guarantee the safety and efficacy instead of absolute identity to the original biologics. Biosimilars' regulatory approval applications will be categorized separately from conventional generic drugs. In general, the applications should be submitted, as the new drug applications, with data from clinical trials, manufacturing methods, long-term stability and information on overseas use. The MHLW will assess the data on absorption, distribution, metabolism and excretion (ADME) on a case-by-case basis. The applications do not need to provide data on accessory pharmacology, safety pharmacology and genotoxicity.

Biosimilars are already thriving in Eastern Europe and Asia, where regulatory and intellectual property (IP) standards for biosimilars are more liberal. Biosimilars developed in these regions are primarily sold domestically. These markets are considered less controlled. The quality of the biosimilars may not be in full compliance with ICH guidelines although they are often developed through comparative quality testing and clinical trials against the biologics which are already approved in Western countries.

CMC Development

The CMC requirements for biosimilars in the EU are those described in the ICH Common Technical Document (CTD) Quality Module 3 with supplemental information demonstrating comparability or similarity on quality attributes to the reference medicine product. Since the US is a member of ICH and encourages submission using CTD format, once the legal framework for approving biosimilars is established in the US, the

CMC development will be similar to those in the EU.

Biosimilar manufacturers will have no access to the manufacturing process and product specifications of the innovator's products because these are proprietary knowledges. To develop a biosimilar, a biosimilar manufacturer will need to first identify a marketed biologic product to serve as the reference biologic product. Then a detailed characterization of the reference biologic product will be performed. The information obtained from the characterization of the reference biologic product will be utilized to direct the process development of the biosimilar product and comparative testing to demonstrate bioequivalence between the biosimilar product and the reference biologic product. A biosimilar will be manufactured from a completely new process, which may be based on different host/vector system with different process steps, facilities and equipment.

A flow chart for a typical work flow from production to drug use is shown in Figure 2. The CMC development starts with establishment of the expression system. A cell-line will be selected among bacterial, yeast and mammalian host strains and then the correct DNA sequence will be inserted. Elaborate cell-screening and selection methods are then used to establish a master cell bank. Extensive characterization on the master cell bank needs to be carried out to provide microbiological purity or sterility and identity [19].

Bulk protein production involves developing robust and scalable fermentation and purification processes. The goals for fermentation are to increase the expression level and efficiency without compromising the correct amino acid sequence and post translational modification. Achieving high expression requires optimizing culture medium and growth conditions, and efficient extraction and recovery procedures. Correct amino acid sequence and post translational modification

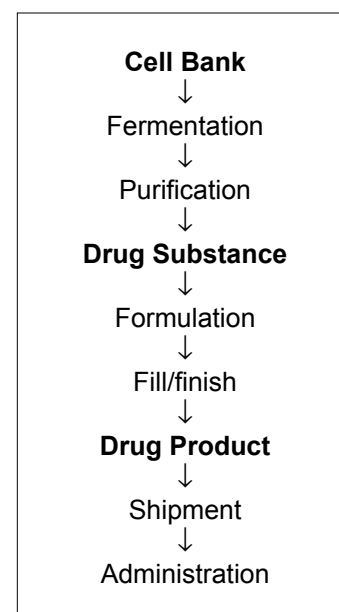


Figure 2: Typical flow chart for a biologics from production to drug use

will need to be verified. Solubilization and refolding of insoluble proteins are sometimes necessary for proteins which have tendency to aggregate under the processing condition. Differences in the cell bank and production processes may create impurities that are different from the innovator's product. The purification process needs to remove impurities such as host-cell proteins, DNA, medium constituents, viruses and metabolic by-products as much as possible. It is important for biosimilar manufacturers to accept appropriate yield losses to achieve high purity, because any increase in yield at the expense of purity is unacceptable and can have clinical consequences.

The final product is produced by going through formulation, sterile filtration and fill/finish into the final containers. Selection of formulation components starts from basic buffer species for proper pH control and salt for isotonicity adjustment. Surfactants may be needed to prevent proteins from being absorbed onto container surface or water-air interface or other hydrophobic surfaces. Stabilizers are required to inhibit aggregation, oxidation, deamidation and other degradations. The container and closure system can be glass vials, rubber stoppers and aluminum seals or pre-filled syringes or IV bags. The container and closure integrity needs to be verified by sterility or dye-leak test.

Biologics are not pure substances. They are heterogeneous mixtures. Each batch of a biologic product for clinical or commercial use needs to be produced in compliance with current Good Manufacturing Practice (cGMP) and is typically tested by a panel of assays (Table 4) to ensure the product meets pre-defined specifications for quality, purity, potency, strength, identity and safety. The product purity is often measured by multiple assays, which measure different product related variants (biologically active) or product related impurities (biologically inactive). Biologics are parenteral drugs and filled into the final containers through the aseptic process so that microbiological control is critical. It is advisable to set up product specifications for a biosimilar within the variation of the reference biologic product.

Product characterization can be performed on selected

batches for primary sequence, high order structures, isoform profiles, heterogeneity, product variants and impurities and process impurity profiles. Physicochemical characterization tests include IEF, CE, HIC, LC-MS, carbohydrate analysis, N & C terminal sequencing, amino acid analysis, analytical ultracentrifugation, CD and DSC [20,21].

Table 4: Product release assays for biologics

Type	Assays
Quality	Appearance, particulates, pH, osmolality
Purity	SDS-PAGE, SEC-HPLC, IEX-HPLC, RP-HPLC
Potency	In vitro or in vivo bioactivity assays
Strength	Protein concentration by A280
Identity	Western blot, peptide mapping, isoelectric focusing
Safety	Endotoxin, sterility, residual DNA, host cell proteins

Biologics are highly sensitive to environmental influences during storage, shipment and handling. Temperature excursion, movement, and exposure to UV light can lead to protein degradation. Product expiry needs to be based on the real time stability data. Stability program should also include accelerated or stress studies to gain insight of the degradation profiles. In-use stability studies are carried out to verify shipping conditions or handling procedures cause no detrimental effect to the drug product.

Comparability Demonstration

A comparability exercise based on the ICH guideline [22] needs to be performed to demonstrate that the biosimilar product and the reference biologic product have similar profiles with respect to product quality, safety, and efficacy. This is accomplished by comparative testing of the biosimilar product and the reference biologic product to demonstrate they have comparable molecular structure, in vitro and in vivo biological activities, pre-clinical safety and pharmacokinetics, and safety and efficacy in human patients.

Comparison of quality attributes between the biosimilar and the reference biologic product employs physicochemical and biological characterization. Comparability

on physical properties, amino acid sequence, high order structures, post-translationally modified forms are evaluated by physicochemical tests. In vitro receptor-binding or cell-based (binding) assays or even the in vivo potency studies in animals need to be performed to demonstrate comparable activity despite they are often imprecise. Levels of product related impurities (aggregates, oxidized forms, deamidated forms) and process related impurities and contaminants (host cell proteins, residual genomic DNA, reagents, downstream impurities) need to be assessed and quantified.

Stability profiles of the biosimilar product and the reference biologic product also need to be studied by placing the products under stressed conditions. The rate of degradation and degradation profiles (oxidation, deamidation, aggregation and other degradation reactions) will be compared. If unknown degradation species are detected, they need to be studied to determine if they affect safety and efficacy.

If differences on product purities and stability profiles are present between the biosimilar product and the reference biologic product, these differences need to be justified using scientific knowledge or preclinical or clinical studies. Changes in the impurity profile should be justified as well.

The demonstration of comparability in quality attributes does not necessarily mean that the biosimilars and the reference biologics are identical, but that they are highly similar. In many cases, the relationship between specific quality attributes and safety and efficacy has not been fully established. For example, physicochemical characterization cannot easily predict immunogenicity and slight changes in manufacturing processes or product composition can give rise to unpredicted changes in safety and efficacy. Changes in bioavailability, pharmacokinetics, bioac-

tivity, and immunogenicity are the main risks associated with the manufacturing of biosimilars. In vivo studies should be designed to measure the pharmacokinetics and pharmacodynamics relevant to clinical studies. Such in vivo studies should be designed to detect response differences between the biosimilar and the reference biologic not just responses per se. In vivo studies of the biosimilar's safety in animals may be used to research any concerns into the safety of the biosimilar in human patients.

Although extensive clinical testing is not necessary for biosimilars, some degree of clinical testing is needed to establish therapeutic comparability on efficacy and safety between the biosimilar and the reference biologic product [23,24]. This includes using surrogate markers of specific biologic activity as endpoints for demonstrating efficacy, and showing that patients didn't develop immunogenic responses to the product. In general, the approval of biosimilars will be based on the demonstration of comparable efficacy and safety to an innovator reference product in a relevant patient population. Clinical data requirement for each individual product will be different and will be determined on a case-by-case basis.

Conclusion

Biosimilars are defined as biological products similar, but not identical, to the reference biological products that are submitted for separate marketing approval

Table 5: *Small-molecule generics versus biosimilars*

Small-molecule Generics	Biosimilars
Approval based on "sameness"	Approval based on "high similarity"
Replicate the innovator's process and product and perform a bioavailability study demonstrating similar pharmacokinetic properties	Full CMC development with comparative testing, conduct substantial clinical trials for efficacy and safety including immunogenicity
Abbreviated registration procedures in Europe and US	Regulatory pathway is defined in EU on "Comparability" status, no pathway yet in US under BLA
Therapeutically equivalent, thus interchangeable	Lack of automatic substitutability
\$1 to \$5 million to develop	\$100-\$200 million to develop
Brand-to-generic competition	Brand-to-Brand competition

following patent expiration of the reference biological products. As one of the ICH members, the US needs to catch up with the EU and Japan as those two countries have already issued regulatory guidelines for biosimilars. Once Congress establishes a legal framework, FDA is expected to set up a biosimilar approval pathway which will be similar to those in the EU and Japan and harmonized under ICH. The biosimilar will need a full CMC development package plus demonstration of comparable quality attributes and comparable efficacy and safety to the innovator's product. Table 5 provides a comparison summary between small-molecule generics and biosimilars. It will take a much bigger effort to develop a biosimilar than a generic drug. Automatic substitution between the innovator product and a biosimilar is not appropriate as a biosimilar is not a generic version of the innovator product and is approved based on comparability to the innovator product.

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