# Guidance for Industry

# Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

## *DRAFT GUIDANCE*

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**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Drug Evaluation and Research (CDER)**
**Center for Biologics Evaluation and Research (CBER)**

**February 2012**
**Biosimilarity**

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**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Drug Evaluation and Research (CDER)**
**Center for Biologics Evaluation and Research (CBER)**

**February 2012**
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# Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

## I. INTRODUCTION

This guidance describes the Agency's current thinking on factors to consider when demonstrating that a proposed protein product is highly similar to a reference product licensed under section 351(a) of the Public Health Service Act (PHS Act) for purposes of submitting a marketing application under section 351(k) of the PHS Act. Specifically, the guidance is intended to provide recommendations to applicants on the scientific and technical information of the chemistry, manufacturing, and controls (CMC) section of a marketing application for a proposed biosimilar product submitted under section 351(k) of the PHS Act.

The Biologics Price Competition and Innovation Act of 2009 (BPCI Act) amends the PHS Act and other statutes to create an abbreviated licensure pathway in section 351(k) of the PHS Act for biological products shown to be biosimilar to, or interchangeable with, an FDA-licensed biological reference product (see sections 7001 through 7003 of the Patient Protection and Affordable Care Act (Pub. L. 111-148) (Affordable Care Act)). The BPCI Act also amended the definition of biological products to include "protein (except any chemically synthesized polypeptide)" (see section 351(i)(1) of the PHS Act). A 351(k) application for a proposed biosimilar product must include information demonstrating biosimilarity, based on data derived from, among other things, "analytical studies that demonstrate that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components."[2]

Although the 351(k) pathway applies generally to biological products, this guidance focuses on therapeutic protein products and provides an overview of analytical factors to consider in demonstrating biosimilarity between a proposed protein product and the reference product.

[1] This guidance has been prepared by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration (FDA).

[2] See section 351(k)(2)(A)(i)(I)(aa) of the PHS Act.

This guidance is one in a series of guidances that FDA is developing to implement the BPCI Act. The guidances will address a broad range of issues, including:[3]

- Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product
- Scientific Considerations in Demonstrating Biosimilarity to a Reference Product
- Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009

When applicable, references to information in these guidances are included in this guidance.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

## II. BACKGROUND

In the 1980s, FDA began to receive marketing applications for biotechnology-derived protein products, mostly for recombinant DNA-derived versions of a naturally sourced product. In light of these applications, FDA established a regulatory approach for the approval of recombinant DNA-derived protein products, which it announced in a policy document published on June 26, 1986 (51 FR 23309), in conjunction with a 1985 document titled *Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology*. The policy requires the submission of an investigational new drug application (IND) to FDA for evaluation before initiation of clinical investigations in human subjects and submission and approval of a new drug application (NDA) or biologics license application (BLA) "before marketing products made with recombinant DNA technology, even if the active ingredient in the product is thought to be identical to a naturally occurring substance or a previously approved product" (51 FR 23309). The policy set forth in those documents was developed in part because of the challenges in evaluating protein products solely by physicochemical and functional testing and because the biological system in which a protein product is produced can have a significant effect on the structure and function of the product itself. Due to the complexities of protein products, FDA has, as a matter of policy, generally required submission of an NDA in accordance with section 505(b)(1) of the FD&C Act or a BLA in accordance with section 351(a) of the PHS Act containing product-specific full safety and efficacy data for recombinant DNA-derived protein drugs. FDA has recognized, however, that "[i]n some instances complete new applications may not be required." (51 FR 23309).

Improvements in manufacturing processes, process controls, materials and product testing, as well as characterization tests and studies, have led to a gradual evolution in the regulation of

[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

protein products. For example, in 1996, FDA provided recommendations in its *FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology Products*, which explains how an applicant may demonstrate, through a combination of analytical testing, functional assays (in vitro and/or in vivo), assessment of pharmacokinetics (PK) and/or pharmacodynamics (PD) and toxicity in animals, and clinical testing (clinical pharmacology, safety, and/or efficacy) that a manufacturing change does not adversely affect identity, purity, or potency of its FDA-approved product.

Since 1996, FDA has approved many manufacturing process changes for licensed biological products, based on a demonstration of product comparability before and after the process change, as supported by quality criteria and analytical testing and without the need for additional nonclinical data and clinical safety and/or efficacy studies. In some cases, uncertainty about the effect of the change and/or the results of the biochemical/functional comparability studies has necessitated assessment of additional data, including nonclinical and/or clinical testing, to demonstrate product comparability.

These concepts were further developed in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and resulted in the Q5E guidance on *Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process*. Although the scope of ICH Q5E is limited to an assessment of the comparability of a biological product before and after a manufacturing process change made by the same manufacturer, certain general scientific principles described in ICH Q5E are applicable to an assessment of biosimilarity between a proposed biosimilar protein product and its reference product. However, demonstrating that a proposed protein product is biosimilar to an FDA-licensed reference product manufactured by a different manufacturer may require more extensive and comprehensive data than assessing the comparability of a product before and after a manufacturing process change made by the product's sponsor. Unlike a manufacturer who modifies its own manufacturing process with extensive knowledge and information about the product and the existing process, including established controls and acceptance parameters, the manufacturer of a proposed biosimilar product will likely have a different manufacturing process (e.g., different cell line, raw materials, equipment, processes, process controls, acceptance criteria) from that of the reference product and no direct knowledge of the manufacturing process for the reference product.

In October 1999, FDA issued a draft guidance for industry on *Applications Covered by Section 505(b)(2)*, which, among other things, stated that FDA may accept an application submitted through the approval pathway described by section 505(b)(2) of the FD&C Act for a drug product containing an active ingredient(s) derived from natural sources or recombinant DNA technology. For example, FDA approved a 505(b)(2) application for a follow-on recombinant DNA-derived human growth hormone product in May 2006. Greater knowledge due to advances in science and technology, and improvements in manufacturing processes, process controls, materials and product testing, as well as characterization tests and studies, facilitate the use of an abbreviated pathway for the approval of a protein product.

The BPCI Act was enacted as part of the Affordable Care Act on March 23, 2010.[4] The BPCI Act creates an abbreviated licensure pathway for biological products demonstrated to be biosimilar to, or interchangeable with, a reference product. Section 351(k) of the PHS Act (42 U.S.C. 262(k)), added by the BPCI Act, sets forth the requirements for an application for a proposed biosimilar product and an application or a supplement for a proposed interchangeable product.

Section 351(i) of the PHS Act defines biosimilarity to mean that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components and that there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product (see section 351(i)(2) of the PHS Act).

To meet the higher standard of "interchangeability," an applicant must provide sufficient information to demonstrate biosimilarity, and also to demonstrate that the biological product can be expected to produce the same clinical result as the reference product in any given patient and, if the biological product is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between the use of the biological product and the reference product is not greater than the risk of using the reference product without such alternation or switch (see section 351(k)(4) of the PHS Act).

Analytical studies provide the foundation for an assessment of the proposed protein product intended for submission in a 351(k) application under the PHS Act and whether it is highly similar to the reference product.

## III. SCOPE

This document provides guidance on analytical studies that may be relevant to assessing whether the proposed biosimilar protein product and a reference product are highly similar, which is part of the biosimilarity assessment. This document is not intended to provide an overview of FDA's approach to determining interchangeability because FDA is continuing to consider the type of information sufficient to enable FDA to determine that a biological product is interchangeable with the reference product. Although this guidance applies specifically to therapeutic protein products, the general scientific principles may be informative for the development of other proteins, such as in vivo protein diagnostic products. If the reference product and the proposed protein product cannot be adequately characterized with state of the art technology as recommended by this guidance, FDA recommends that the sponsor consult FDA for guidance on whether an application for the proposed protein product is appropriate for submission under section 351(k) of the PHS Act.

All product applications should contain a complete and thorough chemistry, manufacturing and controls (CMC) section that provides the necessary and appropriate information (*e.g.*, characterization, adventitious agent safety, process controls, and specifications) for the product

[4] The BPCI Act appears in title VII, subtitle A of the Affordable Care Act.

to be adequately reviewed. This guidance describes considerations for additional CMC information that may be relevant to the assessment of biosimilarity between two protein products. This guidance should be used as a companion to other guidances available from FDA that describe the CMC information appropriate for evaluation of protein products.[5] We encourage early interaction with FDA to discuss specific CMC issues that may arise for an applicant's proposed biosimilar product.

In addition to comparative analytical studies, an assessment of whether a proposed product is biosimilar to a reference product generally will include animal studies (including the assessment of toxicity) and a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics and/or pharmacodynamics).[6]

This guidance applies to applications submitted under section 351(k) of the PHS Act. However, some scientific principles described in this guidance may be informative for the development of certain biological products under section 505(b)(2) of the FD&C Act.[7] Section 505(b)(2) of the FD&C Act and section 351(k) of the PHS Act are two separate statutory schemes. This guidance is not intended to describe any relationship between the standards for approval under these schemes.

## IV. DEFINITIONS

For the purpose of this document, the following definitions are applicable:

*Protein* means any alpha amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size.

*Chemically synthesized polypeptide* means any alpha amino acid polymer that is (a) made entirely by chemical synthesis, and (b) is less than 100 amino acids in size.

[5] For CMC requirements for submission of a marketing application, applicants should consult current regulations, the *Guidance for Industry for the Submission on Chemistry, Manufacturing, and Controls Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal Antibody Product for In-vivo Use* (issued jointly by CBER and CDER, August 1996) and other applicable FDA guidance documents.

[6] For a discussion of the Agency's current thinking on animal and clinical studies relevant to demonstrating biosimilarity, see Draft Guidance for Industry on Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (issued jointly by CDER and CBER, February 2012).

[7] A 505(b)(2) application is an NDA that contains full reports of investigations of safety and effectiveness, where at least some of the information required for approval comes from studies not conducted by or for the applicant and for which the applicant has not obtained a right of reference or use (e.g., the Agency's finding of safety and/or effectiveness for a listed drug or published literature). A 505(b)(2) application that seeks to rely on a listed drug (i.e., the reference product) must contain adequate data and information to demonstrate that the proposed product is sufficiently similar to the listed drug to justify reliance, in part, on FDA's finding of safety and/or effectiveness for the listed drug. Any aspects of the proposed product that differ from the listed drug must be supported by adequate data and information to show that the differences do not affect the safety and effectiveness of the proposed product.

*Biosimilar or biosimilarity* means that "the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components," and "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product."[8]

*Product*, when used without modifiers, is intended to refer to intermediates, drug substance, and/or drug product, as appropriate. The use of the term "product" is consistent with the use of the term in ICH Q5E.

*Reference product* means the single biological product licensed under section 351(a) of the PHS Act against which a biological product is evaluated in a 351(k) application.

## V. GENERAL PRINCIPLES

Advances in analytical sciences (both physicochemical and biological) enable some protein products to be characterized extensively in terms of their physicochemical and biological properties. These analytical procedures have improved the ability to identify and characterize not only the desired product but also product-related substances and product- and process-related impurities.[9] Advances in manufacturing science and production methods may enhance the likelihood that a product will be highly similar to another product by better targeting the original product's physiochemical and functional properties.

In addition to a complete CMC data submission as required under section 351(a) of the PHS Act, the applicant should assess the analytical similarity to the reference product. The rationale for the analytical similarity assessment should be clearly described with consideration for the known quality attributes and performance characteristics of the specific reference product. Extensive, robust comparative physicochemical and functional studies (these may include bioassays, biological assays, binding assays, and enzyme kinetics) should be performed to evaluate whether the proposed biosimilar product and the reference product are highly similar. A meaningful assessment as to whether the proposed biosimilar product is highly similar to the reference product depends on, among other things, the capabilities of available state-of-the-art analytical assays to assess, for example, the molecular weight of the protein, complexity of the protein (higher order structure and post-translational modifications), degree of heterogeneity, functional properties, impurity profiles, and degradation profiles denoting stability. The capability of the methods used in the analytical assessment, as well as their limitations should be described by the applicant. Physicochemical and functional characterization studies should be sufficient to establish relevant quality attributes including those that define a product's identity, quantity, purity, potency, and consistency. The product-related impurities, product-related substances, and process-related impurities should be identified, characterized as appropriate, quantified, and compared to those of the reference product to the extent feasible and relevant, as part of an assessment of the potential impact on the safety, purity, and potency of the product.

[8] Section 7002(b)(3) of the Affordable Care Act, adding section 351(i)(2) of the PHS Act.

[9] The use of the terms "product-related substances" and "product- and process-related impurities" is consistent with their use and meaning in ICH Q6B.

Primary structure of some protein products can be highly heterogeneous and could affect the expected clinical performance of a protein product. In addition to the typically low level of replication errors in the DNA encoding the protein sequence and amino acid misincorporation that occurs during translation, most protein products undergo some post-translational modification that can alter the functions of the protein: by attaching it to other biochemical groups such as a phosphate, various lipids and carbohydrates; by proteolytic cleavage following translation; by changing the chemical nature of an amino acid (e.g., formylation); or by many other mechanisms. Such modifications can result from intracellular activities during cell culture or by deliberate modification of the protein, for example, PEGylation. Other post-translational modifications can be a consequence of manufacturing process operations — for example, glycation may occur with exposure of the product to reducing sugars. In other cases, storage conditions may be permissive for certain degradation pathways such as oxidation, deamidation, or aggregation. As all of these product-related variants may alter the biological properties of the expressed recombinant protein, identification and determination of the relative levels of these protein variants should be included in the comparative analytical characterization studies.

The three dimensional conformation of a protein is an important factor in its biological function. Proteins generally exhibit complex three-dimensional conformations (tertiary structure and, in some cases, quaternary structure) due to their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be absolutely required for functional activity. These rotations are often dependent on low-energy interactions, such as hydrogen bonds and van der Waals forces, which may be very sensitive to environmental conditions. Current analytical technology is capable of evaluating the three-dimensional structure of many proteins. Methods such as X-ray crystallography and multi-dimensional nuclear magnetic resonance (NMR) spectroscopy can help define tertiary protein structure and, to varying extents, quaternary structure, and can add to the body of information supporting biosimilarity. At the same time, a protein's three-dimensional conformation can often be difficult to define precisely using current physicochemical analytical technology. Any differences in higher order structure between a proposed biosimilar and a reference product should be evaluated in terms of a potential effect on protein function. Thus, functional assays are also critical tools for evaluating the integrity of the higher order structures.

A scientifically sound characterization that provides a comprehensive understanding of the chemical, physical, and biological characteristics of the proposed biosimilar product is essential to the design of the manufacturing process and to the conduct of development studies. The body of knowledge that emerges will serve to support product quality during development, at approval, and over the postapproval life of the product. Manufacturers should perform in-depth chemical, physical, and bioactivity comparisons with side-by-side analyses of an appropriate number of lots of the proposed biosimilar product and the reference product and, where available and appropriate, a comparison with the reference standard for specific suitable attributes (e.g., potency). For a discussion of reference standards, see section VI.G of this guidance. The evaluation of multiple lots of reference product and biosimilar product enables determination of product variability across lots and/or range of heterogeneity within a lot of drug product. Identification of the specific lots of the reference product used in the biosimilar studies together

with expiration dates and timeframes of actual use would also be of value. This information will be useful in justifying acceptance criteria used for specifications to ensure product consistency, in addition to assessing similarity. However, acceptance criteria should be based on the totality of the analytical data and not simply the observed range of product attributes of the reference product. For example, some product attributes act in combination to define a product's safety, purity, and potency profile and therefore their potential interaction should be considered when evaluating similarity and setting specifications. Thus, for some glycoproteins, the content and distribution of tetraantennary and N-acetyl lactosamine repeats can affect in vivo potency and should not be evaluated totally independently of each other. Additionally, data obtained for lots used in nonclinical and clinical studies and relevant information on the relationship between an attribute and the performance of the drug product (see ICH Q8) can also be used to help establish acceptance criteria.

An extensive analytical characterization may also reveal differences between the reference product and the proposed biosimilar product, especially when using analytical techniques capable of discriminating qualitative or quantitative differences in product attributes. Emphasis should be placed on developing orthogonal, quantitative methods to more definitively distinguish any differences in product attributes. If the results show highly similar functional and physicochemical characteristics, including, for example, higher order structure, post-translational modifications, and impurity and degradation profiles, the sponsor may have an appropriate scientific basis for a selective and targeted approach to subsequent animal and/or clinical studies to support a demonstration of biosimilarity. It may be useful to compare differences in the quality attributes of the proposed protein product with those of the reference product using a meaningful fingerprint-like analysis algorithm that covers a large number of additional product attributes and their combinations with high sensitivity using orthogonal methods. Advances in manufacturing science and Quality-by-Design approaches may facilitate production processes that can better match a reference product's fingerprint.[10] Such a strategy could further quantify the overall similarity between two molecules and may lead to additional bases for a more selective and targeted approach to subsequent animal and/or clinical studies.

The type, nature, and extent of any differences between the proposed biosimilar product and the reference product, introduced by design or observed from comprehensive analytical characterization of multiple manufacturing lots, should be clearly described and discussed. The discussion should include identification and comparison of relevant quality attributes from product characterization, as this is an important factor in assessing whether the proposed biosimilar product is highly similar to the reference product. The potential effect of the differences on safety, purity, and potency should be addressed and supported by appropriate data.

The type and extent of nonclinical or clinical studies that are needed to demonstrate biosimilarity of the proposed biosimilar product can be influenced by several factors, especially the ability to discern differences and their potential effect on safety, purity, and potency. For example, factors such as the ability to robustly characterize the proposed biosimilar product or the reference product (*e.g.*, lack of suitable or sufficiently discriminative analytical techniques) or availability of a relevant drug substance derived from the reference product could impact the nature of the subsequent nonclinical or clinical studies. In addition, if the proposed biosimilar product or

[10] See ICH Q8(R2) for guidance.

reference product cannot be adequately characterized, the sponsor should consult FDA for guidance on whether an application for such a protein product is appropriate for submission under section 351(k) of the PHS Act.

In general, a sponsor needs to provide information to demonstrate biosimilarity based on data directly comparing the proposed protein product with the reference product. Analytical studies intended to support a demonstration of biosimilarity for purposes of section 351(k) of the PHS Act must as a scientific matter include an adequate comparison to the reference product licensed under section 351(a). However, under certain circumstances, a sponsor may seek to use data derived from animal or clinical studies comparing a proposed protein product with a non-U.S.-licensed product to address, in part, the requirements under section 351(k)(2)(A) of the PHS Act. In such a case, the sponsor should provide adequate data or information to scientifically justify the relevance of this comparative data to an assessment of biosimilarity and to establish an acceptable bridge to the U.S.-licensed reference product.[11] The scientific bridge between the non-U.S.-licensed product and the U.S.-licensed reference product is likely to include comparative physico-chemical characterization, bioassays/functional assays, and comparative clinical and/or nonclinical PK and/or PD data, as appropriate, and data to address any differences in formulation or primary packaging. Sponsors are encouraged to discuss with FDA during the development program the adequacy of the scientific justification and bridge to the U.S.-licensed reference product; a final determination of the adequacy of the information will be made by FDA during review of the 351(k) application.

## VI. FACTORS FOR CONSIDERATION IN ASSESSING WHETHER PRODUCTS ARE HIGHLY SIMILAR

When assessing whether products are highly similar, manufacturers should consider a number of factors, including the following.

### A. Expression System

Therapeutic protein products can be produced by microbial cells (prokaryotic, eukaryotic), cell lines of human or animal origin (e.g., mammalian, avian, insect), or tissues derived from animals or plants. It is expected that the expression construct for a proposed biosimilar product will encode the same primary amino acid sequence as its reference product. However, minor modifications, such as N or C terminal truncations that will not have an effect on safety, purity, or potency, may be justified by the applicant. Differences between the chosen expression system of the proposed biosimilar product and that of the reference product should be carefully considered because the type of expression system and host cell will significantly affect the types of process- and product-related substances and impurities (including potential adventitious agents) that may be present in the protein product. For example, the expression system can have a significant effect on the types and extent of translational and post-translational modifications

[11] Please refer to the Draft Guidance for Industry on Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (issued jointly by CDER and CBER, February 2012).

that are imparted to the proposed protein product, something that may complicate an effort to demonstrate that the proposed biosimilar product is highly similar to the reference product (and thus, for example, affecting the type and extent of nonclinical and clinical data that are needed for demonstrating biosimilarity). Minimizing differences between the proposed and reference expression systems to the extent possible can enhance the likelihood of producing a highly similar protein product. The characterization of the expression construct, including its genetic stability, should be demonstrated in accordance with principles recommended in ICH Q5B.

### B. Manufacturing Process

A comprehensive understanding of all steps in the manufacturing process for the proposed biosimilar product should be established during product development. Characterization tests, process controls, and specifications that will emerge from information gained during process development must be specific for the proposed biosimilar product and manufacturing process. The use of Quality-by-Design approaches to pharmaceutical development, along with quality risk management and effective quality systems, will facilitate the consistent manufacturing of a high-quality product. A type II Drug Master File (DMF) would not be acceptable for a 351(k) application because, as with 351(a) BLAs, the license holder needs to have knowledge of and control over the manufacturing process for the biological product.[12] Other types of contract manufacturing arrangements can be considered if the applicant does not intend to manufacture the product for licensure.[13]

### C. Assessment of Physicochemical Properties

Physicochemical assessment of the proposed biosimilar product and the reference product should consider all relevant characteristics of the protein product (*e.g.*, the primary, secondary, tertiary, and quaternary structure, post-translational modifications, and functional activity(ies)). The objective of this assessment is to maximize the potential for detecting differences in quality attributes between the proposed biosimilar product and the reference product.

The applicant should address the concept of the desired product (and its variants) as defined in ICH Q6B when designing and conducting the characterization studies. Thus, it will be important to understand the heterogeneity of the proposed biosimilar product and the reference product (*e.g.*, the nature, location, and levels of glycosylation) and the ranges of variability of different isoforms, including those that result from post-translational modifications.

[12] A type II DMF may, however, be used to support an Investigational New Drug Application (IND) for a biosimilar product. Assurance of product quality should be provided on each lot of material produced by the DMF holder. Procedures should also be in place to ensure that the IND sponsor is notified by the DMF holder of significant changes to the DMF potentially affecting product quality. The sponsor is expected to provide notification to the Agency of any relevant change in the IND in order to initiate a reevaluation of the DMF.

[13] See FDA's guidance on Cooperative Manufacturing Arrangements for Licensed Biologics (2008).

Particular analytical methodologies can be used to assess specific physicochemical characteristics of proteins.[14] These methodologies are described in published documents, including scientific literature, regulatory guidelines, and pharmacopeial compendia. Some techniques provide information on multiple characteristics. It is expected that appropriate analytical test methods will be selected based on the nature of the protein being characterized and knowledge regarding the structure and heterogeneity of the reference and the proposed biosimilar product, as well as those characteristics that are critical to product performance. To address the full range of physicochemical properties or biological activities adequately, it is often necessary to apply more than one analytical procedure to evaluate the same quality attribute. Methods that use different physicochemical or biological principles to assess the same attribute are especially valuable because they provide independent data to support the quality of that attribute (*e.g.*, Size Exclusion Chromatography and Analytical Ultracentrifugation or Field Flow Fractionation for the determination of aggregates). In addition, the use of complementary analytical techniques in series, such as peptide mapping or capillary electrophoresis combined with mass spectrometry of the separated molecules, should provide a meaningful and sensitive method for comparing products.

Tests used to characterize the product do not necessarily need to be validated for routine quality control purposes, but should be scientifically sound, fit for their intended use, and provide results that are reproducible and reliable. In selecting these tests, it is important to consider the characteristics of the protein product, including known and potential impurities. Information regarding the ability of a method to discern relevant differences between a proposed biosimilar product and a reference product should be submitted as part of the comparison.

Tests chosen to detect and characterize these post-translational protein modifications should be demonstrated to be of appropriate sensitivity and specificity to provide meaningful information as to whether the proposed biosimilar product and the reference product are highly similar.

### D. Functional Activities

Functional assays serve multiple purposes in the characterization of protein products. These tests act to complement physicochemical analyses and are a quality measure of the function of the protein product.

Depending on the structural complexity of the protein and available analytical technology, the physicochemical analysis may be unable to confirm the integrity of the higher order structures. Instead, the integrity of such structures can be inferred from the product's biological activity. If the clinically relevant mechanism(s) of action are known for the reference product or can reasonably be determined, one or more of the functional assays should reflect these mechanisms of action to the extent possible. The assessment of functional activity is also useful in providing an estimate of the specific activity of a product, as an indicator of manufacturing process consistency, as well as product purity and stability.

[14] In some cases, in vivo immunogenicity studies may be able to detect subtle differences in structure or impurities not detected by other methods.

If a reference product exhibits multiple functional activities, manufacturers should perform a set of relevant assays designed to evaluate the range of activities. For example, with proteins that possess multiple functional domains that express enzymatic and receptor-mediated activities, manufacturers should evaluate both activities. For products where a single functional activity can be measured by more than one, but related, parameter (*e.g.*, enzyme kinetics or interactions with blood clotting factors), comparative characterization of each parameter between products should be used to provide additional valuable information.

The manufacturer should recognize the potential limitations of some types of functional assays, such as high variability, that might preclude detection of small but significant differences between the proposed biosimilar product and the reference product. As a highly variable assay may not provide a meaningful assessment as to whether the proposed biosimilar product is highly similar to the reference product, applicants are encouraged to develop assays that are sensitive to changes in the functional activities of the product. In addition, in vitro bioactivity assays may not fully reflect the clinical activity of the protein. For example, these assays generally do not predict the bioavailability (PK and biodistribution) of the product. These factors can impact PD and clinical performance. Also, bioavailability can be dramatically altered by subtle differences in glycoform distribution or other post-translation modifications. Thus, these limitations should be taken into account when assessing the robustness of the quality of data supporting biosimilarity and the need for additional information. Finally, functional assays are critical in assessing the occurrence of neutralizing antibodies in nonclinical and clinical studies.

### E. Receptor Binding and Immunochemical Properties

When binding or immunochemical properties are part of the activity attributed to the protein product, analytical tests should be performed to characterize the product in terms of these specific properties (*e.g.*, if binding to a receptor is inherent in protein function, this property should be measured and used in comparative studies, see ICH Q6B for additional details). Various methods such as surface plasmon resonance, microcalorimetry, or classical Scatchard analysis can provide information on the kinetics and thermodynamics of binding. Such information can be related to the functional activity and characterization of the proposed biosimilar product's higher order structure.

### F. Impurities

The applicant should characterize, identify, and quantify impurities (product- and process-related as defined in ICH Q6B) in the proposed biosimilar product and the reference product. If comparative physicochemical analysis reveals comparable product-related impurities at similar levels between the two products, pharmacological/toxicological studies to characterize potential biological effects of specific impurities may not be necessary. However, if the manufacturing process used to produce the proposed biosimilar product introduces different impurities or higher levels of impurities than those present in the reference product, additional pharmacological/toxicological or other studies may be necessary. As discussed in ICH S6, "[i]t

is preferable to rely on purification processes to remove impurities . . . rather than to establish a preclinical testing program for their qualification."[15]

Process-related impurities arising from cell substrates (*e.g.*, host cell DNA, host cell proteins), cell culture components (*e.g.*, antibiotics, media components), and downstream processing steps (*e.g.*, reagents, residual solvents, leachables, endotoxin, bioburden) should be evaluated. The potential impact of differences in the impurity profile upon safety should be addressed and supported by appropriate data. In all cases, the chosen analytical procedures should be adequate to detect, identify, and accurately quantify biologically significant levels of impurities (see ICH Q2B). In particular, the results of the immunological methods used to detect host cell proteins depend on the assay reagents and the cell substrate used. Such assays should be validated using the product cell substrate and orthogonal methodologies to ensure accuracy and sensitivity. This should be done across both products to the extent relevant and feasible.[16]

The safety of the proposed biosimilar product, as with any biological product, with regard to adventitious agents or endogenous viral contamination should be ensured by screening critical raw materials and confirmation of robust virus removal and inactivation achieved by the manufacturing process (see ICH Q5A for guidance).

### G. Reference Product and Reference Standards

A thorough physicochemical and biological assessment of the reference product should provide a base of information from which to develop the proposed biosimilar product and justify reliance on certain existing scientific knowledge about the reference product. Sufficient evidence that the proposed biosimilar product is highly similar to the reference product must be demonstrated in an appropriate time frame to support a selective and targeted approach in early product development (e.g., reduced nonclinical studies, and/or dose-finding clinical studies).[17] To justify a selective and targeted approach to a clinical program, a comprehensive physicochemical and functional comparison to the reference product should be performed during early product development and discussed with the appropriate FDA staff. An analytical similarity assessment should support the use of lots that demonstrate the biosimilarity of the proposed biosimilar product used in the principal clinical trial to the reference product and the proposed commercial product. The biosimilar application should include a thorough analytical comparison between the proposed biosimilar product and the reference product. In addition, even when multiple approved products are on the market, a sponsor must demonstrate that the proposed product is biosimilar to a single reference product that previously has been licensed by FDA.

If the drug substance has been extracted from the reference product in order to assess analytical similarity, the applicant should describe the extraction procedure and provide support that the procedure itself does not alter product quality. This undertaking would include consideration for

[15] See ICH S6, page 2.

[16] This may be limited by the availability of high levels of reference product host cell proteins or differences in product and reference substrate.

[17] See 21 CFR 312.23 for Investigational New Drug (IND) application content and format.

alteration or loss of the desired products and impurities and relevant product-related substances, and it should include appropriate controls that ensure the relevant product characteristics of the reference product are not significantly altered by the extraction procedure.

If there is a suitable, publicly available and well-established reference standard for the protein, then a physicochemical and/or functional comparison of the proposed biosimilar product with this standard should also be performed. For example, if an international standard for calibration of potency is available, a comparison of the relative potency of the proposed biosimilar product with this potency standard should be performed. As is recommended in ICH Q6B, an in-house reference standard(s) should always be qualified and used for control of the manufacturing process and product.

In summary, analytical studies carried out to support the approval of a proposed biosimilar product should not focus solely on the characterization of the proposed biosimilar product in isolation. Rather, these studies should be part of a broad comparison that includes, but is not limited to, the proposed biosimilar product, the reference product, applicable reference standards, and consideration of relevant publicly available information.

### H. Finished Drug Product

Product characterization studies should be performed on the most downstream intermediate best suited for the analytical procedures used. The attributes evaluated should be stable through any further processing steps. For these reasons, characterization studies are often performed on bulk drug substance.[18] However if bulk drug substance is reformulated and/or exposed to new materials in the finished dosage form, the impact of these changes should be considered.

If the finished drug product is best suited for a particular analysis, the characterization should compare the proposed finished biosimilar product and the finished reference product. If an analytical method more sensitively detects specific attributes in the drug substance, but the attributes it measures are critical and/or may change during manufacture of the finished drug product, comparative characterization may be called for on both the isolated drug substance and the finished drug product.

The acceptability of the type, nature, and extent of any differences between the proposed finished biosimilar product and the finished reference product should be evaluated and supported by appropriate data and rationale. Additionally, different excipients in the proposed product should be supported by existing toxicology data for the excipient or by additional toxicity studies with the formulation of the proposed biosimilar product. Excipient interactions as well as direct toxicities should be considered. Proteins are very sensitive to their environment. Therefore, differences in excipients or primary packaging may affect product degradation and/or clinical performance. Differences in formulation between the proposed biosimilar product and the reference product are among the factors that may affect whether subsequent clinical studies may take a selective and targeted approach.

[18] See 21 CFR 207.3.

### I. Stability

An appropriate physicochemical and functional comparison of the stability of the proposed biosimilar product with that of the reference product should be initiated. Accelerated and stress stability studies, or forced degradation studies, should be used to establish degradation profiles and provide direct comparison of the proposed biosimilar product with the reference product. These comparative studies should be conducted under multiple stress conditions (e.g., high temperature, freeze thaw, light exposure, and agitation) that can cause incremental product degradation over a defined time period. Results of these studies may reveal product differences that warrant additional evaluation and also identify conditions under which additional controls should be employed in manufacturing and storage (see ICH Q5C and Q1A(R) for guidance). Sufficient real time, real condition stability data should be provided to support the proposed dating period.

## VII. CONCLUSION

The foundation for an assessment of biosimilarity between a proposed biosimilar product and its reference product involves the robust characterization of the proposed biosimilar product, including comparative physicochemical and functional studies. The information gained from these studies is critical to the overall product assessment that as a scientific matter is necessary for the development of a proposed biosimilar product. In addition, a 351(k) application for a proposed biosimilar product must contain, among other things, information demonstrating biosimilarity based upon data derived from animal studies (including the assessment of toxicity) and a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics), unless the Agency determines that an element is unnecessary in a particular 351(k) application. The ability to discern relevant differences between the proposed product and its reference product will depend on the available analytical technology and complexity of the product. Any information regarding differences between the proposed product and the reference product should be considered to determine whether the statutory standard for biosimilarity can be met.

## VIII. RELEVANT GUIDANCES

The following guidance documents may be relevant to sponsors developing or considering development of a biosimilar product candidate. All Agency guidance documents are available on FDA's Web page (http://www.fda.gov/RegulatoryInformation/Guidances/default.htm).

1. *Draft Guidance for Industry on Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (issued jointly by CDER and CBER, February 2012)

2. *Draft Guidance for Industry, Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009* (issued jointly by CDER and CBER, February 2012)

3. *FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products* (issued jointly by CDER and CBER, April 1996)

4. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (issued by CBER, February 1997)

5. *Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal Antibody Product for In Vivo Use* (issued jointly by CDER and CBER, August 1996)

6. *FDA Guidance on* Cooperative Manufacturing Arrangements for Licensed Biologics (issued jointly by CDER and CBER, November 2008).

7. ICH M4Q *The Common Technical Document*

8. ICH Q2 *Text on Validation of Analytical Procedures*

9. ICH Q2B *Validation of Analytical Procedures: Methodology*

10. ICH Q3A *Impurities in New Drug Substances*

11. ICH Q5A *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origi*n

12. ICH Q5B *Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products*

13. ICH Q5C *Stability Testing of Biotechnological/Biological Products*

*14.* ICH Q5D *Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products*

*15.* ICH Q5E *Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process*

*16.* ICH Q6B *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*

*17.* ICH Q7 *Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients*

*18.* ICH Q8 *Pharmaceutical Development*

*19.* ICH Q9 *Quality Risk Management*

*20.* ICH Q10 *Pharmaceutical Quality System*

*21.* ICH S6 Preclinical *Safety Evaluation of Biotechnology-Derived Pharmaceuticals*