Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product

Guidance for Industry

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)

April 2015
Biosimilarity

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Contains Nonbinding Recommendations

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Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not create any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance describes the Agency's current thinking on factors to consider when demonstrating that a proposed therapeutic protein product (hereinafter *proposed product* or *proposed biosimilar product*) is highly similar to a reference product licensed under section 351(a) of the Public Health Service Act (PHS Act) for the purpose of submitting a marketing application under section 351(k) of the PHS Act. Specifically, this guidance is intended to provide recommendations to sponsors on the scientific and technical information for the chemistry, manufacturing, and controls (CMC) section of a marketing application for a proposed 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 (Affordable Care Act) (Public Law 111-148). The BPCI Act also amended the definition of biological products to include "protein (except any chemically synthesized polypeptide)."[2] 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."[3]

[1] This guidance has been prepared by the Office of Medical Policy in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration.

[2] See section 351(i)(1) of the PHS Act.

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

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 product and the reference product.

This guidance is one in a series of guidances that FDA is developing to implement the BPCI Act.[4] These guidances address a broad range of issues, including:

- Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference 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
- Formal Meetings Between the FDA and Biosimilar Biological Product Sponsors or Applicants
- Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product

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

In general, FDA's guidance documents 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 naturally sourced products. Consequently, FDA established a regulatory approach for the approval of recombinant DNA-derived protein products, which was announced in the Federal Register (51 FR 23302, June 26, 1986), 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.* This approach addresses 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. 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

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

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. Because of 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 Federal Food Drug and Cosmetic Act (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 products. FDA has recognized, however, that "[i]n some instances complete new applications may not be required" (51 FR 23309, June 26, 1986).

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 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 a sponsor 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 the 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 (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use and resulted in the ICH guidance for industry *Q5E 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 product and its reference product. However, demonstrating that a proposed product is biosimilar to an FDA-licensed reference product manufactured by a different manufacturer typically will be more complex and will likely 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. A manufacturer that modifies its own manufacturing process has extensive knowledge and information about the product and the existing process, including established controls and acceptance parameters. By contrast, the manufacturer of a proposed 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 the draft guidance for industry *Applications Covered by Section 505(b)(2)*, which, among other things, states 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 as a result of advances in science and technology and improvements in manufacturing processes, process controls, materials, and product testing, as well as characterization tests and studies, may support 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.[5] 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 a biosimilar product application.

Biosimilarity is defined in section 351(i) of the PHS Act 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[6] 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). Comparative analytical data provide the foundation for a development program for a proposed biosimilar product intended for submission under section 351(k) of the PHS Act.

III. SCOPE

This document provides guidance on analytical studies that are relevant to assessing whether the proposed product and a reference product are highly similar to support a demonstration of biosimilarity. This document is not intended to provide an overview of FDA's approach to determining interchangeability, which will be addressed in a separate guidance document. Although this guidance applies specifically to therapeutic protein products, the general scientific principles may be informative for the development of other protein products, such as in vivo protein diagnostic products. If the reference product or the proposed product cannot be adequately characterized with state-of-the-art technology as recommended by this guidance, the application may not be appropriate for submission under section 351(k) of the PHS Act. FDA recommends that the sponsor consult FDA for guidance on the appropriate submission pathway.

This guidance describes considerations for additional CMC information that are relevant to assessing whether the proposed product and the reference product are highly similar. All product applications should contain a complete and thorough CMC section that provides the necessary and appropriate information (e.g., characterization, adventitious agent safety, process controls,

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

[6] For more information on *clinically meaningful differences*, see the guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*.

and specifications) for the product to be adequately reviewed. 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.[7] We encourage early interaction with FDA to discuss specific CMC issues that may arise for a sponsor's proposed 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).[8]

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.[9] 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. 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.[10] Advances in manufacturing science and production methods, as well as advances in analytical sciences, may enhance the likelihood that a proposed product can be demonstrated to be highly similar to a reference product by better targeting the reference product's physiochemical and functional properties. In addition, advances in analytical sciences may

[7] For CMC requirements for submission of a marketing application, sponsors should consult current regulations and see the guidance for industry *Submission on Chemistry, Manufacturing, and Controls Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal Antibody Product for In-vivo Use,* as well as other applicable FDA guidance documents.

[8] For a discussion of the Agency's current thinking on animal and clinical studies relevant to demonstrating biosimilarity, see the guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*.

[9] 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 sponsor and for which the sponsor 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.

[10] The use of the terms *product-related substances* and *product- and process-related impurities* is consistent with their use and meaning in the ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.*

enable detection and characterization of differences between the protein products. These differences should be further assessed to understand the impact on product performance.

Despite improvements in analytical techniques, current analytical methodology may not be able to detect or characterize all relevant structural and functional differences between the two protein products. A thorough understanding of each analytical method's limitations will be critical to a sponsor's successful identification of residual uncertainties and, in turn, to the design of subsequent testing. In addition, there may be incomplete understanding of the relationship between a product's structural attributes and its clinical performance. Sponsors should use appropriate analytical methodology that has adequate sensitivity and specificity to detect and characterize differences between the proposed product and the reference product. Accordingly, FDA encourages the use of widely available state-of-the-art technology.

In addition to a complete CMC data submission as required under section 351(a) of the PHS Act, an application submitted under section 351(k) of the PHS Act is required to include data supporting the analytical similarity of the proposed biosimilar product 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.

Comparative analytical data provide the foundation for a biosimilar development program and can influence decisions about the type and amount of animal and clinical data needed to support a demonstration of biosimilarity. Such analytical data should be available early in product development and will permit more detailed discussion with the Agency because known quality attributes can be used to shape biosimilar development and justify certain development decisions. Thus, in addition to the preliminary comparative analytical similarity data that should be submitted to support an initial advisory meeting, FDA encourages sponsors to submit comprehensive analytical similarity data early in the development process: at the pre-IND stage; with the original IND submission; or with the submission of data from the initial clinical studies, such as pharmacokinetic and pharmacodynamic studies. FDA will best be able to provide meaningful input on the extent and scope of animal and additional clinical studies for a proposed biosimilar development program once the Agency has considered the analytical similarity data.

Extensive, robust comparative physicochemical and functional studies (these may include biological assays, binding assays, and enzyme kinetics) should be performed to evaluate whether the proposed product and the reference product are highly similar. A meaningful assessment as to whether the proposed 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 posttranslational modifications), degree of heterogeneity, functional properties, impurity profiles, and degradation profiles denoting stability. The capability of the methods used in these analytical assessments, as well as their limitations, should be described by the sponsor. Physicochemical and functional characterization studies should be sufficient to establish relevant quality attributes including those that define a product’s identity, quantity, safety, purity, and potency. The product-related impurities, product-related substances, and process-related impurities should be identified, characterized as appropriate, quantified, and compared with

multiple lots of the proposed product to multiple lots 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.

Primary structure of some protein products can be highly heterogeneous, which could affect the expected clinical performance of a protein product. Protein heterogeneity may arise in a number of ways. Replication errors in the DNA encoding the protein sequence and amino acid misincorporation may occur during translation, although the level of these errors is typically low. In addition, most protein products undergo some posttranslational modification that can alter the functions of the protein by attaching other biochemical groups such as phosphate and 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 posttranslational modifications can be a consequence of manufacturing process operations; for example, glycation may occur with exposure of the product to reducing sugars. Also, storage conditions may be permissive for certain degradation pathways such as oxidation, deamidation, or aggregation. All of these product-related variants may alter the biological properties of the expressed recombinant protein. Therefore, 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) because of 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 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. Using multiple, relevant, state-of-the-art methods 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 product and a reference product should be evaluated in terms of a potential effect on protein function and stability. 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 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 a demonstration of product quality and the effectiveness of a suitable control system during development and approval 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 product and the reference product and, where available and appropriate, a comparison with a reference standard for

suitable attributes (e.g., potency). For a discussion of reference standards, see section V.G of this guidance. The evaluation of multiple lots of a reference product and multiple lots of a proposed product enables estimation of product variability across lots. The number of lots needed to understand and estimate the lot-to-lot variability of both the reference and proposed products may differ on a case-by-case basis and should be scientifically justified by the sponsor. FDA encourages sponsors to consult with the Agency to ensure that an appropriate number of lots are evaluated. Identification of specific lots of a reference product used in analytical similarity studies, together with expiration dates and time frames and when the lots were analyzed and used in other types of studies, should be provided. This information will be useful in justifying acceptance criteria 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 on the observed range of product attributes of the reference product. This is because some product attributes act in combination to affect a product's safety, purity, and potency profile; and therefore, their potential interaction should be considered when evaluating similarity and setting specifications. For example, for some glycoproteins, the content and distribution of tetra-antennary and N-acetyl lactosamine repeats can affect in vivo potency and should not be evaluated 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(R2))[11] can also be used to help establish acceptance criteria.

An extensive analytical characterization may also reveal differences between the reference product and the proposed 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 definitively distinguish any differences in product attributes. Based on the results of analytical studies assessing functional and physicochemical characteristics, including, for example, higher order structure, posttranslational 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 product with those of the reference product using a meaningful fingerprint-like analysis algorithm[12] that covers a large number of additional product attributes and their combinations with high sensitivity using orthogonal methods. Enhanced approaches in manufacturing science, as discussed in ICH Q8(R2), may facilitate production processes that can better match a reference product's fingerprint.[13] 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.

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[11] ICH guidance for industry *Q8(R2) Pharmaceutical Development.*

[12] For more information on fingerprint-like analysis, refer to Kozlowski S, Woodcock J, Midthun K, Sherman RB, 2011, Developing the Nation's Biosimilars Program, N Engl J Med; 365:385-388.

[13] See the ICH guidances for industry *Q8(R2) Pharmaceutical Development* (ICH Q8(R2)), *Q9 Quality Risk Management* (ICH Q9), *Q10 Pharmaceutical Quality System* (ICH Q10), and *Q11 Development and Manufacture of Drug Substances* (ICH Q11) for guidance on enhanced approaches in manufacturing science.

The type, nature, and extent of any differences between the proposed 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 product is highly similar to the reference product. The potential clinical effects of observed structural and functional differences between the two products should be assessed and supported by animal or clinical studies, if necessary.

The type and extent of animal or clinical studies that are needed to demonstrate biosimilarity of the proposed product can be influenced by several factors, especially the ability to discern differences between the proposed product and reference product and their potential effect on safety, purity, and potency. For example, factors such as the ability to robustly characterize the proposed 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 affect the nature and extent of subsequent animal or clinical studies.

In general, a sponsor needs to provide information to demonstrate biosimilarity based on data directly comparing the proposed product with the reference product. Under certain circumstances, a sponsor may use a non-U.S.-licensed comparator product in certain studies to support a demonstration that the proposed product is biosimilar to the U.S.-licensed reference product. However, as a scientific matter, analytical studies and at least one clinical PK study and, if appropriate, at least one PD study intended to support a demonstration of biosimilarity must include an adequate comparison of the proposed product directly with the U.S.-licensed reference product unless it can be scientifically justified that such a study is not needed. If a sponsor seeks to use data from an animal study or a clinical study comparing its proposed product to a non-U.S.-licensed product to address, in part, the requirements under section 351(k)(2)(A) of the PHS Act, the sponsor should provide adequate data or information to scientifically justify the relevance of these comparative data to an assessment of biosimilarity and establish an acceptable bridge to the U.S.-licensed reference product.

As a scientific matter, the type of bridging data needed will always include data from analytical studies (e.g., structural and functional data) that directly compares all three products (i.e., the proposed product, the U.S.-licensed reference product, and the non-U.S.-licensed comparator product) and is likely to also include bridging clinical PK and/or PD study data for all three products. All three pairwise comparisons should meet the pre-specified acceptance criteria for analytical and PK and/or PD similarity. The acceptability of such an approach will be evaluated on a case-by-case basis and should be discussed in advance with the Agency. For certain complex biological products, a modified approach may be needed.

Issues that a sponsor may need to address to use a non-U.S.-licensed comparator product in a biosimilar development program include, but are not limited to, the scientific bridge between the non-U.S.-licensed comparator product and the U.S.-licensed reference product, including comparative physicochemical characterization, biological assays/functional assays, degradation profiles under stressed conditions, and comparative clinical PK and, when appropriate, PD data, to address the impact of any differences in formulation or primary packaging on product

performance.[14] 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.[15] A final determination of the adequacy of the information will be made by FDA during review of the 351(k) application.

V. 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 in microbial cells (prokaryotic or eukaryotic), cell lines (e.g., mammalian, avian, insect, plant), or tissues derived from animals or plants. It is expected that the expression construct for a proposed product will encode the same primary amino acid sequence as its reference product. However, minor modifications, such as N- or C-terminal truncations (e.g., the heterogeneity of C-terminal lysine of a monoclonal antibody) that are not expected to change the product performance, may be justified and should be explained by the sponsor. Possible differences between the chosen expression system (i.e., host cell and the expression construct) of the proposed product and that of the reference product should be carefully considered because the type of expression system will affect the types of process- and product-related substances, impurities, and contaminants (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 posttranslational modifications that are imparted to the proposed product, which may introduce additional uncertainty into the demonstration that the proposed product is highly similar to the reference product.

Minimizing differences between the proposed and reference expression systems to the extent possible can enhance the likelihood of producing a highly similar protein product. Use of different expression systems will be evaluated on a case-by-case basis.

B. Manufacturing Process

A comprehensive understanding of all steps in the manufacturing process for the proposed 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 product and manufacturing process. The use of

[14] For a more complete discussion of the Agency's current thinking on issues related to bridging data to support use of a non-U.S.-licensed comparator product in certain studies, see the guidance for industry *Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009*.

[15] Please refer to the guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*.

enhanced approaches[16] to pharmaceutical development, along with quality risk management and effective quality systems, will facilitate the consistent manufacturing of a high-quality product. As a scientific matter, as with 351(a) BLAs, a type II Drug Master File (DMF) for a drug substance, drug substance intermediate, or drug product would not be acceptable for a 351(k) application because a license holder is expected to have knowledge of and control over the manufacturing process for the biological product for which it has a license.[17] Other types of contract manufacturing arrangements can be considered if the sponsor does not intend to manufacture the product for licensure.[18]

A sponsor considering manufacturing changes after completing the initial analytical similarity assessment or after completing clinical studies intended to support a 351(k) application will need to demonstrate comparability between the pre- and post-change proposed product and may need to conduct additional analytical studies. The nature and extent of the changes may determine the extent of these additional similarity studies. The analytical similarity studies should include a sufficient number of lots of the proposed biosimilar product used in clinical studies as well as from the proposed commercial process if the process used to produce the material used in the clinical studies is different.

C. Assessment of Physicochemical Properties

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

The sponsor should address the concept of the desired product (and its variants) as discussed in ICH Q6B when designing and conducting the characterization studies. Thus, it will be important to understand the heterogeneity of the proposed 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 posttranslational modifications.

Particular analytical methodologies can be used to assess specific physicochemical characteristics of proteins. 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

[16] See the guidances for industry ICH Q8(R2), ICH Q9, ICH Q10, and ICH Q11 for guidance on enhanced approaches in manufacturing science.

[17] 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.

[18] See the guidance for industry *Cooperative Manufacturing Arrangements for Licensed Biologics*.

regarding the structure and heterogeneity of the reference product and the proposed 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., orthogonal methods to assess aggregation). 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.

Unlike routine quality control assays, tests used to characterize the product do not necessarily need to be validated. But the tests used to characterize the product 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 product and a reference product should be submitted as part of the comparison.

Tests chosen to detect and characterize posttranslational protein modifications should be demonstrated to be of appropriate sensitivity and specificity to provide meaningful information as to whether the proposed 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 qualitative 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 usually 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, the functional assays should reflect these mechanisms of action to the extent possible. Multiple functional assays should, in general, be performed as part of the analytical similarity assessments. 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, potency, and stability.

If a reference product exhibits multiple functional activities, sponsors should perform a set of appropriate assays designed to evaluate the range of relevant activities for that product. For example, with proteins that possess multiple functional domains expressing enzymatic and receptor-mediated activities, sponsors should evaluate both activities. For products where functional activity can be measured by more than one parameter (e.g., enzyme kinetics or

interactions with blood clotting factors), the comparative characterization of each parameter between products should be assessed.

The sponsor 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 product and the reference product. Because a highly variable assay may not provide a meaningful assessment as to whether the proposed product is highly similar to the reference product, sponsors are encouraged to develop assays that are less variable and 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 (pharmacokinetics and biodistribution) of the product, which can affect pharmacodynamics and clinical performance. Also, bioavailability can be dramatically altered by subtle differences in glycoform distribution or other posttranslational 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 that may address residual uncertainties. Finally, functional assays are important 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 proposed product in terms of these specific properties, (e.g., if binding to a receptor is inherent to 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 product's higher order structure.

F. Impurities

The sponsor should characterize**,** identify, and quantify impurities in the proposed product and the reference product, to the extent feasible.[19] A risk-based assessment should be performed on any differences in process-related impurities identified between the proposed product and the reference product. If a 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 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 the ICH guidance for industry *S6(R1*) *Preclinical Safety Evaluation of Biotechnology-Derived*

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

Pharmaceuticals, "[i]t is preferable to rely on purification processes to remove impurities . . . rather than to establish a preclinical testing program for their qualification."[20]

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 process-related impurities in the proposed product are not expected to match those observed in the reference product. However, process-related impurities in the proposed product should be assessed side by side with the impurities in the reference product. The potential impact of the differences in the impurity profile upon safety should be addressed and supported by appropriate data. FDA will apply a product-specific evaluation approach toward differences in impurities between the proposed product and the US-licensed reference product and consider and evaluate the sponsor's assessment of the potential impact of these differences for biosimilar products. In all cases, the chosen analytical procedures should be adequate to detect, identify, and accurately quantify biologically significant levels of impurities (see the ICH guidance for industry *Q2B Validation of Analytical Procedures: Methodology*). In particular, results of 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.[21]

The safety of the proposed 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 the ICH guidance for industry *Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin*).

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 product and justify reliance on certain existing scientific knowledge about the reference product. Sufficient evidence that the proposed product is highly similar to the reference product must be provided in an appropriate time frame to support a selective and targeted approach in early product development (e.g., selected animal studies and/or additional clinical studies).[22]

The analytical similarity assessment submitted with the marketing application should support the demonstration of biosimilarity of the proposed product used in principal clinical study(ies), as well as the proposed commercial product, to the reference product. The biosimilar marketing application should include a thorough analytical comparison between the proposed product and a

[20] See ICH S6(R1), page 2.

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

[22] See 21 CFR 312.23 for IND application content and format.

single reference product previously licensed by FDA. As stated above in section V.B, a sponsor considering manufacturing changes after completing the initial analytical similarity assessment or after completing clinical studies intended to support a 351(k) application may need to conduct additional analytical similarity studies. The nature and extent of the changes may determine the extent of these additional similarity studies.

If the drug substance has been extracted from the reference product to assess analytical similarity, the sponsor should describe the extraction procedure and provide support that the procedure itself does not alter relevant product quality attributes. This undertaking would include consideration for alteration or loss of the desired products and impurities and relevant product-related substances, and it should include appropriate controls to ensure that 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, a physicochemical and/or functional comparison of the proposed product with this standard may also provide useful information. Although studies with such a reference standard may be useful, they do not satisfy the BPCI Act's requirement to demonstrate the biosimilarity of the proposed product to the U.S.-licensed reference product. For example, if an international standard for calibration of potency is available, a comparison of the relative potency of the proposed product with this potency standard should be performed. As 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 product should not focus solely on the characterization of the proposed product in isolation. Rather, these studies should be part of a broad comparison that includes, but is not limited to, the proposed 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 a bulk drug substance.[23] However, if a bulk drug substance is reformulated and/or exposed to new materials in the finished dosage form, the impact of these changes should be considered. Whenever possible, if the finished drug product is best suited for a particular analysis, the sponsors should analyze the finished drug product. The characterization should compare the proposed finished 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 extracted protein and the finished drug product.

[23] See 21 CFR 207.3.

Sponsors should clearly identify excipients used in the proposed product that differ from those in the reference product. The acceptability of the type, nature, and extent of any differences between the proposed finished 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 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 stability and/or clinical performance. Differences in formulation and primary packaging[24] between the proposed product and the reference product are among the factors that may affect whether or how subsequent clinical studies may take a selective and targeted approach.[25]

I. Stability

As part of an appropriate physicochemical and functional comparison of the stability profile of the proposed product with that of the reference product, accelerated and stress stability studies, as well as forced degradation studies, should be used to establish degradation profiles and to provide a direct comparison of the proposed 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 evaluations and also identify conditions under which additional controls should be employed in manufacturing and storage (see ICH guidances for industry *Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* and *Q1A(R2) Stability Testing of New Drug Substances and Products*). Sufficient real time, real condition stability data from the proposed product should be provided to support the proposed shelf life.

VI. CONCLUSION

The foundation for an assessment and demonstration of biosimilarity between a proposed product and its reference product includes analytical studies that demonstrate that the proposed product is highly similar to the reference product notwithstanding minor differences in clinically inactive components. The demonstration that the proposed product is highly similar to the reference product involves robust characterization of the proposed product, including comparative physicochemical and functional studies with the reference product. 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 product as a biosimilar. In addition, a 351(k) application for a proposed product must contain, among other things, information demonstrating biosimilarity based on data derived from animal studies (including the assessment of toxicity) and a clinical study or studies (including the assessment of immunogenicity and

[24] See *ICH Q8(R2) Pharmaceutical Development.*

[25] For more discussion on *selected and targeted approaches*, please refer to the guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*.

pharmacokinetics or pharmacodynamics), unless the Agency determines that an element is unnecessary in a particular 351(k) application.[26] The ability to discern and understand the impact of relevant analytical 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.

VII. 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. Guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*

2. Guidance for industry *Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009*

3. Guidance for industry *Biosimilars: Additional Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009*

4. Guidance for industry *Formal Meetings Between the FDA and Biosimilar Biological Product Sponsors or Applicants*

5. Guidance for industry *Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product*

6. *Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products*

7. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use*

8. 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*

9. Guidance for industry *Cooperative Manufacturing Arrangements for Licensed Biologics*

10. ICH guidance for industry *M4Q: The CTD —Quality* (ICH M4Q)

[26] Section 7002(a)(2) of the Affordable Care Act, adding section 351(k)(2)(A)(i)(I) of the PHS Act.

11. ICH guidance for industry *Q1A(R2) Stability Testing of New Drug Substances and Products* (ICH Q1A(R2)

12. ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and Methodology* (ICH Q2(R1)

13. ICH guidance for industry *Q2B Validation of Analytical Procedures: Methodology* (ICH Q2B)

14. ICH guidance for industry *Q3A Impurities in New Drug Substances* (ICH Q3A)

15. ICH guidance for industry *Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (ICH Q5A)

16. ICH guidance for industry *Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products* (ICH Q5B)

17. ICH guidance for industry *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (ICH Q5C)

18. ICH guidance for industry *Q5D Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products* (ICH Q5D)

19. ICH guidance for industry *Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process* (ICH Q5E)

20. ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (ICH Q6B)

21. ICH guidance for industry *Q7(A) Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients* (ICH Q7A)

22. ICH guidance for industry *Q8(R2) Pharmaceutical Development* (ICH Q8(R2))

23. ICH guidance for industry *Q9 Quality Risk Management* (ICH Q9)

24. ICH guidance for industry *Q10 Pharmaceutical Quality System* (ICH Q10)

25. ICH guidance for industry *Q11 Development and Manufacture of Drug Substances* (ICH Q11)

26. ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (ICH S6(R1))

GLOSSARY[27]

For the purpose of this document, the following definitions apply:

Biosimilar or biosimilarity means "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."[28]

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

Product, when used without modifiers, is intended to refer to the 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.

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

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.

[27] For a discussion of the Agency's current thinking on certain definitions relevant to implementation of the BPCI Act, see the guidance for industry *Biosimilars: Questions and Answers Regarding Implementation of the* Biologics *Price Competition and Innovation Act of 2009.*

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