Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations

Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact (CDER) Sandra Benton, 301-796-1042, or (CBER) Office of Communication, Outreach and Development, 800-835-4709 or 240-402-8010.

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Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

May 2019
Biosimilars

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Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish 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 recommendations on the design and evaluation of comparative analytical studies intended to support a demonstration that a proposed therapeutic protein product is biosimilar to a reference product licensed under section 351(a) of the Public Health Service Act (PHS Act). Additionally, this guidance is intended to provide recommendations to sponsors on the scientific and technical information for the chemistry, manufacturing, and controls (CMC) portion 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 reference product (see sections 7001 through 7003 of the Patient Protection and Affordable Care Act (ACA) (Public Law 111-148). Although the 351(k) pathway applies generally to biological products, this guidance focuses on therapeutic protein products and provides an overview of recommendations for the comparative analytical assessment and other important scientific considerations to support a demonstration of biosimilarity between a proposed therapeutic

[1] This draft guidance has been prepared by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) at the Food and Drug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

protein product (referred to as a *proposed biosimilar*[2] or *proposed biosimilar product*) and the reference product. [3]

This guidance is one in a series of guidances that FDA is developing to facilitate implementation of the BPCI Act.

Relevant final guidance documents[4] issued to date address a broad range of issues, including:

- *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (April 2015)
- *Questions and Answers on Biosimilar Development and the BPCI Act* (December 2018)
- *Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product* (December 2016)
- *Labeling for Biosimilar Products* (July 2018)
- *Considerations in Demonstrating Interchangeability With a Reference Product* (May 2019)

In addition, FDA has published draft guidance documents related to the BPCI Act, which, when finalized, will represent FDA's current thinking. These draft guidance documents include:

- *Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products* (June 2018)
- *Reference Product Exclusivity for Biological Products Filed Under Section 351(a) of the PHS Act* (August 2014)
- *New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 2)* (December 2018)

[2] In this guidance, the following terms are used to describe biological products licensed under section 351(k) of the PHS Act: (1) "biosimilar" or "biosimilar product" refers to a product that FDA has determined to be biosimilar to the reference product (see sections 351(i)(2) and 351(k)(2) of the PHS Act) and (2) "interchangeable biosimilar" or "interchangeable product" refers to a biosimilar product that FDA has determined to be interchangeable with the reference product (see sections 351(i)(3) and 351(k)(4) of the PHS Act).

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

[4] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

When applicable, references to information in these final and draft 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*.[5] 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 potential 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 because the biological system in which such a protein product is produced can have a significant effect on the structure and function of the product itself.

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 the *FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived 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 safety, identity, purity, or potency of its FDA-approved product.

[5] For more information, this document is available on FDA's Other Recommendations for Biologics Manufacturers web page at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/other-recommendations-biologics-manufacturers.

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 collection and 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 ICH guidance for industry *Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process* (June 2005).

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 biosimilar will have no direct knowledge of the manufacturing process for the reference product and will have its own manufacturing process (e.g., different cell line, raw materials, equipment, processes, process controls, acceptance criteria).

Therefore, comprehensive comparative analytical data are necessary to build the foundation for a development program for a proposed biosimilar product intended for submission under section 351(k) of the PHS Act.

The BPCI Act

The BPCI Act, enacted as part of the (ACA) on March 23, 2010, amends the PHS Act and other statutes to create an abbreviated licensure pathway for biological products shown to be biosimilar to, or interchangeable with, an FDA-licensed biological reference product (see sections 7001 through 7003 of the ACA). 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 or a proposed interchangeable product. An application submitted under section 351(k)

must contain, among other things, information demonstrating that "the biological product is biosimilar to a reference product" based upon data derived from:

- Analytical studies that demonstrate that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components;
- Animal studies (including the assessment of toxicity); and
- A clinical study or studies (including the assessment of immunogenicity and PK or PD) that are sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biological product.[6]

FDA has the discretion to determine that an element above is unnecessary in a 351(k) application.[7]

The term *biosimilar* or *biosimilarity* is defined in the PHS Act "in reference to a biological product that is the subject of an application under [section 351(k)]" 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" (section 351(i)(2) of the PHS Act). The term *reference product* is defined in the PHS Act as 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 (section 351(i)(4) of the PHS Act).

Section 351(k)(4) of the PHS Act provides that upon review of an application submitted under section 351(k) or any supplement to such application, FDA will determine the biological product to be interchangeable with the reference product if FDA determines that the information submitted in the application (or a supplement to such application) is sufficient to show that the biological product "is biosimilar to the reference product" and "can be expected to produce the same clinical result as the reference product in any given patient"[8] and that "for a biological product that is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between 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."[9]

The term *interchangeable* or *interchangeability* is defined in the PHS Act, in reference to a biological product that is shown to meet the standards described in section 351(k)(4) of the PHS

[6] Section 351(k)(2)(A)(i)(I) of the PHS Act.

[7] Section 351(k)(2)(A)(ii) of the PHS Act.

[8] Section 351(k)(4)(A) of the PHS Act.

[9] Section 351(k)(4)(B) of the PHS Act.

Act, to mean that "the biological product may be substituted for the reference product without the intervention of the health care provider who prescribed the reference product" (section 351(i)(3) of the PHS Act).

III. SCOPE

This document provides guidance on the use of comparative analytical studies that are relevant to assessing whether the proposed product is biosimilar to a reference product for purposes of submission of a marketing application under section 351(k) of the PHS Act. This document is not intended to provide an overview of FDA's approach to determining interchangeability, which is addressed in a separate guidance document.[10] Although this guidance applies specifically to therapeutic protein products, the general scientific principles may be informative for the development of proposed biosimilars to other protein products, such as in vivo protein diagnostic products. If the reference product cannot be adequately characterized for the purpose of demonstrating that a proposed product is biosimilar to the reference product as recommended in this guidance, the application may not be appropriate for submission under section 351(k) of the PHS Act.

This guidance also describes considerations for CMC information that is relevant to assessing whether the proposed product is biosimilar to the reference product. It is critical that all product applications contain a complete and thorough CMC section that provides the necessary and appropriate information (e.g., characterization, adventitious agent safety, process controls, and specifications) to support that the manufacturing process consistently delivers a product with the intended quality characteristics. 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.[11] We encourage early interaction with FDA to discuss specific CMC issues that may arise for a sponsor's proposed product.

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

[10] See FDA's guidance for industry, *Considerations in Demonstrating Interchangeability With a Reference Product* (May 2019).

[11] 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* (August 1996), as well as other applicable FDA guidance documents.

not only the desired product but also product-related substances and product- and process-related impurities.[12] Advances in manufacturing science and production methods 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 enable detection and characterization of differences between the protein products. These differences should be further assessed to understand the impact on the biosimilar product clinical performance relative to the reference product.

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. FDA encourages the use of available state-of-the-art technology. Sponsors should use appropriate analytical methodologies that have adequate sensitivity and specificity to detect and characterize differences between the proposed product and the reference product.

As part of a complete CMC data submission, an application submitted under section 351(k) of the PHS Act is required to include analytical studies that demonstrate that the biological product is highly similar to the reference product.[13] The rationale for the approach to the comparative analytical assessment should be clearly described, with consideration of the characteristics, known mechanism of action(s), and function of the reference product.

Comparative analytical data provide the foundation for the development of a proposed product for submission in an application under section 351(k) of the PHS Act 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 data that should be submitted to support an initial advisory meeting,[14] FDA encourages sponsors to submit comprehensive comparative analytical data early

[12] 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* (August 1999).

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

[14] See the draft guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products* (June 2018), which provides recommendations to industry on all formal meetings between the FDA and sponsors or applicants for proposed biosimilar products or proposed interchangeable products intended to be submitted under 351(k) of the PHS Act. When final, this guidance will represent FDA's current thinking on this topic.

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 PK and PD 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 comparative analytical data.

Comprehensive, robust comparative physicochemical and functional studies (these may include biological assays, binding assays, and enzyme kinetics) should be performed to evaluate the proposed product and the reference product. A meaningful comparative analytical assessment 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 and product-related substances should be identified, characterized as appropriate, quantified, and compared using multiple lots of the proposed product and 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.

Because therapeutic proteins are made in living systems, there may be heterogeneity in certain quality attributes of these products. Heterogeneity in therapeutic proteins may arise in a number of ways and may affect the expected clinical performance of a protein product. 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 posttranslational modifications 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 (e.g., 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, certain storage conditions may be more or less 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 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, among other things. 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 extent, 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 for all biological products. 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 support approval of the product.

Proposed biosimilar 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. 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 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 comparative analytical studies, together with expiration dates and time frames and when the lots were analyzed and used in other types of studies (nonclinical or clinical studies), should be provided. This information will be useful in justifying acceptance criteria to ensure product consistency, as well as to support the comparative analytical assessment of the proposed product and the reference product. 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; therefore, their potential interaction should be considered when conducting the comparative analytical assessment and setting specifications. For example, for some glycoproteins, the content and distribution of tetra-antennary and N-acetyllactosamine 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))[15] can also be used to help establish acceptance criteria.

An extensive analytical characterization may 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 identify 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[16] 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.[17] 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 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. 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.

V. FACTORS FOR CONSIDERATION IN PERFORMING THE COMPARATIVE ANALYTICAL ASSESSMENT

When performing the comparative analytical assessment to support a demonstration of biosimilarity, manufacturers should consider a number of factors, including the following:

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[15] See the ICH guidance for industry *Q8(R2) Pharmaceutical Development* (November 2009).

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

[17] See the ICH guidances for industry *Q8(R2) Pharmaceutical Development* (November 2009), *Q9 Quality Risk Management* (June 2006), *Q10 Pharmaceutical Quality System* (April 2009), and *Q11 Development and Manufacture of Drug Substances* (November 2012) for guidance on enhanced approaches in manufacturing science.

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 biosimilar to the reference product.

Minimizing differences between the proposed product and reference product expression systems to the extent possible can enhance the likelihood of producing a biosimilar 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. As a scientific matter, 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 enhanced approaches[18] 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 biological products originally licensed under section 351(a) of the PHS Act, an application for a biological product submitted for licensure under section 351(k) of the PHS Act may not incorporate by reference drug substance, drug substance intermediate, or drug product information contained in a Master File (MF) because a license holder is generally expected to have knowledge of and control over the manufacturing process for the biological product for which it has a license.[19] Other types of contract

[18] See the ICH guidances for industry *Q8(R2) Pharmaceutical Development* (November 2009), *Q9 Quality Risk Management* (June 2006), *Q10 Pharmaceutical Quality System* (April 2009), and *Q11 Development and Manufacture of Drug Substances* (November 2012) for guidance on enhanced approaches in manufacturing science.

[19] A MF for drug substance, drug substance intermediate, or drug product information for a biological product may be referenced to support an investigational new drug application (IND) for a proposed biosimilar product. Assurance of product quality should be provided on each lot of material produced by the MF holder. Procedures

manufacturing arrangements can be considered if the sponsor does not intend to manufacture the product for licensure.[20]

A sponsor considering manufacturing changes after completing the initial comparative analytical 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 studies. The nature and extent of the changes may determine the extent of these additional studies. The comparative analytical 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. 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[21] 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 regarding the structure and heterogeneity of the reference product and the proposed product, as well as characteristics critical to product performance.

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should also be in place to ensure that the IND sponsor is notified by the MF holder of significant changes to the MF 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 MF.

[20] See the guidance for industry *Cooperative Manufacturing Arrangements for Licensed Biologics* (November 2008).

[21] See the ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (August 1999).

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; however, 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. The methods 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 quantitative 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 such mechanism(s) of action to the extent possible. Multiple functional assays should, in general, be performed as part of the comparative analytical 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 to the extent that these activities are relevant to product performance. 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 (PK and biodistribution) of the product, which can affect PD 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. Target Binding

When binding is part of the activity attributed to the protein product, analytical tests should be performed to characterize the proposed product in terms of its specific binding 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 product-related impurities in the proposed product and the reference product, to the extent feasible.[22] 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 Pharmaceuticals* (May 2012), "[i]t is

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

preferable to rely on purification processes to remove impurities . . . rather than to establish a preclinical testing program for their qualification."[23]

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 and are not included in the comparative analytical assessment. The chosen analytical procedures should be adequate to detect, identify, and accurately quantify biologically significant levels of impurities.[24] 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.

As with any biological product, the safety of the proposed 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.[25]

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 to support a selective and targeted approach in early product development (e.g., selected animal studies and/or additional clinical studies).[26]

The comparative analytical assessment submitted with the marketing application to support the demonstration of biosimilarity of the proposed product to the reference product should include lots of the proposed product used in principal clinical study(ies), as well as the proposed commercial product. As stated earlier in section V.B, a sponsor considering manufacturing changes after completing the initial comparative analytical assessment or after completing clinical studies intended to support a 351(k) application may need to conduct additional

[23] See the ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (May 2012), page 2.

[24] See the ICH guidance for industry *Q2B Validation of Analytical Procedures: Methodology* (May 1997).

[25] See the ICH guidance for industry *Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin* (September 1998).

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

comparative analytical studies of the proposed product and the reference product. The nature and extent of the changes may determine the extent of these additional analytical studies.

If the drug substance has been extracted from the reference product to conduct analytical studies, 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 of alteration or loss of the desired products and impurities and relevant product-related substances, and it should include appropriate controls to ensure that relevant characteristics of the protein 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.[27] 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.

An in-house reference standard is typically developed from early development lots or lots used in a clinical study(ies). Additional reference standards may be qualified later in development and for a BLA submission. Ideally, a sponsor will have established and properly qualified primary and working reference standards that are representative of proposed product lots used in clinical studies that support the application.

For the development of a proposed product, a reference product lot or a lot of a non-U.S.-licensed comparator product (see section VI.A.4 of this guidance) is typically qualified as an initial reference standard. Once clinical lots of the proposed product have been manufactured, it is expected that one of these lots will be properly qualified (including bridging to previous reference standards) for use as a reference standard for release and stability, as well as comparative analytical testing. If possible, once an in-house reference standard is properly qualified, there should be sufficient quantities to use throughout the development of the proposed product. All lots of reference standards used during the development of a proposed product should be properly qualified. In addition to release testing methods, the qualification protocol for reference standards should include all analytical methods that report the result relative to the reference standard.

For all methods where the result is reported relative to the reference standard, the assignment of a potency of 100% should include a narrow acceptable potency range and ensure control over product drift. For example, a sponsor should consider the use of a pre-determined two-sided confidence interval (CI) of the mean of the replicates, where the mean relative potency and the 95% CI are included within a sufficiently narrow range (e.g., 90-110%). There should be an

[27] Although studies with such a reference standard may be useful, they are not sufficient to satisfy the BPCI Act's requirement to demonstrate the biosimilarity of the proposed product to the U.S.-licensed reference product.

evaluation across the history of multiple reference standard qualifications to address potential drift.

A sponsor generally should not use a correction factor to account for any differences in, for example, potency or biological activity between reference standards.

Use of reference standards inadequately qualified for analytical methods that report results relative to the reference standard is likely to raise concerns regarding the comparative analytical assessment. One approach to address these concerns, if applicable, may be to store the reference product and non-U.S.-licensed comparator product lots under conditions that maintain stability long term, if feasible. Prior to submission of a 351(k) application, the prospective applicant should conduct a reevaluation of all proposed product, reference product, and non-U.S.-licensed comparator product lots using the same reference standard for those methods that report the result relative to the reference standard. Data supporting the stability of the reference product and non-U.S.-licensed comparator product beyond the expiration date under these conditions should be included in the submission.

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, and, where applicable, a non-U.S.-licensed comparator, applicable reference standards, and consideration of relevant publicly available information.

H. Finished Drug Product

Product characterization studies of a proposed product 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 the drug substance. However, if a 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. 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.

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[28] 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

[28] See the ICH guidance for industry *Q8(R2) Pharmaceutical Development* (November 2009).

targeted approach.[29] 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 finished proposed 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.

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 stability 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.[30] Sufficient real time, real-condition stability data from the proposed product should be provided to support the proposed shelf life.

VI. COMPARATIVE ANALYTICAL ASSESSMENT

A thorough understanding of the reference product is critical for a successful biosimilar development program. The Agency recommends that sponsors approach the comparative analytical assessment by first understanding the physicochemical and biological characteristics of the reference product. A full characterization of the reference product, in addition to consideration of publicly available information, will form the basis of product understanding. As described previously, protein products are complex molecules that generally are manufactured in living cells and purified using a variety of technologies; therefore, they have a certain degree of inherent lot-to-lot variability in terms of quality characteristics. The observed lot-to-lot variability may derive from manufacturing conditions and from analytical assay variability. Factors that contribute to lot-to-lot variability in the manufacture of a protein product include the source of certain raw materials (e.g., growth medium, resins, or separation materials) and different manufacturing sites. Therefore, the comparative analytical assessment, it is important to adequately characterize the lot-to-lot variability of the reference product and the proposed biosimilar product.

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[29] For more discussion on *selective and targeted approaches*, please refer to the guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (April 2015).

[30] See ICH guidances for industry *Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (July 1996) and *Q1A(R2) Stability Testing of New Drug Substances and Products* (November 2003).

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665 **A. Considerations for Reference and Biosimilar Products**
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667 *1. Reference Product*
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669 To ensure that the full range of product variability is accurately captured, sponsors should
670 acquire multiple reference product lots throughout the development program of a proposed
671 biosimilar in sufficient quantity to conduct multiple physiochemical and functional assays.
672 Considering the inherent heterogeneity present in protein products and the expected lot-to-lot
673 variability stemming from manufacturing processes, the Agency recommends that a sponsor
674 include at least 10 reference product lots (acquired over a time frame that spans expiration dates
675 of several years), in the analytical assessment to ensure that the variability of the reference
676 product is captured adequately. The final number of lots should be sufficient to provide adequate
677 information regarding the variability of the reference product. In cases where limited numbers of
678 reference product lots are available (e.g., for certain orphan drugs), alternate flexible comparative
679 analytical assessments plans should be proposed and discussed with the Agency.
680
681 *2. Proposed Product*
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683 The Agency recommends that a sponsor include at least 6 to 10 lots of the proposed product in
684 the comparative analytical assessment, to ensure 1) adequate characterization of the proposed
685 product and understanding of manufacturing variability, and 2) adequate comparison to the
686 reference product. These should include lots manufactured with the investigational- and
687 commercial-scale processes, and may include validation lots, as well as product lots
688 manufactured at different scales, including engineering lots. These lots should be representative
689 of the intended commercial manufacturing process. If there is a manufacturing process change
690 during development, it may be possible, with adequate scientific justification, to use data
691 generated from lots manufactured with a different process. However, data should be provided in
692 the 351(k) BLA to support comparability of drug substance and drug product manufactured with
693 the different processes and/or scales. The extent of process development design (as described in
694 guidelines *ICH Q8 (R2) Pharmaceutical Development* and *ICH Q11 Development and*
695 *Manufacture of Drug Substances*) and process understanding should be used in support of the
696 number of proposed biosimilar product lots proposed for inclusion in the comparative analytical
697 assessment in the 351(k) application.
698
699 To the extent possible, proposed biosimilar lots included in the comparative analytical
700 assessment described in section VI.B, Considerations for Data Analysis, should be derived from
701 different drug substance batches to adequately represent the variability of attributes inherent to
702 the drug substance manufacturing process. Drug product lots derived from the same drug
703 substance batch(es) are not considered sufficiently representative of such variability, except for
704 use in testing certain drug product attributes for which variability is mostly dependent on the
705 drug product manufacturing process (e.g., protein concentration). Although it may be preferable
706 to compare the proposed product lots to the reference product lots, it may be acceptable to also

include independent drug substance batches (if the drug substance was not used to make drug product), if needed, to attain a sufficient number of lots for the comparative analytical assessment.

3. Accounting for Reference Product and Proposed Product Lots

Sponsors should account for all the reference product lots acquired and characterized. The 351(k) BLA should include data and information from all reference product and proposed product lots that were evaluated in any manner, including the specific physicochemical, functional, animal, and clinical studies for which a lot was used. When a lot is specifically selected to be included in or excluded from certain analytical studies, a justification should be provided. The date of the analytical testing as well as the product expiration date should be provided in the application. In general, expired reference product lots should not be included in the comparative analytical assessment because lots analyzed beyond their expiration date could lead to results outside the range that would normally be observed in unexpired lots, which may result in overestimated reference product variability. Testing of lots past expiry may be acceptable if samples are stored under long term conditions (e.g., frozen at -80°C) provided that sponsors submit data and information demonstrating that storage does not impact the quality of the product (see section V.G).

The same type of information and data described above to be collected for reference product lots should also be provided on every manufactured drug substance and drug product lot of the proposed product.

Reference product and proposed product lots used in the clinical studies (e.g., PK and PD, if applicable, similarity, and comparative clinical study) should be included in the comparative analytical assessment.

4. Reference Product and Non-U.S.-Licensed Comparator Products

As described in other guidances, a sponsor that intends to use a non-U.S.-licensed comparator in certain studies should provide comparative analytical data and analysis for all pairwise comparisons (i.e., U.S.-licensed product versus proposed biosimilar product, non-U.S.-licensed comparator product versus proposed biosimilar product, and U.S.-licensed product versus non-U.S.-licensed comparator product).

The acceptance criteria used to support a demonstration that a proposed biosimilar product is highly similar to the reference product should be derived from data generated from a sponsor's analysis of the reference product. The comparative analytical assessment should be based on a direct comparison of the proposed product to the reference product. As a scientific matter, combining data from the reference product and non-U.S.-licensed comparator product to determine the acceptance criteria or to perform the comparative analytical assessment to the proposed product would not be acceptable to support a demonstration that the proposed product

is biosimilar to the reference product. For example, combining data from the reference product and non-U.S.-licensed products may result in a larger range and broader similarity acceptance criteria than would be obtained by relying solely on data from reference product lots. Sponsors are encouraged to discuss with FDA, during product development, any plans to submit data derived from products approved outside of the U.S. in support of a 351(k) application.

B. Considerations for Data Analysis

Sponsors should develop a comparative analytical assessment plan and discuss the approach with the Agency as early as practicable. A final comparative analytical assessment report should be available at the time a 351(k) BLA is submitted.

The Agency recommends development of a comparative analytical assessment plan using a stepwise approach. The first step is a determination of the quality attributes that characterize the reference product in terms of its structural/physicochemical and functional properties. These quality attributes are then ranked according to their risk to potentially impact activity, PK/PD, safety, efficacy, and immunogenicity. Finally, the attributes are evaluated using quantitative analysis, considering the risk ranking of the quality attributes, as well as other factors. It should be noted, however, that some attributes may be highly critical (e.g., primary sequence) but not amenable to quantitative analysis.

1. Risk Assessment

FDA recommends that sponsors develop a risk assessment tool to evaluate and rank the reference product quality attributes in terms of potential impact on the mechanism(s) of action and function of the product. Certain quality evaluations of the reference product (e.g., its degradation rates, which are determined from stability or forced degradation studies) generally should not be included in the risk ranking. However, these evaluations should still factor into the comparative analytical assessment of the proposed biosimilar and reference product.

Development of the risk assessment tool should be informed by relevant factors, including:

- Potential impact of an attribute on clinical performance: Specifically, FDA recommends that sponsors consider the potential impact of an attribute on activity, PK/PD, safety, efficacy, and immunogenicity. Sponsors should consider publicly available information, as well as the sponsor's own characterization of the reference product, in determining the potential impact of an attribute on clinical performance.

- The degree of uncertainty surrounding a certain quality attribute: For example, when there is limited understanding of the relationship between the degree of change in an attribute and the resulting clinical impact, FDA recommends that that attribute be ranked as having higher risk because of the uncertainty raised.

FDA recommends that an attribute that is a high risk for any one of the performance categories (i.e., activity, PK/PD, safety, efficacy, and immunogenicity) be classified as high risk. Ideally, the risk assessment tool should result in a list of attributes ordered by the risk to the patient. The risk scores for attributes should, therefore, be proportional to patient risk. The scoring criteria used in the risk assessment should be clearly defined and justified, and the risk ranking for each attribute should be justified with appropriate citations to the literature and data provided.

2. Quantitative and Qualitative Data Analysis

Appropriate analyses of the comparative analytical data are necessary to support a demonstration that the proposed product is highly similar to the reference product notwithstanding minor differences in clinically inactive components. One approach to data analysis would be the use of descriptive quality ranges for assessing quantitative quality attributes of high and moderate risk, and the use of raw data/graphical comparisons for quality attributes with the lowest risk ranking or for those quality attributes that cannot be quantitatively measured (e.g., primary sequence). The acceptance criteria for the quality ranges (QR) method in the comparative analytical assessment should be based on the results of the sponsor's own analysis of the reference product for a specific quality attribute. The QR should be defined as $(\hat{\mu}_R - X\hat{\sigma}_R, \hat{\mu}_R + X\hat{\sigma}_R)$, where $\hat{\mu}_R$ is the sample mean, and $\hat{\sigma}_R$ is the sample standard deviation based on the reference product lots. The multiplier (X) should be scientifically justified for that attribute and discussed with the Agency. Based on our experience to date, methods such as tolerance intervals are not recommended for establishing the similarity acceptance criteria because a very large number of lots would be required to establish meaningful intervals. The sponsor can propose other methods of data analysis, including equivalence testing.

The objective of the comparative analytical assessment is to verify that each attribute, as observed in the proposed biosimilar and the reference product, has a similar population mean and similar population standard deviation. Comparative analysis of a quality attribute would generally support a finding that the proposed product is highly similar to the reference product when a sufficient percentage of biosimilar lot values (e.g., 90%) fall within the QR defined for that attribute. The Agency recommends that narrower acceptance criteria of the QR method in the comparative analytical assessment (e.g., a lower X value) be applied to higher risk quality attributes.

In addition to risk ranking, other factors should be considered in determining which type of quantitative data analysis should be applied to a particular attribute or assay. Some additional factors that should be considered when determining the appropriate type of data evaluation and analysis of results include:

- Nature of the attribute: Attributes that are known to be of high risk should be prioritized over attributes with unknown but potentially high risk (i.e., attributes with a high-risk ranking due to uncertainty).

- Distribution of the attribute: In general, the Agency recommends that sponsors develop the manufacturing process to target the centers of distribution of the quality attributes of the reference product as closely as possible. Therefore, the QR, which assumes that the population mean and standard deviation are similar, is an appropriate approach to demonstrate that the proposed product is highly similar to the reference product. If there are concerns with the distribution, additional information or analyses may be needed to support the QR method or to support a different analysis approach. For example, the distribution of an attribute in the proposed biosimilar product that is biased towards one side of the reference product distribution may raise concerns depending on the nature of the attribute and the role the attribute plays in, for example, the mechanism of action of the product. If such a distribution is observed, appropriate justification may be needed, as a scientific matter, to support the comparative analytical assessment of the products. In cases where an attribute in the reference product is not normally distributed, sponsors should consult with the Agency.

- Abundance of the attribute: Because of the inherent heterogeneity present in protein products, an attribute of the reference product that may pose a high risk when the attribute is present in high abundance (e.g., percent aggregation or percent oxidation) may pose a significantly lower risk (or negligible risk) if the attribute is low-abundance. The abundance of the attribute should be confirmed in both the reference product (as determined by the proposed product sponsor's analysis of the reference product) and the proposed product. Limit assays do not necessarily need to be evaluated using QR; however, the selected limits regarding the amount of an attribute should be defined and justified. The justification should also include consideration of how the amount of the attribute changes over time.

- Sensitivity of assay used for assessing an attribute: Although multiple, orthogonal assays are encouraged for assessing an attribute, not all assays assessing the attribute need to be evaluated in the same manner. While the most sensitive assay for detecting product differences should be evaluated using QR, it may be appropriate to evaluate the results of other assays for the same attribute using a graphical comparison. A justification should be provided for the method of evaluation used for each type of assay.

- Types of attributes/assays: Quantitative analyses may not be applicable to some attributes, (e.g., protein sequence or certain assays used for higher order structure evaluation, or to assays that are only qualitative). The comparative analytical assessment plan should clearly define specific assays where quantitative data analyses would not be applied, and the rationale for that decision.

- Publicly available information: Publicly available information may be relevant to the appropriate type of data analysis and acceptance criteria in the comparative analytical assessment. A sponsor should seek additional advice from the Agency on the inclusion of any publicly available information in the comparative analytical assessment.

For qualitative analyses of lower risk attributes, FDA recommends side-by-side data presentation (e.g., spectra, thermograms, graphical representation of data), to allow for a visual comparison of the proposed product to the reference product.

The final comparative analytical assessment plan should include the risk ranking of attributes, the type of data evaluation to be used for each attribute/assay, and the final data analysis plan. The plan should specify the anticipated availability of both proposed biosimilar and reference product lots for evaluation of each attribute/assay and should include a rationale for why the proposed number of lots should be considered sufficient for the evaluation. The comparative analytical assessment plan should be discussed with the Agency as early in the biosimilar development program as possible so that agreement can be reached on which attributes/assays should be evaluated. The final comparative analytical assessment plan should be submitted to the Agency prior to initiating the final analytical assessments; typically, this occurs in a meeting with the Agency.

C. Comparative Analytical Assessment Conclusions

In the comparative analytical assessment, risk ranking and data analysis are used to evaluate a large number of attributes, often using multiple orthogonal assays. FDA evaluates the totality of the analytical data; if the results of a particular assay do not meet pre-specified criteria, this alone does not preclude a demonstration of high similarity. For example, if differences between products are observed as part of the comparative analytical assessment (including the components of the assessment that were not included in the risk ranking), the sponsor may provide additional scientific information (risk assessment and additional data) and a justification for why these differences do not preclude a demonstration that the products are highly similar.

In certain situations, changes to the manufacturing process of the biosimilar product may be needed to resolve differences observed in the comparative analytical assessment. Data should be provided demonstrating that the observed differences were resolved by any manufacturing changes, and that other quality attributes were not substantially affected. If other attributes were affected by the manufacturing change, data should be provided to demonstrate that the impact of the change has been evaluated and addressed.

VII. CONCLUSION

The foundation for an assessment and a 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 biosimilar to the reference product thus involves robust characterization of the proposed product, including comparative physicochemical and functional studies with the reference product. The information gained from these studies 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 PK or PD), unless the Agency determines that an element is unnecessary in a particular 351(k) application.[31] A sponsor's ability to discern and understand the impact of relevant analytical differences between the proposed product and its reference product is critical to determine whether the statutory standard for biosimilarity can be met.

VIII. RELEVANT GUIDANCES

The following draft and final guidance documents may be relevant to sponsors developing or considering development of a proposed biosimilar product. All Agency guidance documents are available on FDA's web page (https://www.fda.gov/regulatory-information/search-fda-guidance-documents).

1. Guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (April 2015)

2. Guidance for industry *Questions and Answers on Biosimilar Development and the BPCI Act* (December 2018)

3. Draft guidance for industry *New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 2)* (December 2018)

4. Draft guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA Products* (June 2018)

5. Guidance for industry *Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product* (December 2016)

6. *Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products* (April 1996)

7. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (February 1997)

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* (August 1996)

[31] Section 351(k)(2)(A)(i)(I) of the PHS Act.

9. Guidance for industry *Cooperative Manufacturing Arrangements for Licensed Biologics* (November 2008)

10. ICH guidance for industry *M4: The CTD —Quality* (ICH M4Q) (August 2001)

11. ICH guidance for industry *Q1A(R2) Stability Testing of New Drug Substances and Products* (ICH Q1A(R2)) (November 2003)

12. ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and Methodology* (ICH Q2(R1) (November 2005)

13. ICH guidance for industry *Q2B Validation of Analytical Procedures: Methodology* (ICH Q2B) (May 1997)

14. ICH guidance for industry *Q3A(R) Impurities in New Drug Substances* (ICH Q3A(R)) (June 2008)

15. ICH guidance for industry *Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (ICH Q5A) (September 1998)

16. ICH guidance for industry *Q5B Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products* (ICH Q5B) (February 1996)

17. ICH guidance for industry *Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (ICH Q5C) (July 1996)

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) (September 1998)

19. ICH guidance for industry *Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process* (ICH Q5E) (June 2005)

20. ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (ICH Q6B) (August 1999)

21. ICH guidance for industry *Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients* (ICH Q7) (September 2016)

22. ICH guidance for industry *Q8(R2) Pharmaceutical Development* (ICH Q8(R2)) (November 2009)

23. ICH guidance for industry *Q9 Quality Risk Management* (ICH Q9) (June 2006)

24. ICH guidance for industry *Q10 Pharmaceutical Quality System* (ICH Q10) (April 2009)

25. ICH guidance for industry *Q11 Development and Manufacture of Drug Substances* (ICH Q11) (November 2012)

26. ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (ICH S6(R1)) (May 2012)

GLOSSARY[32]

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."[33]

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.[34]

[32] For additional information on the Agency's interpretation of certain terms relevant to implementation of the BPCI Act, see the draft guidance for industry *New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 2)* (December 2018). When final, this guidance will represent FDA's current thinking on this topic.

[33] Section 351(i)(2) of the PHS Act.

[34] Section 351(i)(4) of the PHS Act.