
Guidance for Industry Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

DRAFT GUIDANCE

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

**February 2012
Biosimilarity**

Guidance for Industry Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

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1 **Guidance for Industry¹**
2 **Quality Considerations in Demonstrating Biosimilarity to a**
3 **Reference Protein Product**
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6
7 This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current
8 thinking on this topic. It does not create or confer any rights for or on any person and does not operate to
9 bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of
10 the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA
11 staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call
12 the appropriate number listed on the title page of this guidance.
13

14
15
16 **I. INTRODUCTION**
17

18 This guidance describes the Agency's current thinking on factors to consider when
19 demonstrating that a proposed protein product is highly similar to a reference product licensed
20 under section 351(a) of the Public Health Service Act (PHS Act) for purposes of submitting a
21 marketing application under section 351(k) of the PHS Act. Specifically, the guidance is
22 intended to provide recommendations to applicants on the scientific and technical information of
23 the chemistry, manufacturing, and controls (CMC) section of a marketing application for a
24 proposed biosimilar product submitted under section 351(k) of the PHS Act.
25

26 The Biologics Price Competition and Innovation Act of 2009 (BPCI Act) amends the PHS Act
27 and other statutes to create an abbreviated licensure pathway in section 351(k) of the PHS Act
28 for biological products shown to be biosimilar to, or interchangeable with, an FDA-licensed
29 biological reference product (see sections 7001 through 7003 of the Patient Protection and
30 Affordable Care Act (Pub. L. 111-148) (Affordable Care Act)). The BPCI Act also amended the
31 definition of biological products to include "protein (except any chemically synthesized
32 polypeptide)" (see section 351(i)(1) of the PHS Act). A 351(k) application for a proposed
33 biosimilar product must include information demonstrating biosimilarity, based on data derived
34 from, among other things, "analytical studies that demonstrate that the biological product is
35 highly similar to the reference product notwithstanding minor differences in clinically inactive
36 components."²
37

38 Although the 351(k) pathway applies generally to biological products, this guidance focuses on
39 therapeutic protein products and provides an overview of analytical factors to consider in
40 demonstrating biosimilarity between a proposed protein product and the reference product.
41

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

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

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

- 44
- 45 • Quality Considerations in Demonstrating Biosimilarity to a Reference Protein
- 46 Product
- 47 • Scientific Considerations in Demonstrating Biosimilarity to a Reference Product
- 48 • Biosimilars: Questions and Answers Regarding Implementation of the Biologics
- 49 Price Competition and Innovation Act of 2009
- 50

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

52

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

58

59

60 **II. BACKGROUND**

61

62 In the 1980s, FDA began to receive marketing applications for biotechnology-derived protein
63 products, mostly for recombinant DNA-derived versions of a naturally sourced product. In light
64 of these applications, FDA established a regulatory approach for the approval of recombinant
65 DNA-derived protein products, which it announced in a policy document published on June 26,
66 1986 (51 FR 23309), in conjunction with a 1985 document titled *Points to Consider in the*
67 *Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA*
68 *Technology*. The policy requires the submission of an investigational new drug application
69 (IND) to FDA for evaluation before initiation of clinical investigations in human subjects and
70 submission and approval of a new drug application (NDA) or biologics license application
71 (BLA) “before marketing products made with recombinant DNA technology, even if the active
72 ingredient in the product is thought to be identical to a naturally occurring substance or a
73 previously approved product” (51 FR 23309). The policy set forth in those documents was
74 developed in part because of the challenges in evaluating protein products solely by
75 physicochemical and functional testing and because the biological system in which a protein
76 product is produced can have a significant effect on the structure and function of the product
77 itself. Due to the complexities of protein products, FDA has, as a matter of policy, generally
78 required submission of an NDA in accordance with section 505(b)(1) of the FD&C Act or a BLA
79 in accordance with section 351(a) of the PHS Act containing product-specific full safety and
80 efficacy data for recombinant DNA-derived protein drugs. FDA has recognized, however, that
81 “[i]n some instances complete new applications may not be required.” (51 FR 23309).

82

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

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

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

92
93 Since 1996, FDA has approved many manufacturing process changes for licensed biological
94 products, based on a demonstration of product comparability before and after the process change,
95 as supported by quality criteria and analytical testing and without the need for additional
96 nonclinical data and clinical safety and/or efficacy studies. In some cases, uncertainty about the
97 effect of the change and/or the results of the biochemical/functional comparability studies has
98 necessitated assessment of additional data, including nonclinical and/or clinical testing, to
99 demonstrate product comparability.

100
101 These concepts were further developed in the International Conference on Harmonisation of
102 Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and resulted
103 in the Q5E guidance on *Comparability of Biotechnological/Biological Products Subject to*
104 *Changes in their Manufacturing Process*. Although the scope of ICH Q5E is limited to an
105 assessment of the comparability of a biological product before and after a manufacturing process
106 change made by the same manufacturer, certain general scientific principles described in ICH
107 Q5E are applicable to an assessment of biosimilarity between a proposed biosimilar protein
108 product and its reference product. However, demonstrating that a proposed protein product is
109 biosimilar to an FDA-licensed reference product manufactured by a different manufacturer may
110 require more extensive and comprehensive data than assessing the comparability of a product
111 before and after a manufacturing process change made by the product's sponsor. Unlike a
112 manufacturer who modifies its own manufacturing process with extensive knowledge and
113 information about the product and the existing process, including established controls and
114 acceptance parameters, the manufacturer of a proposed biosimilar product will likely have a
115 different manufacturing process (e.g., different cell line, raw materials, equipment, processes,
116 process controls, acceptance criteria) from that of the reference product and no direct knowledge
117 of the manufacturing process for the reference product.

118
119 In October 1999, FDA issued a draft guidance for industry on *Applications Covered by Section*
120 *505(b)(2)*, which, among other things, stated that FDA may accept an application submitted
121 through the approval pathway described by section 505(b)(2) of the FD&C Act for a drug
122 product containing an active ingredient(s) derived from natural sources or recombinant DNA
123 technology. For example, FDA approved a 505(b)(2) application for a follow-on recombinant
124 DNA-derived human growth hormone product in May 2006. Greater knowledge due to
125 advances in science and technology, and improvements in manufacturing processes, process
126 controls, materials and product testing, as well as characterization tests and studies, facilitate the
127 use of an abbreviated pathway for the approval of a protein product.

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129 The BPCI Act was enacted as part of the Affordable Care Act on March 23, 2010.⁴ The BPCI
130 Act creates an abbreviated licensure pathway for biological products demonstrated to be
131 biosimilar to, or interchangeable with, a reference product. Section 351(k) of the PHS Act (42
132 U.S.C. 262(k)), added by the BPCI Act, sets forth the requirements for an application for a
133 proposed biosimilar product and an application or a supplement for a proposed interchangeable
134 product.

135
136 Section 351(i) of the PHS Act defines biosimilarity to mean that the biological product is highly
137 similar to the reference product notwithstanding minor differences in clinically inactive
138 components and that there are no clinically meaningful differences between the biological
139 product and the reference product in terms of the safety, purity, and potency of the product (see
140 section 351(i)(2) of the PHS Act).

141
142 To meet the higher standard of “interchangeability,” an applicant must provide sufficient
143 information to demonstrate biosimilarity, and also to demonstrate that the biological product can
144 be expected to produce the same clinical result as the reference product in any given patient and,
145 if the biological product is administered more than once to an individual, the risk in terms of
146 safety or diminished efficacy of alternating or switching between the use of the biological
147 product and the reference product is not greater than the risk of using the reference product
148 without such alternation or switch (see section 351(k)(4) of the PHS Act).

149
150 Analytical studies provide the foundation for an assessment of the proposed protein product
151 intended for submission in a 351(k) application under the PHS Act and whether it is highly
152 similar to the reference product.

153

154

III. SCOPE

156

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

169

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

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

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

179
180 In addition to comparative analytical studies, an assessment of whether a proposed product is
181 biosimilar to a reference product generally will include animal studies (including the assessment
182 of toxicity) and a clinical study or studies (including the assessment of immunogenicity and
183 pharmacokinetics and/or pharmacodynamics).⁶

184
185 This guidance applies to applications submitted under section 351(k) of the PHS Act. However,
186 some scientific principles described in this guidance may be informative for the development of
187 certain biological products under section 505(b)(2) of the FD&C Act.⁷ Section 505(b)(2) of the
188 FD&C Act and section 351(k) of the PHS Act are two separate statutory schemes. This guidance
189 is not intended to describe any relationship between the standards for approval under these
190 schemes.

191

192

193 IV. DEFINITIONS

194

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

196

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

199

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

202

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

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

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

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203 *Biosimilar or biosimilarity* means that “the biological product is highly similar to the
204 reference product notwithstanding minor differences in clinically inactive components,”
205 and “there are no clinically meaningful differences between the biological product and
206 the reference product in terms of the safety, purity, and potency of the product.”⁸
207

208 *Product*, when used without modifiers, is intended to refer to intermediates, drug
209 substance, and/or drug product, as appropriate. The use of the term “product” is
210 consistent with the use of the term in ICH Q5E.
211

212 *Reference product* means the single biological product licensed under section 351(a) of
213 the PHS Act against which a biological product is evaluated in a 351(k) application.
214

V. GENERAL PRINCIPLES

215
216
217
218 Advances in analytical sciences (both physicochemical and biological) enable some protein
219 products to be characterized extensively in terms of their physicochemical and biological
220 properties. These analytical procedures have improved the ability to identify and characterize
221 not only the desired product but also product-related substances and product- and process-related
222 impurities.⁹ Advances in manufacturing science and production methods may enhance the
223 likelihood that a product will be highly similar to another product by better targeting the original
224 product’s physicochemical and functional properties.
225

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

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

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

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

261

262 The three dimensional conformation of a protein is an important factor in its biological function.
263 Proteins generally exhibit complex three-dimensional conformations (tertiary structure and, in
264 some cases, quaternary structure) due to their large size and the rotational characteristics of
265 protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein
266 conformation over time, some of which may be absolutely required for functional activity.

267 These rotations are often dependent on low-energy interactions, such as hydrogen bonds and van
268 der Waals forces, which may be very sensitive to environmental conditions. Current analytical
269 technology is capable of evaluating the three-dimensional structure of many proteins. Methods
270 such as X-ray crystallography and multi-dimensional nuclear magnetic resonance (NMR)
271 spectroscopy can help define tertiary protein structure and, to varying extents, quaternary
272 structure, and can add to the body of information supporting biosimilarity. At the same time, a
273 protein's three-dimensional conformation can often be difficult to define precisely using current
274 physicochemical analytical technology. Any differences in higher order structure between a
275 proposed biosimilar and a reference product should be evaluated in terms of a potential effect on
276 protein function. Thus, functional assays are also critical tools for evaluating the integrity of the
277 higher order structures.

278

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

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

303
304 An extensive analytical characterization may also reveal differences between the reference
305 product and the proposed biosimilar product, especially when using analytical techniques
306 capable of discriminating qualitative or quantitative differences in product attributes. Emphasis
307 should be placed on developing orthogonal, quantitative methods to more definitively distinguish
308 any differences in product attributes. If the results show highly similar functional and
309 physicochemical characteristics, including, for example, higher order structure, post-translational
310 modifications, and impurity and degradation profiles, the sponsor may have an appropriate
311 scientific basis for a selective and targeted approach to subsequent animal and/or clinical studies
312 to support a demonstration of biosimilarity. It may be useful to compare differences in the
313 quality attributes of the proposed protein product with those of the reference product using a
314 meaningful fingerprint-like analysis algorithm that covers a large number of additional product
315 attributes and their combinations with high sensitivity using orthogonal methods. Advances in
316 manufacturing science and Quality-by-Design approaches may facilitate production processes
317 that can better match a reference product's fingerprint.¹⁰ Such a strategy could further quantify
318 the overall similarity between two molecules and may lead to additional bases for a more
319 selective and targeted approach to subsequent animal and/or clinical studies.

320
321 The type, nature, and extent of any differences between the proposed biosimilar product and the
322 reference product, introduced by design or observed from comprehensive analytical
323 characterization of multiple manufacturing lots, should be clearly described and discussed. The
324 discussion should include identification and comparison of relevant quality attributes from
325 product characterization, as this is an important factor in assessing whether the proposed
326 biosimilar product is highly similar to the reference product. The potential effect of the
327 differences on safety, purity, and potency should be addressed and supported by appropriate data.

328
329 The type and extent of nonclinical or clinical studies that are needed to demonstrate biosimilarity
330 of the proposed biosimilar product can be influenced by several factors, especially the ability to
331 discern differences and their potential effect on safety, purity, and potency. For example, factors
332 such as the ability to robustly characterize the proposed biosimilar product or the reference
333 product (*e.g.*, lack of suitable or sufficiently discriminative analytical techniques) or availability
334 of a relevant drug substance derived from the reference product could impact the nature of the
335 subsequent nonclinical or clinical studies. In addition, if the proposed biosimilar product or

¹⁰ See ICH Q8(R2) for guidance.

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

339
340 In general, a sponsor needs to provide information to demonstrate biosimilarity based on data
341 directly comparing the proposed protein product with the reference product. Analytical studies
342 intended to support a demonstration of biosimilarity for purposes of section 351(k) of the PHS
343 Act must as a scientific matter include an adequate comparison to the reference product licensed
344 under section 351(a). However, under certain circumstances, a sponsor may seek to use data
345 derived from animal or clinical studies comparing a proposed protein product with a non-U.S.-
346 licensed product to address, in part, the requirements under section 351(k)(2)(A) of the PHS Act.
347 In such a case, the sponsor should provide adequate data or information to scientifically justify
348 the relevance of this comparative data to an assessment of biosimilarity and to establish an
349 acceptable bridge to the U.S.-licensed reference product.¹¹ The scientific bridge between the
350 non-U.S.-licensed product and the U.S.-licensed reference product is likely to include
351 comparative physico-chemical characterization, bioassays/functional assays, and comparative
352 clinical and/or nonclinical PK and/or PD data, as appropriate, and data to address any differences
353 in formulation or primary packaging. Sponsors are encouraged to discuss with FDA during the
354 development program the adequacy of the scientific justification and bridge to the U.S.-licensed
355 reference product; a final determination of the adequacy of the information will be made by FDA
356 during review of the 351(k) application.

357
358 **VI. FACTORS FOR CONSIDERATION IN ASSESSING WHETHER PRODUCTS**
359 **ARE HIGHLY SIMILAR**

360
361 When assessing whether products are highly similar, manufacturers should consider a number of
362 factors, including the following.

363
364 **A. Expression System**

365
366 Therapeutic protein products can be produced by microbial cells (prokaryotic, eukaryotic), cell
367 lines of human or animal origin (e.g., mammalian, avian, insect), or tissues derived from animals
368 or plants. It is expected that the expression construct for a proposed biosimilar product will
369 encode the same primary amino acid sequence as its reference product. However, minor
370 modifications, such as N or C terminal truncations that will not have an effect on safety, purity,
371 or potency, may be justified by the applicant. Differences between the chosen expression system
372 of the proposed biosimilar product and that of the reference product should be carefully
373 considered because the type of expression system and host cell will significantly affect the types
374 of process- and product-related substances and impurities (including potential adventitious
375 agents) that may be present in the protein product. For example, the expression system can have
376 a significant effect on the types and extent of translational and post-translational modifications

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

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

B. Manufacturing Process

384
385
386
387 A comprehensive understanding of all steps in the manufacturing process for the proposed
388 biosimilar product should be established during product development. Characterization tests,
389 process controls, and specifications that will emerge from information gained during process
390 development must be specific for the proposed biosimilar product and manufacturing process.
391 The use of Quality-by-Design approaches to pharmaceutical development, along with quality
392 risk management and effective quality systems, will facilitate the consistent manufacturing of a
393 high-quality product. A type II Drug Master File (DMF) would not be acceptable for a 351(k)
394 application because, as with 351(a) BLAs, the license holder needs to have knowledge of and
395 control over the manufacturing process for the biological product.¹² Other types of contract
396 manufacturing arrangements can be considered if the applicant does not intend to manufacture
397 the product for licensure.¹³

C. Assessment of Physicochemical Properties

398
399
400
401 Physicochemical assessment of the proposed biosimilar product and the reference product should
402 consider all relevant characteristics of the protein product (*e.g.*, the primary, secondary, tertiary,
403 and quaternary structure, post-translational modifications, and functional activity(ies)). The
404 objective of this assessment is to maximize the potential for detecting differences in quality
405 attributes between the proposed biosimilar product and the reference product.

406
407 The applicant should address the concept of the desired product (and its variants) as defined in
408 ICH Q6B when designing and conducting the characterization studies. Thus, it will be important
409 to understand the heterogeneity of the proposed biosimilar product and the reference product
410 (*e.g.*, the nature, location, and levels of glycosylation) and the ranges of variability of different
411 isoforms, including those that result from post-translational modifications.

412

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

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

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

429
430 Tests used to characterize the product do not necessarily need to be validated for routine quality
431 control purposes, but should be scientifically sound, fit for their intended use, and provide results
432 that are reproducible and reliable. In selecting these tests, it is important to consider the
433 characteristics of the protein product, including known and potential impurities. Information
434 regarding the ability of a method to discern relevant differences between a proposed biosimilar
435 product and a reference product should be submitted as part of the comparison.

436
437 Tests chosen to detect and characterize these post-translational protein modifications should be
438 demonstrated to be of appropriate sensitivity and specificity to provide meaningful information
439 as to whether the proposed biosimilar product and the reference product are highly similar.

D. Functional Activities

440
441
442
443 Functional assays serve multiple purposes in the characterization of protein products. These tests
444 act to complement physicochemical analyses and are a quality measure of the function of the
445 protein product.

446
447 Depending on the structural complexity of the protein and available analytical technology, the
448 physicochemical analysis may be unable to confirm the integrity of the higher order structures.
449 Instead, the integrity of such structures can be inferred from the product's biological activity. If
450 the clinically relevant mechanism(s) of action are known for the reference product or can
451 reasonably be determined, one or more of the functional assays should reflect these mechanisms
452 of action to the extent possible. The assessment of functional activity is also useful in providing
453 an estimate of the specific activity of a product, as an indicator of manufacturing process
454 consistency, as well as product purity and stability.

455

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

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

463
464 The manufacturer should recognize the potential limitations of some types of functional assays,
465 such as high variability, that might preclude detection of small but significant differences
466 between the proposed biosimilar product and the reference product. As a highly variable assay
467 may not provide a meaningful assessment as to whether the proposed biosimilar product is
468 highly similar to the reference product, applicants are encouraged to develop assays that are
469 sensitive to changes in the functional activities of the product. In addition, *in vitro* bioactivity
470 assays may not fully reflect the clinical activity of the protein. For example, these assays
471 generally do not predict the bioavailability (PK and biodistribution) of the product. These
472 factors can impact PD and clinical performance. Also, bioavailability can be dramatically
473 altered by subtle differences in glycoform distribution or other post-translation modifications.
474 Thus, these limitations should be taken into account when assessing the robustness of the quality
475 of data supporting biosimilarity and the need for additional information. Finally, functional
476 assays are critical in assessing the occurrence of neutralizing antibodies in nonclinical and
477 clinical studies.

E. Receptor Binding and Immunochemical Properties

478
479
480
481 When binding or immunochemical properties are part of the activity attributed to the protein
482 product, analytical tests should be performed to characterize the product in terms of these
483 specific properties (*e.g.*, if binding to a receptor is inherent in protein function, this property
484 should be measured and used in comparative studies, see ICH Q6B for additional details).
485 Various methods such as surface plasmon resonance, microcalorimetry, or classical Scatchard
486 analysis can provide information on the kinetics and thermodynamics of binding. Such
487 information can be related to the functional activity and characterization of the proposed
488 biosimilar product's higher order structure.

F. Impurities

489
490
491
492 The applicant should characterize, identify, and quantify impurities (product- and process-related
493 as defined in ICH Q6B) in the proposed biosimilar product and the reference product. If
494 comparative physicochemical analysis reveals comparable product-related impurities at similar
495 levels between the two products, pharmacological/toxicological studies to characterize potential
496 biological effects of specific impurities may not be necessary. However, if the manufacturing
497 process used to produce the proposed biosimilar product introduces different impurities or higher
498 levels of impurities than those present in the reference product, additional
499 pharmacological/toxicological or other studies may be necessary. As discussed in ICH S6, “[i]t

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500 is preferable to rely on purification processes to remove impurities . . . rather than to establish a
501 preclinical testing program for their qualification.”¹⁵

502
503 Process-related impurities arising from cell substrates (*e.g.*, host cell DNA, host cell proteins),
504 cell culture components (*e.g.*, antibiotics, media components), and downstream processing steps
505 (*e.g.*, reagents, residual solvents, leachables, endotoxin, bioburden) should be evaluated. The
506 potential impact of differences in the impurity profile upon safety should be addressed and
507 supported by appropriate data. In all cases, the chosen analytical procedures should be adequate
508 to detect, identify, and accurately quantify biologically significant levels of impurities (see ICH
509 Q2B). In particular, the results of the immunological methods used to detect host cell proteins
510 depend on the assay reagents and the cell substrate used. Such assays should be validated using
511 the product cell substrate and orthogonal methodologies to ensure accuracy and sensitivity. This
512 should be done across both products to the extent relevant and feasible.¹⁶

513
514 The safety of the proposed biosimilar product, as with any biological product, with regard to
515 adventitious agents or endogenous viral contamination should be ensured by screening critical
516 raw materials and confirmation of robust virus removal and inactivation achieved by the
517 manufacturing process (see ICH Q5A for guidance).

G. Reference Product and Reference Standards

518
519
520
521 A thorough physicochemical and biological assessment of the reference product should provide a
522 base of information from which to develop the proposed biosimilar product and justify reliance
523 on certain existing scientific knowledge about the reference product. Sufficient evidence that the
524 proposed biosimilar product is highly similar to the reference product must be demonstrated in
525 an appropriate time frame to support a selective and targeted approach in early product
526 development (*e.g.*, reduced nonclinical studies, and/or dose-finding clinical studies).¹⁷ To justify
527 a selective and targeted approach to a clinical program, a comprehensive physicochemical and
528 functional comparison to the reference product should be performed during early product
529 development and discussed with the appropriate FDA staff. An analytical similarity assessment
530 should support the use of lots that demonstrate the biosimilarity of the proposed biosimilar
531 product used in the principal clinical trial to the reference product and the proposed commercial
532 product. The biosimilar application should include a thorough analytical comparison between
533 the proposed biosimilar product and the reference product. In addition, even when multiple
534 approved products are on the market, a sponsor must demonstrate that the proposed product is
535 biosimilar to a single reference product that previously has been licensed by FDA.

536
537 If the drug substance has been extracted from the reference product in order to assess analytical
538 similarity, the applicant should describe the extraction procedure and provide support that the
539 procedure itself does not alter product quality. This undertaking would include consideration for

¹⁵ See ICH S6, page 2.

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

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

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540 alteration or loss of the desired products and impurities and relevant product-related substances,
541 and it should include appropriate controls that ensure the relevant product characteristics of the
542 reference product are not significantly altered by the extraction procedure.
543

544 If there is a suitable, publicly available and well-established reference standard for the protein,
545 then a physicochemical and/or functional comparison of the proposed biosimilar product with
546 this standard should also be performed. For example, if an international standard for calibration
547 of potency is available, a comparison of the relative potency of the proposed biosimilar product
548 with this potency standard should be performed. As is recommended in ICH Q6B, an in-house
549 reference standard(s) should always be qualified and used for control of the manufacturing
550 process and product.
551

552 In summary, analytical studies carried out to support the approval of a proposed biosimilar
553 product should not focus solely on the characterization of the proposed biosimilar product in
554 isolation. Rather, these studies should be part of a broad comparison that includes, but is not
555 limited to, the proposed biosimilar product, the reference product, applicable reference standards,
556 and consideration of relevant publicly available information.
557

H. Finished Drug Product

558
559
560 Product characterization studies should be performed on the most downstream intermediate best
561 suited for the analytical procedures used. The attributes evaluated should be stable through any
562 further processing steps. For these reasons, characterization studies are often performed on bulk
563 drug substance.¹⁸ However if bulk drug substance is reformulated and/or exposed to new
564 materials in the finished dosage form, the impact of these changes should be considered.
565

566 If the finished drug product is best suited for a particular analysis, the characterization should
567 compare the proposed finished biosimilar product and the finished reference product. If an
568 analytical method more sensitively detects specific attributes in the drug substance, but the
569 attributes it measures are critical and/or may change during manufacture of the finished drug
570 product, comparative characterization may be called for on both the isolated drug substance and
571 the finished drug product.
572

573 The acceptability of the type, nature, and extent of any differences between the proposed finished
574 biosimilar product and the finished reference product should be evaluated and supported by
575 appropriate data and rationale. Additionally, different excipients in the proposed product should
576 be supported by existing toxicology data for the excipient or by additional toxicity studies with
577 the formulation of the proposed biosimilar product. Excipient interactions as well as direct
578 toxicities should be considered. Proteins are very sensitive to their environment. Therefore,
579 differences in excipients or primary packaging may affect product degradation and/or clinical
580 performance. Differences in formulation between the proposed biosimilar product and the
581 reference product are among the factors that may affect whether subsequent clinical studies may
582 take a selective and targeted approach.
583

¹⁸ See 21 CFR 207.3.

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584 **I. Stability**

585
586 An appropriate physicochemical and functional comparison of the stability of the proposed
587 biosimilar product with that of the reference product should be initiated. Accelerated and stress
588 stability studies, or forced degradation studies, should be used to establish degradation profiles
589 and provide direct comparison of the proposed biosimilar product with the reference product.
590 These comparative studies should be conducted under multiple stress conditions (e.g., high
591 temperature, freeze thaw, light exposure, and agitation) that can cause incremental product
592 degradation over a defined time period. Results of these studies may reveal product differences
593 that warrant additional evaluation and also identify conditions under which additional controls
594 should be employed in manufacturing and storage (see ICH Q5C and Q1A(R) for guidance).
595 Sufficient real time, real condition stability data should be provided to support the proposed
596 dating period.

597 598 599 **VII. CONCLUSION**

600
601 The foundation for an assessment of biosimilarity between a proposed biosimilar product and its
602 reference product involves the robust characterization of the proposed biosimilar product,
603 including comparative physicochemical and functional studies. The information gained from
604 these studies is critical to the overall product assessment that as a scientific matter is necessary
605 for the development of a proposed biosimilar product. In addition, a 351(k) application for a
606 proposed biosimilar product must contain, among other things, information demonstrating
607 biosimilarity based upon data derived from animal studies (including the assessment of toxicity)
608 and a clinical study or studies (including the assessment of immunogenicity and
609 pharmacokinetics or pharmacodynamics), unless the Agency determines that an element is
610 unnecessary in a particular 351(k) application. The ability to discern relevant differences
611 between the proposed product and its reference product will depend on the available analytical
612 technology and complexity of the product. Any information regarding differences between the
613 proposed product and the reference product should be considered to determine whether the
614 statutory standard for biosimilarity can be met.

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VIII. RELEVANT GUIDANCES

617
618
619 The following guidance documents may be relevant to sponsors developing or considering
620 development of a biosimilar product candidate. All Agency guidance documents are available
621 on FDA's Web page (<http://www.fda.gov/RegulatoryInformation/Guidances/default.htm>).
622

- 623
624 1. *Draft Guidance for Industry on Scientific Considerations in Demonstrating Biosimilarity*
625 *to a Reference Product* (issued jointly by CDER and CBER, February 2012)
626
627 2. *Draft Guidance for Industry, Biosimilars: Questions and Answers Regarding*
628 *Implementation of the Biologics Price Competition and Innovation Act of 2009* (issued
629 jointly by CDER and CBER, February 2012)
630
631 3. *FDA Guidance Concerning Demonstration of Comparability of Human Biological*
632 *Products, Including Therapeutic Biotechnology-Derived Products* (issued jointly by
633 CDER and CBER, April 1996)
634
635 4. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for*
636 *Human Use* (issued by CBER, February 1997)
637
638 5. *Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls*
639 *Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal*
640 *Antibody Product for In Vivo Use* (issued jointly by CDER and CBER, August 1996)
641
642 6. *FDA Guidance on Cooperative Manufacturing Arrangements for Licensed Biologics*
643 (issued jointly by CDER and CBER, November 2008).
644
645 7. ICH M4Q *The Common Technical Document*
646
647 8. ICH Q2 *Text on Validation of Analytical Procedures*
648
649 9. ICH Q2B *Validation of Analytical Procedures: Methodology*
650
651 10. ICH Q3A *Impurities in New Drug Substances*
652
653 11. ICH Q5A *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of*
654 *Human or Animal Origin*
655
656 12. ICH Q5B *Quality of Biotechnological Products: Analysis of the Expression Construct in*
657 *Cells Used for Production of r-DNA Derived Protein Products*
658
659 13. ICH Q5C *Stability Testing of Biotechnological/Biological Products*
660

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- 661 14. ICH Q5D *Quality of Biotechnological/Biological Products: Derivation and*
662 *Characterization of Cell Substrates Used for Production of Biotechnological/Biological*
663 *Products*
664
- 665 15. ICH Q5E *Comparability of Biotechnological/Biological Products Subject to Changes in*
666 *Their Manufacturing Process*
667
- 668 16. ICH Q6B *Specifications: Test Procedures and Acceptance Criteria for*
669 *Biotechnological/Biological Products*
670
- 671 17. ICH Q7 *Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients*
672
- 673 18. ICH Q8 *Pharmaceutical Development*
674
- 675 19. ICH Q9 *Quality Risk Management*
676
- 677 20. ICH Q10 *Pharmaceutical Quality System*
678
- 679 21. ICH S6 *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*