
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DRAFT GUIDANCE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.

**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)**

**May 2019
Biosimilars**

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

36
37 This guidance is one in a series of guidances that FDA is developing to facilitate implementation
38 of the BPCI Act.

39
40 Relevant final guidance documents⁴ issued to date address a broad range of issues, including:

- 41
42 • *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*
43 (April 2015)
- 44 • *Questions and Answers on Biosimilar Development and the BPCI Act* (December
45 2018)
- 46 • *Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a*
47 *Reference Product* (December 2016)
- 48 • *Labeling for Biosimilar Products* (July 2018)
- 49 • *Considerations in Demonstrating Interchangeability With a Reference Product*
50 (May 2019)

51
52 In addition, FDA has published draft guidance documents related to the BPCI Act, which, when
53 finalized, will represent FDA’s current thinking. These draft guidance documents include:

- 54
55 • *Formal Meetings Between the FDA and Sponsors or Applicants of BsUFA*
56 *Products* (June 2018)
- 57 • *Reference Product Exclusivity for Biological Products Filed Under Section*
58 *351(a) of the PHS Act* (August 2014)
- 59 • *New and Revised Draft Q&As on Biosimilar Development and the BPCI Act*
60 *(Revision 2)* (December 2018)

61

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>.

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62 When applicable, references to information in these final and draft guidances are included in this
63 guidance.

64
65 In general, FDA’s guidance documents do not establish legally enforceable responsibilities.
66 Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only
67 as recommendations, unless specific regulatory or statutory requirements are cited. The use of
68 the word *should* in Agency guidances means that something is suggested or recommended, but
69 not required.

70
71

72 **II. BACKGROUND**

73

74 In the 1980s, FDA began to receive marketing applications for biotechnology-derived protein
75 products, mostly for recombinant DNA-derived versions of naturally sourced products.
76 Consequently, FDA established a regulatory approach for the approval of recombinant DNA-
77 derived protein products, which was announced in the *Federal Register* (51 FR 23302, June 26,
78 1986), in conjunction with a 1985 document titled *Points to Consider in the Production and*
79 *Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology*.⁵ This
80 approach addresses the submission of an investigational new drug application (IND) to FDA for
81 evaluation before initiation of clinical investigations in human subjects and submission and
82 potential approval of a new drug application (NDA) or biologics license application (BLA)
83 before marketing products made with recombinant DNA technology, even if the active
84 ingredient in the product is thought to be identical to a naturally occurring substance or a
85 previously approved product. The policy set forth in those documents was developed in part
86 because of the challenges in evaluating protein products solely by physicochemical and
87 functional testing and because the biological system in which such a protein product is produced
88 can have a significant effect on the structure and function of the product itself.

89

90 Improvements in manufacturing processes, process controls, materials, and product testing, as
91 well as characterization tests and studies, have led to a gradual evolution in the regulation of
92 protein products. For example, in 1996, FDA provided recommendations in the *FDA Guidance*
93 *Concerning Demonstration of Comparability of Human Biological Products, Including*
94 *Therapeutic Biotechnology-derived Products*, which explains how a sponsor may demonstrate,
95 through a combination of analytical testing, functional assays (in vitro and/or in vivo),
96 assessment of pharmacokinetics (PK) and/or pharmacodynamics (PD) and toxicity in animals,
97 and clinical testing (clinical pharmacology, safety, and/or efficacy), that a manufacturing change
98 does not adversely affect the safety, identity, purity, or potency of its FDA-approved product.

99

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>.

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100 Since 1996, FDA has approved many manufacturing process changes for licensed biological
101 products based on a demonstration of product comparability before and after the process change,
102 as supported by quality criteria and analytical testing and without the need for additional
103 nonclinical data and clinical safety and/or efficacy studies. In some cases, uncertainty about the
104 effect of the change and/or the results of the biochemical/functional comparability studies has
105 necessitated collection and assessment of additional data, including nonclinical and/or clinical
106 testing, to demonstrate product comparability. These concepts were further developed in the
107 International Conference on Harmonisation of Technical Requirements for Registration of
108 Pharmaceuticals for Human Use (ICH) and resulted in the ICH guidance for industry *Q5E*
109 *Comparability of Biotechnological/Biological Products Subject to Changes in Their*
110 *Manufacturing Process* (June 2005).

111
112 Although the scope of ICH Q5E is limited to an assessment of the comparability of a biological
113 product before and after a manufacturing process change made by the same manufacturer, certain
114 general scientific principles described in ICH Q5E are applicable to an assessment of
115 biosimilarity between a proposed product and its reference product. However, demonstrating
116 that a proposed product is biosimilar to an FDA-licensed reference product manufactured by a
117 different manufacturer typically will be more complex and will likely require more extensive and
118 comprehensive data than assessing the comparability of a product before and after a
119 manufacturing process change made by the product's sponsor. A manufacturer that modifies its
120 own manufacturing process has extensive knowledge and information about the product and the
121 existing process, including established controls and acceptance parameters. By contrast, the
122 manufacturer of a proposed biosimilar will have no direct knowledge of the manufacturing
123 process for the reference product and will have its own manufacturing process (e.g., different cell
124 line, raw materials, equipment, processes, process controls, acceptance criteria).

125
126 Therefore, comprehensive comparative analytical data are necessary to build the foundation for a
127 development program for a proposed biosimilar product intended for submission under section
128 351(k) of the PHS Act.

129
130 *The BPCI Act*

131
132 The BPCI Act, enacted as part of the (ACA) on March 23, 2010, amends the PHS Act and other
133 statutes to create an abbreviated licensure pathway for biological products shown to be
134 biosimilar to, or interchangeable with, an FDA-licensed biological reference product (see
135 sections 7001 through 7003 of the ACA). Section 351(k) of the PHS Act (42 U.S.C. 262(k)),
136 added by the BPCI Act, sets forth the requirements for an application for a proposed biosimilar
137 product or a proposed interchangeable product. An application submitted under section 351(k)

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

- 140
- 141 • Analytical studies that demonstrate that the biological product is highly similar to the
142 reference product notwithstanding minor differences in clinically inactive components;
 - 143 • Animal studies (including the assessment of toxicity); and
 - 144 • A clinical study or studies (including the assessment of immunogenicity and PK or PD)
145 that are sufficient to demonstrate safety, purity, and potency in one or more appropriate
146 conditions of use for which the reference product is licensed and intended to be used and
147 for which licensure is sought for the biological product.⁶
- 148

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

151

152 The term *biosimilar* or *biosimilarity* is defined in the PHS Act “in reference to a biological
153 product that is the subject of an application under [section 351(k)]” to mean “that the biological
154 product is highly similar to the reference product notwithstanding minor differences in clinically
155 inactive components” and that “there are no clinically meaningful differences between the
156 biological product and the reference product in terms of the safety, purity, and potency of the
157 product” (section 351(i)(2) of the PHS Act). The term *reference product* is defined in the PHS
158 Act as the single biological product licensed under section 351(a) of the PHS Act against which a
159 biological product is evaluated in a 351(k) application (section 351(i)(4) of the PHS Act).

160

161 Section 351(k)(4) of the PHS Act provides that upon review of an application submitted under
162 section 351(k) or any supplement to such application, FDA will determine the biological product
163 to be interchangeable with the reference product if FDA determines that the information
164 submitted in the application (or a supplement to such application) is sufficient to show that the
165 biological product “is biosimilar to the reference product” and “can be expected to produce the
166 same clinical result as the reference product in any given patient”⁸ and that “for a biological
167 product that is administered more than once to an individual, the risk in terms of safety or
168 diminished efficacy of alternating or switching between use of the biological product and the
169 reference product is not greater than the risk of using the reference product without such
170 alternation or switch.”⁹

171

172 The term *interchangeable* or *interchangeability* is defined in the PHS Act, in reference to a
173 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.

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174 Act, to mean that “the biological product may be substituted for the reference product without
175 the intervention of the health care provider who prescribed the reference product” (section
176 351(i)(3) of the PHS Act).

177

178

179 III. SCOPE

180

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

192

193 This guidance also describes considerations for CMC information that is relevant to assessing
194 whether the proposed product is biosimilar to the reference product. It is critical that all product
195 applications contain a complete and thorough CMC section that provides the necessary and
196 appropriate information (e.g., characterization, adventitious agent safety, process controls, and
197 specifications) to support that the manufacturing process consistently delivers a product with the
198 intended quality characteristics. This guidance should be used as a companion to other
199 guidances available from FDA that describe the CMC information appropriate for evaluation of
200 protein products.¹¹ We encourage early interaction with FDA to discuss specific CMC issues
201 that may arise for a sponsor’s proposed product.

202

203

204 IV. GENERAL PRINCIPLES

205

206 Advances in analytical sciences (both physicochemical and biological) enable some protein
207 products to be characterized extensively in terms of their physicochemical and biological
208 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.

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209 not only the desired product but also product-related substances and product- and process-related
210 impurities.¹² Advances in manufacturing science and production methods may enhance the
211 likelihood that a proposed product can be demonstrated to be highly similar to a reference
212 product by better targeting the reference product's physiochemical and functional properties. In
213 addition, advances in analytical sciences may enable detection and characterization of
214 differences between the protein products. These differences should be further assessed to
215 understand the impact on the biosimilar product clinical performance relative to the reference
216 product.

217
218 Despite improvements in analytical techniques, current analytical methodology may not be able
219 to detect or characterize all relevant structural and functional differences between the two protein
220 products. A thorough understanding of each analytical method's limitations will be critical to a
221 sponsor's successful identification of residual uncertainties and, in turn, to the design of
222 subsequent testing. In addition, there may be incomplete understanding of the relationship
223 between a product's structural attributes and its clinical performance. FDA encourages the use of
224 available state-of-the-art technology. Sponsors should use appropriate analytical methodologies
225 that have adequate sensitivity and specificity to detect and characterize differences between the
226 proposed product and the reference product.

227
228 As part of a complete CMC data submission, an application submitted under section 351(k) of
229 the PHS Act is required to include analytical studies that demonstrate that the biological product
230 is highly similar to the reference product.¹³ The rationale for the approach to the comparative
231 analytical assessment should be clearly described, with consideration of the characteristics,
232 known mechanism of action(s), and function of the reference product.

233
234 Comparative analytical data provide the foundation for the development of a proposed product
235 for submission in an application under section 351(k) of the PHS Act and can influence decisions
236 about the type and amount of animal and clinical data needed to support a demonstration of
237 biosimilarity. Such analytical data should be available early in product development and will
238 permit more detailed discussion with the Agency because known quality attributes can be used to
239 shape biosimilar development and justify certain development decisions. Thus, in addition to the
240 preliminary comparative analytical data that should be submitted to support an initial advisory
241 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.

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242 in the development process: at the pre-IND stage; with the original IND submission; or with the
243 submission of data from the initial clinical studies, such as PK and PD studies. FDA will best be
244 able to provide meaningful input on the extent and scope of animal and additional clinical studies
245 for a proposed biosimilar development program once the Agency has considered the comparative
246 analytical data.

247
248 Comprehensive, robust comparative physicochemical and functional studies (these may include
249 biological assays, binding assays, and enzyme kinetics) should be performed to evaluate the
250 proposed product and the reference product. A meaningful comparative analytical assessment
251 depends on, among other things, the capabilities of available state-of-the-art analytical assays to
252 assess, for example, the molecular weight of the protein, complexity of the protein (higher order
253 structure and posttranslational modifications), degree of heterogeneity, functional properties,
254 impurity profiles, and degradation profiles denoting stability. The capability of the methods used
255 in these analytical assessments, as well as their limitations, should be described by the sponsor.
256 Physicochemical and functional characterization studies should be sufficient to establish relevant
257 quality attributes, including those that define a product's identity, quantity, safety, purity, and
258 potency. The product-related impurities and product-related substances should be identified,
259 characterized as appropriate, quantified, and compared using multiple lots of the proposed
260 product and multiple lots of the reference product, to the extent feasible and relevant, as part of
261 an assessment of the potential impact on the safety, purity, and potency of the product.

262
263 Because therapeutic proteins are made in living systems, there may be heterogeneity in certain
264 quality attributes of these products. Heterogeneity in therapeutic proteins may arise in a number
265 of ways and may affect the expected clinical performance of a protein product. Replication
266 errors in the DNA encoding the protein sequence and amino acid misincorporation may occur
267 during translation, although the level of these errors is typically low. In addition, most protein
268 products undergo posttranslational modifications that can alter the functions of the protein by
269 attaching other biochemical groups such as phosphate and various lipids and carbohydrates; by
270 proteolytic cleavage following translation; by changing the chemical nature of an amino acid
271 (e.g., formylation); or by many other mechanisms. Such modifications can result from
272 intracellular activities during cell culture or by deliberate modification of the protein (e.g.,
273 PEGylation). Other posttranslational modifications can be a consequence of manufacturing
274 process operations; for example, glycation may occur with exposure of the product to reducing
275 sugars. Also, certain storage conditions may be more or less permissive for certain degradation
276 pathways such as oxidation, deamidation, or aggregation. All of these product-related variants
277 may alter the biological properties of the expressed recombinant protein. Therefore,
278 identification and determination of the relative levels of these variants should be included in the
279 comparative analytical characterization studies.

280
281 The three-dimensional conformation of a protein is an important factor in its biological function.
282 Proteins generally exhibit complex three-dimensional conformations (tertiary structure and, in
283 some cases, quaternary structure) because of their large size and the rotational characteristics of
284 protein alpha carbons, among other things. The resulting flexibility enables dynamic, but subtle,
285 changes in protein conformation over time, some of which may be required for functional

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286 activity. These rotations are often dependent on low-energy interactions, such as hydrogen
287 bonds and van der Waals forces, which may be very sensitive to environmental conditions.
288 Current analytical technology is capable of evaluating the three-dimensional structure of many
289 proteins. Using multiple, relevant, state-of-the-art methods can help define tertiary protein
290 structure and, to varying extent, quaternary structure, and can add to the body of information
291 supporting biosimilarity. At the same time, a protein's three-dimensional conformation can often
292 be difficult to define precisely using current physicochemical analytical technology. Any
293 differences in higher order structure between a proposed product and a reference product should
294 be evaluated in terms of a potential effect on protein function and stability. Thus, functional
295 assays are also critical tools for evaluating the integrity of the higher order structures.

296
297 A scientifically sound characterization that provides a comprehensive understanding of the
298 chemical, physical, and biological characteristics of the proposed product is essential to the
299 design of the manufacturing process and to the conduct of development studies for all biological
300 products. The body of knowledge that emerges will serve to support a demonstration of product
301 quality and the effectiveness of a suitable control system during development, and support
302 approval of the product.

303
304 Proposed biosimilar product, manufacturers should perform in-depth chemical, physical, and
305 bioactivity comparisons with side-by-side analyses of an appropriate number of lots of the
306 proposed product and the reference product and, where available and appropriate, a comparison
307 with a reference standard for suitable attributes (e.g., potency). For a discussion of reference
308 standards, see section V.G of this guidance. Evaluation of multiple lots of a reference product
309 and multiple lots of a proposed product enables estimation of product variability across lots. The
310 number of lots needed to understand the lot-to-lot variability of both the reference and proposed
311 products may differ on a case-by-case basis and should be scientifically justified by the sponsor.

312
313 FDA encourages sponsors to consult with the Agency to ensure that an appropriate number of
314 lots are evaluated. Identification of specific lots of a reference product used in comparative
315 analytical studies, together with expiration dates and time frames and when the lots were
316 analyzed and used in other types of studies (nonclinical or clinical studies), should be provided.
317 This information will be useful in justifying acceptance criteria to ensure product consistency, as
318 well as to support the comparative analytical assessment of the proposed product and the
319 reference product. However, acceptance criteria should be based on the totality of the analytical
320 data and not simply on the observed range of product attributes of the reference product. This is
321 because some product attributes act in combination to affect a product's safety, purity, and
322 potency profile; therefore, their potential interaction should be considered when conducting the
323 comparative analytical assessment and setting specifications. For example, for some
324 glycoproteins, the content and distribution of tetra-antennary and N-acetyllactosamine repeats
325 can affect in vivo potency and should not be evaluated independently of each other.

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327 Additionally, data obtained for lots used in nonclinical and clinical studies and relevant
328 information on the relationship between an attribute and the performance of the drug product
329 (see ICH Q8(R2))¹⁵ can also be used to help establish acceptance criteria.
330

331 An extensive analytical characterization may reveal differences between the reference product
332 and the proposed product, especially when using analytical techniques capable of discriminating
333 qualitative or quantitative differences in product attributes. Emphasis should be placed on
334 developing orthogonal quantitative methods to definitively identify any differences in product
335 attributes. Based on the results of analytical studies assessing functional and physicochemical
336 characteristics, including, for example, higher order structure, posttranslational modifications,
337 and impurity and degradation profiles, the sponsor may have an appropriate scientific basis for a
338 selective and targeted approach to subsequent animal and/or clinical studies to support a
339 demonstration of biosimilarity. It may be useful to compare differences in the quality attributes
340 of the proposed product with those of the reference product using a meaningful fingerprint-like
341 analysis algorithm¹⁶ that covers a large number of additional product attributes and their
342 combinations with high sensitivity using orthogonal methods. Enhanced approaches in
343 manufacturing science, as discussed in ICH Q8(R2), may facilitate production processes that can
344 better match a reference product's fingerprint.¹⁷ Such a strategy could further quantify the
345 overall similarity between two molecules and may lead to additional bases for a more selective
346 and targeted approach to subsequent animal and/or clinical studies.
347

348 The type, nature, and extent of any differences between the proposed product and the reference
349 product, introduced by design or observed from comprehensive analytical characterization of
350 multiple manufacturing lots, should be clearly described and discussed. The discussion should
351 include identification and comparison of relevant quality attributes from product
352 characterization. The potential clinical effects of observed structural and functional differences
353 between the two products should be assessed and supported by animal or clinical studies, if
354 necessary.
355

V. FACTORS FOR CONSIDERATION IN PERFORMING THE COMPARATIVE ANALYTICAL ASSESSMENT

356
357
358
359
360 When performing the comparative analytical assessment to support a demonstration of
361 biosimilarity, manufacturers should consider a number of factors, including the following:

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.

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364

A. Expression System

365 Therapeutic protein products can be produced in microbial cells (prokaryotic or eukaryotic), cell

366 lines (e.g., mammalian, avian, insect, plant), or tissues derived from animals or plants. It is

367 expected that the expression construct for a proposed product will encode the same primary

368 amino acid sequence as its reference product. However, minor modifications, such as N- or C-

369 terminal truncations (e.g., the heterogeneity of C-terminal lysine of a monoclonal antibody) that

370 are not expected to change the product performance, may be justified and should be explained by

371 the sponsor. Possible differences between the chosen expression system (i.e., host cell and the

372 expression construct) of the proposed product and that of the reference product should be

373 carefully considered because the type of expression system will affect the types of process- and

374 product-related substances, impurities, and contaminants (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 posttranslational modifications

377 that are imparted to the proposed product, which may introduce additional uncertainty into the

378 demonstration that the proposed product is biosimilar to the reference product.

379

380 Minimizing differences between the proposed product and reference product expression systems

381 to the extent possible can enhance the likelihood of producing a biosimilar protein product. Use

382 of different expression systems will be evaluated on a case-by-case basis.

383

384

B. Manufacturing Process

385

386 A comprehensive understanding of all steps in the manufacturing process for the proposed

387 product should be established during product development. As a scientific matter,

388 characterization tests, process controls, and specifications that will emerge from information

389 gained during process development must be specific for the proposed product and manufacturing

390 process. The use of enhanced approaches¹⁸ to pharmaceutical development, along with quality

391 risk management and effective quality systems, will facilitate the consistent manufacturing of a

392 high-quality product. As a scientific matter, as with biological products originally licensed under

393 section 351(a) of the PHS Act, an application for a biological product submitted for licensure

394 under section 351(k) of the PHS Act may not incorporate by reference drug substance, drug

395 substance intermediate, or drug product information contained in a Master File (MF) because a

396 license holder is generally expected to have knowledge of and control over the manufacturing

397 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

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

400
401 A sponsor considering manufacturing changes after completing the initial comparative analytical
402 assessment or after completing clinical studies intended to support a 351(k) application will need
403 to demonstrate comparability between the pre- and post-change proposed product and may need
404 to conduct additional studies. The nature and extent of the changes may determine the extent of
405 these additional studies. The comparative analytical studies should include a sufficient number
406 of lots of the proposed biosimilar product used in clinical studies as well as from the proposed
407 commercial process if the process used to produce the material used in the clinical studies is
408 different.

409 410 **C. Physicochemical Properties**

411
412 Physicochemical assessment of the proposed product and the reference product should consider
413 all relevant characteristics of the protein product (e.g., the primary, secondary, tertiary, and
414 quaternary structure; posttranslational modifications; and functional activity(ies)). The objective
415 of this assessment is to maximize the potential for detecting differences in quality attributes
416 between the proposed product and the reference product.

417
418 The sponsor should address the concept of the desired product (and its variants) as discussed in
419 ICH Q6B²¹ when designing and conducting the characterization studies. Thus, it will be
420 important to understand the heterogeneity of the proposed product and the reference product
421 (e.g., the nature, location, and levels of glycosylation) and the ranges of variability of different
422 isoforms, including those that result from posttranslational modifications.

423
424 Particular analytical methodologies can be used to assess specific physicochemical
425 characteristics of proteins. These methodologies are described in published documents,
426 including scientific literature, regulatory guidelines, and pharmacopeial compendia. Some
427 techniques provide information on multiple characteristics. It is expected that appropriate
428 analytical test methods will be selected based on the nature of the protein being characterized
429 and knowledge regarding the structure and heterogeneity of the reference product and the
430 proposed product, as well as characteristics critical to product performance.

431

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).

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432 To address the full range of physicochemical properties or biological activities adequately, it is
433 often necessary to apply more than one analytical procedure to evaluate the same quality
434 attribute. Methods that use different physicochemical or biological principles to assess the same
435 attribute are especially valuable because they provide independent data to support the quality of
436 that attribute (e.g., orthogonal methods to assess aggregation). In addition, the use of
437 complementary analytical techniques in series, such as peptide mapping or capillary
438 electrophoresis combined with mass spectrometry of the separated molecules, should provide a
439 meaningful and sensitive method for comparing products.

440
441 Unlike routine quality control assays, tests used to characterize the product do not necessarily
442 need to be validated; however, the tests used to characterize the product should be scientifically
443 sound, fit for their intended use, and provide results that are reproducible and reliable. In
444 selecting these tests, it is important to consider the characteristics of the protein product,
445 including known and potential impurities. Information regarding the ability of a method to
446 discern relevant differences between a proposed product and a reference product should be
447 submitted as part of the comparison. The methods should be demonstrated to be of appropriate
448 sensitivity and specificity to provide meaningful information as to whether the proposed product
449 and the reference product are highly similar.

450 451 **D. Functional Activities**

452
453 Functional assays serve multiple purposes in the characterization of protein products. These tests
454 act to complement physicochemical analyses and are a quantitative measure of the function of
455 the protein product.

456
457 Depending on the structural complexity of the protein and available analytical technology, the
458 physicochemical analysis may be unable to confirm the integrity of the higher order structures.
459 Instead, the integrity of such structures can usually be inferred from the product's biological
460 activity. If the clinically relevant mechanism(s) of action are known for the reference product or
461 can reasonably be determined, the functional assays should reflect such mechanism(s) of action
462 to the extent possible. Multiple functional assays should, in general, be performed as part of the
463 comparative analytical assessments. The assessment of functional activity is also useful in
464 providing an estimate of the specific activity of a product as an indicator of manufacturing
465 process consistency, as well as product purity, potency, and stability.

466
467 If a reference product exhibits multiple functional activities, sponsors should perform a set of
468 appropriate assays designed to evaluate the range of relevant activities for that product. For
469 example, with proteins that possess multiple functional domains expressing enzymatic and
470 receptor-mediated activities, sponsors should evaluate both activities to the extent that these
471 activities are relevant to product performance. For products where functional activity can be
472 measured by more than one parameter (e.g., enzyme kinetics or interactions with blood clotting
473 factors), the comparative characterization of each parameter between products should be
474 assessed.

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475
476 The sponsor should recognize the potential limitations of some types of functional assays, such
477 as high variability, that might preclude detection of small but significant differences between the
478 proposed product and the reference product. Because a highly variable assay may not provide a
479 meaningful assessment as to whether the proposed product is highly similar to the reference
480 product, sponsors are encouraged to develop assays that are less variable and are sensitive to
481 changes in the functional activities of the product. In addition, in vitro bioactivity assays may
482 not fully reflect the clinical activity of the protein. For example, these assays generally do not
483 predict the bioavailability (PK and biodistribution) of the product, which can affect PD and
484 clinical performance. Also, bioavailability can be dramatically altered by subtle differences in
485 glycoform distribution or other posttranslational modifications. Thus, these limitations should be
486 taken into account when assessing the robustness of the quality of data supporting biosimilarity
487 and the need for additional information that may address residual uncertainties. Finally,
488 functional assays are important in assessing the occurrence of neutralizing antibodies in
489 nonclinical and clinical studies.

E. Target Binding

490
491
492
493 When binding is part of the activity attributed to the protein product, analytical tests should be
494 performed to characterize the proposed product in terms of its specific binding properties (e.g., if
495 binding to a receptor is inherent to protein function, this property should be measured and used
496 in comparative studies) (see ICH Q6B for additional details). Various methods such as surface
497 plasmon resonance, microcalorimetry, or classical Scatchard analysis can provide information on
498 the kinetics and thermodynamics of binding. Such information can be related to the functional
499 activity and characterization of the proposed product's higher order structure.

F. Impurities

500
501
502
503 The sponsor should characterize, identify, and quantify product-related impurities in the
504 proposed product and the reference product, to the extent feasible.²² If a comparative
505 physicochemical analysis reveals comparable product-related impurities at similar levels
506 between the two products, pharmacological/toxicological studies to characterize potential
507 biological effects of specific impurities may not be necessary. However, if the manufacturing
508 process used to produce the proposed product introduces different impurities or higher levels of
509 impurities than those present in the reference product, additional pharmacological/toxicological
510 or other studies may be necessary. As discussed in the ICH guidance for industry *S6(R1)*
511 *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.

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512 preferable to rely on purification processes to remove impurities . . . rather than to establish a
513 preclinical testing program for their qualification.”²³

514
515 Process-related impurities arising from cell substrates (e.g., host cell DNA, host cell proteins),
516 cell culture components (e.g., antibiotics, media components), and downstream processing steps
517 (e.g., reagents, residual solvents, leachables, endotoxin, bioburden) should be evaluated. The
518 process-related impurities in the proposed product are not expected to match those observed in
519 the reference product and are not included in the comparative analytical assessment. The chosen
520 analytical procedures should be adequate to detect, identify, and accurately quantify biologically
521 significant levels of impurities.²⁴ In particular, results of immunological methods used to detect
522 host cell proteins depend on the assay reagents and the cell substrate used. Such assays should
523 be validated using the product cell substrate and orthogonal methodologies to ensure accuracy
524 and sensitivity.

525
526 As with any biological product, the safety of the proposed product with regard to adventitious
527 agents or endogenous viral contamination, should be ensured by screening critical raw materials
528 and confirmation of robust virus removal and inactivation achieved by the manufacturing
529 process.²⁵

530

G. Reference Product and Reference Standards

531

532
533 A thorough physicochemical and biological assessment of the reference product should provide a
534 base of information from which to develop the proposed product and justify reliance on certain
535 existing scientific knowledge about the reference product. Sufficient evidence that the proposed
536 product is highly similar to the reference product must be provided to support a selective and
537 targeted approach in early product development (e.g., selected animal studies and/or additional
538 clinical studies).²⁶

539

540 The comparative analytical assessment submitted with the marketing application to support the
541 demonstration of biosimilarity of the proposed product to the reference product should include
542 lots of the proposed product used in principal clinical study(ies), as well as the proposed
543 commercial product. As stated earlier in section V.B, a sponsor considering manufacturing
544 changes after completing the initial comparative analytical assessment or after completing
545 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.

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

548
549 If the drug substance has been extracted from the reference product to conduct analytical studies,
550 the sponsor should describe the extraction procedure and provide support that the procedure
551 itself does not alter relevant product quality attributes. This undertaking would include
552 consideration of alteration or loss of the desired products and impurities and relevant product-
553 related substances, and it should include appropriate controls to ensure that relevant
554 characteristics of the protein are not significantly altered by the extraction procedure.

555
556 If there is a suitable, publicly available, and well-established reference standard for the protein, a
557 physicochemical and/or functional comparison of the proposed product with this standard may
558 also provide useful information.²⁷ For example, if an international standard for calibration of
559 potency is available, a comparison of the relative potency of the proposed product with this
560 potency standard should be performed. As recommended in ICH Q6B, an in-house reference
561 standard(s) should always be qualified and used for control of the manufacturing process and
562 product.

563
564 An in-house reference standard is typically developed from early development lots or lots used in
565 a clinical study(ies). Additional reference standards may be qualified later in development and
566 for a BLA submission. Ideally, a sponsor will have established and properly qualified primary
567 and working reference standards that are representative of proposed product lots used in clinical
568 studies that support the application.

569
570 For the development of a proposed product, a reference product lot or a lot of a non-U.S.-
571 licensed comparator product (see section VI.A.4 of this guidance) is typically qualified as an
572 initial reference standard. Once clinical lots of the proposed product have been manufactured, it
573 is expected that one of these lots will be properly qualified (including bridging to previous
574 reference standards) for use as a reference standard for release and stability, as well as
575 comparative analytical testing. If possible, once an in-house reference standard is properly
576 qualified, there should be sufficient quantities to use throughout the development of the proposed
577 product. All lots of reference standards used during the development of a proposed product
578 should be properly qualified. In addition to release testing methods, the qualification protocol
579 for reference standards should include all analytical methods that report the result relative to the
580 reference standard.

581
582 For all methods where the result is reported relative to the reference standard, the assignment of
583 a potency of 100% should include a narrow acceptable potency range and ensure control over
584 product drift. For example, a sponsor should consider the use of a pre-determined two-sided
585 confidence interval (CI) of the mean of the replicates, where the mean relative potency and the
586 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.

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

589
590 A sponsor generally should not use a correction factor to account for any differences in, for
591 example, potency or biological activity between reference standards.

592
593 Use of reference standards inadequately qualified for analytical methods that report results
594 relative to the reference standard is likely to raise concerns regarding the comparative analytical
595 assessment. One approach to address these concerns, if applicable, may be to store the reference
596 product and non-U.S.-licensed comparator product lots under conditions that maintain stability
597 long term, if feasible. Prior to submission of a 351(k) application, the prospective applicant
598 should conduct a reevaluation of all proposed product, reference product, and non-U.S.-licensed
599 comparator product lots using the same reference standard for those methods that report the
600 result relative to the reference standard. Data supporting the stability of the reference product
601 and non-U.S.-licensed comparator product beyond the expiration date under these conditions
602 should be included in the submission.

603
604 In summary, analytical studies carried out to support the approval of a proposed product should
605 not focus solely on the characterization of the proposed product in isolation. Rather, these
606 studies should be part of a broad comparison that includes, but is not limited to, the proposed
607 product, the reference product, and, where applicable, a non-U.S.-licensed comparator,
608 applicable reference standards, and consideration of relevant publicly available information.

609 **H. Finished Drug Product**

610
611
612 Product characterization studies of a proposed product should be performed on the most
613 downstream intermediate best suited for the analytical procedures used. The attributes evaluated
614 should be stable through any further processing steps. For these reasons, characterization studies
615 are often performed on the drug substance. However, if a drug substance is reformulated and/or
616 exposed to new materials in the finished dosage form, the impact of these changes should be
617 considered. Whenever possible, if the finished drug product is best suited for a particular
618 analysis, the sponsors should analyze the finished drug product. If an analytical method more
619 sensitively detects specific attributes in the drug substance but the attributes it measures are
620 critical and/or may change during manufacture of the finished drug product, comparative
621 characterization may be called for on both the extracted protein and the finished drug product.

622
623 Proteins are very sensitive to their environment. Therefore, differences in excipients or primary
624 packaging may affect product stability and/or clinical performance. Differences in formulation
625 and primary packaging²⁸ between the proposed product and the reference product are among the
626 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).

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627 targeted approach.²⁹ Sponsors should clearly identify excipients used in the proposed product
628 that differ from those in the reference product. The acceptability of the type, nature, and extent
629 of any differences between the finished proposed product and the finished reference product
630 should be evaluated and supported by appropriate data and rationale. Additionally, different
631 excipients in the proposed product should be supported by existing toxicology data for the
632 excipient or by additional toxicity studies with the formulation of the proposed product.
633 Excipient interactions as well as direct toxicities should be considered.

I. Stability

634
635
636
637 As part of an appropriate physicochemical and functional comparison of the stability profile of
638 the proposed product with that of the reference product, accelerated and stress stability studies,
639 as well as forced degradation studies, should be used to establish degradation profiles and to
640 provide a direct stability comparison of the proposed product with the reference product. These
641 comparative studies should be conducted under multiple stress conditions (e.g., high
642 temperature, freeze thaw, light exposure, and agitation) that can cause incremental product
643 degradation over a defined time period. Results of these studies may reveal product differences
644 that warrant additional evaluations and also identify conditions under which additional controls
645 should be employed in manufacturing and storage.³⁰ Sufficient real time, real-condition stability
646 data from the proposed product should be provided to support the proposed shelf life.

VI. COMPARATIVE ANALYTICAL ASSESSMENT

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649
650 A thorough understanding of the reference product is critical for a successful biosimilar
651 development program. The Agency recommends that sponsors approach the comparative
652 analytical assessment by first understanding the physicochemical and biological characteristics
653 of the reference product. A full characterization of the reference product, in addition to
654 consideration of publicly available information, will form the basis of product understanding. As
655 described previously, protein products are complex molecules that generally are manufactured in
656 living cells and purified using a variety of technologies; therefore, they have a certain degree of
657 inherent lot-to-lot variability in terms of quality characteristics. The observed lot-to-lot
658 variability may derive from manufacturing conditions and from analytical assay variability.
659 Factors that contribute to lot-to-lot variability in the manufacture of a protein product include the
660 source of certain raw materials (e.g., growth medium, resins, or separation materials) and
661 different manufacturing sites. Therefore, the comparative analytical assessment, it is important
662 to adequately characterize the lot-to-lot variability of the reference product and the proposed
663 biosimilar product.

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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A. Considerations for Reference and Biosimilar Products

1. Reference Product

To ensure that the full range of product variability is accurately captured, sponsors should acquire multiple reference product lots throughout the development program of a proposed biosimilar in sufficient quantity to conduct multiple physiochemical and functional assays. Considering the inherent heterogeneity present in protein products and the expected lot-to-lot variability stemming from manufacturing processes, the Agency recommends that a sponsor include at least 10 reference product lots (acquired over a time frame that spans expiration dates of several years), in the analytical assessment to ensure that the variability of the reference product is captured adequately. The final number of lots should be sufficient to provide adequate information regarding the variability of the reference product. In cases where limited numbers of reference product lots are available (e.g., for certain orphan drugs), alternate flexible comparative analytical assessments plans should be proposed and discussed with the Agency.

2. Proposed Product

The Agency recommends that a sponsor include at least 6 to 10 lots of the proposed product in the comparative analytical assessment, to ensure 1) adequate characterization of the proposed product and understanding of manufacturing variability, and 2) adequate comparison to the reference product. These should include lots manufactured with the investigational- and commercial-scale processes, and may include validation lots, as well as product lots manufactured at different scales, including engineering lots. These lots should be representative of the intended commercial manufacturing process. If there is a manufacturing process change during development, it may be possible, with adequate scientific justification, to use data generated from lots manufactured with a different process. However, data should be provided in the 351(k) BLA to support comparability of drug substance and drug product manufactured with the different processes and/or scales. The extent of process development design (as described in guidelines *ICH Q8 (R2) Pharmaceutical Development* and *ICH Q11 Development and Manufacture of Drug Substances*) and process understanding should be used in support of the number of proposed biosimilar product lots proposed for inclusion in the comparative analytical assessment in the 351(k) application.

To the extent possible, proposed biosimilar lots included in the comparative analytical assessment described in section VI.B, Considerations for Data Analysis, should be derived from different drug substance batches to adequately represent the variability of attributes inherent to the drug substance manufacturing process. Drug product lots derived from the same drug substance batch(es) are not considered sufficiently representative of such variability, except for use in testing certain drug product attributes for which variability is mostly dependent on the drug product manufacturing process (e.g., protein concentration). Although it may be preferable to compare the proposed product lots to the reference product lots, it may be acceptable to also

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

710

711 3. *Accounting for Reference Product and Proposed Product Lots*

712

713 Sponsors should account for all the reference product lots acquired and characterized. The
714 351(k) BLA should include data and information from all reference product and proposed
715 product lots that were evaluated in any manner, including the specific physicochemical,
716 functional, animal, and clinical studies for which a lot was used. When a lot is specifically
717 selected to be included in or excluded from certain analytical studies, a justification should be
718 provided. The date of the analytical testing as well as the product expiration date should be
719 provided in the application. In general, expired reference product lots should not be included in
720 the comparative analytical assessment because lots analyzed beyond their expiration date could
721 lead to results outside the range that would normally be observed in unexpired lots, which may
722 result in overestimated reference product variability. Testing of lots past expiry may be
723 acceptable if samples are stored under long term conditions (e.g., frozen at -80°C) provided that
724 sponsors submit data and information demonstrating that storage does not impact the quality of
725 the product (see section V.G).

726

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

730

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

734

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

736

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

742

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

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750 is biosimilar to the reference product. For example, combining data from the reference product
751 and non-U.S.-licensed products may result in a larger range and broader similarity acceptance
752 criteria than would be obtained by relying solely on data from reference product lots. Sponsors
753 are encouraged to discuss with FDA, during product development, any plans to submit data
754 derived from products approved outside of the U.S. in support of a 351(k) application.
755

B. Considerations for Data Analysis

756
757
758 Sponsors should develop a comparative analytical assessment plan and discuss the approach with
759 the Agency as early as practicable. A final comparative analytical assessment report should be
760 available at the time a 351(k) BLA is submitted.
761

762 The Agency recommends development of a comparative analytical assessment plan using a
763 stepwise approach. The first step is a determination of the quality attributes that characterize the
764 reference product in terms of its structural/physicochemical and functional properties. These
765 quality attributes are then ranked according to their risk to potentially impact activity, PK/PD,
766 safety, efficacy, and immunogenicity. Finally, the attributes are evaluated using quantitative
767 analysis, considering the risk ranking of the quality attributes, as well as other factors. It should
768 be noted, however, that some attributes may be highly critical (e.g., primary sequence) but not
769 amenable to quantitative analysis.
770

I. Risk Assessment

771
772
773 FDA recommends that sponsors develop a risk assessment tool to evaluate and rank the reference
774 product quality attributes in terms of potential impact on the mechanism(s) of action and function
775 of the product. Certain quality evaluations of the reference product (e.g., its degradation rates,
776 which are determined from stability or forced degradation studies) generally should not be
777 included in the risk ranking. However, these evaluations should still factor into the comparative
778 analytical assessment of the proposed biosimilar and reference product.

779 Development of the risk assessment tool should be informed by relevant factors, including:
780

- 781 • Potential impact of an attribute on clinical performance: Specifically, FDA recommends
782 that sponsors consider the potential impact of an attribute on activity, PK/PD, safety,
783 efficacy, and immunogenicity. Sponsors should consider publicly available information,
784 as well as the sponsor's own characterization of the reference product, in determining the
785 potential impact of an attribute on clinical performance.
786
- 787 • The degree of uncertainty surrounding a certain quality attribute: For example, when
788 there is limited understanding of the relationship between the degree of change in an
789 attribute and the resulting clinical impact, FDA recommends that that attribute be ranked
790 as having higher risk because of the uncertainty raised.
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792 FDA recommends that an attribute that is a high risk for any one of the performance categories
793 (i.e., activity, PK/PD, safety, efficacy, and immunogenicity) be classified as high risk. Ideally,
794 the risk assessment tool should result in a list of attributes ordered by the risk to the patient. The
795 risk scores for attributes should, therefore, be proportional to patient risk. The scoring criteria
796 used in the risk assessment should be clearly defined and justified, and the risk ranking for each
797 attribute should be justified with appropriate citations to the literature and data provided.

798

799 2. *Quantitative and Qualitative Data Analysis*

800

801 Appropriate analyses of the comparative analytical data are necessary to support a demonstration
802 that the proposed product is highly similar to the reference product notwithstanding minor
803 differences in clinically inactive components. One approach to data analysis would be the use of
804 descriptive quality ranges for assessing quantitative quality attributes of high and moderate risk,
805 and the use of raw data/graphical comparisons for quality attributes with the lowest risk ranking
806 or for those quality attributes that cannot be quantitatively measured (e.g., primary sequence).

807 The acceptance criteria for the quality ranges (QR) method in the comparative analytical
808 assessment should be based on the results of the sponsor's own analysis of the reference product
809 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
810 the sample mean, and $\hat{\sigma}_R$ is the sample standard deviation based on the reference product lots.

811 The multiplier (X) should be scientifically justified for that attribute and discussed with the
812 Agency. Based on our experience to date, methods such as tolerance intervals are not
813 recommended for establishing the similarity acceptance criteria because a very large number of
814 lots would be required to establish meaningful intervals. The sponsor can propose other methods
815 of data analysis, including equivalence testing.

816

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

825

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

830

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

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- 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.
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879 For qualitative analyses of lower risk attributes, FDA recommends side-by-side data presentation
880 (e.g., spectra, thermograms, graphical representation of data), to allow for a visual comparison of
881 the proposed product to the reference product.
882

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

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896 In the comparative analytical assessment, risk ranking and data analysis are used to evaluate a
897 large number of attributes, often using multiple orthogonal assays. FDA evaluates the totality of
898 the analytical data; if the results of a particular assay do not meet pre-specified criteria, this alone
899 does not preclude a demonstration of high similarity. For example, if differences between
900 products are observed as part of the comparative analytical assessment (including the
901 components of the assessment that were not included in the risk ranking), the sponsor may
902 provide additional scientific information (risk assessment and additional data) and a justification
903 for why these differences do not preclude a demonstration that the products are highly similar.
904

905 In certain situations, changes to the manufacturing process of the biosimilar product may be
906 needed to resolve differences observed in the comparative analytical assessment. Data should be
907 provided demonstrating that the observed differences were resolved by any manufacturing
908 changes, and that other quality attributes were not substantially affected. If other attributes were
909 affected by the manufacturing change, data should be provided to demonstrate that the impact of
910 the change has been evaluated and addressed.
911

VII. CONCLUSION

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914 The foundation for an assessment and a demonstration of biosimilarity between a proposed
915 product and its reference product includes analytical studies that demonstrate that the proposed
916 product is highly similar to the reference product notwithstanding minor differences in clinically
917 inactive components. The demonstration that the proposed product is biosimilar to the reference
918 product thus involves robust characterization of the proposed product, including comparative
919 physicochemical and functional studies with the reference product. The information gained from
920 these studies is necessary for the development of a proposed product as a biosimilar. In addition,

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921 a 351(k) application for a proposed product must contain, among other things, information
922 demonstrating biosimilarity based on data derived from animal studies (including the assessment
923 of toxicity) and a clinical study or studies (including the assessment of immunogenicity and PK
924 or PD), unless the Agency determines that an element is unnecessary in a particular 351(k)
925 application.³¹ A sponsor's ability to discern and understand the impact of relevant analytical
926 differences between the proposed product and its reference product is critical to determine
927 whether the statutory standard for biosimilarity can be met.

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930 VIII. RELEVANT GUIDANCES

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932 The following draft and final guidance documents may be relevant to sponsors developing or
933 considering development of a proposed biosimilar product. All Agency guidance documents are
934 available on FDA's web page

935 (<https://www.fda.gov/regulatory-information/search-fda-guidance-documents>).

936

- 937 1. Guidance for industry *Scientific Considerations in Demonstrating Biosimilarity to a*
938 *Reference Product* (April 2015)
- 939
- 940 2. Guidance for industry *Questions and Answers on Biosimilar Development and the BPCI*
941 *Act* (December 2018)
- 942
- 943 3. Draft guidance for industry *New and Revised Draft Q&As on Biosimilar Development*
944 *and the BPCI Act (Revision 2)* (December 2018)
- 945
- 946 4. Draft guidance for industry *Formal Meetings Between the FDA and Sponsors or*
947 *Applicants of BsUFA Products* (June 2018)
- 948
- 949 5. Guidance for industry *Clinical Pharmacology Data to Support a Demonstration of*
950 *Biosimilarity to a Reference Product* (December 2016)
- 951
- 952 6. *Demonstration of Comparability of Human Biological Products, Including Therapeutic*
953 *Biotechnology-derived Products* (April 1996)
- 954
- 955 7. *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for*
956 *Human Use* (February 1997)
- 957
- 958 8. Guidance for industry for the *Submission of Chemistry, Manufacturing, and Controls*
959 *Information for a Therapeutic Recombinant DNA-Derived Product or a Monoclonal*
960 *Antibody Product for In Vivo Use* (August 1996)
- 961

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

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- 962 9. Guidance for industry *Cooperative Manufacturing Arrangements for Licensed Biologics*
963 (November 2008)
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- 965 10. ICH guidance for industry *M4: The CTD —Quality* (ICH M4Q) (August 2001)
966
- 967 11. ICH guidance for industry *Q1A(R2) Stability Testing of New Drug Substances and*
968 *Products* (ICH Q1A(R2)) (November 2003)
969
- 970 12. ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and*
971 *Methodology* (ICH Q2(R1)) (November 2005)
972
- 973 13. ICH guidance for industry *Q2B Validation of Analytical Procedures: Methodology* (ICH
974 Q2B) (May 1997)
975
- 976 14. ICH guidance for industry *Q3A(R) Impurities in New Drug Substances* (ICH Q3A(R))
977 (June 2008)
978
- 979 15. ICH guidance for industry *Q5A Viral Safety Evaluation of Biotechnology Products*
980 *Derived from Cell Lines of Human or Animal Origin* (ICH Q5A) (September 1998)
981
- 982 16. ICH guidance for industry *Q5B Quality of Biotechnological Products: Analysis of the*
983 *Expression Construct in Cells Used for Production of r-DNA Derived Protein Products*
984 (ICH Q5B) (February 1996)
985
- 986 17. ICH guidance for industry *Q5C Quality of Biotechnological Products: Stability Testing*
987 *of Biotechnological/Biological Products* (ICH Q5C) (July 1996)
988
- 989 18. ICH guidance for industry *Q5D Quality of Biotechnological/Biological Products:*
990 *Derivation and Characterization of Cell Substrates Used for Production of*
991 *Biotechnological/Biological Products* (ICH Q5D) (September 1998)
992
- 993 19. ICH guidance for industry *Q5E Comparability of Biotechnological/Biological Products*
994 *Subject to Changes in Their Manufacturing Process* (ICH Q5E) (June 2005)
995
- 996 20. ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria*
997 *for Biotechnological/Biological Products* (ICH Q6B) (August 1999)
998
- 999 21. ICH guidance for industry *Q7 Good Manufacturing Practice Guidance for Active*
1000 *Pharmaceutical Ingredients* (ICH Q7) (September 2016)
1001
- 1002 22. ICH guidance for industry *Q8(R2) Pharmaceutical Development* (ICH Q8(R2))
1003 (November 2009)
1004

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- 1005 23. ICH guidance for industry *Q9 Quality Risk Management* (ICH Q9) (June 2006)
1006
1007 24. ICH guidance for industry *Q10 Pharmaceutical Quality System* (ICH Q10) (April 2009)
1008
1009 25. ICH guidance for industry *Q11 Development and Manufacture of Drug Substances* (ICH
1010 Q11) (November 2012)
1011
1012 26. ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-*
1013 *Derived Pharmaceuticals* (ICH S6(R1)) (May 2012)
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GLOSSARY³²

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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.