U.S. FOOD & DRUG ADMINISTRATION
Center for Drug Evaluation and Research

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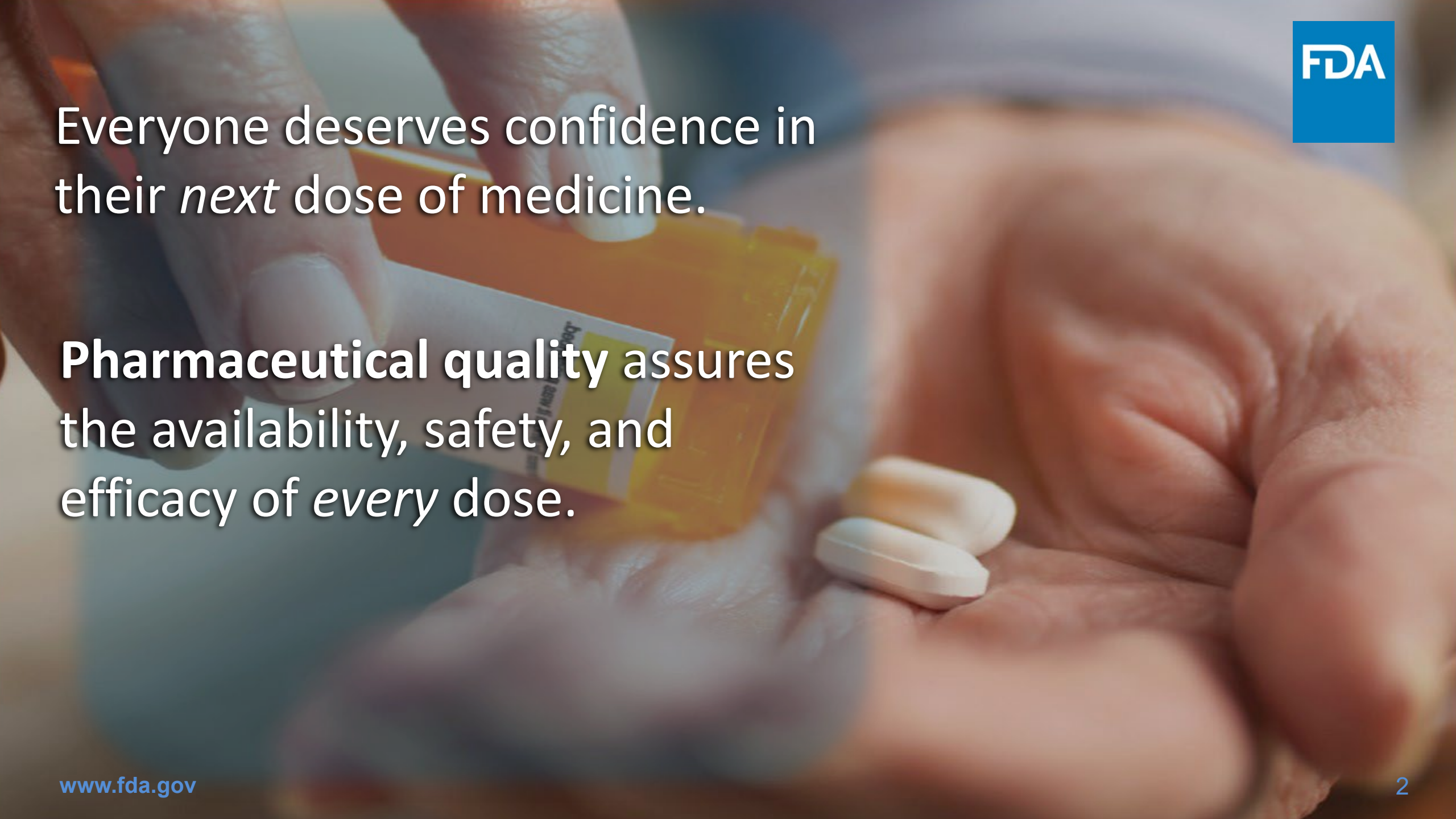
LEVERAGING ADVANCED ANALYTICAL TECHNOLOGIES TO STREAMLINE BIOSIMILAR DEVELOPMENT

GRx+Biosims 2026

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Everyone deserves confidence in their *next* dose of medicine.

Pharmaceutical quality assures the availability, safety, and efficacy of *every* dose.

Outline

I. Regulatory Framework for Biosimilars

II. Comparative Analytical Assessment

III. Evolution of the Clinical Program Expectations

   I. Switching Studies Interchangeability

   II. Comparative Efficacy Studies

   III. PK comparisons to Non-U.S. licensed biological product

IV. Considerations and Best Practices

Regulatory Framework for Biosimilars

The BPCIA creates an **abbreviated licensure pathway for biological products shown to be biosimilar to or interchangeable with** an FDA-licensed reference product.

A 351(k) application must include information demonstrating that the biological product:

- Is **biosimilar** to a reference product;

  Highly similar to and has no clinically meaningful differences from the FDA-approved reference product.

- Utilizes the **same mechanism(s) of action** for the proposed condition(s) of use -- but only to the extent the mechanism(s) are known for the reference product;

- **Condition(s) of use** proposed in labeling **have been previously approved** for the reference product;

- Has the **same route of administration, dosage form,** and **strength** as the reference product; *and*

- Is manufactured, processed, packed, or held in a facility that **meets standards** designed to assure that the biological product continues to be **safe, pure, and potent**.

Reference Product

A reference product is the single biological product, already approved by FDA, against which a proposed biosimilar product is compared.

Interchangeable Product

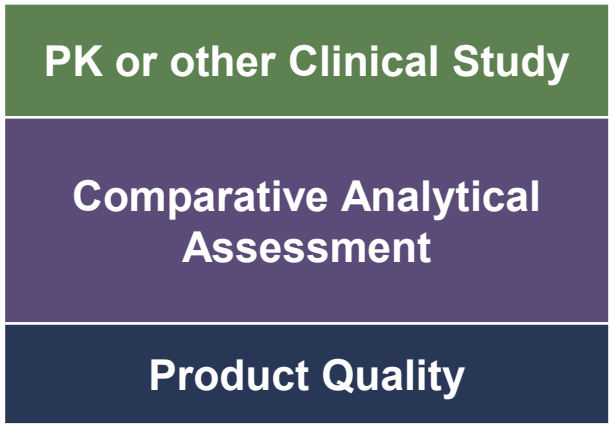
- Is a biosimilar.
- Is expected to produce the same clinical result as the reference product (RP) in any given patient.
- Switching between the proposed product and the RP ***does not ↑safety risks or ↓effectiveness*** compared to using the RP without switching

Adapted from a slide prepared by OTBB

Comparative Analytical Assessment: The Foundation of the 351(k) Pathway

- The CAA is the foundation of FDA's assessment of biosimilarity.
- The goal of the CAA is to be as comprehensive as possible to **maximize the potential for detection of differences in quality attributes** between the proposed biosimilar and the reference product.
- Analytical studies are sensitive to detect small differences between the proposed biosimilar and the reference product that can't often be identified through clinical studies.
- The assessments that follow **closely evaluate whether the differences identified through analytical studies have the potential to impact PK, safety, efficacy, or immunogenicity** based on scientific understanding of attributes and their clinical relevance.

351(k) "Abbreviated" Pathway
Biosimilar Product BLA

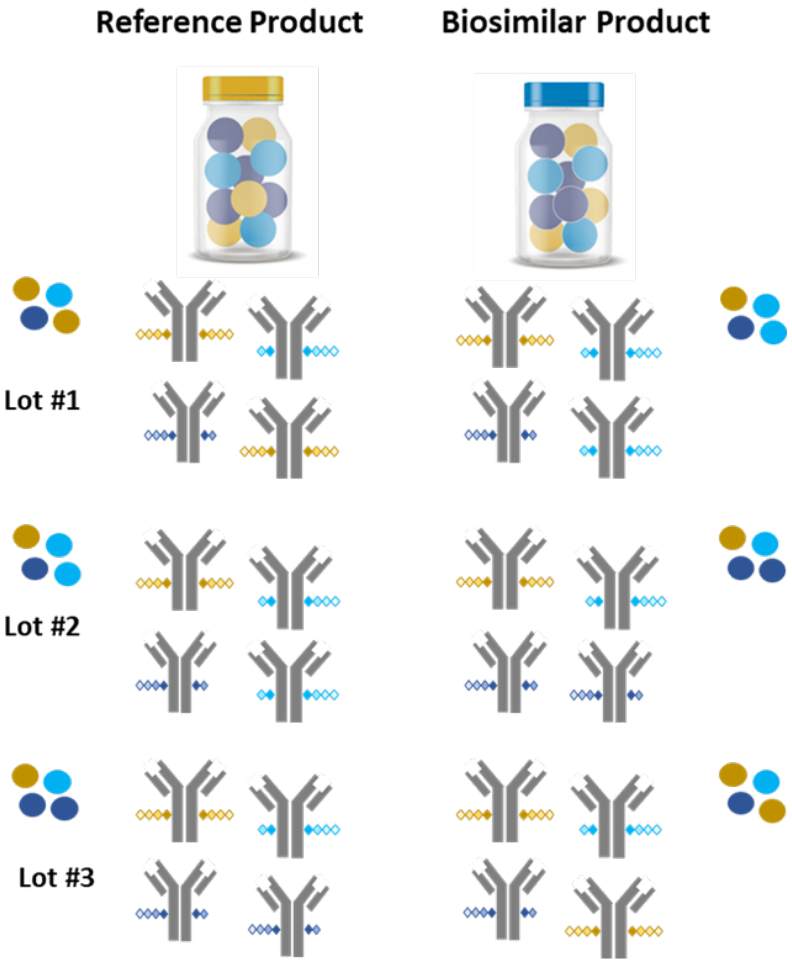


Goal: To demonstrate biosimilarity to a reference product based on comparative assessments

A biosimilar product that is highly analytically similar to its reference product is expected to behave the same clinically.

Adapted from a slide prepared by OTBB

Biological Product Lot-to-Lot Variability



Colored diamonds on the therapeutic protein (e.g., monoclonal antibody) represent various glycans with minor variations in their composition.

Because biologics are generally made in cells, there are inherent variations that result from the manufacturing process.

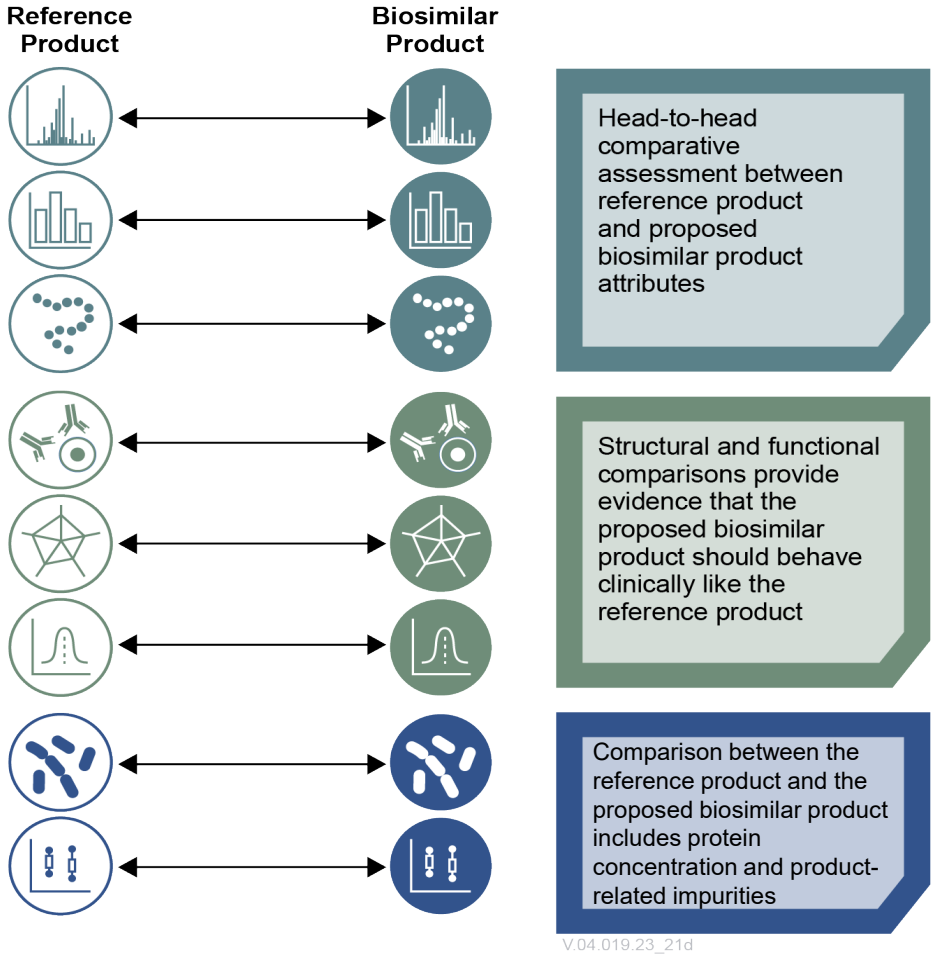
- Inherent variation may also exist within lots and between different lots of reference products and biosimilars.
- The variability in proteins recombinantly produced using clonal cell lines is much smaller compared to proteins derived from natural resources
- As part of the approval process for both reference products and biosimilars, FDA assesses a manufacturer's strategy to control for the pattern and degree of variations between different lots to help ensure consistent safety, purity and potency.
  - The pattern and degree of variations is also maintained during the lifecycle of a biological product (reference product and biosimilar) after manufacturing changes through comparability assessments (ICHQ5E) and the control strategy

Adapted from slide prepared by OTBB

Identification of Product Quality Attributes

Quality attributes evaluated for the CAA are chosen based on their known or potential impact on product quality and clinical performance.

- Quality attributes are properties or characteristics that, when controlled within a defined limit, range, or distribution, ensure the desired product quality.
- Attributes are measured using physicochemical and biological assays and compared in a head-to-head comparative assessment.
- Multiple lots of the reference product and biosimilar are analyzed a head-to-head to identify and characterize CQAs and to help capture manufacture variability.
- To meet the highly similar standard, FDA expects that biosimilar product quality attributes **should be within an appropriate limit, range, or distribution compared to the reference product**.



Slide prepared by OTBB

Examples of Critical Quality Attributes in the CAA

The specific quality attributes included in the CAA depend on the molecule

Structure

Primary Structure
- Sequence
- Sequence Variant
- Deamidation
- Oxidation
- Phosphorylation
- Isoaspartate
- Conjugation Site

Higher Order Structure
- Free Thiols
- Disulfide Bonds
- Thioether
- Thermal Stability
- Secondary, Tertiary, and Quaternary Structure

Size Variants
- Monomer
- High MW – All
- High MW – Dimer
- Residual Homodimer
- Low MW – All
- Low MW – Individual

Linked Non-protein Polymer
- N-glycosylation
- O-glycosylation
- Conjugation

Active Ingredient

Charge
- Acidic Variants
- Basic Variants
- Neutral Main Peak
- Profile
- pI

Mass
- Intact MW
- Non-glycosylated MW

Absorbance
- Extinction Coefficient

Function

Cellular Evaluation
- ADCC
- ADCP
- Apoptosis
- Neutralization
- CDC
- Proliferation
- Inhibition
- Cytotoxicity

Binding
- FcRn
- C1q
- FcγRIa
- FcγRIIa
- FcγRIIIa
- Target Binding

Enzymatic Evaluation
- Kcat
- Km
- Activity

Material Properties

General Attributes
- Protein Concentration

Process-related impurities, formulation (excipients), and additional material properties are evaluated to establish biosimilar product quality and safety but are not included in the CAA.

Categories and subcategories adapted from Welch et al. Bulleted subcategories should not be considered exhaustive. For complete details of taxonomy see: Welch et. al., 2024 (AAPS Open). A controlled vocabulary and taxonomy for the submission of quality attributes for therapeutic proteins

Slide prepared by OTBB

Functional Quality Attributes Reflect the Reference Product's Mechanisms of Action

All known and potential mechanisms of action (MoAs) of the reference product are expected to be reflected in the CAA regardless of the indications being sought for the biosimilar.

- To the extent clinically relevant MoA(s) of the reference product are known or can reasonably be determined, the functional assays conducted in the CAA should reflect such MoAs to the extent possible.

- This is true regardless of the specific indications for which approval is being sought for the biosimilar product:

  - By definition, a biosimilar is **highly similar to an FDA-approved reference product not withstanding minor differences in clinically inactive components**.

  - FDA will only approve a product which it is confident that it is biosimilar in any and every indication approved for the reference product; this should be the case given highly similar structure and function.

Characterizing Quality Attributes of the Reference and Biosimilar Product: Methods

The CAA:

- Comprises a comprehensive battery of robust physicochemical and functional studies (QAs)
- Includes different orthogonal methods (based on different physicochemical principles) to evaluate the same attribute to minimize method specific bias and provide independent data to corroborate the proper characterization of that attribute.
- Expected to use **state-of-the-art** analytical methods, that are shown to be sensitive, reliable, and capable of detecting differences

Note: Process related impurities are not part of the CAA; they are addressed as part of the control strategy.

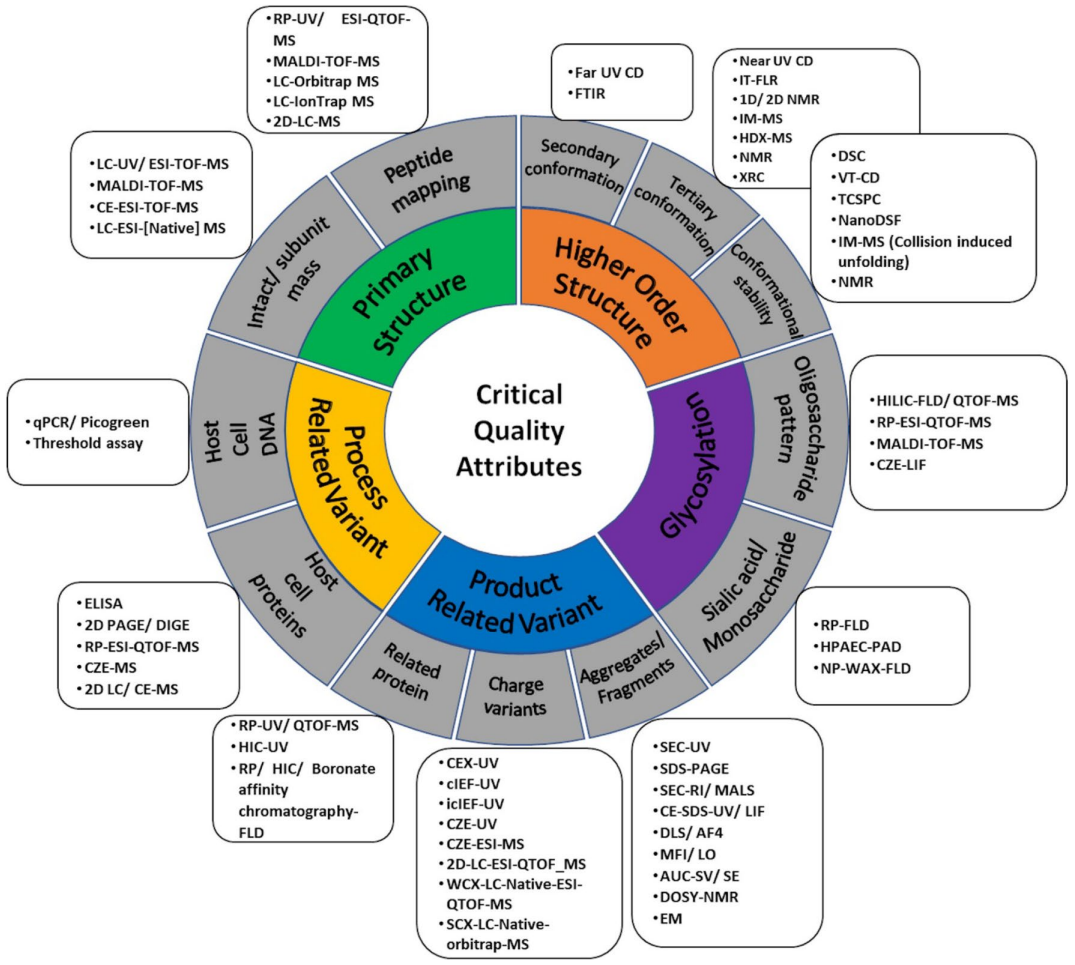


Figure from: Nupur N, et al (2022). Analytical Similarity Assessment of Biosimilars: Global Regulatory Landscape, Recent Studies and Major Advancements in Orthogonal Platforms. *Front. Bioeng. Biotechnol.*, 10:832059.

Emerging Analytical Technologies as Additional Alternatives for Robust QA Characterization

FDA

Each panel: CQA | Examples of Key Analytical Advancement | Examples of Specific Improvements

Primary Structure

LC-ESI-Native MS | MAM | 2D-LC-MS

- Preserves labile PTMs via new fragmentation techniques, increase sequence coverage
- High mass accuracy (<5-2 ppm): distinguishes sequence variants (e.g., deamidation +0.984 Da) and isobaric species, allowing unambiguous characterization of PTMs
- High resolution: separates co-eluting peptides, detects low-abundance sequence variants
- Speed & automation: multi-attribute, native intact mass & HOS

HOS (Secondary)

CD & FTIR Deconvolution Software

- Quantitative secondary structure assessment (not just qualitative)

New techniques with high resolution, sensitivity, quantitative accuracy, method standardization and simpler platforms offer alternatives to comprehensively characterize biologic products and identify differences.

HOS (Tertiary)

2D NMR | HDX-MS | IM-MS

- Atomic-level structure, dynamics & flexibility
- PGSTE NMR: better resolution in formulation buffers
- NIST-led standardized interlaboratory NMR methods

Conformational Stability

nanoDSF | Time-Correlated Single Photon Counting

- Label-free, low-volume, rapid orthogonal stability tools

Glycosylation

RapiFluor-MS | Lectin Arrays | Middle-down NMR

- Faster prep, better signal & ionization; more glycoforms detected
- Domain-specific: structure, linkage position, anomeric configuration

Size Variants

DOSY-NMR

- Hydrodynamic size measurements in solution

Charge Variants

icIEF | Native CEX-MS | microfluidic CE-MS

- Real-time imaging of charge heterogeneity
- Structural ID of individual charge species

Nupur N, et al (2022). Analytical Similarity Assessment of Biosimilars: Global Regulatory Landscape, Recent Studies and Major Advancements in Orthogonal Platforms. *Front. Bioeng. Biotechnol.*, 10:832059.

Investigating Potential Differences in Quality Attributes

- Detection of differences between products using one or more methods may indicate that supplemental studies are needed to further investigate the magnitude and functional impact of the difference. These studies may include:
  - **Fractionation Studies:** Isolate different chromatographic protein fractions to enable evaluation of different molecular variants or species (e.g., structural variants) that could affect therapeutic performance, and
  - **Structure-Activity Relationship (SAR) Studies**: Characterize the relationship between specific molecular structural features and biological activity to assess whether differences between the biosimilar and reference product affect functional activity (e.g., binding activity, target neutralization)
- Detection of differences does not necessarily preclude a demonstration of biosimilarity; however, sponsors must provide robust, data-driven scientific justification supported by literature or other CAA data to support that observed differences are not of concern and are unlikely to impact the safety, efficacy, or clinical performance of the biosimilar relative to its reference product

FDA evaluates the totality of the analytical data; therefore, the results of a particular assay do not define the demonstration of high similarity

Adapted from a slide prepared by OTBB

Analytical Differences in Approved Biosimilar mAbs (EMA/PMDA): Justification & Resolution

Analytical Domain / Quality Attribute	Examples Key Observed Differences (Biosimilar vs. Reference)	Mechanistic Link to Clinical Function	Resolution / Regulatory Acceptance
Glycosylation (Afucosylation & High Mannose)	• Increased afucosylated glycans • Variable high-mannose and sialylation levels	Afucosylation ↑ → ADCC ↑ High-mannose ↑ → ↑ mannose-receptor clearance	Functional ADCC/CDC assays + PK trial confirm no clinically meaningful impact
Size Variants (Aggregates & HMW Species)	• Increased HMW species/particles • Elevated fragments	HMW aggregates → adjuvant effect → ADA formation ↑ → immunogenicity risk	Minor levels accepted; larger differences require manufacturing correction + PK/safety data
Charge Variants (C-term Lys, Deamidation)	• Increased/decreased acidic or basic species • C-terminal lysine and deamidation differences	CDR deamidation → ↓ affinity C-term Lys: cleared in serum <2 h; no impact	C-term Lys universally accepted; other variants resolved by biological activity assays
Fc Effector Functions (FcγR, ADCC, CDC, FcRn)	• FcγRIII binding differences; some ↓ ADCC • FcRn binding shifts observed	FcγRIIIa ↑ → ADCC ↑ FcRn ↓ → ↑ clearance → ↓ half-life	Cell-based assays + PK trial; 1 CES required for a rituximab product (PMDA) — only case identified
Post-Translational Modifications (Deamidation, Oxidation, etc.)	• CDR deamidation; Met oxidation (Fc) • Free thiols; glycation differences	CDR PTMs → ↓ binding affinity Met ox → ↑ clearance; thiols → aggregation	HOS & bioactivity assays confirm function; all resolved at quality level.
Higher-Order Structure & Conformational Stability	• Minor thermal stability (Tm) differences • Tertiary structure diffs by NMR, HDX-MS	HOS defines folding and binding geometry Alterations → ↓ efficacy or ↑ immunogenicity	Orthogonal methods mandatory (CD, FTIR, NMR, HDX-MS, DSC); clinical data cannot substitute

Bielsky MC, et al. Streamlined approval of biosimilars: moving on from the confirmatory efficacy trial. *Drug Discov Today.* 2020;25(10):1910–1918. Guillen E, et al. A data driven approach to support tailored clinical programs for biosimilar monoclonal antibodies. *Clin Pharmacol Ther.* 2023;113(1):108–123. Kirsch-Stefan N, et al. Do the outcomes of clinical efficacy trials matter in regulatory decision-making for biosimilars? *BioDrugs.* 2023;37:855–871. Goto K, et al. Survey on the role of comparative efficacy studies required for biosimilar monoclonal antibody approval in Japan to justify the quality attribute differences between biosimilars and their reference products based on the PMDA assessments. *Pharm Med.* 2025;39:477–491

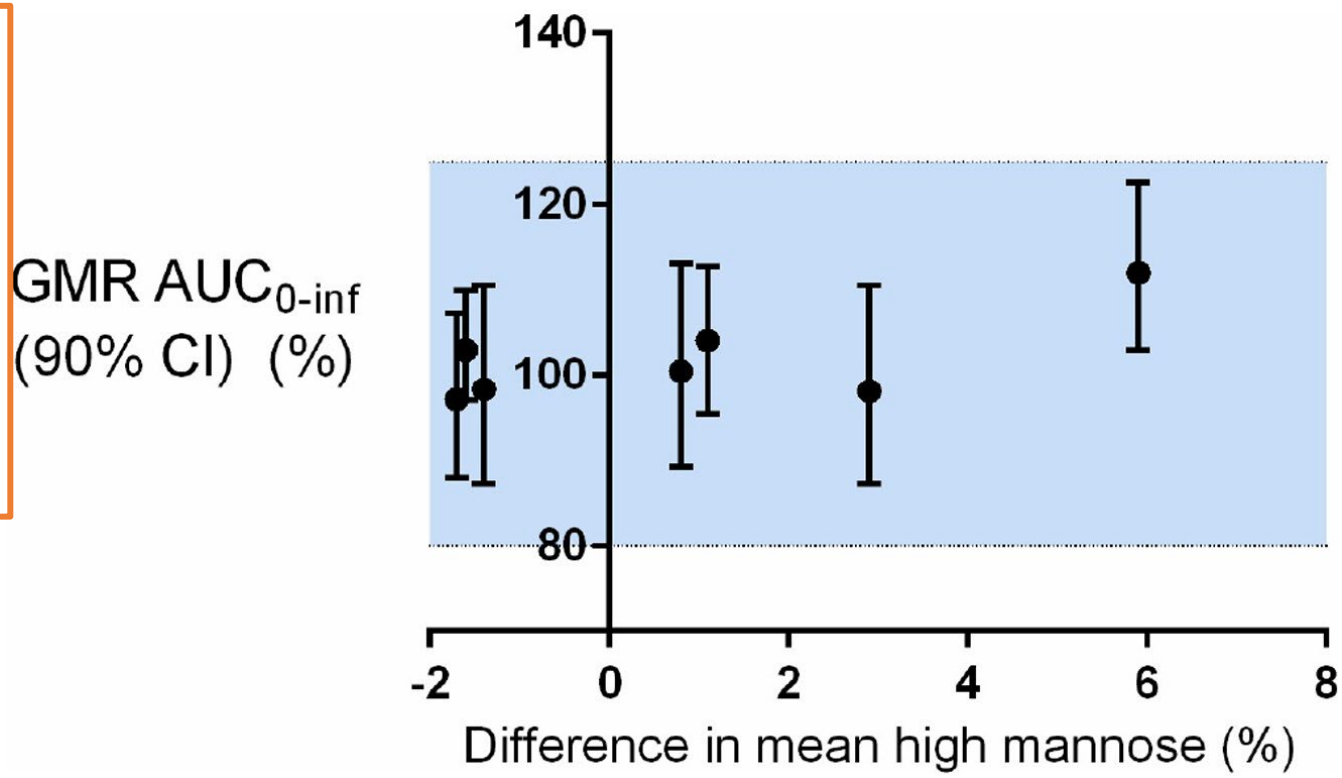
CAA: Analytical Sensitivity

REVIEW

The Mannose in the Mirror: A Reflection on the Pharmacokinetic Impact of High Mannose Glycans of Monoclonal Antibodies in Biosimilar Development

Joel Welch[1]*, Cristina Ausin[2], Nina Brahme[2], Emanuela Lacana[2], Stacey Ricci[2] and Marlene Schultz-DePalo[1]

Biosimilar development has a well-documented foundation of product quality and extensive comparative analytics providing the bulk of the "totality of the evidence" that a proposed product is biosimilar to its reference product. This work provides a retrospective evaluation of a single critical quality attribute—high mannose glycans for monoclonal antibody biosimilars. Given the well-established conclusion that high mannose glycans can impact pharmacokinetic



- High-mannose glycans are known to shorten half-life for monoclonal antibodies.
- Analytical data can detect subtle differences in high-mannose content that are not distinguishable by pharmacokinetic profile.

Clin Pharma and Therapeutics, Volume: 113, Issue: 5, Pages: 1003-1010, First published: 02 November 2022, DOI: (10.1002/cpt.2783)

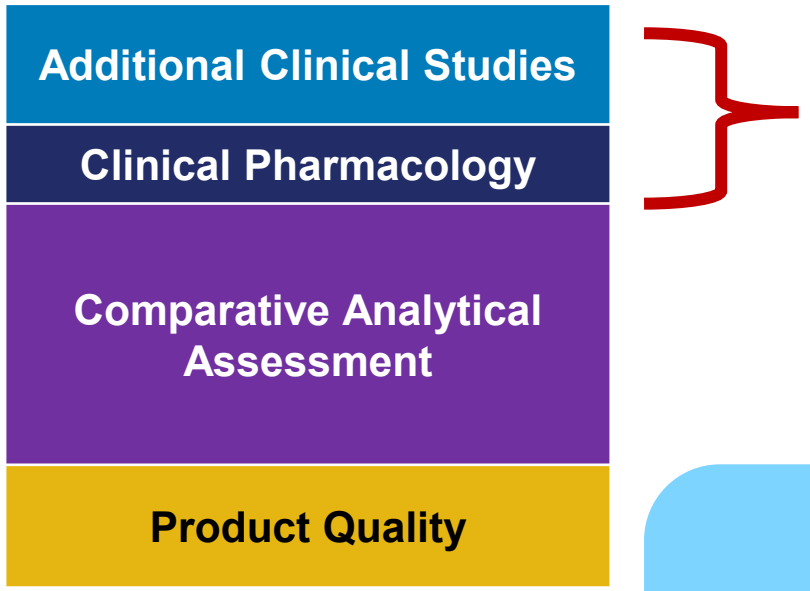
Adapted from slide presented by Joel Welch, FDA Workshop: Increasing the Efficiency of Biosimilar Development Programs, September 19, 2022

CAA – Key Takeaways

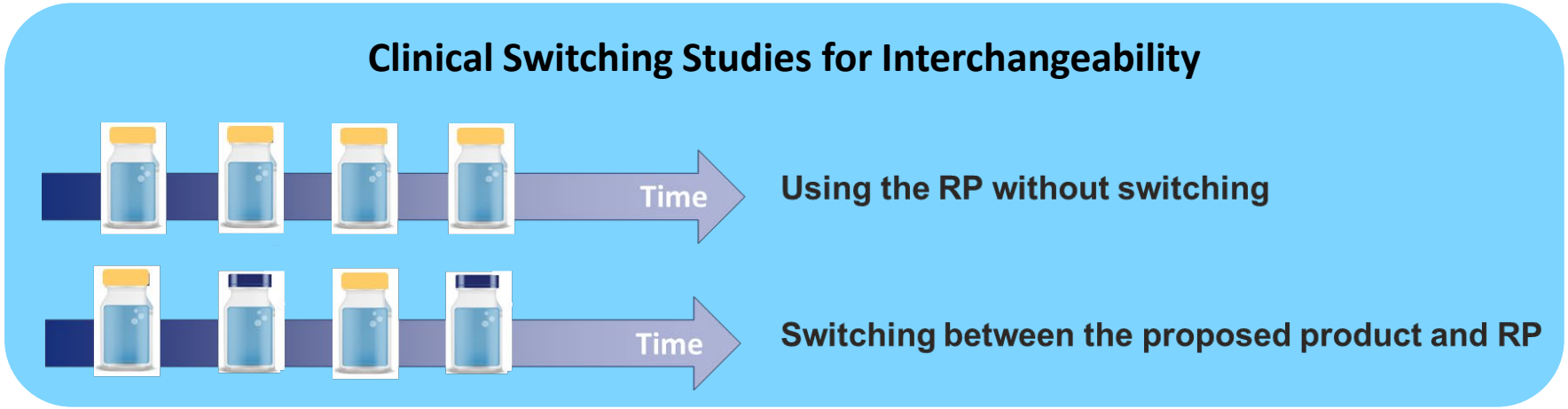
- Comparative analytical assessment is the foundation of biosimilarity.
- Multiple independent analytical methods provide broad molecular characterization, enable robust comparison to the reference product, and minimize blind spots.
- Batch-to-batch variability in a reference biologic is analogous to differences observed between a biosimilar and its reference product.
- Comparative analytical assessment for biosimilars mirrors — but is more comprehensive than — comparability assessment for reference biologics.
- Residual uncertainty can be addressed through orthogonal methods, fractionation, and structure-activity relationship studies.

Overall, a rigorous, comprehensive CAA is a system for building scientific confidence in the similarity of the products.

Historical Clinical Expectations to Support Biosimilarity and Interchangeability



- PK comparison to the US reference product
- Additional clinical studies
- Justify a non-US comparator product

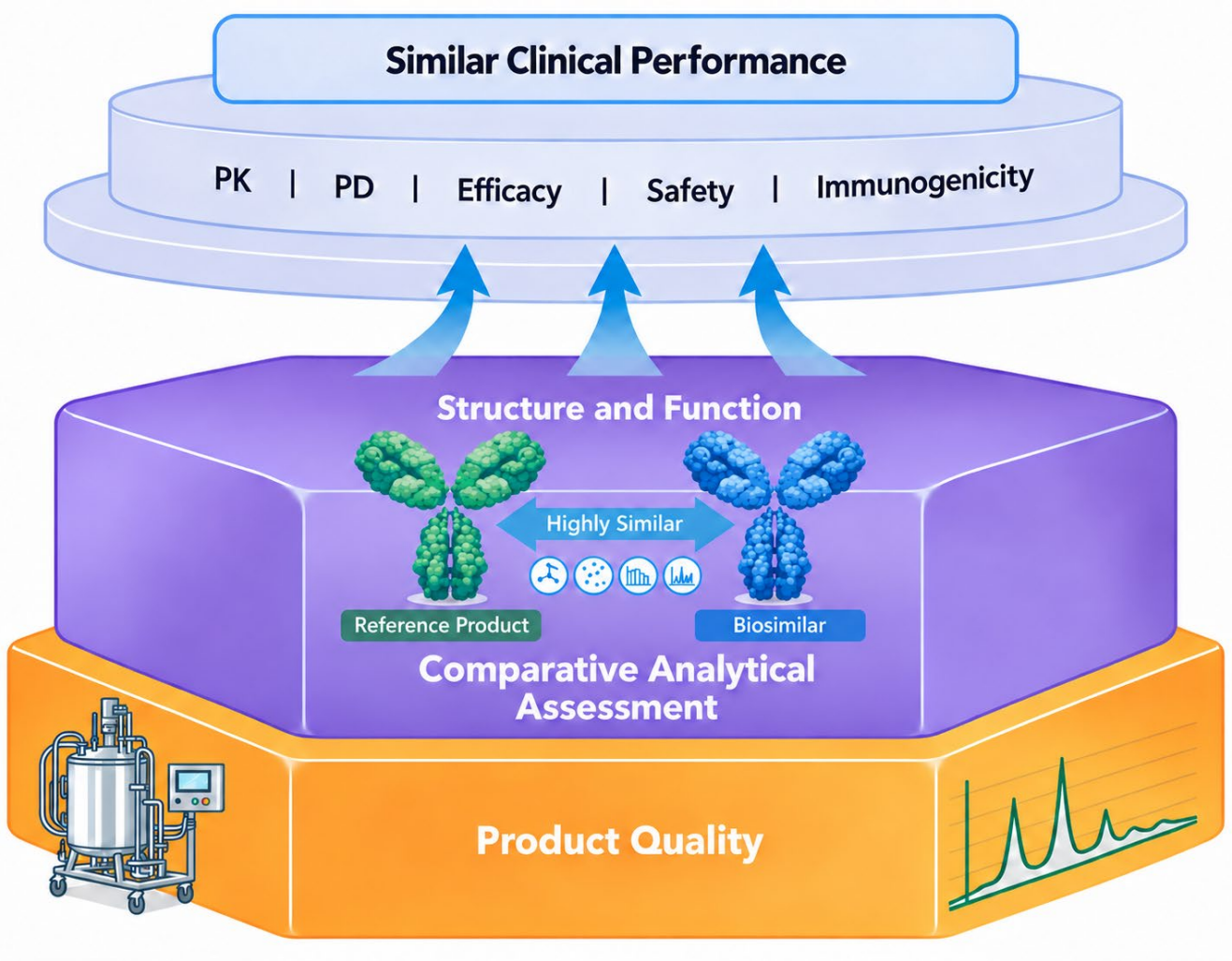


Reflecting on Experience

- Controlled clinical studies for FDA-approved biosimilars do not show safety or immunological concerns with switching between a biosimilar and its reference product
- Multiple reviews of comparative clinical study data have shown that they did not identify true or meaningful differences in efficacy, safety, PK, or immunogenicity between the proposed biosimilar and the reference product.
- Analytical tools can accurately evaluate the structure, biological activity and other physicochemical properties with more precision and sensitivity than clinical studies



Analytical Comparisons Inform Clinical Performance



- Methods are state-of-the-art, sensitive, reliable, and capable of detecting differences
- Functional Assays reflect known or potential mechanism(s) of action
- Results strengthened when different (orthogonal) methods used to evaluate same attribute

Guiding Principles

Improving the efficiency of the biosimilar and interchangeable biosimilar product development and approval process

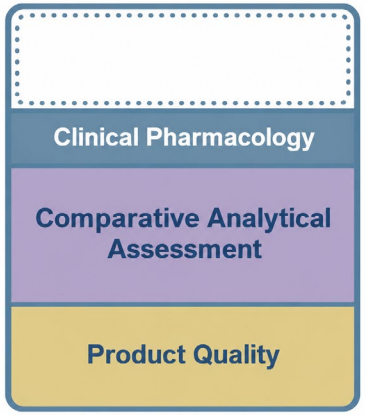
Maximizing scientific and regulatory clarity for the biosimilar product development community



Refine review-related process and practice

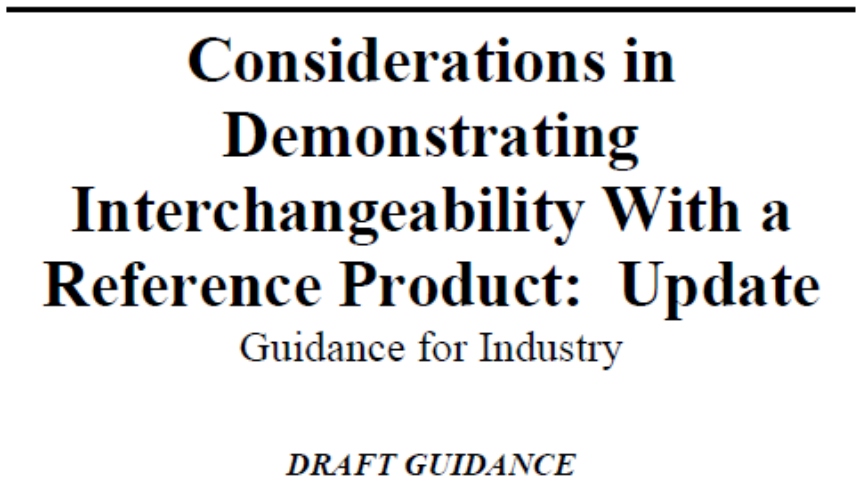


Enhance communication and consistency of development and review



Streamline clinical development programs

Revised Recommendations about Switching Studies June 2024



Considerations in Demonstrating Interchangeability With a Reference Product: Update

Guidance for Industry

DRAFT GUIDANCE

https://www.fda.gov/media/179456/download

"Applicants may choose to provide an **assessment** of why the comparative analytical and clinical data provided in the application or supplement support a showing that the switching standard set forth in section 351(k)(4)(B) of the PHS Act has been met."

Consider A Streamlined Approach Regarding Clinical Studies in Certain Circumstances October 2025

Scientific Considerations in Demonstrating Biosimilarity to a Reference Product: Updated Recommendations for Assessing the Need for Comparative Efficacy Studies

Guidance for Industry

DRAFT GUIDANCE

https://www.fda.gov/media/189366/download

- Products are manufactured from clonal cell lines, are highly purified, and can be well-characterized analytically
- Relationship between quality attributes and clinical efficacy is generally understood and can be evaluated by assays in the CAA
- A human PK similarity study is feasible and clinically relevant

Refining Expectations for Comparative Efficacy Studies (CES)

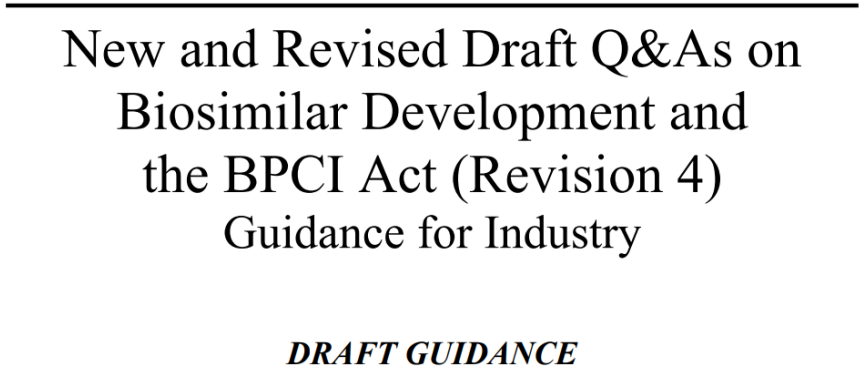
- IPRP BWG Workshop and Concept paper Refined considerations for a CES



- Updated guidance from multiple other health authorities (MHRA, EMA, HealthCanada, PMDA, MFDS, SFDA, NPRA, ANMAT)
- ICH M18 Topic: Framework for Determining Utility of Comparative Efficacy Studies in Biosimilar Development (final concept paper)

ICH
harmonisation for better health

PK Comparisons to Non-US Product may be Justifiable in Certain Circumstances March 2026


New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 4)
Guidance for Industry

DRAFT GUIDANCE

https://www.fda.gov/media/119278/download

- Products are highly purified and can be structurally and functionally characterized
- Show that the US and non-US products have the same or only minor differences in quantitative and qualitative composition of inactive ingredients
- Scientifically justify any analytical differences identified between the US and non-US products

Product Quality Considerations

- Product quality standards for biosimilars and interchangeable biosimilars are **the same** as reference products and CMC development activities need careful planning
  - Commercial facilities, final commercial process (scale up, transfers) and control strategy, and process validation
  - Ensure facilities are inspection-ready
  - Stability data for the commercial shelf-life

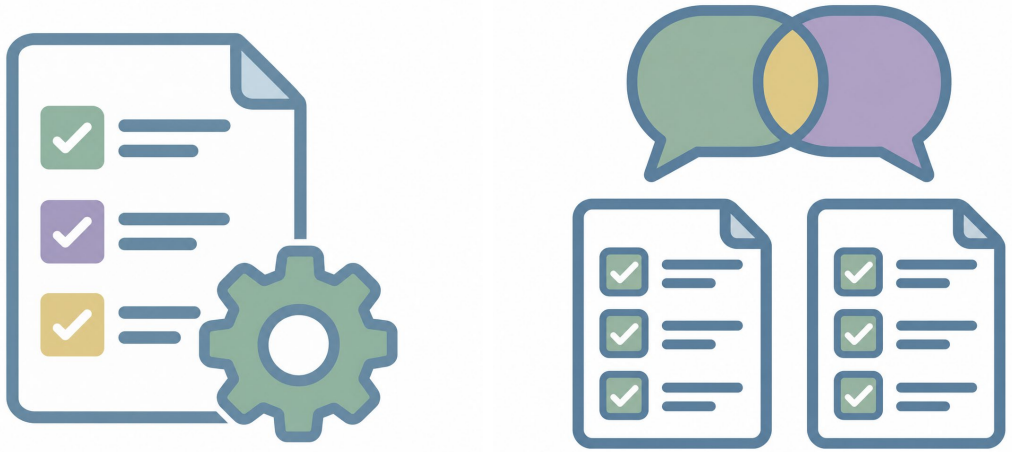
- To help an efficient review, ensure your application is well-organized, links work properly, ensure justifications are clear and scientifically supported





Best Practices for Development Meetings and Select Proposed BsUFA IV Review Efficiencies

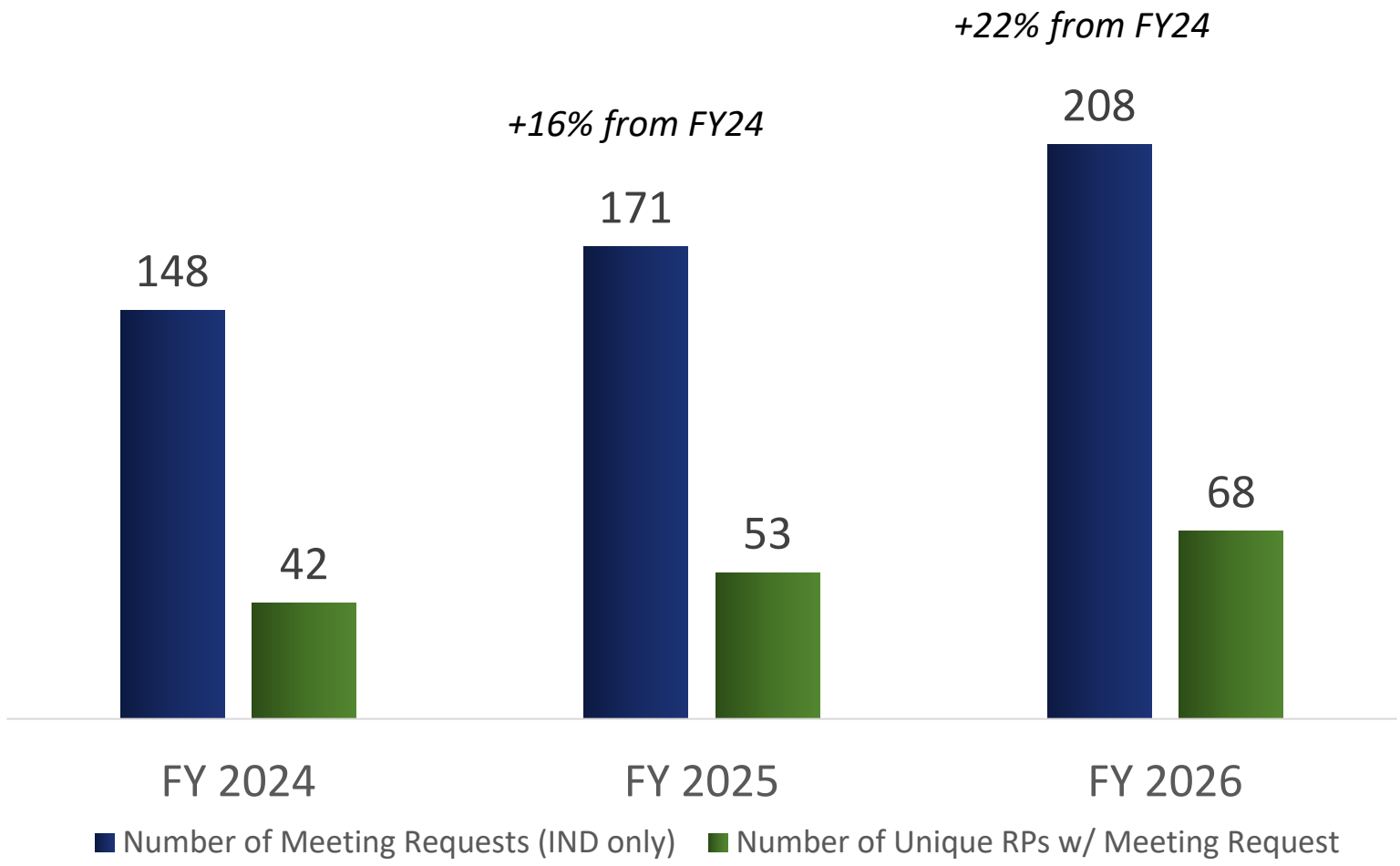
- Thorough, thoughtful Biosimilar Product Development meeting packages explaining overall plan and getting early FDA feedback as needed
  - Be clear about the plans for each of your proposed strengths, dosage forms, routes of administration, and presentations
  - Some discussions may be iterative needing multiple meetings
- Select BsUFA IV Proposals
  - CMC Facility Lifecycle Program
  - BPD meeting updates
  - Revised supplement categories,
  - Imminent Action,
  - Efficiency for submitting iPSPs



State of the Program and BPD Program Activity: FY 2024 – FY 2026

As of **October 27, 2026**:

- CDER received meeting requests to discuss the development of **189** biosimilars for **96** different reference products (RPs)
- FDA has approved **90** 351(k) BLAs for biosimilar products to **21** different RPs, **47** of which are interchangeable; **65** are marketed



Thank You

Visit **www.FDA.gov/biosimilars**