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REGULATORY AND ANALYTICAL SCIENCE OF BIOSIMILARS: A PHARMACEUTICAL CHEMISTRY PERSPECTIVE

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ABSTRACT

The development and approval of biosimilars represent a significant advancement in pharmaceutical science, offering cost-effective therapeutic alternatives to originator biologics. From a pharmaceutical chemistry perspective, the regulatory and analytical evaluation of biosimilars demands a rigorous, multidisciplinary approach to ensure comparable quality, safety, and efficacy. This abstract explores the critical scientific and regulatory frameworks that govern biosimilar development, emphasizing the role of advanced analytical techniques in structural and functional characterization. It discusses key regulatory guidelines from global authorities such as the FDA and EMA, highlighting the stepwise approach in demonstrating bio similarity through quality attributes, non-clinical, and clinical studies. Analytical science plays a central role in deciphering complex molecular structures, glycosylation patterns, and post-translational modifications, using techniques such as mass spectrometry, chromatography, and bioassays. This perspective also addresses the challenges in establishing interchangeability, immunogenicity assessment, and manufacturing consistency. By integrating regulatory requirements with cutting-edge analytical methodologies, pharmaceutical chemists contribute crucially to the reliable development and approval of biosimilars, ensuring therapeutic equivalence and patient safety.

KEYWORDS: Biosimilars, CMC, Regulatory guidelines.

INTRODUCTION

Biologics (biological drugs) are a class of drugs that are produced using a living system such as microorganisms, plant cells or animal cell.[1] Like all drugs, biologics are regulated by United States Food And Drug Administration (FDA).[2] They are generally larger, more complex molecules.[3] Biologics are usually administered via injections and infusions.[3] Biologics are made byreproducing or growing, copies of a specially engineered living cell. The process begins by growing cells in a controlled facility.[4]

BIOSIMILARS

A **biosimilar** (also known as **follow-on biologic** or **subsequent entry biologic**) is a biologic medical product that is almost an identical copy of an original product that is manufactured by different company.[5] Unlike with generic drugs of the more common small-molecule type, biosimilar drugs generally exhibit high molecular complexity and may be quite sensitive to changes in manufacturing processes.[6] They are safe and effective and are developed to make biological drug more affordable.[7] Their development and evaluation involve a rigorous step by step process that include analytical, functional and non clinical and clinical trials.[8]

MOLECULAR AND STRUCTURAL BASIS OF BIOSIMILAR

A biosimilar is defined as a biopharmaceutical drug designed to elicit clinical performance that is similar to that of an already licensed reference product.[1] Unlike their small molecule counterparts, monoclonal antibodies (mAbs) are more complex in nature due to their large size (150 kDa) and multi-chain structure (tetramer, IgG)(2). Further, mAbs demonstrate significant micro-heterogeneity and batch-to-batch variability.[3]

Structural analysis of a biopharmaceutical product, be it a biosimilar or new drug, inevitably requires the use of a wide range of techniques and technologies.[4] The ICH Q6B guidelines for structural investigation, which are invoked by the regulatory authorities as the document detailing their expectations for structural characterization of biosimilars , state that all areas of biomolecular structure must be investigated.[9] This requirement covers primary amino acid sequence and post-translational modifications including glycosylation (if present), through to secondary and tertiary structure and aggregation assessments, as well as an assessment and characterization of product related impurities.[6] Structural analysis is therefore the starting point in the development of a drug product. It goes hand in hand with developing the product itself, as well as the manufacturing process, to produce the expected

end product suitable for use in pharmacokinetics (PK), pharmacodynamics (PD) and clinical –trials.[4]

Structural investigations are performed at the drug development stage to examine and indeed confirm that the product has been made correctly, and also to assess the nature and levels of product related impurities prior to any clinical-based work.[5] It is therefore understandable that analytical methods are liable to evolve as structural data are generated and interpreted, and any further specifically targeted analytical studies are carried out.

Biosimilar structure comparability analysis should describe the primary (amino acid sequence) and higher order structures (HOS), including secondary, tertiary, and quaternary structures. The EMA stresses the importance of comparability of the biosimilar's and the originator antibody's HOS.[8] Detailed structural characterization is highly relevant to the process. This is understandable, considering the importance of the molecule's overall conformation, especially the conformation of the compatibility determining and framework regions (CDR and FR), which are essential for recognizing the antigen. Alterations in these structures may affect the antibody's ability to recognize its antigen and reduce its efficacy.[10]

GLOBAL REGULATORY GUIDELINES

WHO guidelines for biosimilars

1. **Quality**: The comparison showing molecular similarity between the biosimilars and the RP provides the essential rationale for predicting that the clinical safety and efficacy profiles of the RP apply to the biosimilar.[1] The development of a biosimilar requires a high degree of analytical and functional similarity between the biosimilar and the RP. A full quality dossier for both drug substance and drug product is always required.[2] The relevant guidelines for each class of product, such as those issued by the The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology and the WHO Guidelines on evaluation of monoclonal antibodies as similar biotherapeutics products (SBPs).The manufacturer of the biosimilar should additionally carry out a comprehensive and comparative state-of-the-art physicochemical and biological characterization of the biosimilar and the RP and document the results in the submitted dossier.[9]

2. Non Clinical Evaluation: The focus of this section is on the pharma co toxicological evaluation of the biosimilar. To design an appropriate nonclinical study program, one must have a clear understanding of the RP's characteristics. The extent of nonclinical studies required to confirm bio similarity will be influenced by the nature and complexity of the RP. In addition, any differences observed between the biosimilar and RP in the physicochemical and biological analyses will also guide the planning of the nonclinical studies. These include: invitro and in vivo studies.[8]

3. Clinical Evaluation: The main clinical data should be generated using the biosimilar product derived from the final manufacturing process, and which reflects the product for which marketing authorization is being sought. Any deviation from this recommendation needs to be justified and additional data may be required. It comprises of pharmacodynamic and pharmacokinetic studies.[10]

4. Pharmacovigilance: Both RP and biosimilar manufacturers are responsible for ensuring that their products remain safe and efficacious throughout their lifecycle by preventing significant changes to individual products. In this context, it is important to emphasize that the required data can be obtained only by having robust pharmacovigilance systems in place that allow for the collection of product-specific data.[3]

5. **Labelling and prescribing information:** The biosimilar should be clearly identifiable by a unique trade name together with the INN. Biosimilars are assigned INNs using the process and rules used for all biological products. In many cases, the INN for a biosimilar is the same as that for its RP.[11]

6. Roles and Responsibilities of NRA'S: One of the responsibilities of an NRA is to set up appropriate regulatory oversight for the licensing and post-marketing surveillance of biosimilars that are developed and/or authorized for use in its area of jurisdiction. The experience and expertise of the NRA in evaluating biological products are a key prerequisite for appropriate regulatory oversight of these products. The NRA is responsible for clearly defining a suitable regulatory framework for licensing biological product including biosimilars.[12]

ICH guidelines for biosimilars

- Q1A-Q1F: Stability

- Q2: Analytical validation
- Q3-Q3E: Impurities
- Q4A-Q4B: Pharmacopoeias
- Q5A-Q5E: Quality of biotechnological products
- Q6A-Q6B: Specification
- Q7: good manufacturing practice
- Q8: pharmaceutical development
- Q9: Quality risk management
- Q10: Pharmaceutical Quality System
- Q11: Development and Manufacture of Drug Substances
- Q12: Lifecycle Management
- Q13: Continuous Manufacturing of Drug Substances and Drug products
- Q14: Analytical Procedure Development.

INTELLECTUAL PROPERTY RIGHTS IN BIOSIMILARS

Patent Challenges and Litigation: Biosimilar developers have to contend with extensive patent litigation often instigated by reference product sponsors. Numerous originator companies assemble large patent portfolios—not only covering the core biologic molecule but also regulatory data, manufacturing processes, and even ancillary technologies.[2] This "patent thicket" is often a deliberate strategy to delay biosimilar entry into the market. Investigations into litigations have shown that while around half of all patents asserted in biosimilar litigation are manufacturing patents, other composition and formulation patents are more strongly associated with delays in market launch. The prolonged legal process not only increases development costs but also delays the time at which biosimilars can contribute to a competitive market.[7]

Market Entries and Regulatory Pathway: The regulatory pathway for biosimilars is intrinsically linked with IP rights. To gain market approval, biosimilars must undergo a "comparability exercise" demonstrating that any differences from the reference biologic are not clinically meaningful. However, the reliance on data from the innovator's clinical trials and manufacturing processes means that biosimilar developers must negotiate the boundaries of data exclusivity and trade secret protection.[3] From a global perspective, regulatory pathways vary widely. In Europe, the European Medicines Agency (EMA) has been successful in establishing a streamlined biosimilar approval process based on robust

comparability exercises, a framework that has been generally accepted worldwide. Thus, the interplay between regulatory requirements and existing IP rights plays a critical role in determining the speed and success of biosimilars.[1]

Economical and Market Implications: The objectives of IP rights go far beyond securing claims over an invention or a process; they also directly influence pricing strategies, market competition, and ultimately patient access to life-saving therapies. The economic and market outcomes of heavy IP protection in biologics and subsequent biosimilar dynamics are far reaching.[6]

Pricing and Competition: Once biosimilars do enter the market, they tend to generate significant price competition. While they are still not 100% identical to their reference biologics, the introduction of biosimilars forces originator Thus, the economic interplay between IP rights, legal disputes, and the pricing strategies adopted by both innovator and biosimilar companies creates a dynamic market structure that directly affects healthcare expenditure.

Access and Availability: The access of patients to biologics is often hampered by high costs resulting from prolonged IP protection periods. When originators enjoy long exclusivity periods, patients may face limited access to these expensive therapies, especially in markets with constrained healthcare budgets. In contrast, once biosimilars enter the market and competition increases, there is potential for improved patient access thanks to lower prices and more sustainable health economics.[8]

Future Trends and Challenges: Looking forward, the role of IP rights in both biologics and biosimilars is poised to continue evolving in response to technological advances, changing regulatory frameworks, and shifting global market conditions. Ongoing efforts to reform patent strategies, improve litigation processes, and harmonize regulatory guidelines are set to impact the entire biosimilar industry.[5]

INTRODUCTION TO CMC

Sponsors face significant challenges in the development of biosimilars, particularly in the chemistry, manufacturing, and controls (CMC) aspects.[3] Key areas of biosimilar CMC development include establishing a reliable and consistent manufacturing process, controlling critical quality attributes, and developing analytical methods to assess product quality.[3] To

prove bio similarity between the biosimilar and the reference medicinal product (RMP), sponsors must conduct extensive characterisation studies, which include physicochemical and functional assays.[4] Chemistry, Manufacturing, and Controls (CMC) make sure that your biosimilars both meet regulatory requirements and are distinct in the market.[1]

Early CMC development

- **Regulatory Strategy Development:** regulatory strategies considering regional requirements (US, EU, Canada, Japan, Australia).
- **Implementing Quality by Design (QbD) principles in product development for enhanced quality and efficiency.**
- **Manufacturing Process Characterization:** Development, optimization, scale-up, and validation of manufacturing processes ensuring reproducibility and scalability.
- **Risk Management:** Implementing risk management strategies to identify, analyze, and mitigate risks associated with biosimilar development and manufacturing.
- **Analytical Method Development:** Support in developing robust and reliable analytical methods suitable for biosimilar analysis.

Clinical development

- **Regulatory Pathways:** Guidance on regulatory pathways and requirements for high regulated markets.
- **Manufacturing Scale-Up:** Assistance in scaling up manufacturing processes from laboratory to commercial scale.
- **GMP Compliance:** Support in ensuring compliance with Good Manufacturing Practice (GMP) and other relevant regulatory guidelines.

Marketing authorization

- **Regulatory Submissions:** Preparation of CMC sections for regulatory submissions (MAAs, BLAs) and support in electronic submissions.
- **Analytical Comparability:** Guidance on designing and conducting analytical comparability exercises pre- and post-manufacturing changes.
- **CMC Change Control:** Preparation, assessment, and management of CMC change controls.

Post approval activities

- **Post-Approval Changes:** Strategy, writing, and submission of post-approval changes including variations and supplements.
- **Analytical Comparability Exercises:** Guidance on conducting analytical comparability exercises related to proposed post-approval changes.

General CMC support

- **Gap Analysis and Due Diligence:** Assessments and due diligence to identify gaps and ensure compliance.
- **Scientific Advice and Pre-Submission Meetings:** Assistance in scientific advice meetings and pre-submission meetings with regulatory authorities.
- **Project Management:** Comprehensive project management to ensure timelines and deliverables are met.
- **Tailored Training:** Customized training sessions and workshops on biosimilar CMC regulatory topics.

CMC Compliance and Its Importance: CMC compliance involves adhering to the regulations and guidelines established by health authorities such as the FDA, EMA, and ICH. Ensuring compliance is critical for.

1. **Regulatory Approval:** CMC documents are pivotal for drug approval processes. Non-compliance can result in delays, additional costs, or outright rejection of applications.

2. **Product Safety and Efficacy:** Consistency in manufacturing processes ensures that drugs remain safe and effective for consumers.

3. **Market Access and Global Expansion:** Adhering to CMC regulations facilitates international market entry by meeting country-specific requirements.

4. **Lifecycle Management:** CMC compliance extends beyond initial approval, encompassing post-approval changes, product updates, and manufacturing modifications.

Developing a Successful CMC Strategy: A proactive and comprehensive CMC strategy is critical for long-term regulatory success. Steps to develop an effective strategy include.

1. **Conducting a Gap Analysis**

- Identify gaps between existing processes and regulatory requirements.
- Address deficiencies before submission to prevent delays or rejections.

2. **Building a Skilled Team**

- Establish a multidisciplinary team with expertise in manufacturing, quality assurance, and regulatory affairs.
- Leverage external consultants for specialized knowledge.

3. **Investing in Technology**

- Use digital tools for data analytics, document management, and regulatory intelligence.
- Implement systems to track regulatory updates and changes.

4. **Monitoring Post-Approval Changes**

- Maintain a robust change management system to handle variations in manufacturing processes or site transfers.
- Engage with regulators early to discuss significant changes and avoid compliance issues.

CASE EXAMPLES AND REGULATORY BASIS OF BIOSIMILARS

Case study of rituximab

Rituximab was the first mAb approved for treatment of cancer (B cell lymphoma), and it is also approved for immune-mediated and inflammatory diseases, e.g., rheumatoid arthritis, Wegener's granulomatosis. An IgG1k chimeric mAb produced in Chinese hamster ovary (CHO) cells, rituximab targets the B-cell surface receptor CD20.. Twelve additional cysteine bridges are intramolecular and delimit six different globular domains: one variable (V_L) and one constant for the LC (C_L); and, one variable V_H) and three constant for the HCs (C_H1, C_H2 and C_H3). Common post-translational modifications (PTMs) include a conserved N-glycosylation site within its Fc (Asn297) region, N-terminal glutamine to pyroglutamate (pyroGlu) cyclization, and partial C-terminal lysine loss during its synthesis in CHO cells. The primary mechanism of action of rituximab comprises binding of its antigen-binding fragment (Fab) domains to CD20+ B-lymphocytes for induction of apoptosis by either antibody-dependent cell-mediated cytotoxicty (ADCC) and complement-dependent cytotoxicity (CDC).

Case study of Etanercept

Etanercept has been widely used in clinical practice for more than 15 years, and thus it has well-characterized pharmacological, efficacy, and safety profiles. It is produced by the Chinese hamster ovary (CHO) cell expression system as a homodimer of the chimeric protein consisting of the extracellular ligand-binding domain of human tumor necrosis factor receptor

2 (TNFR2) and the Fc domain of human IgG1. The TNFR2 domain contains 4 cysteine-rich domains, 2 *N*-glycosylation sites, and 13 potential *O*-glycosylation sites. The Fc domain contains one *N*-glycosylation site, the IgG hinge, and the CH2 and CH3 domains. Etanercept binds to circulating TNF with high affinity and acts as a natural antagonist to TNF by preventing the TNF molecule from binding to a cell-bound receptor. The Fc region of IgG as a fusion element of etanercept prolongs its serum half-life. Analytical methods were developed based on these MOAs.

SB4 was developed as a biosimilar of etanercept, in accordance with ICH guidelines and the current FDA and EMA guidelines on the development of biosimilar products. These documents provide guidance on test procedures and acceptance criteria for biotechnological/biological products, as well as guidance on quality considerations when similarity is assessed. We describe herein a subset of the 42 state-of-the-art methods of structural and physicochemical analysis and the 19 methods of biological analysis that were performed to evaluate the degree of similarity between SB4 and the reference product.

Case study of Adalimumab

FKB327 w was approved by the European Medicines Agency as a biosimilar to European-authorized adalimumab (Humira®; AbbVie Inc). Adalimumab is a monoclonal antibody, binding and inhibiting tumor necrosis factor (TNF)-α with use indicated for several immune-mediated, chronic, and inflammatory disorders. The approval is based on high similarity in the physicochemical properties between FKB327 and adalimumab. The objective of this study is to assess the biological similarity, with regard to Fab- and Fc-associated functions, and describe the relationship between physicochemical and biological characterization and functional activity. State-of-the-art orthogonal techniques were implemented to assess the structure and function of FKB327. Peptide mapping with liquid chromatography and mass spectrometry, capillary electrophoresis–sodium dodecyl sulfate, ultraviolet circular dichroism, size-exclusion high-performance liquid chromatography (HPLC), and cation exchange HPLC were the techniques used to assess structure. Functional activity was assessed with enzyme-linked immunosorbent assay, surface plasmon resonance, and cell-based assays. The polypeptide sequence of FKB327 was identical to that of adalimumab. FKB327 also was demonstrated to have a similar secondary and tertiary structure to adalimumab. Posttranslational heterogeneities, along with size and charge variants, were not clinically meaningful. FKB327 binds to TNF-α, FcγR, the neonatal Fc receptor, and C1q, and

induces apoptosis, antibody-dependent cellular cytotoxicity, and complement-dependent cytotoxicity. The binding and activity of FKB327 were similar to that of adalimumab. . FKB327 shares similar structure and activity with adalimumab. Based on characterization of physicochemical and biological properties, FKB327 is expected to have a similar safety, immunogenicity amd efficacy profile to adalimubab.

ANALYTICAL STRATEGY AND STEPWISE APPROACH TO BIOSIMILAR

In the very early stages of biosimilar development, it is important to carefully analyse a series of lots of the originator product to determine the protein sequence, identify and quantify enzymatic and non-enzymatic post-translational modification (PTM), analyse biological functionality, and establish variability in product quality attributes against which the biosimilar product in development will be measured.[1] Once lots of the biosimilar product become available, these lots should be tested against the reference product for physical attributes; primary, secondary, and tertiary structural properties; purity and presence of impurities, including those related to the product and its manufacture; and biological activity, all using orthogonal analytical methods that have sufficient sensitivity.[4] Enhanced analytical efforts entail physical, chemical, and biological characterization of a biosimilar product compared with an originator reference product. The development pathway of a biosimilar is specialized, with much greater requirements for physicochemical and biological analytics at early stages than for novel biotherapeutics.[2]

The stepwise approach begins with the analysis of functional and structural characterization at different stages of the manufacturing process of the proposed biosimilar product.[8] Analytical similarity assessment entails the identification of critical quality attributes (CQAs) that are important for clinical outcomes.[6]

Potential Analytical Tools

- Amino acid sequence and modifications: MS, peptide mapping, chromatography
- Glycosylation: Anion exchange, enzymatic digestion, peptide mapping ,CE , MS
- Folding: MS S-S bride determination, calorimetry, and ion mobility MS, NMR, circular dichroism, Fourier transform spectroscopy, fluorescence.
- PEGylation and isomers: Chromatography, peptide mapping
- Proteolysis: Electrophoresis, chromatography, MS.
- Subunit interactions: Chromatography, ion mobility MS.

Calorimetric techniques: Besides spectroscopic methods, calorimetric techniques such as differential scanning calorimetry (DSC) should also be applied for the characterization of biosimilars and comparability studies. DSC provides information on the structural stability of the folded polypeptide, given that the temperature (Tm) at which denaturation occurs is characteristic of the protein stability. Because the denaturation transitions should be the same for a protein drug product and its biosimilar, one can use the DSC thermograms to demonstrate that two products derived from different manufacturing processes are structurally comparable.[6]

Alternative techniques: Other techniques that would complement optical spectroscopic methods include hydrogen/deuterium exchange mass spectrometry, antibody array mapping, and ion-mobility mass spectrometry. Unlike the spectroscopic methods, these alternatives require greater expertise, are likely to be more time consuming, and require significantly costlier instrumentation. However, they are expected to be much more informative than optical spectroscopy alone.[1]

PRIMARY AND HIGHERORDER STRUCTURAL CHARACTERIZATION FOR BIOSIMILARS

The development pathway of a biosimilar is unlike that of a novel biotherapeutic. Analytical requirements are greatly increased before a product enters clinical testing. Enhanced analytical efforts entail physical, chemical, and biological characterization of a biosimilar product compared with an originator reference product. The development pathway of a biosimilar is specialized, with much greater requirements for physicochemical and biological analytics at early stages than for novel biotherapeutics.[13]

Initially, the target originator molecule must be intensively studied to determine the variability of its quality attributes. Multiple originator batches are studied to determine the amino-acid sequence and related PTMs. It is a crucial step because the resulting data form the basis of a quality target protein profile (QTPP) for a biosimilar.[3] There is also a requirement to identify product and process-related variants and impurities.

Specification guidelines from the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) cover physicochemical characterization, with a useful appendix for ensuring that all of a molecule's physical attributes are covered.

Primary Structural Characterization: This focuses on the amino acid sequence and post-translational modifications **(PTMs)**

- **Mass spectrometry (MS)**: Confirms the molecular weight and sequence.
- **High Performance Liquid Chromatography(HPLC):**Analyzes purity and degradation.
- **Edman degradation**: Used for N-terminal sequencing.
- **Peptide mapping**: Identifies modifications like oxidation or glycosylation.

Higher-Order Structural Characterization: This assesses secondary, tertiary, and quaternary structures, which influence biological activity**.**

- **Circular dichroism (CD) spectroscopy**: Evaluates secondary structure.
- **Fourier-transform infrared (FTIR) spectroscopy**: Detects structural changes.
- **Differential scanning calorimetry (DSC):** Measures thermal stability.
- **X-ray crystallography & NMR spectroscopy**: Provide detailed 3D structures**.**

GLYCOSILATON AND POST TRANSLATIONAL MODIFICATION

Glycosylation

Over the past few decades, protein biopharmaceuticals have emerged as one of the most important and innovative fields of human therapeutics, since these drugs are able to fulfill the increasing demand for clinical efficacy and target specificity better than small-molecule drugs. This has enabled the treatment of multiple diseases, such as arthritis, cancer, diabetes and cardiovascular diseases. the increasing expenditures on biologic drugs create a major burden for many healthcare systems, which are forced to introduce rationing of high-cost treatments and prevent patients from accessing the correct treatment Prices for biopharmaceutical therapies greatly exceed the costs for conventional small molecule therapies.

Limitations in developing and manufacturing biosimilars is the structural complexity of biological products, which is inherently related to their biological expression in living systems. Glycosylation is considered as one of the most important CQAs because of its major role in immunogenicity and clinical efficacy of therapeutic proteins. Monoclonal antibody function is dramatically influenced by glycosylation and therefore is a critical quality attribute that must be assessed in therapeutic development. Specifically, some key glycosylation profiles that impact mAb function are.

1. ADCC (Antibody Dependent Cellular Cytotoxicity)
2. Presence of galactose (i.e. “glycosylated”) glycans affect both cell dependent cytotoxicity or CDC and increase clearance
3. High mannose glycans increase clearance considerably
4. The presence of the terminal sialic acid n-glycolylneuraminic acid increases immunogenicity
5. Gallili antigen or the gal alpha gal, is an epitope present in the carbohydrate region in many non-primate mammals and elicits an immunogenic response.

Methods to analyse N-glycans include analysis of released glycans and glycopeptides18 or the analysis of intact or fragmented proteins.[19] The latter option is capable of determining glycan content along with site occupancy of the glycans, however, it requires much more rigorous data interpretation and there are no universal methods to perform this analysis.. More recent advances to accelerate the labelling step proceed through reaction with an activated carbamate (Instant™, for example) to generate a more stable urea. In addition, these newer reagents contain a tertiary amine rendering them capable of MS analysis.

Post translational modification

The majority of therapeutic proteins display one or more post-translational modifications (PTMs). These modifications normally influence the biochemical and therapeutic properties of such proteins. Choosing an expression system capable of generating an appropriate product PTM profile remains one of the most crucial decisions a drug developer must make. The PTMs most commonly associated with currently licensed therapeutic proteins include carboxylation and hydroxylation, amidation and sulfation, disulfide bond formation and proteolytic processing, as well as glycosylation.

γ-Carboxylation and β-hydroxylation: γ-Carboxylation and β-hydroxylation are PTMs characteristic of a small number of proteins, mainly a subset of proteins involved in blood coagulation 13, 14, 15. These PTMs are undertaken by specific carboxylase and hydroxylase enzymes, with conversion of t. arget glutamate residues in the protein backbone to γ-carboxyglutamate (Glu → Gla) and target aspartate residues to β-hydroxy aspartate (Asp → Hya). These modifications are important in facilitating calcium binding.

Amidation and sulfation: Amidation refers to the replacement of a protein's C-terminal carboxyl group with an amide functional group ($CONH_2$). It is a PTM characteristic of many

bioactive peptides, although far less frequently associated with polypeptides 16, 17. In many cases, its exact biological role or roles remain(s) to be fully elucidated; however, it often contributes to peptide stability and/or activity.[12]

CHROMATOGRAPHIC AND ELECTROPHORESIS TECHNIQUE FOR BIOSIMILARS

The higher order structure (secondary, tertiary, and quaternary structures) of a protein must be assessed during therapeutic development and various analytical techniques are available to acquire the appropriate data. Most commonly, high resolution techniques including nuclear magnetic resonance (NMR)8 and X-ray crystallography9 are deployed as the most powerful tools to acquire the finest of details at the atomic level. In the above high resolution analyses the protein is not denatured and kept in its native state which provides information on the overall structure of the protein. Chromatographic analyses that are employed for native state protein assessment are size exclusion chromatograph (SEC)10 and ion-exchange chromatography (IEX),11 which are used to determine aggregation and charge variants, respectively. This section will cover these two chromatographic methods in the analysis of infliximab and biosimilar.[14]

FUNCTIONAL CHARACTERIZATION AND IN VITRO BIOACTIVITY OF BIOSIMILAR

Well established and comprehensive in vitro functional characterization is crucial for the development and production of high quality biosimilar therapeutics. *In vitro* functional and biological characterization support the biosimilar development and production process in a multifaceted manner.[15] In vitro bioactivity studies focus on assessing the biological response of biosimilars in controlled laboratory conditions. These studies help determine whether the biosimilar exhibits comparable pharmacological effects to the reference product.[15] For example, assays measuring cytokine inhibition, receptor binding, and enzymatic activity are commonly used The most important objectives include.

i) characterization of the reference product and definition of the biosimilar quality target profile
ii) supporting successful scale-up
iii) confirming bio similarity of the candidate

iv) characterizing relevant product variants and forced degradation products. Additional aspects, such as supporting clone selection or the extrapolation of indications may arise as well.

Functional characterization methods can be grouped in many ways, *e.g.* based on the antibody region involved, cell-free *vs.* cell-based methods, binding assays *vs.* potency assays. Binding assays vs potency assays classification is followed in the present reviews.[16]

Functional characterization involves evaluating key attributes such as binding affinity, potency, and mechanism of action.[16] This process often includes advanced analytical techniques like mass spectrometry, glycosylation analysis, and surface plasmon resonance to assess structural integrity and biological function. Several advanced analytical techniques are used for biosimilar characterization to ensure structural and functional comparability with the reference biologics. Some of the effective methods are.

- **Mass Spectrometry:** Provides detailed molecular characterization including peptide mapping and post translational modifications.
- **Chromatographic Techniques:** High performance liquid chromatography and capillary electrophoresis help analyze purity charge variants and glycosylation patterns.
- **Surface Plasmon Resonance:** Measures binding affinity and kinetics, crucial for assessing bioactivity.
- **Circular Dichroism (CD) Spectroscopy**: Evaluates secondary and tertiary protein structures.
- **Analytical Ultracentrifugation (AUC)**: Determines molecular weight and aggregation states.
- **Bioassays**: Functional assays that assess potency and biological activity.

STABILITY STUDIES AND FORCED DEGRADATION OF BIOSIMILARS

Stability studies and forced degradation testing are critical components in the development of biosimilars, ensuring their safety, efficacy, and comparability to reference biologics.

Stability Studies: Stability studies of biosimilars are crucial to demonstrate their similarity to the reference biologic, ensure product quality, and support regulatory approval. These studies assess how the biosimilar's characteristics (potency, purity, molecular structure) change over time and under various environmental conditions, like temperature and humidity. The goal is

to establish a shelf life and storage conditions that guarantee the biosimilar's safety and efficacy throughout its use.[17]

Stability studies assess how biosimilars maintain their structural and functional integrity over time under various storage conditions. These studies follow **ICH guidelines** and include.

- **Long-term stability testing** (e.g., refrigerated at 2–8°C or controlled room temperature at 25°C ± 2°C).
- **Accelerated stability testing** (higher temperatures and humidity to predict shelf-life).
- **Batch comparability** (testing multiple biosimilar batches alongside reference biologic batches).
- **Analytical methods** such as potency assays, aggregation analysis, and charge variant profiling.

Examples

1. Adalimumab Biosimilars (e.g., Amgevita, Hulio, Hyrimoz)

- Used for autoimmune diseases like rheumatoid arthritis.
- Stability testing includes forced degradation studies under oxidative stress and freeze-thaw cycles.

2. Rituximab Biosimilars (e.g., Truxima, Ruxience)

- Used for non-Hodgkin’s lymphoma and rheumatoid arthritis.
- Stability studies focus on maintaining monoclonal antibody integrity over time.
  Forced Degradation Studies: Forced degradation studies on biosimilars are intentionally designed to induce degradation, often under conditions more severe than typical storage, to identify potential degradation pathways and the resulting products. This helps assess the similarity of a biosimilar to the reference product, ensuring stability and quality, and aids in establishing stability-indicating analytical methods.
- **Comparability Assessment:** Forced degradation studies help determine the similarities and differences between a biosimilar and the reference product. By subjecting both products to stress conditions, researchers can identify any differences in how they degrade, which can reveal potential differences in manufacturing processes or quality.

- **Stability Characterization:** These studies provide insights into the intrinsic stability of the molecule and the degradation pathways. This information is crucial for determining the shelf life of the product and developing appropriate storage conditions.
- **Development of Stability-Indicating Methods:** By identifying the degradation products, researchers can develop analytical methods that are sensitive and specific to these products, ensuring that stability testing is reliable.
- **Regulatory Requirements:** Forced degradation studies are often required by regulatory agencies, such as the FDA, as part of the biosimilar approval process. They provide a strong foundation for demonstrating the similarity of the biosimilar to the reference product.

IMPURITY PROFILINGANDHOSTCELLPROTEIN(HCP)ANALYSIS

Impurity profiling of biosimilars is a critical process to ensure their safety, efficacy, and regulatory compliance. It involves identifying, characterizing, and quantifying impurities that may arise during manufacturing, storage, or degradation.[17] Types of Impurities in Biosimilars are

1. Product-related impurities: Variants of the therapeutic protein, such as misfolded forms or aggregates.
2. Process-related impurities: Residuals from the production process, including host cell proteins (HCPs), DNA fragments, and purification reagents.
3. Degradation-related impurities: Impurities formed due to environmental factors like temperature, pH, or oxidation.

Impurity Profiling: Impurity profiling plays a **critical role** in regulatory approvals for biosimilars and pharmaceuticals. Regulatory agencies like the **FDA**, **EMA**, and **ICH** require thorough impurity analysis to ensure drug safety, efficacy, and stability.

Analytical Techniques for Impurity Profiling

- Liquid Chromatography-Mass Spectrometry (LC-MS): Highly sensitive method for detecting and quantifying impurities.
- High-Performance Liquid Chromatography (HPLC): Used for separating and analyzing different impurity components.[18]
- Enzyme-Linked Immunosorbent Assay (ELISA): Commonly used for detecting host cell proteins.

- 2D Gel Electrophoresis: Helps visualize protein contaminants.

  Host Cell Protein (HCP): Host Cell Protein (HCP) analysis is a **critical quality control** step in biosimilar development. Since biosimilars are produced using living cells, residual HCPs from the host system can remain in the final product, potentially affecting **drug efficacy, stability, and immunogenicity.**[19]

Key Methods for HCP Analysis in Biosimilars

Enzyme-Linked Immunosorbent Assay (ELISA)

1. **Liquid Chromatography-Mass Spectrometry (LC-MS)**
2. **2D Gel Electrophoresis & Western Blotting**

ANALYTICALSIMILARITYASSESSMENT: STATISTICAL APPROACHES

Analytical similarity assessment is a **key step** in biosimilar development, ensuring that the biosimilar closely matches the reference biologic in terms of **quality, safety, and efficacy.** Analytical similarity assessment in biosimilar studies utilizes statistical approaches to determine the resemblance between a proposed biosimilar and a reference product. This involves identifying critical quality attributes (CQAs), ranking them by risk, and then applying appropriate statistical methods to compare the biosimilar and reference product. A tiered approach, where CQAs are categorized by risk and assessed with varying levels of statistical rigor, is often employed.

***General** Principles For Evaluating Analytical Similarity*

A. Analytical Similarity Assessment Plan

The analytical similarity assessment plan be developed in four stages, corresponding to the following activities

• Development of the risk ranking of the reference product's quality attributes based on the potential impact on the clinical performance categories (i.e., the product's activity as well as pharmacokinetic/pharmacodynamic (PK/PD), safety, and immunogenicity profiles)

• Determination of the statistical methods to be used for evaluating each quality attribute based on the risk ranking and on other factors

• Development of the statistical analysis plan

• Finalization of the analytical similarity assessment plan.

B. Statistical Methods for Evaluation

1. *Tiered Approach for Analytical Similarity Assessment.*

- ***Tier 1: Equivalence Testing***

For high-risk CQAs, statistical equivalence tests are used to demonstrate that the biosimilar's performance is within an acceptable range of the reference product. This can be done using confidence interval or two one-sided t-tests (TOST) methods.

a. *Hypotheses and statistical tests*

Analytical similarity of the quality attributes determined to have the highest potential clinical impact should be evaluated through formal statistical tests of equivalence. Equivalence of attributes measured on a continuous scale can be assessed by testing the difference in means between the proposed biosimilar and reference product.

- ***Tier 2: Quality Range Statistical Approach***

For other high-ranked quantitative CQAs, the quality range approach can be used to assess analytical similarity.

- ***Tier 3: Side-by-Side Comparisons***

For less critical CQAs, side-by-side comparisons can be used to assess similarity.

Attributes to be evaluated in Tier 3 should correspond either to those of lowest risk for potential clinical impact or those attributes which are important but not amenable to formal tests of hypotheses or quantitative evaluation. The number of lots needed for the Tier 3 evaluation can depend upon a number of factors, including, for example, the expected lot-to-lot variability of the attribute. In cases where limited lot-to-lot variability is expected, a single lot of the proposed biosimilar and reference product for the Tier 3 evaluation may be acceptable.

2. Other methods used

- **Descriptive Statistics**
- **Inferential Statistics**
- **Confidence Interval Approach**
- **Two One-Sided t-tests (TOST)**
- **Quality Range Statistical Approach**
- **Side-by-Side Comparisons.**

NONCLINICAL IN VIVO STUDIES

Non-Clinical Studies is a regulatory phase of drug development. To different regulatory agencies it has to be proven that the biosimilar product is similar that of reference product in

terms of its biological and therapeutic characteristics such as quality safety and efficacy before they proceed. Pharmacological Nonclinical biosimilar comparison uses other procedures to ascertain similarity to the reference biologic.[l2]

Principal Methodologies

1. Invitrostudies

Binding Assays : Assess the capacity of the biosimilar to bind to the receptor target, usually performed by the ELISA and surface plasmon resonance.

Functional bioassays: Compare assessments of biological activity (e.g., cell growth, cytokine release.[10]

Structural characterization: Molecular structure is determined by methods such as mass spectrometry, X-ray crystallography and circular dichroism.

Forced Degradation studies: API stability under stressing conditions to predict shelf-life and degradation routes.[15]

2. InVivoStudies

• Pharmacokinetics (PK) and Pharmacodynamics (PD): Animal models allow one to compare absorption, distribution, metabolism, and excretion.

•Toxicology Studies: Assess acute and chronic toxicity, including risk of immunogenicity.

• Extrapolation Studies: Validate the justification of using biosimilars in several indications.[8]

Pharmacokinetics (PK) analysis is quantitative analysis of the kinetics and steady-state relations of the biosimilars. Pharmacodynamics (PD) analysis is quantitative analysis of the drug concentration at an effect site to biosimilar effect relation.

- Critical parameters

1.Areaunderthecurve(AUC)

2.Clearance

3.Half-life.

- Associated assays:

1.Safetypharmacology

2.Reproductivetoxicology

3.Mutagenicityandcarcinogenicitystudies.

Models For In vivo Non-Clinical Studies

Non-clinical studies in the target animal model are conducted for the assessment of similarity of in vivo activity and toxicokinetic. The conventional animal species are rodent (rat/mouse) and non-rodent animals (dog/cat) or non-human primates. These are conducted with a view to further determining comparability or establishing potential differences between the biosimilar and the reference product.

• Particular research objective and research strategy must be clearly considered and designed to reduce the number of animals and not carried out in a tick-box manner.

• Mice or rats, and not a non-human primate species, are usually sufficient to assess PK properties of mAbs. Even if pharmacologic activity is not apparent in rodents, the binding of mAbs to FcRn can be assessed – see above discussion on alternative formulations, novel excipients or higher concentration of existing excipients.

• Whenever feasible, one sex should be tested wherever possible.

• Recovery assessment is not typically necessary.

• A single dose level will usually be sufficient for testing. It ought to be one of those used by the innovator, and not so elevated as to make differences between the intended biosimilar and innovator so readily impossible to discern. In practice, this will usually be the dose that provides exposure within the range of interest clinically, and not the highest dose used by the innovator that is more likely to be saturating.

• The control which can be used to a biosimilar (for in vivo and in vitro and for comparison with the reference product to determine high similarity) need not be a vehicle control group.

• One reference product testing is sufficient.

• Where the innovator used a surrogate (usually because of pharmacologic inertness of the innovator molecule in traditional nonclinical species), it is not recommended to conduct any new tests with a surrogate because already there is a vast quantity of data in humans for the reference product on influencing the target of interest.

PHARMACOKINETICS AND PHARMACODYNAMICS OF BIOSIMILARS

Pharmacokinetics studies

• Target mediated drug disposition (clearance): clearance by target binding is saturable due to the finite number of targets on the cell surface (or circulating soluble targets). Target mediated clearance comparison is of critical importance in the practice of bio similarity but may be difficult to perform in patients due to temporal and widespread variability in target expression.[12]

•Batch selection: Comparative PK study should be conducted using representative batches of biosimilar and reference product and it should be explained how the batches utilized were chosen. In selecting batches, kindly take into account:

•Protein batch content: Delivered volume, wherein pre-filled syringes, injection pens, etc. are utilized.[8]

• Adjustment of protein content: The protein content of selected biosimilar and reference product batches should be previously known and compared by the same analytical method .Well-justified and pre-determined protein content adjustment can be acceptable, provided the variation in dose delivered is not indicative of a systematic difference between the biosimilar and reference product. Similarly, proportional adjustment of protein content in case of non-linear pharmacokinetics should be described in detail.

Alternatives to guaranteeing delivery of the same dose of protein should be addressed

• PK design: Parallel design with extended half-life (t1/2) in mind.

Target density to be kept in mind in the event of target mediated Cl. ADA responses to be taken into consideration in cross over studies, even beyond washout period due to sensitisation. Parallel design is therefore to be preferred. Recombinant forms will not enhance ADA responses. Washout period to be taken into consideration for cross over studies with PD biomarkers. Balanced groups to be ensured (post pre-specified exclusions) and if not, see if this can affect results.[15]

• PK sampling: Should be long enough to avoid >20% extrapolations in AUC0-data. Nonlinear will overestimateAUC0inf because NCA is assumed to be on the basis of linear Cl.

•AUC estimation (trapezoidal rule).: At least three to four samples should be available in the terminal log-linear phase in order to make the terminal rate constant estimate with certainty. AUC extra < 20% of AUC0-data.

Limitations of the study

In comparison to conventional BE approach for small molecules, comparative PK study cannot be utilized to bypass qualitative differences in quality, nonclinical or efficacy and safety studies. The PK study results always must be interpreted and weighed against all other known information.

To what extent the potential differences in disposition between biosimilar and reference product may happen depends on the nature of molecular differences between two drugs. It is

therefore necessary to consider also information on quality attributes and binding features when evaluating the potential for potential differences in pharmacokinetics.

Pharmacodynamic studies

Pharmacodynamic(PD)studies in biosimilars are crucial to establish similarity of biological effect compared to reference biologics. PD studies determine the drug's effect on the body, and PD biomarkers are often used to quantify pharmacologic effect.[20]

Key Features of PD Studies

1.PDBiomarkersBiological markers of drug effect, e.g., receptor binding or cytokine concentrations.

2. Dose-Response Relationship – Considering how various doses affect biomarker response.

3.ComparativePDStudies– Direct comparison of reference product and biosimilar responses between healthy volunteers or patients.

4. Integration with PK Studies – PD data are typically combined with pharmacokinetic (PK) studies to establish similarity.

5. Regulatory Implications – Regulatory agencies like the FDA and EMA are targeting PD biomarkers to enable biosimilar approval.

Pharmacodynamic (PD) biomarkers are crucial biosimilar tools for assessing drug effect and biological response. They are of significant value in a variety of uses.

1. Demonstrating Bio similarity

PD biomarkers provide quantitative evidence that the biosimilar is pharmacologically indistinguishable from the reference biologic. This can have the potential to expedite regulatory approval by reducing the need for costly large-scale clinical trials.

2. Improving Study Effectiveness

Rather than depending on clinical endpoints alone, PD biomarkers enable short, inexpensive studies. They enable dose-response relationships to be determined, i.e., biosimilar assessments can be more accurate.

3. Enabling Regulatory Submissions: Regulatory authorities like the FDA and the EMA prefer the use of PD biomarkers to establish pharmacological similarity. This can lead to faster approval and wider access to biosimilars.

4. ImprovingPatientSafety: By monitoring biological responses, PD biomarkers quantify unforeseen fluctuations in drug action, making biosimilars demonstrate stable safety and efficacy.[20]

COMPARATIVE SAFETY AND EFFICACY STUDIES

Comparative safety and efficacy studies of biosimilars are crucial for demonstrating their similarity to reference biologics. These studies typically involve analytical, functional, and clinical evaluations to ensure that biosimilars provide the same therapeutic benefits without compromising safety.[21]

The study examined adverse event reports focusing on biosimilars of adalimumab, etanercept, infliximab, and rituximab. Findings indicated that biosimilars generally had comparable safety profiles to their reference products, though certain adverse events—such as injection site pain, arthralgia, and fatigue—were reported more frequently for biosimilars. The study concluded that biosimilars remain safe alternatives to reference biologics.[20]

Biosimilar safety studies employ a range of methodologies to assess their risk profiles compared to reference biologics.[14] These include

• Comparative Pharmacokinetics (PK) and Pharmacodynamics (PD) Studies: Evaluating absorption, distribution, metabolism, and elimination to ensure similar exposure and response.

• Immunogenicity Assessments: Measuring anti-drug antibodies (ADA) and neutralizing antibodies (NAb) to detect potential immune reactions.

• Post-Marketing Pharmacovigilance: Continuous monitoring of adverse events through databases like EudraVigilance and FDA's FAERS.

• Switching Studies: Investigating the impact of transitioning patients from reference biologics to biosimilars, ensuring no unexpected safety concerns.

• Real-World Evidence (RWE) Studies: Using observational data to assess long-term safety and effectiveness.

• Extrapolation Studies: Evaluating whether biosimilars can be safely used across multiple indications without additional clinical trials.

Comparative efficacy studies of biosimilars assess their therapeutic equivalence to reference biologics, ensuring they provide similar clinical benefits. These studies typically involve pharmacodynamic assessments, clinical trials, and real-world evidence analyses. Some

agencies, like the UK's MHRA and the WHO, have revised their guidelines to allow biosimilar approval without confirmatory clinical efficacy trials—provided the biosimilar is thoroughly characterized and proven structurally and functionally similar to its reference product. The EMA is also exploring similar revisions, while the FDA remains cautious about reducing efficacy trial requirements.[22] Efficacy studies employ various methodologies to assess the effectiveness of treatments, interventions, or biosimilars. Some common approaches include.

• **Randomized Controlled Trials (RCTs)**: Considered the gold standard, RCTs compare a treatment group with a placebo or control group under controlled conditions.

• **Equivalence and Non-Inferiority Trials**: These trials determine whether a biosimilar performs similarly to the reference biologic within a predefined margin.

• **Pharmacodynamic (PD) Assessments**: Evaluating biomarkers and biological responses to confirm similar efficacy.

• **Real-World Evidence (RWE) Studies**: Observational studies analysing long-term efficacy in diverse patient populations.

• **Extrapolation Studies:** Assessing whether biosimilars can be used across multiple indications without additional trials.

• **Meta-Analyses and Systematic Reviews**: Aggregating data from multiple studies to provide a broader perspective on efficacy.

Statistical Equivalence Testing: Using advanced statistical methods to compare efficacy endpoints.

CLINICAL IMMUNOGENICITY TESTING

Clinical immunogenicity testing of biosimilars is a crucial step in ensuring their safety and efficacy. Biosimilars must demonstrate that they do not induce unexpected immune responses compared to their reference biologics. This involves assessing anti-drug antibodies (ADA), which can impact pharmacokinetics, pharmacodynamics, efficacy, and safety.[23]

Key aspects of immunogenicity testing include

- Comparative clinical evaluations to assess immune response differences between thebiosimilar and reference product.
- Tiered assay systems for detecting and characterizing ADAs.
- Regulatory guidelines from agencies like the FDA and EMA, which outline best practicesfor immunogenicity assessment.

- Single-assay vs. two-assay approaches for evaluating immunogenic similarity.[21]

Immunogenicity testing involves several methodologies to detect and characterize **anti-drugantibodies (ADA)**, ensuring biosimilars do not trigger unexpected immune responses.

Here are the key methodologies.

1. **Screening Assays** – Typically **ELISA** or **electrochemiluminescence (ECL)** assays, used to detect the presence of ADAs in patient samples.
2. **Confirmatory Assays** – Competitive binding assays that verify ADA specificity by testing samples in the presence of excess drug.
3. **Titer Assays** – Quantitative assays that determine the concentration of ADAs in a sample.
4. **Neutralizing Antibody (NAb) Assays** – Functional assays that assess whether ADAs inhibit the biological activity of the drug.
5. **Isotyping and Epitope Mapping** – Techniques like **surface plasmon resonance** or **biolayer** to interferometry characterize ADA binding properties.
6. **Cell-Based Assays** – Used to evaluate the impact of ADAs on drug efficacy in a biological system.

The FDA and EMA have established guidelines for immunogenicity testing to ensure biosimilars and therapeutic proteins do not trigger unexpected immune responses.[23]

FDA Guidelines

The FDA's guidance focuses on developing and validating assays for detecting anti-drug antibodies (ADA). Key aspects include

• Multi-tiered testing approach: Screening, confirmatory, and neutralizing antibody assays.

• Assay validation: Ensuring sensitivity, specificity, and reproducibility.

• Risk-based assessment: Evaluating immunogenicity based on patient population and drug characteristics.

• Comparative immunogenicity studies: Required for biosimilars to demonstrate similarity to reference biologics.[29]

EMA Guidelines

The EMA emphasizes a risk-based approach to immunogenicity assessment. Key points include

• Factors influencing immune response: Patient-related and product-related variables.

• Assay strategy: Binding and neutralizing antibody detection.

• Clinical consequences: Impact on efficacy and safety.

• Integrated immunogenicity summary: Required for regulatory submissions.[30]

POST MARKETING SURVEILLANCE

Post-marketing surveillance of biosimilars is essential for monitoring long-term safety, efficacy, and immunogenicity in real-world settings. Regulatory agencies like the FDA and EMA require ongoing pharmacovigilance to ensure biosimilars maintain their expected performance.[24] The main aspects of post marketing surveillance are.

1. Pharmacovigilance Programs – Regulatory agencies like the FDA, EMA, and WHO require continuous monitoring of biosimilars to track adverse drug reactions (ADRs) and immunogenicity concerns.

2. Real-World Evidence (RWE) – Observational studies, registry data, and electronic health records help assess biosimilar performance outside controlled clinical trials.

3. Interchangeability & Substitution – Surveillance helps evaluate whether biosimilars can be safely substituted for reference biologics without compromising efficacy or safety.

4. Long-Term Safety Monitoring – Since biosimilars are complex biologics, long-term studies are essential to detect rare or delayed adverse effects.

5. Regulatory Frameworks – Agencies like the FDA use systems such as the FAERS (FDA Adverse Event Reporting System) to collect and analyse post-marketing safety data.

Methodologies used for post marketing surveillance

1. Passive Surveillance (Spontaneous Reporting Systems)

o Relies on healthcare professionals and patients voluntarily reporting adverse drug reactions (ADRs).

Examples: FDA's FAERS, EMA's EudraVigilance.

o Strengths: Cost-effective, widely used.

o Limitations: Underreporting and potential bias.

2. Active Surveillance

o Involves systematic data collection to detect safety signals proactively.

o Methods include

- **Cohort Studies** – Following a group of patients using biosimilars over time.
- **Case-Control Studies** – Comparing patients with adverse events to those without.
- **Electronic Health Records (EHR) Analysis** – Mining healthcare databases for trends.

o Strengths: More reliable than passive methods.

o Limitations: Requires significant resources.

3. Real-World Evidence (RWE) & Registries

o Uses observational studies, patient registries, and insurance claims data.

o Helps assess long-term safety, effectiveness, and interchangeability.

o Strengths: Provides large-scale, real-world insights.

o Limitations: Data quality and confounding factors.

4. Pharmacovigilance Programs

o Continuous monitoring through structured frameworks.

o Includes risk management plans (RMPs) and periodic safety update reports (PSURs).

o Strengths: Regulatory-driven, ensures compliance.

o Limitations: Requires ongoing commitment.

5. Innovative Approaches (AI & Big Data)

o AI-driven signal detection and predictive analytics.

o social media and patient-reported outcomes for early warnings.

o Strengths: Faster detection of emerging safety concerns.

o Limitations: Data validation challenges

CASE STUDY OF BIOSIMILARS

1. **Filgrastim (Zarxio)** – This was the first biosimilar to be approved by the FDA in 2015Sandoz's Zarxio is a biosimilar of Neupogen and is designed to prevent infections by boosting white blood cell counts in cancer patients undergoing chemotherapy. Its approval was a landmark in U.S. biosimilar regulation, validating the prospects of abbreviated clinical pathways and yet demonstrating overall safety and effectiveness.

- **Trastuzumab** (Herzuma, Ogivri) – Biosimilars to Herceptin, used in HER2-positive breast cancer, have undergone extensive comparative clinical trials. These studies emphasize the importance of demonstrating equivalent efficacy in neoadjuvant settings and monitoring for cardiac safety.
- **Infliximab** (Inflectra, Renflexis)- Infliximab biosimilars have been at the heart of the conversations on interchangeability and real-world evidence, and are indicated for autoimmune diseases such as rheumatoid arthritis and Crohn’s disease. The latter has

been instrumental in increasing prescriber confidence through its post-marketing surveillance programme.

- **Darfloximab-swnt–** A recently introduced biosimilar example in chronic disease treatment. A prescriber case study by the FDA describes how a prescriber assessed its use by reviewing labeling, safety information, and regulatory materials such as thePurple book.

CTD DOSSIER PREPARATION AND REVIEW

The CTD is divided into five modules, but only Modules 2–5 are harmonized across ICH regions. Module 1 is region-specific.

Module 1: Regional Administrative Information

• Application forms, cover letter, labeling, and prescribing information

•Specific to the regulatory authority (e.g., CDSCO in India, EMA in Europe).

Module 1 of the Common Technical Document (CTD) is region-specific and contains administrative and product-specific information required by the regulatory authority where the application is being submitted. While Modules 2–5 are harmonized across ICH regions, Module 1 varies depending on the country or region (e.g., CDSCO in India, EMA in Europe, FDA in the U.S.).

Key Components of Module 1

1. Application Forms
2. Cover Letter & Administrative Documents
3. Product Information
4. Regional Summaries.

Module 2: CTD Summaries

Module 2 of the Common Technical Document (CTD) is the summary and integration hub of the entire dossier. It distills the detailed data from Modules 3 (Quality), 4 (Nonclinical), and 5 (Clinical) into structured, reviewer-friendly narratives. For biosimilars, this module is especially critical—it's where you articulate the scientific justification for biosimilarity using the "totality of evidence" approach.

Module 3: Quality

Module 3 of the Common Technical Document (CTD) is the Quality section, and for biosimilars, it's where the foundation of similarity is established through detailed Chemistry, Manufacturing, and Controls (CMC) data. This module is often the most voluminous and technically dense, but also the most critical in demonstrating that the biosimilar is highly similar to its reference product.

Structure of Module 3

3.2. S – Drug Substance

• General Information: Nomenclature, structure, physicochemical properties

• Manufacture: Cell line history, upstream/downstream processes, control of materials

• Characterization: Primary and higher-order structure, post-translational modifications (e.g., glycosylation), impurity profiling

• Control of Drug Substance: Specifications, analytical methods, validation

• Reference Standards or Materials

• Container Closure System

• Stability: Real-time and accelerated data, forced degradation studies.

3.2. P – Drug Product

• Description and Composition

• Pharmaceutical Development: Formulation rationale, comparability with reference product

• Manufacture: Batch formula, process validation, in-process controls

• Control of Critical Steps and Intermediates

• Specifications and Analytical Procedures

• Stability: Shelf-life justification, container compatibility.

Module 4: Non-Clinical Study Reports

Module 4 of the Common Technical Document (CTD) houses the Nonclinical Study Reports, and for biosimilars, it plays a supporting—but still important—role in the totality-of-evidence approach. While the emphasis is often on analytical and clinical comparability, nonclinical data can help bridge any residual uncertainty.

Structure of Module4

1. Table of Contents

2. Study Reports.

Module 5: Clinical Study Reports

Module 5 of the Common Technical Document (CTD) contains the Clinical Study Reports, and for biosimilars, it's where the clinical comparability to the reference biologic is demonstrated. This module supports the final layer of the "totality of evidence" approach, following analytical and nonclinical data.

Structure of Module 5: Clinical Documentation

1. Table of Contents
2. Tabular Listing of Clinical Studies
3. Clinical Study Reports.

LABELLING, NAMING AND INTERCHANGEABILITY POLICIES

Biosimilars are regulated through independent regulatory pathways to decide upon their safety and effectiveness. The following are the basics about their policy on naming, labeling and interchangeability.

1. Labelling

The FDA and regulatory bodies suggest that biosimilar labels

- Issue a biosimilarity statement, irrespective of interchangeability designation (e.g., "This product is biosimilar to [reference product].").
- Emphasize the scientific data needed to establish confidence for the safety and efficacy of the product administration, i.e., dosing, administration, and storage.
- Refrain from duplicating clinical information from the reference product unless absolutely necessary in order to explain.[25]

2. Naming

- FDA: Couples root name with four-letter suffix (e.g., adalimumab-atto) to enable traceability and minimize substitution errors.
- EMA: Utilizes the same INN (International Non-proprietaryName) of the original product, with brand names and batch numbers utilized for the pharmacovigilance.
- India (CDSCO): Requests different brand names and manufacturer details but not suffixes.[26]

FDA: Biosimilars are typically given a name by adding the suffix to the root name of the reference biologic. For example

• Reference: infliximab

• Biosimilar: infliximab-dyyb

This naming policy:

• Helps track adverse events.

•Avoids accidental substitution.

• Enables pharmacovigilance operations.

3. Interchangeability

- In the United States, interchangeability is a regulatory term, not a clinical one. A biosimilar that is found interchangeable.
- o. May be substituted on the pharmacy level. without intervention by the prescriber (if state law permits their substitution).
- Should demonstrate that there is no added risk in terms of safety or efficacy when switching between the biosimilar and the reference product.
- New FDA advice suggests switch studies may no longer be required for all biosimilars to be interchangeable, especially if robust analytical and clinical evidence already demonstrate similarity.
- International Comparison: CDSCO and EMA do not offer official interchangeability designations. Substitution is national policy or physician determined.[26]

TRENDS AND INNOVATIONS IN BIOSIMILARS

The biosimilar environment is transforming rapidly due to scientific, regulatory, and market drivers. Some of the most significant trends and innovations influencing the sector include.

1. Advanced Analytical Technologies

• High-resolution mass spectrometry, multi-attribute methodologies (MAM), and artificial intelligence-based analytics are driving biosimilar characterization.

• These technologies enhance detection of subtle structural differences and facilitate strong comparability exercises.[27]

2. AI and Machine Learning Integration: AI is applied in the optimization of cell line development, process control, and clinical trial design. Predictive modelling facilitates identification of CQAs and biosimilar development timelines.[25]

3. Real-World Evidence (RWE) and Post-Marketing Surveillance

• Regulators increasingly rely on RWE to back biosimilar safety and efficacy, particularly for interchangeability and switching studies.

• Digital health technologies and pharmacovigilance platforms enhance traceability and monitoring of patients.[28]

4. Personalized and Targeted Biosimilars

• Innovations are arising on the horizon of personalized biosimilars, custom-designed for particular patient subgroups or disease types.

• This may improve therapeutic efficacy and decrease immunogenicity risk.

5. Regulatory Harmonization and Fast-Track Approvals

Regulators such as the FDA, EMA, and WHO are harmonizing guidelines to facilitate faster global approvals. The FDA's revised policy on interchangeability and the EU's track record of automatic substitution are redefining access channels.

CONCLUSION

The development of biosimilars is a highly complex but essential process that ensures affordable access to life-saving biologic therapies while maintaining the same quality, safety, and efficacy as reference products. It involves rigorous structural, functional, and analytical characterization, guided by international regulatory frameworks such as WHO, EMA, FDA, and ICH, along with strict compliance to CMC requirements. Advanced analytical tools, stability studies, impurity profiling, and statistical approaches for similarity assessment all play a crucial role in proving bio similarity. Despite challenges related to intellectual property rights and manufacturing, biosimilars offer significant economic and clinical benefits by reducing treatment costs, enhancing patient access, and fostering global healthcare sustainability.

Their development involves rigorous analytical, nonclinical, and clinical studies to establish similarity in structure, function, pharmacokinetics, pharmacodynamics, safety, and immunogenicity. Post-marketing surveillance and real-world evidence further ensure long-term safety, efficacy, and patient confidence.

With rapid advancements in analytical technologies, AI integration, and global regulatory harmonization, biosimilars are transforming the healthcare landscape. These innovations not

only strengthen comparability assessments but also accelerate approvals, improve accessibility, and pave the way for personalized biosimilars. Ultimately, biosimilars hold great potential to expand patient access to life-saving therapies while maintaining the highest standards of quality and safety.

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REFERENCES

1. Carney RF, Gill RJ, Lakos Z, Zuluaga D, Snyder JL. Biosimilars and their structural characterization. *mAbs*., 2021; 13(1): 1874126. doi:10.1080/19420862.2021.1874126.
2. Babu S, Kunda R, Saidarao K, Jagga D, LoCastro S, Tomar V, et al. Biosimilars CMC development considerations from the sponsor perspective. *Biologicals*, 2021; 69: 1–8. doi:10.1016/j.biologicals.2020.10.001.
3. DiPaola M. Analytical strategy in the development of biosimilars. *American Pharmaceutical Review*, 2019; 22(3): 28–33. doi:10.1080/19420862.2019.1607243.
4. Greer FM. Primary and higher-order structural characterization strategy for biosimilarity assessment. *BioDrugs*, 2018; 32(3): 193–206. doi:10.1007/s40259-018-0280-5.
5. Cho IH, Lee N, Song D, Jung SY, Bou-Assaf G, Sosic Z, et al. Evaluation of the structural, physicochemical, and biological characteristics of SB4, a biosimilar of etanercept. *mAbs*., 2016; 8(6): 1136–55. doi:10.1080/19420862.2016.1193659.
6. Walsh G. Post-translational modifications of protein biopharmaceuticals. *Drug Discovery Today*, 2010; 15(17–18): 773–80. doi:10.1016/j.drudis.2010.06.009

7. Schreiber S, Yamamoto K, Muniz R, Iwura T. Physicochemical analysis and biological characterization of FKB327 as a biosimilar to adalimumab. *mAbs*., 2020; 12(1): 1709332. doi:10.1080/19420862.2019.1709332.
8. Láng J, Cselovszkiné Balogh Z, Nyitrai MF, Juhász C, Baráné Gilicze AK, Iliás A, et al. In vitro functional characterization of biosimilar therapeutic antibodies. *Front Immunol*., 2021; 12: 617514. doi:10.3389/fimmu., 2021; 617514.
9. International Council for Harmonisation (ICH). Specifications: Test procedures and acceptance criteria for biotechnological/biological products Q6B. *ICH Harmonised Guideline*. 1999. doi:10.1163/2210-7975_HRD-9959-0085.
10. Schreiber S, Yamamoto K, Muniz R, Iwura T. Physicochemical analysis and biological characterization of FKB327 as a biosimilar to adalimumab. *mAbs*., 2020; 12(1): 1709332. doi:10.1080/19420862.2019.1709332
11. Schreiber S, Yamamoto K, Muniz R, Iwura T. Physicochemical analysis and biological characterization of FKB327 as a biosimilar to adalimumab. *mAbs*., 2020; 12(1): 1709332. doi:10.1080/19420862.2019.1709332.
12. European Medicines Agency (EMA). Guideline on similar biological medicinal products. *EMA/CHMP/437/04 Rev., 1*. 2014; doi:10.1163/2210-7975_HRD-2014-0156.
13. Greer FM. Primary and higher-order structural characterization strategy for biosimilarity assessment. *BioDrugs*, 2018 Jun; 32(3): 193–206. doi:10.1007/s40259-018-0280-5
14. Láng JA, Balogh ZC, Nyitrai MF, Juhász C, Gilicze AKB, Iliás A, Zólyomi Z, Bodor C, Rábai E. In vitro functional characterization of biosimilar therapeutic antibodies. Drug Discov Today Technol., 2020 Dec; 38: 39–46. doi: 10.1016/j.ddtec.2020.11.010.
15. Wooding KM, Peng W, Mechref Y. Characterization of pharmaceutical IgG and biosimilars using miniaturized platforms and LC-MS/MS. Curr Pharm Biotechnol., 2016; 17(9): 788–801.
16. Mirasol F. Assessing biosimilarity with analytical advancements. BioPharm Int., 2023 Jun; 36(6): 30–31.
17. Venkatesan P, Valliappan K. Impurity profiling: theory and practice. J Pharm Sci Res., 2014; 6(7): 254–259.
18. Dimpal D, Rutuja D, Ganesh S, Kajal P, Dhananjay P. A review on the impurity profile of pharmaceuticals. Open Access J Pharm Res. 2024 Mar 4; 8(1): 000302. doi: 10.23880/oajpr-16000302.

19. Martínez VPM, Espinosa-de la Garza CE, Pérez NO. Opportunities in the field of host-cell proteins Part 1: Their sources and implications for protein-drug efficacy. BioProcess Int., 2022 Sep 28.
20. U.S. Food and Drug Administration. Pharmacodynamic biomarkers: their role in biosimilar product development. FDA., 2023 Apr 3.
21. Kurki P. Generics and Biosimilars Initiative Journal (GaBI Journal)., 2024; 13(1): 23–6. doi: 10.5639/gabij., 2024; 1301.004.
22. Nikitina V, Santi Laurini G, Montanaro N, Motola D. Comparative safety profiles of biosimilars vs. originators used in rheumatology: a pharmacovigilance analysis of the EudraVigilance database. J Clin Med., 2025 Feb 28; 14(5): 1644. doi: 10.3390/jcm14051644.
23. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. MAbs., 2010; 2(3): 256–65.
24. Hashmi IK, Shaik MA. BIOSIMILARS: A complete review on manufacturing, FDA approval, case studies. Int J Pharm Sci Res., 2024 Oct 1; 15(10): 2963–72. doi: 10.13040/IJPSR.0975-8232.15(10): 2963-72.
25. Hashmi IK, Shaik MA. Biosimilars: a complete review on manufacturing, FDA approval, case studies. Int J Pharm Sci Res., 2024 Oct 1; 15(10): 2963–72.
26. Poore D. Breaking down biosimilar barriers: interchangeability. Center for Biosimilars. 2024 Nov 14.
27. Whelan S. Top trends shaping pharma in 2024: AI, gene editing, biosimilars and real-world data. Technology Networks. 2024.
28. Rao MA, Vibhavari A, Pachika V, Sindhuri VG. Review on biosimilars: innovations, challenges, and future prospects. J Emerg Technol Innov Res (JETIR)., 2025 Feb; 12(2).
29. Seidl A, Hainzl O, Richter M, Fischer R, Böhm S, Deutel B, Hartinger M, Windisch J, Casadevall N, London GM, Macdougall I. Tungsten-induced denaturation and aggregation of epoetin-alfa during primary packaging as a cause of immunogenicity. Pharm Res., 2012; 29(6): 1454–67.
30. Filipe V, Jiskoot W, Basmeleh AH, Halim A, Schellekens H, Brinks V. Immunogenicity of different stressed IgG monoclonal antibody formulations in immune tolerant transgenic mice. MAbs., 2012; 4: 740–52.