

Analytical Approach for Biosimilar Development: Special Focus on Monoclonal Antibody Biosimilars

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Abstract

Long-term safety and efficacy of biosimilars are approved by using abbreviated methods and clinical studies may not always adequate to ensure comparability of biosimilar mAbs comparing to its reference product. Therefore, the analytical strategy of the physicochemical comparison of a biosimilar to its reference product becomes an important data to indicate clinical similarity in safety and efficacy. FDA recommended that demonstration of biosimilarity between reference and biosimilar versions is based upon data derived from analytical studies to show "high similarity" to the reference product not withstanding minor differences in clinically inactive components. Therefore, the physicochemical analytical comparison between biosimilar and its reference product is the primary consideration during biosimilar development. In these review, the approach for physicochemical characterization, biological activity and impurities assessment were reviewed.

Keywords

Biosimilar; Monoclonal Antibody; Cytotoxicity

Abbreviations

Mab: Monoclonal Antibody
HPLC: High Performance Liquid Chromatography
LC-MS: Liquid Chromatography-Mass Spectrometry
FTIR: Fourier Transform Infra Red
DSC: Differential Scanning Calorimetry
CD: Circular Dichroism
UV280: Ultraviolet 280nm
MOA: Mechanism of Action
ELISA: Enzyme-Linked Immunosorbent Assay
SPR: Surface Plasmon Resonance
ADCC: Antibody-Dependent Cell Mediate Cytotoxicity
CDC: Complement-Dependent Cytotoxicity
GP: Gel Permeation
SE: Size Exclusion
CE-SDS: Capillary Electrophoresis-Sodium Dodecyl Sulfate
CZE: Capillary Zone Electrophoresis
cIEF: Capillary IsoElectric Focusing
2-AB: 2-AminoBenzamide
HCP: Host Cell Protein
qPCR: Quantitative Polymer Chain Reaction
CQA: Critical Quality Attribute

Introduction

Biosimilars offer an attractive possibility for health care cost reduction, and this are becoming an attractive consideration for most health care systems [1,2]. Long-term safety and efficacy of biosimilars are approved by using a limited number of analytical methods, and clinical studies may not always be adequate to ensure comparability of biosimilar monoclonal antibodies (mAbs) compared to its reference product [3-5]. Therefore, the analytical strategy for the physicochemical comparison of a biosimilar to its reference product becomes an important procedure to indicate clinical similarity in safety and

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efficacy. Therefore, biosimilars are raising new analytical challenges concerning product characterization and immunogenicity profiling [6-10]. Here we reviewed a general strategy for biosimilar products.

FDA recommend that demonstration of biosimilarity between reference and biosimilar versions is based upon data derived from analytical studies to show "high similarity" to the reference product from originator not withstanding minor differences in clinically inactive components [11-13]. Therefore, the physicochemical analytical comparison between biosimilar and its reference product within the acceptable statistical ranges is the primary consideration during biosimilar development [14,15]. These acceptable range are defined by measuring different lots of the reference products over a period of time [15-17]. Moreover, these analytical data provide significant insight into the cell line expression system, manufacturing process, and scale up stability. It also provides the comparability of physicochemical properties, functional activities, target binding and immunochemical properties, impurities, and finished drug product stability between reference product and reference standards [1,18-20].

Structural and Physicochemical Assessment

Structural and physicochemical assessment of the biosimilar product and the reference product should include all relevant characteristics (e.g., the primary, secondary, tertiary, and quaternary structure; and post translational modifications). Any significant detected differences in quality attributes should be scientifically justified during preclinical studies and clinical trials. The amino acid sequence of the biosimilar product should be identical to the reference product [6,21]. Peptide mapping by mass spectrometer provides information on the primary structure of the biosimilar product as well as the in-depth information of post-translational modifications of different isoforms (Figure 1). These isoforms then require multiple analytical methods to assess their physicochemical characteristics. These analytical methods are based on the structure, heterogeneity, and critical aspects of product performance of the protein being characterized [22-24]. For understanding the full range of physicochemical properties, orthogonal analytical methods are necessary to evaluate quality attributes. Orthogonal methods that use different physicochemical principles to assess the same attribute can provide independent data to support the quality of a specific attribute. Complementary analytical techniques in series, such as peptide mapping or capillary electrophoresis combined with mass spectrometry of the separated molecules, are other ways to provide a meaningful comparison between products [14]. Some examples of structural and physicochemical methods are listed in Table 1. These

methods should be scientifically sound, fit for their intended use, and provide results that are reproducible and reliable. In selecting these tests, it is important to consider the characteristics of the protein product, including known and potential impurities (Table 1).

Biological Activity Assessment

Biological assays are used to demonstrate the mechanism of action (MOA) of the product, as well as to predict its clinical effects. However, the data from biological assays are should only be considered as supplemental to physicochemical analysis. It is a qualitative rather than a quantitative measure of the protein product [15,25,26]. Since structural complexity may disallow physicochemical analysis to confirm the integrity of the higher order structures, the integrity of such structures can be extrapolated from the product's biological activity. If the MOA is known for the reference product, the biological assays can also demonstrate these mechanisms of action. Multiple biological assays should be performed as part of the analytical similarity assessments. Moreover, biological activity is also indicative of the manufacturing processes ability to maintain consistency, product purity, potency, and stability [14,19,23].

The potential caveats of biological assays should be acknowledged. For example, if the biological assays have high variability, they cannot be used to show biosimilarity between the biosimilar product and the reference product. Additionally, bioactivity assays may not fully reflect the clinical activity since bioactivity assays generally do not predict the bioavailability of the product [8,9,19,23,27]. Thus, these limitations should be considered when assessing the robustness of the quality of data supporting biosimilarity and the need for additional information that may address residual uncertainties [22,24,28]. Some representative functional assays are listed in Table 2.

Impurities Assessment

It is required to characterize, identify, and quantify impurities in biologic products. A risk-based assessment should be performed on any differences in process-related impurities identified between the biosimilar product and the reference product. The manufacturer should define the pattern of heterogeneity of the desired product and demonstrate lot-to-lot consistency used in preclinical and clinical studies. Additional pharmacological/toxicological or other studies may be necessary if the manufacturing process produces different impurities or higher levels of impurities than those present in the reference product [1,23,28-30]. Therefore, it is much preferable to remove impurities and contaminants in the downstream process rather than to establish a preclinical testing program for their

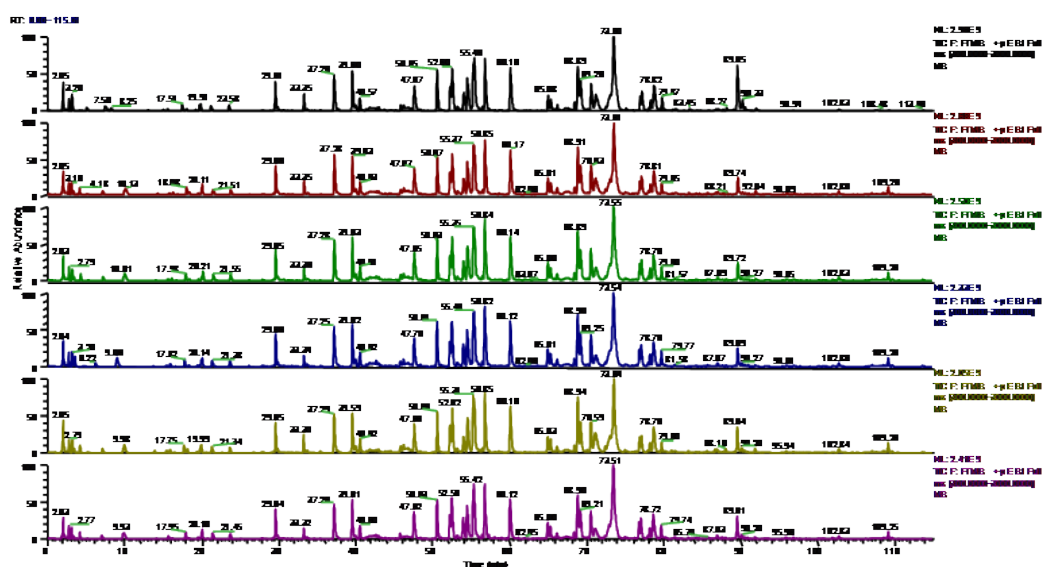


Figure 1: Representative Peptide Mapping of a Mab digested by trypsin

qualification and classification. Common process-related impurities are cell substrates (e.g., host cell DNA, host cell proteins), cell culture components (e.g., antibiotics, media components), and materials in downstream processing steps (e.g., reagents, residual solvents, leachables, endotoxin, bioburden) [19,23,31]. The common analytical techniques for impurities are listed in Table 3.

Conclusion

In this review, the approach for physicochemical characterization, biological activity and impurities assessment were reviewed. The analytical strategy for a biosimilar typically starts with extensive

structural and functional characterization to identify critical quality attributes (CQAs) and clinically active components. Experiments are then used to provide insight into the relationship between CQAs and the clinical safety & efficacy profile; and to predict expected “clinical similarity” from the quality data. Multiple, orthogonal analytical methods of characterization should be chosen specifically to establish quality comparability to the reference product, and certain attributes (e.g. product aggregation and charge heterogeneity) as well as breakdown products during shelf life [32]. Therefore, the comprehensive strategy integration of bioanalytical analysis should

Structural and Physicochemical Methods for Reference Product Comparison	
Primary Structure	
»	Peptide map by visual inspection (Peptide Mapping (HPLC))
»	Molecular weight by mass spectrometry (Intact/Reduced Mass (LC-MS))
»	PTMs including N-terminal, C-terminal Sequencing; Oxidation; Amidation; Deamidation; Sequences coverage; Disulfide bonds by mass spectrometry (Peptide Mapping (LC-MS))
»	Glycosylation including comparison of oligosaccharide patterns (e.g. G0, G0F, G1F and G2F); N-linked oligosaccharide structures, attachment sites and distribution; Glycation ratio and attachment sites; Sialic acid content; Neutral and amino sugar composition (oligosaccharide profiling, N-linked glycan analysis, sialic acid analysis, monosaccharide analysis, and glycation)
»	Amino acid composition (Amino Acid Analysis)
Higher Order Structure	
»	Secondary structures (FTIR)
»	Thermal stability and determination of thermal transition temperatures (DSC)
»	Secondary and tertiary structures (CD)
»	Amount of free sulfhydryl groups (free thiol analysis)
»	Disulfide bond location (Disulfide Bond)
»	Protein conformational change (Antibody Array)
»	Evaluation of the crystal structures of the Fc region (Comparative Evaluation of Fc Structure Using X-ray Crystallography)
Protein Content	
»	Protein Concentration (UV280)
»	Determination of protein concentration (Product specific ELISA)

Table 1: Structural and Physicochemical Methods for Reference Product Comparison

Biological Methods for Reference Product Comparison	
Binding	
»	Target binding affinity (cell based binding affinity (ELISA), soluble receptor-based SPR)
»	All Fcγ receptors binding affinity (SPR/ ELISA)
»	FcRn binding affinity (SPR/ ELISA)
»	Complement binding affinity (SPR/ ELISA)
Function	
»	Fab-associated (<i>In vitro</i> target neutralization/receptor activation/receptor blockade)
»	Fc-associated (ADCC, CDC, complement activation)

Table 2: Biological Methods for Reference Product Comparison

Impurities Assessment Methods for Reference Product Comparison	
Product related variants and impurities	
»	HMW and LMW (gel permeation (GP)/size exclusion (SE) HPLC/CE-SDS)
»	Charge variants (ion exchange HPLC/CZE)
»	Heavy & light chain modifications (reversed phase HPLC)
»	Isoelectric point heterogeneity (cIEF)
»	Carbohydrate analysis (glycans are released from the protein enzymatically, labeled with fluorescence reagent (e.g.2-AB) and analyzed using MS and or LC based techniques)
Process related impurities	
»	Host cell protein analysis (HCP ELISA/MS)
»	Host cell DNA analysis (qPCR)
»	Residual Protein A (if applicable) (ELISA)
»	Endotoxin (Kit)

Table 3: Impurities Assessment Methods for Reference Product Comparison

provide understanding of physicochemical properties; biological activity; purity of the product and impurities from the manufacturing process [33-36]. The biological and immunological methods, such as animal studies, will be needed to further demonstrate biosimilarity in *in vivo* environment before clinical studies can be performed [8]. Depend on the regulatory agency requirements, clinical studies will usually be required to show similar safety profile and pharmacokinetics to the originator's drugs before the biosimilar can be launched to the market. Nevertheless, the body of information from analytical studies not only supports successful CMC and nonclinical development, but also provides insights into the underlying absorption, distribution, metabolism, excretion (ADME) and in-clinical development, and ultimately translated into animal and clinical studies success.

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