GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(REVIEW ARTICLE)

Check for updates

Characterization of biosimilars: Description of the analytical approach

Hichem NAAS [1,*], Sarra MEHENNAOUI [1] and Abdelaziz GHARBI [2]

[1] *Mohammed Lamine DEBAGHINE University Hospital Center, ALGIERS, ALGERIA.*
[2] *Analytical Chemistry laboratory, Faculty of Medicine, BLIDA, ALGERIA.*

GSC Biological and Pharmaceutical Sciences, 2023, 25(02), 215–221

Publication history: Received on 04 October 2023; revised on 12 November 2023; accepted on 15 November 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.2.0482

Abstract

Biological drugs stand out from chemically synthesized drugs by their complexity. The latter puts developers, manufacturers, and controllers of drugs facing really scientific and technological challenges to ensure a safe, effective, and high-quality product. Regulatory agencies evaluate biosimilars based on their level of similarity to the innovative drug, rather than their exact resemblance to it. The recommendations of the Food and Drugs Administration indicate that, when evaluating the product, "The totality of the evidence" will be considered.

With the aim of providing access to cheaper treatment options for patients, the development of biosimilar now focuses on characterization analytical by comparing structural and functional similarities with the product reference biological as it has already undergone the efficacy tests required for its initial approval. Advances in analytical techniques allow for detailed characterization and the identification of differences that may affect the purity, safety, and efficacy of biological product potentially similar to the reference product.

Comparative clinical trials will only take place when the regulatory authority considers them necessary as they are expensive and require more development time, which will against the aim of creating more available and affordable treatment options for patients. In this presentation, the notion of comparability of these agents and their analytical characterization will be presented.

Keywords: Biosimilar; Comparability; Analytical characterization; Critical quality attributes

1. Introduction

The arrival of biosimilars has created competition in the biologics market by offering an attractive opportunity for cost reduction[1][2]. Nevertheless, the persistent uncertainty surrounding their efficacy and safety leaves some healthcare providers reluctant to use them, which limits their use. This uncertainty can be countered through a detailed understanding of the development process.

The FDA has implemented a biosimilar-specific procedure that allows manufacturers to establish the full proof of similarity to the reference product. Analytical tests, non-clinical and clinical information form the basis of this proof. Thus, it will be possible to identify any differences that could impact the purity, efficacy and safety profile[3][4]. Here we will look at a general strategy for biosimilar products.

2. Biological Medicines: Overview

Biological medicines contain active substances from a biological source such as living cells or organisms. Biologic drugs are well established in clinical practice and are, in many cases, essential for the treatment of serious and chronic

* Corresponding author: Hichem NAAS

conditions. Their highly targeted action has revolutionized the treatment of rheumatoid arthritis, psoriasis and certain cancers (lymphomas….etc).

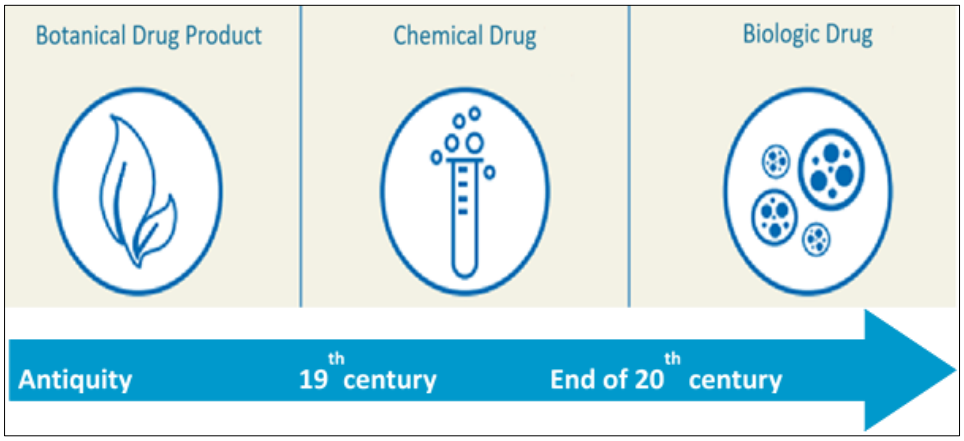

Figure 1 Drug evolution from antiquity to today.

2.1. Large molecular structure

Active substances composed of proteins can vary in size and structural complexity, from simple proteins such as insulin or growth hormones to more complex proteins such as clotting factors or monoclonal antibodies.

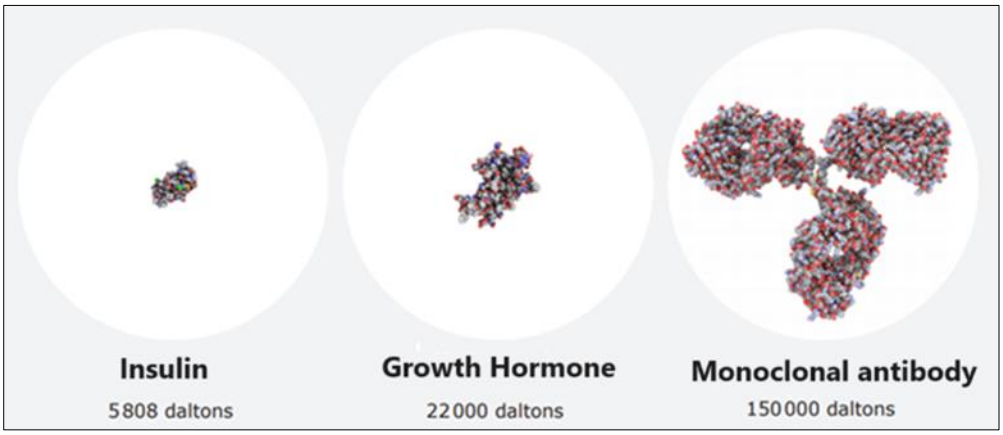

Figure 2 Examples of Proteins in Biologic Medicines

2.2. Inherent degree of variability

Biological medicines are produced by living organisms, which naturally exhibit variations[5]. As a result, the active substance of the final biological drug exhibits a low degree of intrinsic variability "microheterogeneity". This low variability must be within the acceptable range to ensure consistent safety and efficacy. This low degree of variability can be observed within the same batch or between various batches of the same biological medicinal product (Figure 2), particularly in the event of changes in manufacturing processes during the commercial life of the medicinal product (e.g., increase in the scale of production)[5].

Natural variability is inherent in all biological medicines and strict controls are always in place during manufacturing to ensure that this does not influence the way the medicine works or its safety. These include examinations of physicochemical properties, biological activity, purity, sterility, and stability.

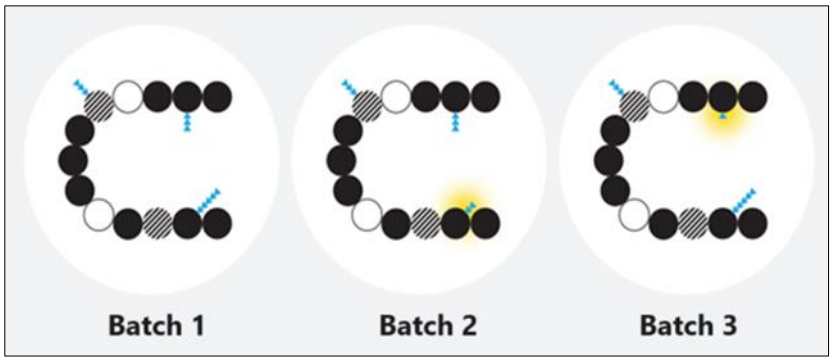

Figure 3 Example of variability between different batches of a biological drug

2.3. Potential immunogenicity

Antibodies targeting the biologic drug could neutralize the drug's activity and reduce its efficacy. Potential immunogenicity should therefore always be assessed for all biological drugs [5].

3. Biosimilar drugs: Definition and characteristics

A biosimilar drug is a biological drug that bears a strong resemblance to another reference biological drug (RBD) and has been demonstrated to have no clinically significant differences in terms of safety, efficacy, and purity.

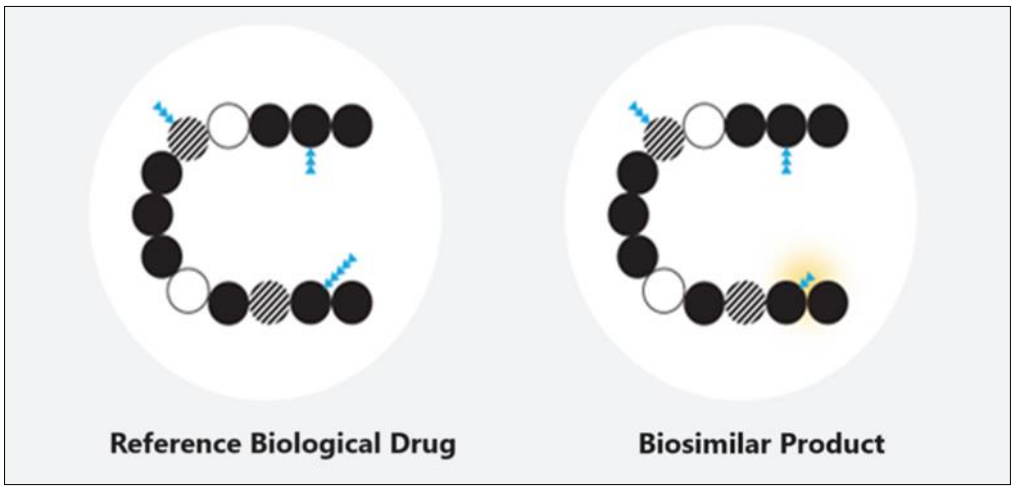

Figure 4 Example of variability between Reference Biological Drug and Biosimilar Product

4. Development and approval of biosimilar drugs

4.1. Approval based on totality of evidence.

Over the years, regulatory authorities have issued guidelines to help developers comply with strict regulatory requirements for approval of biosimilar drugs. These guidelines have evolved to adapt to the rapid advances in biotechnology and analytical sciences, and to take account of experience accumulated during clinical use.

For any biological medicinal product containing a new active substance, the positive benefit/risk ratio is established mainly on the basis of data demonstrating safety and efficacy obtained in pivotal studies carried out in humans, supplemented by data attesting to pharmaceutical quality and by non-clinical data.

Biosimilarity is established through comparative studies with RBD and based on pharmaceutical quality data. The clinical and non-clinical data required for the approval of a biosimilar medicine are different from those required for a biological medicine with a new active substance. These different requirements are explained by the fact that, the biosimilar medicinal product benefits from the experience acquired in terms of safety and effectiveness with the

reference medicinal product.

4.2. A strong regulatory framework

In the USA, the simplified procedure for demonstrating that the product candidate is biosimilar is found in section 351(K) of the Public Health Service act. The characterization will conclude with one of the results mentioned below.

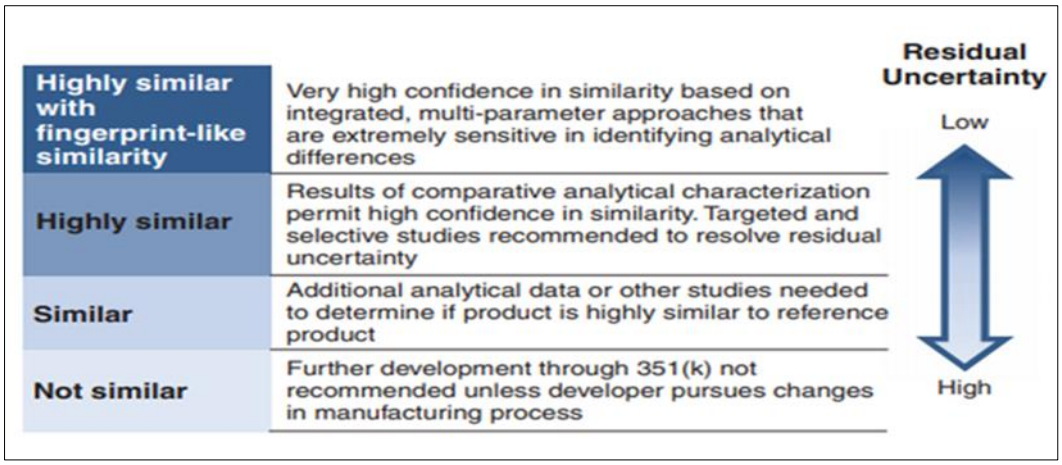

Figure 5 Evaluation of the similarity of a biological drug after comparative analytical characterization. Reproduced from reference 6

Biosimilarity is established by means of comparative studies with the reference medicinal product and based on data attesting to pharmaceutical quality. The objective is not to establish proof of safety and effectiveness independently as is the case with the reference section (351a) but rather to demonstrate that the product is highly similar to the reference through analytical characterization comparative.

4.3. Comparative study: the cornerstone of biosimilar drug development

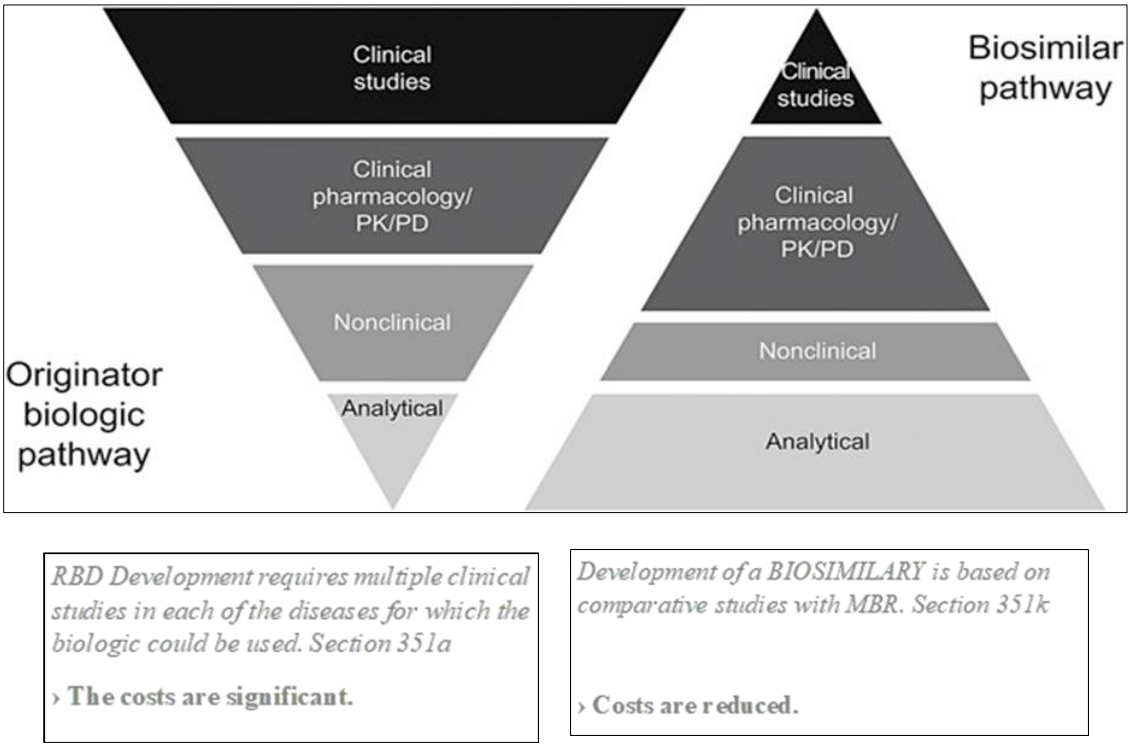

Figure 6 The stepwise approach to the development of reference and biosimilar products

This is a well-established scientific principle in regulatory science: comprehensive quality comparison studies demonstrate the strong similarity of physicochemical properties and biological activity. It is therefore not necessary to repeat the entire clinical development program of the reference drug upon approval of the biosimilar. This is the basic principle of section 351(k) created by the FDA to approve the Biosimilarity candidate.

Comparability in no way means that the quality profile is identical but rather highly similar and that the evidence provided is sufficient to predict that the changes will not affect clinical outcome.

Historically, extensive preclinical and clinical studies have never been defined by the FDA as standard elements of comparability. They are only designed to answer any outstanding questions arising from previous analytical or functional studies.

5. Analytical characterization

Advances in knowledge and analytical techniques have enabled the description of the physicochemical and biological properties of not only the biologically active substance but also the excipients and impurities. These advances coupled with pre-existing data on the reference product make characterization the main axis of the facilitated path to the development of biosimilars.

Therefore, the physicochemical analytical comparison between biosimilar and its reference product within the acceptable statistical ranges is the primary consideration during biosimilar development [7,8]. These acceptable range are defined by measuring different lots of the reference products over a period [8-9]. 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 [1,10-11].

The FDA noted that in addition to criticality, other factors such as the sensitivity of a test and an understanding of the limitations of each type of statistical analysis performed must be considered when adopting a criterion and statistical evaluation tests.

To reduce the risk of error, the FDA recommends orthogonal methodology using 2 or more analytical tests based on different principles to ensure reproducibility and reliability of results. The characterization must include samples from the US and European reference batches, American Pharmacopoeia standards as well as samples from the batches to be tested.

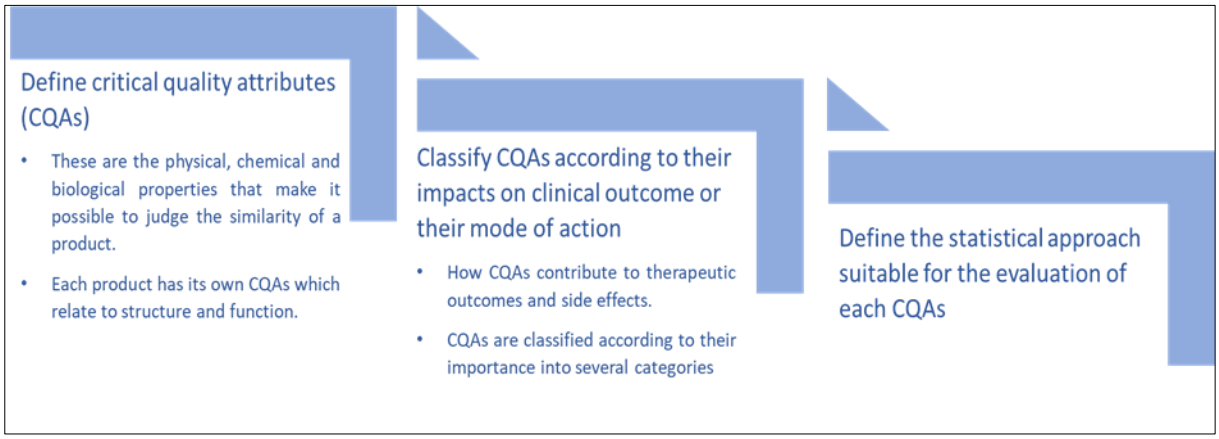

Figure 7 Comparative study of ZARXIO and of his reference

Example: Comparative study of ZARXIO and of his reference

Filgrastim is a recombinant, non-pegylated human granulocyte colony-stimulating factor (G-CSF) analog. It is marketed under the brand name Neupogen® (Amgen).

Granulocyte colony-stimulating factor analogs including Filgrastim, are the mainstays of treatment and prevention of chemotherapy-induced neutropenia and have been found to reduce the risk of neutropenia in various settings, reduce the risk of incidence of febrile, and accelerate the neutrophil recovery.

Zarxio® (Sandoz) has been approved by the FDA as a biosimilar to Neupogen® under procedure 351(k). The manufacturer conducted comparative studies on 6 batches of Zarxio, 4 batches of Neupogen marketed in the United States, and 2 batches of Neupogen from Europe.

Table 1 The most important CQAs selected for Zarxio

Criticality and attributes	**Clinical Relevence**	**Analytic tests**
Very High		
Primary amino acid structure	Efficacy, safety, and immunogenicity	Peptide mapping, and tandem mass spectroscopy
Potency	Efficacy and safety	Bioassay
Target binding	Efficacy and safety	Surface plasmon resonance
Protein concentration	Efficacy	Content determination
High		
Higher order structures	Efficacy and Immunogenicity	Circular dichroism spectroscopy
High molecular Wight aggregates	Immunogenicity	Size-exclusion chromatography
Oxidized variants	Efficacy	Reverse-phase chromatography
Subvisible particles	Immunogenicity	Light obscuration

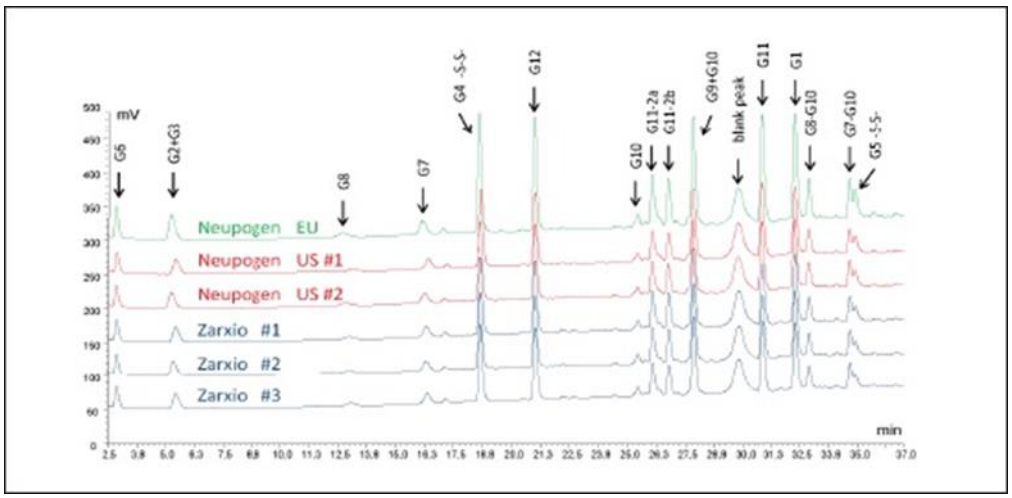

Figure 8 Amino acid sequencing. The proteins were enzymatically digested into peptide fragments. The latter were analysed by reverse phase chromatography. Reproduced from reference 6

In addition to the assessment of structural similarity, the manufacturer established the impurity profile by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE).

The biological activity was evaluated by an in vitro proliferation test.

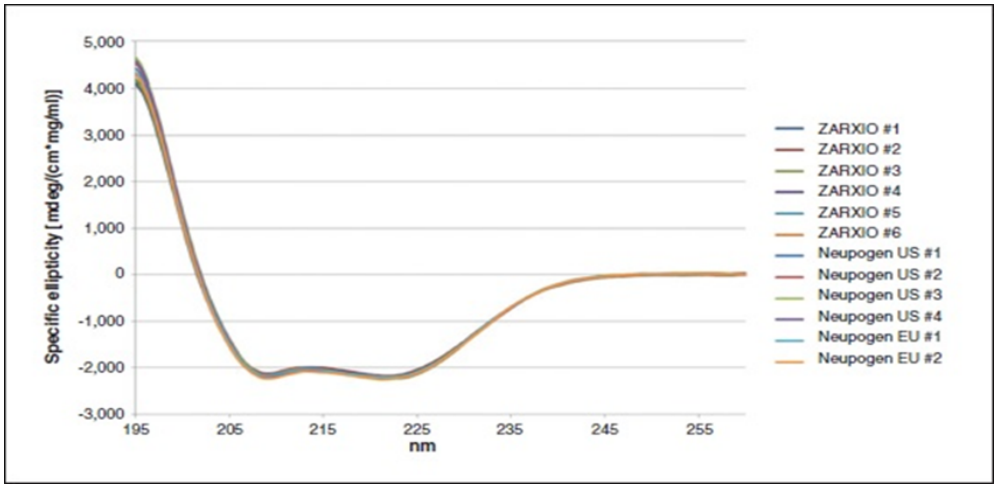

Figure 9 Evaluation of the secondary structure by circular dichroism spectroscopy. This technique measures the absorption of polarized light based on chiral structure. Reproduced from reference 6

6. Conclusion

The regulation of biosimilar drugs in the USA has influenced the development of these drugs around the world, establishing approval requirements that are based on rigorous scientific logic.

Compliance with ethical standards

Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in relation to this document.

References

[1] Khraishi M, Stead D, Lukas M, Scotte F, Schmid H. Biosimilars: A Multidisciplinary Perspective. Clin Ther. 2016 May; 38(5):1238-1249.

[2] Cortés J, Curigliano G, Diéras V. Expert perspectives on biosimilar monoclonal antibodies in breast cancer. Breast Cancer Res Treat.2014 Apr; 144(2):233-239.

[3] Smolen JS, Cohen SB, Tony HP, Scheinberg M, Kivitz A, et al. A randomised, double-blind trial to demonstrate bioequivalence ofGP2013 and reference rituximab combined with methotrexate inpatients with active rheumatoid arthritis. Send to Ann Rheum Dis.2017Sep; 76(9):1598-1602.

[4] Azevedo VF, Galli N, Kleinfelder A, D'Ippolito J, Urbano PC. Etanercept biosimilars. Rheumatol Int. 2015 Feb; 35(2):197-209.

[5] European Medicines Agency. Biosimilar medicines in the EU Information guide for healthcare professionals, October 2019

[6] Peter M.Sullivan, Lisa M.DiGrazia. Analytic characterization of biosimilars.AM J HEALTH-SYST PHARM, Avril 2017.

[7] Sullivan PM, DiGrazia LM. Analytic characterization of biosimilars. Am J Health Syst Pharm. 2017 Apr; 74(8):568-579.

[8] Tan Q, Guo Q, Fang C, Wang C, Li B, et al. Characterization and comparison of commercially available TNF receptor 2-Fc fusion protein products. MAbs. 2012 Nov-Dec; 4(6):761-774.

[9] Storz U. Of patents and patent disputes: The TNFα patent files. Part1: Humira. Hum Antibodies. 2017; 25(1-2):1-16.

[10] Kuhlmann M, Covic A. The protein science of biosimilars. Nephrol Dial Transplant. 2006 Oct; Suppl 5:v4-8.

[11] Jacobs I, Petersel D, Shane LG, Ng CK, Kirchhoff C, et al. Monoclonal Antibody and Fusion Protein Biosimilars Across Therapeutic Areas: A Systematic Review of Published Evidence. BioDrugs. 2016Dec; 30(6):489-523.