MLS - HEALTH & NUTRITION RESEARCH (MLSHNR)
http://mlsjournals.com/Health&nutritionrsearch-Journal
ISSN: 2603-5820
(2024) MLS-Health & Nutrition Research, Vol 3(1) 53-72 doi.org/10.60134/mlshn.v3n1.2654

The scientific overview of the status of biosimilars worldwide and their conception of biosimilarity versus their quality attributes

El panorama científico del estado de los biosimilares a nivel mundial y su concepción de la biosimilaridad frente a sus atributos de calidad

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Manuscript information:

Received/Received: 21/03/24
Revised/Reviewed: 05/04/24
Accepted: 09/05/24

Keywords:
biosimilars, quality attributes, harmonization.

ABSTRACT

The attempt to bring closer biological treatments, with high cost for citizens, has boosted the birth and growth of biosimilar drugs. Molecules whose production is focused on being copies of the active ingredients of drugs of biological origin catalogued as innovative. As they are biological molecules, the fact of being copies of the active ingredient becomes complex, as small variations in their biochemical composition can affect their safety and efficacy. Unlike innovators, whose marketing rationale is aimed at the safety of the drug through clinical studies, the basis for being marketed under safe conditions, however, biosimilar drugs focus on ensuring that their quality attributes are as close as possible to the molecule they are intended to replace. For this reason, by studying the critical quality attributes and the modulators that affect them, it is possible to establish a classification of these attributes that will allow harmonization of the biosimilar concept. The attributes that characterize the molecules are antagonistic or complementary to each other, making it possible to establish a range of acceptance that allows the development of a system for grading the comparability between innovators and biosimilars, bringing the concept, which to date has been theoretical, closer to a quantitative aspect. But always taking into consideration fundamental aspects such as the incidence of laboratory error in its assessment. Therefore, based on a harmonized model of the concept of the quality attribute, this should be reformulated towards a term that unifies the concept with its intrinsic error, so that it can be assessed in a harmonized way.

RESUMEN

El intento de acercamiento de tratamientos biológicos, con elevado coste para los ciudadanos, ha impulsado el nacimiento y crecimiento de los

Palabras clave:
biosimilares, atributos de calidad, armonización

medicamentos biosimilares. Moléculas cuya producción está enfocada a ser copias de los principios activos de los medicamentos de origen biológico catalogados como innovadores. Al ser moléculas biológicas, el hecho de ser copias del principio activo se hace complejo, pues pequeñas variaciones en su composición bioquímica pueden afectar a su seguridad y eficacia. A diferencia de los innovadores, cuyo razonamiento de comercialización está dirigido a la seguridad del medicamento mediante estudios clínicos, base para ser comercializado en condiciones seguras, sin embargo, los medicamentos biosimilares, se centran en que sus atributos de calidad sean los más próximos a la molécula que pretenden sustituir. Por ese motivo, mediante el estudio de los atributos críticos de calidad, y los moduladores que le afectan, es posible establecer una clasificación de los mismos que permitan la armonización del concepto biosimilar. Los atributos que caracterizan a las moléculas son antagonistas o complementarios entre sí, permitiendo establecer un rango de aceptación que permita el desarrollo de un sistema de graduación de la comparabilidad entre innovadores y biosimilares, acercando el concepto hasta la fecha teórico, a un aspecto cuantitativo. Pero siempre tomando en consideración aspectos fundamentales como la incidencia del error del laboratorio en su valoración. Por lo que, basándose en un modelo armonizado del concepto del atributo de calidad, este debe ser reformulado hacia un término que unifique el concepto con su error intrínseco, de manera que pueda ser valorado de forma armonizada.

Introduction

In the last decade, biosimilars have experienced exponential growth, transforming the treatment paradigm for various diseases (1).

They are biologic drugs that have become increasingly popular worldwide (2) and are similar to other biologic drugs already on the market, known as reference biologics. However, unlike these, biosimilars are not identical in terms of molecular structure (3) and aim to provide more affordable and accessible alternatives to reference biologics (4). These drugs have been shown to be equally effective and safe compared to reference biologics in numerous clinical studies.

Currently, biosimilars are available in many countries around the world, but their regulations are diverse and in many cases diffuse. The European Union has been a pioneer in the approval of biosimilars since 2006 (5), and has established a solid regulatory framework to guarantee their quality, efficacy and safety. Other countries, such as the United States and Japan, have also developed their own regulatory frameworks for the approval of biosimilars. One of the most important milestones for biosimilars was the approval of the first biosimilar by the World Health Organization (6).

In terms of therapeutic indications, these drugs cover a wide range of therapeutic areas, such as oncology, rheumatology and diabetes. These drugs have been shown to be effective in treating a variety of diseases and conditions, making them an attractive option for many patients and healthcare professionals.

However, despite advances in the field of biosimilarity, there are still challenges to be overcome. One of the main challenges is public education and awareness about biosimilars, as many patients and healthcare professionals may still have doubts or concerns about the quality and efficacy of biosimilars compared to reference biologics (5).

In addition, access to biosimilars may vary from country to country due to national regulations and policies. Some countries have implemented policies of automatic substitution of reference biological products for biosimilars, which has contributed to greater use and access; however, in other countries, the adoption of biosimilars may be slower due to legal or economic barriers (7).

Based on this, this article aims to critically examine the scientific status of biosimilars, highlighting regulatory progress and the evolution of research in this area, relying on statistics as a means of establishing a definition of the term biosimilar with greater standardization.

Current status of biosimilars in the world..:

1. Global Regulation:

The regulatory frameworks of the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) have been key pillars in the development of biosimilars. As mentioned above, the global regulation of biosimilars is constantly evolving and varies between different regions and countries. However, there are certain key aspects that are considered in the regulation of biosimilars worldwide (8).

In general, international regulators consider the comparability, quality, efficacy and safety of biosimilars to ensure that these drugs are equivalent to reference biologics. Some of the key elements of the global regulation of biosimilars are as follows (9):

a. Benchmarking: Regulators require comparative studies between biosimilars and reference biologics, which include analyses of physicochemical, functional, pharmacokinetic and pharmacodynamic characteristics. This is essential to demonstrate that biosimilars are similar in terms of structure and function to reference biologics.

b. Clinical studies: Biosimilars must also undergo comparative clinical studies that demonstrate their efficacy and safety compared to reference biologics. These studies may include Phase III clinical trials or bioequivalence studies, depending on the requirements of each national or regional regulation.

c. Pharmacovigilance: Regulators emphasize the importance of adequate pharmacovigilance to monitor and evaluate the side effects and long-term safety of biosimilars once they are on the market. This is done through the monitoring and analysis of data on the safety and efficacy of biosimilars.

d. Change of manufacturer: The global regulation also addresses the switching of biosimilar manufacturers and the need to demonstrate equivalence between the different versions of the drug in terms of quality, efficacy and safety.

e. Labeling and nomenclature: The regulation also includes requirements for the labeling of biosimilars, ensuring that patients and healthcare professionals can clearly identify the medicines and distinguish them from reference biologics. In addition, the adoption of an appropriate and distinctive nomenclature for biosimilars is considered important.

While there are international guidelines and regulations for biosimilars, each country or region has its own approach and approval process that must be scrupulously followed by pharmaceutical companies. Some regions, such as the European Union, the United States and Japan, have developed specific regulations and more comprehensive regulatory frameworks for drugs in this category. However, a greater effort is still required to achieve greater harmonization and convergence in the global regulation of biosimilars, since qualitative criteria prevail in their definition.

2. Scientific Developments:

Scientific progress in the characterization and manufacture of biosimilars is essential to guarantee their quality and efficacy.

The scientific development of biosimilars is a steadily growing field, driven by technological advances and scientific knowledge in molecular biology, genomics and biotechnology. The following are some highlights of the current scientific development of biosimilars (10,11):

a. Analytical characterization: Thorough characterization of biosimilars is essential to demonstrate their similarity to reference biologics. Advanced molecular biology techniques, chromatography, mass spectrometry and protein folding and aggregation analysis are used to evaluate the structure, purity and biological activity of biosimilars.

b. Modeling and simulation: Computational modeling and simulation are used in the development of biosimilars to predict and optimize pharmacokinetic and pharmacodynamic properties. This helps to establish the development strategy and identify the critical characteristics of biosimilars.

c. Preclinical studies: Preclinical studies play an important role in the evaluation of the toxicity and biological activity of biosimilars. In vitro and in vivo studies are conducted to demonstrate the similarity between biosimilars and reference biologics.

d. Clinical studies: Comparative clinical studies are crucial to establish equivalence in terms of efficacy, safety and immunogenicity between biosimilars and reference biologics. These studies usually involve patients with specific diseases and evaluate clinical and pharmacokinetic parameters.

e. Innovations in production: The production of biosimilars has undergone significant technological advances, which has improved the quality and efficiency of their manufacture. For example, improvements in cell culture processes, purification

and formulation have allowed for greater reproducibility and consistency in the production of biosimilars.

f. Precision medicine: Precision medicine, which is based on the identification of specific molecular characteristics of patients, is also influencing the development of biosimilars. Approaches such as biosimilar drugs tailored to a specific biomarker profile are being investigated, which could further improve efficacy and safety in the treatment of diseases.

In general, the scientific development of biosimilars continues to evolve with the aim of improving the quality, efficacy and safety of these drugs, through advances in analytical characterization, modeling and simulation, preclinical and clinical studies, innovation in production and the use of precision medicine.

3. Adoption and Challenges:

Despite successes, widespread adoption of biosimilars faces persistent challenges. Despite advances in the field of biosimilars, there are still several challenges that will need to be addressed in the future. These challenges include (12):

a. Change of mentality and education: One of the main challenges is to change the mentality and educate both healthcare professionals and patients about biosimilars. Many still have doubts or concerns about the quality, efficacy and safety of these drugs compared to reference biologics. Increasing awareness and understanding is essential to ensure wider adoption and confidence in biosimilars.

b. More consistent regulations and policies: While many countries have established regulatory frameworks for the approval of biosimilars, there is still some variability among national regulations and policies. It is important to promote greater harmonization and consistency in the evaluation and approval of biosimilars, which would facilitate their entry and access globally.

c. Economic sustainability: As biosimilars enter the market, they may provide more affordable options for patients and healthcare systems. However, due to the costs associated with the production and development of biologic drugs, there is still a need to address the economic sustainability of biosimilars. This involves balancing drug prices to ensure affordability and maintaining investment in research and development.

4. Strengthening the supply chain and quality: The quality and integrity of biosimilars are essential to ensure their efficacy and safety. It is important to strengthen the supply chain (13) and maintain high quality standards in the manufacture, storage and distribution of these drugs. This implies establishing and maintaining effective quality control and regulatory oversight mechanisms.

5. Development of new formats and technologies: As the field of biosimilars continues to evolve, it is also important to invest in research and development of new formats and technologies (14). This includes the formulation of biosimilars in different presentations, such as tablets or inhalers, to provide additional options and convenience to patients and reduce the need to visit medical centers or the training required for administration. New technologies also include the development of molecules that allow several diseases to be treated in a single administration, including research to develop molecules with specificity for two or three different antigens, and thus obtain more specific treatments or broaden the range of action (14).

6. Search for a standardized element of the biosimilar concept: The current definition of biosimilar used to standardize the concept of biocomparability between an innovative drug and a generic drug is reinforced by the fact that these are highly complex molecules, and therefore the comparison between them must be made from a qualitative point of view. The available studies on how to catalog biosimilars always present a comparison between the different attributes on a case-by-case basis (15-18).

If individualized studies of quality attributes are grouped together for globalized comparison, it is possible to establish comparability as a key concept.

From a statistical point of view, and based on the numerous individual studies of quality attributes (19), it is possible to establish whether these attributes are complementary or antagonistic.

One of the main difficulties encountered by regulatory agencies when approving a dossier of a drug that claims to be biocomparable to a reference drug is that the analytical results of the different critical quality attributes (CQA) are presented on an individualized basis. This way of characterizing a biosimilar may cause loss of knowledge or a masking of knowledge by not discussing the complementarity between the same quality attributes together with other CQA. This classification of attributes is intended to ensure that the drug can be interchanged, without any harm to the patient, the treatment with the innovator, maintaining the health benefit, but reducing the cost of treatment or, on the contrary, negatively impacting the outcome of treatment. It is necessary to understand that a biosimilar drug, or any biological drug, may present health risks that are highly variable in both time and effect (8,20)

It is easy to argue that the different attributes independently may be comparable against an innovator drug, but these same attributes found in the different comparisons attached to the approval dossiers (21), do not facilitate understanding for the patient or for untrained personnel.

Therefore, the industry must move towards an evolution of the concept of biosimilar, which will allow the establishment of a clear and understandable definition for every individual. This can provide answers to several of the problems that have been raised throughout the article, such as awareness and education, as well as the definition itself.

One of the simplest forms of presenting any information is the graph (22), as it is the statistical concept demanded by industry and regulatory agencies, and requires little training to elucidate the result provided. Since attributes can exhibit complementarity and antagonism, it is an easily presentable form of comparability and should be part of the definition of the biocomparable antibody. This article establishes the methods for the valuation of the quality attributes, since they form the main structure of the definition.

Method

In order to address the definition of biosimilar and achieve harmonization of the definition, the classification of biosimilars into different phases must be taken, the subject of this article being the analytical or preclinical phase, as it is the most relevant when submitting pre-approval dossiers to any regulatory agency or to clients.

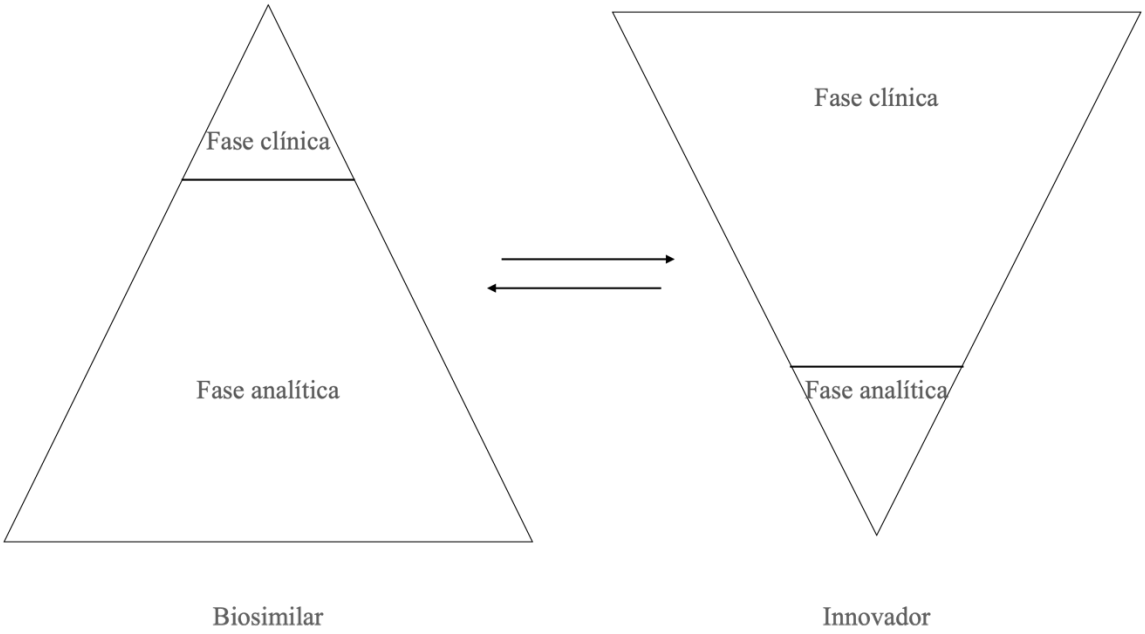

Figure 1. Approach to the development of a biosimilar vs. an innovator

As can be seen in Figure 1, the analytical or preclinical phase is the most relevant phase when establishing a biosimilar, since, as indicated in the most prestigious international guidelines, the fact of being analytically similar for some or analytically comparable for others is a compelling reason for approval. This concept forms part of the basis of the methodology as it is part of the hypotheses identified. For this reason, the starting point of the methodology is the quality attributes. Since the starting point of the quality attributes is so broad, the objective of this article is to delimit the classification of the attributes according to their criticality and the rest of the variables that directly or indirectly influence the quality attributes themselves and how to value them.

A. Quality attributes

From the guidelines published by the agencies, it can be concluded that, for similarity and comparability, they include the common attributes of potency, biological activity and function. But then we find separate structure, properties and stability, with influence on similarity and immunogenicity and pharmacology on comparability. All together should determine a biosimilar.

Based on the terminology provided by Kwon et al. (23) on the critical quality attributes and characteristics that positively and negatively influence the structure of an antibody indicated by BWG (12), a basic equation can be developed that brings together all the aspects required by the agencies and by science.

The authors cited above classify the quality attributes under a 3-level ranking, determining which, according to their criteria, are considered critical. Relying mainly on the functionality of the characteristic indicated in their writings, they divide the attributes into seven major groups, implying their structure and nature.

- Primary protein structure.
- Higher order structures.
- Variants loaded.
- Mass variants.

- Oligosaccharides.
- Biological activity.
- Content.

These attributes are given a score of three levels of criticality compared to their biological or clinical function.

It is true that the attributes indicated are the most relevant in a monoclonal antibody, and it is true for any of its variants that may be developed in the future, but the agglutination in only three levels causes overestimation of the criticality of some attributes and underestimation of others, since the three levels are associated to high, medium and low. And it is true that the worst case is an immunogenicity derived from the molecule, but it should not be at the same level of an attribute that influences a mode of action, since from studies performed in the laboratory by *in vitro* or *in vivo* techniques, sufficient information is obtained to determine the extent of the influence of that attribute.

In response to one of the issues raised, namely the importance of attributes, it is necessary to redefine the criticality of the attributes that currently exist in the field of mAbs (monoclonal antibodies), since they do not exclusively influence one factor. An attribute such as amino acid sequence cannot be included only with influence on biological potency. The amino acid sequence, classified as level 3 criticality by the studies of Kwon et al. (23), it cannot be evaluated together with the rest of the quality attributes because it implies an underestimation of the true complexity. Changes in specific regions of the amino acid structure of the protein can completely modify its conformational structure by charge interactions, even changing the function produced, indicating that it can not only affect potency, but can become a potential immunogenic attribute or simply destabilize the protein. For this reason, it was determined that the study of this attribute should be extracted from the group of critical quality attributes and, in particular, the weight should be placed on the regions determining complementarity (CDR), since these are the most variable regions and the ones directly related to complementarity. These regions are identified in protein databases such as UniProt and can be automatically identified (24). But in the case of biosimilars, being copies of an original molecule, these regions are already pre-sequenced, and it is only necessary to perform a comparative BLAST-type analysis.

The comparative analysis of these characteristics in particular can determine if it presents a complementarity against the same antigen, or if it is a new function for the protein, which would be due to the fact that it is a new molecule. (25) Thus it is determined that not all quality attributes can be compared under the same umbrella, nor under the same classificatory characteristics. It is important to establish a higher level of characterization and to establish groups that are not based exclusively on their function, but on all the interactions they have in a living environment.

From the body of information it is understood that it is necessary to re-classify the critical quality attributes, from a broader view, starting from the knowledge bases provided by Kwon and collaborators or those published by BWG at the Fimea conference 2017.

In such a way that the relationship of an analytical variable, versus antagonist or enhancer, represent a complex biological system through the results of the laboratory analysis itself.

Results

From the study of the quality attributes, a series of characteristics or variables of relevance were obtained when assessing the importance of the different characteristics of the biosimilars and, therefore, what relevance should be applied when classifying their comparability. These attributes can be grouped into the following groups:

A. General quality variables

The general quality attributes can be broken down into the following classification:

- Lower order (primary to tertiary structure):
  - Amino acid sequences: They define the primary structure of the protein. From the initial research study of this parameter, the need to work on this variable independently from the rest of the parameters was obtained due to the influence it has on the rest of the parameters. The variable is controllable by international databases and will be compared by BLAST. Even so, it will be subject to the matrix criterion that will be developed during the subsequent phases.
  - N-terminal pyroglutamate: This variable should initially be studied independently to obtain the scope of its results, techniques with which results can be obtained, influence of the analyst's work, etc
  - C-terminal lysine: as with the previous variable, the influence of this variable and the techniques with which results are obtained should be studied. In turn, its influence on the structure of the antibody and its effect on other non-structural variables must be identified.
  - Disulfide bridges: They are involved in the definition of the tertiary structure of the protein. Their influence on protein stability will be studied independently.
  - Other free amino acids: The stability of this variable in the structure of a monoclonal antibody will be studied.
- Higher order (Quaternary structure): It will be evaluated whether the study of this variable is relevant in the final packaging of a biosimilar, knowing its amino acid structure.
- Variants of loads: Of the variables mentioned below, the analysis techniques used will be studied and the quantification of those identified as qualitative will be assessed.
  - De-amidation.
  - Isomerization.
  - Oxidized forms.
  - Sialized forms.
- Mass variants: As the variables of variant loads, the quantification of techniques that by their nature are qualitative in nature will be obtained and a comparison will be made with respect to their influence on structure, function and immunogenicity.
  - Aggregates.
  - Fragmentations or degradation products.
  - Truncated forms.
  - Monomers.
  - PEGylations.
- Oligosaccharides: As with other variables that make up the critical quality attributes, their influence on the biosimilar concept and, in turn, the

influence of the techniques applied to obtain consistent data should be studied in matrix form. All values are available from field studies for different molecules.

- Fucose or galactose.
- Non-human glycans.
- Glycans *High* manosas.
- Non-glycosylated forms.

- Other post-translational modifications: Changes in the protein during different phases of its development can modify the initial design of an antibody, being a fundamental part in determining comparability and biosimilarity. For this reason, these variables must be studied under the criticality matrix and their influence by the human factor when obtaining results. Since these are qualitative variables, their values will be quantified by normalizing them in order to be able to compare all the critical data.
    - Phosphorylation: addition of phosphate groups to the antibody.
    - Deamidation: removal of an amino group from the antibody.
    - Oxidation: initial modification of amino acid side chain groups by ROS and subsequent conversion to carbonyl and other derivatives.
    - Glycation: modification of amino groups by the action of reducing sugars.
    - Glycosylation: addition of carbohydrates to an antibody.
    - Sulfation: addition of a sulfur trioxide group.
    - Isomerization Succinylation: transformation of one molecule into another.
- Glycan forms: They have an identified immunogenic effect, but of different influence depending on their nature. In turn, they can act on the structure and function of proteins.
    - Manosa: Increases the elimination of antibodies and acts on biological functions.
    - Fucose: They have a direct influence on biological functions, improving ADCC and *binding* if found in lower amounts.
    - Galactose: In the case of exposure, it increases the clearance of antibodies.
    - GlcNAc: They influence the elimination and biological functions of antibodies. Special relevance if bisecting.
    - NANA sialic acid: It has anti-inflammatory activity and is critical in the elimination of fusion proteins. Their influence on mAbs will be studied.
    - NGNA sialic acid: It interferes with the biological functions of antibodies and is immunogenic in humans.
    - Galalpha-3Galbeta1-GlcNAc-R: It is highly immunogenic in humans and produces anaphylaxis.
- Biological activity: this is the name given to the set of variables whose results are obtained from *in vitro* experimentation.
    - ADCC: From this variable we obtain a potency result that is part of the performance of the drug in a living organism. This indicates the ability to neutralize the antigen.

- ADCP: From this variable we obtain a potency result that is part of the performance of the drug in a living organism. Compatibility with the rest of the variables and the scope will be studied.
- CDC: From this variable we obtain a potency result that is part of the performance of the drug in a living organism.
- Apoptosis: The scope of this variable should be studied since not all drugs have the same function. It will be studied how to assess in case of not having influence on all antibodies.
- *Binding*: Variable that highlights the binding capacity against an antigen, its influence on the antibody structure and its effect against potency values will be determined.
- Union FcgR: It responds to binding specificity criteria and is directly related to *binding* and potency. The scope of this variable in a biosimilarity study should be clarified.
- Union C1q: Variable relating one of the functions of antibodies in living complement-binding systems. Being a characteristic not present in all antibodies, its relevance and how to include it in the final equation developed during the research will be studied.
- Union FcRn: It also responds to binding specificity criteria and is directly related to *binding* and potency. The scope of this variable in a biosimilarity study should be clarified.

- Impurities:
  - Host impurities: Since this is one of the characteristics that all parenteral pharmaceutical products have in common, we will study whether its inclusion is relevant when determining whether a monoclonal antibody is a biosimilar. Within this variable are:
    - hcDNA, which refers to the DNA fragments of the host cell that produces the monoclonal antibody. It is measured in particles per billion by PCR and its values must be negligible to be approved. Its elimination occurs in the different stages of purification of a monoclonal antibody.
    - Insulin: a molecule necessary in the development of a culture, but which may not be present in the final formulation.
    - Protein A: Protein used in the first chromatographic columns in the purification of an antibody due to its high affinity to regions of the antibody and used to remove the remaining residues from a culture. Treatment of these columns to remove the antibody may result in the release of protein A and is immunogenic. Therefore, it is eliminated in subsequent treatments.
    - HCP: proteins that are part of the structure and metabolism of host cells. The values of this variable should be negligible in the final form of a biological drug.
  - Leachables/Extractables: impurities that have an adjuvant effect on folding. Its influence and the techniques that detect it should be studied, since they are valued in the critical quality parameters.
- Protein concentration: This variable will be studied in the influence of the clinical phase, since the concentration that the drug must have in market format must be identical to that of the innovator to be compared. It is a value included in the technical dossiers of the products, as well as in the medical vademecums.

B. Quality variables by structure

- Variable region attributes: This variable will be studied together with the parameters detected during the investigation that are directly related to this region of an antibody.
- Attributes constant region: The relevance of this region of the antibody for the characterization of a biosimilar will be studied. Quality analyses will be determined for this region in order to establish the critical quality attributes.
- Physicochemical characterization: This variable will be part of the critical quality attributes characterization matrix. Different levels will be determined by means of statistical techniques or Tier ranking.
- Biological and functional characterization: This variable will be used for the development of the critical quality attributes matrix. Its value will be determined by statistical methods or Tier ranking during the development of the research.

C. Other non-structural quality variables

- Osmolarity: the concentration of the protein in the solution and the rest of the components that make up the buffer in which the active ingredient is embedded can act on the rest of the quality variables, making this comparison indispensable. In proteins where the composition may be comparable, buffer changes may cause the properties to be altered.

From the study of the individual quality attributes and their analytical techniques, it was found that there is an intrinsic error in the techniques and in the laboratories themselves, which are not considered as part of the relevance of the attributes from a statistical point of view:

- Laboratory error: This variable will be developed based on the available methodologies and adapted to the needs of the analysis and development laboratories of the biotechnology industry under GxP methodology. It will act as a modulator of the analysis of each critical quality parameter selected and based on the techniques used to obtain them.

Within this variable, it was found that they should be assessed from two points of view:

- Method errors: The difference between methods that have automation versus methods that are mainly manual or whose critical analysis steps depend on human interaction will be studied
- Uncertainty of the method: The systematic error or bias present in the design of the analytical techniques or in the instrumentation used to assess the attributes will be studied, as opposed to the random error resulting from unpredictable causes and from working with population samples.

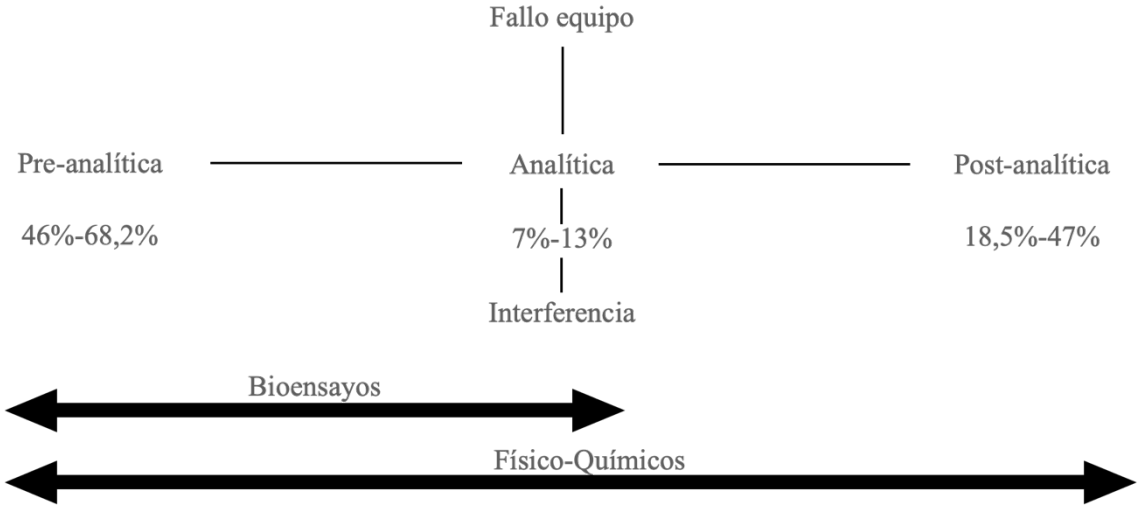

Figure 2. Errors in laboratory tests.

The study of the classification of the quality attributes has been developed taking into account the three main normative guidelines established for the assessment of the biosimilarity of a monoclonal antibody. Since the consideration of biosimilarity must be accompanied by comparability, these attributions have been taken into account at the time of the assessment.

The guidelines established by the major regulatory agencies with international prestige establish qualitative principles for assessing the statistical relevance of the quality attributes of a monoclonal antibody. Taking into account that these agencies have similar regulations and that they are recognized by other agencies and associations as the basis for establishing the internal standards of their respective countries, the study began with a comparison of the major standards and their points in common. The World Health Organization establishes in its international guidelines the following principles as critical points for the establishment of biosimilarity of a monoclonal antibody:

- The physicochemical properties including the structural properties of the antibody
- The biological activity of the antibody
- Impurities present in the antibody preservation medium
- The immunochemical properties of the antibody against the target organism
- and technical specifications of innovative antibody

For their part, the European Medicines Agency and the *Food and Drug Administration* of the United States establish certain attributes for physicochemical properties as critical, in particular, when establishing the characteristic of biosimilarity. Among these characteristic attributes would be the structure and function of the antibody, impurities, class and subclass, amino acid structure, N- and C-terminal amino acids, disulfide bridges, carbohydrate content and other post-translational modifications that may influence the structure of the antibody. *In vitro* assays carry great weight in most agencies such as those mentioned above, since binding capacity and binding strength of antigen and antibody form a major part of *in vitro* studies for biosimilarity. Another common point presented by all the agencies is that the purity of the antibody, in

terms of its composition, the presence of impurities in its medium and the absence of contaminations are critical in patient safety.

It can be concluded that in order to determine the biosimilarity of a monoclonal antibody, it is not only possible to opt for a statistical quantification of the relevance of each attribute measured in the Certificates of Analysis (CoA) and which therefore form part of the final specifications of the monoclonal antibodies, but also to have the attributes widely recognized by government agencies so that the equation, and therefore the ITEM of quality attributes that is being developed, is widely recognized and admitted.

The study of the quality attributes item was developed taking into account the three fields analyzed in the methodology. Within these fields are the quality attributes recognized by the scientific literature, the quality attributes considered critical by international regulatory agencies for the establishment of biosimilarity and biocomparability, and the technical specifications of marketed monoclonal antibodies for which relevant documentation is available.

The following results were obtained for the quality attributes recognized by the scientific literature.

The studies developed to establish the critical quality attributes in monoclonal antibodies intended to be a biosimilar antibody are based on establishing qualitative criticality to their different attributes. In the studies of Kwon et al. (23), a score of 3º of confidence is established for the primary structure and higher structures, charged variants, mass variants, oligosaccharides, biological attributes and content. the different degrees of criticality granted by this score for the different impacts that may be suffered on the biological or clinical function of the drug itself do not take into account the analytical methods by which this criticality can be determined by carrying out a more detailed study of each of the attributes that belong to the classification developed by Kwon et al (23), it can be seen that the same impact on the biological or clinical function is attributed a different criticality even though the same analytical technique can yield specific results.

Table 1 shows the comparison between the variables to be taken into account for a score of the criticality of a quality attribute versus an impact and the analytical method most commonly used in the pharmaceutical industry to obtain specific results.

Table 1. Relationship of quality attributes and their criticality from a qualitative point of view.

Quality attribute	Criticality	Impact on biological/clinical function	Analytical method
Structure 1st			
Amino acid sequence	+++	Power	
N-terminal	++	*In vivo* pharmacokinetics	
Pyroglutamate			Peptide mapping,
C-terminal lysine	+	No influence on biological activity *in*	Edman degradation
elimination		*vivo*	
Bisulfate bonds	+++	Power	
Higher order structure	++	Potency and receptor-antigen binding	FT-IR spectrophotometry, fluorescence, circular dichroism
Variants loaded			
De-rolled shapes	+	Biological activity *in vitro*	Liquid chromatography
Oxidized forms	++	Immunogenic aggregates	under IEX, IEF, CE,
Sialized forms	+++	*In vivo* thinning or clearing	HPAEL-PAD, Mass techniques.
Mass variants			
Aggregates	+++	Immunogenicity	
Truncated shapes	+	Biological activity	

Quality attribute	Criticality	Impact on biological/clinical function	Analytical method
Monomers	+	Biological activity	Liquid chromatography under SEC, Mass, HIC techniques. SDS-PAGE
PEGilations	+++	*In vivo* clearance	
Oligosaccharides			
Fucose or galactose	+++	Influence on Fc-effector activity	HPAEL-PAD, Liquid Chromatography (LC), Capillary Electrophoresis (CE), Mass Spectrometry (LC-MS)
Non-human glycans	+++	Immunogenicity	
High-mannose glycans	++	Immunogenicity	
Non-glycosylated forms	++	Influence on Fc-effector function, ADCC and clearance *in vivo*	
Biological activity			
ADCC	+++	Mode of action	Cell-based assays, ELISA, SPR
ADCP	++	Mode of action	
CDC	+++	Mode of action	
Apoptosis	++	Mode of action	
Antigen-Antibody Binding	+++	Mode of action	
Union FcγR	++	ADCC	
Union C1q	++	CDC	
Union FcRn	++	*In vivo* clearance	
Content	+++	Pharmacokinetics	UV-Vis spectrophotometry

Note: Consideration of critical quality attributes in the assessment of analytical comparability of biosimilar products (23)

As can be seen in the table above, the classification of quality attributes in terms of their criticality is based on their impact on biological or clinical function. None of the studies carried out to test the criticality of the quality attributes of monoclonal antibodies in the organism, whether by in vitro, in vivo or in silico methods, take into consideration the different interactions that may occur between the quality attributes themselves, the medium in which the drug itself is embedded, and the problems associated with the performance of laboratory tests.

The pharmaceutical industry establishes critical quality attributes against the target antigen profile. This profile is catalogued in a specific study called QTPP (*Quality Target Product Profile*). It should be considered a compelling reason why the assays included in the QTPP should have greater weight than other assays that do not influence this profile.

It is also necessary to consider the presence or absence of anti-drug antibodies, known as *anti-drug antibodies* (ADAs), as part of a detailed study of the critical quality attributes for monoclonal antibodies.

During the development of the concept of the quality variable item or critical quality attribute coined in this dissertation work, the need to classify the different attributes used in the fields of biosimilarity assessment and comparability of a monoclonal antibody against its reference molecule versus the attributes themselves was observed. Table 2 breaks down which attributes favor biosimilarity and which attributes favor comparability.

Table 2. Classification of attributes with respect to their biosimilarity or comparability

Demonstrate biosimilarity	Demonstrate comparability
Primary structure	Analytical studies
Higher order structures	Non-clinical studies
Immunochemical properties	Pharmacological clinical studies (PK/PD)
Attachment to the receiver	Clinical safety
Stability	Clinical efficacy
Biological function	Immunogenicity
General properties of the antibody	

Demonstrate biosimilarity	Demonstrate comparability
Excipients	

On the other hand, during the study of the different quality attributes that were to be called items for the evaluation of the definitive critical quality attributes in the conception of the biosimilar concept, it was required to establish, in comparison with the studies prepared by the European Medicines Agency, the U.S. Medicines Agency and pharmaceutical industry associations such as Fimea, regions within the superior structure of the monoclonal antibody that influence each of the characteristics and attributes.

For this reason, when faced with a simplified structure of the antibody, it is established which attributes are of greater relevance in the case of the absence or presence of modifications in the biosimilar antibody compared to the innovator antibody.

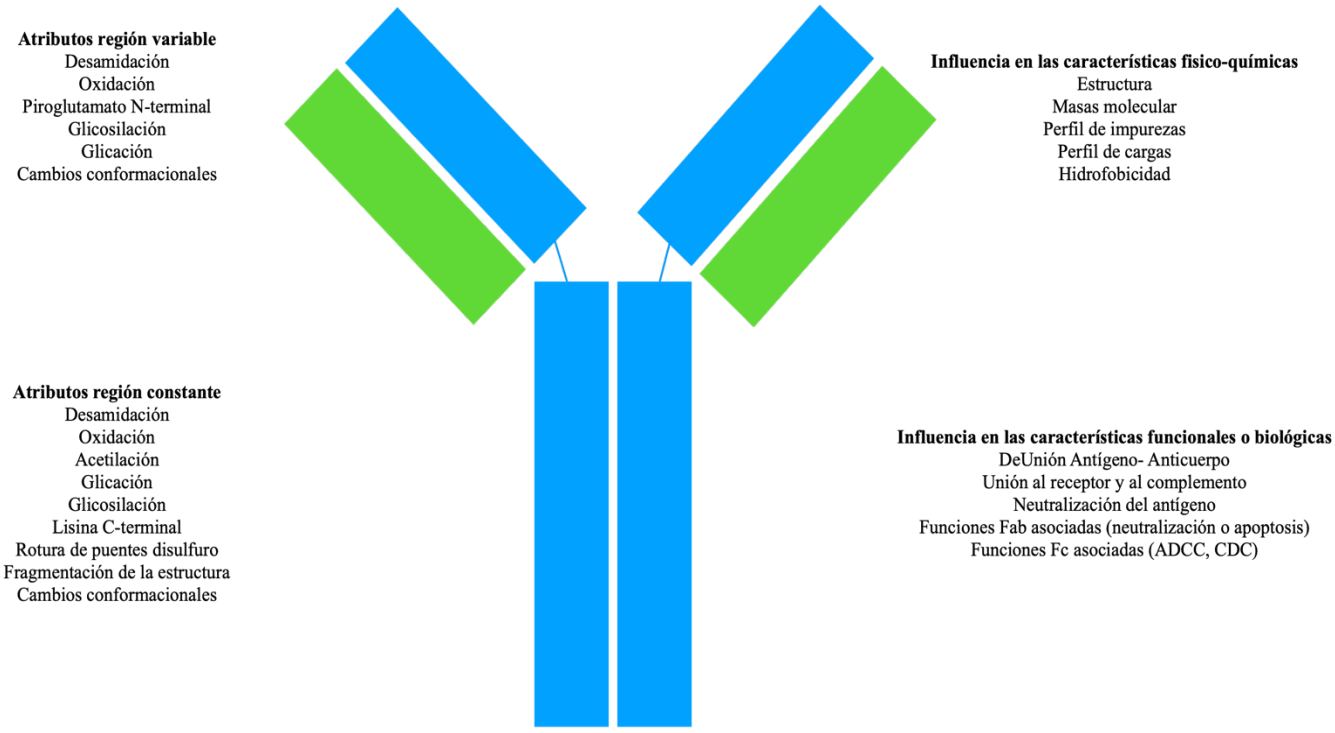

Figure 3. Antibody-attribute relationship

Therefore, it can be concluded that the variables are currently studied individually and not considered as a whole within the definition of biosimilar. A new structure of the definition should be adopted to bring together all the concepts, the proposed unit being the quality ITEM, since it should be composed of the quality attribute and the laboratory error.

Discussion and conclusions

The current status of biosimilars reveals a constantly evolving scientific landscape, supported by regulatory advances and technical developments that have enabled the simplification of analytical techniques as well as the representation of results. Definitions, whatever the concept, must allow a clear and precise understanding of what is being explained. In the case of biosimilars, the current definition presents a qualitative basis that allows free interpretation of the results for both pharmaceutical laboratories and regulatory agencies. This range of possibilities, radically opposed to the conception of a definition, does not allow the biosimilar term to be understood without prior knowledge of the subject. In turn, the definition is focused on the characterization of critical quality attributes on an individual basis. The error in the conception of the current definition of biosimilarity stems from its comparison to a generic drug, which can be directly compared by therapeutic dose and active ingredient. In contrast, in biosimilarity, the drug interacts with a living organism and it is part of this living organism that performs the therapeutic functions, which requires not only pharmacological but also immunological factors.

Scientific advances have made it possible to establish a rigorous connectivity between the critical attributes and biological functions of a living organism. For this

reason, the concept of biosimilarity must go through the process of restructuring to the concept of item promulgated in this document, since it must be assessed in a globalized manner and not in an individualized manner. The restructuring of the definition of biosimilar should not stop exclusively at the critical quality attributes, but should take under the term item, other variables of great relevance for the conception of a definition of greater accuracy and quantifiable basis, to establish in a single sentence the reality of the functioning of a biological drug in an organic system with which it interacts.

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