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Characterization and comparability of biosimilars: A filgrastim case of study and regulatory perspectives for Latin America

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ABSTRACT

Background: Developing countries have an estimate of ten times more approved biosimilars than developed countries. This disparity demands the need of an objective regulation that incorporates health policies according to the technological and economical capabilities of each country. One of the challenges lies on the establishment of comparability principles based on a physicochemical and biological characterization that should determine the extent of additional non-clinical and clinical studies. This is particularly relevant for licensed biosimilars in developing countries, which have an extensive clinical experience since their approval as generics, in some cases more than a decade. To exemplify the current status of biosimilars in Mexico, a characterization exercise was conducted on licensed filgrastim biosimilars using pharmacopeial and extended characterization methodologies.
Results: Most of the evaluated products complied with the pharmacopeial criteria and showed comparability in their Critical Quality Attributes (CQAs) towards the reference product. These results were expected in accordance with their equivalent performance during their licensing as generics. Accordingly, a rational approval and registration renewal scheme for biosimilars is proposed, that considers the proper identification of CQAs and its thoroughly evaluation using selected techniques.
Conclusions: This approach provides support to diminish uncertainty of exhibiting different pharmacological profiles and narrows or even avoids the necessity of comparative clinical studies. Ultimately, this proposal is intended to improve the accessibility to high quality biosimilars in Latin America and other developing countries.

1. Introduction

Since their introduction, biotherapeutic products have transformed modern medicine by bringing novel and targeted therapies for several life-threatening and chronic diseases, providing healing opportunities and improving the quality of life of patients, while reducing the incidence and severity of side effects. This biotechnology-based field has grown in the last decades, allowing the continuous development and commercialization of similar products in terms of quality, safety and efficacy with respect to a licensed product whose innovation patents had expired.

However, the introduction of these biosimilar products and their increasing worldwide manufacture has been received with controversy, mainly because of the absence of a consensus about the scientific and regulatory requirements needed to confirm their similarity, in spite of the proved quality of biosimilars and their positive impact, intended to increase health coverage and diminish the treatment costs [1].

In 2003 the European Medicines Agency (EMA) became the first regulatory organization that established initial requirements to approve biosimilars, followed by other regulatory and health agencies around the globe [2]. A current concern is the regulatory situation in developing countries, including Latin America, where almost 80% of deaths related to non-communicable diseases occur [3]. Particularly, chronic and degenerative diseases had caused 50% of the disease burden in developing countries along with an estimated loss of $84 USD billions of their income in 2015 [4], becoming practically unaffordable for their patients and health systems.

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In this regard, one of the main focuses of the Pan-American Health Organization (PAHO) is to develop a harmonized regulation for biotherapeutic products across Latin America in order to improve equity in health and quality of life. In response, guidelines for biological medicines have been issued in some countries; nevertheless, the requirements for the approval of each type of product (*e.g.*: vaccines, hemoderivatives, allergenic extracts or biotherapeutics) are frequently non-differentiated. Even though the guidance established by the World Health Organization (WHO), the Food and Drug Administration (FDA), the International Conference on Harmonization (ICH) and the EMA were often used as a reference, 12% of Latin American countries have licensed biosimilar products issued as generics without a specific clinical evaluation [5].

Since 2012, Mexico is a leader in Latin America by issuing an updated regulation aimed for biosimilars approval, published by the Mexican Ministry of Health (SALUD) and the Federal Commission for the Protection against Sanitary Risk (COFEPRIS) [6,7,8]. Other regulatory agencies which have issued similar regulations are the Brazilian Health Surveillance Agency (ANVISA) [9], the National Administration of Drugs, Foods and Medical Devices (ANMAT) from Argentina [10], the National Food and Drug Surveillance Institute (INVIMA) from Colombia [11], the National Drug Agency (ANAMED) from Chile [12], the Department for Regulation and Control of Pharmaceutical and Related Products (DRCPFA) from Guatemala [13], the Ministry of Health Directorate-General of Medical Supplies and Drugs (DIGEMID) from Peru [14] and the Ministries of Health from Costa Rica [15] and Ecuador [16].

According to the updated guidelines, the pathway for biosimilars' approval begins with an exhaustive characterization and a comprehensive comparability study of the Critical Quality Attributes (CQAs), strongly related to the functionality and safety of the biopharmaceutical. These CQAs should comprise the attributes already recognized by the reference product, either found in characterization exercises or during the experience with equivalent molecules. The guidance states that a clinical comparative study should be followed, whose extension is defined by the characterization results and designed to assess meaningful differences, if they exist [17]. In effect, the higher the comparability the less pharmacological studies needed to evidence similarity [6,18,19].

However, one of the major challenges related to the CQA's characterization, between the biosimilar and its reference product, is their proper selection, including the definition of their comparability principles, which, ultimately, allows a rational evaluation of the biosimilar towards their licensing and continuous surveillance. A proper selection is particularly relevant, given that a successful characterization and comparability exercise supports the selection of in-process controls and quality specifications for biosimilars.

1.1. Regulatory challenges in Latin America towards characterization and comparability

For the first-generation biopharmaceuticals (synthesized as analogous of human endogenous proteins), scientific consensuses have allowed the establishment of pharmacopeial monographs stating the minimum attributes to be evaluated. Since licensed products have been safe and effective, the compliance of the control limits specified in these monographs had proved to be useful to ensure quality for human use. The aforementioned, along with an active pharmacovigilance program, set the basis for the comparability studies used for the first biosimilars registration in Europe [20]. This scheme would be adequate for the registration or license renewal of biosimilars in Latin America; nonetheless, pharmacovigilance programs are not fully incorporated or are still in process of being regulated.

Hence, a demonstrated comparability sustained on a comprehensive characterization and the clinical record during the commercialization period of an approved biosimilar, should be considered as the major contributors for their licensing renewal. Whereas, the completion of clinical trials, with narrowed extension as long as CQAs comparability is demonstrated, must be considered for new biosimilar applications in order to diminish the uncertainty of exhibiting an altered pharmacological behavior.

In summary, comparability studies must include pharmacopeial methodologies and selected state-of-the-art-technologies to thoroughly evaluate CQAs, accompanied by the clinical record or narrowed trials as appropriate. The design of the analytical characterization strategy should be planned around this purpose, and the technical capabilities to detect relevant modifications.

1.2. A case of study: filgrastim in Mexico

Filgrastim is a non-glycosylated recombinant human granulocyte colony-stimulating factor (rhG-CSF) indicated for treatment of neutropenia. It was first approved in 1991 by the FDA and belongs to the first generation of biotherapeutic products after the registration of insulin in 1982. Since 2008, nine biosimilars containing filgrastim have been approved by the EMA, one of them recently approved by the FDA. However, over 50 filgrastim biosimilars are available in developing countries, most of them licensed as generic drugs since the early 2000s. For instance, in Mexico eight filgrastim biosimilars are currently commercialized. These latter had demonstrated to be safe and effective, by the absence of adverse events for more than seven years since their licensing, being Filatil® the only product manufactured in this country by Probiomed S.A. de C.V (Mexico City, Mexico) from the drug substance to the drug product. It is important to notice that the other products are commercialized using imported active pharmaceutical ingredients from Korea, Lithuania, India, Cuba, Argentina, Austria, among other countries. Also, Zarzio® (Sandoz International GmbH; Holzkirchen, Germany) obtained its approval in 2014, being in the Mexican market for less than two years.

To illustrate the current status of filgrastim biosimilars in Mexico and propose a rational scheme for their licensing renewal according to the updated regulation, a comparability analysis was performed considering the identified CQAs for this molecule (Table 1). The physicochemical and biological properties were evaluated in comparison to the reference product, Neupogen® (Amgen Inc.; Thousand Oaks, *CA*) [21] using pharmacopeial and extended methodologies which were selected according to their sensitivity, specificity, cost and the need of specialized personnel and infrastructure (Table 2). Extended methodologies were chosen to evaluate each identified CQA, based on the premise that not all the analytical techniques that a manufacturer could afford should be assessed, as their outcomes are not always linked to any functional or pharmacological behavior.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used for the analyses were at least ACS grade and were obtained from J.T. Baker (Avantor Performance Materials, Inc.; Center Valley, PA) or Sigma Aldrich (St. Louis, MO). All assays were performed using ultrapure Milli-Q water (Millipore, Billerica, MA).

2.2. Filgrastim samples

Filgrastim biosimilars commercialized in Mexico included: Filatil® from Probiomed S.A. de C.V., Dextrifyl® from Laboratorios Pisa S.A. de C.V., Inmunef® from Lemery S.A. de C.V., Biocilin® from Representaciones e Investigaciones Médicas, S.A. de C.V., Ior LC® from Alvartis Pharma S.A. de C.V., and Biofilgran® from Landsteiner

Table 1
Identified CQAs of filgrastim.

Attribute		Critically	Impact		
			Efficacy		Safety
			Pharmacodynamics	Pharmacokinetics	Immunogenicity
Amino acid sequence		Very High	⇳Affinity and biological potency	⇳Volume of distribution and clearance	Inherent of the molecule. Differential response to different sequences
Biological potency		Very High	⇳Biological activity	Non determined	Non determined
Affinity towards G-CSFR		Very High			
Higher order structure		High	⇳Affinity and biological potency	⇳Volume of distribution and clearance	Inherent of the molecule. Differential response to different epitopes
Aggregates and degradation isoforms	High-molecular weight variants	High	⇩Specific biological potency	⇩Bioavailability	⇧Anti-drug antibodies
	Truncated variants	Low	Non-determined		Non determined
Charge heterogeneity	Oxidized variants	High	⇳Affinity and biological potency	⇳Volume of distribution and clearance	
	Deamidation	Very Low	Non-determined		

Scientific S.A. de C.V.; Neupogen® from Amgen Inc. was used as the reference product.

2.3. Pharmacopeial analysis

Peptide mapping, reverse phase (RP) and size exclusion chromatographies (SEC) as well as polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF) and *in vitro* potency were performed according to the filgrastim pharmacopeial monograph [22].

2.4. Extended characterization analysis

Higher order structure was evaluated by differential scanning calorimetry (DSC), circular dichroism (CD) and fluorescence lifetime using time correlated single photon counting (TCSPC). Operation and sample treatment conditions were performed as previously described for DSC, by Flores-Ortiz et al. [23]; CD and TCSPC by Perez-Medina et al. [24].

Whole-molecule exact masses and tryptic peptide mappings were analyzed by reverse phase ultra-performance-liquid-chromatography coupled to a tandem quadrupole/time-of-flight mass spectrometer (RP-UPLC-MS/MS) using an ESI source on a SYNAPT G2 HDMS (Waters Corp.; Manchester, UK) and an ACQUITY UPLC H-Class Bio System (Waters Corp., Milford, MA). Acquisition was carried out in the 400–3500 m/z range employing a positive ion mode. Data was analyzed using BiopharmaLynx software (Waters Corp., Milford, MA). Amino acid sequence was confirmed by alignment to the theoretical filgrastim sequence.

Table 2
Analytical methodologies evaluation.

CQA	Analytical tool	Qualified personnel, specialized infrastructure	Cost	Standardization, sensitivity, specificity	Relationship towards CQAs	Score
Amino acid sequence	Multiangle Light Scattering	1	2	2	1	0
	SDS-PAGE	1	1	1	1	0
	Mass spectrometry	2	3	3	2	0
	Peptide mapping MS/MS	2	3	3	3	1
	Peptide mapping UV	1	2	2	1	0
	Edman Degradation	1	2	2	2	1
High order structure	TCSPC Lifetime spectroscopy	1	2	2	2	1
	Free Thiol Analysis	1	1	2	1	1
	Differential Scanning Calorimetry	1	2	3	3	3
	Nuclear Magnetic Resonance Spectroscopy	3	3	2	2	-2
	X-ray crystallography	3	3	3	3	0
	Circular Dichroism	1	2	3	3	3
	Fourier Transform Infrared Spectroscopy	1	2	2	2	1
	Hydrogen Deuterium Exchange	3	3	3	3	0
Charge heterogeneity	Hydrophobic Interaction Chromatography	1	2	2	2	1
	Capillary Isoelectrofocusing	1	2	3	3	3
	Capillary Zone Electrophoresis	1	2	3	2	2
	Isoelectrofocusing	1	1	2	2	2
	Cation Exchange Chromatography	1	2	3	2	2
	Anion Exchange Chromatography	1	2	3	2	2
Aggregates and degradation isoforms	Capillary Gel Electrophoresis	1	2	2	3	2
	Size Exclusion Chromatography	1	2	2	3	2
	Reverse Phase Chromatography	1	2	2	3	2
	SDS-PAGE	1	1	2	3	3
	Analytical Ultracentrifugation	2	3	2	3	0
Affinity towards target molecule	ELISA (cell based)	2	1	2	3	2
	Flow Cytometry (cell based)	3	3	3	3	0
	Surface Plasmon Resonance	3	3	3	3	0
	Biolayer Interferometry	2	2	3	3	2
	Isothermal Titration Calorimetry	2	2	3	3	2
Biological potency	Depends on the mechanism of action of the biopharmaceutical					

1 = Low, 2 = Medium, 3 = High.
Score = (Standardization, sensitivity, specificity + Relationship towards CQAs) − (Qualified personnel, specialized infrastructure + Cost).

Filgrastim affinity towards its receptor was assessed using a cell based ELISA assay. M-NFS-60 murine myeloid leukemia cells (ATCC CRL-1838TM) disposed in 96 well plates, were incubated at 4°C during 2 h with serial dilutions of each filgrastim sample, ranging from 300 g/mL to 0.37 g/mL. Cells were washed with a 0.1% tween 20 Tris buffered saline solution, to be incubated for 1 h with a 0.4 ng/mL solution of human anti-G-CSF antibody (R&D Systems; McKinley Place, MN). Afterwards, cells were washed and incubated for 1 h with a 0.1 ng/mL solution of goat anti-IgG horseradish peroxidase-conjugated antibody (HRP) from R&D Systems (McKinley Place, MN). Cells were washed again to add 100 L of TMB substrate (Thermo Fisher Scientific; Carlsbad, *CA*). After 20 min, a 0.01 N hydrochloric acid solution was added to quench the reaction. UV–Vis absorbance was acquired at 450 nm on a SpectraMax M3 plate reader from Molecular Devices (Sunnyvale, *CA*). Dose–response curves were adjusted using a 4 parameter logistic model. Relative affinity was calculated by comparing the half maximal effective concentration (EC50) of the sample against the reference product.

3. Results

3.1. Filgrastim comparability results

Pharmacopeial analysis results revealed comparability towards the reference product and compliance with the pharmacopeial acceptance criteria, except for one product (Table 3).

For all the evaluated products a similar peptide mapping profile (number and abundance of peaks) towards the reference product was obtained. For the main isoform, differences below 0.07 units in the isoelectric point (pI) were revealed by cIEF in comparison to the reference value of 6.22. Also SDS-PAGE showed correspondence in position and intensity for the observed bands under both reducing and non-reducing conditions. Additionally, no species with different molecular masses other than filgrastim were observed during the SDS-PAGE analyses for all biosimilars. Regarding purity, the aggregate level was under the pharmacopeial limit in all cases whilst the content of related proteins was below the limit of 3.5%, with the exception of one product, whose higher content of oxidized and deamidated isoforms did not showed an impact on its biological potency (Table 3). Collectively, resulting in a biological potency that lied within the expected acceptance range.

These results constitute the basis to demonstrate physicochemical and biological similarity. Further, this characterization overview of filgrastim products, as stated, was strengthened by orthogonal techniques and the evaluation of other CQAs not considered in the pharmacopeial monograph, such as higher order structure and receptor affinity (Table 4).

Mass spectrometry (MS), a well-known high-resolution technique for protein characterization, confirmed the identity of filgrastim through intact mass analysis and sequence verification. MS revealed coverage over 92% for all products against the theoretical sequence, and differences below 0.5 Da for the main isoform against the reference product and the theoretical molecular mass, with a similar content of related variants with different masses (Table 4, Fig. 1).

Circular dichroism (CD) and fluorescence lifetime spectroscopy by Time-Correlated Single Photon Counting (TCSPC) were employed to assess higher order structure. Both techniques are useful to evaluate the spatial arrangement of specific residues within the molecule by their absorbance and fluorescence properties, respectively. Fluorescence lifetimes (τ) showed differences below 7% for all the assessed biosimilars against the reference product, attributable to the dependence of the fluorescence lifetime to the formulation properties of each product. Likewise, CD measurements revealed a similar secondary and tertiary structure, with comparable profiles among the biosimilars and the reference product. Additionally, differential scanning calorimetry (DSC) was used to determine the thermal transitions, as a measure of the structural integrity, thermostability and solubility of each biosimilar product at their specific formulation. A comparable behavior was observed among all products, with differences lower than 2°C in their respective transition temperatures against the reference product.

Finally, receptor affinity assays were performed to complete CQA's evaluation, since the interaction of filgrastim with its receptor represents the onset for its mechanisms of action and reflects an adequate three-dimensional structure and chemical composition. Most of the biosimilar products had a comparable affinity towards the granulocyte colony-stimulating factor receptor (G-CSFR). However, two products showed mean measured affinities outside the observed confidence interval for the reference product of 81 to 123% ($n = 5$).

Table 3
Pharmacopeial analyses.

CQA	Pharmacopeial attribute	Methodology	Acceptance criteria	Neupogen®	Biosimilar product					
					Filatil®	2	3	4	5	6
Amino acid Sequence	Identity	Peptide mapping	Correspondence in chromatographic profiles	The profile of all the biosimilars correspond by showing similar number and abundance of peaks against the reference product						
Charge heterogeneity	Identity and Impurities with different charge	Isoelectric focusing	Isoelectric point between 5.7 to 6.3	6.2 ± 0.01	6.2 ± 0.01	6.2 ± 0.01	6.2 ± 0.00	6.2 ± 0.03	6.2 ± 0.00	6.2 ± 0.01
			Charge variants content <10.0%	1.4 ± 0.16	5.5 ± 0.24	5.6 ± 0.12	4.0 ± 0.69	4.6 ± 0.20	2.7 ± 0.27	3.8 ± 0.66
Aggregates and degradation isoforms	Identity and Impurities with different molecular masses	Polyacrylamide gel electrophoresis	Relative molecular weight is 18.8 kDa Impurities <2.0% Correspondence in electrophoretic profile	The SDS-PAGE gels for all the biosimilars showed a principal band around 18.8 kDa with similar position and intensity against the reference product. No bands with different molecular masses were detected						
	Identity and Impurities with higher molecular masses	Size-exclusion chromatography	Aggregates content <2.0%	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.03	0.5 ± 0.08	0.9 ± 0.49	0.1 ± 0.02	0.4 ± 0.31
			Correspondence in retention time	The retention time for all the biosimilars showed undistinguishable differences						
	Related proteins	Reverse phase liquid chromatography	Total impurities content <3.5%	1.6 ± 0.33	2.8 ± 0.54	3.5 ± 0.24	1.0 ± 0.23	1.9 ± 0.85	1.6 ($n = 1$)	4.3 ± 7.07
			Highest impurity content <2.0%	0.7 ± 0.07	1.3 ± 0.47	1.2 ± 0.06	0.5 ± 0.12	1.1 ± 0.28	0.7 ($n = 1$)	3.0 ± 7.23
Biological Potency	Potency	Cell proliferation assay	Induces cell proliferation (80–125%)	–	92.7 ± 2.58	91.0 (n = 1)	88.0 (n = 1)	92.3 ± 4.24	85.0 (n = 1)	98.5 ± 3.16

Table 4
Extended analyses.

CQA	Methodology		Neupogen®	Biosimilar product					
				Filatil®	2	3	4	5	6
Amino acid sequence	Mass spectrometry	Intact molecule mass (Da)	18,798.2 ± 0.19	18,798.2 ± 0.06	18,798.7 ± 0.06	18,798.6 ± 0.03	18,798.3 ± 0.04	18,798.2 (n = 1)	18,798.4 ± 1.55
		Sequence coverage (%)	98.3 ± 0.00	98.9 ± 1.65	99.4 ± 1.65	98.3 ± 0.00	93.0 ± 18.11	98.3 (n = 1)	98.3 ± 0.00
High order structure	Circular dichroism	Secondary structure	The profile of all the biosimilars corresponds with the reference product						
	Fluorescence spectroscopy	Fluorescence lifetime (s)	3.35E-09 ± 0.02	3.14E-09 ± 0.19	3.12E-09 ± 0.22	3.24E-09 ± 0.02	3.39E-09 ± 0.00	3.36E-09 ($n = 1$)	3.19E-09 ± 0.35
	Differential scanning calorimetry	Transition temperature (°C)	68.1 ± 1.61	66.9 ± 1.50	66.8 ± 1.07	67.4 ± 0.19	68.9 ± 3.72	67.7 ($n = 1$)	66.3 ± 7.07
		Transition enthalpy (kJ/mol)	1002.5 ± 111.85	1110.4 ± 159.02	1000.7 ± 170.91	925.6 ± 10.45	939.9 ± 161.21	851.8 ($n = 1$)	962.2 ± 282.07
Affinity towards G-CSFR	Sandwich ELISA	Receptor affinity (%)	98.9	105.6	99.9	Non determined	63.5	73.8	118.2

4. Discussion

The comparability exercise revealed that most of the evaluated filgrastim products are physicochemically and biologically similar to the reference product. Although, differences were observed on the content of oxidized and deamidated isoforms and the relative affinity towards G-CSFR in some products, no impact on the biological potency was observed as measured by cell proliferation. In such cases, additional non-clinical or clinical comparative studies are needed to reduce the uncertainty regarding altered *in vivo* safety and efficacy profiles.

For instance, biosimilars with an increased amount of oxidized and deamidated isoforms would require additional non-clinical PK studies (*i.e.*: *in vivo*) to assure comparability, as these isoforms may lead to changes in the electrical charge of the molecule, altering their interactions against cells and tissues, resistance to proteolysis and receptor affinity [25]. Whereas, biosimilars with differences in receptor affinity would require additional clinical PK studies as these modified affinities could affect the receptor-mediated clearance of filgrastim [26].

In summary, when physicochemical differences are found on an approved product, additional experimental models should be used to demonstrate comparability as long as they can be regarded as relevant to the pathophysiological conditions in patients [23]. Affinity tests, activity assays, or even a higher number of participants (*i.e.*: stringent statistics) and endpoints during clinical trials, could be included to fulfill this purpose and ultimately obtain registration renewal.

On the other hand, products with extensive market experience that proved to be comparable after a thorough CQA evaluation can be recognized as biosimilars through a proper analysis of their wealth of accumulated clinical evidence over time. This evidence includes investigator initiated trials or IITs, real world evidence or RWE, electronic health records or EHRs, and pharmacovigilance/safety reports, of sufficient quality to generate valid scientific conclusions to

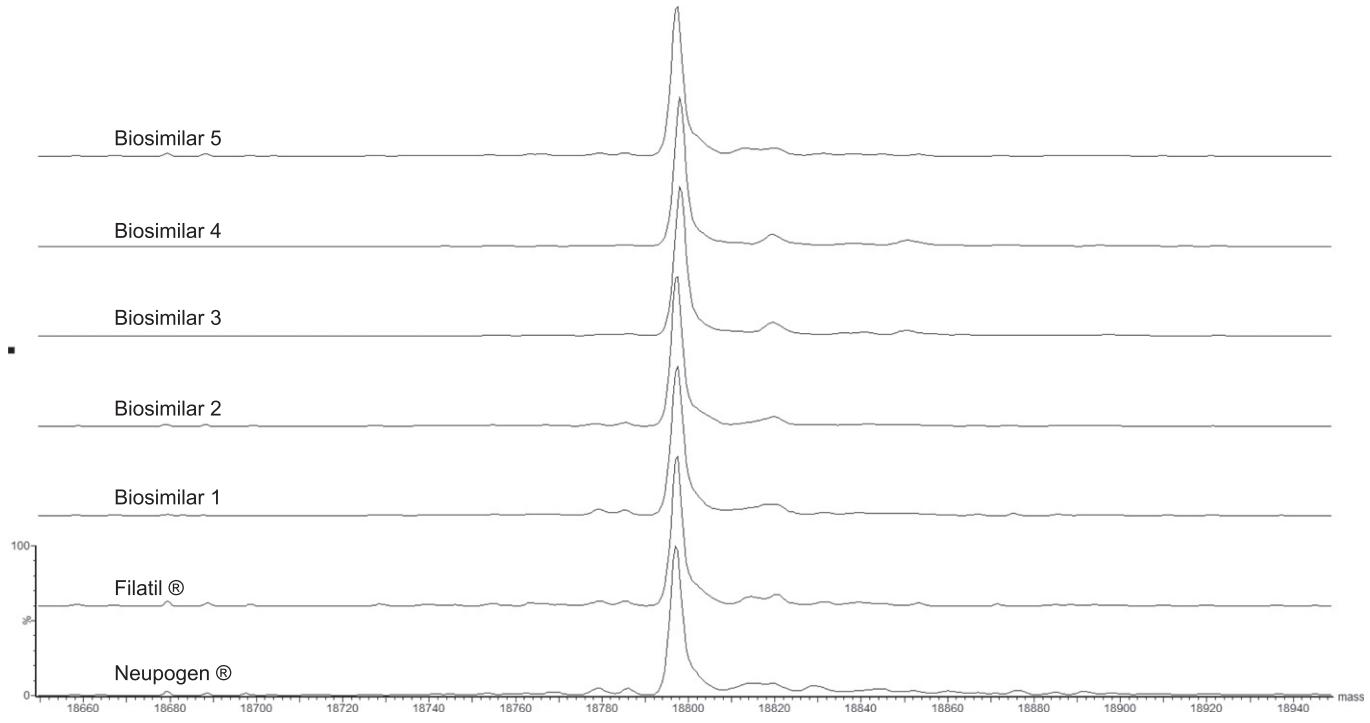

Fig. 1. MS profiles of the filgrastim samples.

demonstrate a comparable efficacy and safety profile with respect to the reference product.

Evoking the proposed regulatory approach, manufacturers of biosimilars approved as generics should formally complete the presented characterization exercise using several batches of their product and its reference. This would finally allow to establish comparability and to determine the source of any observed differences, either intrinsic to the biosimilar or due to the variability of the reference product, and the necessity of additional studies. Moreover, this exhaustive exercise will evidence the biosimilar manufacturing process consistency in each case, which should be complemented with the product lifecycle management process and annual product reviews. The aforementioned along with the analyzed clinical evidence obtained during the commercialization period of a product can support their registration renewal.

In this sense, Mexico has published specific guidelines for the registration of biosimilars and the update of products approved as generics to a biosimilar-status [8]. The recommendations involve a case-by-case analysis of the information submitted during the biosimilar application, including CQA's comparability, defining the need and extension of additional studies. In all cases, Good Manufacturing Practices (GMP) compliance is mandatory, regardless of the product origin.

4.1. Perspectives: innovation and industrial progress in Latin America

As has been mentioned, healthcare systems in developing countries demand access to affordable and specialized biopharmaceutical therapies, encouraging the advent of biosimilar products. As a result, more than 100 biosimilars had been approved since the late 1990s worldwide, even before any specific regulation appeared.

A reasonable approach to address this need, with long-term benefits for developing countries, would stimulate scientific progress, contribute to gain experience in biotechnological manufacturing processes and allow competitiveness with market and price equilibrium. For instance, Brazilian authorities have successfully promoted a scheme that has boosted the local industry towards self-sufficiency. Their strategies involved incentivizing the local manufacturing of biosimilars by imposing high import taxes, official promotion of their usage, investment in higher education and the creation of R&D institutes. The partnerships between multinational, academia, private and public institutions could also improve the development of national technological capabilities. Thus enabling the foundation of competitive companies and reducing biopharmaceutical monopolies, giving equity to the health conditions among developed and developing countries.

It must be highlighted that occasionally the benefits of the relationships of international companies with developing countries willfully rely on the reduction of expenses due to lower scientific labor costs. Sometimes the lower entry barriers of these countries allow the entrance of products (even those with different physicochemical properties with respect to the reference product), intending to obtain safety and efficacy information that minimizes the manufacturer's risks for more stringent markets. Thus making imperative to promote a functional and rational regulatory scheme with authentic mutual benefits. In this sense, a truly fruitful relationship between each country and biosimilar producers would benefit the drug quality and accessibility, and ultimately stimulate local scientific and socioeconomic progress.

Stimuli to a biosimilar market would force to overcome the current challenges in Latin American biopharmaceutical industries, including those related to the development, implementation and maintenance of GMPs and the improvement of their pharmacovigilance programs.

For instance, this characterization overview highlights the similarity of most licensed generic products favoring an updated registration program based on a rational approach, which considers comparability of the identified CQAs and the analyzed clinical evidence obtained during the commercialization period as the major contributors for their registration renewal. This exercise is intended to provide certainty to the regulatory authorities towards the approval process, thus strengthening the confidence and acceptance of physicians and patients.

5. Concluding remarks

A regulatory framework is the first step to address the current uncertainty regarding biosimilars approval. This could be achievable through rational foundations that incorporate scientific knowledge. Accordingly, the physicochemical and biological comparability exercise of these molecules is the major player to evidence their similarity and to reduce risks associated to a different pharmacological behavior with respect to the reference product.

CQAs must be the primary target of comparability studies, properly assessed by the selection of specific analytical techniques, whose results can be coherently or meaningfully linked to drug functionality. This would narrow, with respect to the full set of studies conducted by the reference product manufacturer, the extent of comparative clinical studies for biosimilar products, avoiding redundant demonstration of safety and efficacy. Furthermore, a comprehensive comparability study along with the clinical record accumulated during the commercialization period of products approved under a non-specific biosimilar regulation should be considered sufficient evidence for their registration renewal as biosimilars.

We believe that the implementation of a rational approach by regulatory agencies and health authorities based on the concepts presented herein is the keystone to improve the accessibility to high quality biosimilars in Latin America and other developing countries. Furthermore, promoting the investment in research and development of local biopharmaceutical companies by reducing costs and time to satisfy the necessity of specialized therapies.

Conflict of interest

Alexis J. Romero-Diaz, Mariana P. Miranda-Hernandez, Victor R. Campos-Garcia, Nancy D. Ramirez-Ibañez, L. Carmina Juarez-Bayardo, Karen Moreno-Duran, Miriam S. Cedillo-Robles, Nestor O. Perez, Emilio Medina-Rivero and Luis F. Flores-Ortiz are employees of Probiomed S.A. de C.V., which is developing, manufacturing and marketing biosimilar products. All the authors, including Karina Mendoza-Macedo and Helgi Jung-Cook declared no conflict of interest.

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