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PERSPECTIVE DE CERCETARE ȘI FABRICAȚIE A BIOSIMILARULUI SOMATROPINA ÎN REPUBLICA MOLDOVA

PERSPECTIVES OF RHGH BIOSIMILAR RESEARCH AND MANUFACTURING IN REPUBLIC OF MOLDOVA

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Rezumat. În Moldova nu există precedent în producerea biosimilarelor. Elucidarea structurii biochimice și clonarea genei hormonului de creștere uman (hGH) au dus la dezvoltarea farmaceutică a rhGH la mijlocul anilor 1980 în SUA. Odată cu expirarea perioadei de protecție a brevetului pentru hGH de referință, a apărut oportunitatea dezvoltării produselor medicamentoase biosimilare. Proiectul de inovare și transfer tehnologic „Procedeu de cultivare a materiei prime pe bază de Pichia pastoris cu expresia hormonului de creștere" a demarat în ianuarie 2021, în cadrul Universității de Stat de Medicină și Farmacie „Nicolae Testemițanu", Centrul Științific al Medicamentului din Republica Moldova. Proiectul este finanțat de Agenția Națională pentru Cercetare și Dezvoltare din Moldova, iar cofinanțator este Întreprinderea Farmaceutică Balkan Pharmaceuticals. Scopul proiectului este transferul tehnologic al procedeului de cultivare a vectorului Pichia pastoris cu expresia hGH: crearea băncii de celule de lucru, prepararea inoculului și creșterea culturii Pichia pastoris, precum și elaborarea documentației aferente acestor activități. Fabricarea autohtonă a rhGH oferă un avantaj farmacoeconomic deosebit față de concurentul existent astăzi pe piața Republicii Moldova - Saizen® pulbere pentru soluție injectabilă, Ares Trading SA, Elveția (producător Merck Serono SPA, Italia).

Cuvinte cheie: biosimilar, rhGH, transfer tehnologic, cultura Pichia pastoris, bancă de celule de lucru.

Summary. In Moldova there is no precedent in biosimilars production. Elucidation of the biochemical structure and cloning of the hGH gene led to the pharmaceutical development of rhGH in the mid-1980s in the USA. With the expiry of patent protection period of the reference hGH, the opportunity arose for the development of biosimilar medicinal products. The project for innovation and technology transfer "Cultivation process of raw material based on Pichia pastoris with the expression of growth hormone" has started in January 2021, within the State University of Medicine and Pharmacy "Nicolae Testemitanu", the Scientific Center of Medicine from Republic of Moldova. The project is funded by the National Agency for Research and Development from Moldova and co-financier is the Pharmaceutical Enterprise Balkan Pharmaceuticals. The overall goal of the project is the technology transfer of cultivation process of the vector Pichia pastoris with the expression of hGH: creation of working cell bank, preparation of inoculum and growth of Pichia pastoris culture, and elaboration of documentation related to these activities. The local manufacture of the rhGH offers an incomparable pharmacoeconomic advantage over the competitor existing today on the market of Republic of Moldova - Saizen® powder for injection solution, Ares Trading SA, Switzerland (manufacturer Merck Serono S.P.A., Italy).

Keywords: biosimilar, rhGH, technological transfer, Pichia pastoris culture, working cell bank.

INTRODUCTION

The EMA defines a biosimilar as *"a biological medicinal product that contains a version of the active substance of an already authorized original biological medicinal product (reference medicinal product) in the European Economic Area (EEA). Similarity to the reference medicinal product in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise needs to be established"*. The pioneer of this drug class is Omnitrope® (Somatropin) and it was approved by the European Medicines Agency (EMA) in 2006. Since 2006, several general and product-specific biosimilar guidelines were developed by the EMA and it is mandatory to mention that EU was the first region in the world to develop a legal, regulatory, and scientific framework for approving biosimilar medicines. In comparison the first biosimilar was approved in the USA only in 2015. To date, EMA has recommended the approval of 77 biosimilars within the product classes of: 1) human growth hormone; 2) granulocyte colony-stimulating factor; 3) erythropoiesis stimulating agent; 4) insulin; 5) follicle-stimulating hormone (FSH); 6) parathyroid hormone; 7) tu-

mour necrosis factor (TNF)-inhibitor; and 8) monoclonal antibodies [1].

Pharmaceutical enterprises worldwide have at least three good reasons to move to a biologics-oriented industry. Firstly, the distinctive capability of biologics to bind with target sites that are difficult to achieve or even inaccessible to small molecule drugs which is mainly due to protein-protein interactions that involve flat surfaces with fewer charged regions. Secondly, another attractive feature of biologics consists in its most auspicious commercial potential [2]. It is estimated that around $194 billion worth sales of biologics are at risk in the period between 2017 and 2022. This anticipates the beginning of another patent cliff era of blockbuster biologic drug products being challenged by biosimilars. Thirdly, the overall economic return assured by biologics is significantly higher than that of small molecule drugs.

The expiration of the term of patent protection (generally this period is 20 years from the date of filing the relevant patent application) of the original biosimilar products is a key factor in the decision of companies to develop biosimilars [3]. Most innovators also depend on supplementary protection certificates (SPCs), that represents the intellectual property right that serves as a five years' extension to the patent right, and data exclusivity to extend the term of protection. The European Commission recently issued a proposal to amend the rules on SPCs to include "Waiver of export production" to third parties for patented products, in order to promote the competitiveness of generic and biosimilar industries in global markets. This change may encourage the expansion of the market and the export of locally manufactures biosimilars [4].

Because it is by definition easier to reproduce than to generate a product *de novo*, biosimilar has a higher chance of conquering the market and denotes fewer pharmacoeconomic risks than the reference biological products. In addition, investments in original biologic products are incomparably higher than in the case of biosimilar products, with a much lower chance of success [5]. Reports from the research and development departments of pharmaceutical companies show that about 95% of all drug projects never reach the market. The investments needed to develop and market a biosimilar is considerably larger than the amount between $ 1 million and $ 4 million attributable to generic drugs [6]. Between 7 and 8 years at a cost of between 100 and 250 million dollars is needed to develop a biosimilar. However, IMS Health estimates that the use of biosimilars has resulted in savings of up to € 100 billion in the United States (US) and the five major European Union (EU) countries by 2020.

DISCUSSIONS

Biologics manufacture involves a fundamentally different approach from traditional chemical synthesis. Involving living systems makes nearly impossible following an exact science as chemistry. The design of complex multistep processes utilizing mammalian and microbial cell cultures brings to life the paradigm of the biologic manufacturing process "The process is the product". Conventional preparation process of biologics assumes sequential steps like desired gene isolation, insertion into vector, expression of host cell, cell culture, cell bank creation and characterization, production of protein, purification of protein, analysis and finally formulation. The biosimilar development process unfolds based on several strategic considerations [7]:

(1) Defining the target—This involves detecting any variability in the reference target molecule and any corresponding changes in qualitative properties of the drug.
(2) Development based on target—The engineering process of the biosimilar is designed to match the criteria of the reference, including factors like choice of cell line, biological processes, and others.
(3) Similarity confirmation of the biosimilar—Degree of biosimilarity with the reference assessed via physical, chemical, and biological analyses of the biosimilar.
(4) Regulatory authorization—Co-operating with relevant regulatory authorities to determine the minimum amount of clinical information required for biosimilarity approval.
(5) Clinical assessment—Conducting clinical trials to confirm biosimilarity and compiling any other information required for commercialization of the biosimilar.

Biosimilars face a more stringent regulatory assessment than generics, including the need for clinical trials, which significantly increases costs and timelines to market entry of biosimilars. Establishing appropriate standards for biosimilarity remains one of the main and the most prolific topics for scientific, legislative, and regulatory debate. There are five generally accepted starting points fundamental to the assessment of biosimilar products:

(1) we cannot try on the generics template for biosimilars, therefore the approach should be individual and specific to this drug class;
(2) similarity should be assessed in terms of quality, safety, efficacy;
(3) a step-wise comparability approach that proves the similarity of the similar to reference product in terms of quality is a prerequisite for the reduction of nonclinical and clinical data submitted;
(4) the assessment of each biosimilar is based on a case-by-case approach;
(5) the importance of pharmacovigilance is stressed.

At first sight, the process of assessing the biosimilarity of products is similar to that of assessing bioequivalence for generic medicinal products. If the development of a generic focuses on the demonstration of bioequivalence, then in order to demonstrate biosimilarity, a series of comparability studies are carried out aiming at juxtaposition of each characteristic of the biosimilar medicinal product with that of the reference medicinal product. The process includes endpoint selection; biosimilarity criteria; study design; statistical methods for data analysis [8]. Biosimilar

products are obtained from living cells or organisms with mixed, complicated structures that are difficult, if not impossible, to fully characterize. Thus, standard methods for assessing the bioequivalence of generic small molecule medicinal products cannot be properly and directly applied to assess biosimilarity.

The studies performed for demonstrating pharmaceutical quality must provide detailed data on: structural characterization and other physicochemical properties; purity (traces of residues resulting from the manufacturing process must be controlled and must not exceed acceptable levels); biological activity; excipients and raw materials; concentration and pharmaceutical form; control of the manufacturing process (in order to ensure compliance of the active substance and the finished medicinal product within the accepted intervals for the technical specifications);the stability of the active substance and the finished medicinal product during the shelf life, under the defined storage conditions [15]. It is known that biosimilar products are sensitive to environmental factors, such as light and temperature, and a minor change or variation in any critical stage of the manufacturing process could lead to a drastic change in clinical outcomes.

Factors influencing the number and types of clinical trials to be performed include: molecule complexity and available comparability data, availability of a final efficacy pharmacodynamic evaluation criterion, safety profile of the reference drug or pharmacological class, potential for immunogenicity, the possibility of extrapolation to other indications.

In case the originally authorized medicinal product has more than one indication, the efficacy and safety of biosimilar must be justified or demonstrated separately for each of the therapeutic indications [14]. At least one comparative trial (efficacy and safety) in a "sensitive" population with relevant clinical endpoints should be held. It is possible the extrapolation to other indications of the reference product, not studied during the development of biosimilar, based on the overall evidence of comparability. The acceptance of indications extrapolation is decided on a case-by-case basis, depending on the strength of scientific demonstration of comparability.

Although doctors can confidently use biologics in all their approved indications, assuming that they are given based on scientific evidence, the reference to the principle of extrapolation can only be made after sound comparability studies. Comparability is designed as a step-by-step process, tailored to each drug; stage 1 comprises the knowledge gained from initial quality comparability studies that are used to determine the extent and type of non-clinical (stage 2) and clinical (stage 3) studies required in the next stage of development, always aiming to rule out clinical performance differences between the biosimilar and the reference medicine.

In terms of interchangeability, the intervention of both medical practitioners and healthcare authorities is needed. Interchangeability refers to the possibility of replacing one drug with another that is expected to have the same clinical effect [9]. The decision to allow interchangeable use and substitution of the reference biological medicine with the biosimilar one shall be taken at national level. Starting from the existing legal framework, the respective regulations, guidelines and recommendations are issued. As with any medication, healthcare professionals must choose carefully when prescribing, considering the patient's medical history. Therefore, any decision to replace a medicinal product with another medicinal product that has the same indication should be taken by the physician in consultation with the patient considering possible national policies on the prescription and use of biologic medicinal products. Reimbursement of biosimilars instead of originators, more national bodies dedicated to biosimilars, the reassuring data from switching studies, increased awareness of health professionals, a growing number of publications confirming the clinical similarity of biosimilars with originators and finally the accumulated positive clinical experience with biosimilars speak in favor of the increasing confidence in biosimilars [10].

In Moldova there is no precedent in biosimilars production. In March 2013, the order on the introduction of good manufacturing practice (GMP) came into force. As a result, only 16 of the 28 existing pharmaceutical companies remained. Currently Moldovan manufacturers produce over 400 generic drugs, it is the main and in fact the only focus of the domestic pharmaceutical industry. The local manufacture of the biosimilars obviously would offer an incomparable pharmacoeconomic advantage over all the existing and potential competitors.

Local biosimilars production involves reducing the time and costs for developing internal know-how and the prospect of creating contagion effects in other areas, including access to wider distribution networks and new business opportunities [13]. Consistent is the reduction of state budget expenditures for the acquisition of biosimilars and the redirection of resources to the development and implementation of national health programs. Broadening the profile of local pharmaceutical plants implies the emergence of new jobs. An inconceivable advantage is the increase of the production volume and the turnover of the national pharmaceutical industry through the export of biosimilars, the increase of the sales market. Manufacturing biosimilars in Republic of Moldova represents an excellent opportunity for increasing the operating profit margin for local manufacturers, for traditional generic drugs it's roughly 20, but for biosimilars around 30. That goes without saying that reputational benefits cannot be ignored.

Somatropin is a polypeptide consisting of 191 amino acids, by composition and chemical structure it is identical to human growth hormone of pituitary origin in terms of consistency and composition, as well as the map of peptides, isoelectric point, molecular mass, isomeric structure and biological activity. The first data confirming the effectiveness of human growth hormone (rhGH) were obtained in 1958, just 2 years after its isolation from the human pituitary gland. However, before the development

of recombinant DNA technology, hGH for the purpose of substitution therapy could only be obtained by extraction and purification from human cadaveric pituitary glands. Therefore, the market supply was very low and hGH replacement therapy was reserved only for the most severe cases of growth hormone deficiency (GHD). Elucidation of the biochemical structure and cloning of the hGH gene led to the pharmaceutical development of recombinant human growth hormone (rhGH) in the mid-1980s in the United States. With the expiry of the period of protection of the reference medicinal product on the market, the opportunity arose for the development of biosimilar medicinal products.

Omnitrope® (biosimilar rhGH; Sandoz, Kundl, Austria) approved by EMA in 2006, has since been approved worldwide, including in the US, and the product is now available in over 50 countries. Since its launch, more than 40.000 patients have been treated with biosimilar rhGH, generating a total experience of almost 107 million days of inpatient treatment [11]. The appearance of an abundant amount of rhGH allowed the treatment of several children, later and adults, as well as the extension of the area of therapeutic indications. RhGH is currently indicated in growth retardation in children due to reduced or lack of secretion of endogenous growth hormone or chronic renal failure, in case of gonadal dysgenesis (Turner syndrome), growth disorders in children born small for gestational age (SGA), and in adults in the accentuated growth hormone deficiency, also in severe burns. 14 years on the European market for the first biosimilar rhGH may be characterized by the absence of unexpected or unique adverse events, the absence of signs of increased risk of cancer or glucose homeostasis disorder compared to the reference biological medicine and other rhGH products, and the immunogenicity of biosimilar rhGH is, also similar to that of other products in that class [12].

Currently on the market of Republic of Moldova only one medicinal product from the group of anterior pituitary lobe hormones is authorized, namely Saizen®, powder and solvent for injection solution, 8 mg, marketing authorization holder Ares Trading SA, Switzerland, manufacturer Merck Serono S.P.A., Italy. Thus, we can conclude that the domestic pharmaceutical market does not face the phenomenon of competition regarding medicinal products from the ATC group H01AC01. Because rhGH medical therapy usually has a recommended duration of several years and an individualized dosing regimen with a dose calculation based on the patient's body weight, a priority issue is to increase patient adherence and adherence to treatment.

The project for innovation and technology transfer "Cultivation process of raw material based on Pichia pastoris with the expression of growth hormone", with registration number 21.80015.8007.244T has started in January 2021, within the State University of Medicine and Pharmacy "Nicolae Testemitanu", the Scientific Center of Medicine from Republic of Moldova. The project is funded by the National Agency for Research and Development from Republic of Moldova and co-financier is the Pharmaceutical Enterprise Balkan Pharmaceuticals, Republic of Moldova.

The project has the following objectives:

- perform the technological transfer for the manufacturing process of a new biosimilar rhGH product and improve the innovation capacity of pharmaceutical manufacturer Balkan Pharmaceuticals;
- obtain scientific results during the technological transfer process;
- improve the collaboration among the co-financier Pharmaceutical manufacturer Balkan Pharmaceuticals, the scientific research institutes and the National Agency for Research and Development of the Republic of Moldova;
- increase the capacity of assimilation into manufacturing process of the research results obtained during the project;
- create the WCB (working cell bank) of Pichia pastoris culture for short and long term storage, with subsequent inoculum preparation and growth of Pichia pastoris culture with rhGH expression.

The final result of the project is the technology transfer of cultivation process of the vector Pichia pastoris with the expression of rhGH: creation of working cell bank, preparation of inoculum and growth of Pichia pastoris culture, and elaboration of documentation related to these activities.

The excellence of the project consists not only in the manufacture of the first biosimilar in the Republic of Moldova - an exhaustive goal in itself, but also in the unprecedented production of recombinant human growth hormone (rhGH) in Republic of Moldova. This ensures the empirical exploration of the most promising segment of the global pharmaceutical industry with the fastest growth rate. The realization of the project will involve the endowment of the local plant with appropriate equipment and machinery and improving the manufacturing facilities, implementing
highly performant analytical and control methods as well as increasing employees' qualification. Per se the field of biosimilars is eminently boundless for various local and international scientific researches in the huge range of the disciplines of medicine and pharmacy.

The relevance of the project "Cultivation process of raw material based on Pichia pastoris with the expression of growth hormone" is ensured by:

- strengthening the innovation capacity of the beneficiary economic agent (project co-financier) and increasing the product portfolio;
- obtaining in perspective a new biosimilar product, corresponding to regulation requirements in the field of biological medicinal products for human use;
- the implementation of a biosimilar rhGH in the manufacturing of the project co-financier who is at the same time the beneficiary of the results obtained from the research activity;

- the novelty resulting from the complexity of biosimilar cultivation methods, and complex analytical testing, using high performance methods and high-technological equipment for Pichia pastoris culture;
- obtaining a working cell bank and the cultivation of Pichia pastoris with the expression of rhGH for the first time in Republic of Moldova;
- partners who have specialists and necessary equipment for the documentation works and implementation into manufacturing process of the new elaborated technologies.

The implementation of the project "Cultivation process of raw material based on Pichia pastoris with the expression of growth hormone" involves the development of activities in the frontier fields: biochemistry, genetics, pharmaceutical chemistry, pharmaceutical technology, pharmacology, clinical medicine. The activities in the project are supported by the partnership with a university and a research center, involving specialists in several fields: chemistry, biology, genetics, biochemistry, pharmacy, medicine, computer science. The results of the interdisciplinary collaboration will be documentation works, scientific research, cultivation and conditioning technologies, genetic studies, technological production regulations, compartments of the authorization documentation, which will demonstrate the multidisciplinary character of the proposed topic.

The local manufacture of the rhGH obviously offers an incomparable pharmacoeconomic advantage both over the only competitor existing today on the market of Republic of Moldova - Saizen® and over the potential ones.

Given that recombinant human growth hormone (rhGH) is purchased from the state budget according to the criterion - at the lowest price without VAT, in accordance with all requirements, including national registration in the State Nomenclature of Medicines of the Republic of Moldova, the priority of the domestic product is obvious based only on the classic formula of price calculation by summing the cost price and the manufacturer's margin.

The realization of the project "Cultivation process of raw material based on Pichia pastoris with the expression of growth hormone" involves the establishment of partnership and collaboration relations with the most important and promising players worldwide on the biosimilars market.

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