Progress in biopharmaceutical development

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Abstract

Since its introduction in 1982, biopharmaceutical drugs have revolutionized the treatment of a broad spectrum of diseases and are increasingly used in nearly all branches of medicine. In recent years, the biopharmaceuticals market has developed much faster than the market for all drugs and is believed to have great potential for further dynamic growth because of the tremendous demand for these drugs. Biobetters, which contain altered active pharmaceutical ingredients with enhanced efficacy, will play an important role in the development of biopharmaceuticals. Another significant group of biopharmaceuticals are biosimilars. Their introduction in the European Union and, recently, the Unites States markets will reduce the costs of biopharmaceutical treatment. This review highlights recent progress in the field of biopharmaceutical development and issues concerning the registration of innovative biopharmaceuticals and biosimilars. The leading class of biopharmaceuticals, the current biopharmaceuticals market, and forecasts are also discussed.
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Keywords: *biobetter, biopharmaceutical, biosimilar, drug market, monoclonal antibodies, recombinant vaccines*

1. Biopharmaceuticals

Biopharmaceuticals represent some of the best accomplishments of modern science. These drugs are increasingly being used in practically all branches of medicine and have become one of the most effective clinical treatment modalities for a broad range of diseases, including cancers and metabolic disorders.

The term "biopharmaceuticals" was coined in the 1980s and refers to pharmaceuticals produced in biotechnological processes using molecular biology methods. Thus, this group of products was distinguished from the broad category of biologics, which are pharmaceuticals produced using conventional biological methods [1].

Biopharmaceuticals have many advantages. For instance, they target only specific molecules, rarely causing the side effects associated with conventional small-molecule drugs [2]. Additionally, compared with conventional drugs, biopharmaceuticals exhibit high specificity and activity [3]. The application of biopharmaceuticals has facilitated the treatment of patients who respond poorly to traditional synthetic drugs.

1.1. Biopharmaceuticals and synthetic drugs

Biopharmaceuticals differ from synthetic drugs in all respects. The differences between these two categories of drugs include the nature of the product, the source of the active agent, bioequivalence criteria, identity, structure, manufacturing methods, composition, dosing, formulation, handling, intellectual property rights, legal regulations, and marketing [1].

Biopharmaceuticals are produced in living cells, whereas synthetic drugs are the products of chemical processes.

Most synthetic drugs are small molecules. For example, a molecule of acetylsalicylic acid is composed of 21 atoms. In contrast, biopharmaceuticals are typically 100–1000 times larger [4]. The active pharmaceutical ingredient of such a drug may contain 2000–25,000 atoms. Biopharmaceuticals are also structurally much more complex because of the formation of polymeric chains, which vary greatly in their structure.

Abbreviations: *ADC, antibody–drug conjugate; bsAbs, bispecific antibody; CFPS, cell-free protein synthesis; CRISPR, clustered regularly interspaced short palindromic repeats; EPO, erythropoietins; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; G-CSF, granulocyte colony stimulating factor; mAb, monoclonal antibody; scFv, single-chain variable fragment; TNF, tumor necrosis factor; VLPs, virus like particles.*

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The purity of the active ingredient in a pharmaceutical drug and the composition of the final product can be verified relatively easily. Highly pure chemical substances from various sources, including those composed of a mixture of isomers, can be generally considered similar or even identical for practical purposes [1]. The situation is different for biopharmaceuticals. Because of the biological differences between the expression systems and the conditions of the applied manufacturing process, a certain degree of variability may occur, even between different batches of the same product [5]. Therefore, batch-to-batch variations must be monitored to ensure conformance within a specific range. The properties of active pharmaceutical ingredients in biopharmaceuticals, other than their primary structure (e.g., the amino acid sequence), significantly depend on the manufacturing method. For this reason, it is assumed that "the process defines the product" for biopharmaceuticals [6, 7].

Other characteristics of biopharmaceuticals that distinguish them from synthetic drugs are their sensitivity to degradation in the alimentary system and limited penetrability through the intestinal epithelium [8]. As a result, they are typically administered parenterally via direct injection rather than orally [3]. Biopharmaceuticals also require complex stabilization systems because of their temperature sensitivity.

Unlike synthetic drugs, biopharmaceuticals exhibit much more complex mechanisms of action. For example, interferon affects the expression of more than 40 genes. Such extensive complexity often makes it difficult to determine these pharmaceuticals' complete mechanisms of action [9].

Moreover, in contrast to synthetic drugs, biopharmaceuticals are potentially immunogenic. Even relatively small differences in the structure of the active ingredient may considerably affect the immunogenicity of a drug [10–12]. Process-related impurities may also be immunogenic [13, 14].

1.2. Generics versus biosimilars

Generics are defined as drugs that are equivalents of the innovative reference drugs containing the same active pharmaceutical ingredient. The term refers to substitutes for synthetic drugs. For these drugs, because of their characteristics, the production of a preparation containing an exact copy of the active pharmaceutical ingredient is relatively rapid, simple, and inexpensive. According to the data of the American Federal Trade Commission, the development of a generic drug requires 3–5 years and costs $1–5 million [15]. Additionally, a generic version may be 80–90% cheaper than the innovative reference drug [16].

The term "generic" is not used in reference to biopharmaceuticals. The European Medicines Agency (EMA) decided that the term "biosimilars" should be used in the European Union (EU) to refer to biological medical products containing a version of the active pharmaceutical ingredient found in previously registered reference biological medicinal products [17, 18]. The U.S. Food and Drug Administration (FDA) use the term follow-on biologics. Moreover, both of these agencies determined that biosimilars may have different actions than the reference drug [19, 20].

These differences are related to the application of different expression systems and different manufacturing and purification processes in the production of biosimilars. No biopharmaceutical can be duplicated completely, even if the expression systems used in its manufacture are identical (e.g., mammalian cells or bacteria). Biosimilars may potentially differ from the innovative reference drugs in their glycosylation pattern or the electrical potential of the active pharmaceutical ingredient. These differences may influence the quality, strength, and safety of the drug [21, 22]. As a result, pharmacokinetic and pharmacodynamic properties of biosimilars and reference biopharmaceuticals may also differ. However, the tremendous progress made in bioproduction and analytical methods has made it possible to produce proteins and glycoproteins that are similar to the reference product [23].

1.2.1. Registration of biosimilars and generics

The fact that biosimilars are not generics affects their registration procedures. The registration requirements for biosimilars, although less stringent than those for innovative biopharmaceuticals, are much stricter than those imposed on generics. Biosimilars are registered based on their confirmed biosimilarity to the corresponding, previously registered innovative drug. These pharmaceuticals may be registered after the presentation of the required documentation, including a comparison with the reference drug, or after the presentation of complete documentation, such as that required for an innovative drug.

The relevant European guidelines [17] were adopted in 2005, and several years later, in 2010, a similar set of guidelines was introduced in the United States [24].

The first biosimilar, somatotropin (brand name Omnitrope), was registered in the EU in 2006. Currently, in the EU, 23 biosimilars have been registered with the EMA, including five erythropoietins (EPO) used to treat anemia caused by dialysis and chemotherapy, seven filgrastim-granulocyte colony stimulating factors (G-CSF) administered to treat leucopenia caused by chemotherapy, one human growth hormone administered to treat growth disorders, two folliculotropic hormones used to treat fertility disorders, two insulin glargine, two enoxaparin sodiuman—anticoagulant used to prevent blood clots, and four antibodies, including infliximab and etanercept [25]. The next wave of biosimilars is expected to be monoclonal antibodies [26]. The first biosimilar monoclonal antibody (infliximab) was registered in the EU in 2013. Infliximab is an antibody against tumor necrosis factor (anti-TNF) and is used to treat autoimmune disorders, such as rheumatoid arthritis and Crohn's disease. This drug was registered as two individual products under the brand names Inflectra and Remsima because the active pharmacological agent produced by one company is converted into the final drug by two independent manufacturers.

In the United States, the first biosimilar, filgrastim-sndz (brand name Zarxio), was approved by FDA in March 2015

[27]. Zarxio is produced by the European company Sandoz and is a biosimilar of filgrastim (brand name Neupogen), which was originally developed and manufactured by Amgen Inc. Zarxio is registered for the treatment of the same conditions as Neupogen [27]; however, it was approved as a biosimilar, not a substitute.

Considering that the patent protection period has already expired or will soon expire for several important biopharmaceuticals (Fig. 1), it might be possible to register other biosimilars in the near future.

1.3. Biobetters

Another group of biopharmaceuticals are biobetters. Biobetters are biopharmaceuticals that have been structurally and/or functionally altered to achieve an improved or different clinical performance, compared to approved reference products [18]. Biobetters are treated by regulators as different from the existing products and, therefore, are evaluated as new drugs, in a standard approval procedure.

Biobetters represent the next stage in the development of biopharmaceuticals in which proteins were purposefully altered equivalents of existing drugs. The changes introduced were aimed to improve the proteins, i.e., obtain stronger clinical effects, require less frequent administration, achieve better targeting, and/or be better tolerated compared with their equivalents [28]. For this purpose, these proteins were optimized for favorable biodistribution and pharmacokinetic and pharmacodynamic properties. The introduced modifications included changes in the amino acid sequence and the glycosylation pattern of a given protein. In the case of monoclonal antibodies, pegylation or combination with a cytotoxic drug are strategies to enhance their efficacy tor change their half-life. The first such biopharmaceutical produced with an altered amino acid sequence was the fast-acting insulin analog Lispro (brand name Humalog), which was registered in the United States in 1996. Another example of such an improvement is the extension of the pharmacokinetic half-life of equivalents of rituximab, trastuzumab, and bevacizumab, which was achieved by introducing two or three amino acid mutations in the Fc domain [29]. In this case, the extension of intervals between the drug administration or reduction in the dose is expected to lower the treatment costs.

Another example of a biobetter is the ado-trastuzumab emtansine (brand name Kadcyla), an antibody–drug conjugate produced by Roche and an improved equivalent of the trastuzumab antibody (brand name Herceptin) manufactured by the same company [30]. Kadcyla slows down disease progression by almost twofold in patients with HER2-positive advanced breast cancer, extending the median total survival time by 5.8 months relative to other treatment methods [31].

Both biosimilars and biobetters are natural alternatives for the reference biopharmaceuticals and therefore compete for the same market. This competition is influenced by the costs of developing and launching the drug in the market, legal regulations connected with the registration of individual groups of drugs, and the time required to proceed from introduction to turnover. As a rule, the development of a biosimilar is faster and cheaper than that of a reference drug because a biosimilar is an equivalent of an existing drug. Additionally, the risk of failure during trials is much lower for a biosimilar. For this reason, biosimilar drugs may potentially replace the reference drug on the market after the expiration of its patent protection. Manufacturers of reference drugs may attempt to prevent this potential replacement by introducing a biobetter, which is an improved version of the reference drug. If the additional advantages of a biobetter are so significant that it may be preferable in therapy, then the development of a biosimilar may not be viable or the introduction of such a drug will bring much lower profits. Thus, biobetters may limit the market share for biosimilars. In contrast, the development of biobetters requires more extensive research than biosimilars, greatly increasing the costs of drug development. For this reason, it is crucial for a drug's success for that drug to have therapeutic benefits that are significant enough to justify its broad application, despite its potentially higher price.

One example of the competition described above is the case of the drugs used to treat chronic myeloid leukemia. The reference biopharmaceutical rituximab (brand name MabThera) was patented in 1993 in Europe and 4 years later in the United States. However, in 2014, 2 years before the expiration of rituximab's patent protection, the companies producing this drug introduced the biobetter obinutuzumab (brand name Gazyva/Gazyvarol) into the market. Rituximab is a monoclonal anti-CD20 antibody and shows greater efficacy than the reference biopharmaceutical. Additionally, it must be stressed that Gazyva had been registered before the studies on a biosimilar were completed [32].

1.4. Role of cytochrome P450 enzymes in drug development

Metabolism of drugs is a complex process in which many different enzymes are involved. Among these enzymes are cytochromes P450, a large superfamily of ubiquitous heme-containing monoxygenases [33, 34]. These enzymes are essential for metabolism of 80% of clinically used drugs. In the drug metabolism, multiple products may be obtained from the same drug and one drug may be metabolized by more than one cytochrome P450 enzyme. Moreover, each enzyme acts on more than one drug. The substances resulting from a drug metabolism can be biologically active and may cause adverse drug reactions [33]. Therefore, in the drug discovery and development process it is of key importance to investigate the metabolism of drug candidates. This has led to increased demand for drug metabolites to facilitate evaluation of their possible adverse effects in animals and humans as well as drug's efficacy and pharmacokinetics.

The use of human cytochromes P450 to produce drugs, drug metabolites, and intermediates is mainly limited by their poor solubility, stability, and low coupling [35]. By contrast, P450 BM3, bacterial P450 enzyme from *Bacillus megaterium*,

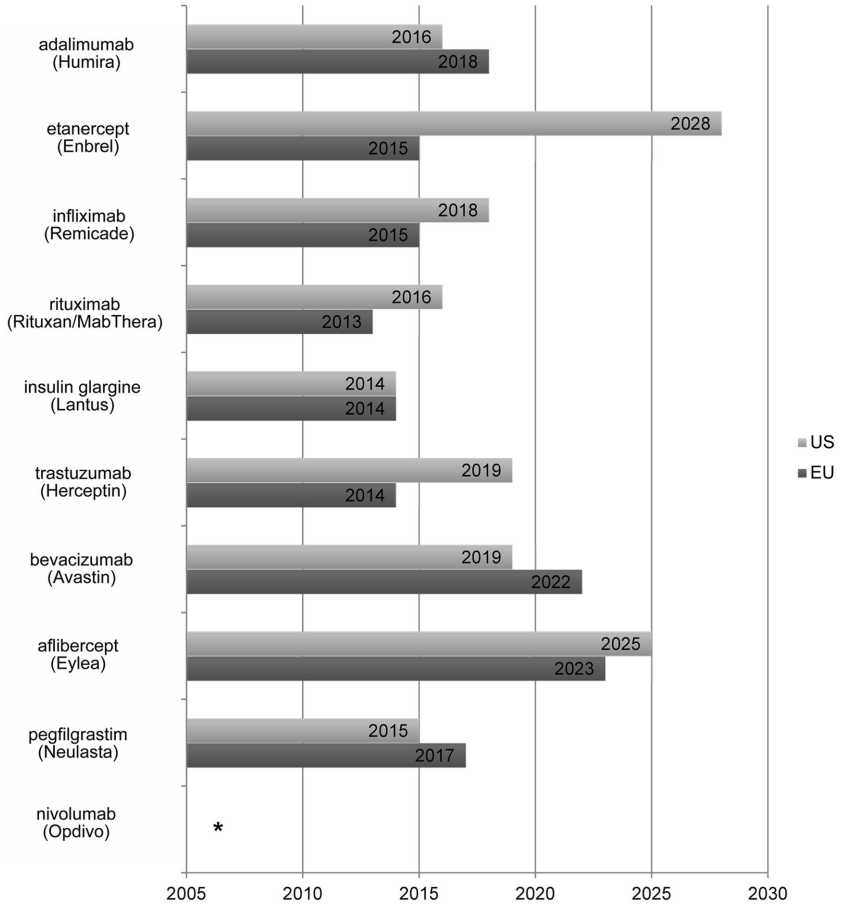

FIG. 1 *Dates of patent expiry for the 10 best-selling biopharmaceuticals in 2016. * Data not available.*

has been shown to be able to produce drug metabolites typical of the human enzymes with a high coupling efficiency [33, 36]. Owing to P450 BM3 characteristics, multiple protein engineering studies have been performed on this enzyme to widen its catalytic abilities [37–40]. Moreover, several constructions have been already reported including different fusion human P450 enzymes, engineered by connecting the P450 BM3 reductase domain with human cytochromes P450 3A4, 2C9, 2C19 [41], 2A6, CYP2C6, and CYP4F11 [42], monkey 2C20 [43], and dog CYP2D15 [44, 45]. Also the catalytic performance of one of the created chimeric proteins was improved in terms of coupling efficiency and enzyme turnover by engineering the loop connecting the two domains [46]. The published results were an important factor in engineering catalytically self-sufficient human P450 for applications in biocatalysis [46].

2. Systems for the Production of Biopharmaceuticals

Unlike synthetic drugs, the active pharmaceutical ingredients in biopharmaceuticals include recombinant proteins and nucleic acids. Currently, the vast majority of commercially available biopharmaceuticals contain recombinant proteins as their active pharmaceutical ingredient. These proteins are produced in prokaryotic systems, mainly *Escherichia coli*, or eukaryotic systems based on fungi (*Saccharomyces cerevisiae* and *Pichia pastoris*), mammalian cells, or insect cell lines. The use of cell-free expression systems (*in vitro* systems), which greatly facilitates modifying synthesis conditions, has also been studied [47].

The production of biopharmaceuticals in each of the aforementioned systems has advantages and drawbacks. For these reasons, many different expression systems are used based on the specific properties of a given recombinant protein.

2.1. Mammalian expression system

Mammalian expression systems are generally the preferred platform for manufacturing biopharmaceuticals. In recent years, a steady increase in the use of these expression systems has been observed. This is because of the growing interest in the production of large, complex molecules that require specific posttranslational modifications (most notably glycosylation) that occur only in mammalian expression systems [48]. Additionally, in the case of mammalian cell lines and animal cell lines in general, most recombinant proteins can be secreted and do not require cell lysis to extract with subsequent protein refolding (as is the case with bacteria) [48].

However, protein production in cell lines raises potential safety concerns due to the possibility of contaminants with

animal viruses. Other drawbacks of protein production in cell lines include the complex nutritional requirements, slow growth and fragility, and a relatively high production time and cost [49]. Currently available mammalian expression systems include Chinese hamster ovary cells, rodent cell lines (e.g., NS0, BHK, and Sp2/0), and human cell lines (e.g., HEK293, PER.C6, HT-1080, and CAP) [50]. Among available mammalian cell lines, Chinese hamster ovary cell line is the primary choice for recombinant protein production, with 7 of the top 10 best-selling biopharmaceuticals from 2016 being produced in these cells. In general, the number of recombinant protein products produced in mammalian systems that are approved for use as drugs in humans increased over 2010–2014 to approximately 60% [49].

2.2. Bacterial expression system

Nevertheless, bacteria remain the dominant expression system, facilitating the production of large quantities of active pharmaceutical ingredients used in biopharmaceuticals. According to the data provided by BioProcess Technology Consultants, in 2010, the total production of pure proteins as active pharmaceutical ingredients in biopharmaceuticals amounted to 26.4 tons. Of this, 68% were produced in bacterial systems and 32% in mammalian systems. The predominant group of proteins produced in bacteria comprised insulins, and the vast majority of those produced in mammalian systems were monoclonal antibodies [51].

The bacteria of choice for heterologous protein expression are *E. coli*. Its attractiveness for industrial applications results from its well-understood genetics, cell biology, and easy handling. Expression systems based on *E. coli* allow for rapid growth, high product yield, cost-effectiveness, easy process scale-up, and short turnaround time [52, 53]. The limitations of this expression host for the production of complex recombinant biopharmaceuticals include the absence of mammalian-like posttranslational modifications, such as glycosylation, phosphorylation, and proteolytic processing [54]. Hence, *E. coli* is the expression host of choice in the biotechnology industry for the large-scale production of small recombinant proteins that do not require posttranslational modifications [49]. Another limitation results from *E. coli's* inability to produce correct disulfide bonds, protein solubility issues, and the presence of endotoxins (lipopolysaccharide) [55]. Currently, several strategies are applied to improve protein expression, such as the use of mutated *E. coli* strains to promote protein disulfide bond formation [52].

2.3. Yeast expression system

Additional favorable microbial recombinant protein production systems are the eukaryotic microorganisms *S. cerevisiae and P. pastoris* [56]. Both of these hosts are capable of producing recombinant proteins with proper folding and posttranslational modifications [57]. Therefore, they are considered better than prokaryotes where posttranslational modification of the target protein is needed. The *S. cerevisiae* yeast expression system is frequently used due to their rapid growth in protein-free media and ability to secrete the product extracellularly. However, posttranslational modifications that occur within the cells often lead to the production of undesired hypermannosylation [56], which can result in altered protein binding activity, and potentially yield an altered immunogenic response in therapeutic applications. In *P. pastoris*, oligosaccharides have much shorter chain lengths and the strain has been reported to produce complex, terminally sialylated or "humanized" glycoproteins [58]. *P. pastoris* is an expression system that is appreciated for its growth to very high cell densities, for its available strong and tightly regulated promoters, and for the possibility to produce gram amounts of recombinant proteins per litre of culture both intracellularly and in a secretory fashion [59]. However, protein yields can be remarkably lower, particularly if the expressed complex proteins are heterooligomers, membrane-attached or prone to proteolytic degradation [59].

2.4. Insect cell line expression system

Insect cell-based recombinant protein production system represents a compromise between bacterial and mammalian expression systems. Its advantage over the bacterial system is that it allows for posttranslational modifications but unlike mammalian system it does not preserve the original glycosylation pattern. [60]. Another advantage of use of insect cells is that they are less demanding and they grow to higher densities compared to mammalian cells.

An insect cell line expression system was used to produce Cervarix, a vaccine against certain types of cancer-causing human papillomavirus. This vaccine was approved by the EMA in 2007.

2.5. Transgenic animals

In addition to commonly used prokaryotic and eukaryotic expression systems, there is an increasing interest in the use of transgenic animals for recombinant protein production. This is due to the low cost of producing large amounts of complex proteins in these systems [61, 62]. Transgenic animals offer the opportunity to produce human recombinant proteins with posttranslational modifications that closely match human proteins. However, there are issues with the generation of transgenic founders. Although many strategies have evolved over the past decades, transgenesis in animals is relatively inefficient and time consuming. Attempts to improve transgenesis by various methods have had limited success, mainly due to random transgene integration and the control over transgene copy number [63].

The first biopharmaceutical, ATryn, whose active pharmaceutical ingredient was produced in transgenic animals (goats), was launched on the market in 2006 in the European Union (2009 in the United States). This drug is an anticoagulant and contains a plasma protein, human alfa antithrombin [64]. Since then, other proteins produced in transgenic rabbits have been approved for use. Thus, conestat alfa (brand name Ruconest), a recombinant analogue of the human esterase inhibitor C1, was approved for the treatment of hereditary angioedema [65]. The

recombinant proteins produced by these animals are secreted into their milk [66].

2.6. Plant expression system

The production of biopharmaceuticals derived from plants has attracted great interest. Transgenic plants have the potential to become cost-effective systems for the large-scale production of human therapeutic proteins. The use of plants eliminates potential contamination of the therapeutic drug with animal pathogens, as plant cell cultures are not susceptible to mammalian viral pathogens and, conversely, plant viruses do not infect human cells [67]. Another advantage is that orally immunogenic recombinant proteins expressed in an edible plant may be orally administered without processing, including expensive purification steps [68]. Moreover, plant expression systems are able to produce proteins with complex glycosylation patterns; however, the glycan structures produced are significantly different from those produced in humans.

Drawbacks of plant-based expression system for recombinant protein production are related to long production timelines that render this technology unsuitable for the rapid production of pharmaceuticals to combat emerging diseases [69]. Another issue is that current methods in plant biotechnology cannot precisely control transgene expression levels in plants in a consistent manner [70].

As an alternative to using whole plants as bioreactors, there has been considerable progress in the use of plant cell cultures, such as carrot suspension culture and tobacco BY-2 cells [71]. Until now, the main classes of biopharmaceutical proteins successfully produced and correctly folded in plants have been subunit vaccines and virus-like particles (VLPs), antibodies, and therapeutic enzymes, including several products that have completed phase II trials and are close to commercialization [70]. In 2012, a protein produced in plant cell lines (carrot root cells) was allowed to enter the drug market. This protein, recombinant human glucocerebrosidase (brand name Elelyso), is an active pharmaceutical ingredient in the drug used to treat Gaucher's disease and became the first plant-produced biopharmaceutical approved for human administration by the FDA [72, 73]. Since Gaucher's disease is a rare disease, treatment of this disease with an orphan drug is costly (US$200,000 annually per patient for life). The use of a carrot cell production system reduced the cost to US$150,000 per patient per year [70].

2.7. Cell-free protein synthesis

Cell-free protein synthesis (CFPS), also called *in vitro* expression, is an innovative and promising alternative to the expression of recombinant proteins in living cells. CFPS is the production of recombinant proteins using translation machinery extracted from cells. In this system, the enzymes required for the transcription and translation processes are present in a cell extract instead of a live organism. Several obstacles initially limited CFPS as a protein production technology, including low protein production rates, high reagent costs, small reaction scales, and limited ability to correctly fold proteins containing multiple disulfide bonds.

Currently, because of the significant progress made to automate and optimize reaction conditions, cell-free systems have become an attractive protein production platform offering several advantages over traditional cell-based expression methods [74]. First, CFPS environment is not limited by the presence of a cell wall or homeostasis conditions to maintain cell viability. CFPS enables direct access, and therefore control of the translation environment, and manipulation of the reaction composition and conditions, which is advantageous for the optimization of protein production. As a result, new components can be added/synthesized and maintained at precise concentrations [75]. Other advantages of CFPS over cell-based systems include the ability to produce difficult to express proteins, e.g., membrane proteins as well as toxic proteins [76]. Unlike systems based on living organisms, it is believed that the protein synthesis conditions in CFPS are analogous to those of chemical reactions, which is promising from the perspective of technical scalability.

Compared to expression methods based on bacterial or tissue culture cells, CFPS is considerably faster because it does not require gene transfection, cell culture, or extensive protein purification. Moreover, the speed and ease of protein production in CFPS results from the possibility of direct expression from PCR-generated templates without requiring fragment cloning. Despite of the made progress CFPS still suffers from the lower yield of target proteins and relatively high cost compared to the other expression systems.

Recently, Sutro Biopharma has developed STRO-001, an antibody–drug conjugate (ADC). It was developed via proprietary cell-free protein synthesis and site-specific conjugation platforms, which facilitate multiple rounds of antibody and ADC optimization. STRO-001 is a novel CD74-targeting ADC composed of a *p*-azido-methyl-phenylalanine-containing anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a noncleavable dibenzocyclooctyne-maytansinoid linker-warhead. [77]. STRO-001 has been shown to eradicate tumors in human xenograft models of non-Hodgkin lymphoma and multiple myeloma diseases. The company plans to submit an Investigational New Drug application to the FDA at the end of 2017 and initiate STRO-001 clinical testing in the first quarter of 2018. If it passes the tests, it will be the first commercial biopharmaceutical produced in a cell-free expression system, which would prove commercial viability for this technique.

3. First Gene Therapies

In addition to recombinant proteins, nucleic acids may also be biopharmaceuticals' active pharmaceutical ingredients. Most studies on gene therapy have focused on the induction or inhibition of cellular processes underlying diseases. Gene therapy is based on the introduction of genetic material into an organism or patient, either directly or using viruses.

A breakthrough in the biopharmaceutical sector was the registration of a DNA-based drug (the first gene therapy). The first drug used in gene therapy (Alipogene tiparvovec, brand name Glybera) was approved for use in the EU in 2012 [78]. This therapy compensates for the lipoprotein lipase deficiency found in rare hereditary disorders that leads to severe pancreatitis. Unfortunately, Glybera administration provides only temporary relief [51]. Initially, the cost of a single treatment was estimated at $1.6 million [79]. In 2015, this figure decreased to $1 million [80], yet this therapy remains the most expensive in the world. Glybera failed to achieve recognition of benefit in two EU countries (France and Germany) and is unlikely to ever be commercialized in other European countries. Moreover, plans for the commercialization of this therapy in the United States have been abandoned. Thus, over 4 years after the European regulatory approval, the first commercial gene therapy drug has been used in only one case [81].

A few years earlier, in 2003, another drug used in human gene therapy, Gendicine, was approved in China for the treatment of head and neck squamous cell carcinoma [82]. In 2011, Neovasculgen was registered in Russia as a first-in-class gene-therapy drug for the treatment of peripheral artery disease, including critical limb ischemia.

Recently, three other gene therapies (Imlygic, Strimvelis, and Invossa) were approved. Imlygic (Talimogene laherparepvec) was approved by the EMA and the FDA in October 2015. It is a modified form of the herpes simplex virus type 1 for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with recurrent melanoma after initial surgery [83].

In April 2016, the EMA approved the first *ex vivo* stem cell gene therapy (Strimvelis) with indications to treat patients with adenosine deaminase-deficient severe combined immunodeficiency [84]. Strimvelis consists of autologous gene-corrected hematopoietic stem cells and is prepared from the patient's own bone marrow hematopoietic stem cells, which are genetically modified using a gamma-retroviral vector to insert a functional copy of the adenosine deaminase gene [85].

In July 2017, South Korea's Ministry of Food and Drug Safety approved the country's first gene therapy drug. The drug, Invossa, is the world's first cell-medicated gene therapy for osteoarthritis. Invossa uses allogeneic human cartilage cells engineered to express transforming growth factor TGF-ß1. The drug was approved for sales in the domestic market.

3.1. The future of gene therapy

Between 1989 and April 2017, 2,463 gene therapy clinical trials have been completed, are ongoing or have been approved worldwide [86]. So far, most of them have been aimed at the treatment of cancer (64.4% of all gene therapy trials) [86].

In July 2017, the FDA advisory panel recommended approval of tisagenlecleucel-T, a chimeric antigen receptor T-cell therapy. The treatment would be directed for children and young adults with B-cell acute lymphoblastic leukemia [87]. Chimeric antigen receptor T-cell therapy treatment approval is expected by the end of 2017. If the FDA accepts the recommendation, this treatment will be the first gene therapy to ever reach the market in the United States.

While most gene therapy trials have addressed cancer, a significant number of gene therapy trials have targeted rare inherited monogenic diseases (10.5% of all gene therapy trials) [86]. Monogenic diseases receive much attention as they hinge on the conceptually simple idea that diseases caused by a known single gene defect could be potentially cured by the insertion and expression of a single correct copy of the mutant or deleted gene in host cells. One monogenic disease that may be curable in the near future using gene therapy is the inherited bleeding disorder hemophilia B. After receiving a single dose of an experimental gene therapy (SPK-9001) in a clinical trial, patients with hemophilia produced near-normal levels of clotting factor IX, allowing them to stop clotting factor infusions and to pursue normal daily life activities without disabling bleeding episodes [88].

Over 77% of all gene therapy clinical trials performed to date are phase I or I/II. Ninety-three gene therapy clinical trials were in phase III. It has been predicted that by 2020, some 5–10 gene therapies will be available. The first gene therapies are expected to be used to treat a rare form of blindness, Leber's congenital amaurosis, sickle-cell anemia, beta-thalassemia, and a spectrum of rare cancers and genetic diseases [89, 90]. However, given the lessons learned from the development of Glybera gene therapy, the high costs and the temporary nature of its beneficial effects, we will likely not see the widespread use of such therapies in the near future. Nevertheless, the changes brought about by the introduction of gene therapies will spark an era of targeted and personalized treatment [90].

Recent research shows that the development of gene therapy can be accelerated by a new, revolutionary genome-editing tool: clustered regularly interspaced short palindromic repeats (CRISPR) [91]. It was successfully used for *in vitro* CRISPR-based genome editing to correct defective genotypes [91]. Moreover, several studies have also shown that CRISPR therapies can be successfully implemented *in vivo* [92]. There are currently two clinical trials involving CRISPR-Cas9 for targeted cancer therapies that have been approved in China and the United States [91]. On October 2016 as a part of clinical trials, a group of Chinese researchers used immune cells that contain a gene (PD-1) edited using this technique. The cells were removed from the blood of a person with lung cancer, the gene was disabled, and the cells were injected back into the patient. In 2017, another Chinese group plans to start three clinical trials of drugs developed using the CRISPR technique. These therapies target bladder, prostate, and renal cell cancers [93]. In June 2016, the U.S. National Institutes of Health approved the first CRISPR clinical trial in the United States to help augment cancer therapies.

As CRISPR-based treatments have made enormous progress since their beginning only a few years ago, there is great hope that this tool will strongly accelerate the development of gene therapies. Nonetheless, more

investigations are needed to fully harness the power of this technique.

4. Therapeutic Antibodies

Monoclonal antibodies (mAbs) are the largest class of biopharmaceuticals and are currently utilized in therapies for cancer, inflammatory diseases, cardiovascular diseases, organ transplantations, infections, respiratory diseases, and ophthalmologic diseases. This group of biopharmaceuticals includes mAbs and derivative antibodies, such as bispecific antibodies (bsAbs), antibody–drug conjugates, radiolabeled antibody conjugates, antigen-binding fragment Fab, and Fc-fusion proteins [94].

The availability of fully human and humanized mAbs increased the therapeutic efficacy for oncology and hematooncology and for inflammatory and autoimmune diseases. The first monoclonal antibody–based biopharmaceutical was muromonab-CD3 (sold under the brand name Orthoclone OKT3), which is administered during acute kidney transplant rejection [95]. This drug was registered in 1986; however, the dynamic development of the antibody market began in the late 1990s, when the first chimeric mAb was registered. As of March 2017, in the EU and the United States, a total of 71 monoclonal antibody-based drugs have been registered [96]. Currently, fully human antibodies are growing as a proportion of mAbs in the clinic. In 2002, the FDA approved the first fully human mAb, adalimumab. Since that time, approximately 40% of all of the marketed mAbs are fully human.

4.1. Bispecific antibodies

Most registered antibodies are monospecific antibodies that are capable of interacting with a single target. However, complex diseases, such as cancers or inflammatory disorders, are frequently multifactorial in character. In these cases, the inhibition of many different pathogenic factors and signaling pathways may enhance the therapeutic efficacy. For this purpose, bsAbs were designed [97]. These antibodies are artificial proteins composed of fragments of two different monoclonal antibodies and thus bind to two different types of antigens. bsAbs are most commonly used in cancer immunotherapy, where they simultaneously bind to two targets, e.g., a tumor cell and cytotoxic cells (via a receptor, such as CD3) [98, 99]. bsAbs efficiently stimulate the host immune system, facilitating the destruction of cancer cells [100].

Initially, bsAbs were generated via the chemical conjugation of two different antibodies, producing a single molecule equipped with four antigen-binding regions (four Fab fragments), with each of the fragment pairs binding a different molecule [101]. Another approach to generating bsAbs is the fusion of two hybrids, producing antibodies with differing specificities. Currently, more than 50 different technological platforms are available for bsAbs production [102].

So far, the FDA has approved two bsAbs, catumaxomab and blinatumomab [103]. Catumaxomab (brand name Removab), a rat-mouse hybrid IgG2 monoclonal antibody produced in a rat-mouse hybrid cell line (hybridoma), was the first approved bsAb. This antibody is used in the intraperitoneal treatment of malignant ascites. Catumaxomab was registered in 2009 in the EU and is in clinical trials in the United States. The other bsAb, Blinatumomab (trade name Blincyto), was registered in 2014 by FDA for the treatment of relapsed or refractory Ph-negative acute lymphoblastic leukemia in adults. Blinatumomab is currently undergoing multiple phase II and phase III clinical trials for other B-cell related malignancies [104]. Currently, there are two bsAbs (Emicizumab and MEDI-565) in phase III clinical trials [103]. Emicizumab is a bsAb for prophylactic use to reduce the number of bleeding episodes in patients with hemophilia A and factor VIII inhibitors. MEDI-565 (also known as MT111 and AMG 211) is a bsAb for the treatment of patients with cancers expressing carcinoembryonic antigen.

Currently, there are increased numbers of clinical trials being performed on novel, bsAb-based drugs. Therefore, the introduction of new drugs of this type on the market is expected in the near future.

4.2. Radiolabeled antibody conjugates, antibody–drug conjugates

Another group of monoclonal antibody-based drugs includes cancer antigen-specific mAbs conjugated with isotopes emitting radiation or a highly potent drug. Both of these strategies facilitate the specific destruction of cancer cells. Currently, two radio-immunoconjugates are registered for the treatment of non-Hodgkin's lymphoma, 131I-Tositumab (brand name Bexxar) and 90Y-ibrytumomab tiuksetanem (brand name Zevalin) [105, 106].

In recent years, ADCs have become powerful tools in the treatment of cancer. An ADC is a bioconjugate that contains a mAb that specifically binds to a tumor virus surface antigen and a highly potent drug that is attached to the antibody via cleavable or noncleavable linkers. This design ensures specificity and efficacy in targeting cancer cells and allows the healthy tissues to remain generally unaffected. Recently, the FDA approved two such drugs, brentuximab vedotin (brand name Adcetris) and ado-trastuzumab emtansine (brand name Kadcyla).

In 2015, more than 50 new ADCs were in clinical trials all over the world [107]. As of January 2017, there are 37 ADCs in phase I trials. Three ADCs entered phase I/II development in 2016, increasing the total number of ADCs in this stage to eight. Four ADCs (AGS-16C3F, Anetumab Ravtansine, SAR566658, and Rova-T) progressed toward phase II, yielding 11 phase II ADCs. Two drugs (IMGN853 and SGN-CD33A) entered phase III trials, which doubled the number of ADCs in this clinical phase [108]. In August 2017, FDA approval could become a reality for inotuzumab ozogamicin [108], the anti-CD22 antibody–drug conjugate for the treatment of patients with relapsed or refractory acute lymphoblastic leukemia.

Overall, it is estimated that approximately 10 new ADCs will come onto the clinical market in the next decade [107].

Despite the tremendous progress in ADC development, further studies are necessary to enhance their safety and efficacy [109]. A major challenge in this regard is ensuring the homogeneity of ADC molecules [110].

4.3. Single-chain variable fragment

An important focus of studies on mAbs relates to the reduction of the active particle size to enhance antibody penetration following their administration and facilitate their production. This line of research addresses the development of single-chain variable fragments (scFvs).

4.4. Glycoengineered monoclonal antibodies

Another technological innovation was the development of the first glycoengineered mAb, afutuzumab (brand name Gazyva), which was registered in 2013 in the United States [111]. The introduced modifications enhanced the antibody-dependent cell-mediated cytotoxicity. This antibody is an immunomodulator used to treat lymphoid malignancies [112].

4.5. The future of therapeutic antibodies

Currently, most registered mAb-based drugs are used to treat cancers and autoimmune disorders. According to World Health Organization estimates, the number of new cancer cases will increase to 27 million in 2030 [113] because of the growing number of elderly individuals. Taking into account these epidemiological data and the resulting huge demand for anticancer therapy, it is expected that anticancer drugs will be the leading group among all registered drugs. This is also confirmed by data on the number of mAbs that are entering clinical trials. During 2014–2016, pharmaceutical companies initiated the first in-human studies for an average of approximately 80 mAbs-based therapeutics annually, of which more than 60% were designed to treat cancer [114]. In 2016, antibodies for cancer represented approximately 55% of the overall clinical pipeline of therapeutic antibodies. Anticancer mAbs that entered the clinical pipeline in 2017 include two (CX-072 and KN035) that target the programmed death-1 receptor ligand, and one each (CBT-501 and FLYSYN) that target programmed death-1 receptor and Fms-like tyrosine kinase, respectively [114]. Among the five mAbs that have recently entered late-stage clinical studies, three (utomilumab, isatuximab, and SHR-1210) are being evaluated as treatments for cancer. The other two mAbs, crizanlizumab and olokizumab, are being studied in patients with sickle cell disease (for the prevention or reduction of the occurrence of pain crises) and rheumatoid arthritis, respectively [115].

Extensive research efforts are currently focused on the development of improved antibodies against known molecular targets associated with asthma, leukemia, nonsmall-cell lung carcinoma, and multiple sclerosis. In the near future, next-generation antibodies with improved properties, including ADCs and bsAbs, are expected to gain popularity as biobetter antibody therapeutics [103].

Additionally, studies are being conducted to develop mAb biosimilars that are already used in therapy. High-specificity mAbs against the antigens present in specific pathological cell types are increasingly being used in both therapy and clinical diagnostics. Because of the large number of mAb candidates undergoing evaluation in late-stage clinical studies (over 50 as of December 2016), a trend toward first marketing approvals of at least six to nine mAbs per year is expected to be observed in the near-term. This prediction indicates that the market for mAbs is likely to develop dynamically, which will be manifested in further increases in the sales of these drugs.

5. Vaccines

Another potential area of biopharmaceutical growth is vaccine development. Any given vaccine may be classified as biopharmaceutical if molecular biology methods are used in its development. An example could be live attenuated vaccines where recombinant DNA technology was used to alter the pathogen's genome. Another group of vaccines that can be classified as biopharmaceuticals are subunit vaccines, which are based on specific, highly purified recombinant protein antigens.

5.1. Subunit vaccines

Subunit vaccines contain only defined antigens instead of whole pathogens, and, therefore, their application does not introduce the risk of infection. However, a major challenge for current subunit vaccine development is the fact that many new subunit vaccines are poorly immunogenic and mobilize insufficient immune responses for protective immunity. Therefore, effective adjuvants are needed to enhance, direct, and maintain the immune response to vaccine antigens [116]. New adjuvants are designed to not only boost immunological response but also to increase cross-protection against different strains or variants of the same pathogen. The new generation of rationally designed vaccine adjuvants target specific innate immune receptors such as toll-like receptor (TLR) 4 and TLR9. These novel adjuvants reached human clinical trials stage [117]. Several studies are also conducted on the synergistic effects of different adjuvants to identify new beneficial effects of vaccine efficiency [118].

Another crucial aspect of development of next-generation vaccines is the optimal presentation of the antigen to the immune system to achieve desirable immune response. In the quest for novel and effective presentation methods as well as delivery strategies, VLPs offer several promises. VLPs can elicit strong T and B cell immune responses because they contain repetitive displays of viral surface proteins that present conformational viral epitopes. VLPs are not infectious but have similar properties to virions, enabling them to be used as both particulate carriers and adjuvants in vaccine development. VLPs were successfully used in approved vaccines for hepatitis B and human papillomavirus.

5.2. New technologies in vaccine development

Another important subject in biopharmaceutical development is the emergence of new technologies, which have the potential to revolutionize the vaccine field. These technologies include

reverse vaccinology, structural vaccinology, and synthetic vaccines.

5.2.1. Reverse vaccinology

The concept of reverse vaccinology involves using bioinformatics tools to screen the entire genome of a pathogen to identify genes encoding proteins with the attributes of good vaccine targets. Current reverse vaccinology approaches include comparative *in silico* analyses of multiple genome sequences, which enable the identification of conserved antigens within a heterogeneous pathogen population and the identification of antigens present in pathogenic but not commensal strains [119]. Moreover, transcriptomic and proteomic data sets are integrated into a selection process that allow for the accelerated identification of vaccine targets to be tested in animal models. Reverse vaccinology was successfully applied against serogroup B meningococcus [120]. This technology was also used in advanced preclinical and clinical vaccine studies against several pathogens, including those resistant to antibiotics [121].

5.2.2. Synthetic vaccines

Synthetic vaccines were developed to accelerate vaccine availability for future pandemics. This technology enables the rapid generation of vaccine viruses from sequence data. Dormitzer et al. [122] used an enzymatic assembly from chemically synthesized oligonucleotides and improved *in vitro* error correction for the rapid, accurate gene synthesis of two major influenza virus surface glycoproteins (hemagglutinin and neuraminidase). This synthetic approach allowed for the development of vaccine seeds in a matter of days instead of the typical 2–3 months needed when conventional technologies are used.

5.2.3. Structural vaccinology

Structural vaccinology is emerging as a promising platform for the identification of effective protective antigens to facilitate the development of optimized and possibly broadly protective vaccines. In this technology, the domains within an immunogenic protein that contain epitopes inducing protective immune responses are identified and expressed in a recombinant form. These domains can be used as potent immunogens devoid of the regions of the immunogenic protein that are irrelevant from a vaccine standpoint [123]. Recently, it was shown that, with epitope from respiratory syncytial virus, structural vaccinology enabled to generate small, thermally and conformationally stable protein scaffolds that accurately mimic the viral epitope structure and induce neutralizing antibodies [124].

5.3. Vaccines pipeline

Research on molecular biology methods in vaccine development has discovered several promising results. There are a number of novel vaccines at various stages of the drug acceptance process. They include vaccines against major infectious diseases such as HIV and tuberculosis, a universal flu vaccine, and vaccines against noninfectious diseases.

5.3.1. HIV

Two HIV vaccines, HVTN 702 and Ad26, are currently part of efficacy clinical trials in humans. The HVTN 702 vaccine regimen consists of two experimental vaccines, a canarypox vector-based vaccine called ALVAC-HIV and a two-component 120 HIV glycoprotein subunit vaccine with MF59 adjuvant to enhance the body's immune response to the vaccine. The results of HVTN 702 clinical trials are expected in late 2020 [125]. The second study (Ad26) is based on "mosaic" vaccines designed to induce immunological responses against a wide variety of HIV subtypes responsible for HIV infections globally. The vaccine uses a strain of adenovirus serotype 26 as a vector to deliver three (trivalent) or four (tetravalent) mosaic antigens for HIV variant genes and Clade C 140 HIV glycoprotein adjuvanted with aluminum phosphate. The results from these clinical trials are expected in late 2017 [126].

5.3.2. Tuberculosis

Thirteen different tuberculosis vaccine candidates are currently in clinical trials; eight of them are subunit vaccines, six of which contain or express one of *Mycobacterium tuberculosis* antigen 85 complex proteins (either the Ag85A or Ag85B) [127]. The current test evaluates the potency of new candidates in animal models by measuring the reduction in the bacillary load in the lungs during the acute phase of the infection. However, so far, none of the candidates has been able to prevent the establishment of the infection [128].

5.3.3. Malaria

Substantial progress has been made in the development of malaria vaccines during the past decade. In 2015, recombinant protein-based malaria vaccine, RTS,S/AS01 (trade name Mosquirix), has received a positive opinion from the EMA [129]. RTS,S/AS01 is the world's first licensed malaria vaccine.

5.3.4. Universal flu vaccine

The progress in the field of biopharmaceuticals may enable development of a universal flu vaccine. Such vaccine, in contrast to currently available vaccines, would be able to provide long lasting and broad protection against flu infections [130]. Some very promising results were obtained from the structure-based development of an influenza virus H1 hemagglutinin stem-only immunogen. The immunogen confers heterosubtypic protection in mice and ferrets. In this study, vaccination of mice and ferrets elicited broadly cross-reactive antibodies that completely protected mice and partially protected ferrets against lethal heterosubtypic H5N1 influenza virus challenge despite the absence of detectable H5N1 neutralizing activity *in vitro*. Protection against H5N1 challenge indicates that vaccine-elicited hemagglutinin stem-specific antibodies can protect against diverse group 1 influenza strains [131].

5.3.5. Respiratory syncytial virus and Group B Streptococcus

There is a public health need for vaccines against respiratory syncytial virus and Group B Streptococcus. For both pathogens,

maternal immunization could reduce the risk of neonatal infection and death by passive placental transfer of maternal antibodies. New vaccines against these conditions may establish a precedent for maternal immunization as the initial indication [132]. Respiratory syncytial virus is a pathogen for which there is significant clinical pipeline activity, and the likelihood of a candidate emerging for licensure soon. One company is considering phase III study designs for their Group B Streptococcus candidate vaccine [132].

5.4. Noninfectious disease vaccines

As life expectancy has increased in the recent past, noninfectious diseases such as ischemic heart disease, stroke, and cancer are the leading cause of death. Some other noninfectious diseases, including diabetes, Alzheimer's disease, and other neurodegenerative diseases, are becoming leading causes of morbidity [133]. The development of therapeutic vaccines will lead to opportunities to manage these diseases at early stages. Vaccines to treat chronic diseases would be expected to extend life expectancy. Currently, studies of this type are in the discovery stage. However, in 2010, the FDA approved Sipuleucel-T, the first therapeutic vaccine that represents a milestone and may pave the way for the wider use of cancer vaccine immunotherapies. Sipuleucel-T is an autologous active cellular immunotherapy used for treating men with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer [134].

Effective therapeutic cancer vaccines may rely on the development of personalized vaccines tailored to match a person's particular cancer mutations. Two clinical studies are the first to report that the approach could combat melanoma skin cancer in humans [135]. The ideas for these vaccines are based on using unique cancer cell components that are combined with agents that stimulate an immune response. The vaccine is injected into the patient to trigger an immune attack against cancer.

6. The Growth of Biopharmaceuticals Market

Currently, the industry consisting of the development, manufacturing, and marketing of biopharmaceuticals is a multibillion dollar industry. The common practice in market reports is to separately present information about vaccines and other biopharmaceuticals.

Vaccine research costs continue to grow. One of the major contributing factors to this growth is the use of state-of-the-art vaccine development techniques. On the other hand, society expectation is that a vaccine must be affordable for everyone. Because of that implied price cap, pharma industry generally regards vaccines as not the most profitable market segment. However, this perception of vaccine market is changing.

In 2015, worldwide vaccination market generated 27.6 billion U.S. dollars and is projected to total around 39.0 billion U.S. dollars in 2022 [136]. The major factors contributing to the expected growth of the vaccines market include high prevalence of diseases, rising government and nongovernment funding for vaccine development, and increasing focus on immunization programs [137].

For example, market for vaccines against shingles is predicted to more than double by 2022 [138]. Currently, Zostavax, live, attenuated virus vaccine, dominates this market. However, it is anticipated that Shingrix, a recombinant subunit vaccine, which is under review by regulators in the United States and Europe, may offer greater protection in older patients [138].

The global vaccines market is segmented based on technology, type, disease indication, end-users, and regions. Based on increasing company investments, the highest growth rate in the vaccines market is expected to be registered in the conjugate vaccines segment. Two conjugate vaccines against *Streptococcus pneumoniae*, Penavnar and Prevnar 13, already succeeded in the marked. The combined sales of these vaccines accounted for approximately $6,3 billion in 2015 which places them on the top of best-selling vaccines list (Fig. 2).

In 2016, biopharmaceutical sales (excluding vaccines) reached U.S.$163 billion, a 5.8% increase since 2015 and a 102% increase since 2008 [139]. It is believed that this market has great potential for further dynamic growth. According to the report "Global Protein Therapeutics Market Outlook 2020," this market may reach U.S.$208 billion by the end of 2020 [140]. The increase is a result of the growing number of innovative biopharmaceuticals launched on the market, their therapeutic efficacy, and the high prices of this group of drugs compared with conventional drugs. Since 1995, approximately 50 biopharmaceuticals have been registered every 4 years. By the end of 2014, a total 212 biopharmaceuticals were registered and approved in the United States and the European Union.

However, most profits from biopharmaceutical sales are generated by one group of these drugs: monoclonal antibodies.

6.1. Therapeutic antibodies

Since the registration of the first mAb in 1986, the sales of the mAbs have grown every year and in 2016 it reached U.S.$106.9 billion [139]. mAbs sales thus account for nearly 66% of the total sales of biopharmaceuticals (excluding vaccines) (Fig. 3).

This corresponds to a 205% increase since 2008, when the sales of these preparations amounted to nearly U.S.$35 billion. For comparison, the sales of all biopharmaceuticals increased by 102% in this period.

The top 10 best-selling biopharmaceuticals in 2016 included eight Abs (six mAbs and two Fc-based fusion proteins) (Fig. 4). The monoclonal antibody adalimumab (brand name Humira), a TNF-α inhibitor used to treat rheumatoid arthritis and related disorders, ranked first on this list, generating revenue of $16.486 billion.

In addition to mAbs, the most profitable biopharmaceuticals in the group of the top 10 best-selling products include insulin glargine (long-acting basal insulin analogue) and pegfilgrastim (a factor-stimulating granulocyte colony formation)

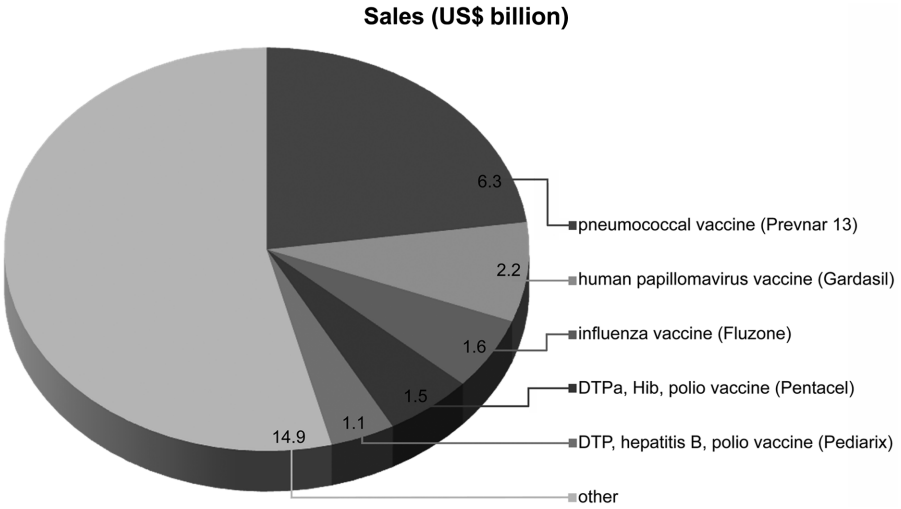

FIG. 2 *Sales (US$ billion) of the five best-selling vaccines in 2015. Prepared based on the data published by EvaluatePharma 2016 [136].*

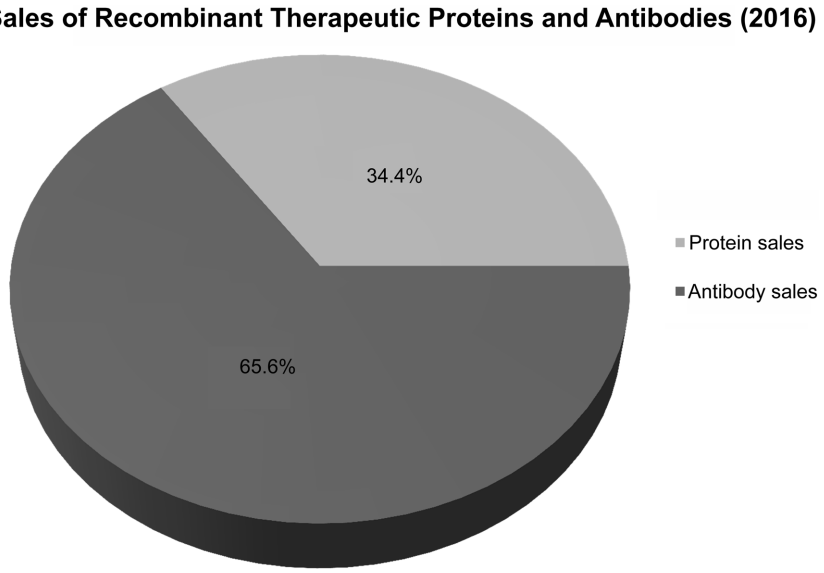

FIG. 3 *The shares of recombinant proteins and mAbs in the world market of therapeutic proteins sales for 2016. Prepared based on the data published by La Merie in 2017 [139].*

(Fig. 3). In 2016 similarly to 2015, these drugs generated total profits of U.S.$11.6 billion [139].

6.2. Biosimilars

Therapy using biopharmaceuticals is costly. For example, in 2009, the annual cost of breast cancer therapy with Herceptin was $37,000 in the United States, whereas that of Gaucher disease therapy with Cerezyme was $200,000 [24]. To reduce the costs of biopharmaceutical treatment, it is crucial to register biosimilars based on the documentation of their biosimilarity. When compared with reference biopharmaceuticals, development of biosimilars reduces time and cost required (Fig. 5). The benefits associated with the introduction of biosimilars include reduced therapy costs, increased availability of the therapy, and, consequently, more balanced health care expenditure [4, 141].

However, the total savings resulting from the administration of biosimilars will not be as significant as that resulting from of the replacement of original synthetic drugs with generics. This is because the manufacture and introduction of biosimilars require considerable outlays. It is estimated that the total cost for the development of a biosimilar drug that meets the formal requirements for its approval, including the cost of manufacturing, could be as high as U.S.$75–250 million [142], and the whole process could take 7–8 years [143]. These are barriers against the introduction of biosimilars on the

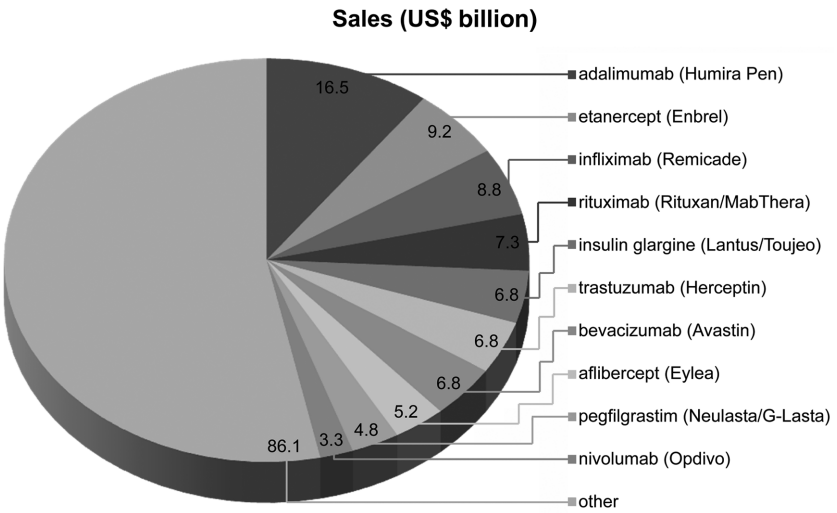

FIG. 4 *Sales (US$ billion) of the 10 best-selling biopharmaceuticals in 2016. Prepared based on the data published by La Merie in 2017 [139].*

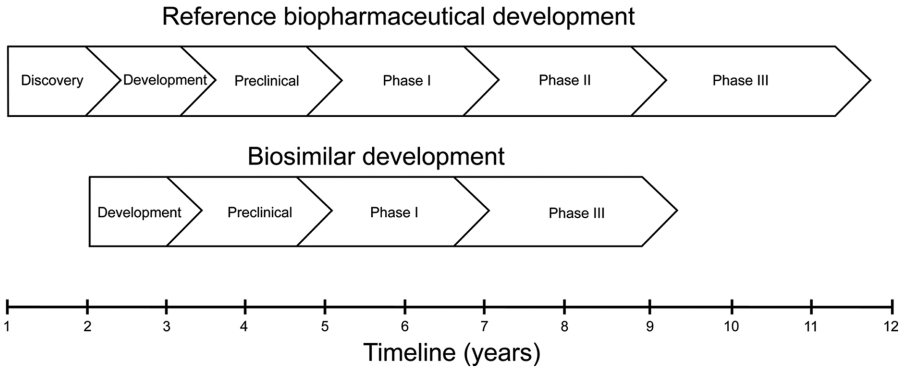

FIG. 5 *Biosimilars and reference biopharmaceuticals development timeline [142, 143].*

market. Additionally, it is difficult for biosimilars to gain access to the market. For example, within two years of its launch on the market, a biosimilar version of erythropoietin gained a 37% share in the European market. For comparison, a typical generic drug gains a 90% share in the market within 1 year of its launch [144]. Despite these problems, according to the data of the European Generic Medicines Agency, biosimilars generated savings of approximately 1.4 billion EUR in the EU in 2009. The median decrease in the market prices of the reference biopharmaceuticals caused by the introduction of biosimilars amounted to 35% (data for 2006–2013). Moreover, it is estimated that in 2007–2020, in eight EU countries (France, Germany, Italy, Poland, Romania, Spain, Sweden, and the UK), biosimilars of erythropoietins, G-CSFs, and monoclonal antibodies will generate between 11.8 and 33.4 billion EUR in savings [145].

However, it must be emphasized that the greatest numbers of biosimilar registrations in the EU were recorded in 2006–2008. Since then, there has been a decrease in the number of registered drugs of this group [51]. The lower-than-expected number of registered preparations may have resulted, among other factors, from the scope of the data that must be presented for biosimilar approval. This scope is closer to that required for the registration of an innovative drug, rather than a conventional generic. Moreover, in accordance with the regulations adopted in most EU countries, pharmacists are not allowed to automatically replace biopharmaceuticals with biosimilars. The reduction in the number of registered biosimilars may also, at least partially, result from distrust on the parts of both patients and doctors [149]. Therefore, the success of biosimilars will depend not only on the quality of the preparations but also on developing the trust of doctors and patients.

6.3. Biosimilar market forecast

According to Allied Market Research, the revenue of the world biosimilar market will increase from U.S.$2.55 billion in 2014 to U.S.$26.55 billion in 2020, growing at a CAGR of 49.1% from 2015 to 2020 [147]. This increase in the market value will be influenced by the sales of biosimilars, which will likely

occur after the expiration of the patents for the reference drugs that currently bring the highest profits, among other factors.

7. Future Prospects for Biopharmaceuticals

In recent years, the biopharmaceutical market has been developing at a faster rate than the market for all drugs. According to analysts, this market will continue to grow. The recently observed and anticipated steady increases in the sales of biopharmaceuticals are associated, among other factors, with the growth of the elderly population and the consequent increase in the number of chronic diseases, the growing number of diabetes and cancer patients, and an increase in the incidence of autoimmune diseases. Insight into the mechanisms underlying various medical conditions has facilitated the identification of specific factors and processes triggering the pathological changes [148]. This has inspired continued research on the applicability of biopharmaceuticals in new clinical situations.

The confirmed efficacy of biopharmaceutical drugs and their acceptance as therapeutic solutions by doctors and patients all contribute to the growing demand for new biopharmaceuticals. One advantage of their application is that they offer targeted therapies rather than symptomatic treatment [146].

In many cases, they have facilitated the treatment of previously incurable diseases.

The ability to produce proteins with properties superior to those of native proteins has played a major role in this regard. The growth rate of the biopharmaceuticals market may be significantly influenced by the development of molecular biology methods and their automation, increases in the knowledge about expression systems, and better understanding of the operational processes and technological factors related to the scale-up of recombinant protein production. The highly promising prospects of the biopharmaceutical market are related to breakthrough innovations, such as the development of immunotherapy, antibody–drug conjugates, and gene therapies.

Factors hindering the development of this market include the high costs of implementing the developed biopharmaceuticals.

In the area of novel biopharmaceuticals, we may see a trend in the active pharmaceutical ingredients toward the enhancement of naturally found ones' therapeutic efficacies. Considering the data on the preparations being currently tested in clinical trials, we can expect a steady increase in the numbers of newly registered mAbs and their dominant presence in the biopharmaceutical market. Because the patent protections of many of the best-selling biopharmaceuticals will expire soon, it appears logical to forecast the introduction of a significant number of biosimilars, which will be their equivalents.

Author Contribution

Malgorzata Kesik-Brodacka was responsible for the conception and development of this review article, critically revised the content of the manuscript at all stages, and provided final approval of the submitted version. Malgorzata Kesik-Brodacka is the guarantor for the overall content.

Conflict of Interest

MKB is a coinventor of patents and patent applications pertaining to insulin analogs, avian influenza flu vaccine, and recombinant proteins expression systems.

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