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BIOLOGICS AND BIOSIMILARS- A MEDICINE FROM THE LIFE AND FOR THE LIFE: THE COMPLETE REVIEW

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ABSTRACT: In this review, we are completely discussing different eras in pharmaceutical drugs/medicines, *i.e.*, Biologics and biosimilars. These biologics can be called life drugs because they are deriving from living organisms, microbes, yeast, bacteria, and human blood products. These biologics are biopharmaceutical products that are derived from living organisms and are very complex in molecular structure, and do have very high molecular weight. The synthesis of these biologics is completely different from the small molecule drugs that are chemically synthesized. These biologics are synthesized by recombinant DNA technology. As these biologics are patented, they won't allow the copying of these products. But they are now expiring their patents and leading their way to the development of their close copies, *i.e.*, Biosimilars. Biosimilars are the copies of the biological which has to be proven as highly similar to that of their branded biologics for their efficacy and safety and has to be developed after the expiring of the patent term period of the biologics, like the generic drugs for the branded pharmaceutical drugs. This biologics development costs around >$1Billion and the development of biosimilars costs around $100-200M. Highly similarity of biosimilars to that of their biologics can be determined by the "totality of evidence", which may involve 2-300 analytical tests for the assessment of similarity of biosimilars to the biologics. The approval of these involves the various regulatory authorities and Acts like • European Medicine Agency, Europe • Pathway of Biosimilars Act, 2009, the USA, *etc.*

INTRODUCTION: Biologics, the term which can define by itself, is the word originating from biology (the science of living organisms). In view of pharmaceuticals, these biologics are the class of the medicines/drugs that are derived from the living organisms (plants, animals, microorganisms, yeast, bacteria and also from humans as from the blood products).

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But the medicines that are produced from these plants are not considered biologics. This is because the plants produce a myriad of molecules with a well-established pharmaceutical value that is conventionally termed as “natural products.

Products like penicillin, salicin, quinine, digoxin, and paclitaxel are considered natural products, not biologics. This is because; these natural products have the chance of commercialization that implies their bulk manufacturing using industrial-scale chemical synthesis. Thus they can be considered as medicines that can be chemically synthesized, which cannot be considered as “Biologics”. This is because of these biologics should be derived from

living organisms and cannot be synthesized by means of chemical formulas or modifications. These biologics can be decided based on their two properties like

- Their relative molecular size of API
- Their compositional uniformity

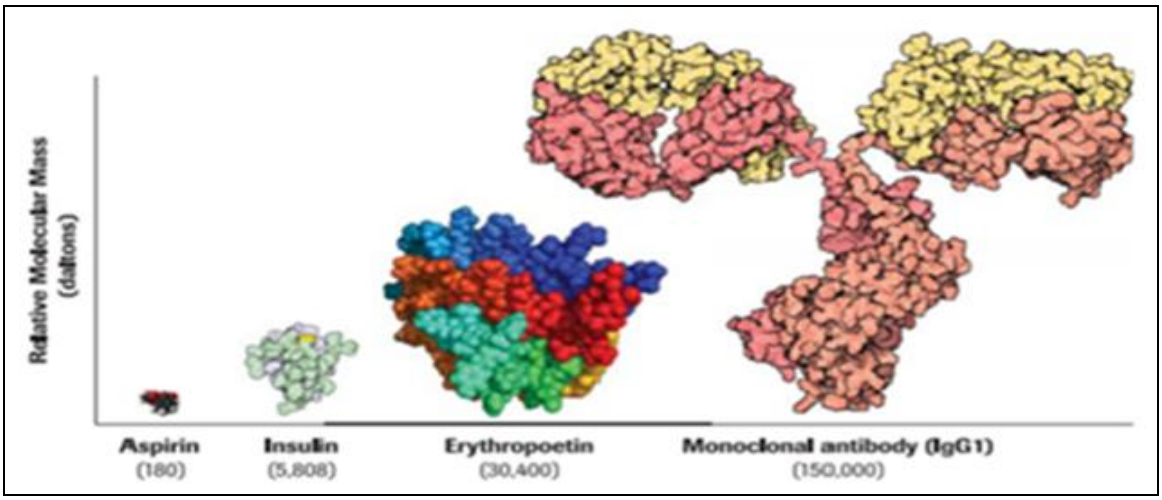


FIG. 1: MOLECULAR SIZE COMPARISON OF ASPIRIN. *relative molecular masses are shown in parentheses. Images molecular of the month, adapted with permission from the RCSB protein data bank website, with attributions to David good sell.

History of Biologics:

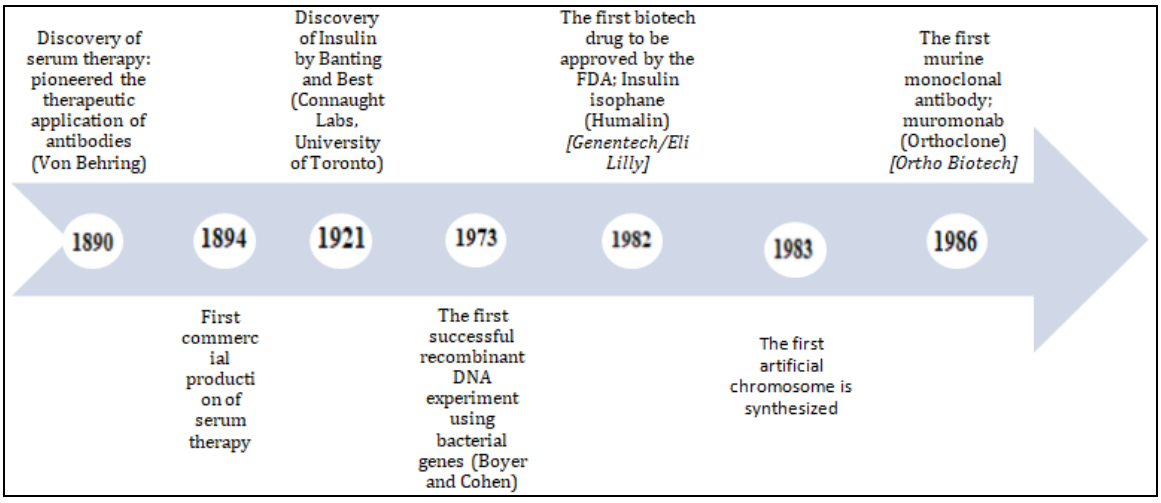
- Biologics are 20th-century medicines. The first biological that is isolated is INSULIN, which is a hormone that is isolated from the islets of Langerhans in the pancreas, by Frederick Banting and Charles Best in Toronto in 1921.

  They isolated this hormone from the pancreatic drugs of dogs and were then used in inborn insulin deficiency patients (*i.e.*, type-1 diabetes).

- Later on, in 1973, Herbert Boyer and Stanley Cohen and colleagues described the advent of recombinant DNA technology in the production of insulin.

  This breakthrough attracted venture capitalist Robert Swanson and ameeting was held between them, leading to the establishment of the world's first biotechnology company, Genentech.

- Using their breakthrough recombinant DNA technology, Genentech introduced the human insulin gene into bacteria, enabling the isolation of biosynthetic insulin, which was as same as human insulin and no differences were seen between the biosynthetic insulin and human insulin.



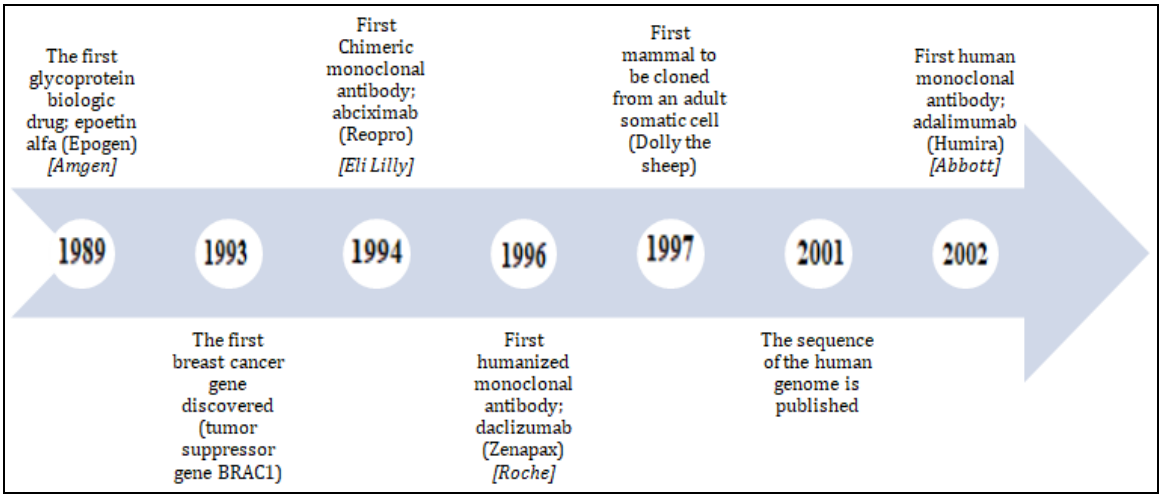


FIG. 2: TIMELINE: BIOLOGICS DEVELOPMENT

Biologics: Biologics can be thus defined as the products that can be a drug or vaccine which are mainly derived from the living organisms either from the microorganisms, animals, or humans. They may be cells or tissues which can be used in transplantation as well. As these biologics are derived from living organisms, they are mainly comprised of proteins, carbohydrates, nucleic acids [1]. Biologics can be produced by means of biotechnology (that is either recombinant DNA technology, controlled gene expression or antibody technologies), which involves a recombinant hormones, recombinant vaccines, gene therapy products, cell therapy products. These advancements in biologics production have introduced many new ways in the treatment of cancer, diabetes, anemia, rheumatoid arthritis, and multiple sclerosis. These biologics have a variety of products that include enzymes, vaccine, blood or blood components, monoclonal antibodies, proteins like cytokines, thrombolytic agents which plays an important role in treating different disease conditions in humans [2]. Unlike the chemically synthesized drugs with simple and low molecular structure, these biologics are heterogeneous in their size and structure. They are very complex and large in their molecular structure. For example, Filgrastim, which is a human granulocyte colony-stimulating factor (a relatively small biologic), weighs approximately 18,800 Da and is 100 times larger than the small molecule drug, aspirin, having a molecular weight of 180 Da.2

- Advantages:
- Effective in treating their respective diseases
- Safety data from clinical trials and post-marketing data
- Manufacturing and cell line changes are closely monitored to prevent large changes in drug
- Disadvantages:
- High cost
- Changes in manufacturing and cell culture conditions can lead to batch-to-batch heterogeneity
- No comparative clinical trials to show if batch differences affect efficacy and safety [2].

TABLE 1: DIFFERENCE BETWEEN THE SMALL-MOLECULE DRUGS AND BIOLOGICS

Small molecule drugs	Biologics
Chemically synthesized	Derived from the living organisms
Having low molecular weight and simple structures	Having high molecular weight and complex in nature
Physicochemical properties can be defined clearly	Physicochemical properties cannot be clearly defined because of complexity
Single entity	Heterogeneous mixture
Stable	Sensitive to heat and shear
Often non-antigenic	Usually antigenic
Can have different routes of administration	Generally administered parenterally
Easy to purify and simple processes	It is difficult to purify and lengthy purification process

Not affected by slight changes in production process and environment	Highly susceptible to slight changes in production process and environment
Distribution to any organ/ tissue is possible	Distribution is generally limited to plasma/extracellular fluid
Any contamination can be easily detected and can be avoided and can be removable	High possibility of contamination and detection is harder and removal is often impossible
It Can be stored in room temperature and refrigerator	Biologics and biosimilars frequently require special handling (such as refrigeration) and processing to avoid contamination by microbes or other unwanted substances
Different route of administration	They are usually administered to patients via injection or infused directly into the bloodstream.
The cost is not so high	The cost of specialty drugs, including biologics, can be extremely high
Example	
Aspirin with nine carbon, eight hydrogen and four oxygen atom	Biologic drug contain 6000 carbon, almost 10000 hydrogen atom and about 2000 oxygen atom
Naming	
Brand Name: Dolo 650	Proprietary name – Neupogen
Generic name: Paracetamol	Non-proprietary name - Filgrastim

What makes the Biologic more Complex than the Small-Molecule Drugs: The small-molecule drugs are uncomplicated drug substances having their own, well-defined structure that can be accessed by means of synthetic organic chemistry that provides a way for these compounds to transform in a sequential manner to their well-defined structure. In this, the side products and impurities can be readily identified and can be easily removed by various analytical means. Biologics generally have high molecular weight because of their high number of proteins that are generally linked by means of the peptide linkages. These peptide linkages are responsible for the 3D shape and structure of proteins in those biologics, which are responsible for their pharmaceutical functions. The slight modification or change in that structure may lead to remarkable changes in the biologics and their functions as well. Thus, these biologics are structurally demanding to be prepared effectively by organic synthesis. These biologics need to be maintained at cold chain conditions when working with them. This is because of their structural importance of having proteins that are unstable in the presence of proteases and may also lead to bacterial contamination in the solutions of proteins. Thus, biologics should be carried over in chilled conditions and can be stored and shipped in the form of freeze-dried powdered forms, which can be reconstituted with a suitable solvent while using it, and the reconstituted drug should be used within 24 h.

TABLE 2: RECENTLY APPROVED BIOLOGICS BY FDA [3]

Trade Name	Indications for Use	Approval Date
Luxturna Voretigene Neparvovec	Treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy	12/19/2017
HEPLISAV-B Hepatitis B Vaccine (Recombinant), Adjuvanted	Hepatitis B Vaccine (Recombinant), Adjuvanted is indicated for immunization against infection caused by all known subtypes of hepatitis B virus. Hepatitis B Vaccine (Recombinant), Adjuvanted is approved for use in adults 18 years of age and older	11/9/2017
Fibrin Sealant (Human)	The Fibrin Sealant (Human) is indicated for use as an adjunct to hemostasis for mild to moderate bleeding in adults undergoing surgery when control of bleeding by standard surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical	11/1/2017
Shingrix Zoster Vaccine Recombinant, Adjuvanted)	Prevention of herpes zoster (shingles) in adults aged 50 years and older.	10/20/2017
YESCARTA axicabtagene ciloleucel	Indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy,	10/18/2017
Anti-Human Globulin (Rabbit/Murine	Anti- Human Globulin (Rabbit/Murine Monoclonal) is intended for use in the direct antiglobulin test to detect the *in-vivo* coating of human red blood	10/15/2017

Monoclonal)	cells with IgG and/or C3b and/or C3d components and the indirect antiglobulin test to detect the *in-vitro* coating of human red blood cells with IgG and/or C3b and/or C3d components	
Anti-Human Globulin	A bundled submission for the following 3 products Anti-IgG, -C3d Polyspecific (Rabbit Polyclonal and Murine Monoclonal), product code Z350U - (FDA's name: an Globulin (Rabbit/Murine Monoclonal) Anti-IgG (Rabbit Polyclonal), product code Z356U - (FDA's name: Anti-Human Globulin) Anti-C3d ALBAclone (Murine Monoclonal IgG), product code Z360U - (FDA's name: Anti-Human Globulin (Murine Monoclonal))	9/21/2017

Manufacturing of Biologics: Unlike the small-molecule drugs manufacturing by the use of chemical processes, the biologics are manufactured by using the host cells. In the manufacturing process of these biologics is a sequential method which starts from the selection of DNA sequence that is responsible for the protein in the biologics and cloning of the sequence into the suitable DNA vector, followed by the trans-fection of this DNA expression into a cell that produces the desired quality and quantity of the biologic products. This process can be scaled up using large bioreactor cells for growing these recombinant cells in order to commercialize them.

The purification plays an important role as it clears all the unwanted proteins, substrates that mat degrade the quality of the product, which can be done by using a multi-step down-streaming process. This purified protein (biologics) can be then transferred into a suitable delivery device that is suitable for their transportation, storage and application to the patients [2].

The manufacturing process of these biologics is generally divided into 7 steps. They are:

- Host-cell development,
- Master cell bank establishment,
- Protein production,
- Purification,
- Analysis,
- Formulation and
- Storage and handling

Each step in this process may affect the characteristics of the biologic product.

Host-cell Development: This host cell is used for the production of the desired product by the insertion of the DNA sequence that is responsible for the coding of the desired protein. The type of host cell used here (can be bacteria or eukaryotic) and the exact sequence of genes used can influence product characteristics.

Master Cell Bank Establishment: This master cell bank is unique and it cannot be exactly similar to the other cell bank. This cell the bank can be developed by using elaborate cell screening and selection process.

Protein Production: The cells are then cultured in large bioreactor cells under the specified optimized conditions that produce the desired quality and quantity protein structures.

The type of bioreactor cells used, the components in the solution (like serum, growth factors, nutrients, carbohydrates) the fermentation, and the physical conditions that are maintained in the bioreactor vessel can influence the protein characteristics that alter the biologics action.

Purification: The proteins thus produced should be processed further for its purification, which purifies the desired protein of interest with the related proteins, DNA, and other impurities, which can be done by a series of steps that are designed in such a way that it produces the optimum yield and purity.

Analysis: The purified protein structures are then analysed for its purity, potency, and its uniformity in their sequence. These proteins are analysed in order to check the amino acid sequences, glycosylation patterns, aggregation, isoform profiles, heterogeneity, and potency which can be the reason for any changes in the biologics action in the body.

Formulation, Storage and Handling: Formulated product is then transferred into a suitable delivery device that is suitable for their transportation, storage and application to patients.

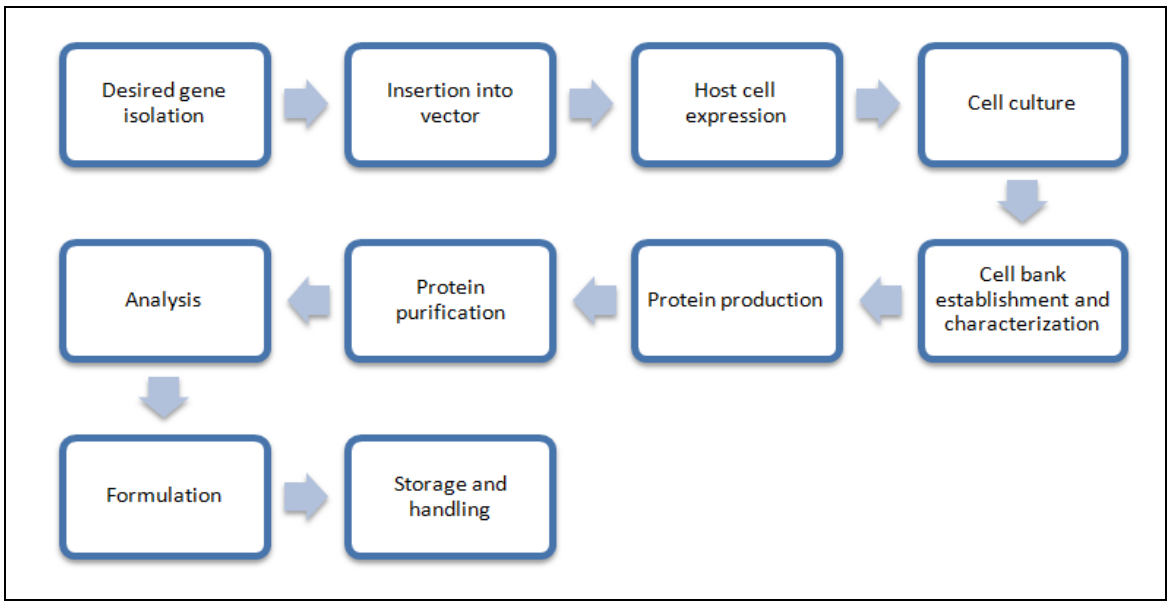


FIG. 3: TYPICAL STEPS INVOLED IN MANUFACTURING OF BIOLOGICS PRICE OF BIOLOGICS

Price of Biologics: The prices of biologics are generally very high. There are a variety of reasons that can explain why these biologics are more expensive.

- The research and development costs are extremely high and E testing and manufacturing process is lengthy and complicated, and there exists an eight-stage developmental process for the development of a new biologic product.
- The chances of success of the selected molecules for developing a new product are extremely low, *i.e.*, one in every 5000 new selected molecules make it through from the discovery stage to the approval stage.
- This process takes a long time, usually 12 years or more, and that costs billions.
- There is a high chance of failure rate in the manufacturing process due to their complex manufacturing process pathway, unlike simple molecule drug manufacturing, which can be synthesized chemically.
- Even their market is small when compared to the small-molecule drugs because they are usually targeted for rare diseases.

These reasons and their parents made the biologics more expensive. But it also creates a way for their generics, *i.e.*, generic biologics, which can be called as BIOSIMILARS. Biosimilars can be generally developed when a patent of the particular biologic is expired; then their formulation data is free to use for other competitors to produce the generic biologics. These biosimilars are copies of the biologics, which can be produced by the same formulation methods used by their reference branded biologics and can be produced in such a way that it should be highly similar to that of their branded biologic [4].

Biosimilars:

- Biosimilars are biological products called highly similar biologics *i.e.*, these are not exactly the copy of their biologic reference product but are highly similar to the reference branded biologic product. This highly similarity could be the same sequence of amino acids sequence in their protein structure as that of their reference biologic product. There should not be any structural difference between these biosimilars with that of the biologics. This can be called as generic biologics *i.e.*, generic versions of biologics as that of the generic drugs in case of small-molecule branded drugs. But in these biosimilars, we cannot expect exact copies of their reference biologic, because even in the biologics there can be chance for slight variations from their batch to batch production.

- Thus these can be considered as highly similar to that of their reference branded biologic.

- Biosimilar can be defined as the drugs that are similar to an approved biologics with regard to its quality, safety, and efficacy. These biosimilars should be demonstrated through the comparison of quality and biological activity as well as nonclinical and clinical study data [5]. Similar but not the same. The twin but not the clone"

Biosimilars can be defined in different ways, Such as:

- **US FDA:** A biosimilar is a biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product.

- **EU EMA:** A biosimilar is a biological medicine highly similar to another already approved biological medicine." •

- **WHO:** Similar biotherapeutic product (SBP). A biotherapeutic product that is similar in terms of quality, safety and efficacy to an already licensed reference biotherapeutic product [6].

- These biologics are now taken very important place in the market because these biologics was running out of their patent and making the path to develop these biosimilars development and market [7].

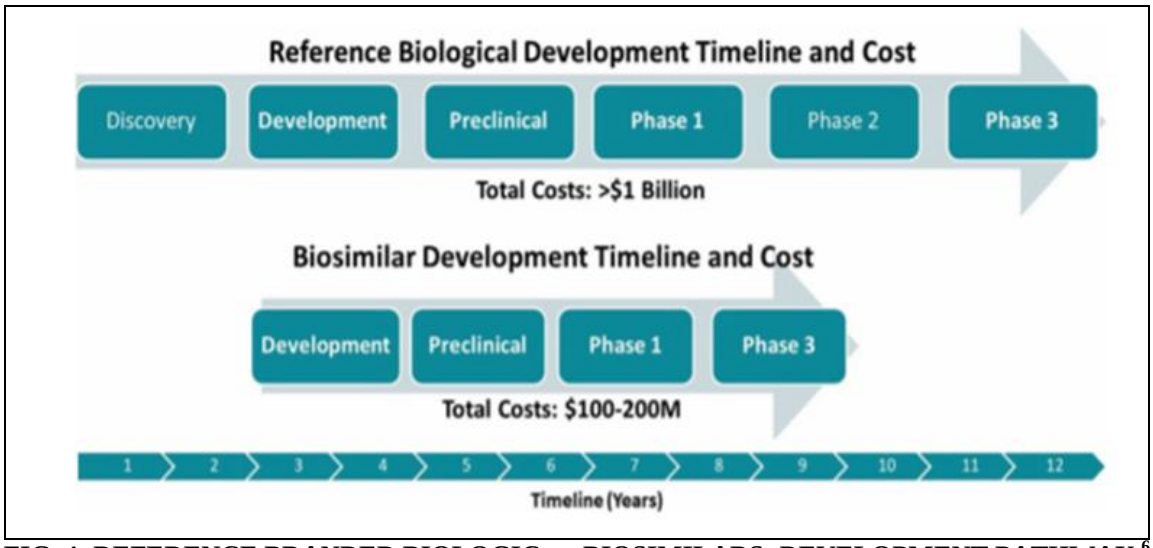


FIG. 4: REFERENCE BRANDED BIOLOGIC *vs.* BIOSIMILARS- DEVELOPMENT PATHWAY [6]

Development of Biosimilars- Target Quality Attributes: Developing the target quality attributes will be the foremost step before developing the biosimilars. These target quality attributes are defined by the characteristics of the reference branded product to which the biosimilar is intended to be highly similar [8].

Main considerations for the resolution of these target quality attributes include:

- An idea of which the quality attributes is important for the biological activity and clinical performance of the protein. Quality attributes for the monoclonal antibodies consist of amino acid sequence, purity, aggregate, charge heterogeneity, higher-order structure, post-translational modifications (including glycosylation profile), and potency/biological activity, but these quality attributes do not only limited to this.

- Target quality control for each mechanism of action should be clearly known, and the mechanism of action for particular indications should be known, if they are having multiple mechanisms of action.

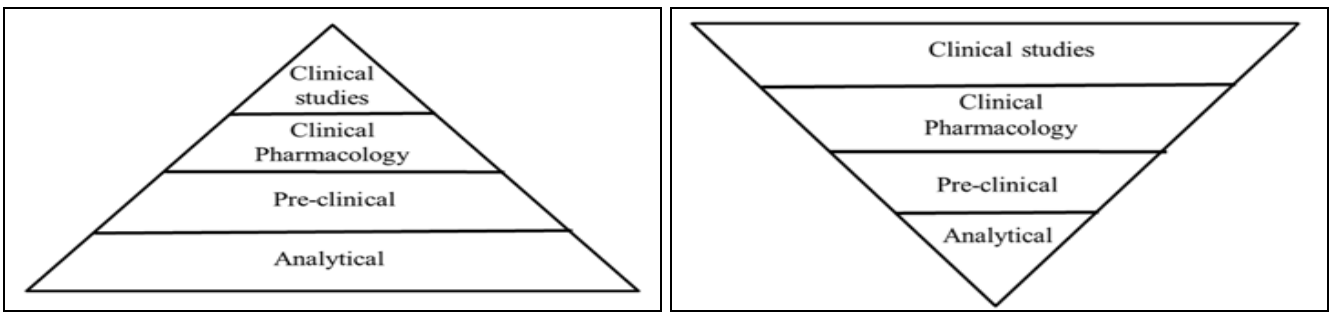
The ranges for the development of biosimilarity should not be confused with those of the manufacturing consistency of a process. For biosimilarity, these ranges can be determined for a biosimilar process using state-of the-art methods

and requirements at the time of process development and stability data collected, according to requirements outlined in ICH Q5C, which produces quality pharmaceutical products.

Regulatory Requirements and Approval of Biosimilars: As these biologics and biosimilars are process dependent products, it is highly difficult to demonstrate the similarity between them, and it would be a complex process that has to be done before getting the market approval as the simple differences may have clinical implications and may pose a significant risk to the patient safety. The analytical tests that are currently available are not sophisticated enough to detect the very small structural differences between the innovator biologic and biosimilar, which may make a vital difference that can affect the characteristics of the product. Therefore these biosimilars must be assessed for their efficacy and safety by valid preclinical and clinical studies. In CANADA, biosimilars are called subsequent entry biologics (SEBs). The approval of these SEBs is still in its fledging, and approving these drugs is complicated by several reasons.

- SEBs are highly similar but not identical.
- SEB manufacturers do not have the innovator's raw drug (*i.e.,* unformulated one), which helps in testing purposes. Thus it is difficult to develop an exactly similar biologic product for the reference and demonstrate the comparability.
- The various analytical tests that are now available are not sophisticated enough to determine the smaller molecular changes in those complex biologics and biosimilar products.

Health Canada published the finalized guidance documents for the approval of SEBs. In April 20, 2009, the first SEB Omnitrope™ was approved in Canada. European Medicine Agency (EMA) had published numerous guidelines to specify the detailed requirements for their market approval and this EMA also produced the product specific guidelines to develop the biosimilars based on the recombinant erythropoietin, somatotropin, human granulocyte colony-stimulating factor, human insulin, recombinant IFN-a and low molecular weight heparins. In the USA, biosimilars are approved based on The Pathway for Biosimilars Act of 2009 and the Patient Protection and Affordable Care Act of 2010. These acts provided a decent way for the US Congress for the FDA to clearly regulate the approval pathway for biosimilars. The approval of biosimilars is the stepwise pathway that starts from the discovery to phase-3 takes almost 12 years. The molecules, after their discovery and preclinical trials, it enters into Phase-1 by applying the Investigational New Drug Application (IND), to evaluate their safety and dosage. After finishing this phase, these molecules go for Phase-2, where the efficacy and side effects are evaluated. This phase is then followed by Phase-3 to evaluate their efficacy and adverse effects in large populations. Biosimilars also follow the same path for their approval, but they have the shortened way like they don't have the discovery phase because they are the copies of the branded biologics and also don't require the initial efficacy required (*i.e.,* Phase-2). This reduces the path development time to 8 years. These biosimilars also needless work when compared to biologics. Thus, this less work and direct progressions from Phase-1 to Pase-3 make this biosimilar at the price of 10-20% of the price of making a new biologic product [6].



BIOSIMILARS **ORIGINAL BIOLOGICS**

FIG. 5: THE CANONICAL DIAGRAM FOR DRUG DEVELOPMENT FOR BIOSIMILARS COMPARED TO ORIGINATOR BIOLOGICS [5, 6]

The Bio Analytical Challenges Involved in Biosimilars: These biosimilars are generally have to be highly similar as that of their original branded biologics. But, it is impossible to produce the exact copies of biosimilars for their original branded drugs, as the biosimilar developers/companies have to reverse engineer the product to develop a process that can produce these highly similar products. It is possible to manufacture the identical imprints of particular small peptides, for which the amino acid sequence has been determined. These small differences in the biosimilars that are produced by recombinant DNA technology may affect the glycosylation and other post-translational modifications, heterogeneity parameters such as C-terminal lysine, or product-related substances and impurities. Thus, the development and manufacturing of this biosimilar should be carefully monitored for any alterations in these compounds, which may result in clinically meaningful differences [8].

Important challenges in the field of biosimilars includes

- Verification of the similarity,
- The interchangeability of biosimilars and innovator products,
- The possible need for unique naming to differentiate the various biopharmaceutical products,
- Regulatory framework,
- Commercial opportunities as well as guidelines to assist manufacturers in product development,
- Intellectual property rights and
- Public safety.

Analytical Similarity Assessment in Biosimilar Studies: The assessment of biosimilarity of biosimilars is one of the major in approving the biosimilars. Thus, for the assessment of a biosimilar's biosimilarity, USFDA recommended a stepwise approach in their recent guidance. This helps in obtaining the totality of evidence for demonstrating biosimilarity between the reference branded biologics and the proposed biosimilar product. Analytical similarity assessment involves identification of structural and functional characteristics of "Critical Quality Attributes (CQAs)". These CQAs are appropriate to clinical outcomes at various steps of the manufacturing process followed by studies in animals for the assessment of toxicity, clinical pharmacology pharmacokinetics (PK) or pharmacodynamics (PD) studies, and which also involves the clinical studies for the assessment of immune-genicity, safety/tolerability, and efficacy [9]. Thus, for the analytical studies, these CQAs are classified into 3 tiers namely,

- **Tier 1:** CQAs that are most relevant to clinical outcomes.
- **Tier 2:** CQAs that are less (mild-to-moderate) relevant.
- **Tier 3:** CQAs that are least relevant to clinical outcomes.

These three tiers were categorized based on mechanism of action (MOA) or Pharmacokinetics using suitable statistical models or methods. The FDA proposes the equivalence tests for CQAs in tier-1, whereas quality range approach for CQAs in tier-2 and raw data graphical presentation for CQAs in tier-3. This proposal are made in order to assist the sponsors in the analytical similarity assessment for the attaining the totality of evidence, which helps in demonstrating the similarity between the reference branded biologics and the proposed biosimilar product [9].

Biosimilars in India: In recent years, the ecosystem and market of biosimilars are becoming more important globally. India has their own development in this biosimilar ecosystem which is much more risen than the other countries, which makes the way to develop more pharmaceutical companies in India [10]. India has their first biosimilar product that was approved much earlier than UAS and Europe. The first biosimilar that has approved in India was Hepatitis-B, which was approved in 2000, even though there was no specific guideline for the approval of the biosimilars [10]. The similar biologics in India are regulated as per the Drug and Cosmetic Act (1940), Drug and Cosmetic Rules, 1945, and Rules for Manufacture, Use Import, Export, and Storage of Hazardous Microorganisms / Genetically

Engineered Organisms or Cells, 1989 (rules, 1989) notified under Environmental (Protection) Act, 1986 [10]. At present, there are nearly 100 Indian pharmaceutical companies involved in the manufacturing of biosimilars.

In India, biosimilars are called "similar biologics", which requires much more data than the normal generics for their approval because there were no such specific guidelines for their approval in early stages. Thus, there occur some challenges in the production of similar biologics, which makes them a quite difficult in approving.

Thus there urges a need for the development of specific guidelines for the approval process. However, in 2012, the Central Drugs Standard Control Organization (CDSCO), in collaboration with the Department of Biotechnology (DBT) has developed "Guidelines on Similar Biologics; Regulatory Requirements for Marketing Authorization in India". This was then amended in 2016 [10]. These guidelines provide the data for the manufacturing of similar biologics and also regulate their quality, safety, and efficacy. It also publishes the pre and post-marketing regulatory requirements for similar biologics [10].

TABLE 3: EXAMPLES OF THE BIOLOGICS THAT ARE APPROVED IN INDIA [10]

Product Name	Active Drug	Indications
Glaritus	Insulin glargine	Diabetes mellitus
Grafeel	Filgrastim	Neutropenia
Epofer	Epoetin alfa	Anemia
Adfar	Adalimumab	Rheumatoid Arthritis, Crohn's disease
Abcixirel	Abciximab	Autoimmune disease
Razumab	Ranibizumab	Wet macular degeneration, macular edema, degenerative myopia
Relipoietin	Epoetin alfa	Anemia, autologous blood transfusion, chronic kidney failure, HIV
Abcixirel	Abciximab	Autoimmune disease

Evaluation of Biologics and Biosimilars: There is no particular analytical technique or non-clinical / clinical study is available for the demonstration of the high similarity of biosimilars with their reference branded biologics on its own; the task of demonstrating molecular equivalence needs a panel.

- **Preclinical Phase:**
  - Analytical studies for structural characterization
  - *In-vitro* studies for functional and immunological characterization
  - Immunological studies
- ***In-vivo* (Nonclinical) Studies:**
  - Clinical phase
  - PK/PD similarity
  - Immunogenicity similarity
  - Efficacy similarity

Preclinical Assessment of Biosimilars: The preclinical assessment generally involves 3 steps, namely [11]

❖ ***In-vitro* Studies:**

- These are designed to get the differences between the biologics and biosimilar products.
- It will be the more sensitive method in detecting the differences and can assess the sufficient batches of product which are intended for use in subsequent clinical trials.
- Assessments include measurement of Target antigen binding, Binding to all Fc gamma receptors, including the neonatal Fc (FcRn) and complement (C1q), Antigen binding (Fab) functions (*i.e.* ligand neutralization, receptor activation and/or blockade), Fc-associated functions *i.e.* complement activation, antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

❖ **Considering Need for Preclinical *In-vivo* Assessment:**

- Here, the various factors that should be considered while determining the need for additional *in-vivo* testing can be detected. They are: Presence of biosimilar attributes that are distinct from the reference biologic product (*e.g.* a new post-translational modification), Presence of any quality attributes that differ in significant amounts between the biosimilar and the reference

biologic product any relevant differences in formulation (*e.g.* different excipients).

- These factors are considered collectively to evaluate the need for additional *in-vivo* testing; each does not necessarily constitute a need for *in-vivo* testing *in-vivo* testing might not be needed if studies in step 1 are satisfactory and no factors of concern are identified If *in-vivo* studies are considered necessary, availability of a relevant animal species must also be considered

❖ **Preclinical *In-vivo* Studies:**

- Depending on the need for additional information, the focus could be on pharmaco-kinetic (PK), Pharmacodynamic (PD), or safety, designed to maximize the information obtained.
- Principles of the 3Rs (animal replacement, refinement, and reduction) should be applied; euthanasia of study animals might not be needed. Justify study duration and observation period based on PK and clinical use of mAb.
- If possible, compare PK and PD quantitatively (*i.e.*, concentration dose assessment) at therapeutic dose.
- Although not predictive of immunogenicity in humans, blood samples can be collected and stored if needed to interpret animal *in-vivo* data.
- If new excipients are used, it might be necessary to evaluate local tolerance.
- Safety pharmacology and reproductive toxicology assessments are not considered relevant for biosimilar mAbs [11].

Immunogenicity: Immunogenicity is a key factor that distinguishes both biopharmaceuticals and biosimilars from low-molecular-weight pharmaceuticals and generic drugs based on their capability of forming an immune response. In general, many biopharmaceuticals don't have any clinical consequences even the antibodies are present for them, but some biopharmaceuticals have antibodies that can be associated with the immune effects (like allergy, anaphylaxis, and serum sickness), a loss of effect, neutralization of the endogenous protein, or, very occasionally, improvement of the activity. So, it is important to evaluate the factors that are responsible for this type of immune response of both the bio-pharmaceuticals and biosimilars. The factors that are responsible for the immune response are majorly classified into two classes, namely.

❖ **Product-Related Factors:**

- Differences in the primary sequence from endogenous protein
- Lack of glycosylation of recombinant proteins that are produced in bacteria can either expose the antigenic sites or decreases the solubility and increase aggregation.
- Hyperglycosylation of recombinant products produced in eukaryotic cells
- Formulation
- Storage and handling, when inappropriate conditions are followed, can induce the denaturation of the protein or may cause the aggregation of them.

❖ **Patient-Related Factors:**

- Route of administration
- Dose and treatment duration
- Depressed immune response
- Congenital deficiency of an endogenous protein

Most biologics are generally approved via a subset of the Public Health Services Act (PHS). The remaining less complex biologics are generally approved *via* the FD & C ACT and their actions will be transported to the PHS Act 351(K) pathway. The biosimilars are approved based on a totality of evidence approach that depends mostly on analytical studies [12].

PHS Act/Biologics Price Competition and Innovation (BCPI) Act of 2009: In recent years, the Biologics Price Competition and Innovation

(BCPI) Act of 2009, which is an amendment to the PHS Act that was subsequently enacted as a part of the Affordable Care Act of 2010. This Affordable Care Act of 2010 creates a path for the abbreviated license for biologics to show biosimilarity, or interchangeable with already FDA-approved reference branded drugs. According to PHS Act, biosimilarity is the way that the biological product is highly similar to the reference product even there exists some small differences in clinically inactive substances, provided there are no clinically meaningful differences between the two products in terms of safety, purity, and potency.

Branded Reference Biologics Approval: It involves the BIOLOGICS License Application (BLA), which includes applicant information, product and manufacturing information, pre-clinical and clinical studies, and labeling. This BLA pathway was the only pathway for the approval o these biologics and biosimilars. But later on, the 351(K) biosimilar pathway was introduced to approve the biosimilars [12].

Biosimilar Pathway: FDA stated that the foundation for the approval of biosimilars is the analytical comparison of these biosimilars with that of their reference branded biologics and that comparison would take place on the basis of the "totality of evidence".

The totality of evidence is the approach that is used by European Medicine Agencies (EMA), which has been approving the biosimilars since 2006 [12].

Totality of Evidence: This is the submission that is generally done in order to compare the analytical studies of both the biosimilar and their original branded biologic product. The company itself must undertake the process, and the risk of getting the reference biologic product should be taken care of by itself and characterization of that reference product to define its target quality attributes, and it should be set standards for the comparison of biosimilar. After making the biosimilar, it must be characterized like the biologics, and both then the products are compared. Generally, 2-300 analytical tests are used in extensively. These tests may assess the structure and post-translational changes (amino acid analysis, LC-MS peptide mapping, Edman degradation, FTIR, circular dichroism, X-ray crystallography), presence of aggregates (size exclusion chromatography), product purity (capillary gel electrophoresis, SEC, LC-MS), charged variants (iso-electric focusing, ion-exchange chromatography), function and bioactivity (binding assays, antibody-dependent cell-mediated cytotoxicity studies, and cell-based bioactivity assays), and thermal stability (DSC). Each quality attribute should be assessed by multiple analytical methods and some analytical methods may be appropriate for the assessment of multiple quality attributes. The primary structure of the potential biosimilar must be identical to that of the reference product, which makes use of multiple mapping techniques critical [12].

Economics of Biosimilar: Even though the market of these biologics is small, their sales are pretty much high because of their cost and effective nature. The sales of several biologics are more than $1 billion annually. For example, in 2011, Remicade (infliximab) had their global sales of almost $7.19 billion, whereas Avastin (bevacizumab) had their global sales of $5.98 billion.

Even the biosimilar development needs an investment of $1 million to $4million, which is far higher than the generic drugs market. It takes 7 to 8 years to develop a biosimilar, at the cost of between $100 million and $250 million.

There were only 16 biosimilars that have been approved, which comes under 3 classes (human growth factor, short-acting erythropoietin, and daily granulocyte colony-stimulating factor (GCSF). But these 3 classes' biosimilars occupy almost 11% of the total patient volume and approximately 18% of all biologic sales.

Barriers to Market Entry: Biosimilars will somehow face struggles for entering into the market in their efforts to compete with biologics. Specifically, biosimilars have to overcome the particular barriers that are associated with manufacturing, marketing, storage (cold) and other distribution issues, delivery devices, immunogenicity (*i.e.,* patient adverse reactions because of live organisms) and special requirements for pharmacovigilance (*i.e.,* post-sale monitoring).

The major barriers that are encountered by the biosimilars when they are trying to compete with the branded biologic in the market are:

- Complexity of Expertise
- Lack of Automatic Substitution
- Clinical Trials

CONCLUSION: Biologics are the biopharmaceutical products that are generally produced from living organisms, which are very having a very complex molecular structure and high molecular weight. These are generally produced by means of recombinant DNA technology using a host cell. They cannot be easily demonstrated, unlike the small-molecule drugs, because of their complex molecular structure, which can be made up of proteins in 3d manner. The biologics are generally manufactured in a sequential process which starts from the selection of DNA sequence that is responsible for the protein in the biologics and cloning of the sequence into the suitable DNA vector, followed by the transfection of this DNA expression into a cell that produces the desired quality and quantity of the biologic products.

This biologics are highly expensive but has a very small market share because it generally targets the rare treatment of rare disease conditions. Thus, their sales are very high even though their market share is less. As these biologics are patented, they are not allowed to copy by the others until the patent term is over. Thus, after the patent term is finished, they are making a pathway for the development of their duplicates, *i.e.*, their generics, which are called as biosimilars. These biosimilars are not the exact copies of their branded biologics but are highly similar to them. They are produced in the same way as biologics are produced, but the exact copies are not possible because of their highly complex structure. Their similarity can be determined by evaluating the primary amino acid sequence in both the biosimilars and the biologics. The FDA, EMA approve these biologics and biosimilars and in India by the specific guidelines that are developed in their own nation and should be complied with all other guidelines if they are marketing globally.

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REFERENCES:

1. Johnson JA: Specialist in Biomedical Policy, Biologics and Biosimilars: Background and key issues, congressional research service, 2017 (www.crs.gov)
2. Patel PK, King CR and Feldman SR: Biologics and biosimilars. J of Dermato Treatment 2015; 26(4): 299-02.
3. www.fda.gov
4. 4.https://www.health24.com/Natural/News/why-are-biologics-so-expensive-20170623
5. Al NAA, Gorial FI and Al-Sulaitti S: Review of biologics, biosimilars and intended copies in rheumatology, and current practice in Iraq, Dovepress Journal https://dx.doi. org/10.2147/OARRR.S176965
6. Agbogbo FK and Ecker DM: Current perspectives on biosimilars. Journal of Industrial Microbiology & Biotechnology 2019; 46: 1297-11.
7. Chan JCN and Chan ATC: Biologics and biosimilars: what, why and how? ESMO Open 2017; 2: e000180.
8. Ahmad AL-Sabbagh MD, Ewa Olech MD and Joseph E: McClellan PhD, MBA, Carol F. Kirchhoff PhD, Development of Biosimilars, Seminars in Arthritis and Rheumatism http://dx.doi.org/10.1016/j. semarthrit.2016. 01.002
9. Shein-Chung C and Song F: Analytical Similarity Assessment in Biosimilar Studies, The AAPS Journal 18(3), May 2016, DOI: 10.1208/s12248-016-9882-5
10. Meher BR, Balan S, Mohanty RR, Jena M and Das S: Biosimilars in India; current status and future perspectives. J Pharm Bioall Sci 2019; 11: 12-5.
11. Bui LA: Key considerations in the preclinical development of biosimilars, Drug Discovery Today Volume 00, Number 00 April 2015, http://dx.doi.org/10.1016/j.drudis. 2015.03.011
12. Nagel KM: Introduction to Biologic and Biosimilar Product Development and Analysis, AAPS Introductions in the Pharmaceutical Sciences, https://doi.org/10.1007/ 978-3-319-98428-5_4
13. Blandizzi C and Meroni PL: Comparing originator biologics and biosimilars: a review of the relevant issues, Elsevier HS Journals, http://dx.doi.org/10.1016/j. clinthera. 2017.03.014

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