ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 8, 2024

Online - 2455-3891
Print - 0974-2441

Review Article

PHARMACEUTICAL AND CLINICAL CHALLENGES OF BIOLOGICAL MEDICINES: ONGOING HURDLES FROM DRUG DEVELOPMENT TO THERAPEUTIC APPLICATIONS

SAYON PAUL[1]*, AVIK SARKAR[2]

[1]Department of Pharmacy, M.J.N. Medical College and Hospital, Coochbehar, West Bengal, India. [2]Department of Pharmacy, ESIC Medical College and Hospital, Kolkata, West Bengal, India.
*Corresponding author: Sayon Paul; Email: sayon808paul@gmail.com

Received: 12 May 2024, Revised and Accepted: 24 June 2024

ABSTRACT

Beyond conventional small drug molecules mostly of synthetic origin, clinical benefits have been well established by administering large complex biomolecules against different diseases including cancer, metabolic disorders, and infectious diseases. From insulin, its different derivatives and dosage forms to cutting-edge messenger ribonucleic acid (mRNA) based vaccines, stem cells, immunotherapy with chimeric antigen receptor T cells for cancer likewise offered novel, pivotal pathways in healthcare and helped in alleviating the corresponding diseases, maintaining the overall quality of life of patients. However, the lifecycle management of these biopharmaceuticals offers stern challenges, namely, the structural complexity of biomedicines impacting drug discovery and formulation development, multifaceted manufacturing processes involving living systems (e.g., mammalian cell lines, microbial agents, plants, fungi, etc.), temperature and humidity sensitive supply chain management, stringent regulatory requirements, invasive drug delivery approaches, monitoring immunogenicity after drug administration, etc. Animal and clinical testing of the biologics are also very challenging. Novel biopharmaceuticals including cell-based medicines, recombinant products, gene therapy products, etc. often face ethical and higher cost-related issues. Proper alignment of regulatory guidelines, innovative bioinformatics, and software-based drug discovery tools, implementation of quality by design approaches to identify critical quality parameters at the drug developmental phase, the suitable training to health-care professionals on usage, safety, immunogenicity, handling and storage of biopharmaceuticals would bestow clinical benefits of biopharmaceuticals to the desired patients. Continual research is going on to market new biopharmaceuticals in a cost-effective manner for difficult-to-treat terminal diseases preferably through peroral administration.

Keywords: Biopharmaceuticals, Structural complexity, Formulation development, Regulatory compliance, Quality by design, Immunogenicity, Bioethics.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i8.51398. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

As per the United States Food and Drug Administration (USFDA), biopharmaceuticals signify the array of biological medicinal products including vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins. Biologics mostly consist of sugars, proteins, nucleic acids moiety or complex combinations of these substances, or can be living entities such as cells and tissues that are isolated from diverse sources of natural origin – human, animal, or microorganism – and can be carefully processed by biotechnology methods and other cutting-edge technologies to obtain desired products of medicinal significance (Table 1). Gene-based and cellular biologics often generate avid interest among scientists, and hence, they are at the forefront of biopharmaceutical research, and they can be clinically utilized to treat a variety of terminal diseases having no other treatment options currently available. Most synthetic drugs are small molecules, a molecule of acetylsalicylic acid is composed of 21 atoms. However, biopharmaceuticals are typically 100–1000 times larger. A biopharmaceutical may contain an active pharmaceutical ingredient of 2000–25,000 atoms. Biopharmaceuticals are also structurally much more complex because of the formation of polymeric chains, which vary greatly in their structure [1,2]. Using recombinant DNA technology, human insulin, became the first biopharmaceutical approved by the US FDA and was introduced in the market in 1982 [2]. The first therapeutic monoclonal antibody awarded USFDA approval for human use was muromonab-CD3 in 1986 for the treatment of acute transplant rejection [3]. Biopharmaceuticals deliver improved specificity, potency, and targeting ability than conventional therapeutic agents [2]. Hence, considerable focus is being imparted on biologicals as active pharmaceutical ingredients to manage, control, and eradicate diseases. A survey conducted between January 2018 and June 2022 in the United States and/or European Union (EU) witnessed the approval of 180 distinct biopharmaceutical active ingredients that entered the market and they could be categorized as recombinant clotting factors; recombinant thrombolytics, anticoagulants, and other blood-related products; recombinant hormones; recombinant growth factors; recombinant interferons, interleukins, and tumor necrosis factor (TNF); vaccines; monoclonal-antibody-based products; and other recombinant products. These biologicals are mostly focused on cancer, followed by inflammation-related conditions, neutropenia, diabetes, viral and bacterial infection, weight control, weight loss (Wegovy), and Alzheimer's disease (Aduhelm). The genre of monoclonal antibodies continues to lead in the number of approvals and sales (Fig. 1), although COVID-19 vaccines are listed as the highest-grossing individual products [4]. Despite continual therapeutic attention, structural complexity and the larger molecular size of biopharmaceuticals add up to difficulty in drug design and formulation development. Hence, subsequent manufacturing and distribution of biopharmaceuticals also face challenges including their storage which often demands refrigeration. Further, regulatory compliance in biopharmaceutical manufacturing is one of the toughest hurdles that often accompany facility inspection. Considering the complex multi-layered manufacturing process and critical in-process quality controls and analysis of finished products, it becomes a challenge for manufacturers to successfully comply with the norms of regulatory science and good manufacturing practices. Beyond pharmaceutical considerations, the clinical administration of biologics also demands attention, especially for immunogenic reactions that need to be monitored in patients following the administration of the biopharmaceuticals.

Considering the diverse nature of biologics, the following common terminologies of biological drugs are discussed in short for thorough understanding.

Biosimilars and interchangeable biosimilars

As per USFDA, a biosimilar can be categorized as a biopharmaceutical that is extremely similar to an existing approved biologic reference product with no clinically meaningful differences from the approved product. Biosimilars are produced with the same types of living sources, are given to the patient in the same way, and have the same strength, dosage, potential treatment benefits, and potential side effects with respect to their corresponding reference product. A biosimilar can be provided to patients who have previously been treated with the reference product (treatment-experienced), as well as in patients who have not previously been treated with the reference product (treatment-naïve). An interchangeable biosimilar is a biosimilar that meets additional federal requirements and a pharmacist may substitute an interchangeable biosimilar for its reference product without consulting the prescriber, depending on state pharmacy laws. USFDA does not evaluate or approve a biosimilar as interchangeable unless a company requests it. To assess the safety of switching, manufacturers generally carry out clinical studies where patients alternate between the interchangeable biosimilar and the reference product and subsequently evaluate for equivalency in clinical response. The results should establish no reduction in efficacy or upsurge in safety risk associated with switching. Regarding interchangeability between biosimilars, the European Medicines Agency (EMA) and the Heads of Medicines Agencies of the EU have issued a joint statement confirming that biosimilar medicine authorized in the region of the EU is interchangeable with the reference medicine or with an equivalent biosimilar medicine. Decisions regarding substitution at the pharmacy-level without consulting the prescriber are monitored by individual member states. The first biosimilar, Omnitrope (human growth hormone from Sandoz), was approved in 2006 by both the USFDA and EMA. However, biosimilars are different than generics. Generics are categorized as groups of drugs that are equivalent to innovative reference drugs, mostly synthetic, containing the same active pharmaceutical ingredient. The production of generic preparation containing an exact copy of the active pharmaceutical ingredient is relatively rapid, simple, and inexpensive [5]. Instead, biopharmaceuticals are practically impossible to duplicate completely, even if the expression systems used in their production are identical (e.g., mammalian cells or bacteria). Biosimilars can potentially vary from the innovative reference drugs in their glycosylation pattern or the electrical potential of the active pharmaceutical ingredient resulting in differences influencing the quality, strength, and safety of the drug [6,7]. Exceptional progress in bioproduction and analytical methods enabled the production of biosimilars such as proteins and glycoproteins that are comparable to the reference product [8]. For a biosimilar, equivalency of physicochemical, biological, and functional characteristics as well as efficacy and safety/immunogenicity with the approved reference product must be established [9].

Bio-betters

The term "bio-better" was first depicted in 2007 by G.V. Prasad, CEO of Dr. Reddy's Laboratories, at a conference of bio-investors in Mumbai, India. It has been widely portrayed since then but yet is not a defined term. It generally means a biologic that is better or superior to the reference molecule in one or more parameters while having a similar target [10]. They have been structurally and/or functionally reformed to attain an improved or different clinical performance, compared to approve reference products [11]. However, they are evaluated as new drugs, in a standard approval procedure [5]. Development of bio-betters involves the process in which molecules such as proteins are purposefully altered as equivalents of existing drugs to improve stronger clinical effects, require less frequent administration, achieve better targeting, and/or be better tolerated compared with their equivalents [12]. Although the term was introduced later modification of biological drugs for better therapeutic outcomes was initiated a few years back. Going by its terminology, it can be interpolated that the first bio-better to receive FDA approval, in 1996, was a fast-acting insulin analog, insulin lispro, produced by altering the amino acid sequence at two positions [5,13]. Another bio-better molecule is an antibody-drug conjugate ado-trastuzumab emtansine (brand name Kadcyla from Roche) which is a better therapeutic equivalent of the trastuzumab antibody (brand name Herceptin, Roche). Kadcyla slows down the progression of HER2-positive advanced breast cancer almost twofold in patients with an improved median total survival time of 5.8 months relative to other treatment methods [14,15].

Value-added medicines (VAM)

As per the updated case studies report on January 2019 for value-added medicines by IQVIA consulting services, VAMs are medications where innovations are applied to off-patent molecules, offering enhanced value to patients and all relevant health-care stakeholders such as new therapeutic use, improved efficacy, better safety and/or tolerability profiles, more convenient route of administration, and/or ease of use [16]. These improvements contribute to the sustainability of health-care systems through better adherence, improved safety, better efficiency of health-care professional resources, and improved cost-effectiveness among others. VAM can be of different origins, for eample, chemical, botanical, or biological likewise. Broadly value-added medicines can be categorized into three groups: (a) Repositioning (new indication), (b) reformulation (change in formulation, strength, and route of administration), and (c) combination (two or more products/offerings into one product, that is, medicine/medicine, medicine/device, and medicine/service) [16]. An example of VAM is by repurposing (new indication) of biological peptide-based anti-diabetic medication, an analog of Glucagon-like peptide (GLP-1), exenatide for Parkinson's disease. Initial data from the randomized, double-blind, placebo-controlled, single-center trial for Parkinson's disease administering exenatide (2 mg) by self-injection (s.c. route) once weekly, showed that patients underwent such treatment were improved compared to the placebo arm with regard to their performance in part 3 of Movement Disorders Society-Unified Parkinson's Disease Rating Scale motor subscale [17]. Parallel group multicenter phase 3, double-blind, randomized, and placebo-controlled trial is ongoing with exenatide to further study details of its effects on Parkinson's disease [18].

The systematic review of different challenges faced by biomedicines was carried out by analyzing the web-based literature repertoires from various databases such as the United States National Library of Medicine, and Embase. Various publicly available documents from the different ministries of health, and international regulatory affairs organizations such as the International Council for Harmonisation (ICH) were also rigorously assessed and extracted the necessary information. The initial literature search was done by applying a set of keywords based on the subject matter. After primary information collection, relevant data were evaluated and considered, and the rest of, the out-of-scope, data were excluded from the study. Now based on the study of primary information, wherever needed, detailed updated information was gathered by a specific terminology-based literature search. These literatures were then further screened following exclusion criteria such as duplicity and non-English papers, and the rest are considered for exclusive review. Finally, relevant updated information gathered from diverse resources (from databases, e-books to biopharma websites, etc.), was thoroughly assessed for the review article. The relevant information was cited accordingly.

In the following sections, we will enlighten the current challenges of biopharmaceuticals impacting drug/formulation development, manufacturing, supply chain management, regulatory hurdles, and also the challenges related to the clinical administration of biological medicines (Fig. 2).

PHARMACEUTICAL CHALLENGES OF BIOMEDICINES

Complexity in molecular structures and drug discovery

Biologics mostly are large complex molecules/combinations that are not easily identified or characterized. Hence, drug discovery and analytical

method development and its validation offer immense challenges to the research scientists. The molecular weights of biological drugs range from a few kDa to 1000 kDa [19] (Fig. 3). For example, monoclonal antibodies have a higher molecular weight (~150 kDa) and complex secondary and tertiary structures that can undergo post-translational modifications [20]. Biologics are often produced from bacteria, yeast, insects, plants, fungi, or mammalian cells engineered with the gene of interest involving complex processes such as protein purification, gene isolation, and recombinant DNA technology [19,21]. The secondary, tertiary, and quaternary structures of biologics such as therapeutic proteins affect the efficacy and safety of the biopharmaceuticals. Factors that influence the functionality of biologics include but not limited to protein folding, denaturation, amino acid substitution, deamidation, N- and C-terminal modifications, protein aggregation, oxidation, O/N-linked glycosylation, truncation, phosphorylation, sulphation, PEGylation, carbamylation/carboxylation/acetylation, multimer dissociation, mismatched S-S bonds, fatty acylation, gamma-carboxyglutamylation, formylation, and methylation [19]. Biological complex molecules require special formulations including stabilizers and preservatives for storage [21]. Because biologics are often heterogeneous in molecular structure, they possess an impurity profile that depends on and may vary with the processes used to manufacture and test each batch [22]. Further recombinant proteins such as growth factors possess short half-life and predictive biomarkers of efficacy are absent/limited for monoclonal antibodies [23]. *In vivo*, the clinical behavior of biological molecules also demands further attention. Poor bioavailability of orally administered biologics is observed due to their restricted penetration across the intestinal epithelium and susceptibility to enzymatic degradation by proteases and peptidases in the intestinal lumen [24]. The limited solubility of antibodies in solution and limitations on the volume of fluid that may be tolerated with i.m. or s.c. injection, these routes are feasible only for antibodies that demonstrate relatively high dose potency [25]. Blood capillaries permit diffusion of biologics with molecular weight <16 kDa to reach systemic circulation from i.m., s.c. injection sites, larger biomolecules enter systemic circulation through lymphatic drainage [24]. Due to the large size and polarity of monoclonal antibodies, diffusion across vascular endothelial cells is very slow and diffusion in tissues by the convective transport through paracellular pores in vascular endothelial cell membranes is thought to be the principal mechanism for the transport of biologicals such as monoclonal antibodies from blood to interstitial fluids of tissue [24,25]. For monoclonal antibodies and other large therapeutic proteins, the reported volume of distribution after IV administration is close to the plasma volume, suggesting limited distribution into tissues [26]. The biopharmaceuticals with molecular weights <69 kDa are principally cleared by renal excretion. Therefore, the clearance of these biologics can be compromised in patients with renal impairment [19]. In gene therapy, it is not possible to predetermine the exact dose, also some diseases are polygenic and multifactorial such as cardiac diseases and gene therapy suffers from unknown kinetics and dynamics as well. Similarly, cell therapy demands monitoring of stem cell engraftment, also precise mechanism of action could remain undefined for cell-based therapies [23]. Pharmacokinetic characteristics of biotherapeutics are often complex, involving linear and non-linear processes. Similarly, pharmacodynamic characteristics of biologics are also complex involving multiple functional features of the molecular structure, such as Fab (fragment antigen-binding) region of monoclonal antibody (mAb) for target engagement, FcgR (fragment crystallizable gamma receptor) binding site for antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity, receptor internalization, and so on [27]. Hence, such complex molecular structures of biopharmaceuticals offer immense challenges to selecting ideal biological drug candidates showing optimum therapeutic action in a validated manner. Bioinformatics and related software-based drug delivery tools are currently being utilized to design biomedicines, hence resulting in a precise number of lead molecules on which physicochemical experiments can be carried out for optimization.

Hurdles to formulation development

The formulation development process can, in general, be compartmentalized broadly into three different but interconnected stages, namely, pre-formulation, formulation, and process development [28]. Formulation development also includes the analytical characterization of the biological molecules, where manufacturers develop assays to test specific physical and quality attributes of their biologics. These tests characterize the complex molecular structure such as protein sequence, peptide map, protein folding, charge heterogeneity, and post-translational modifications, while *in vitro* efficacy assays explore the mechanism of action [29]. Manufacturability also plays a role when defining final formulations, because the product eventually needs to be manufactured at a large and commercially viable scale. For example, filtration using very low pore size filters can be effortlessly performed in the laboratory, but low-volume throughput and the costs of industry-sized filter systems might make such implementation impractical on a large production scale [30].

The following parameters impart challenges in the formulation development of biologics:

Different therapeutic modalities of biologics

Biological therapeutics encompass an array of different modalities such as protein therapeutics (growth factors, monoclonal antibodies, etc.), nucleic acids (e.g., DNA, mRNA, synthetic oligonucleotides, etc.), cell, tissue, and gene-based therapeutics (e.g., gene delivery vectors/viruses, transgenic cells, stem cells, engineered tissues, etc.). Protein therapeutics is large and complex 3D structures requiring proper folding and conformational stability for efficient functionality. Developing stable formulations with these fragile protein/peptide biomolecules adds more criticality [31]. Intrinsic properties of protein-based drugs such as large size, poor membrane permeation, physicochemical instability, and susceptibility to enzymatic degradation create a formidable challenge for the delivery of protein drugs, particularly for non-invasive drug delivery [32]. The surface charge of therapeutic proteins, derived from amino acid sequences of the proteins and the pH of their surroundings, can cause protein drugs to interact with molecules on the cell surfaces or tissue components, thereby affecting the absorption, distribution, and elimination of proteins in the body [32]. A noticeable liability feature of nucleic acids is their high vulnerability to *in vivo* degradation from enzymatic attacks by nuclease, which often necessitate chemical modifications of their backbone to prolong their stability, extend their circulation half-life, and improve their transcription and translation efficiency. Critical features of cell-based therapeutics include their extremely sensitive architecture, pleomorphic shape, and surface-expressed antigens and they are also costlier biopharmaceuticals [31].

Excipients selection

Excipients used in biological medicines are broadly categorized by function as pH modifiers, tonicity agents, bulking agents, wetting and/or solubilizing agents, antioxidants, antimicrobial preservatives, chelating, and/or complexing agents [33]. It is a challenging job for formulation scientists to combine the right excipients in the correct concentration, because a stabilizing excipient potentially displays a destabilizing effect on a different protein instability pathway, and excipients also potentially impact each other's action. For instance, polysorbates are added for protection against interface-related protein aggregation and may contain oxidizing species, which may promote chemical instability. The most commonly used excipient water for injection is a natural solvent for proteins. Still, it may mediate possible protein degradation reactions, which is the reason why many biological products are lyophilized to reduce the water content to minimal amounts [30,34]. Impurities within excipients, such as trace metal ions, hydroperoxides, and bacterial endotoxins can impact the stability and potency of the biologic medicines [31]. Care should be taken to consider the impact on protein stability, osmolality, and pH alteration

while selecting excipients, especially during the development of high-concentration biologic formulation [35]. Biological drug substances are inherently more unstable than small molecules. In addition, the quantity of available drug substances may be limited, especially during early development. This puts additional challenges to formulation development scientists to depend heavily on forced degradation studies using limited analytical techniques which may often result in suboptimal formulation development, ultimately leading to stability and manufacturing challenges during commercial manufacturing. The development of high-concentration antibody formulations demands further attention due to additional constraints of viscosity at high concentration, analytical characterization, and isotonicity of formulation while maintaining the ratio of excipients to antibodies [36]. Significant considerations are to be imparted on processes/techniques used in developmental study and the intrinsic nature of excipients. Certain excipients, which can be beneficial in liquid formulations, should be avoided in lyophilizates (e.g., volatile buffers such as acetate, or salts that lower the glass transition temperature [Tg'] of the maximally freeze-concentrated solution of amorphous formulation), whereas some excipients' functions are specific for lyophilized products, for example, bulking agent and lyoprotector [30].

Routes of administration

Due to lower stability and a greater sensitivity to enzymatic degradation, oral delivery of biologics remains very challenging. The majority of biologics are currently administered through subcutaneous (s.c.) or intramuscular (i.m.) injection or through intravenous (i.v.) infusion [37]. Intravenous administration requires invasive procedures which can be inconvenient and painful and add complexity to patients, health-care professionals, and the overall health-care system [38-40]. Further systemic exposure of biologic drug substances following i.v. delivery can impart additional clinical challenges. Recombinant cytokine therapy, TNF-α is believed to act preferentially on tumor endothelium, inducing hyperpermeability that results in hemorrhagic necrosis of the tumor tissue. However, following i.v. administration, systemic exposure to high levels of TNF-α can cause severe toxicities such as hypotension and septic shock-like syndrome. For this reason, the use of TNF-α in cancer therapy has been limited to isolated limb perfusions, approved in Europe for the treatment of soft-tissue sarcomas and metastatic melanoma in the extremities [37,41,42]. Another disadvantage of i.v. route is the potential for rapid clearance of the therapeutic biologics from the circulation either by renal filtration or non-specific binding and uptake (e.g., endothelial pinocytosis) [43]. Although antibody therapies (whole IgG) can persist in circulation for days or weeks because of their large size and recycling through neonatal Fc receptors (FcRns), recombinant cytokines and antibody fragments commonly persist for much shorter circulation times (typically minutes to hours) [44-46]. The subcutaneous route provides expanded opportunity for self- or caregiver-assisted administration at home or in an office setting (using devices such as pre-filled syringes, autoinjectors, wearable bolus injectors, and pumps) and reduces the frequency of or eliminates hospital visits, thereby improving patient experience and increasing patient compliance, lowering treatment costs [47]. Whereas i.v. administration can deliver large volumes of medication directly into the systemic circulation without volume limitation, s.c. injections have traditionally been restricted to fluid volumes of 1–2 mL, with recent increases to volumes of about 3 mL [47]. Many biological molecules possess an exponential relationship between concentration and viscosity [48] and with a volume limitation of 1–2 mL may require increased solution concentrations to allow for s.c. delivery of high doses which in turn can result in very high viscosities, and affect the stability, and manufacturability [49]. One important consideration for developing subcutaneous delivery is that although a biotherapeutic has been proven to be safe and well-tolerated, home administration in a life-threatening disease carries the risk of under or over-dosing, which can be challenging for patients and caregivers. Hence, formulation development scientists have been focusing on ways to further optimize subcutaneous applications of high-dose biotherapeutics such as the development of high-concentration formulations to reduce the overall volume of a subcutaneous medication [49], coadministration of the dispersion enhancer hyaluronidase facilitates spreading of an injected volume in the subcutaneous interstitial space and, therefore, enables injection of a larger volume at a personalized rate at the patient's favored injection site (e.g., thigh, abdomen, or upper arm) [50]. Infusion pumps can augment such efforts by overcoming the back pressure generated by subcutaneous tissue during injection [51]. Like the subcutaneous, intramuscular route may cause the potential severity of local injection site reactions (and associated pain). Common injection site reactions include erythema, edema, hematoma, swelling, pruritus, local infection, and pain [52-54]. Autoinjectors help to improve patient compliance in a variety of self-administered therapies, for example, SmartJect injectors (for anti-TNF therapy of rheumatoid arthritis patients) have confirmed lower incidences of injection site reactions and reduced pain [52,55]. Hence, the need for alternative routes of delivery of biologics, especially non-invasive delivery systems, is currently being extensively explored.

Stability study

Generation, evaluation, and interpretation of stability data are some of the key aspects of formulation development. Stability data inform several important aspects of formulation development, manufacturing, and transportation such as the selection of formulation type, and primary packaging materials, defining the manufacturing process, and specifying shelf-life likewise [56]. Considering the large size, high structural complexity, compositional variability, and inherent marginal stability, biologics can undergo a variety of different physical and chemical degradations, leading to loss of their stability and activity by various degradation pathways such as physical degradation from conformational (e.g., unfolding), colloidal (e.g., aggregation and particulate formation), interfacial (e.g., adsorption and degradation at interfaces), or morphological instabilities (e.g., disruption of epitope presentation on cell surfaces), whereas chemical degradation involves modifications of the covalent bonds and can include oxidation (e.g., tryptophan and methionine in proteins, and guanine in nucleic acids), deamidation (e.g., asparagine and glutamine in proteins, and cytosine in nucleic acids), isomerization (e.g., aspartate in proteins), disulfide bond shuffling (e.g., strand separation and desulfurization in DNA and mRNA), and clipping of functional groups and/or linkers (e.g., payload deconjugation in anti-body drug conjugate) likewise [31,57-59]. Manufacturing aspects are also to be considered for the stability of biologics. For example, proteolytic enzymes can make their way through the purification steps and can enter into the final product (and stability samples) causing proteolytic degradation and the stability scientist should be aware of any such impurities that may be present and also will take into consideration that the consequence of their presence may only show up in longer-duration studies, in the meantime process and analytical development scientists should simultaneously work on to prevent such enzymes from getting into the final product [60]. The presence of trifluoroacetate in the peptide material affects its physicochemical properties and can change the secondary structure, solubility, and aggregation tendency of synthetic peptides, and even impact the flexibility, ultimate size, and geometry of fibrils formed. Because of the grave consequences of protein fibrillation in neurodegenerative diseases, the prevention of aggregation *in vivo* is a subject of intense research [61]. Many peptides and proteins, under the right conditions, have been shown to self-assemble into highly structured amyloid fibrils containing a cross-β-sheet structure of tightly interacting intermolecular β-sheets [62,63]. Some of the peptides that self-assemble into fibrillar structures have also been shown to form hydrogels [64]. Disordered aggregates or amorphous aggregates are often granular in appearance when imaged by atomic force microscopy. Insoluble amorphous aggregates may result in precipitates, a major stability challenge in biopharmaceutical formulation development [65,66]. Glucagon, the peptide hormone, does not fibrillate in the body, however, can easily fibrillate if mishandled, for example, in extended storage at a concentration <2.5 mg/mL in 0.1 M HCl at 37°C, glucagon molecules were found to be transformed into fibrillar aggregates within 24 h. Further study indicated that glucagon fibrils are found to be toxic to rat pheochromocytoma (PC12) cells [67].

For analysis of aggregation, the formulation can be modified by fusing the peptide glucagon to a green fluorescent protein, which does not display fluorescence when it accumulates in inclusion bodies and thereby specifies loss of biological structure and function [68,69]. Most biologics are parenteral formulations and may require lyophilization more often. In many cases, however, the lyophilized product may require a constitutional step and/or dilution before administration. The compatibility of the diluents as well as all contact materials, for example, stainless steel needles, polyvinyl chloride (PVC), and non-PVC i.v. bags, filters, and associated tubing, all should be considered for developmental study. The constitution step may be executed in the vial by adding the diluent with a syringe. Care needs to be taken not to agitate the protein during this process as this can lead to degradation [70]. Stability studies of biotechnological products should comply with the norms of the ICH Q5C guideline along with the ICH Q1A to Q1F stability guidelines for these products' registration.

Selection of packaging materials

Biological products, mostly parenteral, inevitably encounter various packaging materials during their production, shipment, and storage. They can lose effectiveness from their interaction with packaging materials, in addition to biological degradation from external factors such as extreme temperatures and light [71]. Seidl *et al*. 2012 studied the presence of soluble tungsten in the syringes, most likely derived from the pins used to manufacture the syringes. Spiking of epoetin alfa with sodium poly-tungstate or an extract of tungsten pins used to manufacture the syringes induced the formation of aggregates, both dimers that appeared to be covalently linked by disulfide bonds. Sodium poly-tungstate had also a strong denaturing effect on the protein [72]. Several approaches are being followed by the manufacturers of pre-filled syringes, including the use of proprietary glass coatings or baked-on siliconization processes as well as alternate materials for the pins used in the manufacture of staked needle syringes [73]. One of the most common lubricants used is silicone oil, especially for pre-filled syringes or cartridges. Silicone oil represents a hydrophobic surface, possibly inducing direct protein-protein interactions, which may result in protein denaturation and aggregation [74]. On storage in glass vials and syringes, the glass surface may release heavy metal ions; organic compounds may leach out of the polymer-based materials as used in vial stoppers, syringe plungers, and barrels of polymer-based syringes. The formulator needs to gather such information on the leachable and take appropriate steps, when necessary, e.g., switching packaging material or its vendor, adding suitable coatings, or adjusting formulation features such as the pH [75]. The packaging or container system can influence oxidative stability on biologics as well. For example, there have been reports of oxidation due to the leaching of metals from stainless steel, as in the case of the monoclonal antibody HER2, rhuMAb HER2 [76]. Stoppers and other containers are known to be sources of leachable metals that can cause subsequent oxidation in protein pharmaceuticals [77]. Formulation development needs to address such problems, for example, the addition of chelating agent EDTA to sequester leached iron ions and also useful to prevent metalloprotease activation [78,79]. Yokota *et al*., 2000 studied that amounts of methionine (Met58) oxidized human recombinant interleukin 11 (rhIL-11) were increased when rhIL-11 samples were stored in plastic tubes at 37°C in the dark, oxidation of samples in polypropylene tubes were found to much more than samples stored in polystyrene tubes. Further, the oxidation was greatly enhanced when samples stored in polypropylene tubes were exposed to light [80]. The transition of primary packaging materials should also to be intensely monitored. During the development of a liquid drug product, a new impurity was discovered when the primary packaging material was changed from glass to plastic high-density polypropylene. The leachable impurity obtained from the plastic container and the leachable compound was a hydrophobic molecule, containing a linear chain of 16 or more carbons, and had a molecular weight of 282 Daltons as per chromatographic and spectral analysis [81]. Hence, the focus is given that the packaging system of biologics is relatively inert, rugged, cleanable or disposable, sterilizable, and last but not least, cost-effective.

Manufacturing challenges of biological drugs

The manufacturing processes of biologics are complex and involve living systems (e.g., mammalian cell lines, microbial agents, plants, and fungi) and multiple critical processes (e.g., gene isolation, recombinant DNA engineering, and protein purification) which further require high technological expertise to ensure consistency and quality of the final product [21]. The manufacturing process of biopharmaceuticals can be broadly categorized into up and downstream processes. The upstream process is defined as the microbial growth required to produce biopharmaceutical molecules through the transformation of substrates into the desired metabolic products [82]. This involves a series of events such as cell line selection and development, screening and selection of clones, optimization of media, optimization of feed, and process optimization. The number of factors should be considered in these steps such as the selection of host cells and expression vectors, type of process (batch, fed-batch, continuous, etc.) temperature, pH, oxygen supply control, sterilization of materials and equipment, environmental monitoring for microbial contamination, etc. which adds complexity to the overall manufacturing process [83]. Downstream processing includes all steps necessary to purify a biological product from cell culture broth to the final purified product of desired purity and yield [84]. It involves critical stages to capture the target biomolecule and to eliminate host cell-related impurities (e.g., host cell proteins, DNA, etc.), manufacturing-related impurities (e.g. buffers, leached ligands, antifoam, etc.), and product specific impurities (e.g., aggregates, fragments, clipped species, etc.). Each purification step would remove one or more classes of impurities [85,86]. Since the manufacturing performance of cell-based therapies is highly dependent on the quality of the extracted cells, therefore leads to highly variable critical process parameters – critical quality attributes (CPP-CQA) which adds more criticality to designing and implementation of quality by design (QbD) [87]. Bioreactor complexity and interplay between process conditions and cellular metabolism have made mechanistic modeling of bioreactors difficult [88].

Challenges to QbD implementation

QbD is defined as a systematic risk-based approach to product development that begins with pre-defined objectives and this approach extends through the life-cycle of biopharmaceutical products (Fig. 4). It emphasizes the understanding of product, process, and analytical control. It is based on comprehensive science and quality risk management [89]. As per ICH Q8 (R2), the basic principle of QbD is that the quality of the medicinal products should be built in by design. In general, a biotech process consists of approximately 10–20 unit operations that are executed in a series [86,90]. Each of these unit operations may be regulated by 2–10 process parameters and 5–20 raw materials. Thus, anyone between 20 and 100 process parameters and raw materials can influence the outcome of a biotech process. Hence, it is not feasible for a manufacturer to evaluate the impact of each of these factors [91]. The common tactic encompasses the use of risk analysis tools to recognize the key process parameters and raw materials that may impact process performance. The identified parameters and raw materials may then be evaluated and post-evaluation those parameters, that are found to possess significant effects on process performance and/or product quality, undergo intense examination for their main effects and interactions with each other. The outcome of this exercise assists in the formation of design space for each unit operation and the entire process as well [92,93]. As per ICH Q8, a design space can be described as the multidimensional combination and interaction of the input variables (e.g., material attributes) and process parameters assigned to ensure quality. Regulatory approval is not necessary for movement within a design space since this is not considered a change. However, change within a design space requires through monitoring by the sponsor's quality system. Design space is planned by the applicant and is subject to regulatory evaluation and approval [94]. Roche/Genentech has licensed two therapeutic recombinant monoclonal antibody products, Obinutuzumab (GazyvaÒ) and atezolizumab (TencentriqÒ), in the US using QbD principles, representing the first approvals for biologics that were comprehensively based on QbD information,

including approved design space claims as well as a post-approval lifecycle management plans, contained in the license application [95]. Implementing QbD in development is anticipated to cause an overall rise in quality of products which ultimately will improve the trust and public image of the company [96]. However, only 151 out of 494 medicinal products approved in Europe during 2014–2019 described in their regulatory dossier the usage of QbD during the development of the product. Unfortunately, no significant increase in information about the usage of full QbD in regulatory dossiers is seen from 2014 to 2019, especially for full applications [89]. Internal misalignment, technical barriers due to the unavailability of correct equipment, extra time and money, and management issues are substantial barriers to the execution of QbD [96]. On a joint QbD workshop of EMA and the Parenteral Drug Association in 2014, regulators highlighted variations in terminology and the definitions coined by pharmaceutical companies in their regulatory submissions, or during regulatory discussions on QbD matters. Such aberrations jeopardized the regulatory assessments and resulted in more queries by regulatory auditors, which could discourage companies from introducing QbD terminology into the regulatory dossiers even if QbD was applied in the design & development. This indicated the necessity for international synchronization of regulatory assessments and terminologies [97].

Supply chain challenges of biological drugs

The stability of biopharmaceuticals is highly affected by temperature excursions and shocks. Blood products, conventional vaccines (e.g., live-attenuated viruses), and monoclonal antibodies must be transported and stored under refrigeration conditions of 2–9°C [98]. As per USFDA Vaccines and Related Biological Products Advisory Committee, 2020 genetically engineered products, such as mRNA vaccines, must be stored and handled under temperature conditions at about −70°C. Newer technologies such as chimeric antigen receptor T cells, should be stored and transported either fresh (−80°C) or cryopreserved (−180°C), depending on the manufacturing practice, noting that they are also highly sensitive to shear stress and vibrations, because of their cell-based nature [99]. While all medicinal products are susceptible to the rigors of cross-border shipping, biopharmaceuticals in particular are heat sensitive and vulnerable to contamination. Keeping these biopharmaceutical drugs under specified cold temperature conditions is a crucial part of the supply chains [100]. The International Air Transport Association (IATA) 2021 published a guidance document concerning COVID-19 vaccine transport highlighting the criticality of equipment to transport or hold temperature-sensitive health-care shipments, whether these are aircraft or non-aircraft containers, active or passive Temperature Controlled Containers (TCC), insulated containers, thermal blankets, or ramp "cool" dollies. Further aircraft and non-aircraft TCC with dry ice and lithium batteries should comply with the IATA Dangerous Goods Regulations. Jaffer 2020 described three key packaging solutions for temperature-sensitive biological materials active (dynamic), semi-active, and passive (static). Active packaging depends on an outside power source to preserve a constant temperature. In semi-active solutions, a stationary cold source, such as a phase-change material (PCM), is placed in an isolated section, and heat exchange between the biological material and the cold source is controlled using a system that operates without an electrical power source. Passive packaging encompasses eutectic plates of a PCM within an insulating material. The usage of lightweight vacuum-insulated panels and PCMs provides better dependability in a smaller space for increased payload efficiency. While they are more expensive initially, they have proven to be more cost-effective over the long term [101]. In addition, for real-time monitoring and tracking of the environmental condition, various new age techniques such as integrated data collection and transmission capabilities along with web-based asset management software systems are deployed which also helps in the geolocation of a package as it moves through the supply chain, thus safeguard proper handling and delivery of biopharmaceuticals. Data gathered by such systems permit information-centric logistics decision-making and may also improve packaging design [102]. Another important consideration in the supply chain of biopharmaceuticals is humidity to maintain product quality. Humidity can also bring about damage to the packaging and erase the printed information such as shelf-life. [103]. For the transport of very sensitive biopharmaceuticals, that have short shelf lives or require specific transport conditions, the use of sensors can be fruitful to measure temperature, humidity, shock, and light, with real-time transmission of data [104].

Challenges to regulatory approval

As discussed above, various categories of biologics require different sets of regulatory requirements for submission and subsequent approval for human use. Compliance data in accordance with various guidelines such as current good manufacturing practices, guidelines such as ICH Q5 (Quality of Biotechnological Products), ICH Q9 (Quality Risk Management), ICH Q11 (Development and manufacture of drug substances-chemical entities and biotechnological/biological drugs) signify importance to design proper formulation and manufacturing steps of biopharmaceuticals [105]. Harmonization and alignment of different guidelines and exchange of real-time data between different stakeholders such as manufacturers and regulatory bodies are important for dynamic review processes by regulatory authorities, as happened during the COVID-19 pandemic. Novel technologies, especially in manufacturing, such as continuous and modular manufacturing, use of artificial intelligence models to replace empirical testing, training on the models, and interfacing it with advanced analytics require proper guidelines from a regulatory science perspective for products' quality and patients' safety [106]. To help lower the costs of biologics, regulators portrayed abbreviated approval pathways for biosimilars by nurturing competition. However, intellectual property rights concern for biosimilars, that is, the large numbers of patents along with trade secrets which sprinkle difficulty to the reverse engineering of original biologics to obtain corresponding biosimilars. Compliance with the guidelines for clinical studies of biosimilars also adds challenges to biosimilar approval. USFDA has already issued new guidance to help sponsors develop plans to unroll more participants from under-represented racial and ethnic populations. Furthermore, physicians are pressing for the inclusion of obese patients in such clinical trials since populations of overweight people are significant nowadays [107]. After entry into the market, every new biological product is kept under regulatory monitoring through post-marketing surveillance for any safety issue, or adverse event [108]. Novel biological therapies such as gene therapy and cell therapy require stringent regulatory screening which leads to the huge cost for these drugs. For example, elivaldogene autotemcel (Skysona) received accelerated approval to slow neurologic dysfunction in boys aged 4–17 years with cerebral adrenoleukodystrophy but priced at $3 million. Accelerated approvals are granted based on surrogate markers with limited available clinical data. However, one condition is imposed that sponsors would follow-up with confirmatory trials. Some of them faced issues with the trials. For example, hydroxyprogesterone caproate injection (Makena, Covis Pharma) received accelerated approval in 2011 but failed a confirmatory study. Fecal microbiome-based products such as fecal microbiota transplants offer difficulty in quality control and regulation activities [109]. Hence, manufacturers should remain clear and compliant with the regulatory requirements for the submission of documents, audits, clinical studies, and overall timeline to approval for the effective launch of the biological drugs and also regulatory bodies need to ascertain clear harmonized guidelines, especially for novel biologic therapies to support innovation and easy market entry for minimizing disease burden of patients at affordable cost.

CLINICAL CHALLENGES OF BIOPHARMACEUTICALS

Safety concerns of biological drugs

The most critical safety concern of biopharmaceuticals is immunogenicity. Minimization of immunogenicity must begin at the molecule design stage by reducing or eliminating antigenic epitopes and building favorable physical and chemical properties [110]. The formation of neutralizing anti-drug antibodies (ADA) against the corresponding biologic or immune complexes that trigger proteolytic elimination in the reticuloendothelial system will cause increased

clearance of a biologic [19]. Many factors contribute to the ability of biologics to elicit ADA production. Intrinsic factors affecting immunogenicity are protein sequence (including similarity to endogenous proteins and the presence of T- and B-cell epitopes), post-translational modification (glycosylation and oxidation), and tertiary structure (including aggregation propensity). Extrinsic factors include the route, dose, and type of formulation (that may affect aggregation), production process (that may affect both aggregation and post-translational modifications), impurities, clinical subject characteristics (genetic makeup, inflammation status, concomitant medications, and disease population), as well as drug pharmacology (specifically related to immunosuppression) [111]. Immunogenic responses include anaphylaxis, cytokine release syndrome, and ADA formation; hence patients should be closely monitored following administration of biologics [112]. The outcome of immunogenicity may also vary from little/no impact to serious health implications. In instances, where the impact of immunogenicity was not clearly delineated during clinical development, the importance of post-marketing pharmacovigilance studies becomes immense for further immunogenicity information following chronic dosing. The magnitude of ADA as well as its onset may influence the *in vivo* exposure and the efficacy of the biotherapeutics. The longevity of the response can also impact the overall exposure, especially if the ADA response matures from a binding response to a neutralizing response, due to affinity maturation or potential epitope spreading [27]. One example of immunogenicity took place a couple of years ago when, at the request of the European Health Authorities, Johnson and Johnson made a change in the manufacturing process for its product Eprex, which was synthetic erythropoietin (epoetin alfa) which increased the production of red blood cells and reduces the need for transfusions of red blood cells. The change triggered a serious adverse reaction in a small number of patients. These patients lost their ability to make red blood cells because they, after administering Eprex by a new manufacturing process, produced an antibody that inactivated both the administered erythropoietin and the body's natural protein which is essential for red blood cell production. Following a lengthy and expensive investigation the root cause was identified and correction was carried out accordingly [113]. The subtle differences in glycosylation may impact the patients' experience as changes in glycosylation may ultimately influence binding, immunogenicity, and effector activity [114]. The potential to develop ADA could be higher following s.c. or i.m. administration than i.v. administration of biologics because phagocytes and NK cells, which are responsible for the initial, innate, immune response, are found under the skin and in the mucosal epithelia, and hence immunogenicity demands proper clinical studies when treatment is done with biologics [19].

Challenges faced by patients and health-care professionals

Biological medicinal products offer additional challenges to health-care professionals and patients as well. Biologics such as anti-TNF-α medications, for example, infliximab, etanercept, etc. are effective in rheumatic diseases, which in turn demand attention since they can cause opportunistic infections such as reactivation of tuberculosis and cardiovascular incidents such as induction of left ventricular dysfunction, acute pulmonary edema, and congestive cardiomyopathy [115]. This demands thorough monitoring of the patients following administration of anti-TNF-α medications. Knowledge of biosimilars across different cadres of health-care professionals is also not uniform. Friganović *et al*. 2022 revealed in a study that most nurses are not adequately educated about biosimilars, only nurses with a bachelor's degree possess better knowledge about the advantages of biosimilar drugs. Further, they avoided talking to the patients about biosimilars due to a lack of knowledge irrespective of the academic degree of the nurses [116]. Leonard *et al*. 2019 highlighted that interchangeability and pharmacy-based switching of biologics caused concerns for physicians. Efficacy and safety concerns such as immunogenicity or the inclination of the biosimilar to generate an immune response also played roles in lacking confidence in physicians to prescribe them. <50% of U.S. physicians were found to be unaware of the most recent U.S. biosimilar approvals [117,118]. U.S. pharmacists, on average, were familiar with biosimilars [118-121]. However, regarding interchangeability, 95% of pharmacists believed interchanging biosimilars for original drugs was a joint physician–pharmacist responsibility [122]. Hence, regarding familiarity with biological drugs, proper uniform training, and knowledge updation is a requirement across different stakeholders of healthcare facilities. Management of biomedical wastes derived from biological medicines such as expired/spilled vaccines, and cell-based medications imparts additional challenges. Pre-treatment is required especially for infectious biowaste as per WHO guidelines for log 6 and log 4 reduction [123]. Cost and patients' compliance with biological drugs are also the key determinants of the biopharmaceutical drugs' success. Inhalation delivery of insulin initially received USFDA approval in 2006, but it was soon discontinued in the next year owing in part to high costs as well as poor patient uptake of the bulky device. Continual research studies resulted in a more compact device that can effectively deliver insulin by inhalational route in patients with type 2 diabetes and subsequently got regulatory approval from the USFDA in 2014 [124].

Challenges to clinical studies and bioethics

Biologics should be screened by laboratory analysis and animal testing to ascertain their pharmacologic and toxicologic effects before they can be administered in humans. As per the 21 CFR (US Code of Federal Regulation) part 601, for obtaining a biologic license, the applicant shall submit data from non-clinical laboratory and clinical studies in compliance with the prescribed requirements of safety, purity, and potency. Non-clinical laboratory tests both *in vivo* and *in vitro* study should be directed in compliance with the requirements of GLP as per part 58 of 21 CFR. Appropriate bioanalytical methods are the mainstay of getting proper pharmacokinetic, toxicokinetic, and immunogenicity data in the pre-clinical as well as clinical development of biologics [125]. In general, the adverse effects of biopharmaceuticals are due to exaggerated pharmacology, unintentional tissue cross-reactivity, or immune system-mediated adverse effects [126,127] and these factors are key determinants of pre-clinical safety procedures on a case-by-case basis. For example, the non-clinical development of a biosimilar requires a complex understanding of the selection of the appropriate reference product (or products), the key molecular and quality product attributes (influencing safety and toxicity efficacy), and understanding the range of variability at batch release and throughout its shelf life [128]. Further, biologics require an array of different types of assays for the quantification of the protein itself, also its biological activity. This usually requires more capacity, time, and logistical effort. Ligand-binding assays (immunoassays for quantification of a protein in support of pharmacokinetic studies), the activity assays (bioassay *in vitro* often involves human cell line-based techniques or *in vivo* animal models) require proper validation, evaluation, and monitoring [125,129]. There are various animal models for pre-clinical studies for biological, for example, the murine model for immune-oncology development. However, successful engraftment remains highly inconstant with such a model, and proper development of pre-clinical models consumes a long time [130,131]. Pre-clinical safety of biopharmaceuticals is also being evaluated by studies in non-human primates (NHPs) but these models also suffer from the fact that reproductive and developmental toxicity and carcinogenicity are not easily studied in NHPs [126]. The usage of animals in pre-clinical studies always involves ethical issues. As per the 3R principle, introduced by Russel and Burch, animals should be utilized only when there is a real necessity (Replacement), the minimum possible number of animals should be used (Reduction) and finally minimum animal suffering should be considered during the study (Refinement). To ensure that research on animals is conducted ethically and responsibly, associations and guidelines for the protection of animal rights are developed with the adaptation of guidelines developed by the American Psychological Association for use by psychologists dealing with non-human animals. In this context, the 3D cell culture models are useful to screen for pharmacological activity, preliminary toxicological assessment, and elucidation of cellular pathology and physiology. The primary advantages of such 3D cell culture techniques include a high structural complexity, the simulation of cell-to-cell interactions, the physiological behavior of cells in tissues, and more

Table 1: List of significant novel biological therapies approved by the USFDA between 2022 and 2023

S. No.	Biologic drug (Trade Name)	Manufacturer	Active therapeutic agent	Dosage form and route	Indication
1	Nadofaragene firadenovec-vncg (Adstiladrin)	FinVector Oy, Finland	Adenovirus vector-based gene therapy product, comprised of rAd-IFN, a replication-deficient recombinant type 5 adenovirus (Ad5) vector expressing the interferon alfa2b (IFNα2b) transgene driven by novel polyamide surfactant Syn3.	Suspension, intravesical	High-grade, Bacillus Calmette-Guérin (BCG) unresponsive non-muscle invasive bladder cancer (NMIBC)
2	Fecal microbiota (Rebyota)	Rebiotix Inc., Roseville, Minnesota	Fecal microbiota, live-jslm from donor human stool	Suspension, rectal	Recurrent Clostridioides difficile (CDI) infection, following antibiotic treatment for recurrent CDI
3	Etranacogene dezaparvovec-drlb (Hemgenix)	uniQure, Inc., Lexington, Massachusetts	Recombinant adeno-associated viral vector serotype 5 (rAAV5) vector containing a codon-optimized version of the naturally occurring Padua variant of the FIX gene (hFIXco-Padua)	Suspension, i.v. infusion	Hemophilia B (congenital Factor IX deficiency)
4	Elivaldogene autotemcel (Skysona)	Lonza Houston, Inc., Houston, Texas	Autologous CD34+hematopoietic stem cells (HSCs) that have been transduced with Lenti-D, a lentiviral vector (LVV) encoding ABCD1 complementary DNA (cDNA)	Suspension, i.v. infusion	Neurologic dysfunction in boys 4–17 years of age with early, active cerebral adrenoleukodystrophy
5	Betibeglogene autotemcel (Zynteglo)	Lonza Houston, Inc., Houston, Texas	Autologous CD34+hematopoietic stem cells (HSCs), transduced with a replication-incompetent, self-inactivating lentiviral vector (LVV), BB305, and encoding a modified β-globin gene (βA-T87Q globin or LentiGlobin)	Suspension, i.v. infusion	Transfusion-dependent β-thalassemia
6	Measles, Mumps, and Rubella Vaccine, Live (PRIORIX)	Glaxo SmithKline Biologicals, Rixensart, Belgium	Live viral vaccine composed of three live attenuated viruses (1) measles (Schwarz strain), (2) mumps (RIT 4385 strain), and (3) rubella (Wistar RA 27/3 strain)	Suspension, s.c. injection	Active immunization for the prevention of measles, mumps, and rubella in individuals 12 months of age and older
7	Ciltacabtagene autoleucel (Carvykti)	Janssen Biotech, Inc., Horsham, Pennsylvania	Autologous Human T cells genetically modified *ex vivo* with lentiviral vector encoding Chimeric Antigen Receptor (CAR) for B Cell Maturation Antigen (BCMA or CD269)	Suspension, i.v. infusion	Relapsed or refractory multiple myeloma after four or more prior lines of therapy including a proteasome inhibitor, an immunomodulatory agent, an anti-CD38 monoclonal antibody
8	COVID-19 Vaccine, mRNA (Spikevax)	ModernaTX, Inc., Norwood, MA, and Lonza Biologics Inc., Portsmouth, New Hampshire	Nucleoside-modified mRNA encoding for spike protein of SARS-CoV-2, formulated with lipid to form RNA-encapsulating lipid nanoparticles	Suspension, i.m. injection	Active immunization to prevent COVID-19 disease caused by SARS-COV-2 virus
9	Respiratory Syncytial Virus Vaccine (Abrysvo)	Pfizer Inc., New York	Bivalent recombinant stabilized prefusion F protein subunit vaccine (RSVpreF), consists of equal amounts of prefusion F antigens from the two major RSV subtypes – RSV subtype A, RSV subtype B	Solution, i.m. injection	Active immunization of (a) pregnant individuals at 32 through 36 weeks gestational age against lower respiratory tract disease (LRTD) and severe LRTD by respiratory syncytial virus (RSV) in infants from birth through 6 months and (b) LRTD caused by RSV in adults (60 years and older)
10	Donislecel-jujn (Lantidra)	CellTrans Inc., Chicago, Illinois	Allogeneic Pancreatic Islet Cells	Suspension, Hepatic portal vein infusion	Type 1 diabetes patients, unable to approach target HbA1c
11	Beremagene geperpavec-svdt (Vyjuvek)	Krystal Biotech, Inc., Pittsburgh, Pennsylvania	Replication-defective, non-integrating, engineered herpes simplex virus type 1 (HSV-1)-based vector expressing human type VII collagen (hCOL7)	Suspension mixed with excipient gel for topical application	Wounds in patients (6 months and older) with dystrophic epidermolysis bullosa with mutation (s) in the collagen type VII alpha 1 chain (COL7A1) gene.

Source: Biologics license application (BLA) approval documents from USFDA

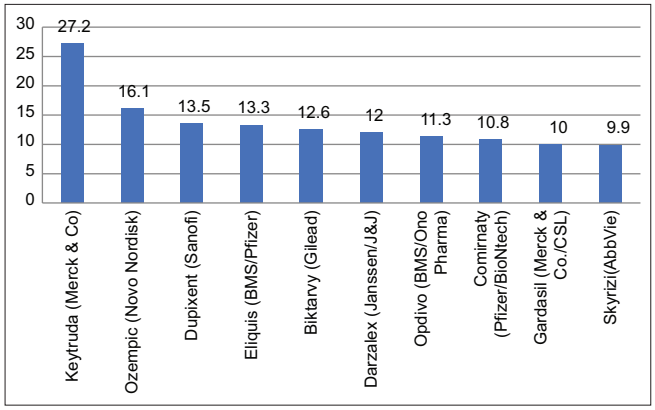

Fig. 1: Projected global sales of leading brands including biologics (with manufacturer) in 2024 in USD Billion
Source: Maximize Market Research (MMR). Report ID:39044. January 2024

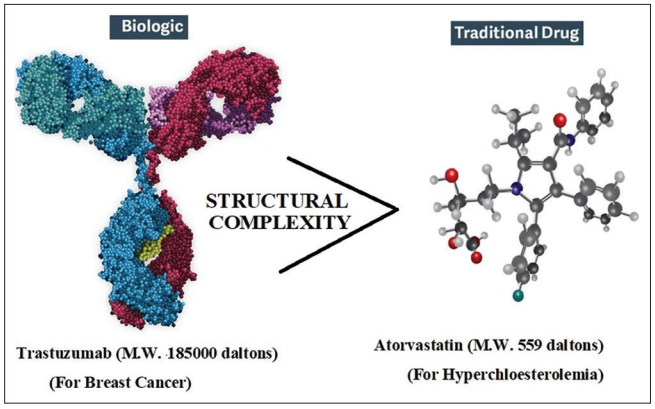

Fig. 3: Structural complexity between biological drug and small molecule drug.
Source: Scientific American, 2017

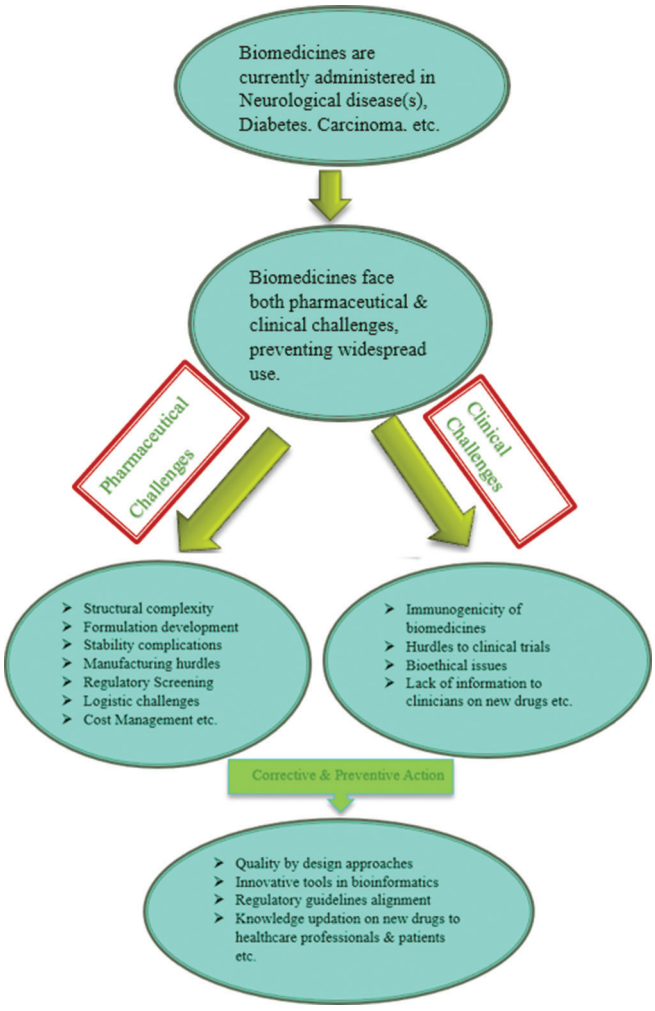

Fig. 2: Pharmaceutical and clinical challenges of biological medicines

Fig. 4: Quality by Design approaches to the life-cycle of biopharmaceuticals

realistic data in-line with *in vivo* animal models [132-135]. Recently organoids have been developed from pluripotent stem cells preserving long-term near-native 3D epithelial organization while holding genetic stability and high heterogeneity [136]. For example, intestinal organoids are developed from normal adult human ileal small intestinal tissue [137]. In parallel alternative animal models are also being developed- Galleria mellonella larvae, Zebrafsh (*Danio rerio*), Brine Shrimp (*Artemia* Sp.), Roundworms (*Caenorhabditis elegans*), Fruit fly (*Drosophila melanogaster*) likewise [135]. The use of *D. melanogaster* is gaining popularity because of the translational relevance of *Drosophila* in drug discovery and drug repurposing, its genomic simplicity, ease of use, minimal ethical issues, and cost-effectiveness. Clinical studies with *D. melanogaster* involve metastatic melanoma (NCT01271907), personalized cancer therapy in metastatic medullary thyroid or metastatic colon cancer (NCT02363647) [131,138]. Bioinformatics also plays an important role in pre-clinical studies. Multiple independent algorithms are utilized cooperatively to successfully predict the miRNA binding sites in protein-coding genes and their associated biological networks. Moreover, bioinformatic tools such as Kegg and IPA/Ingenuity identify presumed biological pathways, and in some cases disease states, targeted by miRNAs [139]. Clinical study of the biomolecules demands further attention, which is especially true in oncological trials where biologics such as immunological agents, plays an important therapeutic role. Heterogeneity within any given tumor type from patient to patient (inter-patient heterogeneity), and within an individual (intra-patient heterogeneity) create hurdles to advancement in cancer treatment outcomes. The usual clinical trial design models are confronted by heterogeneity since they are unable to assess targeted therapeutics against low-frequency genomic

oncological aberrations with satisfactory influence. To cater to these challenges, next-generation biomarker-based clinical trial designs have been recently exercised [140]. For example, exploratory platform – "BATTLE," "I-SPY" [132,133]; Expansion platform Type IIA: Grass-Roots, Holistic and Histology Dependent – Personalized Antibodies for Gastro-Esophageal Adenocarcinoma trials, etc. [134]. In general, these newer trial designs often present regulatory hurdles, concerning biologic drugs and associated diagnostic tools development and approval [140].

CONCLUSION

Biopharmaceuticals are one of the most valuable genres of modern medicines, especially considering the clinical benefits they offer particularly to some of the terminal diseases such as cancer. Continuous innovations in the field such as mMRA technology, immunotherapy, gene therapy, and cell therapy likewise, and their clinical successes offer hope to health-care professionals and patients to alleviate diseases. Alignment of regulatory norms, proper QbD approaches, and adequate training to health-care professionals on the safety, efficacy, immunogenicity, handling, and storage of these large complex biopharmaceuticals bestow clinical benefits to the intended patients. Continual research going on to market new biopharmaceuticals for peroral administration and also, they are extensively studied for new indication(s), rare and difficult-to-treat diseases. Innovations in the field of manufacturing, purification, and analytical methods will augment the therapeutic benefits of biopharmaceuticals to reach out to ailing patients in a timely and cost-effective manner.

ACKNOWLEDGMENT

The authors are thankful to each member of the Department of Pharmacy for their encouragement.

AUTHORS CONTRIBUTION

Sayon Paul is involved in conceptualization, data curation, formal analysis, writing of the original draft, and editing. Avik Sarkar is involved in supervision, reviewing, and editing.

DECLARATION OF CONFLICT OF INTEREST

There is no known conflict of interest or any financial interest or personal relationship that might influence the paper.

FUNDING

The authors declare that no source of funds was allotted to this work.

REFERENCES

1. Misra M. Biosimilars: Current perspectives and future implications. Indian J Pharmacol. 2012;44(1):12-14. doi: 10.4103/0253-7613.91859
2. Leader B, Baca QJ, Golan DE. Protein therapeutics: A summary and pharmacological classification. Nat Rev Drug Discov. 2008;7(1):21-39. doi: 10.1038/nrd2399
3. Leavy O. Therapeutic antibodies: Past, present and future. Nat Rev Immunol. 2010;10(5):297. doi: 10.1038/nri2763
4. Walsh G, Walsh E. Biopharmaceutical benchmarks 2022. Nat Biotechnol. 2022;40(12):1722-60. doi: 10.1038/s41587-022-01582-x
5. Kesik-Brodacka M. Progress in biopharmaceutical development. Biotechnol Appl Biochem. 2018;65(3):306-22. doi: 10.1002/bab.1617
6. Vlasak J, Bussat MC, Wang S, Wagner-Rousset E, Schaefer M, Klinguer-Hamour C, *et al*. Identification, and characterization of asparagine deamidation in the light chain CDR1 of a humanized IgG1 antibody. Anal Biochem. 2009;392(2):145-54. doi: 10.1016/j.ab.2009.05.043
7. Khawli LA, Goswami S, Hutchinson R, Kwong ZW, Yang J, Wang X, *et al*. Charge variants in IgG1. MAbs. 2010;2(6):613-24. doi: 10.4161/mabs.2.6.13333
8. Beck A. Biosimilar, biobetter and next generation therapeutic antibodies. MAbs. 2011;3(2):107-10. doi: 10.4161/mabs.3.2.14785
9. Weise M. From bioequivalence to biosimilars: How much do regulators dare? Z Evid Fortbild Qual Gesundhwes. 2019;140:58-62. doi: 10.1016/j.zefq.2018.12.001
10. Sharma A, Kumar N, Kuppermann BD, Bandello F, Loewenstein A. Biologics, biosimilars, and biobetters: Different terms or different drugs? Eye. 2019;33(7):1032-4. doi: 10.1038/s41433-019-0391-5
11. Weise M, Bielsky MC, de Smet K, Ehmann F, Ekman N, Narayanan G, *et al*. Biosimilars-why terminology matters. Nat Biotechnol. 2011;29(8):690-3. doi: 10.1038/nbt.1936
12. Strohl WR. Fusion proteins for half-life extension of biologics as a strategy to make biobetters. Biodrugs. 2015;9(4):215-39. doi: 10.1007/s40259-015-0133-6
13. Kim H, Alten R, Cummings F, Danese S, D'Haens G, Emery P, *et al*. Innovative approaches to biologic development on the trail of CT-P13: Biosimilars, value-added medicines, and biobetters. MAbs. 2021;13(1):e1868078. doi: 10.1080/19420862.2020.1868078
14. LoRusso PM, Weiss D, Guardino E, Girish S, Sliwkowski MX. Trastuzumab emtansine: A unique antibody-drug conjugate in development for human epidermal growth factor receptor 2-positive cancer. Clin Cancer Res. 2011;17(20):6437-47. doi: 10.1158/1078-0432.CCR-11-0762
15. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, *et al*. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med. 2012;367(19):1783-91. doi: 10.1056/NEJMoa1209124
16. IQVIA Consulting Services. Case Studies for Value Added Medicines Unlocking the Potential of Patient-centric Continuous Innovation; 2019. p. 1-57. Available from: https://www.medicinesforeurope.com/wp-content/uploads/2019/04/iqvia-mfe_case-studies-for-vams_final-word-document_vupdate2019-v3.0.pdf [Last accessed on 2024 May 4].
17. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, *et al*. Exenatide once weekly versus placebo in Parkinson's disease: A randomised, double-blind, placebo-controlled trial. Lancet. 2017;390(10103):1664-75. doi:10.1016/S0140-6736(17)31585-4
18. Vijiaratnam N, Girges C, Auld G, Chau M, Maclagan K, King A, *et al*. Exenatide once weekly over 2 years as a potential disease-modifying treatment for Parkinson's disease: Protocol for a multicentre, randomised, double blind, parallel group, placebo controlled, phase 3 trial: The 'Exenatide-PD3' study. BMJ Open. 2021;11(5):e047993. doi: 10.1136/bmjopen-2020-047993
19. Zhao L, Ren T, Wang DD. Clinical pharmacology considerations in biologics development. Acta Pharmacol Sin. 2012;33(11):1339-47. doi:10.1038/aps.2012.51
20. Mellstedt H. Clinical considerations for biosimilar antibodies. Eur J Cancer Suppl. 2013;11(3):1-11. doi: 10.1016/S1359-6349(13)70001-6
21. Chan JC, Chan AT. Biologics and biosimilars: what, why and how? ESMO Open. 2017;2(1):e000180. doi:10.1136/esmoopen-2017-000180
22. Morrow T, Felcone LH. Defining the difference: What makes biologics unique. Biotechnol Healthc. 2004;1(4):24-9. PMID: 23393437
23. Ciucci G, Colliva A, Vuerich R, Pompilio G, Zacchigna S. Biologics and cardiac disease: Challenges and opportunities. Trends Pharmacol Sci. 2022;43(11):894-905. doi: 10.1016/j.tips.2022.06.001
24. Ovacik M, Lin K. Tutorial on monoclonal antibody pharmacokinetics and its considerations in early development. Clin Transl Sci. 2018;11(6):540-52. doi: 10.1111/cts.12567
25. Wang W, Wang E, Balthasar J. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther. 2008;84(5):548-58. doi: 10.1038/clpt.2008.170
26. Dong JQ, Salinger DH, Endres CJ, Gibbs JP, Hsu CP, Stouch BJ, *et al*. Quantitative prediction of human pharmacokinetics for monoclonal antibodies. Clin Pharmacokinet. 2011;50(2):131-42. doi: 10.2165/11537430-000000000-00000
27. Wang YM, Jawa V, Ma M. Immunogenicity and PK/PD evaluation in biotherapeutic drug development: Scientific considerations for bioanalytical methods and data analysis. Bioanalysis. 2014;6(1):79-87. doi:10.4155/bio.13.302
28. Medi MB, Chintala R, Akhilesh B. Excipient Selection in Biologics and vaccines Formulation Development. Vol. 19. European Pharmaceutical Review; 2014. p. 16-20. Available from: https://www.europeanpharmaceuticalreview.com/article/24136/excipient-selection-biologics-vaccines-formulation-development [Last accessed on 2024 May 04].
29. Courtney KN. Key CMC Considerations for Biologic Development. Vol. 24. American Pharmaceutical Review; 2021. Available from: https://www.americanpharmaceuticalreview.com/featured-articles/581696-key-cmc-considerations-for-biologic-development [Last accessed on 2024 May 04].
30. Weinbuch D, Hawe A, Jiskoot W, Friess W. Introduction into formulation development of biologics. In: Warne N, Mahler HC, editors. Challenges in Protein Product Development. (AAPS Advances in the Pharmaceutical Sciences Series). Cham: Springer; 2018. p. 3-22.

doi: 10.1007/978-3-319-90603-4_1
31. Muralidhara BK, Wong M. Critical considerations in the formulation development of parenteral biologic drugs. Drug Discov Today. 2020;25(3):574-81. doi: 10.1016/j.drudis.2019.12.011
32. Bajracharya R, Song JG, Back SY, Han HK. Recent advancements in non-invasive formulations for protein drug delivery. Comput Struct Biotechnol J. Sep 2019;17:1290-308. doi: 10.1016/j.csbj.2019.09.004
33. Ionova Y, Wilson L. Biologic excipients: Importance of clinical awareness of inactive ingredients. PLOS One. 2020;15(6):e0235076. doi: 10.1371/journal.pone.0235076
34. Kerwin BA. Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: Structure and degradation pathways. J Pharm Sci. 2008;97(8):2924-35. doi: 10.1002/jps.21190
35. Challener CA. Excipients for high-concentration biologics. Biopharm Int. 2022;35(3):17-37.
36. Bhambhani A, Kissmann JM, Joshi SB, Volkin DB, Kashi RS, Russell MC. Formulation design and high-throughput excipient selection based on structural integrity and conformational stability of dilute and highly concentrated IgG1 monoclonal antibody solutions. J Pharm Sci. 2012;101(3):1120-35. doi: 10.1002/jps.23008
37. Irvine DJ, Su X, Kwong B. Routes of delivery for biological drug products. In: Wei W, Manmohan S, editors. Biological Drug Products: Development and Strategies. United States: Wiley; 2013. p. 1-48. doi: 10.1002/9780470571224.pse521
38. Kruse GB, Amonkar MM, Smith G, Skonieczny DC, Stavrakas S. Analysis of costs associated with administration of intravenous single-drug therapies in metastatic breast cancer in a U.S. population. J Manag Care Pharm. 2008;14(9):844-57. doi: 10.18553/jmcp.2008.14.9.844
39. Vescia S, Baumgärtner AK, Jacobs VR, Kiechle-Bahat M, Rody A, Loibl S, *et al*. Management of venous port systems in oncology: A review of current evidence. Ann Oncol. 2008;19(1):9-15. doi: 10.1093/annonc/mdm272
40. Tetteh EK, Morris S. Evaluating the administration costs of biologic drugs: Development of a cost algorithm. Health Econ Rev. Dec 2014;4(1):26. doi: 10.1186/s13561-014-0026-2
41. Van Horssen R, Ten Hagen TL, Eggermont AM. TNF-α in cancer treatment: Molecular insights, antitumor effects, and clinical utility. Oncologist. 2006;11(4):397-408. doi: 10.1634/theoncologist.11-4-397
42. Grünhagen DJ, de Wilt JH, Ten Hagen TL, Eggermont AM. Technology insight: Utility of TNF-α-based isolated limb perfusion to avoid amputation of irresectable tumors of the extremities. Nat Rev Clin Oncol. 2006;3(2):94-103. doi: 10.1038/ncponc0426
43. Thurber GM, Schmidt MM, Wittrup KD. Antibody tumor penetration: Transport opposed by systemic and antigen-mediated clearance. Adv Drug Deliv Rev. 2008;60(12):1421-34. doi: 10.1016/j.addr.2008.04.012
44. Chapman AP, Antoniw P, Spitali M, West S, Stephens S, King DJ. Therapeutic antibody fragments with prolonged *in vivo* half-lives. Nat Biotechnol. 1999;17(8):780-3. doi: 10.1038/11717
45. Kontermann RE. Strategies to extend plasma half-lives of recombinant antibodies. Biodrugs. 2009;23(2):93-109. doi: 10.2165/00063030-200923020-00003
46. Herrington-Symes AP, Farys M, Khalili H, Brocchini S. Antibody fragments: Prolonging circulation half-life special issue-antibody research. Adv Biosci Biotechnol. 2013;4(5):689-98. doi: 10.4236/abb.2013.45090
47. Badkar AV, Gandhi RB, Davis SP, LaBarre MJ. Subcutaneous delivery of high-dose/volume biologics: Current status and prospect for future advancements. Drug Des Devel Ther. 2021;15:159-70. doi: 10.2147/DDDT.S287323
48. Palm T, Eren S, Gandhi R, Khossravi M. The importance of the concentration-temperature-viscosity relationship for the development of biologics. Bioprocess Int. 2015;13:32-4.
49. Garidel P, Kuhn AB, Schäfer LV, Karow-Zwick AR, Blech M. High-concentration protein formulations: How high is high? Eur J Pharm Biopharm. 2017;119:353-60. doi: 10.1016/j.ejpb.2017.06.029
50. Frost GI. Recombinant human hyaluronidase (rHuPH20): An enabling platform for subcutaneous drug and fluid administration. Expert Opin Drug Deliv. 2007;4(4):427-40. doi: 10.1517/17425247.4.4.427
51. Wasserman RL. Recombinant human hyaluronidase-facilitated subcutaneous immunoglobulin infusion in primary immunodeficiency diseases. Immunotherapy. 2017;9(12):1035-50. doi: 10.2217/imt-2017-0092
52. Lugaresi A. Addressing the need for increased adherence to multiple sclerosis therapy: Can delivery technology enhance patient motivation? Expert Opin Drug Deliv. 2009;6(9):995-1002. doi: 10.1517/17425240903134769
53. Genovese MC, Covarrubias A, Leon G, Mysler E, Keiserman M, Valente R, *et al*. Subcutaneous abatacept versus intravenous abatacept: A phase IIIb noninferiority study in patients with an inadequate response to methotrexate. Arthritis Rheumatol. 2011;63(10):2854-64. doi: 10.1002/art.30463
54. Kaiser C, Knight A, Nordström D, Pettersson T, Fransson J, Florin-Robertsson E, *et al*. Injection-site reactions upon Kineret (anakinra) administration: Experiences and explanations. Rheumatol Int. 2012;32(2):295-9. doi: 10.1007/s00296-011-2096-3
55. Di Cantogno EV, Russell S, Snow T. Understanding and meeting injection device needs in multiple sclerosis: A survey of patient attitudes and practices. Patient Prefer Adherence. 2011;5:173-80. doi: 10.2147/PPA.S14903
56. Kuzman D, Bunc M, Ravnik M, Reiter F, Žagar L, Bončina M. Long-term stability predictions of therapeutic monoclonal antibodies in solution using Arrhenius-based kinetics. Sci Rep. 2021;11(1):20534. doi: 10.1038/s41598-021-99875-9
57. Bhatnagar BS, Bogner RH, Pikal MJ. Protein stability during freezing: Separation of stresses and mechanisms of protein stabilization. Pharm Dev Technol. 2007;12(5):505-23. doi: 10.1080/10837450701481157
58. Shire SJ. Formulation and manufacturability of biologics. Curr Opin Biotechnol. 2009;20(6):708-14. doi: 10.1016/j.copbio.2009.10.006
59. Thomas CR, Geer D. Effects of shear on proteins in solution. Biotechnol Lett. 2011;33(3):443-56. doi: 10.1007/s10529-010-0469-4
60. Mazzeo A, Carpenter P. Stability studies for biologics. In: Huynh-Ba K, editor. Handbook of Stability Testing in Pharmaceutical Development-Regulations, Methodologies, and Best Practices. New York: Springer Science+Business Media; 2009. p. 353-69. doi: 10.1007/978-0-387-85627-8_17
61. Murphy RM. Peptide aggregation in neurodegenerative disease. Annu Rev Biomed Eng. 2002;4(1):155-74. doi: 10.1146/annurev.bioeng.4.092801.094202
62. Jahn TR, Makin OS, Morris KL, Marshall KE, Tian P, Sikorski P, *et al*. The common architecture of cross-β amyloid. J Mol Biol. 2010;395(4):717-27. doi: 10.1016/j.jmb.2009.09.039
63. Riek R, Eisenberg DS. The activities of amyloids from a structural perspective. Nature. 2016;539(7628):227-35. doi: 10.1038/nature20416
64. Bowerman CJ, Nilsson BL. Review self-assembly of amphipathic β-sheet peptides: Insights and applications. Peptide Sci. 2012;98(3):169-84. doi: 10.1002/bip.22058
65. Trainor K, Broom A, Meiering EM. Exploring the relationships between protein sequence, structure and solubility. Curr Opin Struct Biol. 2017;42:136-46. doi: 10.1016/j.sbi.2017.01.004
66. Zapadka KL, Becher FJ, Gomes dos Santos AL, Jackson SE. Factors affecting the physical stability (aggregation) of peptide therapeutics. Interface Focus. 2017;7(6):20170030. doi:10.1098/rsfs.2017.0030
67. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, *et al*. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res. 2004;21(7):1274-83. doi: 10.1023/B: PHAM.0000033016.36825.2c
68. Wurth C, Guimard NK, Hecht MH. Mutations that reduce aggregation of the Alzheimer's Aβ42 peptide: An unbiased search for the sequence determinants of Aβ amyloidogenesis. J Mol Biol. 2002;319(5):1279-90. doi: 10.1016/S0022-2836(02)00399-6
69. Frokjaer S, Otzen DE. Protein drug stability: A formulation challenge. Nat Rev Drug Discov. 2005;4(4):298-306. doi: 10.1038/nrd1695
70. Krielgaard L, Jones LS, Randolph TW, Frokjaer S, Flink JM, Manning MC, *et al*. Effect of tween 20 on freeze-thawing- and agitation-induced aggregation of recombinant human factor XIII. J Pharm Sci. 1998;87(12):1597-603. doi: 10.1021/js980126i
71. Wang M, Li Y, Srinivasan P, Hu Z, Wang R, Saragih A, *et al*. Interactions between biological products and product packaging and potential approaches to overcome them. AAPS PharmSciTech. 2018;19(8):3681-6. doi: 10.1208/s12249-018-1184-z
72. Seidl A, Hainzl O, Richter M, Fischer R, Böhm S, Deutel B, *et al*. Tungsten-induced denaturation and aggregation of epoetin alfa during primary packaging as a cause of immunogenicity. Pharm Res. 2012;29(6):1454-67. doi: 10.1007/s11095-011-0621-4
73. Harrison B, Rios M. Big Shot: Developments in Prefilled Syringes. Vol. 31. PharmTech; 2007. p. 50-60. Available from: https://www.pharmtech.com/view/big-shot-developments-prefilled-syringes [Last accessed on 2024 May 04].
74. Jones LS, Kaufmann A, Middaugh CR. Silicone oil induced aggregation of proteins. J Pharm Sci. Apr 2005;94(4):918-27. doi: 10.1002/jps.20321
75. Crommelin DJ, Hawe A, Jiskoot W. Formulation of biologics including biopharmaceutical considerations. In: Crommelin D, Sindelar R, Meibohm B, editors. Pharmaceutical Biotechnology. Cham: Springer

Nature Switzerland AG; 2019. p. 83-103. doi: 10.1007/978-3-030-00710-2_5
76. Lam XM, Yang JY, Cleland JL. Antioxidants for prevention of methionine oxidation in recombinant monoclonal antibody HER2. J Pharm Sci. 1997;86(11):1250-5. doi: 10.1021/js970143s
77. Nguyen TH. Oxidation degradation of protein pharmaceuticals. In: Cleland JL, Langer R, editors. ACS Symposium Series 567, Formulation and Delivery of Proteins and Peptides. Washington DC: ACS; 1994. p. 59-71. doi: 10.1021/bk-1994-0567.ch004
78. Janecki DJ, Reilly JP. Denaturation of metalloproteins with EDTA to facilitate enzymatic digestion and mass fingerprinting. Rapid Commun Mass Spectrom. 2005;19(10):1268-72. doi: 10.1002/rcm.1924
79. Zhou S, Schöneich C, Singh SK. Biologics formulation factors affecting metal leachables from stainless steel. AAPS PharmSciTech. 2011;12(1):411-21. doi: 10.1208/s12249-011-9592-3
80. Yokota H, Saito H, Masuoka K, Kaniwa H, Shibanuma T. Reversed phase HPLC of Met58 oxidized rhIL-11: Oxidation enhanced by plastic tubes. J Pharm Biomed Anal. 2002;24(2):317-24. doi: 10.1016/S0731-7085(00)00419-2
81. Castner J, Benites P, Bresnick M. Leachables and extractables. In: Jameel F, Hershenson S, editors. Formulation and Process Development Strategies for Manufacturing Biopharmaceuticals. New Jersey: John Wiley and Sons; 2010. p. 857-80. doi: 10.1002/9780470595886.ch34
82. Gronemeyer P, Ditz R, Strube J. Trends in upstream and downstream process development for antibody manufacturing. Bioengineering. 2014;1(4):188-12. doi: 10.3390/bioengineering1040188
83. Tripathi NK, Shrivastava A. Recent developments in bioprocessing of recombinant proteins: Expression hosts and process development. Front Bioeng Biotechnol. 2019;7:420. doi: 10.3389/fbioe.2019.00420
84. Roch P, Mandenius CF. On-line monitoring of downstream bioprocesses. Curr Opin Chem Eng. 2016;14:112-20. doi: 10.1016/j.coche.2016.09.007
85. Azevedo AM, Rosa PA, Ferreira IF, Aires-Barros MR. Chromatography-free recovery of biopharmaceuticals through aqueous two-phase processing. Trends Biotechnol. 2009;27(4):240-7. doi: 10.1016/j.tibtech.2009.01.004
86. Rathore AS, Kapoor G. Application of process analytical technology for downstream purification of biotherapeutics. J Chem Technol Biotechnol. 2015;90(2):228-36. doi: 10.1002/jctb.4447
87. Sarkis M, Bernardi A, Shah N, Papathanasiou MM. Emerging challenges and opportunities in pharmaceutical manufacturing and distribution. Processes. 2021;9(3):457. doi: 10.3390/pr9030457
88. Szkodny AC, Lee KH. Biopharmaceutical manufacturing: Historical perspectives and future directions. Annu Rev Chem Biomol Eng. 2022;13(1):141-65. doi: 10.1146/annurev-chembioeng-092220-125832
89. Ter Horst JP, Turimella SL, Metsers F, Zwiers A. Implementation of quality by design (QbD) principles in regulatory dossiers of medicinal products in the European Union (EU) between 2014 and 2019. Ther Innov Regul Sci. 2021;55(3):583-90. doi: 10.1007/s43441-020-00254-9
90. Rathore AS. QbD/PAT for bioprocessing: Moving from theory to implementation. Curr Opin Chem Eng. 2014;6:1-8. doi: 10.1016/j.coche.2014.05.006
91. Eon-Duval A, Broly H, Gleixner R. Quality attributes of recombinant therapeutic proteins: An assessment of impact on safety and efficacy as part of a quality by design development approach. Biotechnol Prog. 2012;28(3):608-22. doi: 10.1002/btpr.1548
92. Rathore AS. Quality: Design space for biotech products. Biopharm Int. 2007;20(4):40-5.
93. Rathore AS, Singh SK, Kumar J, Kapoor G. Implementation of QbD for manufacturing of biologics-has it met the expectations? In: Jagschies G, Lindskog E, Łącki K, Galliher P, editors. Biopharmaceutical Processing - Development, Design and Implementation of Manufacturing Processes. Amsterdam: Elsevier; 2018. p. 1051-73. doi:10.1016/B978-0-08-100623-8.00048-7
94. Kozlowski S, Swann P. Considerations for biotechnology product by design. In: Rathore AS, Mhatre R, editors. Quality by Design for Biopharmaceuticals: Principles and Case Studies. New Jersey: John Wiley and Sons Inc.; 2009. p. 9-30. doi: 10.1002/9780470466315.ch2
95. Kelley B. Quality by Design risk assessments supporting approved antibody products. MAbs. 2016;8(8):1435-6. doi: 10.1080/19420862.2016.1232218
96. Kokil S, Singh S. Current perspective on opportunities and adoption challenges of QbD implementation in pharmaceutical product development. Invent Rapid Pharm Process Dev. 2016;2:1-8.
97. Milmo S. Quality by design bridging the gap between concept and implementation. Biopharm Int. 2014;29(4):40-5.
98. Sykes C. Time-and temperature-controlled transport: Supply chain challenges and solutions. P T. 2018;43(3):154-70. PMID: 29491697
99. Papathanasiou MM, Stamatis C, Lakelin M, Farid S, Titchener-Hooker N, Shah N. Autologous CAR T-cell therapies supply chain: Challenges and opportunities? Cancer Gene Ther. 2020;27(10-1):799-809. doi: 10.1038/s41417-019-0157-z
100. Ned P, Lopez E. Top challenges facing drug supply chains. Biopharmdrive; 2018. Available from: https://www.biopharmadive.com/news/top-challenges-facing-drug-supply-chains/521876 [Last accessed on 2024 May 04].
101. Jaffer G. Pharma's Almanac. Keys to Successful Storage, Management and Transport of Biological Materials; 2020. Available from: https://www.pharmasalmanac.com/articles/keys-to-successful-storage-management-and-transport-of-biological-materials [Last accessed on 2024 May 04].
102. Tetz A. The Evolution of Smart Temperature-controlled Packaging. Packaging Europe; 2017. Available from: https://packagingeurope.com/the-evolution-of-smart-temperature-controlled-packaging/2958.article [Last accessed on 2024 May 04].
103. Ibrahim T, Araujo CA. Biopharmaceutical supply chain challenges in developing countries: an exploratory analysis. Suppl Chain Forum Int J. 2021;22(4):294-309. doi: 10.1080/16258312.2021.1936152
104. Zobel A, Heelan B. Regulatory, Clinical and Logistics Challenges of Advanced Therapy Medicinal Products (ATMPs) in clinical research. European Pharmaceutical Review; 2017. Available from: https://www.europeanpharmaceuticalreview.com/article/81122/regulatory-clinical-and-logistics-challenges-of-advanced-therapy-medicinal-products-atmps-in-clinical-research [Last accessed on 2024 May 04].
105. Penugonda VL, Cherukuri VP, Vaka NS, Juturi RK. Development of biosimilars: An overview of the regulatory framework in India, USA, EU; major challenges and related case studies. Int J Pharm Sci Res. 2021;12(12):6341-52. doi: 10.13040/IJPSR.0975-8232.12(12).6341-52
106. Chisholm O, Critchley H. Future directions in regulatory affairs. Front Med. 2023;9:1082384. doi: 10.3389/fmed.2022.1082384
107. Druedahl LC, Almarsdóttir AB, Sporrong SK, De Bruin ML, Hoogland H, Minssen T, *et al*. A qualitative study of biosimilar manufacturer and regulator perceptions on intellectual property and abbreviated approval pathways. Nat Biotechnol. 2020;38(11):1253-56. doi: 10.1038/s41587-020-0717-7
108. Lokesh C, Sonia K, Kamaraj R. Regulatory requirements for biological product approval process in India. Res J Pharm Technol. 2020;13(2):1050-4. doi: 10.5958/0974-360X.2020.00193.6
109. Senior M. Fresh from the biotech pipeline: Fewer approvals, but biologics gain share. Nat Biotechnol. 2023;41(2):174-82. doi: 10.1038/s41587-022-01630-6
110. Sekhon BS, Saluja V. Biosimilars: An overview. Biosimilars 2011;1:1-11. doi: 10.2147/BS.S16120
111. Vugmeyster Y, Xu X, Theil FP, Khawli LA, Leach MW. Pharmacokinetics, and toxicology of therapeutic proteins: Advances and challenges. World J Biol Chem. 2012;3(4):73-92. doi: 10.4331/wjbc.v3.i4.73
112. Wang J, Iyer S, Fielder PJ, Davis JD, Deng R. Projecting human pharmacokinetics of monoclonal antibodies from nonclinical data: Comparative evaluation of prediction approaches in early drug development. Biopharm Drug Dispos. 2016;37(2):51-65. doi: 10.1002/bdd.1952
113. Acri KM, Lybecker N. Biologics and Biosimilars: A Primer. Fraser Institute; 2020. Available from: https://www.fraserinstitute.org/sites/default/files/biologics-and-biosimilars-a-primer.pdf [Last accessed on 2024 May 04].
114. Schneider CK, Kalinke U. Toward biosimilar monoclonal antibodies. Nat Biotechnol. 2008;26(9):985-90. doi: 10.1038/nbt0908-985
115. Negrusz-Kawecka M. The role of TNF-alpha in the etiopathogenesis of heart failure. Pol Merkur Lekarski. 2002;12(67):69-72. PMID: 11957809
116. Friganović A, Mędrzycka-Dąbrowska W, Krupa S, Oomen B, Decock N, Stievano A. Nurses' knowledge and attitudes towards biosimilar medicines as part of evidence-based nursing practice-international pilot study within the project biosimilars nurses guide version 2.0. Int J Environ Res Public Health. 2022;19(16):10311. doi: 10.3390/ijerph191610311
117. Cohen H, Beydoun D, Chien D, Lessor T, McCabe D, Muenzberg M, *et al*. Awareness, knowledge, and perceptions of biosimilars among specialty physicians. Adv Ther. 2016;33(12):2160-72. doi: 10.1007/s12325-016-0431-5
118. Leonard E, Wascovich M, Oskouei S, Gurz P, Carpenter D. Factors affecting health care provider knowledge and acceptance of biosimilar

medicines: A systematic review. J Manag Care Spec Pharm. 2019;25(1):102-12. doi: 10.18553/jmcp.2019.25.1.102
119. Dylst P, Vulto A, Simoens S. Barriers to the uptake of biosimilars and possible solutions: A Belgian case study. Pharmacoeconomics. 2014;32(7):681-91. doi: 10.1007/s40273-014-0163-9
120. Pasina L, Casadei G, Nobili A. A survey among hospital specialists and pharmacists about biosimilars. Eur J Intern Med. 2016;35:e31-3. doi: 10.1016/j.ejim.2016.07.010
121. O'Callaghan J, Bermingham M, Leonard M, Hallinan F, Morris JM, Moore U, *et al*. Assessing awareness and attitudes of healthcare professionals on the use of biosimilar medicines: A survey of physicians and pharmacists in Ireland. Regul Toxicol Pharmacol. 2017;88:252-61. doi: 10.1016/j.yrtph.2017.06.013
122. Adé A, Bourdon O, Bussières JF. A survey of pharmacists' knowledge and views of biosimilars in Quebec and France. Ann Pharm Fr. 2017;75(4):267-75. doi: 10.1016/j.pharma.2017.01.003
123. Gahlot P, Dhankhar R, Yadav P, Vigarniya MM. Challenges of biomedical waste management. Ann Biol. 2019;35(2):191-200.
124. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: Formulation and delivery strategies. Nat Rev Drug Discov. 2014;13(9):655-72. doi: 10.1038/nrd4363
125. Baumann A. Preclinical development of therapeutic biologics. Expert Opin Drug Discov. 2008;3(3):289-97. doi: 10.1517/17460441.3.3.289
126. Baumann A. Nonclinical development of biopharmaceuticals. Drug Discov Today. 2009;14(23-4):1112-22. doi: 10.1016/j.drudis.2009.09.013
127. Giezen TJ, Mantel-Teeuwisse AK, Meyboom RH, Straus SM, Leufkens HG, Egberts TC. Mapping the safety profile of biologicals. Drug Saf. 2010;33(10):865-78. doi: 10.2165/11538330-000000000-00000
128. Ryan AM. Frontiers in nonclinical drug development. Vet Pathol. 2015;52(2):419-26. doi: 10.1177/0300985814547282
129. DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, *et al*. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharm Res. 2003;20(11):1885-900. doi: 10.1023/B: PHAM.0000003390.51761.3d
130. Bareham B, Georgakopoulos N, Matas-Céspedes A, Curran M, Saeb-Parsy K. Modeling human tumor-immune environments *in vivo* for the preclinical assessment of immunotherapies. Cancer Immunol Immunother. 2021;70:2737-50. doi: 10.1007/s00262-021-02897-5
131. Chakraborty R, Darido C, Liu F, Maselko M, Ranganathan S. Head and neck cancer immunotherapy: Molecular biological aspects of preclinical and clinical research. Cancers. 2023;15(3):852. doi: 10.3390/cancers15030852
132. Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FD. 3D cell culture systems: Advantages and applications. J Cell Physiol. 2015;230(1):16-26. doi: 10.1002/jcp.24683
133. Belfiore L, Aghaei B, Law AM, Dobrowolski JC, Raftery LJ, Tjandra AD, *et al*. Generation and analysis of 3D cell culture models for drug discovery. Eur J Pharm Sci. 2021;163:105876. doi: 10.1016/j.ejps.2021.105876
134. Moysidou CM, Barberio C, Owens RM. Advances in engineering human tissue models. Front Bioeng Biotechnol. 2021;8:620962. doi: 10.3389/fbioe.2020.620962
135. Freires IA, Morelo DF, Soares LF, Costa IS, de Araújo LP, Breseghello I, *et al*. Progress and promise of alternative animal and non-animal methods in biomedical research. Arch Toxicol. 2023;97(9):2329-42. doi: 10.1007/s00204-023-03532-1
136. Tuveson D, Clevers H. Cancer modeling meets human organoid technology. Science. 2019;364(6444):952-5. doi: 10.1126/science.aaw6985
137. Belair DG, Visconti RJ, Hong M, Marella M, Peters MF, Scott CW, *et al*. Human ileal organoid model recapitulates clinical incidence of diarrhea associated with small molecule drugs. Toxicol *In Vitro*. 2020;68:104928. doi: 10.1016/j.tiv.2020.104928
138. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, *et al*. Patient-derived xenograft models: An emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998-1013. doi: 10.1158/2159-8290.CD-14-0001
139. Hanna J, Hossain GS, Kocerha J. The potential for microRNA therapeutics and clinical research. Front Genet. 2019;10:478. doi: 10.3389/fgene.2019.00478
140. Catenacci DV. Next-generation clinical trials: Novel strategies to address the challenge of tumor molecular heterogeneity. Mol Oncol. 2015;9(5):967-96. doi: 10.1016/j.molonc.2014.09.011