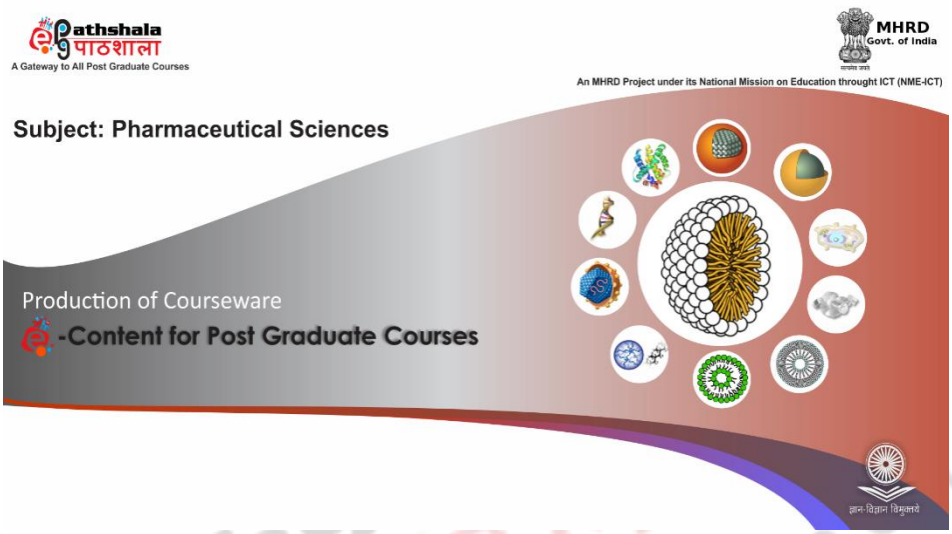

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Pharmaceutical sciences

Biopharmaceutics and Pharmacokinetics

Clinical Trials of Biopharmaceuticals

INTRODUCTION TO BIO-PHARMACEUTICALS

Bio-pharmaceuticals are medical drugs produced using biotechnology

Bio-pharmaceuticals are nucleic acids (DNA, RNA or antisense oligonucleotides) or proteins **(antibodies), used for therapeutic and/or diagnostic purposes. These are prepared from an original non-engineered biological source.** Biopharmaceuticals drugs structurally mimics compounds found within the body and are produced by applying biotechnologies. These have the potential to cure diseases rather than merely treat symptoms, and have fewer side effects because of their specificity.

The large majority of biopharmaceutical products are pharmaceuticals that are derived from life forms. Small molecule drugs are not typically regarded as biopharmaceutical in nature by the industry. However members of the press and the business and financial community often extend the definition to include pharmaceuticals not created through biotechnology. That is, the term has become an oft-used buzzword for a variety of different companies producing new, apparently high-tech pharmaceutical products.

When a biopharmaceutical is developed, the company will typically apply for a patent, which is a grant for exclusive manufacturing rights. This is the primary means by which the developer of the drug can recover the investment cost for development of the biopharmaceutical. The patent laws in the United States and Europe differ somewhat on the requirements for a patent, with the European requirements are perceived as more difficult to satisfy. The total number of patents granted for biopharmaceuticals has risen significantly since the 1970s. In 1978 the total patents

granted was 30. This had climbed to 15,600 in 1995, and by 2001 there were 34,527 patent applications.

Within the United States, the Food and Drug Administration (FDA) exerts strict control over the commercial distribution of a pharmaceutical product, including biopharmaceuticals. Approval can require several years of clinical trials, including trials with human volunteers. Even after the drug is released, it will still be monitored for performance and safety risks.

The manufacture of the drug must satisfy the "current Good Manufacturing Practices" regulations of the FDA. They are typically manufactured in a clean room environment with set standards for the amount of airborne particles.

Biopharmaceuticals are drugs which are produced with the means of biotechnology. There are a number of ways in which such drugs can be made, but the key distinction between them and other drug is that they are not extracted from a native source or synthesized with chemical reactions. Instead, they are created with the use of living organisms which may have been modified to produce the desired compound. This requires the use of specialized equipment and clean rooms for safety which protect the integrity of the pharmaceutical compounds while they are produced and packaged.

One classic method of making biopharmaceuticals involves the use of a bioreactor, a container which is used to create tightly controlled conditions which facilitate the growth of a particular organism. In a bioreactor, drugs can be produced by organisms which generate

biopharmaceuticals as a byproduct of their life cycle, often because these organisms have been modified to produce specific proteins and nucleic acids. Cell cultures and modified microbes can both be used in bioreactors to make drugs and compounds which can be used in the production of pharmaceuticals.

Genetic modification of plants and animals may also be used to make biopharmaceuticals. Transgenic cows may be designed, for example, to secrete a specific compound in their milk. The practice of using transgenic organisms for the production of pharmaceuticals has been controversial in some regions of the world, for reasons varying from ethical concerns to worries that such organisms could cross-breed with conventional organisms and become contaminated.

A variety of substances can be made using biopharmaceutical techniques, including blood factors, interferons, hormones, vaccines, and monoclonal antibodies. When researchers develop new biopharmaceuticals, they typically file for patents to protect their inventions and the process, and go through a series of steps to seek approval so that their drugs can be sold on the open market. These steps involve extensive testing for safety and efficacy, to confirm that the drugs work as claimed.

The first biopharmaceutical to hit the market was artificial human insulin, which was released in 1982 for use by diabetics. The biopharmaceuticals industry exploded after the 1980s, thanks to increasing interest in additional medical treatments, and advancements in laboratory science which made new developments possible. One advantage to such drugs, especially as an

alternative to native compounds, is that they tend to be safer and the dosing is extremely reliable, because the production conditions are very tightly controlled.

BIOSIMILARS

Biosimilar is a biological medical product that is nearly identical to an original product manufactured by a different company. Biosimilars are approved versions of original "innovator" products, and can be manufactured only after the original product's patent expiration. Biosimilars generally exhibit high molecular complexity, and may be sensitive to changes in manufacturing processes, unlike with generic drugs (small molecule type). Follow-on manufacturers have access to the commercialized "innovator" product but not to the originator's original cell bank and molecular clone, nor to the active drug substance, nor to the exact fermentation and purification process. Therefore, it is harder to establish fungibility between innovators and generics among biologics than it is among totally synthesized and semi-synthesized drugs, thereby the name "biosimilar" was coined to distinguish these drugs from small-molecule generics.

Drug related authorities such as Food and Drug Administration (FDA), European Medicines Agency (EMA) and Health Canada hold their own set of criterion for demonstration of the similar nature of two biological products in terms of efficacy and safety. According to these authorities, analytical studies establish that the biological

product is quite similar to the reference product notwithstanding minor differences in animal studies (including the assessment of toxicity), a clinical study/studies (including the assessment of pharmacokinetics or pharmacodynamics and immunogenicity) and clinically inactive components are sufficient to demonstrate purity, safety and potency in one or more suitable conditions of use for which the reference product is approved, licensed and intended to be used.

EMA has granted a marketing authorization for only a few biosimilars since 2006 including a monoclonal antibody. Meanwhile, on March 6, 2015, the FDA approved the United States's first biosimilar product, the biosimilar of Filgrastim (trade name Zarxio) by Sandoz. In case of a monoclonal antibody containing medicinal product, such as Remsima, extensive biological and physicochemical characterization for it and its reference product Remicade was conducted in order to demonstrate their highly similar properties.

BACKGROUND

Development of *in vitro* biological production systems and cloning of human genetic material has permitted the production of any recombinant DNA based biological substance to be developed as a drug. Recombinant DNA technology in combination with Monoclonal

antibody technology has flagged the way for tailor-made targeted medicines. Cell- and gene therapies are evolving as new approaches.

Recombinant therapeutic proteins are of a compound nature (composed of a long chain of amino acids, modified amino acids, folded by complex mechanisms, derivatized by sugar moieties,). These proteins are derived from living cells (animal/human cell lines, bacteria/yeast). The definitive characteristics of a drug containing a recombinant therapeutic protein are determined by the process through which they are produced: development of the genetically modified cell for production, choice of the cell type, production process, formulation of the therapeutic protein into a drug, purification process, etc.

After the expiry of the patent of approved recombinant products (e.g., insulin, interferons, human growth hormone, erythropoietin, monoclonal antibodies and many more) any other company can develop and market these biosimilars. Every biopharmaceutical products or biological display a certain degree of inconsistency, even between different batches of the same product, which is because of the inherent variability of the manufacturing process and the biological expression system. Any kind of reference product has undergone numerous changes in its manufacturing processes, and such changes (ranging from new purification methods, change in the supplier of cell culture media or new manufacturing sites) were verified with proper data and was approved by

the EMA. However, in case of biosimilars, it is mandatory to take a both clinical and non-clinical test that enable the detection of differences between the two products in terms of human pharmacokinetics (PK) and pharmacodynamics (PD), immunogenicity, safety and efficacy.

Currently, the concept of development of biosimilar monoclonal antibodies follow the principle that an extensive state of the art analytical, physico-chemical and functional comparison of the molecules is supplemented by comparative clinical and non-clinical data that demonstrate equivalent safety and efficacy in a clinical "model" that is considered to be most sensitive to detect any minor differences (if any) between biosimilar and its reference monoclonal antibody.

EMA established the fact that, whilst biosimilar products are similar to the original product, they are not exactly the same. Every biological exhibit a certain degree of variability. However, if function and structure, pharmacodynamic effects pharmacokinetic profiles and/or efficacy are comparable for the biosimilar and the reference product, the adverse drug reactions related to exaggerated pharmacological effects can be expected with similar frequencies.

Complexity of biological molecules raised the requests for substantial safety and efficacy data for a biosimilar approval. This has been gradually replaced with a greater dependence on assays that show sensitivity sufficient to detect any significant modification in

dose. However, the safe application of biologics depends on an appropriate and informed use by patients and healthcare professionals. A specific designed pharmacovigilance plan is required for introduction of biosimilars. It is costly and difficult to re-form biologics because the complex proteins are derived from living organisms that are modified genetically. In contrast, small molecule drugs made up of a chemically based compound are considerably less expensive to reproduce and can be easily simulated. Biosimilars must demonstrate close to identity to the parent "innovator" biologic product (based on data compiled through animal, clinical, analytical studies and conformational status) before its release to the general public use.

Once a drug is released in the market by FDA, it should be re-evaluated for its efficacy and safety after every six months for the first and second years. Subsequently, re-evaluations are conducted yearly, and the result of the assessment should be reported to authorities such as FDA. Biosimilars are also required to undergo pharmacovigilance regulations as its reference product. Therefore, biosimilars approved by EMA are required to submit a risk management plan (RMP) along with the marketing application and should provide regular safety update reports after the product is marketed. The RMP includes the prospective pharmacovigilance studies and safety profile of the drug.

Several PK studies, such as studies conducted by CHMP (Committee for Medicinal Products for Human Use) have been conducted under various conditions such as antibodies

from an originator's product versus antibodies from a biosimilar, combination therapy and monotherapy, various diseases, etc. with the purpose to validate comparability in pharmacokinetics of the biosimilar with the reference medicinal product in a sufficiently homogeneous and sensitive population.

Promotion of bio-pharmaceuticals as an avant-garde class of therapeutic agents has been majorly contributed by the advancements in the field of biotechnology and genetic research. The term 'bio-pharmaceutical' was first used in the 1980s to describe the class of **preventive or therapeutic medicines that are formulated by the application of recombinant DNA technology on living cells. The diverse family of bio-pharmaceuticals include peptides, vaccines, hormones, cytokines, antisense drugs, monoclonal antibodies, enzymes and cell therapies.**

In 1982, first product in this category 'human' insulin was developed by Genentech entered the international markets under the trade name of 'Humulin' manufactured and marketed by Eli Lily and Company. High potency and efficacy clubbed with fewer side effects and ability to treat the diseases rather than merely the symptoms, presented an array of advantages that drives the large-scale production of bio-pharmaceuticals. Furthermore, the number of life threatening diseases cured with these bio-pharmaceuticals is increasing day by day. **More than three hundred bio-pharmaceutical products have been approved till date and many more are in different stages of clinical development.** According to International Market Analysis Research and Consulting Group's new report "Global Biopharmaceutical Market Report (2010-2015)", the

global bio-pharmaceutical market is expected to reach levels of sales figure worth more than US$ 167 Billion by 2015.

The European regulatory authorities adopted a specific approval procedure to authorize ensuing versions of previously approved biologics, also termed as “biosimilars” or "similar biological medicinal products". This procedure is based on an exhaustive demonstration of comparability of a "biosimilar" product to an existing approved product. However, in United States, the Food and Drug Administration (FDA) decided that new legislation was required to enable them to approve “biosimilars” to those biologics originally approved through the PHS Act pathway.

On March 23, 2010, President Obama signed Patient Protection and Affordable Care Act as part of which US FDA gained the authority to approve biosimilars. FDA has previously approved biologic products using comparability between the a new biosimilar and an originally approved product, for example, Omnitrope (May 2006), was similar to Genotropin, formerly approved as a biologic drug under the FD&C Act.

Under the BPCI (Biologics Price Competition and Innovation) Act of 2009, a biologic that has been approved as an “interchangeable” may be substituted for the reference product without the intrusion of the health care provider. For example, Sandoz’s Zarxio is biosimilar to Amgen’s Neupogen (filgrastim), which was originally licensed in 1991. This is the first product to be passed under the BPCI Act. But Zarxio was approved as a

biosimilar, not as an interchangeable product by FDA in March 2015. FDA held that its approval of Zarxio is based on review of evidence that included functional and structural characterization, human pharmacokinetic and pharmacodynamics data, animal study data, clinical immunogenicity data and other clinical safety and effectiveness data that signifies Zarxio is biosimilar to Neupogen.

HOW BIO-PHARMACEUTICALS ARE DIFFERENT FROM CHEMICAL-BASED DRUGS

Compared with other types of pharmaceutical products, products derived from a biological source or a biotechnological process are structurally complex and involve manufacturing processes that require tight control to ensure their safety, quality, and efficacy. Biological products, because of their sheer size, are orders of magnitude more complicated than small - molecule drugs. This can be seen by a comparison of molecular weight, which can be used as a measure of the size of a given product. Moreover, the product arising from the manufacturing process is often not a pure, homogeneous mixture. Rather, various forms of these molecules are usually present in the final product.

Biopharmaceuticals are fundamentally different from the conventional small molecule chemical drugs in the average size of the two types of drugs. The chemically synthesized products are known as “small molecules” drugs (e.g. aspirin, molecular weight 180 Da). In general, the biopharmaceuticals are complex macromolecules that are over 100 times larger (e.g. interferon

beta, molecular weight 19,000 Da) with complex structural and appropriate biological activity requirements. Biopharmaceuticals have more potential heterogeneity than small molecule drugs. The large majority of biopharmaceutical products are derived from life forms. Small molecule drugs are not typically regarded as biopharmaceutical in nature by the industry. The nature of the manufacturing process, and the safety and efficacy profile of biopharmaceutical products are also different. **First generation biopharmaceutical products majorly include simple replacement proteins having identical amino acid sequence to that of a native human protein and non-engineered murine monoclonal antibodies. Second generation products comprise of modern biopharmaceuticals that are engineered. To generate novel fusion proteins, several engineering approaches entailing alteration of glycol component of a glycosylated protein, alteration of amino acid sequence or the covalent attachment of chemical moieties such as polyethylene glycol have been applied that could ultimately result into improved pharmacokinetic profile and immunological properties.**

REGULATORY CHALLENGES EMERGING FROM THE ARRIVAL OF BIOSIMILARS

From the above it becomes clear that biopharmaceuticals may be associated with unique safety concerns. The unpredictability of safety concerns of biopharmaceuticals triggered increased regulatory interest as a result of the arrival of so-called ‘biosimilars’. The first wave of biopharmaceuticals that were approved in 1980s and 1990s, including recombinant human

growth hormone, insulin and erythropoietin, have lost, or are about to lose their patent protection. For small molecules the generic paradigm applies, allowing a product to receive regulatory authorization based on an abbreviated dossier demonstrating comparable quality and pharmacokinetics. If two small molecule pharmaceutical products contain the same active ingredient and their bio-availabilities lie within predefined limits, they are considered 1 10 therapeutically equivalent, i.e. it is assumed that they have the same efficacy and safety profile of the originator product and they are deemed interchangeable. In practice this means competing versions of small molecules are authorized after performing comparative quality studies and pharmacokinetic studies. This is possible, because small molecules can be fully characterized and their manufacturing process is well controlled. On the contrary, biopharmaceuticals are complex mixtures of large and intricate molecules that cannot be fully characterized using existing analytical methods. Due to variability in the manufacturing process, it is not possible to produce two identical products. In the European Union, legislation and regulation has been introduced to allow the approval of competing version of these products, so-called biosimilars, based on a reduced dossier. The arrival of biosimilars has posed challenges to regulators, both pre- and post-authorization. All biopharmaceuticals are manufactured under strictly controlled conditions. However, differences in production processes between an innovator product and a biosimilar product, including the use of different cell lines and growth conditions may result in subtle differences between two products that potentially have a major impact on the efficacy and safety of a product. Therefore, besides providing adequate quality studies and comparative

pharmacokinetic data, comparative clinical studies are required to ensure that a biosimilar product has a comparable efficacy and safety profile of the innovator product. However, as biosimilars are not exact copies of the innovator products, uncertainties remain whether biosimilars can be safely exchanged with innovator products and whether current pharmacovigilance systems are capable of identifying specific safety issues associated with biosimilars. Current European regulations require additional post authorization activities for biosimilar products. Every biosimilar needs to prepare a so-called risk management plan detailing risk identification and minimization activities. Nevertheless, uncertainties about safety of biosimilars have caused concerns with prescribing physicians, which may have resulted in an initially slow uptake of biosimilars. However, the use of biosimilars is growing and the interest of developing biosimilars is increasing as the US is finalizing its regulatory requirements for establishing biosimilarity. The growth of biosimilars is expected to increase as several widely used biopharmaceuticals, mainly monoclonal antibodies, are about to lose patent protection. The European biosimilar regulatory framework has been in place now for several years and 13 products have received marketing authorization. As experience in increasing with biosimilars, it is time to evaluate how current regulatory requirements are coping with the challenges posed by these group biopharmaceuticals. Studying the role of regulation in drug innovation Pharmaceutical regulators have a dual responsibility to, on the one hand protect and promote public health and on the other, to facilitate pharmaceutical innovation. These different roles may conflict as different stakeholders require regulators to either take a more stringent or less

stringent approach. Over the years various regulatory tools and methods to manage the benefits and risks of medicinal products throughout their lifecycles have become established. Legislation has laid down requirements to establish quality, efficacy and safety of medicinal products during their development phase. In addition, regulatory oversight of post authorization pharmacovigilance activities is needed to ensure that the benefits of a product continue to outweigh its risks in real life patient populations. All these measures, whether they are scientific guidelines, risk management requirements or product labeling, intend to generate the optimal amount of information needed to assess the benefits and risks of medicinal products, throughout their product lifecycle. While regulatory requirements may be effective in the goals they aim to achieve, they also add to the burden of developing new medicines. The effect of pharmaceutical regulation on pharmaceutical innovation has been the subject of intense debate for many years although in recent years it seems that the debate has intensified. The number of new drugs entering the clinic is not increasing despite increased spending on research and development by the pharmaceutical industry, a situation that has been called the 'innovation paradox'. Several reasons have been brought forward to explain this. The 'low hanging fruit' has been picked, meaning that treatments have been developed for the less complex diseases, whereas the remaining diseases with the most unmet medical need are of great complexity. New drugs have to compete with a high standard of care, making it ever more challenging to develop new approvable drugs. Also, the strategies employed by pharmaceutical companies to develop new drugs may not be suitable to develop the drugs that society requires. Another frequently cited

cause for this situation is the increase in regulatory demands, not only in bringing a drug on the market, but also in keeping it there. Several commentators have accused regulators for becoming too risk averse and of creating too many barriers for pharmaceutical innovation and thus depriving patients of much needed medicines. One of the responses to the criticism on the regulatory system has been an increasing investment in 'regulatory science'. Regulatory science has been defined as the science of developing new tools, standards and approaches to assess the safety, efficacy, quality and performance of [pharmaceutical] products. It facilitates bringing products to the market through the "[development of] new tools, standards, and approaches that efficiently and consistently assess the safety, efficacy, quality, and performance of products". In addition, it has been argued that regulatory science should evaluate the performance of the regulatory system itself in achieving its objectives of improving public health, ensuring patient safety and stimulating innovation.

CLINICAL TRIALS OF BIO-PHARMACEUTICALS

"A clinical trial is an experiment testing a medical treatment on human subjects."--- Piantadosi

The participation of humans means that we must execute every aspect of clinical research to the highest ethical standards, and protection of participants' welfare is our paramount concern. Regulatory agencies worldwide govern how trials are to be conducted. These agencies include the FDA, the European Medicines Agency (EMA), and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA). **ICH (International Conference on Harmonisation of**

Technical Requirements for Registration of Pharmaceuticals for Human Use) provides a single global platform to biopharmaceutical industry from different countries and regulatory agencies to have detailed discussions over technical and scientific aspects for drug registration and market approval. Its mission is "to achieve greater harmonisation to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner." Biopharmaceutical clinical trials are typically classified into various phases, with any given trial being identified as belonging to one of them. A common system includes four temporal phases: phases I, II, III, and IV. However, as ICH Guideline E812 noted, "It is important to recognise that the phase of development provides an inadequate basis for classification of clinical trials because one type of trial may occur in several phases." The guideline provides an alternate 4-item system, one that employs more informative nomenclature relating to study objectives. This system includes human pharmacology, therapeutic exploratory, therapeutic confirmatory, and therapeutic use trials. Guideline E8 hence combines these systems as indicated by the headings of the next 4 sections.

Phase I (Human Pharmacology)

An enormous amount of work spanning several years is completed in the new drug development process before clinical trials commence. This work comprises a drug's nonclinical development program. Nonclinical development comprises *in silico*, *in vitro*, *ex vivo*, and *in vivo* testing, including investigations conducted at the intracellular, cellular, isolated tissue, isolated organ,

and intact animal levels. No animal model is a perfect predictor of the precise effects of a drug in humans, but these data nonetheless constitute the rational basis for determining what drug doses we administer to the individuals participating in first-in-human clinical trials. Human pharmacology trials assess the safety of the drug, obtain a thorough knowledge and understanding of the drug's pharmacokinetic profile and potential interactions with other drugs (drug-drug interactions), and estimate pharmacodynamics activity. Clinical pharmacologists typically conduct these trials. They include relatively small numbers of participants, but a lot of assessments are completed for each one. Acute single-dose studies are conducted first. Short-term studies of various doses are then conducted, followed by longer-term studies of various doses. Eventually, dose-finding studies are conducted to determine the maximum tolerated dose of the drug. Human pharmacology trials are informative about providing answers to questions concerning any side effects, their characteristics, and whether they are consistent to any notable degree across participants.

Phase II (Therapeutic Exploratory)

Participants in these trials have the disease or condition of clinical concern, e.g., hypertension, thus facilitating initial assessments of a drug's safety and efficacy in the intended patient population. They are conducted by researchers trained in clinical trial methodology and operational execution. Often, several hundred participants take part in these trials. Some of them receive the drug being developed (the investigational drug), and some receive a control

treatment, which can be a placebo or an active comparator. The nature of these trials is therefore comparative, since responses to the drug are compared with responses to the control treatment to investigate the drug's comparative efficacy. Some authors have voiced the opinion that these trials provide the most accurate assessment of efficacy, since they are conducted in an extremely tightly controlled manner. While we agree, however, this environment is not typical of those in which the drug will eventually be used if approved. Therapeutic confirmatory and therapeutic use trials provide more realistic assessments regarding the drug's benefit to large numbers of patients in real-world therapeutic settings.

Phase III (Therapeutic Confirmatory)

Therapeutic confirmatory studies are conducted as Randomized Clinical Trials (RCTs). Several thousand participants with the disease or clinical condition for which the drug is being developed take part. These trials are often required to specifically include certain subgroups of participants that are representative of patients who will receive them in clinical practice if the drug is approved. For example, EMA's 2010 guideline for the development of new antihypertensive agents states that the number of participants 75 years and older "should be sufficient to assess both efficacy and safety in this group and specific attention should be paid to them." As for therapeutic exploratory trials, the nature of these trials is comparative. The drug's treatment effect, the representation of the drug's efficacy, is calculated as "mean response to the drug treatment minus mean response to the control treatment." An example of a therapeutic

confirmatory study in the field of hypertension was published by Bakris and colleagues. This study was an RCT comparing the single-pill combination of azilsartanmedoxomil and chlorthalidonevs co-administration of azilsartanmedoxomil and hydrochlorothiazide in participants with stage 2 primary hypertension. It was conducted as part of a phase III program in support of a marketing application for the single-pill combination. Additional safety data are also gained from trials like this, adding to the safety data portfolio already accumulated. Upon completion of therapeutic confirmatory trials, sponsors submit a marketing application to regulatory agencies. If an agency determines that there is compelling evidence of beneficially balanced safety and efficacy, it will approve the drug for use in its jurisdiction.

Phase IV (Therapeutic Use)

Therapeutic use studies are conducted once the drug is on the market. They may be optional studies, or studies required by a regulatory agency as a condition of approving the drug for marketing. In the former case, the biopharmaceutical company sponsoring a trial may wish to know more about the drug's performance in patients who were not well represented in preapproval trials, eg, patients with compromised liver function and patients taking several concomitant medications. In addition, other sponsors such as an institute within the National Institutes of Health may want to explore a drug's place in current treatment practice guidelines, comparing its safety and/or efficacy with other treatment options or combinations of various treatment options. In the latter case, the regulatory agency approving the drug for marketing felt

that, based on the drug's benefit-risk balance as indicated by the data they had to review at that time, the drug could be marketed and hence offer immediate benefit to patients, but it also felt that it would be advantageous to require the sponsor to provide additional safety and/or efficacy information that could be used to refine the drug's label if necessary. In these cases, the drug often receives a restricted initial label.

CURRENT PRACTICES IN THE CLINICAL TRIALS OF BIO-PHARMACEUTICALS

Biopharmaceuticals are being developed to treat various life threatening condition such as viral infections, cancer, diabetes, hepatitis, etc., and these can be grouped into various categories:

1. Hematopoietic Growth Factors

Hematopoietic growth factors are glycoproteins that stimulate the proliferation and development of clonogenic precursor cell populations. Through recombinant technology, hematopoietic growth factors administered at pharmacologic doses can provide significant clinical benefit for the cancer patient undergoing chemotherapy.

As hematopoietic growth factors serve to stimulate the production of mature blood cells, theirclinical application in diseases characterized by sub-optimal production of specific blood celltypes was obvious. Several Colony Stimulating Factor (CSF) preparations have gained regulatory approval, or are currentlybeing assessed in clinical trials. G-CSF and GM-CSF have proved useful in thetreatment of neutropenia. All three CSF types are useful in the treatment

ofinfectious diseases, some forms of cancer and the management of bone marrow transplants, as they stimulate the differentiation/activation of white blood cell types most affected by such conditions.

Recombinant human erythropoietin was purified and cloned in 1977 and then commercially produced as epoetin alfa.3 It was initially approved in 1987 as a treatment for anemia in chronic renal failure patients on dialysis and in 1990 as a treatment for patients with HIV-related anemia. In 1993, the indication was expanded to include chemotherapy-related anemia. The approval was based on a randomized placebo-controlled trial, with three subsets including anemia cancer patients who received platinum-based chemotherapy, non- platinum based chemotherapy, or no chemotherapy. The non-chemotherapy patients received a lower dose of epoetinalfa (100 units per kg) for a shorter period (8 weeks), and the hemoglobin improvement did not reach statistical significance. The chemotherapy groups received 150 units per kg epoetinalfa for 12 weeks, resulting in an improvement in hematocrit of approximately 6% points in cisplatin and in non-cisplatin groups compared with placebo. In addition, a statistically significant reduction was seen in transfusion requirements and overall QoL in the epoetin group compared with placebo. Adverse events were similar in the two groups and largely reflected the underlying disease and chemotherapy.[http://freecontent.lww.com/wp-content/uploads/2014/10/Perry-Ch6 Hematopoietic-Growth-Factors.pdf].

Table 1. Colony Stimulting Factors approved for medical use or in clinical trials

Product	Indication	Company	Status
Nepogen (filgrastim; G-CSF)	Neutropenia caused by Chemotherapy; Bone Marrow transplants	Amgen Inc.	Approved
Leukine (sargramostim; GM-CSF)	Autologous bone marrow transplantation; Neutrophi; recovery after bone marrow transplantation	Immunex	Approved
Neulasta (PEGylatedfilgrastim)	Neutropenia	Amgen	Approved
GM-CSF	Immune stimulation in malignant melanoma; Crohn's Disease	Berlex	In clinical trials
GM-CSF	Myeloid reconstitution	Cangene	In clinical trials

	following stem cell transplantation		

Cytokines

Cytokines are hormone-like molecules that can control reactions between cells. They activate cells of the immune system such as lymphocytes and macrophages. Interferon is potent glycoprotein cytokine that acts against viruses and uncontrolled cell proliferation. Interleukins function as messengers for various steps in the immune process.Several ILs, particularly those capable of modulating transformed cell growth, as well asthose exhibiting immune-stimulatory properties, enjoy significant clinical interest. As with othercytokines, the advent of recombinant DNA technology facilitates production of these moleculesin quantities sufficient to meet actual/potential medical needs.The first IL to be approved for medical use was IL-2, approved in 1992 by the FDA for thetreatment of renal cell carcinoma. Several additional IL preparations are currently in clinicaltrials:

Table 2: Some Interleukin preparations approved or in clinical trials for general medical use

Product	Indication	Company	Status
Proleukin (rIL-2)	Renal Cell	Chiron Corp.	Approved

	Carcinoma		
Neumega (rIL-11)	Prevention of Chemotherapy induced thrombocytopenia	Genetics Institute	Approved
IL-2	HIV	Bayer	In clinical trials
IL-2	HIV and non-Hodgkin's lymphoma	Chiron	In clinical trials
IL-4	Cancer	Schering Plough and National Cancer Institute, USA	In clinical trials
IL-4 and IL-13	Asthama	Regeneron	In clinical trials
Il-10	Inflammatory Disease	Schering Plough	In clinical trials
Il-18	Cancer	GlaxoSmith Kline	In clinical trials
IL-18	Rheumatoid arthritis, Crohn's Disease	Serono	In clinical trials

Cytokines, Cited 19 Feb 2006, Available from: http://www.microvet.arizona.edu/courses/MIC419/Tutorials/cytokine.

(http://www.agls.uidaho.edu/biotech_society/Lecture_Presentations/Lecture17_BiopharmaceuticalsII.ppt)

Enzymes

These are complex proteins that cause a specific chemical change in other substances without being changed themselves.

Table 3: Thrombolytic agents (enzymes) approved for general medical use

Company	**Product**
Genentech	Activase
Galenus-Mannheim	Ecokinase
Boehringer-Mammheim	Retavase
Boehringer-Mammheim	Rapilysin
Boehringer-Ingelheim	Tenecteplace
Genentech	TNKase
Various	Streptokinase
Various	Urokinsae

Various	Staphylokinase

Monoclonal Antibodies

Monoclonal antibodiesare produced from immortal cells with an antibodyproducing spleen cells. Examples include Infliximab, adalimumab, rituximab. Monoclonal antibodies now account forapproximately one third of all new treatments. Their

applications include the treatment of breast cancers,leukemia, asthma, rheumatoid arthritis, psoriasis, chronicgastrointestinal inflammatory disease and transplant rejection. First fully human monoclonal antibody waslaunched in 2003 in UK-removing potentialfor immunogenic reactions. New indications and therapies are emerging all the time. The development of humanantiviral monoclonal antibody therapies regardingantigenic variability of circulating viral strains and theability of viruses to undergo neutralization escape wasreported.[Marasco and Sui, 2007]

Table 4: Examples of Monoclonal Antibodies approved European Union

Product Name	Company	Action	Indication
Avastin	Anti-VFGF Monoclonal Ab	Roche	Colo-rectal Carcinoma
Erbitux	Anti-EGF	Merck	Colo-rectal

	Monoclonal Ab		Carcinoma
Herceptin	Anti HER-2 Monoclonal Ab	Roche	Breast Cancer
Humira	Anti-TNFalpha Monoclonal Ab	Abbott	Rheumatoid Arthritis
Mabcampath	Anti-CD52 Monoclonal Ab	Genzyme	Chronic leukemia
Mabthera	Anti-CD20 Monoclonal Ab	Roche	Rheumatoid arthritis/lymphoma
Raptiva	Anti-CD11 Monoclonal Ab	Serono	Psoriasis
Ramicade	Anti-TNFalpha Monoclonal Ab	Centocor BV	Rheumatoid Arthritis
Simulect	Anti-CD25 Monoclonal Ab	Novartis	Immune suppression
Tysaberi	Anti-alpha4 integrin monoclonal Ab	Elan	Multiple sclerosis
Xolair	Anti-IgE monoclonal Ab	Novartis	Asthma

Vaccines

These are microorganisms or subunitof microorganisms that can be used to stimulateresistance in a human to specific diseases as well as tostimulate immune response. Examples include hepatitis B virus [Baraclude (Entecavir), Adefovirdipivoxil, Lamivudine, AlfaInterferon], Ebola virus. Researchers have identified a protein in infected livercells that is essential for hepatitis C virus replicationInhibiting this protein is highly efficient in blockingvirus replication.[Xao et al, 2009, Kaul et al, 2009]

Antisense Drugs

Antisense drug is a medicationcontaining part of the non-coding strand of messengerRNA (mRNA). Antisense drugs work at the genetic levelto interrupt the process by which disease-causingproteins are produced. Instead of attacking the bacteriaor viruses that cause diseases, antisense drugs will interrupt into the portion of a cell's genetic machinerythat produces disease-related proteins. Among muchnew moleculartherapeutics being explored for cancertherapy, antisense oligonucleotides are emerging as anovel approach to cancer therapy, and used alone or incombination with conventionaltreatments such aschemotherapy and radiation, with numerous antisenseagents being evaluated in preclinical studies and severalanticancer antisense drugs in clinical trials. One ofthe treatments for genetic disorder or infections isantisense therapy. When the geneticsequence of agene is known to be causative of a disease, antisense drugs hybridize with and inactive atmRNA, thereby, restricting a particular gene fromproducing the protein for

which it holds the recipe.Although specificity and selectivity are the key featuresof antisense oligonucleotides, the need to target theright tissues and reach the nucleus remains a challengeto overcome. [Morcos, 2007; Malik et al, 2008]

Peptide Therapeutics

Peptide therapeuticscharacterizes a novel class of therapeutic agents. Currently,only selected cationic antimicrobial peptides have beenlicensed, and only for topical applications. It is nowpossible to produce very large quantities of therapeuticpeptides with tight specifications by using the widepossibilities offered by liquid phase and solid phasetechnologies, alone or in combination depending on thespecific features of a given project. Moreover, manypeptides currently in the preclinical or clinical stagescontain non-natural amino acids (β-amino acids or aminoacids having D configuration) to make them more activeor more stable. Selection methodologies addressingprotease resistance have been developed and whencombined with methods such as pegylation antibodyFc attachment and binding to serum albumin look likelyto finally turn therapeutic peptides into a widely acceptedrug class. [McGregor 2008, Oyston et al, 2009]

Cell Therapies

Cell therapy designates theprocess of introducing new cells into tissues in order totreat a disease. Several stem cell therapies are routinelyused to treat disease today. Adult stem cell transplante.g.

bone marrow stem cells, peripheral blood stem cells and umbilical cord bloodstem cell transplant.

Umbilical cord blood stem celltransplants are less prone to rejection than either bonemarrow or peripheral blood stem cells. The best-knownstem cell therapy to date is the bone marrow transplant,which is used to treat leukemia and other types of cancer,as well as various blood disorders. Regenerative medicine using stem-cell research,tissue engineering and gene therapy is innovativeresearch and it focuses on the repair, replacement andregeneration of cells, tissues or organs to restoredamaged function resulting from diseases and ailments.

Stem cell-based therapies, tools and targets are ourfuture. The big challenge for the stem cell community istherefore to facilitate the best possible interaction withthe population at large i.e. one stem cell world. Stem cell treatments are a type of genetic medicinethat introduces new cells into damaged tissue to treat a disease or injury. Many medical researchersbelieve that stem cell treatments have the potential tochange the face of human disease and alleviate suffering.The ability of stem cells to self-renew and give rise tosubsequent generations that can differentiate offered alarge potential to culture tissues that can replace diseasedand damaged tissues in the body, without the risk ofrejection and side effects. A number of stem celltreatments exist, although most are still experimentaland/or costly, with the notable exception of bone marrowtransplantation. Medical researchers anticipate one daybeing able to use technologies derived from adult andembryonic stem cell research to treat cancer, Type 1diabetes mellitus, Parkinson’s disease, Huntington’sdisease, Celiac Disease, cardiac failure, muscle damageand

neurological disorders, along with many others.[Stem cell therapies today. Available from: http://learn.genetics.utah.edu/content/tech/stemcells/sctoday.]

CHALLENGES FOR SMALL BIOPHARMACEUTICAL COMPANIES

Small biopharmaceutical companies often encounter important challenges in designing and implementing clinical development programs. In a context in which only approximately 10% of clinical programs result in drugs that achieve regulatory approval, small-company clinical programs may have an even lower rate of success than that of large companies owing to limited internal experience in clinical development and limited infrastructure, which may also affect manufacturing and clinical supply. However, these challenges are largely overshadowed by limited resources and funding, which in turn fuel demand for short timelines owing to the need to demonstrate progress to investors. As such, these companies must focus their resources on small, less-costly development programs for very specific targets and often must spearhead new approaches to testing new products in order to survive. Small companies use a variety of approaches to address these challenges, including the use of new technical platforms, the use of new formulations or technologies that enhance the actions of known drugs, or the use of trial designs that take advantage of the specific market they hope to enter. Other companies develop products that are spun off from or licensed from large companies. In fact, many small companies may choose to partner with larger companies to add resources and experience. Many small companies also repurpose drugs or pursue narrow niche markets — such as rare inherited

diseases, uncommon cancers, or specific infectious diseases — to remain viable. Furthermore, many companies turn to rare diseases for an opportunity to successfully negotiate many of the aforementioned issues, though such diseases present their own challenges — in particular, the small number of patients available for clinical trials. Table 1 illustrates some examples of approaches used by small companies that achieved Food and Drug Administration (FDA) approval for their drugs in 2014 and 2015.

The unique challenges of clinical development by small companies are often addressed with the use of smaller clinical development programs than those used by large companies. Development of drugs for rare diseases may be used as a strategy, but the small size of the populations with such diseases and the small samples available for trials require approaches that can maximize the power to detect efficacy, which can include the use of historical controls, new surrogate end points, or enrichment for participants who are likely to have a response. Temptations to use uncontrolled, early, small studies to support further development of products may prove problematic. Small companies with limited resources require both innovative approaches and rigor for success.

Table 5: Examples of small biopharmaceutical companies that achieved regulatory success from independently intiated Phase 3 studies.

Table 1. Examples of Small Biopharmaceutical Companies That Achieved Regulatory Success from Independently Initiated Phase 3 Studies.*

Company	Drug	Indication	Year Approved	Strategic Focus or Advantage
Alkermes	Aristada	Schizophrenia	2015	Modification of existing drug
Human Genome Sciences	Tanzeum	Type 2 diabetes	2014	Modification of existing drug
Retrophin	Cholbam	Bile acid synthesis disorders	2015	Reformulation, rare disease
Kythera Biopharmaceuticals	Kybella	Reduction of fat below the chin	2015	Reformulation
Paladin Labs	Impavido	Leishmaniasis	2014	Reformulation
Orexigen Therapeutics	Contrave	Obesity	2014	Repurposed or combination drug
Synageva BioPharma	Kanuma	Lysosomal acid lipase deficiency	2015	Rare disease, new manufacturing
Wellstat Therapeutics	Xuriden	Hereditary orotic aciduria	2015	Rare disease, genetic
TopoTarget	Beleodaq	Peripheral T-cell lymphoma	2014	Rare disease, oncology
Biomarin	Vimizim	Morquio A syndrome	2014	Rare disease, genetic
Tesaro	Varubi	Chemotherapy-induced nausea	2015	Licensed from other company; niche area: oncology
Medicines Company	Kengreal	Acute coronary intervention	2015	Licensed from other company; niche area: acute care
Furiex Pharmaceuticals	Viberzi	IBS with diarrhea	2015	Licensed from other company; spinoff; niche area: GI disease
Vanda Pharmaceuticals	Heltioz	Sleep–wake disorder in blindness	2014	Licensed from large company, rare disease
InterMune	Esbriet	Idiopathic pulmonary fibrosis	2014	Licensed from large company, rare disease
BioCryst Pharmaceuticals	Rapivab	Influenza	2014	Surrogate end point
Anacor Pharmaceuticals	Kerydin	Fungal infection	2014	New chemical technology
Relypsa	Veltassa	Hyperkalemia	2015	New chemical technology, spinoff
Durata Therapeutics	Dalvance	Acute severe skin infections	2014	Niche area: hospital care

* GI denotes gastrointestinal, and IBS irritable bowel syndrome.

PRODUCTION OF BIOPHARMACEUTICALS

Unlike traditional medicines that are made by chemical synthesis, biologics are made by biosynthesis in living cells. Biologics are generally much larger than traditional synthetic medicinal products and range from highly complex inactivated vaccines and plasmaderived factors to highly purified, well characterised recombinant therapeutic proteins. As new biological therapies come to market, the term biologics may encompass a diverse portfolio and include

therapeutic options such as gene and cellular therapies, therapeutic vaccines, and nucleic acid preparations. The use of therapeutic proteins as the treatment of choice for certain unmet medical needs was enabled by the convergence of two emerging technologies in the 1970s: genetic engineering and the science of cell culture. These technologies provided researchers with the ability to create specific recombinant DNA molecules encoding specific proteins and the methodology to introduce these recombinant DNA molecules into bacterial or animal cells that synthesised the protein. Further advances in cell culture technology permitted the development of high-viability, high-density cell cultures and the ability to scale cultures to larger volumes. Cell cultures, maintained in large, computer-controlled, stainless steel bioreactors enabled large-scale protein production. An interesting illustrative case history in the development of a biologic can be seen with the medicinal product alpha-interferon. In the early 1970s, interferons were heralded as promising therapeutics for a variety of disease conditions from viral infections to cancer. Initially, alpha-interferon was produced by purification of the active protein from human white blood cells. As cell culture technology advanced, a number of groups were successful in producing alpha-interferon in vitro, from cultures of transformed human lymphoblastoid cells that spontaneously produced a range of endogenous interferons. The advent of recombinant DNA technology enabled the creation of DNA vectors containing the alpha-interferon gene and the successful expression of the gene in bacterial cells. In 1986, both non-recombinant and recombinant alpha-interferons gained regulatory approval. The introduction of recombinant expression systems cleared the way for several major protein products to be launched as

therapeutics. Peptide hormones (erythropoietin, growth hormone, beta-interferon, reproductive hormones) [Chu and Robinson 2001] and enzymes (tissue plasminogen activator) were produced. These molecules were used as “replacement therapies” to treat patients with diseases caused by the deficiency of specific molecules; supplementation of endogenous protein levels with the recombinant product provided a therapeutic benefit. Frozen cell banks, containing recombinant cells producing these replacement proteins, provided a readily available supply of the required factor that was not dependent on rare and potentially hazardous raw materials such as human blood and tissue. The next generation of protein therapeutics moved beyond the established strategies of managing disease states by restoring or supplementing endogenous proteins. Recombinant proteins emerged in the 1990s that included antibodies designed to bind to specific antigens or the cells they were attached to, permitting the removal or destruction of the antibody-bound moiety by the immune system or via toxic molecules attached to the antibodies. Antibodies targeting tumor markers (alemtuzumab [CamPath 2005], and trastuzumab)and markers of inflammatory disease (antitumour necrosis factor (TNF) antibodies for rheumatoid arthritis and antiIgE for asthma) were successfully developed and deployed in the clinic, having a profound impact on a range of diseases. Additionally, the ability to screen patients and identify those who would respond to a particular therapy added a further refinement in treatment of various diseases.

PROCESS DEVELOPMENT

The delivery of a new biopharmaceutical to the marketplace requires an extensive and extended period of process development involving the application of advanced techniques in molecular biology, cell culture, separation technology, and formulation science, taking several years to complete. Prior to regulatory approval, development of the manufacturing process consumes considerable resources in terms of equipment and material as well as the people that are needed to prepare and characterise the clinical trial material. Once the manufacturing process is finalised, the process details are transferred to the manufacturing facility where the material is to be made. Details of the manufacturing process and the characterisation of the material produced are provided to the regulatory agencies to provide evidence of a stable and reproducible process, molecule, and product. Process changes after regulatory approval require additional process and molecular analyses as well as additional filings in many cases. The creation of the Master Cell Bank (MCB) is a critical milestone in biopharmaceutical process development. The MCB is a cryopreserved, long-term store of recombinant cells, either bacterial or mammalian, containing the gene that encodes the desired protein. Following transfection of host cells with a DNA plasmid containing the desired gene, the cells are subjected to a cloning procedure to ensure genetic uniformity and then are screened to identify clones that have a stable, high-level expression of the desired protein. Once identified, the desired clone is expanded and cryopreserved in vials, creating the MCB. The MCB is the source of all cells used to manufacture the medicinal product, either directly by thawing MCB vials or via an intermediate working cell bank (WCB) derived from the MCB. Cell banks are usually laid down as hundreds

of vials and stored at multiple redundant locations to ensure security of supply. These cell banks are extensively tested and characterised to ensure that they are fit for purpose, stably express the desired protein over the manufacturing period, and do not contain microbiological contaminants. Another major component of process development is defining the cell culture process that expands the small number of cells in the MCB or WCB vials into the large volumes of cells required to produce economically viable amounts of proteins in a production facility. For example, the process may require expanding 3 million cells in a 1-millilitre vial to a 20,000-litre volume in a stainless steel bioreactor to achieve 10 million cells per millilitre. The cell culture development process establishes the appropriate nutrient media and specific physiological conditions for cell growth including O2 and CO2 levels and the pH of the medium, as well as any specific manipulations required to achieve high levels of protein production.