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THE MODERN ENGINEERING IN BIOSIMILIAR DRUGS DEVELOPMENT AS BIOACTIVE GENE CLONNING

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Abstract: Bio therapeutic product tends to similar properties like- efficacy, Safety and quality to a licensed bio- originator. USFDA guideline clearly said that Bio-Similar drugs are not generic medications nor identical to the innovator medicine and also it's not ensuring therapeutic equivalence with innovator drug. Getting Bio-Similar product marketing approval is a challenging task. To improve access of Bio-Similar drugs within the US market, US-FDA allows abbreviated pathway for their approval. Recently India is becoming a most preferable destination for Bio-Similar manufacturers, because of Make in India program. Introduction of recombinant technique to prepare Monoclonal antibody based Bio-Similar drug becoming popular within pharmaceutical manufactures because of many recent patent expiries of Biologics. The biologies are produced by cell culture method; hence, chances of variability's are more as comparable with the chemically synthesized conventional medicine and various biological medicines has led to developed Bio-Similar drugs across the globe. The biologies are produced by cell culture method; hence, chances of variability's are more as comparable with the chemically synthesized conventional medicine. Therefor it is impossible to produce an identical copy of an innovator product; hence, Bio-Similar is not considered as generic drugs. These drugs are Twin but not a clone of the innovator drug. The Bio-Similar drugs always face challenges regarding verification of the similarity, the interchange ability, unique naming to differentiate the various Bio-Pharmaceutical products, commercial opportunities, IPR and public safety.

Keywords: USFDA, Monoclonal antibody, Biosimilar products,

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INTRODUCTION

The first product developed was HUMULIN by USFDA in 1982 that sales now account for billion by 2015 Bio-Similar is the fastest growing field of pharmaceutical industries. They were produced mainly by (Recombinant DNA technology-antibody/monoclonal body's technologies). This has provided many cures and prevention to some life Dangerous illness such as Diabetes, rheumatoid arthritis, cancer, inflammatory etc. The predominant advantage of biological drugs is higher specificity. For monoclonal antibodies (mAb), there are numerous ways that antibodies could be used for therapy. For example: mAb therapy can be applied to destroy malignant tumor cells or prevent tumor growth by blocking cell-specific receptors, or by delivering a radioactive molecule to a target cell, thereby delivering a lethal chemical dose to the target cell (radio immunotherapy). It is possible to produce a specific mAb to almost any extracellular/cell surface target.

Various standards required for approval of bio-similar product

A. Consistency of process.
B. Conforming manufacturing standard to appropriate regulations.
C. Innovator used should be standardized including
D. Pharmacokinetics/pharmacodynamics and clinical data Table 1.

Table -1 Interpretation of Bio-Similar Products.

Definition and Interpretation of Bio-Similar Products		
Term	**Introduced by**	**Definition**
SBP (Similar Biologic Product)	WHO	Similar to an already licensed reference Biotherapies product in terms of quality, safety, efficacy
FOB (Follow–On Biologic)	US-FDA	Highly similar to the reference product without clinically meaningful differences in safety, purity and potency
SEB (Subsequent Entry Biologic)	Canada	A drug that enters the market subsequent to a version, which is previously authorized in Canada with demonstrated similar to a reference biologic drug

Monoclonal Antibodies

Monoclonal antibodies are non-specific antibodies produced by identical B lymphocytes, called plasma cells, by inducing various conditions. The Mab acts against a specific epitope of the antigen, thereby it can bind specifically to an antigen in a mixture of antigens.

Production

Mab can be produced by including a plasma cell, life span of plasma cell is short, and hence the duration of secretion of antibody is short. It can be produced by employing hybridoma technology. Hybridoma cell clones are produced by the fusion of two deaf cells, the myeloma cell and the plasma cell. The spleen plasma cells are obtained from mouse or rabbit, which is already immunized with antigen of choice. The splenocytes contain polyclonal beta cells, which secrete antigen specific antibodies. The immortal myeloma cell is selected by growing in the culture containing toxic purine analog, 8-Azaguanine and then subjected to an enzyme inhibitor, aminopterin, a folic acid analog. These screens and choose myeloma cells are unable to produce and lack HGPRT (hypoxanthine, guanine, phosphoribosyltransferase) gene and cannot survive in HAT (Hypoxanthine, Aminopterin and Thymidine) medium.

Spleen cell and myeloma cell are fused by using polyethylene glycol as a fusion agent, which produced hybridoma cell. Cells are then transferred to HAT medium. In which the infused myeloma cell dies in HAT medium, the unfused beta cell dies naturally, only hybridoma cells are immortal and survive in HAT medium. In initial purification the 1st step is the separation of clones of hybridoma cells from each other. (Purification is done by chromatography method). Then the subcloning is done for the isolation of single clones. Then the large scale propagation of single clone in tissue culture medium to produced Mab.

Pharmacokinetic

Table 2: Comparative layout of Bio-Similars and traditional drugs.

Comparative Layout	Traditional	Bio-Similars
Molecule structure	Simple	Large & complex
Synthesis	Chemically synthesized	Produced by cell lines (but not identical to reference drug)
Route of administration	Orally	Iv or subcutaneous
Half life	0.5-8 hours	1-6 weeks
Antidrug Antibody formation	Not applicable	Possible

Study designs and analysis method would be enough to meet the objectives. After the long research, they found the successful design for the biosimilars. Table 2. The route of administration of the Bio-Similars is a little bit more complex than the traditional drug. They are complex in nature. Due to their long half-line and potential to elicit immunoresponse.

Some authorities

Some authorities which are included into the process are given below:

A. Institutional Biosafety Committee (I.B.S.C): It is required for the handling of hazardous microorganism and/ or genetically engineered organism.
B. Review Committee on genetic manipulation: It is for the research and development, exchange of genetically engineered cell bank.
C. Genetic Engineering Approval Committee.

It is also for the procedure of the Bio-Similars. It is also included in the pre and post marketing study. CDSCD is the national regulatory authority in India for safety, quality of the drug in the country. The DBT is required for the development and preclinical study for the recombinant DNA. Nowadays, many companies produce the Bio-Similars. In India, the drug is approved by RCGM & CDSCD. The similar biologics are regulated as per the drug and cosmetic act, 1940 and Drug and Cosmetic rule; 1945.

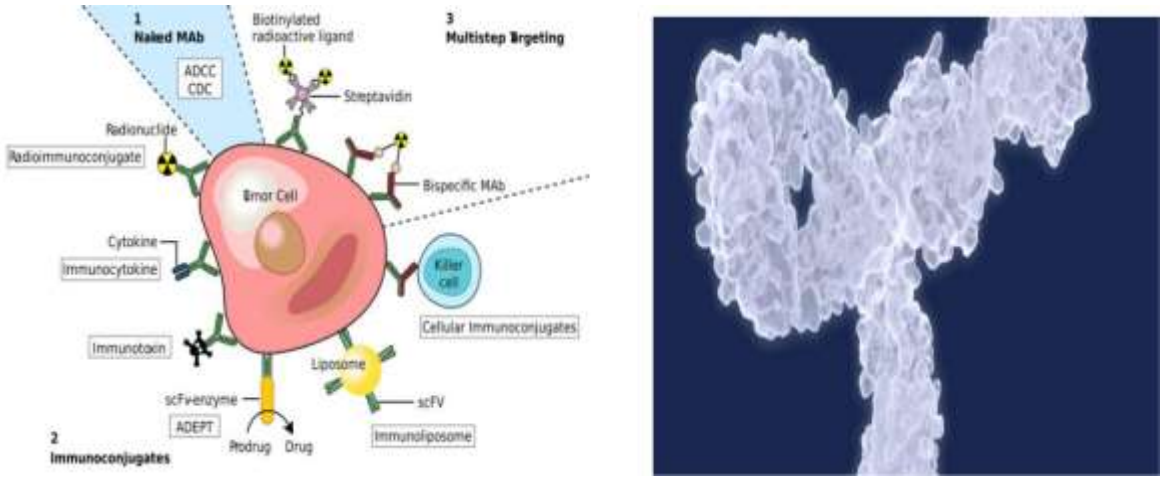

Figure-1: Recombinant DNA

After the expiry of the patent of approved recombinant drugs (e.g., insulin, human growth hormone, interferons, erythropoietin, monoclonal antibodies and more) any other biotech company can develop and market these biologics (thus called biosimilars). Every biological (or biopharmaceutical products) displays a certain degree of variability, even between different batches of the same product, which is due to the inherent variability of the biological

expression system and the manufacturing process. Any kind of reference product has undergone numerous changes in its manufacturing processes, and such changes in the manufacturing process (ranging from a change in the supplier of cell culture media to new purification methods or new manufacturing sites) was substantiated with appropriate data and was approved by the EMA. In contrast, it is mandatory for biosimilars to take a both non-clinical and clinical test that the most sensitive clinical models are asked to show to enable detection of differences between the two products in terms of human pharmacokinetics (PK) and pharmacodynamics (PD), efficacy, safety and immunogenicity.

Its difficult and costly to recreate biologics because the complex proteins are derived from living organisms that are genetically modified. In contrast, small molecule drugs made up of a chemically based compound can be easily replicated and are considerably less expensive to reproduce. In order to be released to the public, biosimilars must be shown to be as close to identical to the parent innovator biologic product based on data compiled through clinical, animal, analytical studies and conformational status. Generally, once a drug is released in the market by FDA, it has to be re-evaluated for its safety and efficacy once every six months for the first and second years afterward re-evaluations are conducted yearly, and the result of the assessment should be reported to authorities such as FDA. Biosimilars are required to undergo pharmacovigilance (PVG) regulations as its reference product. Thus biosimilars approved by EMEA (European Medicines Agency) are required to submit a risk management plan (RMP) along with the marketing application and have to provide regular safety update reports after the product is in the market. The RMP includes the safety profile of the drug and proposes the prospective pharmacovigilance studies.

Biological Products

Many of today‘s important medications are biological products. Biological products are made from living organisms. The material they are made from can come from many sources, including humans, animals and microorganisms such as bacteria or yeast. Biological products are manufactured through biotechnology, derived from natural sources or, in some cases, produced synthetically.

Most biological products are more complex in structure and have larger molecules or mixtures of molecules than conventional drugs (also called small molecule drugs). Conventional drugs are made of pure chemical substances and their structures can be identified. Most biologics, however, are complex mixtures that are more difficult to identify or characterize.[4]

(a) What are biosimilar and interchangeable biological products?: There are two new types of biological products-biosimilar and interchangeable. Biosimilars are a type of biological product that are licensed (approved) by FDA because they are highly similar to an already FDA-approved

biological product, known as the biological reference product (reference product), and have been shown to have no clinically meaningful differences from the reference product. An interchangeable biological product, in addition to meeting the biosimilarity standard, is expected to produce the same clinical result as the reference product in any given patient.

(b) Will biosimilars and interchangeable work in the same way as the biological reference product they were compared to?: Yes, biosimilars have no clinically meaningful differences in terms of safety and effectiveness from the reference product they were compared to. In addition, a biosimilar needs to have the same mechanism of action as the reference product it was compared to, which means it will work in the same way as the reference product. The FDA will only approve a biosimilar product if it has the same mechanism of action, route of administration, dosage form, and strength as the reference product. Additionally, a biosimilar can only be approved for the indications and conditions of use that have been previously approved. .

Aranesp (Darbepoetin alfa: Erythropoietin)

Drug developers: Dr. Reddy‘s Laboratories: Cresp® launched 2010 in India as that country‘s only darbopoetin alfa of any kind, and as world‘s first generic darbopoetin alfa, Merck: MK-2578 development halted in 2010, Stada: Silapo® marketed in EU, where it was authorized December 2007 for anemia that is causing symptoms in patients with chronic renal failure; anemia in adults receiving chemotherapy to treat certain types of cancer and to reduce the need for blood transfusions; and to increase the amount of blood patients with moderate anemia can self-donate before surgery.

Nature and indication: Erythropoiesis-stimulating agent (ESA) for anemia due to chronic kidney disease (CKD) in patients on dialysis and patients not on dialysis; the effects of concomitant myelosuppressive chemotherapy, and upon initiation, there is a minimum of two additional months of planned chemotherapy. 2012 sales: $2.040 billion (Amgen), Patent status: Patent set to expire 2016 in EU; 2024 in U.S.

Epogen® / Procrit® / Eprex / Erypo (Epoetin alfa; Erythropoietin)

Drug developers: Hexal: Epoetin alfa Hexal marketed in EU, where it was authorized August 2007 for anemia, cancer and chronic kidney failure, Hospira: Retacrit® marketed in EU, where it was authorized in December 2007 for anemia associated with chronic renal failure or other kidney problems, adults receiving chemotherapy for some cancers. Also indicated to increase the amount of blood patients with moderate anemia can self-donate before surgery, and to reduce the need for blood transfusions in patients with moderate anemia about to undergo major bone surgery. In U.S., Phase III trial launched last year, Medice: Abseamed® marketed in

EU, where it was authorized August 2007 for anemia, cancer, and chronic kidney failure, Sandoz: Binocrit® marketed in EU, where it was authorized August 2007 for anemia and chronic kidney. In U.S., the company said October 25, 2012, that it has started patient enrolment in a Phase III clinical trial, comparing safety and efficacy of biosimilar with reference product Epogen® /Procrit® in anemia associated with chronic kidney disease.

Nature and indication: Erythropoiesis-stimulating agent for anemia due to chronic kidney disease in patients on dialysis and not on dialysis; due to Zidovudine in HIV-infected patients; and due to effects of concomitant myelosuppressive chemotherapy, where upon initiation, there is a minimum of two additional months of planned chemotherapy. Also, for reduction of allogeneic RBC transfusions in patients undergoing elective, noncardiac, nonvascular surgery.

Enbrel (Etanercept: Tumor necrosis factor inhibitor)

Drug developers: Avesthagen: Avent™ in preclinical studies as of 2012, BioXpress Therapeutics: Biosimilar in active development, Cipla: Launches biosimilar in India on April 17, at a price of Rs. 6,150 ($113.43), 30% less than the innovator product, Hanwha Chemical: HD203 —scheduled for launch,‖ company states on its website without including a date, following submission for marketing approval to South Korea‘s Korea Ministry of Food and Drug Safety following completion of Phase I and Phase III trials. Hanwha has said it will seek a partner to commercialize HD203 and a biosimilar for Herceptin (trastuzumab), LG Life Sciences: LBEC0101 completed Phase I trial in South Korea, Mycenax Biotech: TuNEX in Phase III clinical trials in Japan and South Korea, Protalix Biotherapeutics: PRX-106 in

The preclinical studies, Shanghai CP Goujian Pharmaceutical: Etanar®, marketed in Colombia; Yisaipu, marketed in China.

Genotropin (Somatropin or somatotropin: Growth hormone)

Drug developers: BioPartners: Valtropin® marketed in EU, where it was authorized April 2006, 12 days after Omnitrope, for pituitary dwarfism and Turner syndrome; authorization withdrawn voluntarily —for commercial reasons‖ in October 2011, and withdrawn formally in May 2012, Sandoz: Omnitrope® marketed in EU, where it was authorized April 2006 for pituitary dwarfism, Prader-Willi syndrome, and Turner syndrome; the first biosimilar authorized by the European Medicines Agency. In U.K., was the first biosimilar recommended for approval by the National Institute for Health and Clinical Excellence in 2010.

In Japan, launched October 2009 as that nation‘s first approved biosimilar. Nature and indication: Peptide human growth hormone for children with growth failure due to growth hormone deficiency (GHD), Prader-Willi syndrome, small for gestational age, Turner syndrome, and idiopathic short stature; and for adults with either adult onset or childhood onset GHD.

Pursuing FDA approval since 2009 for additional indication, Replacement of human growth hormone deficiency (Mark VII multidose disposable device); received two complete response letters. —

We are working to address the FDA's requests for additional information,‖ Pfizer stated in its 2012 Financial Report. 2012 sales: $832 million (Pfizer). Patent status: Patents expired 2008 and April 16, 2013 in U.S.

List of approved biosimilars product launched by Indian companies.

Prduct Name	Active substance	Therapeutic area	Year of approval in India	Company
Acellbia	Rituximab	Non- Hodgkin lymphoma chronic B- cell lymphocytic	2017	Biocad
Bevacirel	Bevaciazumab	Colorectal cancer	2016	Reliance Life Sciences (Lupin)
Cizumab	Bevaciazumab	Colorectal cancer	2016	Hetero
Etacept	Etanercept	Ankylosing spondylitis, Psoriatic arthritis, Psoriatic, Juvenile rheumatoid arthritis	2013	Cipla
Filgrastim	Filgrastim	Neutropenia	2013	Lupin
Insugen	Human Insulin	Diabetes mellitus	NR	Biocon
Krabeva	Bevaciazumab	Metastatic colorectal cancer, cervical cancer, overian cancer, brain cancers	2017	Krabeva
Maball	Rituximab	Lymphoma, Non-Hodgkin's Lymphoma	2015	Hetero Group
MabTas	Rituximab	Lymphoma, Non-	2013	Intas

		Hodgkin's Lymphoma		Pharmaceuticals
Pegfilgrastim	Pegfilgrastim	Cancer, Neutropenia	2013	Lupin
Maball	Rituximab	Lymphoma, Non-Hodgkin's Lymphoma	2015	Hetero Group
Zyrop	Erythropoietin	Chronic kidney failure	2010	Cadila Healthcare

Humira (Adalimumab: TNF inhibiting anti-inflammatory biologic medication)

Drug developers: AET BioTech and BioXpress: Biosimilar being co-developed under agreement announced October 25, 2012; companies will be jointly responsible for development, registration, and manufacture of the biosimilar, based on BioXpress technology. AET BioTech will provide further investment in the biosimilar based on committed long-term financing, and oversee any future commercialization of the product, Amgen: Biosimilar in active development, Boehringer Ingelheim: BI695501 completed Phase I trial in New Zealand, studying the biosimilar's safety and pharmacokinetics compared to Humira (adalimumab) in October 2012, Fujifilm and Kyowa Hakko Kirin: Companies announce 50–50 joint venture, Fujifilm Kyowa Kirin Biologics, to develop a biosimilar version of Adalimumab for rheumatoid arthritis.

Herceptin (Trastuzumab: Monoclonal antibody that interferes with the HER2/neu receptor)

Drug developers: Amgen, Synthon, and Watson (now Actavis): Global licensing agreement announced July 18, 2012, for clinical development and testing of biosimilar. Deal followed publication March 2, 2012, of Phase I trial results showing bioequivalence between Synthon's biosimilar and Herceptin, BioXpress: Biosimilar in active development, Hanwha Chemical: Biosimilar in development. Hanwha has said it is seeking a partner to commercialize Herceptin and HD203, a biosimilar for Enbrel, Hospira: Biosimilar in active development, Pfizer: PF-05280014 completed Phase I REFLECTIONS B327-01 trial as of December 2012, to study the safety and pharmacokinetics of the biosimilar compared to Herceptin. The study yielded —positive data,‖ hence the company —is exploring plans to go into Phase III this year, Mikael Dolsten, president of Pfizer's Worldwide Research & Development unit, said on the Q4 2012 earnings conference call January 29, PlantForm: Clinical trials in humans expected to begin in 2014. Biosimilar expected to be launched, —in partnership with a pharmaceutical company,‖ in world markets in 2016, Stada Arzneimittel: Joined with Gedeon Richter in announcing plans

August 2011 to collaborate on biosimilars for trastuzumab and rituximab. Richter agreed to buy from Stada trastuzumab for a —low single-digit million Euros figure,‖ they announce.

Neupogen (Filgrastim: granulocyte colony-stimulating factor (G-CSF) analog used to stimulate the proliferation and differentiation of granulocytes)

Drug developers: Biocon and Celgene: Nufil marketed in India by Biocon; in active development for EU by joint venture ctArzneimittel: Biograstim® marketed in EU, where it was authorized September 2008 for cancer, hematopoietic stem cell transplantation, and neutropenia, Dr. Reddy's Laboratories: Grafeel marketed in India, Hexal: Filgrastim Hexal® marketed in EU, where it was authorized February 2009 for cancer, hematopoietic stem cell transplantation, and neutropenia, Hospira: Nivestim™ marketed in EU, where it was authorized June 2010 for cancer, hematopoietic stem cell transplantation, and neutropenia, Intas/Apotex: Neukine in Phase III development, Merck & Co.: MK-4214 in Phase III clinical development; acquired through acquisition of Insmed in 2009, Ratiopharm: Ratiograstim® marketed in EU, where it was authorized September 2009 for cancer, hematopoietic stem cell transplantation, and neutropenia.

Patent set to expire December 2013 in U.S.; Patent expired 2006 in EU.

Remicade (Infliximab: Chimeric monoclonal antibody biologic drug that works against tumor necrosis factor alpha (TNF-α)

Drug developers: Amgen: Biosimilar in active development, BioXpress: Biosimilar in active development, Celltrion: Ramsima™ (formerly CT-P13) authorized for marketing in Korea on July 20, 2012, for rheumatoid arthritis, ulcerative colitis, Crohn's disease, ankylosing spondylitis, and psoriasis. Applied for marketing authorization in EU, Hospira: Biosimilar in active development.

Nature and indication: Tumor necrosis factor (TNF) blocker for moderately to severely active rheumatoid arthritis in adults, in combination with methotrexate; Crohn's disease in children six years and older, and adults who have not responded well to other medicines; rheumatoid arthritis; ankylosing spondylitis; psoriatic arthritis; chronic, severe, extensive, and/or disabling plaque psoriasis in adults; moderately to severely active ulcerative colitis in children six years and older and adults that have not responded well to other medicines.

Rituxan / MabThera (Rituximab)

Drug developers: Amgen: Biosimilar in active development, BioXpress: Biosimilar in active development, Boehringer Ingelheim: BI695500 in Phase III development in U.S., EU, Brazil, Guatemala, Russia, Norway, Ukraine, Argentina, Peru, New Zealand. U.S. study recruiting participants as of April 17, according to ClinicalTrials.gov (NCT01682512)

Celltrion and Hospira: Conducting Phase I trial in South Korea of CT-P10 for RA and another Phase I trial for lymphoma.

Rituxan/MabThera. ―This year, we expect data,‖ Mikael Dolsten, president of Pfizer's Worldwide Research & Development unit, said on the Q4 2012 earnings conference call January 29. Probiomed: Kikuzubam® marketed in Bolivia, Chile, Mexico, and Peru, Roche: CEO Severin Schwan was quoted in March as pushing back his company's anticipated launch of a rituximab biosimilar beyond the 2016 date he had earlier cited in The Wall Street Journal, until the end of this decade, Sandoz: GP2013 in Phase I/II tria More recent announcements, however, focus on the company's technologies for developing biosimilar and proprietary drugs. On April 26, 2012.

Avastin (Bevacizumab: Angiogenesis inhibitor): Amgen is developing a biosimilar to the breast cancer drug, while Fujifilm and Kyowa Hakko Kirin on October 24, 2012, announce 50–50 joint venture, Fujifilm Kyowa Kirin Biologics, to develop a biosimilar version of Avastin (Roche / Genentech) for cancer indications. Glargine (long-acting basal insulin analogue): Biocon and Mylan on February 14 announced a strategic collaboration to develop a biosimilar to the insulin analog Lantus (Sanofi).

Conclusion:

The Pharmacovigilance is an important soul for the extra limitation of the clinical trials. Traceability and proactive risk management is very important forthe knowledge about the safety of the Bio-Similar and the immunogenicity is important. There is a specific need to use good design clinical trials to establish Bio-Similar drugs. Most importantly due to complexity of Bio-Similar product it is necessary to identify specific product for a specific disease. The molecular complexity of Bio-Similar product, robustness of manufacturing process, structural resemblance to an innovator drug, mechanism of action, innovator experience and clinical data resemblance need to be considered before filing Bio-Similar for marketing approval. Another key concern is interchangeable with Biopharmaceuticals and Bio-Similar product. Nevertheless, physician awareness about the Bio -Similar product could enhance patient safety and efficacy.

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