



Review Article

Recent Progress in Biopharmaceutical Drugs Research and Development

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Abstract: Advances in human health biology and disease have opened up exciting new possibilities for potential new treatments and cures to meet patient needs. Our understanding of these molecular principles has revealed the existence of many regulatory molecules or proteins with medical significance. These proteins are produced naturally within the body only in minute quantities. Developments in recombinant DNA technology and hybridoma technology facilitate the large-scale production of protein of medical interest and are called biopharmaceuticals. This articles attempts to provide an overview of the recombinant biopharmaceutical products available in the market and their contribution towards improving human health. Latest developments within each sectors of biopharmaceuticals is highlighted to provide a greater focus upon actual commercial products thus far manufactured and approved.

Keywords: Biopharmaceuticals, Recombinant Proteins, Monoclonal Antibodies, Growth Factors, Hormones, Blood Factors, Cytokines and Interferons, & Vaccines

1. Introduction

Microorganisms are important to us for many reasons as they produce things of value such as amino acids, nucleotides, vitamins, antibiotics, medicines, toxins, pesticides, solvents, organic acids, and animal & plant growth factors. They are important products as they benefit our health, nutrition and economy. These valuable products are produced only in tiny amounts by microbes as they need for their own benefit. However, they can be manufactured in large quantity by biotechnologists using genetic manipulation.

Recent developments in genomics, proteomics, metabolomics, high-throughput technologies and systems biology [1-3] have helped in elucidating process and mechanisms distributed throughout the genome and cells. Progress in strain development and mathematical models have contributed to identification of targets generation and characterization of economically important industrial microbes. Sequencing projects of hundreds of genomes, the availability of sequences corresponding to model organisms, new DNA microarray and proteomics tools, mutagenesis and

recombination methodology have accelerated microbial strain improvement programs [4-6]. These advances in molecular medicines and powerful tools to enhance computational capacity are enabling researchers to better understand human disease at the molecular level.

2. Recombinant Therapeutic Medicines

2.1. Development of Biotherapeutic Proteins

As our knowledge of disease increases, so does the potential of discovering and developing innovative medicines. Basic research from biopharmaceutical companies has studied the genetics of genes encoding biologically active proteins in detail. Further they demonstrated modification and transferring them from one organism to another in order to obtain highly efficient synthesis of their products (Table 1).

Recombinant

Table 1. Steps involved in development of biotherapeutic medicines.

Steps	Activity
1	Protein identification, isolation, biological characterization, amino acid sequencing, mapping of glycosylation, disulfide bridging and peptide domain
2	Gene identification, isolation, nucleic acid sequencing and amino acid sequence identification
3	Cloning and expression of human gene into plasmid to develop a recombinant plasmid and expression into bacterial, yeast or mammalian cells, research cell bank & master working cell bank developed.
4	Fermentation trials, downstream process, protein purification and characterization, bio-analytical testing, biological activity testing, animal toxicology study in comparison with gold standard.
5	Quality testing, genetic characterization, process validation, bulk product testing, stability test, final product testing, formulation development, human clinical trials, drug approval & product launch.

DNA method [7] has helped to produce biologically active proteins that do not exist in nature, such as chimeric, humanized or fully human monoclonal antibodies, or antibody-related proteins or other engineered biological medicines such as fusion proteins, using a range of different expression systems such as bacteria, yeast, transformed cell lines of mammalian origin, insect and plant cells, as well as transgenic animals and plants. For example, insulin extracted from animals naturally was used to treat diabetes earlier. In 1982, researchers produced human insulin from recombinant *Escherichia coli* containing recombinant plasmid with h-insulin gene [8]. This first approved human insulin benefits the vast majority of diabetic patients today.

Biotherapeutic medicines are an important and integral component of modern medicine that targets many chronic and acute disease areas with highly-specific treatments. Biotherapeutic medicines derive primarily from human or animal sources and they function as replacement therapies. They structurally mimic those found within the body because of their specificity and have a fewer side effects. They have moreover proven to be effective in the treatment of conditions that had not been positively addressed by chemically-synthesized small molecule medicines. These complex medicines improve quality of life of about 400 million

patients worldwide, treating widespread diseases such as cancer and diabetes, hepatitis C, and chronic renal failure – as well as less common ones such as haemophilia, Fabry's disease, growth deficiency, multiple sclerosis and Crohn's disease.

These medicines open new avenues for delivering cutting-edge treatments for numerous diseases and wide patient populations by acting on unique and diverse range of specific targets. Many patients are leading healthier lives as a result of biotherapeutic medicines, often without realizing the source of these products. Biotherapeutic medicines are made using living systems, which are more sensitive to change than the straight forward chemical synthesis process commonly used for small molecule medicines. The end product is therefore determined by a wide range of factors, which include the actual manufacturing process (Table 2). Small changes in manufacturing can alter the final product, as biotherapeutic medicines are composed of larger and more complex molecules which are difficult to characterize. The high complexity of this process requires precision, conformance to good manufacturing practices and defined specifications in order to maintain the safety and efficacy of the product over time [9-11]. About 200 plus in-process tests are carried out for a biotherapeutic medicine, compared to around the 50 done for a chemically-synthesized small molecule medicine.

Table 2. Quality control tests performed during development of biotherapeutic medicines.

Quality tests	Details
Genetic material	Genetic analysis of gene (by PCR and sequencing), screening, stability, etc.,
Bulk protein analysis	Partial GMP, Protein purification, Amino acid sequence, peptide mapping, HPLC analysis, ELISA, Western blot analysis, bioactivity assay, etc.,
Process Validation	Full GMP, protein yield, HPLC analysis, N-terminal & C-terminal sequencing, CD spectra, ELISA, Western analysis, bioactivity assay, protein stability, residual DNA & endotoxin testing, lab animal test, etc.,
Final product analysis	Protein analysis (as above), contamination of endotoxin and nucleic acid, bioactivity assay, etc.,

Biotherapeutic medicines are fundamentally different from the conventional small molecule chemical drugs. There is a fundamental difference in the average size of the two types of drugs. The chemically synthesized products are known as small molecules drugs (e.g. aspirin). In general, biopharmaceuticals are complex macromolecules that are over 100 times larger (e.g. interferon β) 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. The nature of the manufacturing process, and the safety and efficacy profile of biopharmaceutical products are also different. These medicines can be grouped into various

categories such as are growth factors, cytokines, hormones, blood clotting factors, enzymes, vaccines and monoclonal antibodies as discussed below.

2.2. Hematopoietic Growth Factors

G-CSF (Granulocyte colony stimulating factor) is a 21-kDa glycoprotein that functions as a growth and differentiation factor for neutrophils and their precursor cells by activating mature neutrophils. Filgrastim (r-hGCSF) is produced in *E. coli* used for treating chemotherapy-induced neutropenia [12-14]. This 18.8-kDa recombinant product displays biological activity indistinguishable from native GCSF.

GM-CSF (Granulocyte macrophage colony stimulating

factor) is a polypeptide with 127 amino acid exhibiting a molecular mass in the region of 22-kDa. Like GCSF, GM-CSF also proved to be useful in treating neutropenia (Table 3). Both are used in the treatment of infectious 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.

M-CSF (Macrophage colony stimulating factor) serves as a growth, differentiation and activation factor for macrophages and their precursor cells. It is also known as CSF1. This cytokine is produced by various cell types. Three related forms of human M-CSF have been characterized. All are ultimately derived from the same gene and share common C- and N-termini.

Erythropoietin (EPO) is a haemopoietic growth factor responsible for stimulating and regulating erythropoiesis (production of red blood cells) in mammals [15-17]. EPO is found in serum and in urine of anaemic individuals. Expression of r-human erythropoietin in Chinese hamster ovary (CHO) cells facilitated large scale production of r-hEPO (Epogen and Procrit), which has found widespread medical application (Table 3) now.

Thrombocythaemia is a disease characterized by abnormal megakaryocyte proliferation, leading to elevated blood platelet levels that result in the risk of clot formation within blood vessels. Thrombocytopenia is induced by a number of clinical conditions, including bone marrow failure, chemotherapy, and various viral infections. Thrombopoietin (TPO) is the haemopoietic growth factor now shown to be the primary physiological regulator of platelet production [18] and is of first generation. The second-generation thrombopoietic growth factors include peptides, non peptides, and agonistic antibodies are also called thrombopoietin receptor agonists (such as Eltrombopag and Romiplostim), have been approved for treatment.

2.3. Growth Factors

EGF (Epidermal growth factor) influences on endothelial cells, epithelial cells and fibroblasts, to stimulate growth of the epidermal layer. Along with several other growth factors, EGF plays a role in the wound-healing process [19]. After several exploratory and confirmatory clinical trials, the intralesional administration of human recombinant epidermal growth factor has been approved for the treatment of advanced diabetic foot ulcers.

Regranex (Becaplermin), a human platelet-derived growth factor (PDGF) that is indicated for the treatment of lower extremity diabetic neuropathic ulcers that extend into the subcutaneous tissue or beyond and have an adequate blood supply [20-22]. The potent stimulatory effects of PDGF as a chemoattractant and mitogen for mesenchymal cells (including osteogenic cells), along with its ability to promote angiogenesis, have been demonstrated in a variety of preclinical models predicting maxillofacial, spine and appendicular skeletal and soft-tissue applications. The product (Regranex) was approved for general medical use and its active ingredient is manufactured in an engineered strain of *Saccharomyces*

cerevisiae harbouring the PDGF B-chain gene.

TGF (Transforming growth factor) β appears to relate to tissue remodeling, wound repair and haemopoiesis [23]. Such activities render them as potentially useful therapeutic agents. Along with TNF- α and IFN- δ , TGF- β is a physiologically relevant negative regulator of haemopoiesis. TGF- β also inhibits the growth of various human leukaemia cell lines *in vitro*, rendering it of potential interest as a putative anti-cancer agent [24].

2.4. Hormones

Insulin is a peptide hormone produced by the β -cells of the pancreatic islets of Langerhans. It plays a central role in regulating blood glucose levels, generally keeping it within narrow defined limits, irrespective of the nutritional status [8, 25-27]. Failure of the body to synthesize sufficient insulin results in the development of insulin dependent diabetes mellitus (IDDM). This is also known as type 1 diabetes or juvenile-onset diabetes. First approved r-human insulin (Table 3; Humulin, Humalog1, etc.) benefits the vast majority of diabetic patients today.

Glucagon is synthesized by the A cells of the Islets of Langerhans, and also by related cells found in the digestive tract. The major biological actions of glucagon tend to oppose those of insulin, particularly with regard to regulation of metabolism [28]. Glucagon has an overall catabolic effect, stimulating the breakdown of glycogen, lipid and protein. A prominent metabolic effect is to increase blood glucose levels. Indeed, the major physiological function of glucagon (Ultratard) is to prevent hypoglycaemia (Table 3).

Human growth hormone (r-hGH) is a peptide hormone synthesized in the anterior pituitary. It promotes normal body growth and lactation and influences various aspects of cellular metabolism. Mature hGH contains 191 amino acid residues and displays a molecular mass of 22-kDa. GH primarily displays an anabolic activity. It stimulates the growth of bone, muscle and cartilage cells directly. r-hGH (Protropin, Genotropin, etc.) has a potentially wide range of therapeutic uses [29]. To date, its major application has been for the treatment of short stature, Turner's syndrome, idiopathic short stature and chronic renal failure [30].

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) play critical roles in the development and maintenance of male and particularly female reproductive function [31, 32]. Human chorionic gonadotrophin (hCG, Ovidrel) produced by pregnant women, plays a central role in maintaining support systems for the developing embryo during early pregnancy [33, 34]. LH promotes synthesis of testosterone, the major male androgen by the testicular Leydig cells. FSH sensitizes these cells to the activities of LH, probably by increasing LH receptor numbers on the cell surface. Recombinant human FSH (Gonal F) produced from CHO cell lines has been purified and used to treat human sub-fertility or induction of super ovulation in human. Similarly r-hLH (Luvris) produced from CHO cell lines used to induce ovulation in human.

Table 3. FDA approved r-h growth factors and r-h hormones for treating human diseases.

Biotherapy Area	Generic Name	Brand Name
GROWTH FACTORS		
Neutropenia	Filgrastim	Granulocyte & Neupogen
Febrile neutropenia	Pegfilgrastim	Neulasta
Anemia	Epoetin alfa & Eprex	Erypro, Eprex, NeoRecormon, Epogen & Procrit
Myeloid reconstitution	Sargramostim	Leukine & Leucomax
Diabetic foot ulcer	Becaplemin	Regranex
Oral mucositis	Palifermin	Kepivance
HORMONES		
Diabetes mellitus (Insulin dependent)	Human Insulin	Humulin, Novolin, Velosulin BR, Actrapid, Velosulin, Monotard, Insulatard, Protaphane, Mixtard, Actraphane
	Aspartate	Humalog, Novolog
	Detemir	Letemir
	Glargine	Lantus
	Glusuline	Apidra
	Inhalation	Exubera
Hypoglycemia	Glucagon	Ultratard
Diabetes mellitus (type 1 & 2)	Pramlintide	Glucagen
Diabetes mellitus (type 2)	Exenatide	Symlin, Byetta
Short Stature with Turner's syndrome GH deficiency in children & adults	Human Growth Hormone(Somatropin & Somatrem)	Protropin, Humatrope, Nordiotropin, Nutropin, Nutropin Depot, Serostim & Genotropin
AIDS wasting		Saizen & Biotropin
Short bowel syndrome		Zorbtiva
Infertility	Human chorionic gonadotropin	Ovidrel
	Human follicle-stimulating hormone	Gonal F, Follistim, Puregon
	Human luteinizing hormone	Luveris (Lutropin alfa)

Calcitonin is a composed of 32 amino acids that binds to osteoclasts and inhibits bone resorption [35]. Calcitonins from other species are effective in humans, but salmon calcitonin is the one most widely used due to its high affinity (40 times that of human calcitonin) for the human calcitonin receptor and slow rate of clearance. However, human calcitonin is less potent but also less antigenic than salmon calcitonin. Most clinical trials of calcitonin have used salmon calcitonin, but human calcitonin is as effective.

2.5. Cytokine and Interferon Family

Cytokines act upon or are produced by leukocytes (white blood cells). They play a central role in regulating both immune and inflammatory function and related processes, such as haematopoiesis and wound healing. Cytokines constitute the single most important group of biopharmaceutical substances [36, 37] as coordinators of the immune and inflammatory response and manipulation of cytokine activity can have a major influence on the body's response to a variety of medical conditions. Several immunosuppressive and anti-inflammatory drugs are now known to induce their biological effects by regulating the production of several cytokines. (For example, IL (Interleukin)1 to IL 15 & TNF (Tumour necrosis factor) α , Interferon, etc.). Administration of certain cytokines can enhance the immune response against a wide range of

infectious agents and cancer cells. Recent progress in r-DNA technology has contributed to development of several r-cytokine products (Table 4). IL-2 is used to treat cancer and IL-11 is used to treat thrombocytopenia in cancer patients. Etanercept (Enbrel) is a biopharmaceutical that treats autoimmune diseases by interfering with TNF (a soluble inflammatory cytokine) by acting as a TNF inhibitor [38-40]. It has USFDA approval to treat rheumatoid arthritis, juvenile rheumatoid arthritis and psoriatic arthritis, plaque psoriasis and ankylosing spondylitis.

Most species actually produce a whole range of interferons. Humans produce at least three distinct classes, IFN- α , IFN- β and IFN- δ with several subtypes [41-46]. These interferons are produced by a variety of different cell types (macrophages, eosinophils, vascular endothelial cells, keratinocytes & fibroblasts) and exhibit a wide range of biological effects including cellular resistance to viral attack, regulation of immune function, regulation of growth and differentiation of many cell types and sustenance of early phases of pregnancy in some animal species. Due to their biological activities, most interferons are used in the treatment of many medical conditions like augmentation of the immune response against infectious agents (viral, bacterial, protozoan, etc.), treatment of some autoimmune conditions and treatment of certain cancer types (Table 4).

Table 4. FDA approved r-h cytokines and r-h interferons for treating human diseases.

Biotherapy area	Generic name	Brand name
Cytokines		
Cutaneous T-cell lymphoma	Denileukin diftitox	Ontak
Metastatic renal cell carcinoma & metastatic melanoma	Aldesleukin	Proleukin
Thrombocytopenia	Oprelvekin	Neumega
Interferons		
	Interferon α -n1	Wellferon
	PEG-Interferon α 2a	Pegasys
Hepatitis C	PEG-Interferon α 2b	PEG-Intron A, Viraferon PEG
	Interferon α 2b + Ribavirin	Rebetron & Viraferon
Cancer, genital wart & hepatitis	Interferon α 2b	Intron A
Hairy cell leukemia	Interferon α 2a	Roferon A
Hepatitis B & C	Interferon α 2b	Alferon-N
Chronic granulomatous disease	Interferon δ 1b	Actimmune
	Interferon β 1b	Betaferon & Betaseron
Multiple sclerosis	Interferon β 1a	Avonex, Rebif

IFN- α preparation (Table 4) have also proved efficacious in the treatment of additional viral-induced medical conditions. R-IFN- α 2B (Intron A) and r-IFN- α n3 are already approved for the treatment of sexually transmitted genital warts, caused by a human papilloma virus [41-46]. IFN- β , normally produced by fibroblasts, was the first interferon to be purified. Humans synthesize a single IFN- β molecule containing 166 amino acid residues, which exhibits 30% sequence homology to IFN- α . IFN- δ is usually referred to as 'immune' interferon. Several IFN genes have been cloned and their product approved for human (Table 4).

2.6. Blood Factors

The process of blood coagulation is dependent on a large number of blood clotting factors [47-51] which act in a

sequential manner. A recombinant form of factor VIIa (NovoSeven or Eptacog a-activated) is marketed (Table 5). The recombinant molecule is produced in a BHK cell line, and the final product differs only slightly from the native molecule. It has proved effective in the treatment of serious bleeding events in patients. Individuals who display a deficiency of factor IX develop haemophilia B, also known as Christmas disease. Although its clinical consequences are very similar to that of a deficiency of factor VIII, its general incidence in the population is far lower. Persons suffering from haemophilia B are treated by recombinant factor IX (BeneFIX) produced in genetically engineered CHO cells. Similarly, r-human blood factor VIII benefits haemophilia A (Table 5) patients where it complements the defects in blood factor VIII.

Table 5. FDA approved r- human blood factors for treating human diseases.

Biotherapy area	Generic name	Brand name
	Kogenate & Helixate	Octocog alfa
Haemophilia A (Factor VIII)	Recombinant	Rurioctocog alfa
	ReFacto	Morocotocog alfa
Von Willebrand disease	recombinant von Willebrand factor +Factor VIII	von Willebrand factor/factor VIII
Haemophilia B	Factor IX	BeneFIX
Haemophilia (FVII deficiency)	Factor VIIa	Novo-Seven

2.7. Recombinant Enzymes

The natural process of thrombosis functions to plug a damaged blood vessel. Rapid removal of the clot can often minimize the severity of tissue damage. Subsequent to this repair, the clot is removed via an enzymatic degradative process called fibrinolysis. Plasmin catalyses the proteolytic degradation of fibrin present in clots, thus effectively dissolving the clot. Therefore, several thrombolytic agents have found medical application (Table 6).

Tissue plasminogen activator (tPA or fibrinokinase) is a 527 amino acid serine protease represents the most important physiological activator of plasminogen [52-57]. The r-h tPA gene has been cloned which facilitated its large-scale production in CHO cell lines. Activase has proved to be effective in the early treatment of patients with acute myocardial infarction where it significantly increased rates of

survival. Thus, Activase established itself as a first-line option in the management of acute myocardial infarction. Genetically engineered and modified forms of tPA have also been obtained in an effort to develop a product with an improved therapeutic profile (e.g. faster-acting or exhibiting a prolonged plasma half-life). Reteplase is one such modified r-h tPA produced in *E. coli* cells and also sold under the names Ekokinase, Retavase and Rapilysin (Table 6) [54-57].

Urokinase is a serine protease produced by the kidney and is found in both the plasma and urine. Human urokinase [58] has been used to treat acute medical events such as pulmonary embolism, the product is normally administered to the patient at initial high doses (by infusion) for several minutes. This is followed by hourly i.v. injections for up to 12 h.

Streptokinase is an extracellular bacterial protein produced by *Streptococcus haemolyticus* group C. It has the ability to induce blood clot lysis and therefore widely employed as

thrombolytic agent [59]. It is administered to treat a variety of thrombo-embolic disorders, including: pulmonary embolism, deep-vein thrombosis, arterial occlusions (obstruction of an artery) and acute myocardial infarction.

Staphylokinase is a protein produced by *Staphylococcus aureus*, which also displays therapeutic potential as a thrombolytic agent [60, 61]. The staphylokinase gene has been cloned in *E. coli*, as well as various other recombinant systems. The protein is expressed intracellularly in *E. coli* at high levels, representing 10–15% of total cellular protein. Although staphylokinase shows no significant homology with

streptokinase, it induces a thrombolytic effect by a somewhat similar mechanism.

α 1-Antitrypsin is a 394 amino acid, 52-kDa serum glycoprotein. It is synthesized in the liver and secreted into the blood, where it is normally present at concentrations of 2–4 g/l. The α 1-antitrypsin gene has been expressed in a number of recombinant systems including milk of transgenic sheep [62] and used in treating pulmonary emphysema, respiratory infections or a deficiency in the production of serum α 1-antitrypsin.

Table 6. FDA approved r-human enzymes for treating human diseases.

Therapy area	Generic name	Brand name
Acute myocardial infarction, pulmonary embolism, stroke & CVT clot removal	Alteplase	Activase & Retavase
Acute myocardial infarction	Reteplase Tenecteplase	TNKase
Acute coronary syndrome	Tirofiban HCl/Eptifibatide	Aggrastat /Integrilin
Coronary angioplasty & unstable angina	Bivalirudin Lepirudin	Angiomax Refludan
Respiratory complications of cystic fibrosis	Dornase alfa	Pulmozyme
Type 1 Gaucher's disease	Imiglucerase	Cerezyme
Fabry's disease	Algalsidase	Fabrazyme
Mucopolisaccharidosis I (Hurler syndrome)	Laronidase	Aldurazyme
Mucopolisaccharidosis VI	Galsulfase	Naglazyme
Severe combined immune deficiency	PEG-ademase	Adagen
Hyperuricemia	Rasburicase	Fasturtec
Acute lymphoblastic leukemia	PEG-L-asparaginase	Oncospar

DNase preparations have been used in the treatment of cystic fibrosis (CF). Presence of microorganisms in the lung attracts phagocytic neutrophils. They begin to ingest the microorganisms and as a result large quantities of DNA are released from damaged microbes and neutrophils at the site of infection. DNA is extremely viscous and substantially increases the viscosity of the respiratory mucus. Recent innovation in CF therapy is to use Pulmozyme (Table 6) to reduce the viscosity of respiratory mucus [63-66]. Pulmozyme is produced in an engineered CHO cell line harboring a nucleotide sequence coding for r-human DNase.

Gaucher's disease is an inborn error of metabolism characterized by lack of the enzyme glucocerebrosidase, with consequent accumulation of glucocerebrosides, particularly in tissue based macrophages. Cerezyme is produced in a CHO cell line harboring the cDNA coding for human β -glucocerebrosidase and is used for treatment of Gaucher's disease [67, 68].

Recombinant α -galactosidase was also approved for general medical use. α -Galactosidase has been approved for long-term enzyme replacement therapy in patients with Fabry's disease. Like Gaucher's disease, Fabry's disease is a genetic disease of lipid metabolism. Sufferers display little or no liposomal α -galactosidaseA activity. This results in the progressive accumulation of glycosphingolipids in several body cell types. Two recombinant α -galactosidase products (Table 6) are now on the market (Fabrazyme and Replagal) [69].

Purine metabolism in some mammals is characterized by a further oxidation of uric acid to allantoin by the enzyme, urate oxidase [70]. Allantoin is significantly more water-soluble than uric acid and is also freely excreted via the renal route. Administration of urate oxidase to humans suffering from

hyperuricaemia results in the reduction of serum uric acid levels through its conversion to allantoin. A recombinant form of the fungal enzyme (Fasturtec) has been produced from an engineered strain of *S. cerevisiae* (Table 6).

In humans, increased generation of O_2 and/or reduced SOD levels have been implicated in a wide range of pathological conditions, including ageing, asthma, accelerated tumor growth, neurodegenerative diseases and inflammatory tissue necrosis. Administration of superoxide dismutase (SOD) has been found to reduce tissue damage due to irradiation or other conditions that generate O_2 . r-Human SOD has been expressed in several recombinant systems, and is being used to prevent tissue damage [71, 72] induced by exposure to excessively oxygen-rich blood.

2.8. Recombinant Vaccines

A vaccine contains a preparation of antigenic components derived from a pathogen. Normally, an initial dose administration is followed by subsequent administration of one or more repeat doses over an appropriate time scale. Such booster doses serve to maximize the immunological response. In most instances, upon vaccine administration, both the humoral and cell-mediated arms of the immune system are activated. The long term immunological protection induced will normally prevent subsequent establishment of an infection by the same or antigenically-related pathogens. Traditional vaccine preparations have largely been targeted against viral and bacterial pathogens, as well as some bacterial toxins and, to a lesser extent, parasitic agents, such as malaria.

The advent of recombinant DNA technology has rendered possible the large-scale production of polypeptides normally present on the surface of virtually any pathogen. These

polypeptides, when purified from the producing organism (e.g. *E. coli*, *S. cerevisiae*) can then be used as 'sub-unit' vaccines. The first such product was that of hepatitis B surface antigen,

which gained marketing approval from the FDA (Table 7) in 1986. Following this, several vaccines have been developed for various bacteria, virus and malaria [73, 74].

Table 7. List of few approved recombinant vaccines for treating human diseases.

Therapy area	Generic name	Brand name
Hepatitis B	Hepatitis B vaccine	Engerix-B
		Recombivax-B
		Pediarix-B
		Hepacare
Hepatitis A & B prevention	Hepatitis A & B vaccine	Twinrix & Ambirix
Haemophilus influenzae B & Hepatitis B	Haemophilus b & Hepatitis B vaccine	Comvax
Lyme disease	Lyme disease vaccine	LymErix
Hepatitis, Tetanus, Diphtheria & pertussis	Hepatitis B, tetanus, diphtheria & pertussis vaccine	Tritanrix HB
		Infanrix-HepB
Hepatitis, Tetanus & Diphtheria	Diphtheria, tetanus & hepatitis B vaccine	Primavax
Haemophilus influenzae B & Hepatitis B	Haemophilus influenzae B and hepatitis B vaccine	Procomvax
Diphtheria, Tetanus & Pertussis	Diphtheria, tetanus and pertussis vaccine	Triacelluvax

2.9. Monoclonal Antibodies

Köhler and Milstein's method, which is still useful today, involves fusion of a cancerous mouse B-cell myeloma with an immunized mouse plasma cell, creating a hybrid cell, or *hybridoma* where the benefits of both the cells retained. This discovery propelled science and medicine into the modern monoclonal antibody era. The resulting hybrid's immortality is provided by the myeloma cell, and the plasma cell supplies the (monoclonal) antibody secretion function. The major drawbacks of murine monoclonal antibodies are the reduced plasma half-life for example, human IgG normally has a half-life of about three weeks while murine IgG has a half-life of only a few hours. Despite of this, murine monoclonal antibodies (Orthoclone OKT3 and Zevalin) (Table 8) are used for imaging and therapeutic applications [75-78].

Chimeric antibodies (Table 8) are composed of protein sequences from two origins: murine and human. While murine antibodies are 100% murine protein, chimeric antibodies are

typically only about 33% murine proteins with the remainder human protein [79-82]. In chimeric antibodies (Reopro, Remicade, Erbitux, Rituxan etc.), the variable region with the antigenic specificity of murine is retained while the constant regions which dictate the antibody isotype and constant region genes from human are added.

Humanized monoclonal antibodies (Table 8) typically retain only the hypervariable regions of a murine antibody while the remainder of the antibody is human and are less likely to elicit an immune response than murine or chimeric monoclonal antibodies. Thus, humanized antibodies (Synagis, Herceptin, Campath etc.) typically contain only 5-10% murine composition.

Human monoclonal antibodies [83-85] are fully, or nearly 100%, human in composition. This method uses technologies such as genetically engineered knockout or transgenic mice and phage display libraries to develop fully human antibodies (Vecitibix, Simponi, Ilaris, Stelara etc.).

Table 8. FDA approved Monoclonal antibodies.

Biotherapy area	Generic name	Trade name
Metastatic breast cancer	Trastuzumab	Herceptin
Respiratory viral & pneumonia in children	Palivizumab	Synagis
Crohn's disease, rheumatoid arthritis, ankylosing spondylitis & ulcerative colitis	Infliximab	Remicade
Rheumatoid arthritis & psoriatic arthritis	Rituximab	Rituxan
Acute myeloid leukemia	Gemtuzumab	Mylotarg
Chronic lymphatic leukemia	Alemtuzumab	Campath
B-cell non-Hodgkins lymphoma	Ibritumomab	Zevalin
CD20+ follicular non-Hodgkins lymphoma	Tositumomab	Bexxar
Metastatic colorectal cancer & head and neck carcinoma	Cetuximab	Erbitux
Metastatic colon or rectal cancer	Bevacizumab	Avastin
Asthma	Omalizumab	Xolair
Psoriasis	Efalizumab	Raptiva
Multiple sclerosis	Natalizumab	Tysabri
Prevention of blood clots after PTCA or coronary intervention & unstable angina prior to PTCA	Abciximab	ReoPro
Acute allograft rejection in renal transplant patients & heart and liver transplant rejection	Muromomab	Orthoclone OKT3
Kidney transplant acute rejection	Daclizumab	Zenapax
Acute kidney transplant rejection	Basiliximab	Simulect
Sepsis and Gram (+)ve bacteremia	Nebacumab	Centoxin
Malignant ascites	Catumaxomab	Removab

3. Conclusion

Tremendous progress has been made in the research and development of biopharmaceutical drugs. Advances in manufacturing and processing revolutionized the production of biopharmaceuticals using r-DNA technology and hybridoma technology. Thus, much has been learnt from the scientific and clinical experiences of these biological molecules. Further research and development into biotherapeutic medicines continue to expand opportunities to treat an ever increasing number of diseases, and intellectual property rights will remain a crucial incentive for such innovation. Biopharmaceutical drugs play a role in the discovery and development of biomarkers and will go hand in hand with improved diagnostics, treatments and prevention methods. New technologies and new discoveries are always emerging, yet many challenges remain. Identifying and validating new targets, addressing oral delivery of biotherapeutic drugs, and improving phase III success rate, are a few to be named. It is essential that the biomanufacturing process be able to deliver and demonstrate a safe therapeutic drug at each step of the clinical trial, be economically able to reproducibly manufacture sufficient inventory with a stable shelf-life, and to successfully traverse the regulatory and efficacy hurdles in order to obtain regulatory approval.

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