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Pharmacology of Recombinant or Genetically Engineered Drugs

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ABSTRACT

Recombinant technology or genetic engineering is a modern method used for the synthesis of therapeutic agents. The central theme of recombinant technology is the process of "gene cloning" which consists of the production of a defined fragment of DNA and its propagation and amplification in a suitable host cell. Drugs developed by recombinant technology or genetic engineering are known as biologics, biopharmaceuticals, recombinant DNA expressed products, bioengineered, or genetically engineered drugs. A current list of various products developed by recombinant technology includes erythropoietin, coagulation modulators, enzymes, hormones, interferons, interleukins, granulocyte colony-stimulating factors, anti-rheumatoid drugs, and various other agents like TNF, becaplermin, hepatitis-B vaccine, antibodies etc. This article provides general as well as recent pharmacological information on different aspects of recombinant drugs that may be useful for their better understanding by users and health care professionals.

Key words: Biologics, erythropoietin, interferon, interleukins, insulin, thrombolytic enzymes

DOI: 10.4103/0975-1483.55747

INTRODUCTION

Drugs developed using living organisms with the help of biotechnology or genetic engineering are known as biologics, biopharmaceuticals, recombinant DNA expressed products, bioengineered, or genetically engineered drugs. The recombinant technique was developed by Cohen in 1973.[1] The general procedure starts from the identification of the gene responsible for the production of the desired product. The gene is isolated from human cells and inserted into other carrier or vector cells like bacteria (*Escherichia coli*) or yeast (*Saccharomyces cerevisiae*, *Hansenulla polymorpha*, *Pichia pastoris*) that proliferate and produce large amounts of the desired product.[2] The central theme of recombinant DNA technology is the process of "gene cloning" which consists of the production of a defined fragment of DNA and its propagation and amplification in a suitable host cell. Recombinant technology was only possible after the discovery of restriction endonucleases, the enzymes used as cutters for a desired segment[3] of genes known as recognition sequences. There are various other enzymes that have great value in recombinant technology such as DNA polymerases, ligases, kinases, alkaline phosphatases, and nucleases.[4] For thousands of years, living microorganisms have been used to produce gases, leaven bread, ferment alcoholic liquids, and for other desired by-products, but their use in recombinant DNA technology is often described as a relatively new science.[5] Recombinant technology, thus, is a new method for the development of drugs and other life-saving products that involves the blending of discoveries in molecular biology, DNA alteration, gene splicing, immunology,

and immunopharmacology. Now a days, various human diseases are treated with the help of drugs developed by recombinant technology, such as erythropoietin, coagulation modulators, enzymes, hormones, interferons, interleukins, granulocyte colony-stimulating factors, anti-rheumatoid drugs, and various other agents like Tumor necrosis factor (TNF), becaplermin, hepatitis-B vaccine, antibodies etc. This article provides general as well as recent pharmacological information on different aspects like a brief description of recombinant products, pharmacological actions, mechanism of action, adverse effects, doses, contraindications, interactions and therapeutic applications. The pharmacological information of bio-engineered products may be useful for better understanding by users and professionals in the medical and health-related sciences.

MANUFACTURING PROCESS FOR RECOMBINANT PRODUCTS

The following steps are involved in the production of recombinant products:[4]

Extraction of the DNA from a donor organism and the plasmid from bacteria
↓
Cleaved with restriction endonucleases
↓
Joined to form a recombinant DNA molecule or vector
↓
Insert this vector into host cell or expression system (*E. coli* or Yeast)
↓
Multiplication of recombinant DNA molecules
↓
Formation of clone
↓
Scale-up (harvesting or fermentation) of cell to produce recombinant drugs
↓
Purification (centrifugation or filtration)
↓
Preformulation
↓
Preclinical studies (animal testing)
↓
Clinical studies (human testing)
↓
Final product

CLASSIFICATION OF RECOMBINANT PRODUCTS

The following products or biologics are obtained by recombinant or genetic engineering technology:

(I) Recombinant erythropoietin
- Epoetin-α
- Epoetin-β
- Epoetin-ω
- Darbepoetin-α

(II) Recombinant blood coagulation modulators

(a) Coagulants
- Factor-VII A
- Factor-VIII
- Factor-IX

(b) Anticoagulants
- Lepirudin
- Desirudin
- Drotecogin alfa

(III) Recombinant enzymes

(a) Thrombolytic enzymes
- Alteplase
- Anistreplase
- Duteplase
- Reteplase
- Saruplase
- Streptase or streptokinase
- Tenecteplase
- Tissue plasminogen activator
- Urokinase

(b) Therapeutic enzymes
- Agalsidase beta
- Aeromonas aminopeptidase
- Alpha-glactosidase-A
- Dornase alpha
- Imiglucerase or glucocerebrosidase or alglucerase
- Laronidase or alpha-L-iduronidase
- Galsulfase or *N*-acetylgalactosamine-4-sulfatase

(IV) Recombinant hormones

(a) Insulin and its analogs
- Human insulin
- Insulin aspart
- Insulin glargine
- Insulin lispro
- Insulin glulisine
- Insulin-like growth factor-I
- Insulin-like growth factor-II

(b) Growth hormone and its analogs
- Growth hormone
- Growth hormone (ovine)
- Growth hormone binding protein

Growth hormone-20K
Somatrem
Somatropin
Sermorelin

(c) Gonadotropins

Recombinant follitropin-α
Recombinant follitropin-β
Recombinant choriogonadotropin
Follicle-stimulating hormone
Luteinizing hormone

(d) Other hormones

Calcitonin
Glucagon

(V) Recombinant interferons

Interferon alfa-2a
Interferon alfa-2b
Interferon beta-1a
Interferon beta-1b
Interferon-gamma-1a
Interferon-gamma-1b
Interferons-ω
Consensus interferon

(VI) Recombinant interleukins

Interleukin-1 alpha
Interleukin-1 beta
Interleukin-2 (aldesleukin)
Interleukin-3
Interleukin-4
Interleukin-10
Interleukin-11 (oprelvekin)
Interleukin-13
Interleukin-15
Interleukin-17
Interleukin-18

(VII) Recombinant granulocyte colony stimulating factors

Filgrastim
Lenograstim
Molgramostim
Sargramostim
Regrasmostim
Pegfilgrastim

(VIII) Recombinant anti-rheumatoid agents

Infliximab
Adalimumab
Basiliximab
Daclizumab
Anakinra
Etanercept

(IX) Miscellaneous agents

Recombinant antibodies
Recombinant albumin
Fc purified recombinant protein
Becaplermin
Beta-nerve growth factor
Bone morphogenetic protein-2
Brain-derived neurotrophic factor
Ciliary neurotrophic factor
Chymosin
Endostatin human
Enteropeptidase/Enterokinase
Epidermal growth factors
Fibroblast growth factor
Hepatitis-B vaccine
Hepatocyte growth factor
Influenza A, nucleoprotein, recombinant protein
Lung surfactant protein
Relaxin
TNF
Recombinant vaccines
α-antitrypsin
Thrombopoietin

PHARMACOLOGY OF INDIVIDUAL PRODUCTS

Recombinant erythropoietin

Erythropoietin[6] is a 165 amino acid glycoprotein hormone that is synthesized naturally in the kidney and the liver but is now prepared by recombinant technology. It stimulates erythrocyte formation and is available as epoetin-α, epoetin-β, and darbepoetin-α. *Mechanism of action*: It stimulates proliferation and differentiation of erythrocytes. *Dose*: 5-100 units/kg/*s.c.* or *i.v.*/three times a week. *Adverse effects*: Allergic reaction, deficiency of iron and folic acid, disorientation, encephalopathy, flu-like symptoms, headache, mild hypertension, seizures, and thrombosis. *Uses*: It is preferred in cases of anemia due to AIDS, cáncer chemotherapy, chronic renal failure, and anemia in premature babies. It is also useful in hematopoietic disorders and is used to increase the benefits of autologous blood transfusión.

Recombinant blood coagulation modulators

Coagulants[7]

Factor-VII A is a unique hemostatic agent containing 406 amino acids[8,9] that is supposed to activate the extrinsic pathway of the coagulation cascade.[10] *Dose*: 150- 500 μg/ kg of body weight. *Adverse effects*: Factor VIIa may cause adverse effects such as pyrexia, hemorrhage, injection site reactions, arthralgia, headache, hypertension, nausea, vomiting, pain, edema, and rashes. *Uses*: It is preferred in cases of hemophilia-A and B, and in patients with congenital and acquired bleeding abnormalities.

Factor-VIII is an endogenous glycoprotein coagulating factor that is necessary for blood clotting and hemostasis. It is a cofactor essential for factor IX to activate factor X in the intrinsic pathway. It is a 1438 amino acid peptide that is produced in CHO cells. Deficiency of factor VIII causes a common inherited bleeding disorder known as hemophilia-A.[11,12] *ADME*: It is administered intravenously; its $t_{1/2}$ is about 12 hours. *Dose*: 10-50 IU/ kg/ *i.v.*/every 2-3 days. *Adverse effects*: Factor VIII may cause adverse effects like febrile reactions, angioedema, flushing, Urticaria, light-headedness, visual disturbances, nausea, and vomiting. *Uses*: It is preferred in cases of prevention of bleeding during surgery in patients with hemophilia-A.

Factor-IX is a polypeptide containing 415 amino acids; it is a vitamin K-dependent coagulation factor that is synthesized in the liver or by recombinant technology. *Mechanism of action*: Factor IX activated by factor IXa, in combination with factor VIII, activates factor X to Xa and leads to the conversion of prothrombin to thrombin and the formation of a fibrin clot. *Dose*: 75 IU/kg/*i.v.* infusion. *Adverse effects*: Factor IX may cause adverse effects like headache, fever, chills, flushing, burning at injection site, tingling, hives, lethargy, nausea, and vomiting. *Uses:* It is preferred in bleeding conditions such as hemophilia-B.

Anticoagulants

Lepirudin[13] and Disirudin[14] are thrombin-inhibiting agents, developed by recombinant technology and are also known as derivatives of hirudin. *Precautions*: used cautiously in patients with renal failure. *Mechanism of action*: They directly bind to clot-bound and fluid-phase thrombin. *Dose*: 30 mg/kg/*i.v.*/ day. *Uses*: They are preferred in cases of heparin-induced thrombocytopenia and deep vein thrombosis.

Drotrecogin alfa is a recombinant form of human activated protein-C used as an anticoagulant and anti-inflammatory agent. *Mechanism of action*: Inhibition of coagulation by proteolytic inactivation of factors Va and VIIIa. *Dose*: 24 mg/kg/h for a maximum of 96 hours. *Adverse effects*: Drotrecogin alfa therapy may cause bleeding. *Uses*: It is preferred for the treatment of severe sepsis.

Recombinant enzymes

Thrombolytic enzymes

Alteplase[15] is an enzyme that catalyzes tissue plasminogen to plasmin and is commonly used to prevent clot-related myocardial disorders. *Mechanism of action*: It dissolves blood clots by converting plasminogen into plasmin that digests fibrin, fibrinogen, and other proteins. *Interactions*: Its action accelerates in combination with heparin. *Contraindicated in* bleeding, defective homeostasis, trauma, surgical procedures, stroke, acute pericarditis, hypoglycemia and hyperglycemia. *Dose*: 100 mg/*i.v.* infusion. *Adverse effects*: Alteplase may cause adverse effects like nausea, vomiting, fever, arrhythmias, allergy, hypotension, intracranial hemorrhage, and GIT bleeding. *Uses*: It is preferred in cases of angina pectoris, as an anticoagulant, intravascular thrombosis, ischemic diseases, myocardial infarction, pulmonary embolism, and to dissolve thrombi.

Tissue plasminogen activator is fibrin-selective but does not activate systemic plasminogen. It is synthesized by recombinant technology and is also obtained from cultured human melanoma cells. *Mechanism of action*: It induces fibrinolysis of the formed thrombus by preferentially activating plasminogen bound to fibrin. *Dose*: 100 mg/*i.v.*/3 hours. *Uses*: It is preferred in the treatment of deep vein thrombosis.

Streptase or streptokinase[16] is obtained from β-hemolytic Streptococci group-C. It combines with circulating plasminogen to form an activation complex, which then causes limited proteolysis of other plasminogen molecules to plasmin. Antistreptococcal antibodies present due to the initial dose of streptokinase make a loading dose necessary in the beginning. *Mechanism of action*: Streptokinase activates the conversion of plasminogen (profibrinolysin) into plasmin (fibrinolysin), which stimulates the conversion of fibrin (insoluble) into fibrin fragments (soluble). *Dose*: 250,000 units followed by 100,000 units/hour for 1-3 days/*i.v.* *Contraindicated in* active internal bleeding, bleeding diathesis, cerebral tumor. If hemostasis is important, pregnancy, previous cerebrovascular accident, recent cranial trauma, surgery within ten days, and uncontrolled hypertension. *Adverse effects*: Streptokinase may cause anaphylaxis, bronchospasm, hypersensitivity, fever, hypotension, and arrhythmias. *Uses*: It is preferred in cases of acute arterial thromboembolism, acute myocardial infarction, acute thrombotic stroke, deep venous thrombosis, local thrombolysis in the anterior chamber of the eye, myocardial infarction, and unstable angina.

Urokinase[17] is a proteolytic but not antigenic, fibrin-selective, thrombolytic, or fibrinolytic agent. It is developed by recombinant technology as pro-urokinase and is also derived from the human kidney (is present in urine). It is converted to urokinase from pro-urokinase upon its binding to fibrin. *Mechanism of action*: It directly converts plasminogen into plasmin. *Contraindicated in* bleeding risks, vascular aneurysm, endocardial thrombi, and allergy to streptokinase. *Dose*: 3,00,000 Units/h for 12 hours/*i.v.* *Adverse effects*: Urokinase may cause fever, bleeding, GIT bleeding, and hemolytic stroke.

Uses: It is preferred in cases like central deep vein thrombosis such as superior vena cava syndrome and ascending thrombophlebitis. It is also effective in acute coronary thrombosis, myocardial infarction, and multiple pulmonary emboli.

Anistreplase[18] or anisoylated plasminogen-streptokinase activator complex is a human plasminogen and streptokinase. The anisoyl group is removed in blood by a hydrolytic deacylation process. *Dose*: 30 units/*i.v.* infusion over 2-5 minutes. *Adverse effects***:** Anistreplase may cause hypotension and allergy.

Tenecteplase[19] is developed by the recombinant technique and is a mutant of alteplase containing 527 amino acids. It is more fibrin-selective and more resistant to plasminogen activator inhibitor-1. It is given as a single bolus injection of 30-50 mg. It has a longer $t_{1/2}$ and greater efficacy.

Saruplase[20] is a full-length, human, unglycosylated, single-chain polypeptide containing 411 amino acids, urokinase type plasminogen activator and also known as prourokinase. It is obtained by recombinant technology from *E. coli*. It is a fibrin-specific fibrinolytic agent and is effectively used for the treatment of thrombotic disorders such as acute myocardial infarction. *Dose*: 20 mg/*i.v.* bolus followed by a 60 mg infusion for 60 minutes. *Uses*: It is preferred in thrombotic disorders and myocardial infarction.

Therapeutic enzymes[21]

Imiglucerase[22] is administered as enzyme replacement therapy in Gaucher's disease, a familial disorder affecting mainly the liver, spleen, bone marrow, and lymph nodes due to the deficiency of beta-glucocerebrosidase activity, leading to the accumulation of glucocerebrosidase in many body tissues. It improves hematological abnormalities, hepatosplenomegalia and quality of life in patients of Gaucher's disease. *Mechanism of action*: It catalyzes the hydrolysis of glucocerebrosidase to glucose and ceramide. *Dose*: 60 units/kg/*i.v.* infusion/two weeks. *Adverse effects***:** Imiglucerase may cause abdominal cramps, angioedema, diarrhea, dizziness, fatigue, fever, flushing, headache, hypotension, nausea, vomiting, tachycardia, and urticaria. *Uses***:** It is preferred in the treatment of Gaucher's disease.

Agalsidase beta is an enzyme used for the treatment of Fabry's disease; a lysosomal storage disorder caused by a deficiency of alpha-galactosidase, leading to progressive accumulation of glycosphingolipids, particularly GL-3 in many body tissues. *Mechanism of action*: It provides an exogenous alpha-galactosidase-A, and catalyzes the hydrolysis of glycosphingolipids including GL-3. *Dose*: 1 mg/kg/*i.v.* infusion/two weeks for 20 weeks. *Adverse effects*: Agalsidase beta may cause nausea, vomiting, abdominal pain, abnormal tear secretion, anemia, bradycardia, dizziness, drowsiness, edema, fatigue, fever, headache, hypersensitivity reactions, hypertension, injection site pain, myalgia, palpitation, paraesthesia, proteinuria, tachycardia, tremors, and visual disturbances. *Uses*: It is preferred in the treatment of Fabry's disease.

Laronidase is an enzyme used for the treatment of non-neurological manifestations of mucopoly saccharidosis-I, a lysosomal storage disorder caused by the deficiency of alpha-L-iduronidase. *Dose*: 0.58 mg/kg/*i.v.* infusion/once a week. *Adverse effects*: Laronidase may cause flushing, musculoskeletal pain, rashes, headache, and abdominal pain. *Uses*: It is preferred in non-neurological manifestations of mucopolysaccharidosis-I and to improve pulmonary functions.

Dornase alpha[23] is an enzyme prepared from Chinese hamster ovary cells. It is a 260 amino acid, phosphorylated, glycosylated, recombinant human deoxyribonuclease-1 (rhDNase) or is a genetically engineered product of a naturally occurring human enzyme that breaks extracellular deoxyribonucleic acid or DNA. It is useful in the treatment of cystic fibrosis. *Pharmacological actions*: It reduces mucous viscosity in cystic fibrotic patients. *Mechanism of action*: Cleaves extracellular DNA and reduces sputum viscosity. *Precautions*: Breast feeding mothers. *Dose*: 2.5 mg/OD or BD/inhaled by nebulizer. *Adverse effects*: Dornase alpha may cause chest pain, conjunctivitis, laryngitis, pharyngitis, rashes, urticaria, and voice changes. *Uses*: It is preferred in the treatment of cystic fibrosis.

Recombinant hormones

Insulin and its analogs[24]

Insulin is a protein hormone produced by the β-cells of the Islets of Langerhans of the pancreas. It regulates the metabolism of carbohydrates, fats, and proteins. Lack of insulin or resistance to its action may causes diabetes mellitus. It lowers the blood glucose concentration by increasing the uptake of glucose into tissues, enhancing glycogen synthesis from glucose, and inhibition of glyconeogenesis. *Sources of insulin*: Human insulin is prepared by enzymatic modification of porcine insulin or by recombinant technology using *Escherichia coli*. *Routes for insulin administration*: Intraperitoneally, implants, oral, nasal, rectal, and inhalation. *Insulin delivery devices*: External artificial pancreas, implantable pumps, insulin pumps, insulin syringes, jet injectors, and pen devices. *Mechanism*

of action: Insulin interacts with insulin receptors located on the liver and the fat cells, and enhances the transport of glucose from the blood into cells by increasing the activity of glucose transporters. *ADME*: Insulin is administered subcutaneously, degraded by proteolytic enzymes in the liver, kidney, and muscles, and its $t_{1/2}$ is 5-9 min. *Interactions*: Alcohol and β-blockers produce a synergistic hypoglycemic effect. *Contraindicated* with β-blockers which mask the hypoglycemic effect in diabetic patients who are on insulin therapy. *Adverse effects*: Insulin may cause hypoglycemia, local irritation, local hypertrophy, hypersensitivity, and weight gain or obesity. *Uses*: It is preferred in diabetes due to pancreatic disease, diabetes, ketoacidosis or diabetic coma, hyperkalemia, improving appetite and body weight, and schizophrenia. It can also be used to test the completeness of vagotomy, in the treatment of diabetes mellitus, particularly IDDM, and an insulin + glucose infusion may be used in-cyclic vomiting, hyperemesis gravidam, anorexia, and acute alcoholism.

Growth hormones and their analogs[25]

Growth hormones and their analogs are synthesized by recombinant technology, and are commonly used as growth-promoting agents. These agents are not effective in bone disorders, cardiac diseases, cartilage disorders, Down syndrome, families of short stature, GIT problems, and renal complications. They are available as somatrem, somatropin, and sermorelin. These agents are recommended for the treatment of complications due to the deficiency of growth hormone. Growth hormone deficiency may occur due to skull fracture, excessive exposure to radiation, cancer disease, and due to intracranial infections like tuberculosis or meningitis. *Pharmacological actions*: Stimulate the growth of connective tissues and skeletal muscles and enhance protein synthesis and fatty acid metabolism. They also decrease the use of glucose in the body. *Contraindicated in* severe obesity, severe respiratory syndrome, and after renal transplantation. *Adverse effects*: Growth hormones may cause acromegaly, antibody formation, arthralgia, atherosclerosis, diabetes mellitus, fluid retention, gigantism, glucose intolerance, headache, hypertension, hypoglycaemia, hypothyroidism, insulin resistance, myalgia, nausea, vomiting, pain and pruritis at injecting point, paraesthesia, and visual disturbances. *Uses*: They are preferred in anti-aging and performance enhancers, chronic renal deficiency, dwarfism, gonadal dysgenesis, growth hormone deficiency, idiopathic short stature, and Sheehan's syndrome.

Gonadotropins[26]

(i) Recombinant human growth hormone, (ii) Menotropins (FSH+LH) (obtained from urine of menopausal women)-Recombinant follitropin-α and follitropin-β, (iii) Urofollitropin or menotropin (Pure FSH), and (iv) Human chorionic gonadotropin (obtained from urine of pregnant women). Recombinant choriogonadotropin-α and luteinizing hormone. *Uses*: They are preferred in reproductive endocrinology, to restore fertility in males and females, and diagnosis of pregnancy, time of ovulation and several male and female reproductive disorders.

Follicle-stimulating hormone[27] is secreted from the anterior pituitary under the influence of the hypothalamic gonadotropin-releasing factor. It is mainly released in the first phase of the menstrual cycle wherein it induces the growth and maturation of graafian follicles by acting on the ovaries. *Adverse effects*: Follicle stimulating hormone may cause adverse effects like gynecomastia. *Uses*: It is preferred in cryptorchidism, and for the maturation of prepubertal testes, and spermatogenesis.

Other hormones

Calcitonin is a polypeptide hormone containing 32 amino acids. It is prepared by recombinant technology and is also secreted by c-cells of the thyroid gland in mammals and the cells of ultimobranchial bodies in lower vertebrates. Its synthesis and release depend on calcium present in the body. It is involved with parathyroid hormone in the regulation of bone turnover and hence, in the maintenance of the calcium balance and homeostasis. It is used to reduce plasma calcium levels in hypercalcemia. *Mechanism of action*: Prevents bone resorption by inhibiting the action of bone osteoclasts. *Interactions*: Enhances the release of calcitonin glucagon, adrenergic agents, gastrin, prolactin, serotonin, and thyroxin. *Adverse effects*: Calcitonin may cause effects allergic reactions, alteration in taste, abdominal pain, diarrhoea, dizziness, facial flushing, nausea, vomiting, rashes, and tingling sensation in the hands. *Uses*: It is preferred in hypercalcemic states associated with hyperparathyroidism, osteoporosis, Paget's disease, and vitamin-D intoxication.

Recombinant interferons[25,28]

Interferons are naturally occurring, inducible, small glycoproteins or cytokines, host-specific, broad-spectrum, potent, synthetic antiviral agents. They may also be produced by all body cells in response to inducers such as attenuated measles, microbial extracts, viruses, and anionic polysaccharides. They induce protein kinases, oligoisoadenylate synthetase, and phosphodiesterase enzymes. They can now be prepared by recombinant and cell culture techniques. They are nonspecific replication inhibitors of DNA and RNA viruses. The following types of interferons have been developed: Interferon alfa-2a,

interferon alfa-2b, interferon beta-1a, interferon beta-1b, interferon-gamma-1a, interferon-gamma-1b, interferons-ω, and consensus interferon. *Mechanism of action*: They block viral replication by inhibiting the translation of viral mRNA into viral proteins and suppress cell proliferation. *ADME*: They are administered *i.m.* or *s.c*, have a half short $t_{1/2}$, and a small amount have been found to enter cerebrospinal fluid. *Dose*: 3-10 MU/*i.v.* infusion/TDS/ week. *Adverse effects*: Interferons may cause alopecia, anemia, bone marrow depression, cardiovascular disorders, depression, fatigue, fever, GIT disturbances, headache, hepatotoxicity, hyperglycemia, hypersensitivity reactions, hypotension, influenza-like symptoms, leucopenia, myalgias, myelosuppression, nephrotoxicity, neutropenia, suicidal behavior, thrombocytopenia, and weakness. *Uses*: α-interferon is used in hepatitis, AIDS, herpes simplex, herpes zoster, and certain types of cancers, hairy cell leukemia, and viral infections. β-interferons are used in sclerosis (hardening of blood vessels). Interferon-γ has been used in rheumatoid arthritis, chronic granulocytosis, hepatitis, leishmaniasis, leprosy, and psoriasis. Consensus interferon is used in hepatitis-C, neoplastic disorders, and infectious complications.

Recombinant interleukins

Aldesleukin[29] is an interleukin-2 obtained from cultures of *E. coli* and is available in two forms: Alpha and beta. *Mechanism of action*: It stimulates the natural killer cells, T and B cells, TC cells, and enhances cellular immunity. *Dose*: 600,000 units/kg/eight hourly for five days. *Adverse effects*: Aldesleukin may cause nausea, vomiting, diarrhea, fever, capillary leak syndrome, hypotension, arrhythmias, peripheral edema, prerenal azotemia, thrombocytopenia, decreased tissue perfusion, confusion, and can reduce concomitant infection. *Uses*: It is preferred in the treatment of metastatic renal cell carcinoma and melanoma.

Oprelvekin is a recombinant human interleukin-11 cytokine, consisting of 177 amino acids. It stimulates megakaryocytes and their precursors in the bone marrow and thus, prevents severe thrombocytopenia and decreases the need for platelet transfusions in cancer patients receiving highly myelosuppressive chemotherapy.

Pharmacological actions: It stimulates the growth of multiple lymphoid and myeloid cells and consequently, enhances platelet and neutrophil counts. *Mechanism of action*: Suppresses chemotherapy-induced thrombocytopenia by stimulating megakaryocytes and their precursors in the bone marrow. *ADME*: It is administered subcutaneously and its $t_{1/2}$ is seven hours. *Dose*: 25-50 μg/kg/*s.c.*/day. *Adverse effects*: Oprelvekin may cause arthralgia, dizziness, headache, anemia, dyspnea, hypokalemia, atrial arrhythmia, fatigue, immunogenicity, myalgia, palpitations, and peripheral edema. *Uses*: It is preferred in the treatment of chemotherapy-induced thrombocytopenia.

Recombinant granulocyte colony-stimulating factors[30-31]

These are naturally occurring or synthetic gylcoproteins that stimulate the multiplication, differentiation, and actions of neutrophills, monocytes, and macrophages. They are useful in the treatment of aplastic anemia and AIDS.

Filgrastim[32] is a 175 amino acid, recombinant, human granulocyte colony-stimulating factor that enhances the synthesis of neutrophils. *Pharmacological actions*: It enhances neutrophil functions and production, increases phagocytosis, cytotoxic and antibody-dependent killing effect of neutrophils, and enhances bone marrow repopulation after high doses of chemotherapy, radiotherapy, and bone marrow transplants. It also mobilizes hematopoietic stem cells into peripheral blood. *Mechanism of action*: It stimulates proliferation and differentiation of neutrophils. *ADME*: It is administered as an *s.c* or *i.v.* infusión, 20 μg/kg for 30 minutes, $t_{1/2}$ is 3.5 hours. *Contraindicated in* severe congenital neutropenia. *Dose*: 5 μg/kg/*s.c.* or *i.v.*/day. *Adverse effects*: Filtrate may cause anemia, bone tenderness, and pain, breathlessness, cutaneous vasculitis, disuria, epistaxis, exacerbation of rheumatoid arthritis, fever, flushing, hepatomegaly, hypotension, increased uric acid, lethargy, mialgia, nausea, vomiting, osteoporosis, pericardial effusions, lowering of BP, skin rashes, splenic enlargement, tachycardia, thrombocytopenia, and urinary abnormalities. *Uses*: It is preferred in bone marrow suppression or myelosuppression during cáncer chemotherapy, chemotherapy-induced neutropenia, cytotoxic-induced neutropenia in AIDS patients during zidovudine therapy, leucopenia, to increase neutrophil counts, to prevent infections, and to treat congenital, cyclic, and aplastic anemia-induced neutropenia.

Molgramostim is an unglycosylated, 127 amino acid, recombinant, human granulocyte macrophage colony-stimulating factor that is derived from *E. coli*. *Mechanism of action*: It stimulates the production of granulocytes and monocytes. *Dose*: 5-10 μg/kg/*s.c.*/day. *Adverse effects*: Molgramostim may cause hypotension, flushing, bone pain, fever, musculoskeletal pain, and GIT disturbances. *Uses*: It is used to reduce the severity of neutropenia in patients on immunosuppressive therapy and to accelerate myeloid recovery in patients with bone marrow transplantation.

Sargramostim is a 127 amino acid glycoprotein that is produced in a yeast expression system. It enhances myeloid engraftment in autologous bone marrow transplantation, decreases the duration of antibiotic administration, and reduces the incidence of infections. It also enhances the proliferation and differentiation of neutrophils, eosinophils, monocytes, and macrophages. *ADME*: It is administered subcutaneously or intravenously, $t_{1/2}$ is 2-3 hours. *Dose*: 125-500 μg/m^2/day/*s.c* or *i.v.* infusion. *Adverse effects*: Sargramostim may cause pleural and pericardial effusion, fever, hypotension, tachycardia, rigors, flushing, malaise, nausea, vomiting, diarrhea, dyspnea, alopecia, bone pain, myalgia, rashes, and mucositis. *Uses*: It is preferred in bone marrow suppression or myelosuppression during cáncer chemotherapy.

Pegfilgrastim[33,34] is a pegylated polyethylene glycol-substituted filgrastim, recombinant human granulocyte colony-stimulating factor. It has a longer duration of action than filgrastim and enhances WBCs production. *Dose*: 6 mg/*s.c.* or *i.v.*/OD/chemotherapy. *Uses*: It is preferred in febrile neutropenia and infections in chemo patients.

Recombinant antirheumatoid agents[5,31]

Etanercept is a genetically engineered, modified fusion protein that resembles the cell surface receptor to which TNF-alpha normally binds to exert its actions. It is thus, a cytokine-inhibiting agent, inhibiting tumor necrosis factor-alpha, and is hence, used in the treatment of idiopathic arthritis. *Mechanism of action*: Inhibition of inflammatory and immunoregulatory actions of TNF-alpha by blocking its interaction with cell surface receptors. *ADME*: It is administered subcutaneously, $t_{1/2}$ is about 115 hours. *Interactions*: Etanercept enhances the action of methotrexate. *Contraindicated in* active infections, pregnancy, and breast feeding. *Dose*: 25 mg/twice/*s.c.*/week. *Adverse effects*: Etanercept may cause abdominal pain, aplastic anemia, anemia, leucopenia, sepsis, asthma, cholecystitis, dyspepsia, dizziness, GIT hemorrhage, headache, hypertension, hypotension, myocardial or cerebral ischemia, esophagitis, opportunistic infections, pancreatitis, pruritis, venous thromboembolism, nausea, tuberculosis, optic neuritis, vomiting, and worsening heart failure. *Uses*: It is preferred in ankylosing spondylitis, idiopathic arthritis, plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, scleroderma, and Wegener's granulomatosis.

Anakinra[35,36] is a recombinant form of interleukin-1ra which inhibits the interactions of interleukin-1 with immune cells and is used for the treatment of rheumatoid arthritis. *Pharmacological actions*: It inhibits proinflammatory and immunomodulatory effects of interleukin-1. *Mechanism of action*: Inhibition of interleukin functions by blocking interleukin receptors. *Precautions*: In asthma patients. *Interactions*: Risk of infection transmission when given with live vaccines and also produces risk of infections and neutropenia when given with TNF antagonists. *Dose*: 100-150 mg/day/*s.c.* *Adverse effects*: Anakinra may cause redness, rashes, flu-like symptoms, pain, and itching at the injection site. Other reactions like headache, nausea, diarrhea, abdominal pain, URTIs, and neutropenia. *Uses*: It is preferred in the treatment of rheumatoid arthritis.

Adalimumab is a human, recombinant, monoclonal antibody to TNF-alpha. It is similar to infliximab but less antigenic as it does not contain any foreign component. *Pharmacological actions*: It blocks the tumor necrosis factor and also modulates leukocyte migration. *Interactions*: Enhances the risk of infections and cancer. *Dose*: 40 mg/0.8 ml/*s.c.*/OD for two weeks. *Uses*: It is preferred in the treatment of rheumatoid arthritis.

Infliximab is a chimeric monoclonal antibody that inhibits the pro-inflammatory cytokine, TNF-α. *Mechanism of action*: Inhibits T-cell and macrophage functions and the release of pro-inflammatory cytokines by occupying the membrane-bound TNF-alpha receptors. *ADME*: It is administered intravenously, $t_{1/2}$ is 200 hours. *Precautions*: Caution may be taken when used in cases of hepatic impairment, renal impairment, heart failure and demyelinating CNS disorders. *Interactions*: Better results with methotrexate and live-attenuated vaccines. *Contraindicated in* severe infections like tuberculosis, pregnancy and breast feeding. *Dose*: 5 mg/kg/*i.v.* infusion/every eight weeks. *Adverse effects*: Infliximab may cause effects like agitation, alopecia, amnesia, anxiety, arrhythmias, arthralgia, bradycardia, cholecystitis, confusion, constipation, demyelinating disorders, diarrhea, diverticulitis, dizziness, drowsiness, dyspepsia, ecchymosis, endophthalmitis, fatigue, flushing, GIT hemorrhage, hematoma, hepatitis, hyperkeratosis, insomnia, interstitial pneumonitis or fibrosis, myalgia, palpitation, peripheral ischemia, rashes, seizures, skin pigmentation, sweating, syncope, vaginitis, and vasospasm. *Uses*: It is preferred in fistulating Crohn's disease, rheumatoid arthritis, and severe active Crohn's disease.

Basiliximab and daclizumab are immunosuppressive, chimeric, monoclonal anti-CD50 antibodies produced by recombinant DNA technology. *Mechanism of action*: Block the alpha-chain of the interleukin-2 receptor located on the surface of activated T-cells. *Interactions*: Better results when given with glucocorticoids, cyclosporine, or azathioprine. *Dose*: 20 mg/two hours before transplantation surgery and 20 mg again on day 4 following transplantation. *Uses*: It is

preferred for the prophylaxis of organ transplants like liver and heart rejection reactions.

Miscellaneous agents

Becaplermin[21] is a recombinant human platelet-derived growth factor for the treatment of full-thickness neuropathic and diabetic ulcers. It is applied topically as a thin layer daily and covered with gauze dressing moistened with physiological saline for a maximum duration of treatment 20 weeks. *Precautions*: Avoid on sites with infection, malignancy, peripheral arteriopathy, osteomyelitis. *Dose*: 0.01% gel/topically. *Adverse effects*: Becaplermin may cause irritation, bullous eruption, and edema. *Uses*: It is used for the treatment of diabetic pressure ulcers.

Hepatitis-B vaccine[37] contains inactivated hepatitis-B virus surface antigen adsorbed on aluminum hydroxide. *Dose*: 1 ml/*i.m.*/repeated one month and six months later. *Adverse effects*: Hepatitis-B vaccine may cause abdominal pain or cramps, agitation, anorexia, arthralgia, asthma, back pain, bronchospasm, chills weakness, constipation, disturbed liver functions, dizziness, ecchymosis, erythema, fatigue, fever, flushing, headache, hypersensitivity, hypotension, influenza-like symptoms, insomnia, lymphadenopathy, malaise, myalgia, nausea, vomiting, pain, palpitation, pruritis, rashes, soreness and induration at the injection site, stiffness in arms, sweating, tachycardia, tingling, and urticaria. *Uses*: It is used to immunize people who are directly involved with the blood or saliva of patients and for passive immunization by vaccination with hepatitis-B immunoglobulin.

Thrombopoietin is a 332 amino acid, high molecular weight glycoprotein produced by hepatocytes and is a primary regulator of platelet production. It is available in two forms: Human megakaryocyte and development factor, and human thrombopoietin. Recombinant human thrombopoietin also reduces the duration of thrombocytopenia and the need for platelet transfusion in cancer patients receiving carboplatin or cyclophosphamide. *Pharmacological actions*: Stimulates the growth of platelets. *Adverse effects*: Therapy with thrombopoietin may cause immunogenicity. *Uses*: It is preferred in thrombocytopenia and thrombocytopenia due to cancer chemotherapy and radiation-induced myelosuppression

CONCLUSION

Recombinant technology or genetic engineering is a modern method used for the synthesis of therapeutic agents. The technique has provided the means to develop many biologically effective therapeutic agents. Recombinant products, biopharmaceuticals, biologics, or genetically engineered drugs are produced using this technology by inserting the desired product isolated from human cells into other carriers or vector cells. Some of the products of recombinant technology are of human origin including erythropoietin, coagulation modulators, enzymes, hormones, interferons, interleukins, granulocyte colony-stimulating factors, antirheumatoid drugs, and various other agents like TNF, becaplermin, hepatitis-B vaccine, antibodies etc. They are proteins and nucleic acids and are very specific, safe and pure agents.

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Source of Support: Nil, **Conflict of Interest:** None declared.