

An Investigation into the World of Protein-Based Therapeutics- Therapeutic Proteins

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Abstract

Protein based therapeutics have been regarded very highly as they have produced immense results in treating various diseases including genetic disorders, neurodegenerative disorders, sexually transmitted diseases, and autoimmune disorders. This has revolutionized the field of medicine by enabling precise targeting for treating diseases and personalized medicine for different individuals. The ability to target the source of a disease or disorder has made this line of therapeutics a major outbreak for revolutionizing the field of targeted medicine and is currently being pursued for developing novel therapeutics. The production of recombinant protein paved the way for these therapeutics and despite the considerable progress made so far in the study and production of therapeutic proteins, there are still concerns over its off-target effects and ethical considerations, which have spurred the exploration of alternative approaches. In this context, this article is presented on therapeutic proteins, an innovative biological approach that has the potential to cure disorders that were once considered incurable. This article highlights the limitations of current targeted medicine technologies and the need for more targeted and personalized approaches to treat genetic disorders. Employing artificially synthesized therapeutic proteins such as monoclonal antibodies (MAC) to target specific cells, tissues, organs, genes, proteins, and sites of disease progression has proven to be more effective in treating the diseases. This approach provides greater specificity and control over various disorders, making it a highly promising therapeutic option for various diseases such as cancer, cardiovascular diseases, and viral infections. This article discusses the status of therapeutic proteins, their potential prospects, as well as the challenges and opportunities that need to be addressed to fully exploit their potential. Overall, the article aims to shed light on the significance of therapeutic medicine in the field of medicine and its potential to revolutionize treatments for various disorders. By providing an overview of this innovative

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approach, it hopes to inspire further research in this area and pave the way for novel and more effective therapies for genetic disorders.

Key words: Therapeutic proteins; Targeted medicine; Monoclonal antibodies; Recombinant protein; Genetic disorders.

Introduction

Proteins are biological molecules that have a lot of different physical and chemical properties. Every protein has its own nature and function in the human body and proteins generally act as enzymes, hormones, signaling molecules, receptors, and binding molecules [1]. Proteins were later begun to be produced synthetically or isolated from living organisms for commercial and therapeutic usage. The production of insulin, the first therapeutic protein and the recent developments in antibody-based therapeutics have led to the revolutionizing treatments for chronic diseases such as cancer and autoimmune disorders [2,3]. The classes and types of therapeutic proteins based on their chemical and physical properties would be discussed further. There are a lot of recombinant proteins that are currently being produced but not all of them are therapeutic proteins. Over 100 therapeutic proteins have been officially approved by FDA so far.

Methods of production of therapeutic proteins

Transgenic animals and transgenic plants are used to produce therapeutic protein. They are typically produced through microbial fermentation on cell cultures, as well as through transgenic animals and plant genetic engineering.

The amino acid chain is the building block of protein. Cellular functions are carried out by proteins [4]. The information stored in the DNA instructs the cell protein synthesis machinery to produce the specific proteins required for its structure and metabolism. Insulin was the first therapeutic protein used to treat disease. Short chains of chemically synthesized peptides, larger proteins produced by living cells, and in some cases, proteins secreted by cells have been used therapeutically.

Production in transgenic plant

Tobacco

Tobacco is also referred to as the “white mouse” of the plant world. It has a high biomass yield and high soluble protein compared to that of any other plants. In addition to antibodies, vaccines, and cytokines, it has the capacity to create a wide variety of therapeutic proteins.

Human growth hormone, which was expressed in transgenic tobacco in 1986, was the first protein produced in plants [5]. For quick reaction to disease outbreaks, like the current influenza A/H1N1 pandemic, and for the patient-specific therapy of cancer, tobacco can produce substantial amounts of protein in a short period of time.

It is necessary to freeze or dry the leaves for storage or extract the protein as soon as

possible after expression since protein storage in the leaves is not especially stable and the result is susceptible to destruction. The presence of phenols and hazardous alkaloids in tobacco tissues often affects subsequent processing.

Legumes

The generation of therapeutic protein in legumes including soybean, pea, and alfalfa has been scientifically shown. Legumes have the benefit of fixing atmospheric nitrogen, eliminating the need for nitrogen in their fertilizer and so lowering the cost of production. These plants do, however, have less leaf biomass than tobacco. Grain legumes, like peas, are being researched as potential expression systems because of the high protein content of their seeds [6,7]. Faba beans (*Vicia faba*) are an excellent source of antioxidants and chemo-preventive agents in addition to having lipid-lowering properties. Mung beans (*Phaseolus aureus*, *Vigna radiata*) are regarded as a healthy diabetic meal since they have a low glycemic index and are high in antioxidants.

Production in transgenic animals

The most advanced technique for producing recombinant proteins from transgenic organisms at present is milk. Other possible systems include blood, milk, egg white, seminal plasma, urine, silk gland, and insect larvae hemolymph. Recombinant proteins are normally too unstable to be maintained in large amounts in blood. Moreover, bioactive proteins in the blood may harm the animals' health. Milk usually avoids these issues. Many

animal species, such as cows, pigs, sheep, goats, and rabbits, are being researched or utilized in making recombinant proteins, which are then found in their milk. There are a number of benefits to using rabbits, including simple transgenic founder and offspring generation, high fertility, relatively high milk production, resistance to prion diseases, and the absence of significant disease transmission to humans.

Mice

Genetically engineered mice have produced therapeutic proteins for human use. This innovation in biopharmaceuticals has made it possible to produce recombinant proteins in vast numbers at low cost [8]. Transgenic mice provide a few advantages over conventional protein expression methods including bacteria, yeast, and mammalian cells to produce therapeutic proteins. Hemophilia can be treated using human clotting factors like factor VIII and factor IX. Monoclonal antibodies are used in the treatment of cancer. For the treatment of liver disease, human serum albumin is used. Human alpha-₁ antitrypsin is used to treat hereditary diseases. These are the same proteins that are produced in mice.

Pig

Pigs are used as a source of islets of Langerhans for islet isolation and xeno-transplantation. The production of insulin in pigs begins with the synthesis of insulin mRNA in the nucleus of the beta cells. This mRNA is then transported to the ribosome, which are cellular structures responsible for

protein synthesis. In the ribosome, the insulin mRNA is translated into a pro-insulin molecule, which contains a signal peptide that helps to guide the molecule to the endoplasmic reticulum. Pro-insulin is then cleaved into c-peptides and packed into secretory vesicles. This secretory vesicle containing the mature insulin is transported into the cell membrane where they fuse with the membrane and release insulin into the bloodstream through the process called exocytosis.

Insect cell culture

Hemagglutinin is the influenza vaccine protein produced by insect cell culture and the baculovirus expression system.

Baculoviruses are the family of viruses that infect insects and act as vectors for the expression of recombinant protein in insect cells [9]. The baculovirus *Autographa californica* nuclear polyhydrosis virus can be propagated in cell lines. These cells' DNA encodes polyhydriin that was unnecessary for the survival of the virus in the lab and could be replaced by the HA gene.

Finally, HA product will purify by using mild detergent conditions and by using a combination of filtration and column chromatography methods.

Hybridoma technology

Hybridoma technology is used to produce therapeutic proteins such as monoclonal antibodies, which are used to treat a variety of diseases. By injecting a particular antigen into a mouse and obtaining the antigen-specific

plasma cells, one may create a hybridoma, which is a Harry cell. Antibody-producing cells from the mouse spleen and it is fusion with an immune cancer cell known as a myeloma cell.

The resulting hybrid cell can be replicated to create several identical daughter clones. The immune cell product is then secreted by these daughter clones [10]. Since they arise from just one kind of cell, the hybridoma cell, these antibodies are known as monoclonal. The benefit of this method is that it may combine the traits of the two separate cell types, including their capacity for rapid growth and the production of significant quantities of pure antibodies. HAT medium (Hypoxanthine Aminopterin Thymidine) is used to produce the monoclonal antibody. First, a desired antigen that is for isolating an antibody against is presented to laboratory animals (such as mice). After animal splenocyte isolation, immortalized myeloma cells without the HGPRT (Hypoxanthine-guanine phosphoribosyltransferase) gene are fused with B cells using polyethylene glycol or the Sendai virus [11]. The HAT medium is used to incubate fused cells.

Myeloma cells that are exposed to aminopterin perish because they are unable to synthesize nucleotides in the de novo or salvage media. Thus, the unfused D cell perishes. B cells that have not merged perish because they are short-lived.

As the HGPRT gene originates from the B cells and is functional, only the B cell-myeloma hybrids survive. These cells create antibodies and possess immortality, which is

a characteristic of myeloma cells. The incubated liquid is next diluted into multi-well plates until just one cell is present in each well. The chosen antibody may then be verified in each well's supernatant. The antibody in a well is produced by the same B cell known as a monoclonal antibody.

Bacteria

E. coli

E. coli is a commonly used host to produce therapeutic proteins due to its fast growth rate, well-established genetic tools, and relatively low cost of cultivation. Insulin, Human growth hormone, and Erythropoietin are the therapeutic proteins that are produced by *E. coli*.

Using methods like PCR or restriction enzyme digestion, the gene encoding the therapeutic protein is extracted from human DNA. The gene for the therapeutic protein is introduced into a plasmid vector that also has a bacterial promoter, which will cause *E. coli* to express the gene [12].

Techniques like electroporation or chemical transformation are used to introduce the recombinant plasmid into *E. coli* cells. The culture medium in which the transformed *E. coli* cells are being cultured permits the expression of the gene encoding the therapeutic protein. Typically, a substance like IPTG is used to induce the bacterial promoter in this manner.

Methods like chromatography are used to separate therapeutic protein from the growth medium.

Streptomyces

Some strains of the bacterium *Streptomyces griseus* naturally produce the antibiotic streptomycin. *Streptomyces* employs a complex metabolic process to produce streptomycin, starting with the conversion of simple nutrients into raw materials for the antibiotic's synthesis. Under carefully controlled conditions, *Streptomyces* bacteria are grown in a nutrient-rich medium. The precursors of streptomycin are produced by the bacteria and converted into antibiotics through an enzymatic process [13].

The bacteria secrete streptomycin into the medium around them. From the medium, streptomycin is extracted and purified. A complex network of genes and metabolic processes that react to variations in environmental factors including nutrition availability, pH, and temperature control the production of streptomycin by *Streptomyces*. *Streptomyces'* genome can be altered using genetic engineering techniques to increase streptomycin production. Overall, *Streptomyces* is an important natural source of antibiotics, including streptomycin, and studies are currently underway to find ways to enhance and optimize the production of these valuable substances.

Spirulina

Spirulina is a type of blue-green algae that can be used to produce a variety of therapeutic proteins such as phycocyanin, C-phycocyanin, and *spirulina* growth factors. C-phycocyanin can be produced by cultivating

under controlled conditions and extracting the protein from the cells.

In a nutrient-rich *spirulina* medium under controlled conditions, such as temperature, light, and pH, *spirulina* cells are grown. The cells are washed to remove foreign objects after harvesting. The cells are then disrupted to release the protein, typically using techniques like sonication. Combining techniques including centrifugation, filtering, and chromatography are used to purify the protein [14,15]. The C-phycoerythrin that has been purified is collected and examined for purity and activity. The specific *spirulina* strains used, the growing conditions, and the purification techniques used can all affect the production and purity of C-phycoerythrin. C-phycoerythrin may be produced in greater quantities and with greater purity by optimizing these variables. As a natural blue colorant in food and cosmetics, as a nutritional supplement, and as a possible medicinal agent, c-phycoerythrin has many applications. *Spirulina's* production of C-phycoerythrin is a sustainable and environmentally beneficial way to produce this protein.

Staphylococcus

Staphylococcus aureus is a type of bacteria that can produce several therapeutic proteins such as Staphylokinase, and coagulase. Coagulase is an enzyme that is produced by *Staphylococcus aureus*. When *Staphylococcus aureus* cells come into touch with host tissues, for example, they create coagulase, a procoagulant chemical [16]. The bacteria secrete coagulase, which binds to

prothrombin in the blood of the host. Prothrombin transforms into its active form, thrombin when coagulase binds to it. A clot is created when thrombin changes the soluble blood protein fibrinogen into the insoluble fibrin. The clot may also function as a source of nutrition for the bacteria to proliferate while shielding them from the host's immune system.

Yeast

Due to its simplicity in genetic manipulation, rapid rate of growth, and capacity for high protein synthesis, yeast is a promising platform for the generation of therapeutic proteins. Yeast can produce a variety of therapeutic proteins including Insulin, human serum albumin, interferon, blood clotting factors, and growth hormone. Yeast can produce human serum albumin (HSA) using recombinant DNA technology [17]. The HSA gene is introduced into the yeast genome during this process, allowing the yeast to produce the protein. Yeast cells are cultured under controlled conditions in a bioreactor to produce HSA. The HSA protein is then extracted and purified once the cells are harvested. After that, the purified HSA can be applied medically. Compared to conventional manufacturing techniques, which involve extracting protein from human blood, the production of HSA by yeast has a number of benefits. It firstly eliminates the risk of blood-borne infection contamination. Secondly, it makes it possible to produce a lot of HSA in a short period of time.

Finally, it lowers the cost of production, increasing patient access to HSA. Overall, the

production of this essential therapeutic protein by yeast using recombinant DNA technology is a secure, successful, and economical technique.

Chemically synthesize therapeutic protein

Chemically synthesized therapeutic proteins are proteins that are artificially created in the laboratory through chemical synthesis, rather than being produced naturally by living organisms. There are several chemically synthesized therapeutic proteins such as glucagon-like peptide 1, erythropoietin, etc. are produced. The first step is to identify the protein of interest, such as insulin, erythropoietin, or a monoclonal antibody. The next step is to design the peptide sequence of the protein. This involves determining the amino acid sequence and any post-translational modifications, such as glycosylation or phosphorylation [18,19]. The peptide sequence is synthesized using chemical methods, such as solid-phase peptide synthesis. This involves the stepwise addition of protected amino acids to a resin-bound peptide chain. The peptide is then folded and assembled into the correct three-dimensional structure of the protein. This may involve the use of chaperone proteins, disulfide bond formation, and other post-translational modifications. The protein is then purified using various chromatography and filtration techniques to remove impurities and obtain a highly pure product. Purified protein is characterized by using various analytical techniques to confirm its identity, purity, and biological activity. The protein is then formulated into a suitable

dosage form, such as a liquid or lyophilized powder, and delivered to the patient via injection, infusion, or other routes of administration.

Protein extraction and purification

Therapeutic proteins are typically extracted using a combination of techniques that can vary depending on the specific protein and its source.

Chromatography

Chromatography is a widely used technique for the extraction and purification of proteins. Chromatography separates molecules based on their physical and chemical properties such as size, charge, hydrophobicity, and affinity. In protein chromatography, a column is packed with a stationary phase, and the protein mixture is passed through the column. The different components of the protein mixture interact with the stationary phase to different extents, allowing for separation and purification of the desired protein. The first step in chromatography is to select a suitable stationary phase and column. The stationary phase is the material that is packed into the column and interacts with the proteins in the sample [20]. The column size and type of stationary phase are selected based on the characteristics of the protein of interest and the sample matrix. Once the column is selected, the sample containing the protein of interest is loaded onto the column. The sample is usually dissolved in a buffer that is compatible with the stationary phase and column. As the sample is passed through the column, the different components of the

protein mixture interact with the stationary phase to different extents, allowing for the separation of the desired protein. For example, in ion exchange chromatography, the stationary phase carries a charged group that interacts with proteins based on their net charge. Once the protein of interest has been separated, it can be eluted from the column. Elution is achieved by changing the conditions of the buffer, such as the pH, salt concentration, or polarity [21]. This disrupts the interaction between the protein and the stationary phase and allows the protein to be released from the column. After elution, the protein can be analyzed to determine its purity and concentration. The purified protein can then be used for downstream applications such as formulation or crystallization.

Crystallization

Crystallization is the process of transforming a liquid or gaseous substance into a solid form with a highly ordered, repeating atomic or molecular structure. The following are the methods and steps involved in the crystallization process.

The first step in crystallization is to select a suitable solvent that will dissolve the substance to be crystallized. The substance to be crystallized is then dissolved in the selected solvent. The solution is heated if necessary to increase the solubility of the substance [22]. The solution is then cooled slowly to allow the solute molecules to come together and form crystals. As the temperature decreases, the solubility of the solute decreases and the concentration of the

solute increases. A small crystal of the substance called a seed crystal, can be added to the solution to encourage the formation of larger crystals. Once the crystals have formed, the solution is filtered to separate the crystals from the remaining liquid. The crystals are then washed with a small amount of fresh solvent to remove impurities and then dried to remove any remaining solvent. Depending on the purity of the crystals obtained, further purification steps may be necessary, such as re-crystallization or sublimation.

Precipitation

The precipitation method is a common method used in the purification of therapeutic proteins.

Preparation of the protein solution

The protein to be purified is first extracted and isolated from the source material (e.g., cell culture or tissue). The protein solution is then prepared by diluting the protein extract in a buffer solution. A suitable precipitating agent is selected based on the protein properties and the desired purity level. Common precipitating agents include ammonium sulfate, polyethylene glycol (PEG), and ethanol. The precipitating agent is added to the protein solution gradually while stirring [23,24]. The solution is then allowed to stand for a period to allow the protein to precipitate out of solution.

Centrifugation

The protein solution is then centrifuged at high speed to separate the precipitated protein from the remaining solution. The

precipitated protein is then re-suspended in a buffer solution and dialyzed to remove any remaining contaminants or impurities. The purified protein is then quantified and analyzed to determine the yield and purity of the protein. The purified protein is then stored in the appropriate conditions to maintain its stability and activity until it is ready for use.

Formulation

The formulation of therapeutic proteins involves the development of a stable and effective product that can be administered to patients. The first step in protein formulation is to thoroughly characterize the protein, including its physicochemical properties, stability, solubility, and purity. This information will be used to design a formulation that will maintain the protein's stability and activity. The buffer system for the protein must be carefully selected to maintain the protein's stability and activity. The buffer pH, ionic strength, and composition can affect protein conformation and aggregation and must be optimized for the protein of interest.

Excipients are added to the protein formulation to improve stability, and solubility, and protect the protein from degradation [25]. Common excipients include sugars, amino acids, polyols, and surfactants. Once the buffer and excipients have been selected, a formulation design is developed. This includes determining the optimal concentration of each component and evaluating the stability of the formulation under different storage conditions. The

formulation is then optimized by systematically varying the concentrations of each component to identify the optimal formulation that maintains protein stability and activity.

Analytical methods development

Analytical methods are developed to monitor protein stability and activity in the formulation, such as size exclusion chromatography, electrophoresis, and spectroscopic methods [26]. Quality control testing is performed to ensure the safety, purity, and efficacy of the final product. This includes testing for contaminants, product potency, and stability under different storage conditions. The final step in protein formulation is to fill the product into the appropriate dosage form (e.g., vials, prefilled syringes) and package it for distribution.

Types of therapeutic proteins

Protein therapeutics are classified into five groups on the basis of:

- a) Therapeutic protein with regulatory and enzymatic activity which deals with replacing a deficient or abnormal protein (endocrine disorders, immune-deficiencies, metabolic enzyme deficiencies), providing a novel function (haemostasis, thrombosis, enzymatic degradation of macromolecules) and augmenting an existing pathway (growth regulation, immuno-regulation, endocrine disorders, haematopoiesis, and fertility).

- b) Therapeutic proteins with special targeted activity that interferes with a molecule or an organism (immuno-regulation, transplantation, pulmonary disorders) and delivering other compounds such as proteins and drugs.
- c) Therapeutic proteins that serve as vaccines which provides protection against foreign body infection and for the treatment of autoimmune disorders and treating cancers.
- d) Therapeutic proteins as diagnostics which makes use of purified and recombinant proteins for medical diagnosis (imaging agents, in-vivo infectious disease analysis).

Enzymes and regulatory proteins: Group-1

This group consists of endogenous proteins that are deficient and are treated using an exogenous protein, which replaces a particular activity in protein deficiencies or abnormal protein production cases [27]. These proteins are used in wide range of conditions like patients lacking in gastrointestinal enzymes, replacing vital blood-clotting factors such as factor VIII and factor IX in case of hemophiliacs, treatment of cystic fibrosis (use of pancreatic enzyme), metabolic enzyme disease such as mucopolysaccharidosis, Gaucher's disease, Fabry disease.

The most prominent recombinant protein is erythropoietin which aids with erythrocyte production in bone marrow and this protein is synthesized in kidneys, this enzyme is

administered in patients with chemotherapy induced anemia or myelodysplastic syndrome to increase their erythrocyte production and in cases of renal failure. Other examples include granulocyte for the treatment of neutropenic patients, Interleukin-1 for treating thrombocytopenic patients, recombinant follicle stimulating hormones in cases of *in-vivo* fertilization.

Targeted proteins: Group-2

Group 2 therapeutic protein focuses on use of antigen recognition sites of immunoglobulins or receptor binding domains of native protein ligands which guides the immune system to destroy targeted molecules and cells or by physically occupying regions of the molecule which has functional importance [28]. Several proteins have been approved by FDA for treating inflammatory diseases like etanercept which acts as immune-adhesion and is a fusion of two human proteins namely tumor necrosis factor receptor and the Fc region of antibody protein IgG1, this neutralizes the harmful effects of TNF and proves as an effective therapy for inflammatory diseases such as arthritis and psoriasis. Recombinant monoclonal antibody palivizumab has been administered for patients with high-risk severe respiratory syncytial virus infection, that binds to RSV F protein and aids with immune-mediated eradication of virus from the body [29]. Other domain in which group-2 therapeutic proteins used are oncological treatments, for example, rituximab is a chimeric monoclonal antibody which binds to CD20 (transmembrane protein) on B-cell non-Hodgkin's lymphomas and targets the cell

destruction, cetuximab is used to treat colorectal cancer, head, and neck cancers by binding to epidermal growth factor and disrupts cell growth and proliferation.

Protein vaccines: Group-3

Group 3 therapeutic proteins are considered as prophylactic and therapeutic vaccines, this concept focuses on specifically injecting the specific immunogenic protein components of a microorganism without the risk of toxic infection [30]. Hepatitis B vaccine was created by the production of recombinant hepatitis B surface antigen protein, which is a non-infectious protein of hepatitis B virus, vaccines for human papilloma virus is made from combining major capsid protein from four strains of HPV that are said to commonly cause genital warts, cervical cancer. These group 3 therapeutic proteins can also be used to treat patients with autoimmune disorders and pregnancies with Rh factor issues.

Protein diagnostics: Group-4

These are purified recombinant proteins that are used for diagnostic purposes, purified protein derivative test acts as a classic example for *in-vivo* diagnostic to test the exposure for *Mycobacterium tuberculosis* where the non-infectious protein is injected just below the surface of the skin of the immunocompetent individual, an active response to this proves that the individual has previously encountered *M. tuberculosis*. Endocrine disorders can be diagnosed using stimulatory protein hormones such as GHRH can be used to detect growth hormone deficiencies in

patients. Secretin is used to detect pancreatic exocrine dysfunction or gastrinoma which stimulates pancreatic secretions and gastrin release [31]. Recombinant thyroid stimulating hormone is used for diagnosis of residual thyroid cancer cells. Natural and recombinant human immunodeficiency virus antigens serve as the common screening and confirmatory tests for HIV infections, these antigens act as a bait for specific antibodies to HIV genes.

Therapeutic proteins can also be categorized based on their molecular types such as antibody-based drugs, anticoagulants, bone morphogenetic proteins, Fc fusion proteins, engineered protein scaffolds, interferons, interleukins, enzymes, hormones, thrombolytics [32].

They can also be further classified based on their molecular mechanism:

- a) Non-covalent binding to target, example: monoclonal antibodies.
- b) Affecting the covalent bonds of the target, example: enzymes.
- c) Exerting activity without any specific interaction with the target, for example: serum albumin.

Monoclonal antibodies (moAb or mAbs)

These are proteins that are synthesised in the laboratory that act or mimic the antibodies in our system, mAbs tend to stick to antigens or foreign bodies to destroy them and they help in stimulating one's immune system. These antibodies are named monoclonal which depicts that they are clones which are identical copies of an antibody.

Monoclonal antibodies are used for diagnostic purposes, disease treatment, to type tissue and blood for its usage in transplants, they are also used as probes to identify materials in laboratory and home-testing pregnancy kits. The conditions that are administered and treated using monoclonal antibodies include cancer, osteoporosis, eye conditions, migraines, high cholesterol, nervous system disorders, organ transplant rejection, inflammatory and autoimmune disorders, infections [33].

These monoclonal antibodies are grouped into three such naked monoclonal antibodies which are given and administered as a therapy by itself with no carrier molecule, conjugated, tagged, loaded/labelled monoclonal antibody which are made into radioactive particle and is tagged along with another drug, bispecific monoclonal antibodies which are modified to target two specific antigens simultaneously. mAbs can modulate the immune system, killing of infected cells and neutralizing infectious organisms [34]. Their mechanism of action includes blockage of physiological ligand receptor interaction and recruiting proteins and immune cells such as natural killer cells, phagocytes, complement which can kill the targeted cell.

Fc fusion proteins

Fc fusion proteins consist of an IgG antibody's FC region (hinge-CH₂-CH₃) linked to a desired protein; the Fc region in the Fc fusion protein binds to the neonatal Fc receptor to prevent degradation. The first Fc fusion therapeutic protein was discovered in 1989

CD4-Fc-fusion antagonists which inhibited the human immunodeficiency virus invading into the T-cells, ultimately serving as an effective treatment of AIDS [35]. The first step in production of FC fusion proteins is molecular designing as the amino acid residues in Fc region and linked protein molecule of interest like a peptidic antigen against a pathogen, or a protein that acts as a bait for the target molecule to bind, mostly a linked protein has therapeutic potential which has beneficial biological and pharmacological properties that plays a vital role in affinity and bio activity. As of now there are eleven fusion proteins that have been approved by FDA, and several novel Fc fusion proteins in preclinical and clinical development.

As of 2011 there were six fusion protein-based drugs that were introduced in the market, four in phase 3 clinical trials and two in different phases of pre-clinical trials [36]. Fc fusion proteins and mAbs together account for almost 43% of all therapeutic proteins as of 2008. The Fc proteins works either as antagonists to binding of receptor (example- aflibercept, rilonacept, etanercept, belatacept, abatacept) or as an agonist to directly aid receptor function to reduce (example-alefacept), or to increase immune functions and activity (example- romiplostim).

Bone morphogenetic proteins

Bone morphogenetic proteins belong to the largest subgroup of transforming growth factor- β family of ligands which employs canonical effectors Smad 1, 5 and 8 to exert

most of their therapeutic effects. Proper regulation of BMP signalling is required and is critical for homeostasis and the development of many human organ systems. Fault in BMP signalling or fault in their regulations (over activation and under activation) are highly inclined with diverse human pathologies, it requires immediate effective approaches to modulate BMP signalling. The FDA has approved recombinant BMP-2, BMP-7 which are promoted as infuse bone graft and OP-1 for its oral, orthopaedic, and maxillofacial usages [37]. There are several engineered bone morphogenetic protein ligands such as B₂A (B₂A₂-K-NS) Which is a BMP-2 based peptide that has heparin binding domain which augments activity of BMP-2; AB₂o₄ which is a segmental chimera of BMP-2 and activin A, it showcased enhanced activity over BMP-2; BMP-7-E6oK, it is an BMP-6 mutant with reduced noggin binding; BMP-2/6 is a heterodimer which is said to have enhanced activity over BMP-6 and BMP-2.

Clinical significance of BMP-based therapeutics: administration of recombinant BMP-6, BMP-2, BMP-7 for orthopaedic and craniofacial issues, BMP receptor kinase inhibitors can be used to treat heterotopic ossification [38].

Augmentation of BMP signalling by engineered recombinant ligand administration has showed to be useful in procedures that requires bone grafts in cases of skeletal defects arising from tumour resection, pathological degeneration, congenital malformation, severe trauma, administration of engineered BMP-2, BMP

related peptide, or small molecule FK506, reduction in expression of BMP antagonist gremlin which aims at increasing BMP signal transduction can be beneficial in the models of tissue fibrosis. BMP based therapeutics for myocardial infarction, spinal cord injury and other pathologies are under clinical trials and preclinical development.

Engineered protein scaffolds

The engineer protein scaffold or non-Ig protein scaffold consists of small, naturally single-chain proteins with a high stability, folding, and yield when expressed in a practical host. The primary scaffold to be characterized was inspired by the β -sheet sandwich structure of protein that creates the antigen-binding domains of antibodies. Other alternatives were based on the α -helix secondary structure of protein [39]. The kunitz domain has the ability to function as a protease inhibitor and is one among the few non-antibody scaffolds that has advanced to the stage of FDA approval. Drugs and diagnostics like Adnectins, Affibodies, Anticalins, Engineered Kunitz-type inhibitors, DARPins are under preclinical development, and several phases in clinical trials for FDA approval.

These engineered protein scaffolds are split into two categories based on location of amino acids that intercede ligand binding:

- a) Protein scaffolds with ligand-binding residues placed in exposed flexible loops [40]. Examples: Adnectins, Anticalins, Avimers, Kunitz domains and Knottins.

- b) Binding residues placed in protein secondary structures like α -helix. Example: designed ankyrin repeat proteins (DARPs) and β -hairpin mimetic. Most of the engineered protein scaffolds are thermostable and are easily synthesized through microorganisms or can be completely chemically synthesized.

Interleukins

These are derived from cytokines that behave as chemical signals between white blood cells. Interleukin-2 (IL-2) which is secreted by activated T-cells aids immune cells grow and divide rapidly, artificially synthesized IL-2 has been approved by FDA to treat advanced kidney cancer and metastatic melanoma [41].

IL-2 can be used as a single drug or administered in combined chemotherapy or with other cytokines such as Interferon- α , recombinant IL-2 as a treatment for Tuberculosis is under clinical trials. Other interleukins like IL-7, IL-12 and IL-21 are under research for use against cancer as both stand-alone as well as combined drugs.

Interferons

These are chemicals that resist viral infection and cancer in the body. There are three types of interferons: IFN- α , IFN- β , IFN- γ [42]. IFN- α is used to treat cancers like hairy cell leukaemia, follicular non-Hodgkin lymphoma, kidney cancer, chronic myelogenous leukaemia, melanoma, Kaposi sarcoma by boosting the immune cell to kill cancer cells, it can also slow down the growth

of cancer cells as well as disrupts blood vessels that tumours need to grow.

Mechanism of action of therapeutic proteins

Therapeutic proteins are biological protein-based drugs that are designed or created in a way to either mimic or enhance the activity of naturally occurring proteins in the body.

These therapeutics are generally produced using recombinant DNA technology and many therapeutic proteins are currently being used to treat various diseases such as cancer, autoimmune disorders, and genetic diseases.

The mechanism by which a therapeutic protein acts basically depends on two factors; the specific protein and the disease being treated. Therapeutic proteins basically work by binding onto a specific molecule in the body like a receptor or an enzyme, and it either enhances or inhibits their activity [43].

For instance, monoclonal antibodies always work by binding to specific molecules on the surface of cancer cells, this helps to identify and activate the immune system to attack and destroy the targeted cancer cell and erythropoietin is a therapeutic protein used to treat anemia and it works by stimulating the production of red blood cells in the bone marrow.

They can also be used as a replacement or supplement for missing or defective proteins in the body, Insulin is used to treat diabetes by replacing the missing insulin that is normally produced by the pancreas.

Enzyme replacement

The mechanism of action by which enzyme replacement therapy is carried out involves the administration of the therapeutic protein that is supposed to act as a replacement for the missing or deficient enzyme in the patient's body. The therapeutic protein is designed to mimic the activity of the missing enzyme and catalyze the same biochemical reaction. Administration of the therapeutic protein is generally done by intravenous infusion and the protein enters the patient's bloodstream [44]. It then travels along with the bloodstream to reach the target tissues and organs. It then acts on the substrates that are accumulating due to the enzyme deficiency or catalyzes the stopped or restricted biochemical reaction of the target enzyme to be replaced.

The therapeutic protein starts its mechanism after reaching its target tissue or organ by binding to the specific substrate on which the missing or deficient enzyme would normally act upon. Then the therapeutic protein begins to catalyze the biochemical reaction that converts the substrate into its normal end product.

This gradually results in the decrease of the accumulation of the substrate and reduction in the symptoms associated with the enzyme deficiency. For instance, in the case of Gaucher disease, the therapeutic protein imiglucerase is used as an enzyme replacement therapy. Imiglucerase is a recombinant form of the missing enzyme, glucocerebrosidase. When imiglucerase is administered to patients with Gaucher

disease, it binds to the accumulated fatty substance called glucocerebroside and catalyzes its breakdown into glucose and ceramide. This reduces the amount of glucocerebroside in the patient's tissues and can alleviate symptoms such as enlarged spleen and liver, anemia, and bone pain.

Receptor activation or inactivation

Some therapeutic proteins are designed in a way to act as agonists or antagonists of receptors in the body to exactly mimic the receptor's pathway of activation or inhibition. The mechanism of action of a therapeutic protein basically depends on its specific target receptor and the nature of the protein itself but here two cases should be taken into consideration, either the protein can act as an agonist or an antagonist. A therapeutic protein acts as an agonist by binding to the target receptor and then triggering a biological response.

For instance, insulin is a therapeutic protein that acts as an agonist. It binds to the insulin receptors on the surface of cells and stimulates glucose uptake by the cells and erythropoietin is another therapeutic protein that acts as an agonist by binding to EPO receptors on red blood cell precursors and further stimulating their growth and differentiation. On the other hand, a therapeutic protein acts as an antagonist by binding to its target receptor and blocking its activation by interfering with endogenous ligands or other agonists. For instance, antibodies that target tumor necrosis factor alpha act as antagonists by binding to TNF- α and preventing it from activating its receptors

[45]. This reduces inflammation and is used to treat autoimmune diseases such as rheumatoid arthritis. There are also other factors by which the mechanism of action of therapeutic proteins affects the activation and inactivation of targets such as modulation of receptor signaling pathways, induction of receptor internalization, or alteration of receptor expression levels. The precision of this mechanism of action of a therapeutic protein depends on its target receptor and the specific properties of the protein itself.

Immunomodulation

Therapeutic proteins can be used to modulate the immune system and its responses in various ways, however the exact mechanism taken up for immunomodulation depends mainly on the specific protein and its target, but there are some general mechanisms like regulating cytokines. Therapeutic proteins can regulate cytokines and cytokines are signaling molecules that play a crucial role in the immune response [46]. For instance, some therapeutic proteins can inhibit pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α), which can be overproduced in autoimmune diseases. Another one way is by modulating immune cell activation, therapeutic proteins can also affect immune cell activation, including T cells, B cells, and antigen-presenting cells (APCs).

For instance, some proteins can stimulate regulatory T cells, which can suppress immune responses and promote tolerance. Therapeutic proteins can target specific cells

or molecules involved in the immune response, such as CD20⁺ B cells in the case of rituximab or induce apoptosis. Therapeutic proteins can also induce apoptosis, or programmed cell death, in certain immune cells like the drug, alemtuzumab. This can induce apoptosis in lymphocytes, which can be beneficial in autoimmune diseases.

Neutralization

Therapeutic proteins are basically large and complex molecules that are used to treat a variety of diseases. These proteins are generally designed to target specific molecules or cells in the body, and often work by binding to these targets and either blocking their activity or triggering a desired immune response and therapeutic proteins can also trigger immune responses in the body, which can reduce their effectiveness and cause side effects. A way to mitigate these immune responses is through a process called neutralization [47].

Neutralization involves the usage of antibodies or other molecules that bind to the therapeutic protein to block its activity. This helps to prevent therapeutic protein from interacting with the body's immune system and triggering any unwanted response or reaction. The neutralization mechanism of therapeutic proteins directly changes depending on the specific protein and the type of immune response that is being targeted [48]. In some cases, neutralization may involve the use of antibodies that are designed to bind specifically to the therapeutic protein and block its activity. In other cases, neutralization may involve the

use of molecules that bind to the same target as the therapeutic protein, but do not trigger an immune response.

Drug delivery mechanisms

Protein-based drugs are referred to as therapeutic proteins. These proteins are large molecules consisting of long-chain amino acids with a multi-folded structural entity responsible for a variety of biological functions. It can be clinically used in replacing an abnormally behaved protein responsible for a specific disease. They can also be supplemented with the body's supply of a beneficial protein to minimize the impact of diseases or chemotherapy [49]. It refers to the methods and techniques used to transport therapeutic agents to target site in the body where they can exert their intended effects. There are different types of mechanisms, and the choice of mechanism depends on the characteristics of the drug, the target site, and the desired therapeutic outcomes.

Delivery of therapeutic protein: challenges and strategies

Recent advances in genetic engineering and pharmaceutical biotechnology have enabled the efficient delivery of therapeutic proteins to combat life-threatening diseases. Although these advancements have increased the importance of therapeutic proteins in the pharmaceutical market, therapeutic delivery to the targeted site remains a major barrier to achieving desired therapeutic outcomes. Most therapeutic proteins are typically administered orally, posing a few challenges

such as enzymatic degradation, poor solubility, and nonlinear pharmacokinetics [50]. Aside from this route, many others have been used for therapeutic protein delivery, including mucosal, intra-nasal, intra-vaginal, pulmonary, and transdermal. Several strategies have been developed and tested to protect these therapeutic proteins from enzymatic degradation and improve their therapeutic efficacy.

Mechanisms involved in therapeutic drug delivery

Intravenous injection: Intravenous injection is a common method for drug delivery that involves the direct injection of a substance into a vein. It allows drugs to rapidly enter the blood stream and reach their target tissues or organs more quickly, such as oral or topical delivery. It can be used to deliver a variety of drugs, including chemotherapy drugs, antibiotics, pain medications, and fluids. The technique is often used in emergency situations when a rapid response is required, or when the patient cannot tolerate other forms of drug delivery. There are some potential risks associated with intravenous injection, such as infection, bleeding, or allergic reactions. When a drug is injected intravenously, it is quickly transported to the heart and then to the lungs, where it is oxygenated and distributed to the rest of the body through the arterial system [51]. Because the drug is delivered directly into the bloodstream, it bypasses the digestive system and the liver, which can affect the way the drug is metabolized and reduce its effectiveness. This is particularly important for drugs with a narrow therapeutic window

or drugs that need to be delivered at a specific rate or interval.

Subcutaneous injection

It is a common method for the delivery for therapeutic protein drugs, which are large molecules that often require slow and sustained release into the bloodstream. This involves injecting the protein drug into the tissue just under the skin. This subcutaneous injection allows for sustained release of the medication into the bloodstream, which can reduce the frequency of injections needed [52]. Subcutaneous injection of therapeutic protein drugs carries a risk of side effects such as injection site reactions, allergic reactions, and immune responses. Patients should be closely monitored for adverse reactions and instructed on proper injection technique.

Intramuscular injection

This involves injecting the protein drug into a muscle. This method can provide a sustained release of the drug into the bloodstream and is often used for drugs that need to be delivered over a longer period. Some examples of therapeutic protein that can be administered through intramuscular injection include, interferons, growth hormones, insulin, erythropoietin etc. they also require specialized storage and handling to maintain their stability and efficacy.

Transdermal delivery

It is commonly used for medication such as hormones, pain relievers, and nicotine replacement therapy. One of the main advantages of transdermal delivery is that it

provides a convenient and non-invasive method of drug delivery, which can improve patient compliance and reduce the need for frequent dosing [53]. Additionally, transdermal delivery can provide a more stable and consistent level of medication in the bloodstream compared to oral medication, which can be affected by factors such as food intake and digestive adsorption. However, not all medications are suitable for transdermal delivery, some drugs may not be able to penetrate the skin or may cause skin irritation. It is important to consult with a healthcare professional to determine if transdermal delivery is appropriate for a particular medication and medical condition.

Oral delivery

It is generally considered to be a convenient method of drug delivery, as it does not require invasive procedures or specialized equipment. It also allows for accurate dosing and provides a relatively predictable onset and duration of action. Additionally, oral medications can be easily stored and transported [54]. However, oral delivery may have some limitations. Some medications may be destroyed by stomach acid or enzymes before they can be absorbed, while others may be poorly absorbed through the gastrointestinal tract. Additionally, some medications may cause gastrointestinal side effects, such as nausea, vomiting, or diarrhea. Overall, the suitability of oral delivery of drug delivery depends on the medication and the individual patient's medical condition. It is important to consult with a healthcare professional to determine if oral delivery is

appropriate and to ensure proper dosing and administration of the medication.

Topical delivery

This method of drug delivery involves the administration of medication directly to the skin or mucous membranes. This method allows for targeted delivery of medication to a specific area of the body, such as a skin lesion or mucosal surface, without exposing the rest of the body to the medication. It provides a non-invasive method of drug delivery that can be easily applied by the patient [55]. It may have some limitations. Some medications may not be able to penetrate the skin or mucous membrane effectively, while others may cause skin irritation or allergic reactions. It depends on the medication and the individual patient's medical condition.

Gene therapy

Gene therapy is a therapeutic drug delivery mechanism that involves the transfer of genetic material to target cells to treat or prevent a disease. The therapeutic gene is typically delivered using a viral or non-viral vector, which can penetrate the cell membrane and transfer the gene into the cell. There are two types of gene therapy involved:

1. Gene replacement therapy: It involves the replacement of a faulty or missing gene with a functional copy of the gene to restore normal gene function [56]. It can be used to treat genetic disorders, such as sickle cell anemia.
2. Gene editing therapy: It involves the targeted modification of a patient's

DNA to correct genetic mutation or to introduce therapeutic effect. It can be used to treat genetic disorders, such as Huntington's disease and Duchenne muscular dystrophy. The delivery of the therapeutic gene may result in off-target effects, immune responses, or adverse reactions. Additionally, the safety and efficacy of gene therapy are still being studied, and more research is needed to fully understand its potential benefits and risks.

Nanoparticles

Nanoparticles can be used to deliver therapeutic proteins to specific cells or tissues in the body. Nanoparticles have gained significant attention as a potential drug delivery system due to their unique properties, including high surface area, size, and shape [57].

There are some types of nanoparticles used in therapeutic drug delivery, including:

1. Liposome: It is composed of lipid bilayer that can encapsulate hydrophilic or hydrophobic drugs. They can also be functionalized by targeting ligands to improve the specificity of drug delivery.
2. Polymeric nanoparticles: These are composed of biocompatible polymers, such as poly (lactic-co-glycolic acid), it can be used to encapsulate a wide range of drugs.
3. Dendrimers: Dendrimers are being researched as a potential drug

delivery system for cancer therapy since they can be functionalized with targeted ligands and other functional groups.

4. Gold nanoparticles: Gold nanoparticles can also be used for gene therapy, where they can deliver genes to target cells or tissues [58,59]. They have been investigated for use in diagnostic imaging, as they have strong optical properties that can be used for contrast enhancement in imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI).

Delivering nanomedicine using a liposome-protein corona in a physiological setting: opportunities and challenges

Active targeting has become a central concept in therapeutic liposome research, as it has the potential to offer additional versatility to develop safer and more effective therapies than passive mechanisms of drug delivery [60,61].

Active targeting exploits the differential (over) expression of surface receptors on target cells by incorporation of a targeting moiety (e.g., ligand) that simplifies the delivery at, and entry of therapeutic in target liposome cells by a receptor-mediated mechanism. It includes monoclonal antibodies, peptides such as RGD (Arginine-glycine-aspartic acid) and NGR (asparagine-glycine-arginine), or small molecules targeting specific receptors such as folate

and transferrin receptors, growth factor receptors and integrins [62].

Application of carbon-based nanomaterials as drug and gene delivery carrier

Carbon nanomaterials have emerged at the forefront of nanoscale research and applications due to their unique properties [63]. In order to successfully internalize carbon nanoparticles into living cells, it is crucial to comprehend the process of delivery systems based on designed carbon nanomaterials to intracellular targets. Internalization of the cell has always been a preferred mechanism of drug delivery.

FDA approved drugs based on engineered/recombinant/therapeutic proteins

Therapeutic biological products include cytokines, monoclonal antibodies, growth factors, immunomodulators, thrombolytics, replacement proteins, anti-coagulants, anti-diabetics, hormones, enzymes, receptor-based therapeutics, interleukins, anti-viral drugs, and anti-cancer therapeutics which are produced and extracted from animals or microorganisms, few being engineered versions of these products. These play a vital role in every field of medicine as it has several advantages in comparison to small-molecule drugs. Protein therapeutics are highly specific and hence mostly used as targeted therapies and considered as precision medicine, the body tends to naturally produce most of these proteins making it easier for the body to tolerate and less likely to inflict autoimmune

response [64]. In cases of genetic disorders these proteins serve as an effective replacement therapy without the need for gene manipulation or gene therapy. The clinical study and approval from FDA are achieved in a short period of time compared to small molecule drugs. There are around 130 therapeutic protein-based drugs approved by FDA, few among them are discussed below.

Agalsidase β

Agalsidase beta under the brand name fabrazyme was approved by FDA in the year 2003 for the treatment of Anderson fabry disease as an enzyme replacement therapy. Fabry disease is the build-up of globotriaosylceramide (Gb₃) resulting from genetic deficiency (X-linked storage disorder) in the production of a lysosomal hydrolase enzyme (α -galactosidase A). It is the recombinant version of human α -galactosidase A. This recombinant fabrazyme binds itself to mannose-6-phosphate receptor and later, agalsidase β hydrolyses the accumulated globotriaosylceramide (Gb₃) as well as other glycosphingolipids, this prevents the further accumulation of glycosphingolipids which results in reduction of severity associated with fabry disease such as cardiomyopathy, cerebrovascular events, and renal failure [65]. Agalsidase β is said to be more effective than agalsidase α in terms of efficiency in decreasing the accumulated lysoGb₃ concentration. There are several common side effects that occur with the usage of this drug such as chest pain, blurred vision, cough, joint pain, stomach pain, swollen joints, hives, and difficulty in moving. The less common severe side effects include

seizures, decreased cardiac output, unconsciousness, dilated neck veins and sometimes less to no pulse.

Coagulation factor IX

Factor IX also known as the Christmas factor is one among the serine proteases in its inactive form involved in the coagulation process. This factor belongs to peptidase family S₁ after activation and is used to treat haemophilia B or factor IX haemophilia. Coagulation factor IX human is marketed under several brand names like alphanine sd, immune vh, kcentra, octaplex and beriplex. Coagulation cascade depends on this factor which makes it a vital protein in the process of hemostasis and common blood clotting. This factor is a zymogen which is a precursor to enzymatic function in its inactive form that is located within the blood plasma and is activated to serine protease with the presence of vitamin K [66]. The activated factor IX forms a complex with calcium ions, membrane phospholipids as well as coagulation factor VIII which results in the activation of coagulation factor X initiating the blood coagulation cascade. This mechanism allows for phenotypically normal coagulation which involves platelet production for normal blood clotting. Weight gain, loss of appetite, fever, signs of excessive blood clotting, continuous bleeding after treatment and appearance of new bleeding spots [67].

Cyclosporine

Cyclosporine is marketed under various brand names such as cequa, neoral, restasis,

verkazia after the initial manufacturing by Sandoz isolated from the fungus *Beauveria nivea* and approved by FDA in 1983 as a steroid-sparing immunosuppression for organ and bone marrow transplants as well as several inflammatory conditions such as atopic dermatitis, rheumatoid arthritis. The calcineurin inhibitor cyclosporine inhibits T cell activation by binding to the receptor cyclophilin-1 inside the cells, forming a complex known as the cyclosporine-cyclophilin complex [68]. Inhibiting calcineurin, this complex prevents calcineurin from dephosphorylating and activating nuclear factors, which are normally responsible for inflammation. The inhibition of IL-2 plays a vital role in disrupting the activation of T-cell and its proliferation and this mechanism is believed to be responsible for cyclosporine's immunosuppressive actions. Besides prolonging patient survival after organ and bone marrow transplants, this drug also prevents and controls serious immune-mediated reactions, such as allograft rejection, inflammation, and autoimmune disease [69]. Side effects of this drug include hypertrichosis, gingival hyperplasia, hyperlipidemia and is speculated to cause nephrotoxicity.

Enbrel

Enbrel is an immunosuppressive medicine that is used to treat inflammatory diseases like rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, severe non-radiographic axial spondylo-arthritis which is said to contain an active compound called etanercept. This drug is originally a biological tumour necrosis factor inhibitor, which acts

as a soluble TNF receptor that binds TNF- α and TNF- β . In cases of rheumatoid arthritis there is an increased production of a certain protein called tumour necrosis factor which causes inflammation, pain and damages the joints, anti-TNF drugs like etanercept, block TNF production and reduce inflammation [70]. Etanercept was approved by FDA around 2003 and is the only fully developed human anti-TNF receptor approved drug that is used to treat and reduce signs and symptoms of structural damage in patients with moderate to active rheumatoid arthritis for patients above 18 years of age. This drug has several severe side effects including eye-related side effects, adverse nervous system reactions, congestive heart failure and autoimmune reactions.

Erbix

Erbix is the brand name under which cetuximab is marketed. Cetuximab is a synthetic chimeric monoclonal antibody derived from human or mouse origin that functions as an Epidermal Growth Factor Receptor (EGFR) fragment that inhibits Epidermal Growth Factor binding (EGF) [71].

As domain III is the binding site for growth factor ligands, this drug binds to EGFR, preventing EGFR from adopting extended conformations or modifications.

Additionally, this drug inhibits EGFR activation, phosphorylation, and receptor-associated kinase activation, ultimately inhibiting tumor invasion and motility, cell cycle progression, and cell survival pathways. The Epidermal Growth Factor Receptor

(EGFR) is found in both normal and cancerous cells and plays a role in the development of epithelial tissue and maintains homeostasis. Overexpression of EGFR is observed in malignant cells and is often mutated in certain types of cancer and acts as a driver for tumorigenesis.

In vitro, cetuximab has shown anti-tumor effects in several cancer cell lines as well as in human tumor xenografts which shows EGFR positive tumors by promoting antibody dependent cellular toxicity, this drug was approved by FDA in 2004 for the treatment of head and neck cancer, metastatic colorectal cancer with BRAF v600E mutation and KRAS wild-type colorectal cancer and is under clinical trials for advanced colorectal cancer, unresectable squamous cell skin cancer and EGFR-expressing non-small cell lung cancer [72]. Cetuximab is used as a monotherapy or in combination with other chemotherapies including radiation therapy, leucovorin, irinotecan and platinum agents. During clinical trials, this drug was associated with several adverse side effects such as cardiopulmonary arrest, interstitial lung disease and interstitial pneumonitis.

Erythropoietin

Erythropoietin (Epoetin- α) is the recombinant version of human erythropoietin which is a glycoprotein hormone that is produced in the kidney cells (renal cortex peritubular cells) and is known for stimulating red blood cells.

This drug was initially discovered and developed by Amgen Inc and is marketed

under the brand names like epoetin- α hexal, epogen, abseamed, epprex. It is in the usage for treating anemia associated complications such as antiviral drug therapy, chronic renal failure, and high-risk perioperative blood loss post-surgical procedures by increasing differentiation from progenitor cells to red blood cells [73]. Epotein- α was approved by the FDA in the year 1981. The binding of epoetin- α to the surface of CD34⁺ on hematopoietic stem cells stimulates the differentiation and division of erythroid progenitor cells creating a signaling cascade which activates genes and thus promoting cell proliferation and prevent apoptosis. This results in increased hemoglobin and hematocrit in the body by producing more amount of red blood cells. Adverse side effects after administration of erythropoietin are reduced vasodilator effect, ischemic stroke, venous thromboembolism, and myocardial infarction.

Humulin N

Humulin N, which is the first ever engineered version of human insulin produced using recombinant DNA technology with the help of non-pathogenic strain of *Escherichia coli*.

This drug was discovered by Eli Lilly & Co around 1982, that is administered to patients with type 1 and type 2 diabetes mellitus by lowering their blood sugar level within 2 to 4 hours after injection, which is estimated to peak in 4 to 12 hours and keeps working for up to 12 to 18 hours. Humulin N is classified under antidiabetic, insulin as well as antidiabetic, insulin intermediate set of therapeutics [74].

This drug, in its crystalline form is obtained from the combination of human insulin and protamine sulfate under optimal parameters and conditions for crystal production. The amino acid sequence of both Humulin N and human insulin is identical and are preferably administered to people above the age of 12. The side effects that come with the usage of this drug include fluid retention which causes weight gain, shortness of breath and swelling in hands or feet, low potassium levels that causes leg cramps, irregular heartbeats, increased thirst and urination, muscle weakness and constipation.

Interleukin-2

Interleukin-2, manufactured and marketed under the name aldesleukin or proleukin is a form of immunotherapy administered to fight against cancer by stimulating the body's immune system to either kill the neoplastic cells (melanoma cells) or by shrinking the tumour cells as they develop in the body. IL-2 is considered a systemic therapy as it takes its course of action through blood stream reaching all parts of the body to fight metastatic and advanced cancers.

The FDA has approved the use of IL-2 to treat stage IV melanoma, as well as metastatic renal carcinoma patients wherein the cancer has spread to adjacent organs from the origin and to other parts of the body in the year 1992 [75]. It is a naturally occurring protein also known as cytokine that regulates and increases the activity and growth of B cells and T cells under normal body conditions, but in case of treatment administration for metastasis, IL-2 tends to stimulate

immunological memory which helps the body to fight melanomas even after the end of treatment. The catch in usage of this immunotherapy is its toxicity which leads to the development of vascular leak syndrome, chest pain and abnormal heartbeats.

Menotropins

Menotropins (Human menopausal gonadotropin) is a purified version and a combination of follicular stimulating hormone (FSH), and luteinizing hormone (LH) extracted from post-menopausal women, hence the name Menotropins. This combination hormone works by stimulating late follicular maturation and recommencement of meiosis of the oocyte which in-turn initiates the rupture of the pre-ovulatory ovarian follicle. In the absence of LH surge, menotropins bind to luteinizing hormone or follicular stimulating hormone or human chorionic gonadotropin hormone receptors of theca or granulosa cells in the ovary to bring out these changes in patients with infertility [76]. This helps the body to produce multiple eggs in the ovulation phase and prepares for *in-vitro* fertilization as well as induction of ovulation in patients with prior cases of receiving pituitary suppression. It is administered in patients with fertility issues either subcutaneously or intramuscularly, which has two subunits from each FSH and LH respectively. The brand name under which menotropins is marketed is called Menopur which was approved by FDA for public usage in the year 2004. Common side effects that are caused with the usage of this drug include stomach cramps, headache, pain, swelling, nausea, rapid

weight gain and few adverse side effects include development of a condition called ovarian hyperstimulation syndrome (OHSS) and hives [77].

Ribociclib

Ribociclib goes by the name ksqali femara, it is a cyclin-dependent kinase inhibitor administered in cases of advanced breast cancer and metastatic cancer.

This slows down the progression and proliferation of cancer cells by the inhibition of two proteins cyclin dependent kinase 4 and cyclin dependent kinase 6 preventing it from over-activating which enables the tumor cells to grow and proliferate more rapidly.

Targeting CDK4 and CDK6 ensures that the cancer cells don't proliferate more rapidly, slowing down the overall carcinogenesis process [78].

Ribociclib is also considered as a targeted therapy or precision medicine as it focuses on specific tissue types and provides protection against oncogenic processes. It is said to inhibit abnormal carcinogenic cells as it arrests the cells at G₁ checkpoint that prevents them from proliferation.

FDA approval for this drug was given around 2017. Common side effects of this drug are as follows: diarrhea, constipation, stomach pain, hair loss, mouth sores, swelling. A few side effects may be serious such as blistering or peeling of skin, bleeding more easily than normal, dark coloured urine, yellowing of skin.

Rituximab

The brands Rituxan, riabni, mabthera, rituxan hycela has the composition of rituximab which was approved by FDA in the year 1997 initially for B-cell non-Hodgkin's lymphoma and later approved for several conditions like chronic lymphocytic leukemia, pemphigus vulgaris, Wegener's granulomatosis and rheumatoid arthritis.

Rituximab is a recombinant mouse or human monoclonal anti-CD20 antibody which targets CD20 on the surface of normal as well as malignant B cell lymphocytes [79]. It lowers the proliferation as well as the existence of B-cell in cases of non-Hodgkin's lymphoma and chronic lymphocytic leukemia, in cases of rheumatoid arthritis it reduces swelling and decreases joint pains, skin lesions are also reduced with the use of this drug.

This drug follows multiple mechanism of action for killing CD20 cells, hence the mode of action is divided into two broad categories one being the direct effects where in complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity are included and the other being indirect effects which deals with structural changes, sensitization of cancer cells to chemotherapy and chemotherapy [80].

Common side effects that come with the usage of this drug include headache, fever, nausea, constipation. As this drug mainly targets the immune system, the patients are highly prone to getting infected and the illness becoming severe.

Synagis

Synagis is the humanized version of monoclonal antibody (IgG1K) called palivizumab produced by recombinant DNA technology. It is a monoclonal anti respiratory syncytial virus F protein antibody that is administered in patients affected with respiratory syncytial virus and to prevent serious sequelae after the initial infection. Synagis was approved by the FDA for medical use in 1998 for infants and babies to prevent serious lung infections caused by RSV [81]. It acts by binding itself to the fusion glycoprotein of respiratory syncytial virus and in turn blocking the uptake by cellular receptors of the host organism. This drug exhibits neutralizing and fusion inhibitory activity against RSV that inhibits the virus from further replication and spread.

Synagis is a combination of human (domains of human IgG1 and variable framework regions of VH genes) and murine (mouse myeloma cell line) antibody sequences which has two heavy chains and two light chains. The side effects that come along with this drug include fever, swelling, and redness. Palivizumab does not cause any adverse and life-threatening side effects during the treatment as well as post treatment.

Conclusion

Therapeutic proteins have several advantages over many drugs as they are easy to manipulate, mostly extracted from the naturally occurring proteins, synthetic versions are feasible, and they have shorter

approval time for clinical usage compared to other small molecule drugs.

Therapeutic protein being a wonder drug to various fatal diseases and disorders

There are more than 130 therapeutic protein-based drugs that are approved by FDA and are widely used in the treatment of several diseases and disorders [82].

The introduction of recombinant version of erythropoietin has helped treat anaemia related complications, Humulin for diabetes mellitus type 1 and type 2, Cetuximab for the treatment of neck, head, colorectal cancers, Menotropins a hormone based drug isolated from menopausal woman is used for infertility, fabrazyme for the treatment of Anderson Fabry disease, coagulation factor IX for haemophilia B, cyclosporine for immune suppression in organ transplant patients and as well as Ribociclib for breast cancer being the recent approved drug under the category of therapeutic proteins.

Several drugs are under clinical trials and under preclinical development.

Therapeutic proteins as single cell proteins

These are edible naturally found proteins that are isolated, extracted and up scaled using algae, fungi, bacteria, and yeast which are considered as natural medicines for controlling obesity, prevention and reduction of cholesterol as well as helps with the lowering of blood sugar levels.

Shorter research, approval time as they have limited mechanism of action

The therapeutic proteins follow four mechanisms such as enzyme replacement, receptor activation or inactivation, immunomodulation and neutralization which makes it easier for the researchers to study the drug and develop faster compared to several small molecule therapeutics which ultimately results in the shorter duration for the approval for therapeutic usage by FDA.

Therapeutic proteins as precision medicine

The receptor targeted drugs that work by either inhibiting or inactivating the signalling pathway, gene expression like rituximab that targets CD20 in cases of non-Hodgkin's lymphoma, Ribociclib administered in cases of advanced and metastatic breast cancers are few examples that supports therapeutic protein being precision medicine.

Protein therapeutics are highly specific in comparison with small molecule drugs [83].

The drawbacks that come with the usage of therapeutic protein includes ovarian hyper stimulation syndrome with the use of menotropin, IL-2 causes vascular leak syndrome, ischemic shock in cases of erythropoietin treatments, cardiopulmonary arrest with Erbitux usage.

Other downfall of therapeutic proteins is related to their undesired pharmacokinetics behaviour which includes being poorly stable, having low solubility, and low short half-life

period, as well as poor membrane permeability and immunogenicity.

Ethical issues associated with the use of therapeutic proteins:

1. Study conducted with the help of extensive usage of Animal models also known as transgenic animals to analyse the immunogenicity that comes with the administration of therapeutic proteins [84]. Immunogenicity is considered as a major drawback as it produces antibody response and sometimes triggers autoimmunity which could be fatal.
2. Therapies and treatments that involve proteins are highly priced, the reason being protein therapeutics and its counter effects measures that are involved to neutralize the antibody production and associated side effects.
3. Adverse side effects that come with the treatment such as anaphylaxis, cardiopulmonary arrest, ovarian hyper stimulation, type 1 hyper stimulatory response, induction of anti-drug antibodies is highly concerning and could be fatal if left untreated.
4. Lower circulation and half-life of protein therapeutics is another issue that makes it less efficient as the duration of action is less compared to small molecule drugs.

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