REVIEW ARTICLE

Review on monoclonal antibodies – Manufacturing aspects, tactics, and future prospects

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Abstract

Since 1940, the researches related to antibodies have acknowledged beneficial understanding of the antibody formation, its structure and diversity but the experimentation in Hybridoma technology in 1975 instigated the interest in clinical application of monoclonal antibodies. Monoclonal antibodies are emerging pharmaceutical products, used in the treatment of cancer, allergies, auto-immune disease, and inflammation. Although a potent biologic, it has obstacles in regulatory approval process and its approval has been hindered due to a lack of manufacturing consistency or the implementation of manufacturing improvements late in the product development process. The major drawback is not being able to preserve the efficacy, safety, and promote industrialization of the product. This review predominantly emphasizes the complications that underlie in the development of monoclonal antibodies such as its instabilities, determines conceptual actions such as approaches for stabilization, and explains the problems and future prospects of monoclonal antibody therapy and alternative form of antibody delivery. The development of stable formulations and effective clinical implementation of monoclonal antibodies can be used for targeted drug delivery methods in the near future.

Key words: Delivery, formulation, monoclonalantibodies, stability, stabilization

INTRODUCTION

Biologics, also known as biological products, are drugs that are manufactured from living organisms using extremely complicated processing methods. It should be treated and administered under strict medical supervision. It includes a wide range of products such as gene therapy, therapeutic proteins, monoclonal antibodies, and vaccines.

Biologics include several benefits over synthetic drugs, including high specificity and low toxicity. Biologics are concerned with preventing less serious adverse events in terms of safety. Immunogenicity is one significant exception, as it can affect biologic's efficacy, safety, and disposition.

Biosimilar is very similar to the reference product, but they are not identical. In contrast, there will be a difference between dosage, dosing, effectiveness, and safety. Many countries are currently working to develop regulatory pathways for approval of biosimilars.

The biologics and biosimilars market in the United States is constantly developing, and the advantages for patient access and cost containment will continue to increase as more medicines are developed. There are 17 biosimilars on the market in United States as of June 2020, competing against seven reference biologics, with nine more Food and Drug Administration (FDA)-approved biosimilars expected to hit the market soon.

The significance of biologics is already being recognized, resulting in lower costs and savings for patients. Despite the launch of biosimilars, the overall prices of all originator biologics reduced, according to a recent study of market dynamics of biologics and biosimilars.

In the 19th century, Emil von Behring and ShibasaburoKitasato proposed serum therapy, which led to the founding of antibody-based therapy. Antibody-related research conducted since 1940 provides valuable information structure and formation of antibody. Brunet's clonal section hypothesis, which states

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that each cell generates only one particular antibody, was a breakthrough in hybridoma technology in 1975, allowing hybrid cells to secrete an infinite amount of rodent-derived monoclonal antibodies. Hybridoma technology sparked interest in the clinical application of mAbs.

mAbs are one of the rapidly growing research areas in the pharmaceutical sector. There are currently over 200 mAbs in clinical trials, with over 600 in preclinical research. They are effective in the treatment of diseases such as cancer, allergies, and auto-immune disorder. mAbs are now part of an approved class of drugs that are likely to progress from clinical trials to regulatory approval.

Monoclonal antibodies generated in a variety of ways, the most common of which are:

- Murine – 100% mouse protein.
- Chimeric – Therapeutic antibodies with a combination of human and non-human proteins (65% human and 35% of mouse protein).
- Humanized – 95% human and 5% of mouse protein.
- Fully human – 100% of human protein.

Before the approval of full-length mAbs therapeutics, many therapeutic class of protein had low commercialization success rate. In many of the early clinical trials, patients had immune reactions to the administered mAbs, due to the production of their own antibodies to the mAbs. Since the early mAbs were of mouse origin, a lot of this happened. Moreover, dosing was discovered to be quite high, on the order of mg/kg, posing major manufacturing and commercialization challenges. As a result, humanized therapeutic mAbs were favored for long-term administration because the risk of producing human anti-mouse antibodies (HAMA response) was minimized.

Even though the above concerns can be addressed using product expertise and appropriate models to align the drug product with the desired result, regulatory approval remains the most common stumbling block for mAbs. According to a 2004 FDA survey, the rate of success from initial investigational new drug (IND) to successful licensure is about 8%.

GENERIC DRUGS VERSUS BIOSIMILARS

Generic drugs are chemically synthesized and similar to reference product in terms of, quality, safety, and efficacy. The similarity between generic and reference can be proved by bioequivalence analysis in humans.

Biologic medications, on the other hand, are large molecules, most often proteins. Variability is a problem since the production of a biological product is a complicated process that involves living cells. Moreover, since manufacturing methods are typically proprietary, companies engaged in developing a potential biosimilar should design the entire process from the ground up. As a result, creating a biologic that is similar to the originator is nearly impossible. Even minor changes in the manufacturing process may cause structural changes in the product, affecting its biological activity, safety, effectiveness, and immunogenicity. As a result, biologics production must be checked regularly to ensure batch-to-batch accuracy.

This review focuses on the significant complications associated with mAb's development, and explore the questions that should be resolved in the future as the market of biologics is significantly increasing.

GENERAL CONCERNS FOR ANTIBODY FORMULATION

Antibodies, like most protein therapeutics, have formulation problems due to their proteinaceous origin.[1] Extreme temperature, variation in pH, and stress affect protein stability. The ability to determine the physicochemical and thermodynamic stability of antibody medications has enhanced, because of the advances in analytical methods. However, the most difficult part is preparing dosing materials with required protein concentration.

The early preclinical studies play a key role in efficiently finding a new bio-therapeutic product.[2] This study aims to develop a delivery mechanism that can be used in clinical studies by alleviating problems associated with the stability by thorough pre-formulation studies. During pre-formulation, unstable sites and linkage to the attached molecule were identified. These data were frequently analyzed to decide if the protein could be re-engineered to improve stability and folding efficiency.[3]

CHALLENGES IN MABS DEVELOPMENT

Overview of Product Failure

The overall product failure can be attributed to three different reasons, namely, safety, efficacy, and commercialization.[4] The most common causes are that the product fails to demonstrate effectiveness in clinical trials. The researchers aim to detect this failure in early phase of product development through pivotal research. It is crucial to choose the right signal and several popular products have struggled in their first indications. Safety issues raised during the clinical trial could cause the product's approval to be halted or delayed.[5] Even though manufacturing concerns are rarely the cause of product failure, they can cause major delays in the approval of complex biotechnology drugs. Monoclonal antibody product approval has been hindered due to a lack of reproducibility.[6]

Overview of Production of Monoclonal Antibodies

The monoclonal antibody production starts from cells from mammalian culture. The quality of the product and

product-related contaminants is affected by the type of bioreactor, media composition, culture length, and other factors.[4] Other natural sources, such as trans-genic plants or animals, affect the product's characteristics.

Purification of monoclonal antibodies usually involves several chromatography columns. They are chosen based on the desired outcome. Other steps in the purification process, such as low pH incubation or nanofiltration, are intended to isolate or inactivate the endogenous retrovirus (Sofer, 1995). These parameters are crucial for effective viral clearance, but they may also affect purification.[7]

Manufacturing Control

The modern production of monoclonal antibodies and the majority of pharmaceuticals are based on the triad of process management (e.g., raw material, approval requirements, in-process testing, specified set-points, defined process, and hold times), process validation, and product testing.[8,9] This combination, in addition to product information, is crucial for building biochemical comparability across products after a manufacturing change.

Despite the benefits of this manufacturing strategy in getting high-quality monoclonal antibodies to market, modifications in the manufacturing process have caused issues scale-ups in manufacturing that include careful process validation and process conservation to ensure the product's safety and efficacy.[10]

INSTABILITIES OF MAB'S

Antibodies are more stable than other proteins, but they also subject to variety of physical and chemical degradation.[11] This instability can be observed in the liquid and lyophilized form. The degradation is mainly affected by the glycosylation state of antibody. The degradation mechanism can vary depending on the stress. Physical and chemical instabilities are two main types of degradation pathways.[12]

Physical Instabilities

Denaturation, aggregation, and surface adsorption are three main mechanisms through which antibodies become physically unstable.

- Denaturation: Denaturation of antibodies can occur in a variety of ways including temperature changes, shear, and different processing measures. Antibodies seem to be more thermally tolerant than proteins. For example, while mesophilic proteins appear to melt at temperatures below 70°C, antibodies do not melt fully until temperatures rise above 70°C.[13]

A protein may be denatured to varying degrees by lyophilization. After lyophilization, an anti-idiotypic antibody (MMA 383) in a formulation with mannitol, saccharose, sodium chloride, and phosphate lost its *in vivo* immunogenic properties (about 10%–20% of normal response rate).[14]After lyophilization, no signs of deterioration were observed, but it was inactive which may be due to conformational changes. The lyophilized and non-lyophilized antibodies had varying fluorescence properties for tryptophan.[15]

- Aggregation and Adsorption: One of the most prevalent causes of physical instabilities in the antibody is aggregation. The most difficult aspect of designing protein formulations at higher concentrations was the concentration-dependent antibody aggregation.[16] These aggregates have lower activity and, more significantly, higher immunogenicity.

Protein aggregation is usually exacerbated when the concentration of protein is increased. Increased IgG1 shows increase in the aggregation which is evident from the nephelometric unit. Aggregation can also be enhanced by storing in liquid state.[17] It was confirmed from the accelerated aggregation of saline solution of mAb Vinca alkaloid conjugate.[18]

In the case of solid forms, aggregation was discovered to be the primary route of degradation mechanism. During storage of freeze-dried anti-IgE, the number of aggregates increased with increased temperature and relative humidity.[19]

Urea, Guanidinium chloride, amino acids (particularly glycine and arginine), several sugars, polymers (such as PEG and dextran), surface-active agents (polysorbate 20 and 80), and even antibodies themselves were used to lower the rate of protein aggregation.[11] Anti-aggregating agents may, therefore, fall into channels or grooves, whereas large agents may interfere with the protein's lower curvature. In simple terms, small agents would prevent a protein from acquiring a conformation modification that would make it more prone to aggregation, while large agents would minimize the number of surface contacts that would lead to an aggregation occurrence. Antibodies can easily adsorbed on to the variety of surface, thereby decreasing concentration. For example, a mouse monoclonal antibody IgG1 was found to be adsorbed on the glass shake flask's surface, resulting in a reduction in protein concentration. Coating or the addition of Pluronic F127 may help to reduce this loss.[11]

Chemical Instability

Chemical instability can be caused by several factors, including the following.

Deamidation

Antibody deamidation is a popular mechanism for protein degradation and purified antibody preparations can contain a large number of deamidated types. Protein deamidation occurs primarily through the intermediate of succinimide at Asn (more readily asparagine) and Gln (glutamine).[11]

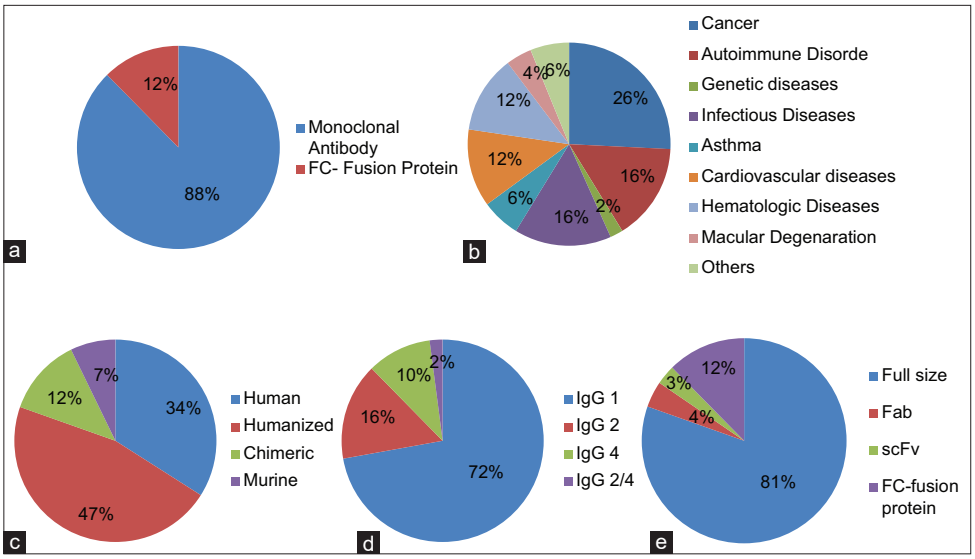

Figure 1: Monoclonal antibodies classified by Food and Drug Administration/European Medicines Agency: (a) Approved drugs. (b) Therapeutic indication. (c) Antibody origin. (d) IgG type. (e) Antibody format

Storage can easily generate a large amount of deamidated products suspected that 4% of the total number of Asn and Gln residues in a chimeric antibody was deamidated on storing the solution at pH = 7.2 at 50°C for 3 weeks.[20] Through the acquisition of additional carboxylic groups, deamidation events cause the antibody to become more acidic.

Tyler-cross and Schirch discovered that the deamidation of Asn residues in neutral and alkaline solutions is dependent on the amino acid residue on the carboxyl side of Asn.[21] Differences in charge distribution or high-performance cation exchange chromatography are usually used to detect deamidation in antibody preparations at the initial stage.

Oxidation

Met, Trp, Tyr, His, and Cys are oxidizable residues in proteins.[22] Deamidation and isomerization are the most common mode of degradation when compared to oxidation. In general, oxidation occurs during storage of antibodies. OKT3 (IgG2a), in solution during storage at 5°C, is the oxidation of non-di-sulfide Cys and several Met residues.[23]

Exposure to light can also intensify the oxidation, for example, light exposure (20,000 lux for 2 weeks at 27°C) to the recombinant humanized monoclonal antibody HER2, rhuMab HER2 corresponds to a 5–10% increase in oxidation in the varying liquid formulation at Met H225 and Met H431 at temperatures 30and 40°C, respectively.[24]

Fragmentation

Some of the most probable sequences contributing to fragmentation in proteins are Asp-Gly, Asp-Pro, and some other sequences like Asn-Ser. Antibodies are readily fragmented, even during the manufacturing process.[11] Antibody fragmentation can be intensified by some processing conditions, including acidic or basic treatment, thermal treatment, freeze-thaw, and storage. Masses with a loss of light chain, loss of F-ab arm, and broken heavy chain and light chain, resulting in both peptide and disulfide bond cleavages are referred to as fragments.

APPROACHES FOR STABILIZATION OF ANTIBODY FORMULATIONS

Liquid antibody formulation has many advantages of easy and quick to prepare and less cost but suffers a disadvantage of instability when compared to other formulation methods. Water is vital in liquid compositions since it facilitates electron transfer while oxidation and deamination reactions.[3] The hydrophobic surfaces of proteins were exposed to water, causing thermodynamic stresses that led to protein aggregation. As a consequence, water is a significant issue in the stabilization of antibody-based products. Water can be minimized in an antibody-drug formulation by lyophilization or incorporating antibodies into hydrophobic polymer systems.

Lyophilization

Antibodies may be freeze-dried to reduce the water content (usually 2–8%) for optimum stabilization in the dry state and to enhance reconstitution stability.[25] Excessive aggregation, asparagine deamination, and isomerization are all caused by a higher moisture content in lyophilized antibody preparations. Solid state characterization helps in detecting the events associated with aggregation and conformation, during the preservation of lyophilized monoclonal antibody preparations.[26]

The addition of carbohydrate adjuvants at adequate levels helps in inhibiting aggregation which eventually leads to improved native protein structure. Sucrose, trehalose, and

mannitol are sources of carbohydrates or polyol compounds that have been shown to provide this stabilizing effect.[27]

The antibody-drug may undergo structural changes when water is removed during various drying stages of the lyophilization cycle and might be replaced by a shell of non-water additives. Therefore, the rate of rehydration is the crucial parameter affecting the stability at the time of reconstitution.[3] During reconstitution, rate of rehydration affects the recovery of native conformation of protein.

Polymer Delivery System

The mAbs can be developed into polymeric delivery system such as microsphere by incorporating hydrophobic polymer matrix. The potential of poly lactyl coglycolide (PLGA) microparticulate formulations to stimulate immunization against embedded proteins is due to their immune system recognition.[28]

The polymeric delivery systems formulated using a specific type of polymer may be used to create an effective antibody formulation which can be delivered to the appropriate sites. For example, local delivery of antibodies directly using a carboxymethyl cellulose aqueous gel may be an efficient anti-infective technique after surgery. The biodegradable polymer hyaluronic acid hydrogel is used to deliver antibodies to specific sites in the CNS for a sustained period.[29]

The release of bioactive protein has also been demonstrated using polyurethane hydrogel with antibody coatings.[30] It is essential to note that all of these methods need a polymer solubilization step with the solvents, which can affect the stability of protein drug conjugates.

Surfactants

Surfactants are categorized as non-ionic (including amphoteric) or ionic (cationic and anionic). Because of their low critical micelle concentration, non-ionic surfactants are often sufficient to prevent protein surface adsorption or aggregation. Tween 20, Tween 80, Triton X-100, Polysorbate 20, Polysorbate 80, Pluronic F68, Pluronic F88, Pluronic F127, and Brij 35 (polyoxy-ethylene alkyl ether) are some of the most widely used non-ionic surfactants.[31]

The polysorbates Tween 20 and Tween 80 are the most widely used surfactants to avoid aggregation and stabilize monoclonal antibody products such as Rituxan, Remicade, and Humira.[32] Pluronic F127 has shown to stabilize recombinant growth hormone (rhGH) by preventing aggregation during the encapsulation phase, which is needed for the preparation of extended-action PLGA microsphere formulations.[33]

Recent attempts to improve protein therapeutics' transmucosal absorption have led to the creation of a new class of alkyl saccharide excipients, such as ProTek® and Aegis therapeutics.[34] Thus, they are non-toxic and considered "generally recognized as safe" (GRAS) by the US FDA. It also greatly improved the transmucosal absorption of proteins smaller than 30 kDa. Since they are water-soluble, they can be used to produce several dosage forms and are compatible with a variety of administration routes.

Sugars and Polyols

Sucrose and trehalose tend to be the most widely used stabilizers for the formulation, though glucose, lactose, ascorbic acid, and maltose can also be used. Sugar's ability to stabilize depends on its concentration. To achieve significant protein stabilization, a concentration of 0.3M (or 5%) sugar or polyols has been suggested as a minimum.[35]

Salts

Depending on the protein and its concentration, salts can stabilize, destabilize, or have no effect on protein stability. Sodium chloride, a common salt, has been discovered to play a key role in the stabilization of proteins like IL-1R. KCL can also be valuable as a protein stabilizer.[36]

Cyclodextrins

The efficiency of various cyclodextrins as protein stabilizing excipients has been examined. Since it serves as an excellent solubilizer and is considered safe for parenteral administration, hydroxypropyl-cyclodextrin (HP-CD) tends to be a beneficial stabilizing excipient.[37] A study demonstrated the use of HP-CD to stabilize monoclonal antibodies and demonstrated its superiority over other additives typically used in protein formulations.

DIFFICULTIES AND POSSIBILITIES OF MAB'S THERAPY

The idea that all therapeutic agents have side effects applies to mAbs as well. Depending on the class and route by which it is delivered, these can range from moderate to serious symptoms.[38] Some of the most known side effects seen in patients taking mAbs are a mild allergic reaction accompanied by fatigue, headache, dizziness, and sometimes high blood pressure. Rashes, intense itching, extreme pain, and drowsiness have all been recorded after receiving FDA-approved mAbs (Raxibazumab®) for the treatment of infectious inhalational anthrax.[39]

According to a 2004 FDA survey, the success rate in the development of mAbs from initial IND to complete licensure is about 8.[40] Just a few mAbs are currently available on the market, with a large number of new mAbs in the development. Patients have always found these medications

to be exorbitant and almost unaffordable. Since there is no generic competition, sales of the first-generation mAbs are indeed very good in terms of safety, price, and demand. As a result, mAbs therapy became a financial strain for patients, necessitating the implementation of certain eminent health plans and step-wise therapies.[41]

Monoclonal antibodies have a positive outlook if they can develop and improve therapeutic mAbs, reducing their negative effects as antibody-based drugs. Through the use of conjugated antibodies with coupling effector molecules and monoclonal antibodies can be further improved to have better effects.[42] The majority of monoclonal antibodies produced in the early transgenic mouse platform are still murine. The Xeno mouse strains were the first to be engineered with the bulk of both human VH and VK repertoires, making them genetically more stable.[43]

The treatment of antibody fragments with polyethylene glycol (PEG) was used to improve the efficacy of mAbs. The binding affinity of an antigen can be enhanced using phage display libraries to isolate antibodies with high antigen affinities, which increases their therapeutic ability.[38]

ALTERNATIVE FORMS OF ANTIBODY DELIVERY

The majority of antibodies produced have mainly focused on injectable routes of administration. A significant proportion of FDA-approved antibody-based cancer drugs are given as an IV infusion to target tumor sites that are difficult to diffuse or inaccessible otherwise.[44] The other routes of administration can also be utilized for antibody delivery. The growing number of antibody therapeutics on the market, as well as increased research into non-life-threatening indications that can be treated with antibodies, is driving force to deliver these agents through other routes. According to research, antibodies given through the IV infusion are more sensitive to proteolysis than antibodies given through the IM injection.[45] Synagis®, the antibody used in treating respiratory syncytial virus (RSV) infection in neonates, was found to be proteolyzed when given intravenously.[46] As a result, it was decided to administer it through intramuscular injection.

Alternative routes of administration (the oral, respiratory tract, transdermal, intracellular targeting, and micro-fluidic pumps) deliver the therapeutic agent directly to the intended site, making it more effective, less invasive, and easier to administer. If fewer active agents can be used, the alternative route of administration is more convenient and potentially less expensive.[3]

Oral Route of Administration

Oral administration is a feasible route for most dosage types, but it possesses certain challenges with antibody delivery. The instability of an antibody in the GI tract and competition from endogenous antibodies at the target receptor sites is the most commonly associated problems of oral antibody delivery.[47] This may have a significant impact on the efficiency of intake following oral delivery. Recent research has shown that fetal FC receptors (FcRn) expressed on the apical surface of enterocytes intake antibodies from the intestinal lumen. This discovery has inspired researchers to look at utilizing this receptor mechanism to deliver antibodies after they have been taken orally. Clinical trials for the oral delivery of chicken-derived (IgY) antibodies for treating and preventing GI tract infectious diseases using this receptor are currently in progress.[48]

Pulmonary Route

Protein therapeutics delivered through intranasal and pulmonary have shown to have better clinical results than those delivered orally.[49] However, this route greatly decreases the residence time of administered protein therapeutic because of the clearance mechanism which eliminates foreign particles. A research proposed a method for topical delivery of therapeutic antibodies to the mucosal surface that offers therapeutic value.

Aerosols containing small particles of liquid or dry powder are used to deliver antibodies at the respiratory tract.[50] The size of droplets or particles in both cases has to be small enough to penetrate the deep lung. Inhaled insulin was approved by the FDA in 2006, but it was phased out in 2007 due to high costs and poor patient compliance. To overcome this inconvenience, a compact device was designed and approved in 2014 which effectively delivered insulin by inhalation (Afrezza®). This shows that delivery of antibodies through the pulmonary route remains a viable option.

Topical Administration

Transdermal patches are a painless alternative to injections, but their use is typically limited to hydrophobic products with a low molecular mass.[51] To deliver macromolecules, peptide chaperon (TD-1), SPACE (skin permeating and cell entering), and cell-penetrating peptides like poly-arginine have been designed to temporarily disrupt the skin structure. Using *in-vivo* phage display, the TD-1 and SPACE peptides were discovered to directly penetrate the skin and deliver large molecules such as insulin and hyaluronic acid.[52]

Microneedles, a promising delivery method that has successfully administered insulin, vaccines, and parathyroid hormones, have proven to be appealing for vaccine delivery because they have been shown to produce stronger immune responses than intramuscular injections.[45] A coin-sized smart insulin patch has recently been created, with microneedles made of glucose sensing polymer that is encapsulated with insulin. The microneedles penetrate under the skin and can

detect blood sugar levels once they are applied to the skin. Each microneedle pierces the skin a few half millimeters below the surface, allowing insulin to reach the body.

Intracellular Targeting

While systemic delivery of antibodies has received a lot of attention, targeted and intracellular delivery is also significant. Intracellular targeting was accomplished using antibody ligands to target receptors on the cell surface of particular tissues after systemic administration. Intracellular targeting has the potential to improve the therapeutic index and efficacy of cytotoxic drugs by preferential delivery to cancerous tissue.[3]

For the intracellular delivery of a variety of biomolecules, a polymeric system with a pH-responsive endosomolytic function has been designed.[53] The pH of these vesicles will decrease from near neutral to below pH 6. This technique was used to covalently bind a monoclonal antibody to make it possible to deliver to the target cell's cytoplasm.

Implantable, Microfluidic Pump

Commercially available implantable pumps deliver biopharmaceutical drugs, especially insulin. The use of implantable insulin pumps increases glycemic regulation as compared to multiple regular injections. Because of their lightweight and ability to be mounted discreetly on the skin, the insulin patch pump overcomes the limitations of implantable pumps. In recent, clinical trial was conducted using an implantable pump which precisely delivered the human parathyroid fragments. Even though, currently available pumps have facilitated successful drug delivery, challenges still exist because pump implantation is invasive and requires regular drug refilling.[45]

MONOCLONAL ANTIBODIES APPROVED TO DATE

Antibodies have contributed a lot to the health-care industry since the first therapeutic antibody was developed as a result of technological advancements. In 2020, here are the top ten best-selling innovative drugs (includes late 2019) consisted of two monoclonal antibodies (mAbs) that produced $3.84 billion in worldwide sales.[54] FDA and EMA have approved a total of 85 monoclonal antibodies and 12 Fc-fusion proteins currently on the market. A total of 15 mAbs were being evaluated, with the estimated PDUFA date mentioned in the table no 2.

The majority of mAbs currently on the market have been formulated to treat diseases such as cancer (26%), autoimmune (16%), infectious (16%), hematologic (12%), cardiovascular (12%), and many others [Figure 1].

All currently accepted mAbs are of the IgG isotypes 1, 2, 4, and hybrid 2/4. As a result, the structure of immunoglobulin (Ig) was critical in determining whether it functioned against itself or foreign antigens. IgG1 is the preferred IgG subclass for the development of therapeutic mAbs, representing 72% of mAbs currently in clinical use, followed by IgG2 (16%), IgG4 (10%), and IgG2/4 (2%). Because of their limited life span compared to other IgG subclasses and their long hinge, which complicates bioprocessing, there are currently no approved IgG3 mAbs [Figure 1].

Antibodies may be viewed as whole molecules or fragments when it comes to size-based structures. Full-size mAbs account for 81% of all mAbs in clinical trials, with Fc-fusion proteins accounting for 12% and antigen-binding fragments (Fab) accounting for 4%. Blinatumomab and Broluzizumab have been approved in 2014 and 2020, respectively, for treating acute lymphoblastic leukemia and neovascular age-related muscular degeneration. Antibody fragments were developed to be highly specific and selective than full length mAbs. It also provides additional benefits such as greater penetration into target tumors or tissues.

CHALLENGES ASSOCIATED WITH PRODUCTION OF ANTIBODIES

Despite of its efficacy, mAbs create certain side effects in a patient. It can cause mild to severe side effects such as anaphylaxis and enhanced drug clearance which affect drug pharmacokinetic properties.[55] Molecular engineering can be used to fine tune mAbs, to reduce its immunogenicity. Epitopes (antigenic determinant) can be engineered into a less immunogenic molecule, once they have detected.[56]

While engineered mAbs have improved functional characteristics, drug stability remains a major concern. There are two widely used strategy adopted to enhance the stability of the monoclonal antibody.[57]

The stability of the formulation can be increased by incorporating stabilizers (surfactants). The improvement in the molecular structure of mAbs can be achieved through protein engineering.[58] Theoretical techniques have been used to forecast unwanted events using structure-based computational design methods, resulting in an emphasis solely on the beneficial substitution. This combined approach results in the development of improved versions of original mAbs.[54]

CONCLUDING THOUGHTS

It is difficult to maintain the stability of monoclonal antibodies, because of their endogenous origin and inherent characteristics. The production of a stable formulation that can be used for site-specific delivery is required for

the successful clinical application of these novel agents. Even though protein engineering has resulted in major improvements in the standards to be attained, the stability and efficacy can only be achieved through formulation modification. Quality by design (QBD) can be employed during the product development stages which can ensure quality of the mAbs.

Monoclonal antibodies lead the top of the biotechnology-derived therapeutics, which opens the doorway to explore it for un-met medical needs. The development of stable formulations and effective clinical implementation of these novel agents can be used for targeted drug delivery methods. Antibody-based medicines are likely to be more complicated in the future than currently available human-approved drugs.

While the biological characterization is incredibly hard, understanding molecular mechanisms through the use of related models can aid in the resolving of toxicity and efficacy issues. Overall, formulation development is needed not only for the final product's stability and shelf life but also to validate the effective manufacturing of the protein drug from drug substance to the final drug product.

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