Monoclonal Antibodies: Limitations & Potential Improvement Strategies

Monoclonal antibodies (mAbs) are one of the most important therapeutic proteins and have been used to treat a variety of diseases such as tumors, inflammatory and autoimmune diseases. Currently, mAb technology is continuously evolving and many mAbs with significantly improved efficacy and safety have been successfully developed.

However, the identification of new drug targets is a huge challenge for antibody development. And mAbs also have limitations that affect their clinical use. Among the most prominent challenges are the short pharmacokinetic properties and stability issues of mAbs during manufacture, transport and storage, which can lead to aggregation and protein denaturation. Researchers are currently developing multiple strategies to improve the formulation and dosage forms of antibodies to improve efficacy and increase the range of clinical applications of mAbs.

Current Research Status of mAbs

mAbs are highly homogeneous antibodies produced by a single B-cell clone and directed only against a specific antigenic epitope, which are usually prepared using the hybridoma technique. Since the world's first murine-derived mAb, Muromonab OKT3, was introduced in 1986, more than two hundred mAbs have been approved and marketed worldwide, and more than one thousand mAbs are in the clinical phase.

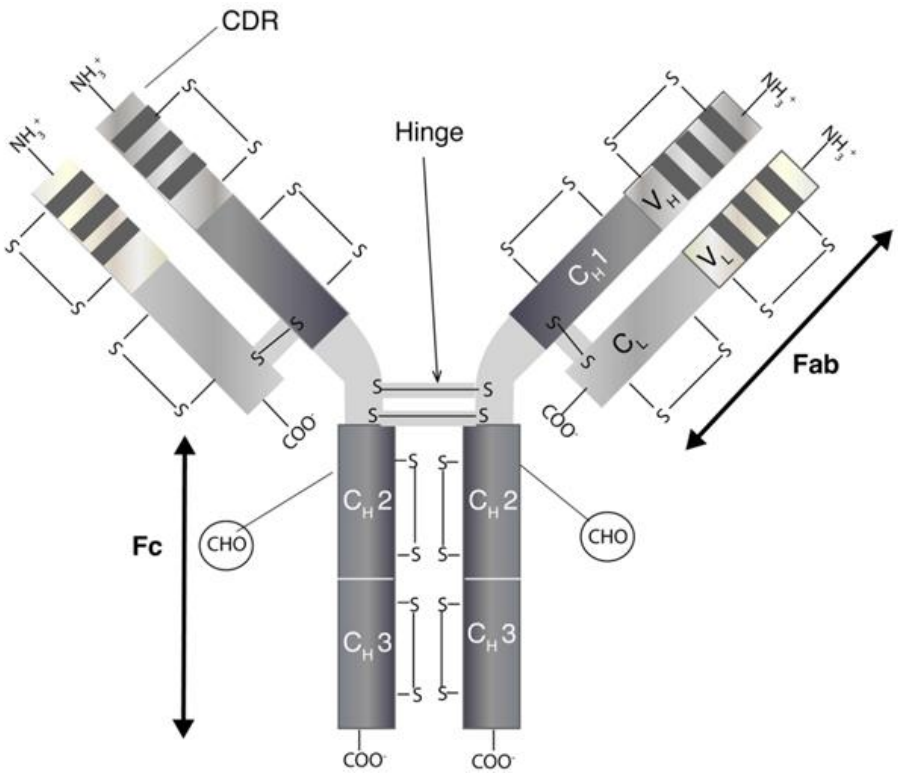

Figure 1. A schematic diagram representing the modular structure of a monoclonal antibody (mAb).

Currently, these approved mAbs are mostly used in oncology, rheumatoid and autoimmune diseases. According to the Cortellis database, mAbs currently in development are mostly focused on cancer, immune diseases, infectious diseases, inflammatory diseases, dermatological diseases and neurological diseases, with cancer accounting for 46%, immune diseases for 12% and infectious diseases for 10%. In addition, mAbs have a good safety profile and enhanced efficacy, with higher success rates reported in early clinical progression.

General Limitations of mAbs

Pharmacokinetic Limitations

While most mAbs can display prolonged circulation times via FCRN-mediated recycling, **many therapeutic proteins have short in vivo half-lives,** typically of hours to days. For example, bevacizumab, a mAb unlicensed for use in neovascularized ocular tissue, is a cost-effective anti-VEGF drug that is clinically equivalent to ranibizumab. The half-life values for intravitreal injectable doses of bevacizumab and ranibizumab were found to be 6.7-10.0 days and 7.2-9.0 days, respectively. Typically, short in vivo half-lives result in frequent dosing, which may increase adverse side effects and may lead to high costs and compliance for patients and the health care system.

Studies have shown that most mAbs have a limited ability to penetrate and accumulate in tissues due to their large size. Therapeutic proteins can be passively distributed from the circulation to peripheral tissues through pores in the capillary wall or through the endothelial cell transcellular pathway. Subcutaneous administration is an ideal route of administration, and macromolecules are likely to be confined to the tissue interstices after injection due to their large size. In general, biologic therapies can reach the circulation by two routes: through capillaries or lymphatic vessels. In contrast, absorption through capillaries is reported to be dependent on passive transport and is limited to compounds with molecular weights below 16 kDa. Therefore, most biotherapeutic drugs cannot be transported via the capillary route and are dependent on the lymphatic system. Therefore, it is challenging to deliver proteins in target tissues to maintain effective therapeutic concentrations.

The pharmacokinetic properties of most classes of protein drugs are further reduced due to **enzymatic degradation mechanisms in the organism**. Protein-based drugs usually act extracellularly (e.g., cell surface receptor interactions and ligand binding). Proteins and mAbs are known to be susceptible to enzymatic degradation. Therefore, unlike most small molecule drugs, the development of oral delivery systems for these bioactive compounds is challenging. Thus, the route of administration of biologics is usually parenteral (intravenous, subcutaneous, intramuscular or intradermal). Currently, most proteins and mAbs are now used for subcutaneous injection.

While humanized proteins may also induce immune responses in humans, humanized and fully human mAbs and other therapeutic proteins are less immunogenic in humans than non-human proteins (e.g., murine antibodies). Thus, the use of animals to predict human responses to protein drug candidates is another challenge in the development of protein therapeutics. Animals generate immune responses to target antigens, however, the level of immunogenicity is relatively different between animal models and humans. The immunogenicity of conventional animal models has been reported to be overestimated, resulting in unreliable prediction of patient immunogenicity due to the different mechanisms of immune response between humans and animals. Therefore, immune responses obtained from animal studies have yet to be studied for corroboration in predicting clinical outcomes and translation to human studies.

It has also been shown that, like most therapeutic protein drugs, antibody-based drugs generally tend to clear more quickly when used at high doses due to the potential for aggregation.

Formulation Challenges

Studies have shown that biological macromolecules, including mAbs, have **a unique three-dimensional structure**. A complex balance of intramolecular and intermolecular interactions between amino acid functional groups and the external environment determines the folded structure. A series of non-covalent interactions are essential for maintaining the native folded structure, such as electrostatic interactions, van der Waals interactions of the backbone and side chain residues, hydrogen bonding and hydrophobic interactions. Since the folded structure is in dynamic equilibrium, any factor that alters the equilibrium of interactions may lead to structural changes, resulting in an unstable state of the macromolecule. It was found that high temperature can lead to protein conformational destabilization or partial/complete unfolding. In addition, changes in pH can lead to aggregation of proteins. For example, one study compared the stability of IgG1 and IgG4. At lower pH and high temperatures, IgG4 formed more soluble aggregates than IgG1 due

to lower unfolding temperatures and changes in tertiary structure that reduced conformational stability.

As the tertiary structure of biologics is susceptible to environmental physical stresses, structural changes in mAbs may occur at any stage of the production process, from initial protein expression to processing to storage. Structural transformation can be attributed to the presence of non-physiological conditions in the process that may drive the adaptation of the finished product to structural variation, which is a major issue and challenge in biopharmaceutical development. Pressures such as buffer selection, manufacturing technology and container selection have also been reported to exacerbate this problem.

Potential Strategies to Overcome Challenges in Antibody-Based Therapies

Use of Excipients to Stabilize Formulations

Several studies have shown that excipients have been widely used to improve the stability of proteins and peptides. Excipients can improve the conformational stability of mAbs by reducing protein dynamics and motion, especially at high concentrations, and inhibiting interface-dependent aggregation. Excipients typically inhibit protein aggregation and protect proteins by adsorbing to the air-liquid interface. Some generally recognized as safe (GRAS) excipients include pluronic F68, trehalose, glycine and amino acids such as arginine, glycine, glutamic acid and histidine, which are present in many commercial protein therapy products.

Introduction of Protein Scaffolds

A scaffold is typically defined as a single-chain peptide framework that contains a highly structured core associated with a highly conformationally tolerant variable fraction that allows for insertions, deletions, and other substitutions. Currently, most protein scaffolds

have been developed for validated targets including TNF-α, CD20, VEGF, CD19 and CD3. scaffolds tend to have lower molecular weights than mAbs and offer enhanced solubility and thermal stability as well as better tissue penetration.

Protein scaffolds do have some advantages over current protein therapies, but they also show some limitations. Scaffold proteins are potentially immunogenic as exogenous proteins. Minimizing immunogenicity is a major issue in the development of non-endogenous therapeutic proteins. Although most protein scaffolds are now derived from naturally occurring human proteins, it still does not completely eliminate adverse immune responses. Furthermore, despite the significant investment in the development of protein scaffolds, only one product (Kalbitor escallantide/DX-88) has been successfully registered for clinical use (Figure 2).

Figure 2. Kalbitor escallantide/DX-88

Monoclonal Antibody Drug Delivery Strategies

Studies have shown that preparing mAb drug delivery release systems can increase the duration of action of protein drugs. Commonly used delivery strategies include microparticulate, liposome, hydrogel, etc. (Figure 3).

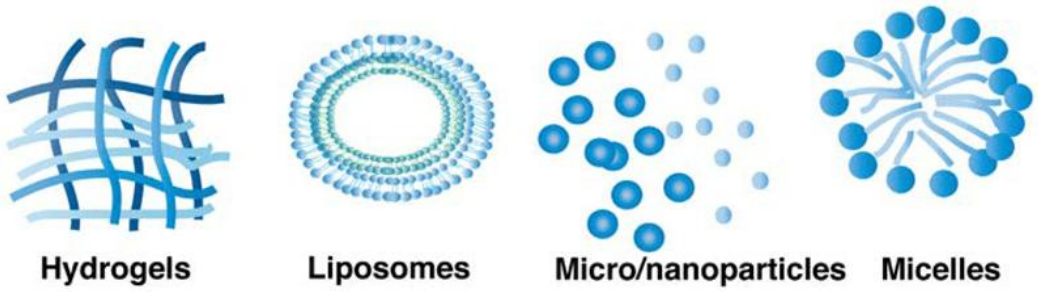

Figure 3. Common formulation strategies used to prolong protein release.

(1) Microparticulate

Microparticles have been considered as one of the most common long-term delivery vehicles for protein drugs, including mAbs and peptides. The most commonly used material in microparticle formulations is a hydrolytically degradable copolymer called poly(lactic-co-glycolic acid) (PLGA). Currently, peptide-loaded particles have been widely reported, such as Exenatide, Sandostatin®, Vivitrol®, Risperdal® Consta®, Zoladex and Lupron. In contrast to therapeutic peptides, encapsulation of proteins into PLGA microspheres is quite challenging and has not been clinically approved due to lack of protein stability.

(2) Hydrogels

The stated goal of hydrogel systems is to release the active form of the protein while maintaining long-term therapeutic concentrations of the drug. Hydrogels are alternatives to particle-related formulations and have been investigated for the delivery of large molecular weight compounds, but there are no clinically approved protein-loaded hydrogel systems.

Hydrogels are polymeric materials that are insoluble in water under physiological conditions and swell considerably in aqueous media. Cross-linking of polymer chains prevents complete dissolution of the polymer. Thus, hydrogels made of hydrophilic polymers can absorb water into their network structure and swell. The high water content

properties of hydrogels make them biocompatible, so they are being tested in tissue regeneration applications. However, the high water content of hydrogels is a challenge in developing extended drug release systems, despite the potential advantages of hydrogels over other drug delivery systems (DDS).

(3) Liposomes

Liposomes are bilayer vesicles composed of phospholipids, where hydrophilic and hydrophobic compounds are encapsulated in the aqueous core of the vesicles or embedded in the bilayer structure, respectively. Studies have shown that biotherapies incorporating liposomes are effective in increasing the bioavailability of drugs. Unlike polymeric particles, conventional liposome systems are less likely to prolong the release of encapsulated proteins. pH effects on bilayer instability could explain the breakdown of liposomes and thus the release of encapsulating agents. Processes such as protonation of phospholipid head groups and acid-catalyzed hydrolysis of the bilayer can lead to in vivo disassembly of the bilayer. In addition, modification of lipid bilayers can alter the kinetic profile of the drug, such as the type of phospholipid or the admixture of cholesterol.

It has also been shown that modification of liposome surface functionalization with polyethylene glycol prevents liposome aggregation and may enhance stability by reducing protein-biofluid interactions.

Protein Modification to Increase Duration of Action

Studies have shown that recombinant and chemical modifications can extend the half-life of proteins. Most strategies have been used for the extension of protein action and can be used in long-acting monoclonal antibody formulations. Common modification strategies include human serum albumin (HSA), Fc fusion and PEGylation, and these strategies have entered or passed clinical trials.

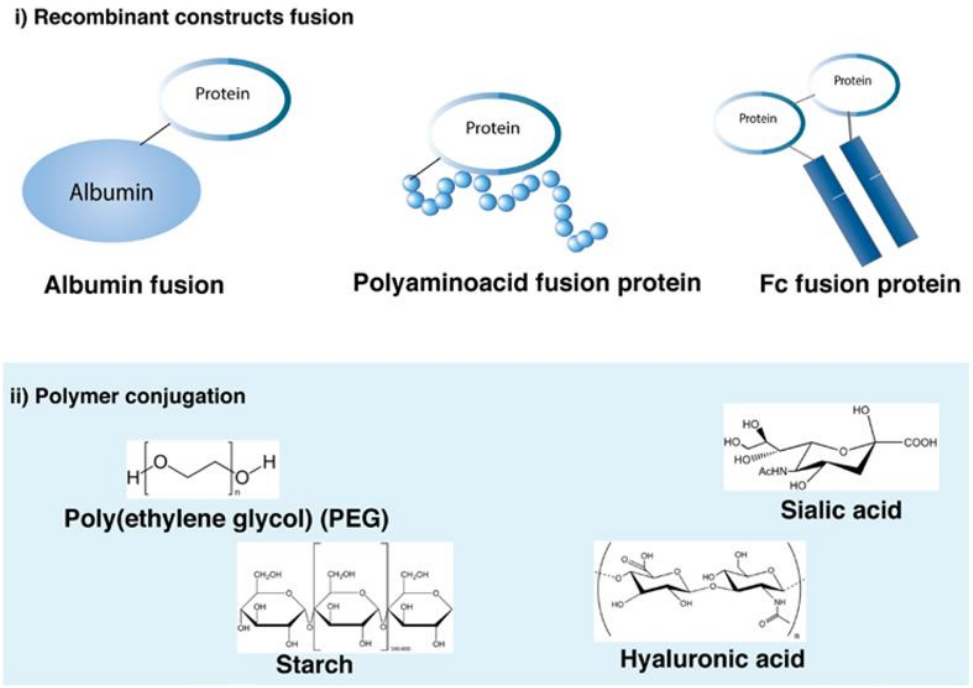

Figure 4. Methods to increase the duration of action of proteins.

(1) Albumin–Protein Fusions

Albumin is the most abundant protein in the blood and it is a multi-binding transporter protein produced by the liver. It has different binding abilities for different insoluble hydrophobic endogenous ligands and exogenous ligands. Albumin has three structural domains (DI, DII, DIII), each with two sub-structural domains (A and B) connected by a flexible loop. These structural domains are biologically active and have seven fatty acid binding sites. The half-life of albumin in humans is 19-22 days, which is achieved through an FCRN-mediated cycle similar to that of monoclonal antibodies. the FCRN binding site is located at DIII of albumin. the protein can be fused to the N-terminal or C-terminal end of albumin to take advantage of the long half-life of albumin. Small fragments (22 kDa) of albumin containing the FCRN binding domain can extend the half-life of protein therapeutics.

Although albumin-peptide fusions have been registered for clinical use, no albumin-protein fusion product has been successful in clinical trials. The albumin-peptide

fusion product Albiglutide (Tanzeum®) is a recombinant fusion protein consisting of two tandem copies of modified human GLP fused to albumin that has been approved for improving glycemic control in adults with type 2 diabetes (Figure 8).Albiglutide has a molecular weight of approximately 73 kDa and acts as a GLP agonist. Albumin is further modified to be resistant to DPP-4-mediated protein hydrolysis. Albiglutide increases the half-life of active GLP-1 from 1-2 minutes to 4-7 days for native GLP-1, allowing for weekly dosing.

Figure 5. Albiglutide (Tanzeum®)

In addition, GlaxoSmithKline (GSK) and Abylnx have developed single structural domain antibodies, such as nanobodies that bind to albumin. For example, GSK2374697 is a novel albumin-binding structural domain antibody reported to have high affinity for HSA, resulting in a prolonged GLP-1 receptor agonist.

(2) Fc Fusion Proteins

Fc fusion proteins are therapeutic proteins or peptides recombinantly fused to Fc in monoclonal antibodies. fc fusion proteins confer IgG-like pharmacokinetic properties and long serum half-lives to peptides or proteins through the FCRN cycle mechanism. Currently, almost all types of Fc fusion proteins are designed for extended half-lives.

Etanercept (Enbrel®, Amgen/Pfizer) is by far the most successful of the first generation products. It was the first Fc fusion therapy approved in 1998 and was the highest selling protein therapy worldwide in 2009 with $6.6 billion in sales. Other first-generation FC fusion proteins include alefacept (Amevive®), abatacept (Orencia®), rilonacept (Arcalyst®), romiplostim (Nplate®), belatacept (Nulojix®), aflibercept (Eylea®) and ziv-aflibercept (Zaltrap®). Most fc -fusion proteins target receptor-ligand interactions, either as antagonists to block receptor binding or as agonists to stimulate receptor function.

(3) Protein PEGylation

PEGylation is a form of covalent binding of polyethylene glycol to proteins. PEG-protein conjugates are considered as a new chemical entity (NCE), often with improved properties.

PEGs can extend the half-life of biological therapies, and the first approved PEGylated product was pegademase (Adagen®) for the treatment of severe combined immunodeficiency disease (SCID) (Figure 10). Currently, all FDA-approved PEGylated products are PEG-conjugated proteins, with the exception of Peginesatide (Omontys®) and Pegaptanib (Macugen®), a pegylated peptide used as a substitute for erythropoietin to avoid PRCA, which is a target for immune response. Unfortunately, after registration, the product was found to have cardiovascular toxicity, leading to its withdrawal. The discontinuation was due to the activity of the peptide and not to any toxicity associated with PEG.

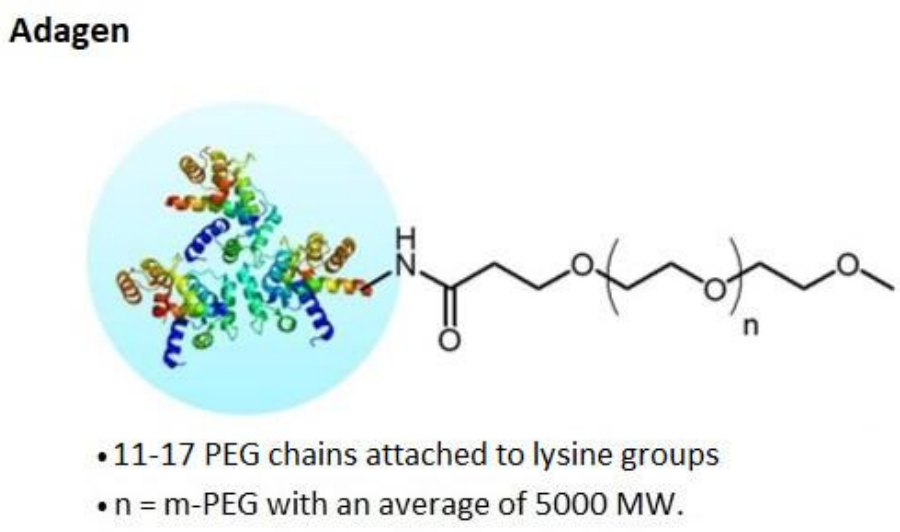

Figure 6. Adagen

In addition, the potential toxicity and immunogenicity of PEG has been of concern. The use of high doses of PEG in animals has been reported to result in the formation of renal vacuoles. These vacuoles disappear when the dose of PEG is stopped. In humans, however, PEG does not appear to have any clinical toxicity. It is clear that high doses of any essentially non-hydrolyzable degradable water-soluble polymer will accumulate in humans, but doses of PEG protein tend to be low (generally less than 1 mg/kg), except for certolizumab pegol at 200 mg, and no PEG toxicity has been reported. And, the only toxicity observed clinically for PEG protein couples is that associated with the protein. In addition, the vast majority of PEGylated proteins have only one PEG molecule bound to the protein, so immunogenicity is rarely observed.

Conclusion

Monoclonal antibodies continue to be one of the fastest growing classes of protein therapeutics. The generation of aggregates and unwanted immunogenicity can lead to various production and clinical challenges. The stability of monoclonal antibodies can be enhanced by the use of excipients (e.g. surfactants and amino acids) or by the production

of more stable structures (e.g. protein scaffolds and bispecific molecules). Many techniques (e.g. Fc fusion and polyethylene glycolization) have also been used to improve the pharmacokinetics and stability of monoclonal antibodies.

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