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A REVIEW ON MONOCLONAL ANTIBODIES AS THERAPEUTIC AGENTS

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ABSTRACT
Monoclonal antibodies are routinely used in several fields but the great challenge has been their use as therapeutic agents for the treatment of diseases. Monoclonal antibodies are protein molecules made in the laboratory from hybridoma cells by recombinant DNA technology. The therapeutic potential of monoclonal antibodies (mAb) was quickly realised after the hybridoma technique allowed their development in the mid-1970s. It has been more than three decades since the first monoclonal antibody was approved by the United States Food and Drug Administration (US FDA) in 1986, and during this time, antibody engineering has dramatically evolved. Current antibody drugs have increasingly fewer adverse effects due to their high specificity. As a result, therapeutic antibodies have become the predominant class of new drugs developed in recent years. Over the past five years, antibodies have become the best-selling drugs in the pharmaceutical market. This review summarizes the therapeutic applications of monoclonal antibodies in treating various disease conditions.

KEYWORDS: Antibodies, Diagnosis, Treatment, Cancer, Infectious disease, Metabolic disorder.

INTRODUCTION
Therapeutic monoclonal antibodies (mAbs), with its great therapeutic success and minimal side effects are now generally acknowledged as beneficial treatment alternatives for patients with rheumatology, cancer, hematology, and dermatology.[1,2] The European Medicines Agency in the EU and the Food and Drug Administration in the USA have currently approved over 79 therapeutic mAbs, and the market for these products has seen significant changes during the previous five years.[3] Since therapeutic mAbs are made biologically from cultured cells like CHO cells, they are heterogeneous. During the manufacturing process, their heterogeneity (glycosylation, N-terminal pyroglutamine cyclization, C-terminal lysine processing, deamidation, isomerization, and oxidation)[4] and ability to form aggregates and multimers[5] are examined. For preclinical pharmacokinetic (PK) research and toxicokinetic (TK) analysis, blood drug concentration analysis, or bioanalysis, of therapeutic monoclonal antibodies is crucial.[6,7] Regardless of the target disease, pharmacokinetic characteristics for a number of therapeutic mAbs have been shown to exhibit significant interpatient variability.[8,9] By binding to particular molecules, monoclonal antibodies offer one of the most significant technological applications for the treatment of numerous diseases. Utilizing these drugs to treat cancer takes advantage of the potential to stop particular cancer biology pathways that are mediated by the targeted antigens. The ability of monoclonal antibodies to carry radioactive isotopes or cytotoxic medicines is another way in which they function.

Monoclonal antibody nomenclature
The United States Adopted Names Council and the American Medical Association have established criteria that regulate the naming of monoclonal antibodies,[10] For monoclonal antibodies or fragments, the suffix - mab is utilized. The source IDs -u-human, - o-mouse, -a-rat, -zu-humanized, -e-hamster, -i-primate, -xi-chimera, -axo-rat/mouse, and -xizu indicate a combination of humanized and chimeric chains and come before the -mab suffix stem. Moreover, the disease, target organ system, or tumour subtype that the antibody is employed against is indicated in the antibody's name: -vir-, or viral -bac- means bacterial, defence mechanism, less infections transmissible, circulatory-cardiovascular, -fungus-antifungal, the neural system's -ner- -kin-interleukin, The musculoskeletal system -mul-os-bone, The toxin target, -toxa-col-colon, -mel-melanoma, -mar-mammary -got-testis, -gov-ovary, -pr(o) stands for prostate, and -tum for other tumors. However, the three stem elements that make up the entire term are target-

source-mab. Nonetheless, drug corporations have the right to begin the agent's name with a naming prefix.

Monoclonal antibodies production

Various methods have been devised to produce monoclonal antibodies. Tissue cultures and mouse ascites fluid are two categories into which these techniques might be divided. Their decision is based on the expense and time commitment. Kohler and Milstein created hybridoma technology to increase the efficiency of producing monoclonal antibodies.[11,12] The monoclonal antibody immunizes mice against the particular antigen that it is targeting. Genetic material from both parent cells is present in the hybrid cells, which are created through the union of mouse myeloma and spleen cells. This characteristic enables them to grow in culture forever and to produce a particular antibody.[13] Later, through chimerization and humanization, the researchers used recombinant DNA technology to create monoclonal antibodies that were safer and more effective.[14] In immunocompromised mice, serum titer levels are used to measure antibody production. As soon as the anticipated level is attained, the spleen cells are removed. These cells, which are generated from mice, are combined with growth factor-cultured immortal myeloma cells. To produce certain antibodies, the hybridoma cells are taken and cloned. The word "monoclonal" suggests that there is only one type of hybridoma cell from which these clones are derived.

Types of monoclonal antibodies

Developments in antibody engineering have produced a variety of mAbs with applications in biomedicine and life sciences. While the ideas behind these antibodies may be identical, their applications and targets may differ. Additionally, a number of considerations, such as the method's effectiveness, availability, and purpose of use, may influence a decision to select one approach over another.

Murine MAbs

Due to immunological system differences between rodents and humans, the use of mouse antibodies generated via hybridoma technology in human therapy (Clinical medicine) is limited. Generally speaking, this leads to treatment failure, with a few exceptions.[15] The effects of murine antibodies on cytotoxicity stimulation are moderate. As a result, when they are continuously provided, the body produces human anti-mouse antibodies (HAMA), which always target the given murine mAb and trigger an allergic reaction.[16-18] This can lead to allergic responses and anaphylactic shock. Specifically, the first therapeutic mAb authorized for clinical use in human medicine was OKT-3, an anti-CD3 mAb derived from mice. Nevertheless, the mAb's efficacy in treating transplant rejection was largely undermined by the patients' significant human anti-mouse antibody (HAMA) reaction.[19] Murine immunogenic components are eliminated with greater effectiveness using a variety of techniques to reduce the immunogenic effects of murine mAbs in human therapy.[20] The bulk of early clinical reagents caused unintended immune reactions in people since mouse mAbs include foreign protein components. The programming of genes *in-vitro* and the expression of these modified sequences in mammalian, bacterial, or fungal cell culture techniques are recent developments in molecular biology. As a result, there is now a greater chance to partially replace the rodent antibody fragment with a comparable human antibody sequence by re-engineering mouse mAb. As a result, the mAb's overall immunogenicity is decreased without compromising the original antibody's capacity for recognition.[21] Humanization-derived antibodies are increasingly being used to treat cancer and inflammatory illnesses; a number of these antibody products are on the market, and others are undergoing clinical testing.[22]

Chimeric MAbs

Specialized therapeutic antibodies, known as "chimeric antibodies," are created by fusing genetic components from mice and humans. Mouse variable areas and human constant regions are manipulated to create them.[23] The composition of these antibodies is around 65% human genetic material to reduce the possibility of undesirable reactions to foreign antibodies. It's interesting to note that certain medications based on chimeric antibodies have been licensed by the Food and Drug Administration for use in human therapy and research. Chimeric monoclonal antibodies are named using the suffix "ximab" at the end, such as Infliximab, Rituximab, and Abciximab.[24]

Humanised MAbs

Human mAbs (HMA) are safe for use in *in-vivo* activities, they have been regarded as natural medicines. Human mAbs are now widely used in the treatment of many diseases and in the creation of cutting-edge immunodiagnostics, thanks to advancements in mAb technology. Over the past few decades, over 20 mAb - drugs - including mAbs from humanized mice - have been approved for use as therapeutic reagents. Additional mAbs are in various phases of clinical trials, overseen by distinct research organizations, and/or working in conjunction with pharmaceutical firms. Human mAb technologies have considerable utility in health economics and are not just confined to strategic research.[25] The hypervariable sections are grafted onto the human variable domain framework in humanized antibodies. Almost 95% of the antibody molecules have a human origin. In terms of affinity with antigens, they can occasionally bind less strongly than the parent murine monoclonal antibodies.[26] Techniques like chain-shuffling randomization can be used to make various modifications to the complementarity-determining region (CDR) in order to boost the affinity of the antibody antigen interaction. Humanized antibodies that have been approved by the FDA include Alemtuzumab, Omalizumab, and Daclizumab.[27]

Fully human MAbs

The stress of keeping immortalized cell lines and human hybridomas alive makes it extremely challenging to produce human mAb using traditional hybridoma procedures. Comparing the use of animal models with *in vivo* immunization of humans against a wide variety of antigens is also impractical.[15] However, the expression of antibody fragments or single-cell variable fragments (Fab or ScFv) in bacteria makes it possible to produce human mAbs. Similarly, antibody libraries can be screened by displaying antibody fragments on filamentous bacteriophages.[28,29] An option for re-engineering murine mAbs with a source of low immunogenic therapeutic antibodies is the generation of completely human mAbs. The majority of these medications were created using phage display platforms or transgenic mice. Even so, it's still difficult to tell them apart. The most popular and well-researched approach for creating novel human antibodies is the phage display technique.[30] An alternative method for producing human mAbs would be to use transgenic mice that have been injected with human immunoglobulins. The immunization of transgenic mice can result in a human antibody response, which can lead to the generation of hybridomas that produce human antibodies. The first medication made entirely of human mAbs, Humira®, was introduced in 2003 to treat rheumatoid arthritis.[31] Currently under development for human clinical testing are a number of therapeutic monoclonal antibodies, including Adalimumab® and Panitimumab®, which are completely human therapeutics. It has been shown that two fundamental platforms can produce effective and well-tolerated therapies for the clinical application of fully human mAbs. Phage display platforms and transgenic mice are two examples of these.[32]

Therapeutic applications of MAbs

Through the discovery of novel targets with increased therapeutic efficacy for use in clinical settings, recent developments in genetic engineering have enabled attempts to enhance the therapeutic application of mAbs.[33] They have been widely used in infectious disease, immunoprophylaxis and immunotherapeutics, either as tools for recognizing, finding, and targeting neoplasms or as carriers for the delivery of lethal chemicals to tumors.[34] Additionally, they have been utilized to treat a variety of malignancies, immunological disorders, rheumatoid arthritis, and metabolic disorders. Their medicinal uses include the treatment of cancer, the treatment of diseases in humans and animals, the creation of vaccines, the inhibition of immunological responses, and the purification of hormones.

Infectious disease

Studies have demonstrated that mAbs may be able to stop Streptococcus mutans from colonizing teeth cavities effectively. Since Streptococcus mutans has novel peptide subunits (epitopes), mAbs may be able to cure these illnesses more successfully. Accordingly, the primary antigen is formed by the colonization of endogenous bacteria (Streptococcus spp., Lactobacillus spp.).[35] The secretory antibodies (sIgA) in saliva primarily mediate the mucosal defense system. Immunization (vaccination) against pure antigen streptococcus mutans aids in the mobilization of salivary glands at induction sites that produce antigen-specific immunoglobulins, especially IgA. Certain B-cells manufacture these immunoglobulins (IgA) as a result of their maturation and differentiation in the saliva. Additionally, some non-human sources of mAbs have been created that don't cause adverse effects such allergic reactions.[36] Protecting against viruses that are circulating worldwide is possible through neutralizing antibodies against extremely changeable viral pathogens.[37] A search of HIV donors' neutralizing antibody repertoires that produced remarkably broad and potent responses produced 17 novel monoclonal antibodies. When compared to the recently reported PG9, PG16, and VRC01 mAbs, many of the novel mAbs are almost ten times more effective; when compared to the original prototype HIV broadly neutralizing mAbs, they are 100 times more potent.[37] With the recognition of additional epitopes on envelope glycoprotein (gp120) by these mAbs, new targets for vaccine development have been identified. Various antibody combinations may provide a more advantageous and varied coverage of circulating viruses, according to an analysis of the neutralization by these currently available mAbs.[37] MAb 9G3 was successfully generated since mAbs that specifically target the C9 binding site of Trichinella spiralis paramyosin (Ts-Pmy) would be required for use in the treatment of Trichinella spiralis infection. It was discovered to be reactive to both native and recombinant Ts Pmy expressed at various stages of Trichinella spiralis. The study revealed that the mAb (9G3) binding to Ts-Pmy effectively hindered Ts-Pmy's binding to human complement C9. This resulted in a notable rise in the *in-vitro* mortality of newly born larvae and a decrease in the infectiousness of Trichinella spiralis in mice treated with mAb.[38] The mAb (9G3) was found to be a useful tool for treating and preventing Trichinella spiralis infections.

Cancer therapy

By using antibody-dependent cell cytotoxicity, monoclonal antibody-mediated treatment attracts cytotoxic cells, such as monocytes and macrophages.[39] By binding complement proteins, monoclonal antibodies (mAbs) induce direct cell cytotoxicity, which is naturally complement dependent.[40] According to reports, some monoclonal antibodies can effectively stop the growth of tumor cells by attaching to and inhibiting growth factor receptors, hence blocking the growth factor.[41] In contrast, Rituximab (IDEC-C2B8) is a chimeric immunoglobulin (IgG) mAb directed against the CD20 antigen that has been shown to be effective against B-cell malignancies.[31] Ibritumomab is a monoclonal antibody against the CD20 antigen on B-cells for the treatment of lymphoma patients. Additionally, monoclonal antibodies are altered to transport poisons, cytokines, radioisotopes

(Used in radioimmunotherapy), and several other active conjugates. The Fab region of bi-specific monoclonal antibodies can be engineered to target both the effector cells and the antigen. Additionally, research reports have demonstrated that leukemic cells can be killed by conjugates, toxins, and medications that contain mAbs.[42] Trastuzumab® is known to block HER-2 receptors in breast cancer, whereas cetuximab® is used to treat specific types of breast cancer and lymphomas by blocking HER-1.[43] Gemtuzumab® and Alemtuzumab® are therapeutic anti-cancer monoclonal antibodies used to treat leukemia; Nimotuzumab® and Cetuximab® are used to treat carcinomas. An additional medication (mAb) that has been given FDA approval for the treatment of colorectal malignancies is bevacizumab. It blocks vascular endothelial growth factor (VEGF) from attaching to its receptors by binding to VEGF. Vitaxin® is a medication that was tested in a clinical trial (phase II) and produced greater results in decreasing solid tumors without any negative side effects. It has been discovered that it binds to a vascular integrin (alpha-v/beta-3) that is lacking in blood vessels found in normal tissues but abundant in blood vessels supplying malignancies.[44]

Auto immune disease

The monoclonal antibodies Infliximab® and Adalimumab®, which are used to treat immunological illnesses, work well against ulcerative colitis, Crohn's disease, and rheumatoid arthritis.[36] As a result of their propensity to bind and block TNF-α, IL-2, and TNF-bound activated T-cells—which are likewise blocked by Daclizumab and Basiliximab—they aid in preventing acute rejection of kidney transplants. Daclizumab® is a powerful monoclonal antibody that is also used to treat T-cell lymphoma, and Omalizumab® is useful in treating different kinds of allergic asthma since it inhibits human IgE.[45] The first FDA-approved therapeutic mAbs (murine IgG2a; CD3-specific) are called OKT3 (Muromonab®, Orthoclone®), and they are presently utilized in steroid-resistant patients who experience rejection following solid organ transplantation. This medication is frequently administered to kidney transplant recipients in order to cause immunosuppression and prevent the foreign tissue from being rejected. It is well known that the (OKT-3) mAb targets the T-cells responsible for rejection.[44] Research has been done to see if treating such illnesses with monoclonal antibodies against immune cell components that cause aberrant immune responses is possible.[46] Since the production of monoclonal antibodies against Escherichia coli endotoxins, mice have been shielded from bacteraemia. Humans have also been examined for them. There is also an anti-T cell monoclonal antibody that eliminates T-cells from a donor's bone marrow before transplantation, which lowers the risk of graft-host illness.[47]

Metabolic disorder

Metabolic illnesses like diabetes and hypercholesterolemia have presented a significant challenge to human medicine. Metabolic diseases are among the conditions for which mAb treatments are used. Numerous metabolic disorders are linked to G-protein-coupled receptors (GPCRs). Scientists have therefore produced monoclonal antibodies (mAbs) to treat metabolic problems by using GPCR membrane fractions as a target.[48] Using a transgenic Xeno Mouse platform, monoclonal antibodies have been generated against the human glucagon receptor (GCGR) from stable cell lines. In a mouse model, it was demonstrated that these candidate mAbs exhibited potential antagonistic activity by lowering blood glucose levels, which led to a long-term suppression of GCGR signalling. As a result, these mAbs were effective in managing hyperglycaemia in diabetics.[49] By using innovative monoclonal antibodies (mAbs) that target the secreted fatty acid-binding protein aP2, researchers at the Harvard School of Public Health have just found a new treatment for type 2 diabetes. This intracellular chaperon protein (aP2FABP4) either stimulates hepatic gluconeogenesis or inhibits peripheral insulin action, or it does both in turn, leading to hyperglycaemia. Numerous metabolic illnesses in humans have been linked to the pathophysiology of fatty acid-binding proteins (aP2). After receiving aP2-mAb in an experimental setting, mice showed improved overall glucose metabolism, increased systemic insulin sensitivity, decreased fasting blood glucose, and decreased fat mass and hepatic steatosis in models of obesity. A promising paradigm for the treatment of type 2 diabetes mellitus has been opened by this strategy.[50] Hypercholesterolemia is one of the main risk factors for cardiovascular illnesses. Statins have mostly been used as first-line therapy to effectively manage this illness; reports of a 50% drop in cholesterol levels have been made.[51] If statins are not tolerated, then other options are advised. In child therapy, diet-based management is the cornerstone. But as of 2010,[52] no research had demonstrated better clinical results. Due to the negative effects of statin-intolerant people, particularly those with an extremely high risk of cardiovascular illnesses, alternative lipid-lowering medications are required.[53] Elevated levels of low-density lipoprotein cholesterol (LDL-C) have been implicated as a primary cause of metabolic diseases. Through the creation of mAbs, studies that aim to lower LDL-C levels by inhibiting the proprotein convertase (Subtilisin/kexin) type 9 (PCSK9) have been conducted. It was discovered that Alirocumab and Evolocumab were both safe and well-tolerated.[54] According to reports, both antibodies significantly lowered LDL-C levels by more than 50%, raised HDL-C levels, and produced positive effects on other lipid levels.[55]

CONCLUSION

Therapeutic monoclonal antibodies represent a significant advancement in medical treatment, offering

effective and well tolerated options for patients across various diseases. They have been utilized to treat a variety of malignancies, immunological disorders, rheumatoid arthritis, and metabolic disorders. Their medicinal uses include the treatment of cancer, the treatment of diseases in humans and animals, the creation of vaccines, the inhibition of immunological responses, and the purification of hormones. With over 79 therapeutic monoclonal antibodies approved for use and significant market growth, they represent a cornerstone of modern medicine, offering hope for better outcomes and improved quality of life for patients worldwide.

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