# Chapter-15

# MONOCLONAL ANTIBODIES: ADVANCE APPLICATION IN CLINICAL AND PHARMACEUTICAL RESEARCH

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The great specificity of MAbs has made them invaluable for studies in immunology and molecular biology. Human therapy, commercial protein purification, suppressing immune response, disease diagnosis, cancer therapy, allergy testing, hormone testing, purifying complex mixtures, elucidating cell membrane structure, identifying specialised cells, preparing vaccines, and boosting the efficacy of medical substances all benefit from their use.

**Diagnostic tools in research and laboratory:** MAbs can be used to assay for the presence of this antigen/substance. Western blotting, immunodot blotting, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), flow cytometry, immunohistochemistry, fluorescence microscopy, electron microscopy, confocal microscopy, and numerous more biotechnological methods all make use of MAbs.

**Gene cloning:** Finding the precise cells that carry the target gene is a major challenge in gene cloning. If a monoclonal antibody (MAb) exists that specifically recognises the target gene product, it can serve as a probe for isolating the cells responsible for making the product and for guiding subsequent therapeutic interventions.

**To identify cell types:** There are many different types of cells involved in the immune response, and MAbs help to identify these cells and decipher the relationships between them. Using monoclonal antibodies (MAbs), researchers have discovered that different types of T-cells may be discriminated from one another based on the cell surface antigens they express. The MAbs were also useful for identifying developmental alterations in T and B cells.

**Protein purification:** By fusing MAbs to a cyanogen bromide-activated chromatography matrix like Sepharose, MAb affinity columns can be quickly and easily manufactured. The level of contamination by undesirable protein species is often quite minimal because the MAbs have high selectivity for the target protein. It is able to elute the target protein in a single, distinct peak because of the singular binding affinity of the MAb-antigen combination. It is possible for the concentration of relative protein in a combination to be quite small when compared to the concentration of total protein. The drawbacks of this approach are also present. Since there is always a tendency for small amounts of immunoglobulin to leak off the immune-affinity column, purifying proteins to a purity of 100% is challenging. Additionally, MAb do not care if the antigenic site is located on an intact protein molecule or a fragment of the molecule.

**Therapeutic tool in clinical medicine**

**Chimeric and humanized antibodies**

At first, hybridoma technique was used to create murine antibodies (ending in -omab). However, these antibodies fail in clinical trials because of the significant differences between the human and mouse immune systems. When given repeatedly, murine antibodies cause minor allergic responses and even anaphylactic shock in humans (HAMA). The host swiftly rids itself of these therapeutic HAMA antibodies. Immune complexes are also formed, and these can cause harm to the kidneys. In an effort to lessen the immunogenicity of murine antibodies, highly effective antibodies were produced by removing their immunogenic component from murine sources.

Because of this need, humanised and chimeric antibodies were created. Therapeutic antibodies called chimeric antibodies are created by fusing the DNA of a nonhuman animal (such a mouse) with human DNA. Chimeric antibodies contain roughly 65% human genetic information to lessen the likelihood of a response to foreign antibodies. The serum half-life of chimeric antibodies is prolonged because they are less immunogenic. The hypervariable regions from murine antibodies are fused to amino acids to create human antibodies.

Almost all of the antibody molecule in this antibody was developed by humans. Humanized antibodies often fail to bind antigen as strongly as the parent murine monoclonal antibody. Techniques like chain-shuffling randomization can induce mutations into the complementarity-determining areas of antibodies and antigens, resulting in increased antibody-antigen binding affinity (CDR). The FDA has approved a number of chimeric antibody-based medications for human use (infliximab, rituximab, abciximab, etc.), and this area of study is rapidly developing. Humanized and chimeric antibodies are gaining popularity for treating inflammatory illnesses and cancer. Examples include daclizumab, omalizumab, alemtuzumab, etc. There are currently a number of humanised and chimeric antibody products available for use in treating a variety of clinical disorders, and there are also some MAbs under clinical trials. Medications derived from chimeric antibodies are denoted by the -ximab suffix, while humanised antibodies are indicated by the -zumab ending. Fully human monoclonal antibodies generated in transgenic mice or by phage display libraries represent the next step in the evolution of antibody synthesis.

This topic is addressed in the review's section on anti-body engineering. When the immunogen is hazardous or when the targeted antigen shares a high degree of similarity with its murine ortholog, humanised MAb are not useful. This is a significant

drawback of humanised Abs. The development of a mouse model for preclinical testing and characterization of a therapeutic target is sometimes quite handy, but is not always relevant.

**Diagnostic applications**

When it comes to testing bodily fluids like blood and urine, monoclonal antibodies have the most cutting-edge diagnostic applications. To detect pregnancy as early as a week or two after conception, MAb is used to react with human chorionic gonadotrophin, a hormone released by the placenta and detectable in the urine of pregnant women. The accessibility of MAbs has also aided in the diagnosis of venereal diseases. There are now MAbs that can diagnose gonorrhoea (caused by Neisseria gonorrhoeae) and Chlamydia infections (induced by Chlamydia trachomatis) in as little as 3-7 days, as opposed to the previous 15-20 minute time frame. Separating the closely related herpes virus 1 and herpes virus is also possible with the help of monoclonal antibodies.

**Identification of cell surface markers**

Different types of immune cells display the cell surface molecules/antigens cluster of differentiation (CD) and human leukocyte antigen (HLA). CD markers and HLA molecules can be recognised by flow cytometry with the help of monoclonal antibodies (MAbs) that target a specific antigen on the cell's surface. Under-representation of CD markers in flow cytometry suggests immunodeficiency illness, while over-production of CD markers suggests malignancy. As a result, a disease's diagnosis can be made by assessing the expression of CD markers. The MAbs have also been useful in elucidating the roles of various immune cell types. Functions of T-cells are revealed by MAbs targeting CD4 markers on TH cells. Patients with a low CD4 count may have acquired immunodeficiency syndrome (AIDS), and the severity of their condition may be indicated by this factor. Therefore, MAbs may be utilised in the study, diagnosis, and treatment of illnesses associated with the immune system.

**In detection and localization of intracellular antigen**

The use of MAbs in flow cytometric analysis is primarily limited to molecules found on the cell surface. Flow cytometry was not used to examine intracellular antigens (enzymes found in the cytoplasm or nucleus, cytokines, oncoproteins, or immunoglobulins). Furthermore, cytometric analyses have not looked at where membrane-associated molecules like CD3 and CD22 end up in the cytoplasm. Intracellular antigens need to be localised and analysed, hence the cells must be fixed and permeabilized (sometimes with detergents). To pinpoint the precise location of the

antigen within the cell, specific antibody conjugates are then utilised. Each intracellular antigen has a unique set of circumstances that need an individual, experimentally derived, best practise. As cytokine levels are often quite low in resting cells, in vitro cell stimulation is required for cytokine detection by flow cytometry. The experimental settings and the type of cell being studied determine which reagent will be most effective in stimulating those cells.

**Cancer diagnosis and therapy**

Antigens expressed on cancer cells can be targeted using MAbs to provoke an immune response against those cells. The diagnostic accuracy of several forms of leukaemia and lymphoma has increased since the release of MAbs that identify antigens on immune cell surfaces. Some solid tumours, like lung, breast, colon, and rectum carcinomas, are being tested using MAbs as well. Blood, sputum, and biopsy samples can be analysed for cancer cells or released compounds from cancer cells using MAbs. Specialized MAbs (monoclonal antibodies) are currently available for the treatment of lung cancer, ovarian cancer, and colorectal cancer. With the use of antibody-dependent cell cytotoxicity, cytotoxic cells including monocytes and macrophages are recruited for MAb-mediated immunotherapy (ADCC).

Direct cell toxicity, known as complement dependent cytotoxicity, is induced by monoclonal antibodies (MAbs) in cancer immunotherapy (CDC). To successfully halt the development of tumour cells, MAbs may also block growth factor receptors, preventing the release of growth factors by tumour cells. The chimeric antibody rituximab (IDEC-C2B8) targets the CD20 molecule and is effective against B-cell malignancies because CD20 antigen is highly expressed on malignant B-cells. Lymphoma patients are treated with ibritumomab, a monoclonal antibody (MAb) directed against the CD20 antigen on B cells (and lymphomas), conjugated to either the radioactive isotope indium-111 (111In) or yttrium-90 (90Y). In addition to ibritumomab, rituximab is used. The lymphoma is treated with tositumomab, a monoclonal antibody (MAb) against CD20. For B-cell lymphoma, 131I-Tositumomab is a reliable, single-treatment drub. These MAbs can be engineered to carry a radioisotope (radio-immunotherapy; RIT), a toxin, a cytokine, or any other active compound. Bi-specific MAbs have a Fab fragment that binds not only to the target antigen, but also to the effector cell.

Bevacizumab, cetuximab, panitumumab, and trastuzumab are only some of the MAbs for cancer that have been approved by the FDA. Trastuzumab, an MAb that blocks human cell-surface epidermal growth factor receptor-2 (HER-2), plays a critical role in the diagnosis of breast cancer. Nearly 20% of breast cancers have overexpressed

HER-2. Some tumour cells express HER-1 (receptor for epidermal growth factor), which can be inhibited by the medicine cetuximab, which is used to treat breast cancer and lymphomas. Clinical experiments have revealed that monoclonal antibodies (MAbs) have generated at least partial remission, and they may be employed for both detection and destruction of cancer cells. Recent research has revealed that MAb conjugates, medicines, and toxins can effectively eliminate leukemic cell populations. Gemtuzumab and alemtuzumab are two therapeutic anti-cancer MAbs used against leukaemia; rituximab is used against non-Hodgkin lymphoma; trastuzumab is used against breast cancer; and nimotuzumab and cituximab are used against carcinomas. By attaching to a molecule (CD52) on lymphocytes, alemtuzumab provides complete remission of chronic lymphocytic leukaemia. In addition, it helps keep kidney transplants from being rejected.

**Conjugated/loaded/labeled MAbs**

The radioactive atoms serve as a delivery channel for medications and poisons that have been attached to conjugated MAbs. MAbs circulate in the body until they encounter cancer cells that express a corresponding antigen, much like a homing device. It transports the poison to the part of the body that needs it the most. This reduces the likelihood of injury to unrelated organs. The chemicals employed in chemotherapy were toxic to both tumour and healthy tissue.

MAbs are referred to as chemo-labeled antibodies since they are conjugated with chemotherapeutic medicines. The Food and Drug Administration (FDA) has approved brentuximab vedotin and ado-trastuzumab emtansine, two chemo-labeled antibodies, for use in cancer treatment. Targeting the CD30 antigen (found on T and B cells), brentuximab vedotin is used to treat Hodgkin lymphoma and anaplastic large cell lymphoma that has not responded to standard chemotherapy. Ado-trastuzumab emtansine is a protein antibody that is linked to the chemotherapy medication DM1 and used to treat advanced breast cancer.

Antibodies are referred to be immune-toxins when they are conjugated with toxins (poisonous substances generated from plants or bacteria) (eg, diptherial toxins). Toxin-bound IL-2 protein is the active ingredient of the cancer medicine denileukin diftitox, which is used to treat a variety of malignancies, including cutaneous T-cell lymphoma (derived from the germ causing diphtheria). The IL-2 molecule generally binds to CD25-positive cells and aids in transporting the poison to these cells.

**Autoimmune diseases**

Infliximab and adalimumab are two examples of MAbs used to treat autoimmune illnesses; they work by binding to and blocking the effects of tumour necrosis factor (TNF)-alpha, a protein involved in the development and progression of rheumatoid arthritis, Crohn's disease, and ulcerative colitis. Basiliximab and daclizumab are used to prevent acute rejection of kidney transplants by blocking the production of IL-2 by activated T cells. Additionally, daclizumab shows promise as a treatment for T-cell lymphoma. Omalizumab is effective in treating moderate to severe allergic asthma because it blocks human IgE.

In steroid-resistant patients experiencing rejection following solid organ transplant, OKT3 (or muromonab, orthoclone) was the first therapeutic MAb (murine IgG 2a CD3-specific) to be authorised by the FDA in 1986. Drugs that decrease the immune system's activity are frequently given to kidney transplant recipients to prevent rejection of the foreign tissue. OKT-3 is an antigen present on CD3 that binds to and destroys T cells, the lymphocytes responsible for the rejection.

An apparent immune system attack on body tissues is at the root of some immunological disorders. Scientists are looking at the possibility of using monoclonal antibodies (MAbs) directed against components of immune cells that play a role in triggering aberrant immune responses to treat autoimmune disorders. Muromonab-CD3 (OKT3), infliximab, adalimumab, omalizumab, and daclizumab are the most often used medications for immune system suppression. Mice are now protected from bacteremia thanks to the development of a human MAb against endotoxin from Escherichia coli. Human trials are also being conducted. In order to lessen the risk of graft-versus-host disease, T-cells from the donor marrow have been removed using an anti-T-cell MAb.

**Dental caries**

The major antigen in dental caries is the colonisation of endogenous bacteria like Streptococcus spp. and lactobacillus spp. Salivary secretory immunoglobulin A (sIgA) antibodies play a major role in the mucosal defence mechanism against dental caries. By administering purified S. mutans antigen at induction sites, mucosal vaccination (or immunisation) promotes the movement of antigen-specific IgA to the salivary glands. Certain B-cells are responsible for making IgA, and then those B-cells must undergo differentiation and maturation before they can be secreted in the saliva. Preventing S. mutans colonisation with monoclonal antibodies (MAbs) or a vaccine has been shown to be successful. More clinical data regarding caries experience is necessary before new peptide subunits or epitopes of S. mutans may be used effectively against dental caries.

A non-human source of MAbs has been established to sidestep possible drawbacks, most notably an undesired antibody response with allergic reactions.

**Other diseases**

In Phase II clinical trials, the medication vitaxin has been effective in reducing the size of solid tumours. There are no negative consequences to using it. It interacts with alpha-v/beta-3, a vascular integrin found in tumor-supplying blood arteries but not in healthy organs. Bevacizumab is an FDA-approved medication for the treatment of colon and rectal cancers that works by blocking vascular endothelial growth factor (VEGF) from attaching to its receptor. Both vetaxin and bevacizumab block the process of angiogenesis. After angioplasty, abciximab is given to patients to keep their coronary arteries from being blocked again. By interacting with platelet receptors, it reduces clumping.

**Antibody Engineering**

The first therapeutic antibody, anti-CD3 murine MAb (OKT-3), was approved in 1986 thanks to humanization of murine antibodies. However, OKT-3 did not seem to be an effective treatment for transplantation rejection since it caused patients to develop high levels of human anti-murine antibody (HAMA). Chimeric antibodies consisting of human constant regions fused to mouse variable regions were developed to lessen the immunogenicity of murine antibodies in humans.

As a further measure, humanised antibodies were developed through protein engineering to lessen the impact of murine antibodies. Humanized antibodies are produced by grafting (through CDR-grafting) only the complementarity determining region (CDR) loops responsible for antigen binding into the human variable domain framework. Antigen binding affinity is often drastically reduced after simple CDR-grafting. Therefore, crucial framework residues that maintain the conformation of CDR loops in the murine antibody are also grafted onto the human template in order to restore the affinity of the parental murine MAb. With the use of computer-guided modelling of the variable domain of antibody, CDR-grafting has been developed.

**Generation of fully human monoclonal antibodies**

Synthesis of human MAbs using traditional hybridoma technique is challenging since immortalised cell lines and human hybridomas do not persistently produce high amounts of antibody, in contrast to the usual production of murine MAbs. Many antigens are not amenable to human in vivo immunisation. Methods for the expression of antibody fragment (Fab or single cell variable fragment, ScFv in bacteria), the display

of antibody fragments on filamentous bacteriophage, and certain powerful techniques for screening of antibody libraries have made the synthesis of human MAbs possible. When it comes to creating novel human antibodies, the phage display technique is by far the most common and well-established method. Another potential method for mass-producing human MAbs is to use transgenic mice that have the human immunoglobulin germ line locus introduced into them. It is possible to make hybridomas that produce human antibodies using conventional hybridoma technology by immunising transgenic mice, which elicits a response similar to that of a human. In 2003, Humira was introduced as the first entirely human monoclonal antibody medication for the treatment of rheumatoid arthritis.

**Other technologies involved**

No animal-free alternative to hybridoma cell generation has been found. The generation of antigen-binding antibody fragments, immunological libraries, naive libraries, synthetic libraries, antibody library screening, cell display, and ribosome display are all recent in vitro advances; nonetheless, these approaches are still experimental and have an unpredictable effect. In recent years, -cells from rabbits have been utilised by researchers. Two or three injections of a pure antigen combined with an adjuvant are given to rabbits before the animal is killed and its spleen cells are harvested. These methods are designed to facilitate the commercialization of monoclonal antibodies, which necessitated the large-scale production of these reagents.

**Pharmaceutical applications of MAbs**

Recent usage of MAbs as a therapeutic agent has increased the success of therapies for some undiagnosed autoimmune disorders or tumours; however, suitable clinical trials and FDA approval for future use must be reviewed at each and every step for its improved use. Antibodies against the CD20 antigen were created in arthritis and B-cell malignancies, while the well-known antibodies against the inflammatory cytokine TNF and against HER-2 were developed in autoimmune illness and breast cancer.

There are four main categories of pharmaceuticals that are made using MAbs. Rituximab, infliximab, and similar medications are examples of the first category, which works by boosting the body's natural defences, whereas the second category, MAb pharmaceuticals coupled with a disruptive substance such a radioisotope, is known as radio-immuno-therapy (RIT). Antibody-directed enzyme prodrug treatment (ADEPT) is the third category, and in the fourth category, MAbs are attached to liposomes (immuno-liposomes) or a nanotechnology drug delivery system. Lymphomas are

particularly sensitive to radiotherapy; hence this is where much of the current research is focused.

Because of their high immunogenicity, which facilitates quick clearance from the body, murine antibodies were specifically selected to reduce radiation exposure. Non-Hodgkin lymphoma, for instance, may be treated with to situ momab. Using MAbs coupled to this drug-activating enzyme is called antibody-directed enzyme pro-drug treatment, and it has been shown to be effective against cancer. If a non-toxic agent is then given systemically, it is converted into a toxic medicine with cytotoxic effects that can be directed at cancer cells. Although the ADEPT clinical trial is still ongoing, the medicines being tested show great promise and should be taken seriously for use in the treatment of cancer in the future.

Antibody coated liposomes (IgG-liposomes) deliver medications or therapeutic nucleotides to cancer cells. When combined with monoclonal antibodies (MAbs), this process is called immuno-targeting. Significant progress has been made, despite the fact that this method is still in its infancy. Therefore, utilising an antibody fragment against the human transferrin receptor, immune-liposomes have been successfully utilised in vivo to tumours to accomplish targeted delivery of tumor-suppressing genes. Both brain and breast cancer tissue have been successfully treated with immune-liposome-mediated tissue-specific gene delivery.

**Side-Effects and Limitations of MAbs**

When opposed to chemotherapy, the negative effects of intravenously administered MAbs are typically minimal. The possibility exists that the initial administration of the medicine will cause a moderate allergic reaction (rash). Low blood pressure, nausea, vomiting, and diarrhoea, as well as fever, headache, weakness, chills, and weakness are all common adverse effects. Some of the negative effects of MAbs are caused by the antigens they are designed to combat. Common adverse reactions to the anti-tumor blood vessel growth drug bevacizumab include kidney damage, hypertension, excessive bleeding, delayed wound healing, and clotting. Anthrax, a potentially devastating kind of bioterrorism, is spread through the inhalation of Bacillus anthracis spores. When other treatments for inhalational anthrax infection have failed, doctors can inject patients with the FDA-approved medication raxibacumab (MAb). Rash with itching, intense discomfort, and sleepiness are common adverse reactions.

More efficient utilisation of treatment-responding medications may be possible as a result of developments in MAb engineering and the creation of cell biomarkers for defining patient subpopulations. Since only a small fraction of the almost 22 MAb

medications licenced by the FDA are currently available, the price of MAb drugs has always been high. There are currently a lot of different MAb medicines in the works. In the absence of generic competition, sales of first-generation safe and cost-effective MAbs are doing fairly well. Some long-term initiatives have been taken to significantly alter the process of developing, commercialising, and marketing MAbs in response to the increasing demand for these types of medications. Patients face high out-of-pocket costs for MAb therapy, but this issue can be mitigated by some health plans and progressive therapies.

In some cases, the established therapeutic product faces competition from a new, enhanced, and more expensive version of the same MAb. It has been discovered that the ranibizumab's precursor medicine, bevacizumab, is more widely available and less expensive following its use by ophthalmologists. In addition, it provides results that are comparable to ranibizumab. Clinical trial data that allows for comparison of existing treatments is necessary in each of these situations. The clinical effectiveness of all these treatments should be trialled and demonstrated, but the benefit to the patient may not always outweigh the cost.

Several billion dollars in profits have been made by the pharmaceutical business thanks to MAbs, a therapeutic agent with a proven track record. Treatment with MAb medicines typically requires greater doses than those necessary by conventional treatments (or products). This necessitates manufacturing procedures that are both efficient and suitable for mass production. Yet the current business faces a persistent struggle from the pressing need to boost production of these products and the push to reduce the price of these costly treatments. The effectiveness of production procedures will increase as a result. Streamlining downstream processes to increase product quantities, implement proper quality with high-concentration product formulations that have sufficient stability, dose-effective products, reduce cost, develop methodologies for time-line MAb production, and develop alternative delivery systems are all ways to overcome these obstacles. Improvements in cell line engineering and cell culture technology, as well as in the degree to which Abs are glycosylated, can boost its effector activity and product efficacy.