International Journal of Research Publication and Reviews, Vol 2, no 12, pp 1619-1628, December 2021

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

A Review on: Monoclonal Antibodies Drug Delivery

Aaditee A Gore, Shashikant N Dhole, Om M Bagade*

Department of pharmaceutics,modern college of pharmacy for ladies moshi,pune,412105,india

A B S T R A C T

The most essential type of drug delivery is monoclonal antibodies. George Kohler of West Germany and Cesar Milstein of Argentina pioneered the manufacturing of monoclonal antibodies utilizing hybridoma technology in 1975. Monoclonal antibodies have lately been utilized to treat cancer, leukemia, arthritis, transplant rejection, asthma, and psoriasis. We will go through 1) the history of monoclonal antibodies in this overview. 2) Therapeutic monoclonal antibodies (therapeutic monoclonal antibodies) 3) the monoclonal antibody manufacturing process 3) Mechanism of Monoclonal Antibodies Instability 4) Factors that influence stability 5) Antibody compositions have a problem with stability. 6) Useful applications The applicability of monoclonal drug delivery is the review's future prospect

Keywords:monoclonal antibodies, immunization, therapeutics, instability, cancer.

Introduction

Antibodies are proteins that the immune system utilizes to recognize and facilitate the identification of foreign entities such as bacteria and viruses. Each antibody recognizes a distinct antigen from that of the target. Monoclonal antibodies (m Ab) are identical antibodies produced by a single type of immune cell, all of which are clones of a single parent cell. Different cell lines have polyclonal antibodies. Their amino acid sequences differ. Antibodies are a type of glycoprotein found in serum. They arise in response to antigens or polysaccharide molecules found outside the body. One of the antibodies generated is unique to the antigen that triggered its synthesis. IgG (monomer), IgA (dimer), IgM (pentamer), IgD (monomer), and IgE are the five major types of antibodies in the immune system (monomer). The immune system is also known as Immunoglobulin (Ig), and the antibody structure is made up of two similar chains: a heavy chain and a light chain, as well as a fixed zone, a flexible area, and a di-sulphide bond, among other things. [14]

Structure

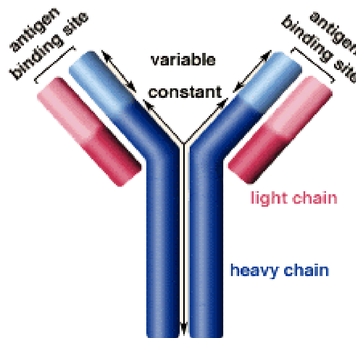


Fig. Structure of antibody

History

Georges Kohler of West Germany and Cesar Milstein of Argentina were the first to use hybridoma technique to produce monoclonal antibodies in 1975. In 1984, they were given the Nobel Prize in Physiology or Medicine. Kohler and Milstein in 1976. The hybrid cells were clones of antibody-producing cells that produced enormous levels of antibody in response to a specific antigen. The hybridoma, like myeloma cells, is capable of rapid proliferation and high antibody secretion rates, allowing it to keep the antibody genes of mouse spleen cells. Greg Winter developed the first humanized m-Abs in 1988 to avoid the reactions seen in patients injected with murine-derived m-Abs [18].

Types of therapeutic monoclonal antibodies

1.Murine m-Abs

Because of the differences between the human and rat immune systems, the use of murine antibodies produced via hybridoma technology in human therapy (clinical medicine) is limited. With the exception of a few exceptional circumstances, this usually ends in treatment failure. [3] Murine antibodies have a minor cytotoxicity-stimulating impact. As a result of the formation of human anti-mouse antibodies (HAMA), which always target the provided murine mAb and, in turn, promote allergic response, their continual administration frequently results in allergic responses and anaphylactic shock. The first therapeutic mAb approved for clinical use in human medicine was anti-CD3 mAb of murine origin (OKT-3). The mAb, on the other hand, failed to address implant rejection because it induces a high number of human anti-mouse antibody (HAMA) responses in patients. Murine immunogenic components are separated with enhanced efficiency by diverse applications in order to decrease immunogenic effects of murine m-Abs in human therapy. Because mouse m-Abs include foreign protein molecules, the majority of previous clinical reagents elicited undesired immune responses in humans. In vitro gene editing and subsequent expression of these modified sequences in mammalian, bacterial, or fungal cell culture techniques have recently been conducted thanks to recent molecular biology advances. As a result, re-engineering mouse mAb to partially replace the rodent antibody fragment with a comparable human antibody sequence is now a superior alternative. As a result, the mAb's overall immunogenicity is lowered without impacting the original antibody's recognition capabilities. Humanization-derived antibodies are becoming more useful in the treatment of inflammatory disorders and cancer, with many antibody products now on the market and others in clinical testing. [2]

2.ChimericmAbs

Chimeric antibodies are therapeutic antibodies that are created by combining genetic components from humans and nonhumans (mice). They're made by tinkering with human sustained regions and mouse variable regions. [8] To limit the danger of negative reactions to foreign antibodies, these antibodies include roughly 65 percent human genetic material. Surprisingly, the Food and Drug Administration has approved numerous chimeric antibody-based medications for use in human therapy and research. The suffix "ximab" is used to name chimeric mAbs, such as nivolumab, Infliximab, Rituximab, and Abciximab. [11]

3.Humanised mAbs

Because of their safety for in vivo actions, human mAbs (HMA) have been classified as natural medicines. Human mAbs are now widely used in the treatment of a variety of diseases, as well as in the creation of innovative immunodiagnostics, thanks to advancements in mAb technologies. Over the last few years, a total of roughly 20 mAb medicines, as well as humanized mouse mAbs, have been approved as therapeutic reagents. Other mAbs are at various stages of clinical trials and are under the supervision of various research institutions and/or pharmaceutical businesses. Human mAb technologies aren't just useful for strategic research; they're also useful in health economics[15]. The excitable variable sections of humanized antibodies are grafted onto the human variable domain framework. The antibody molecules are virtually entirely made up of human DNA. In terms of antigen binding, they are sometimes weaker than the parent murine monoclonal antibodies[4]. Techniques such as chain-shuffling randomization can be used to introduce various modifications within the complementarity determining region to increase antibody-antigen binding affinity (CDR). Daclizumab, omalizumab, and alemtuzumab are examples of humanized antibodies [6].

4.Fully human mAbs

Because of the stress involved in maintaining immortalized cell lines and human hybridomas, producing human mAb using standard hybridoma procedures is relatively difficult. In vivo immunization of humans with a variety of antigens is similarly impractical when compared to animal models. Methods for producing human mAbs, on the other hand, rely on antibody fragments or single cell variable fragment (Fab or ScFv) production in bacteria. Antibody fragments can also be seen on filamentous bacteriophages for antibody library screening. Instead of reengineering murine mAbs with a source of less immunogenic therapeutic antibodies, complete human mAbs can be generated. Transgenic mice and phage display platforms were used to make nearly all of these medications. There is, however, no discernible difference between them. For the generation of novel human antibodies, the phage display approach is a well-established and widely utilized technology. Alternatively, utilizing transgenic mice with human immunoglobulins as a source of human mAbs could be a viable option. A human antibody response resulting from the immunization of transgenic mice can be used to develop

hybriddomas that produce human antibodies. Humira®, the first entirely human mAb medication, was introduced in 2003 to treat rheumatoid arthritis. Adalimumab® and Panitimumab® are two fully human therapeutic mAbs that are currently on the market, with several more in various phases of human clinical development. For the clinical usage of completely human mAbs, two fundamental platforms have shown to deliver active and well tolerated therapies. Transgenic mice and phage display systems are two examples [4].

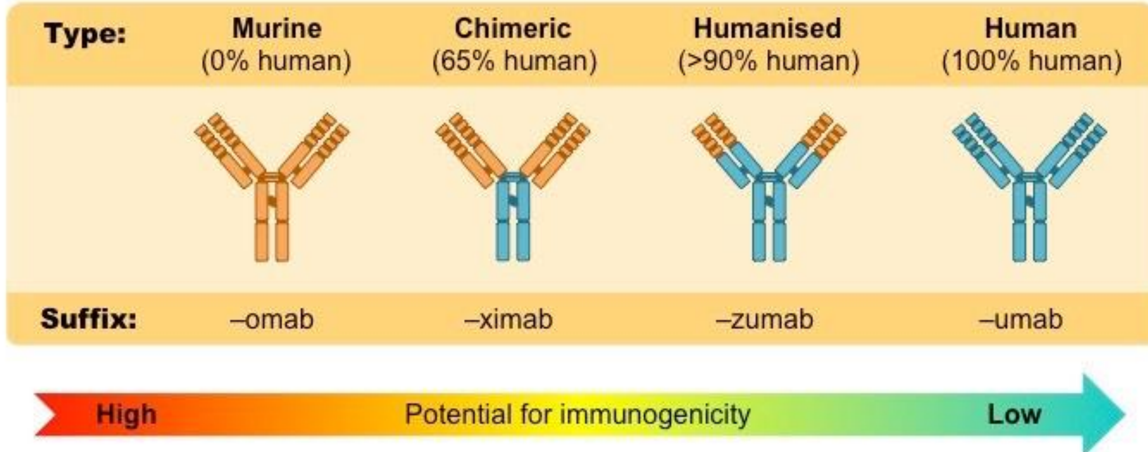


Fig. different types of monoclonal antibodies.

Procedure for production of monoclonal antibodies –

Monoclonal antibody manufacturing has a few fundamental processes.

1. Immunization (the animal is immunized)
2. Cell fusion
3. Selection of hybridomas 4. Product screening
5. Propagation and cloning
6. Storage and characterization

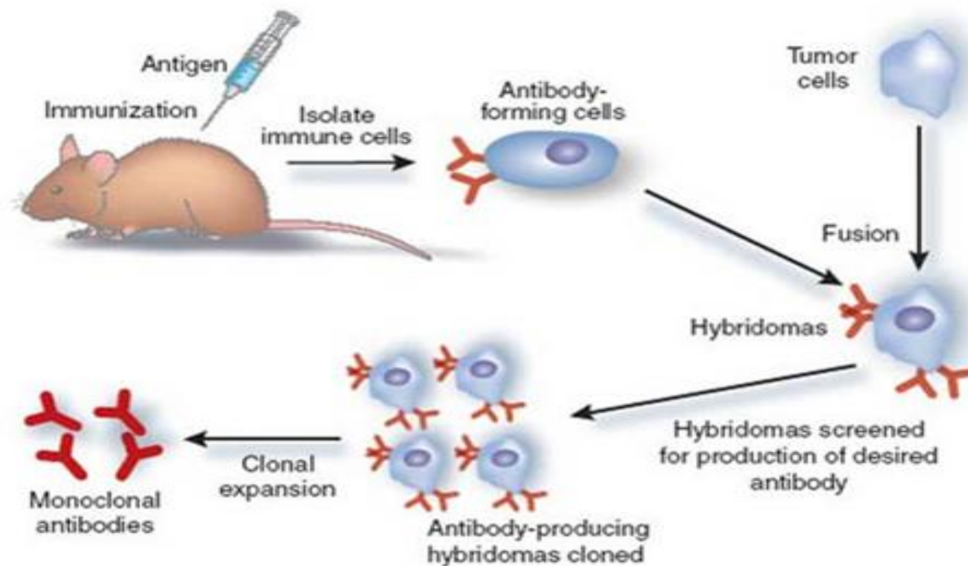


Fig. production of monoclonal antibodies.

1. Immunize the animal

In hybridoma technology, the initial step is to immunize an animal (mouse) with the appropriate antigen. The antigen is injected subcutaneously, coupled with an adjuvant such as Freund's complete or incomplete adjuvant. The injections are done a few times at different locations. Increased stimulation of B-lymphocytes that are reacting to the antigen is possible as a result of this. A final dosage of antigen is given intravenously three days before the animal is killed. This method has ultimately resulted in the growth of immune-stimulated cells for antibody production. During the course of immunization, the concentration of the desired antibodies is measured in the animal's serum at regular intervals. The animal is slaughtered when the antibody serum concentration is ideal. To release the cells, the spleen is aseptically removed and ruptured using mechanical or enzymatic procedures. Density gradient centrifugation separates the lymphocytes of the spleen from the relaxation of the cells.

.

2. Cell Fusion process:

HGPRT negative myeloma cells are combined with completely cleansed lymphocytes (spleen cells). Because polyethylene glycol (PEG) is poisonous, the cells are exposed to it for a brief time (a few minutes). Washing removes the PEG, and the cells are kept in a new medium. Hybridomas (fused cells), free myeloma cells, and free lymphocytes combine to form these cells.

Condition for Fusion Procedure:

earlier carrying out the fusion the following condition should be met for the mouse system:

• Myeloma cells in the logarithmic growth phase
• Ratio of 2-5 lymphocyte per myeloma cell
• 40% PEG (1000dal); PEG should be protested for cell toxicity
• Fusion at 37·c, pH 7.5 -8.0, 3 minutes.

3. Selection of Hybridomas:

When cells are cultivated in HAT media, only the hybridoma cells thrive, while the remainder gradually fade away. This occurs after 7–10 days of cultivation. It's critical to choose a single antibody-producing hybrid cell. If the hybridomas are isolated and cultivated individually, this is achievable. The hybridoma cell slurry is thus diluted that each individual aliquot contains on average one cell. When cultivated in a consistent culture media, these cells produce the appropriate antibody.

4. Screening the Products:

The hybridomas must be hidden in order for the desired specificity antibody to be secreted. Each hybridoma culture's culture medium is checked for the necessary antibody specificity on a regular basis. This condition is usually diagnosed using two techniques: ELISA and RIA. The antibody binds to the specific antigen (typically coated on plastic plates) in both experiments, and the unbound antibody and other medium constituents can be washed away. By screening, the hybridoma cells that produce the appropriate antibody can be identified. Monoclonal antibody refers to the antibody released by hybrid cells.

5. Cloning and Propagation:

Isolate and clone the single hybrid cells that produce the appropriate antibodies. For cloning hybrid cells, two methods are typically used:

• soft agar method • limited dilution method

Limiting dilution method: In this approach, the suspension of hybridoma cells is diluted in steps and aliquots from each dilution are placed in microculture wells. Only one aliquot in each well has a single hybrid cell due to the dilutions. This guarantees that the antibody is monoclonal.

Soft agar method: The hybridoma cells are cultivated in soft agar in this procedure. Many cells can be grown in semisolid media at the same time to create colonies. The nature of these colonies will be monoclonal. Both of the above processes are combined and employed in the final fabrication of MAbs in practice.

6. Characterization and Storage:

To achieve the necessary specificity, the monoclonal antibody must be subjected to biochemical and biophysical characterization. It's also crucial to figure out the MAb's immunoglobulin class or sub-class, the epitope for which it's designed, and how many binding sites it has. It's crucial that the cell lines and mAbs stay stable. The ability of the cells (and MAbs) to tolerate freezing and thawing must be determined. At various stages of cloning and culture, the appropriate cell lines are frozen in liquid nitrogen[14].

Monoclonal antibodies instability mechanism-

Protein degradation can be split into chemical and physical instabilities, each of which has its own set of instability processes. Chemical processes can lead to physical instability, therefore those instabilities are intertwined. Even if the underlying cause of instability is unknown, physical instability may allow chemically sensitive residues to enter or close the distance between residues that may interact[10].

1.Chemical Instabilities

One of the most common chemical degradations is oxidation, which includes the production of disulfide bonds. It can occur with or without the addition of oxidants such as peroxides, light, or metals, and is referred to as auto-oxidation. Methionine, histidine, and cysteine residues, for example, are particularly vulnerable to oxidation. One of the most essential aspects of cysteine oxidation is the creation of disulfide bonds between two oxidized free residues via a thiolate anion intermediate. These spans can be formed intramolecularly or intermolecularly, and their production is aided in a basic environment[12].

2.Physical Instabilities

Denaturation of proteins refers to the loss of higher-order structure as a result of evolution. It could be caused by previously mentioned chemical instabilities, or by severe temperatures or pH levels in the environment. Direct disturbance of the mAb's function, such as a decrease in hinge flexibility or the stimulation of aggregation, can be a result of unfolding. The most significant physical instability is aggregation. Physical instability refers to the formation of high molecular weight species (multimers) from native and folded proteins, regardless of their size or the type of the connections that connect them[10].

Stability Influencing Factors

1.The Structure of Proteins

Protein stability is influenced by all levels of structure. The amino acid sequence (primary structure) provides essential information about whether a protein is prone to aggregation or not. A low isoelectric point (pI) of CDR, for example, appears to favor soluble aggregate formation by increasing electrostatic contacts between mAbs, whereas a high pI of CDR leads to insoluble aggregates, especially when in contact with negatively charged surfaces[5].

2.Protein Concentration and the Potential for Self-Association Aggregation has been demonstrated to be influenced by high protein concentrations. Higher protein concentrations also appear to increase the viscosity of fluids, which may enhance protein-protein interactions and self-association, thereby increasing the aggregation potential of proteins. Given the widespread use of subcutaneous injection of mAbs, which necessitate highly concentrated solutions, this concentration-dependent tendency to aggregation is becoming a growing problem.

3.Temperature mAbs, like other therapeutic proteins, are susceptible to temperature changes during production, storage, and delivery. High temperatures can cause enough disruption in the native protein shape to cause aggregation, but it begins at temperatures much below the protein's equilibrium melting temperature (Tm). At high temperatures, the rate of aggregation increases, as does the rate of other protein processes. In most cases, heat-induced unfolding results in permanent conformational alterations. Deamidation and oxidation are two chemical reactions that are accelerated by high temperatures.

4.Interfaces

Proteins adsorb to hydrophobic surfaces and interfaces because they are surface active molecules. They come across those in a variety of forms throughout their lives.

5.Light

Throughout their lives, protein medicines are exposed to light at various times. They are purified by column chromatography and then exposed (briefly) to the ultraviolet (UV) light of the detector during manufacture, but the main exposure to light happens during storage and administration to patients with IV-bags. If the primary container is not placed within an opaque secondary container, the exposition may be much more significant. Proteins are particularly sensitive to light and to their aromatic residues, causing photodegradation, primarily through photo-oxidation and the production of oxygenated radicals, but also through fragmentation and cross-linking.

6.Excipients

Proteins are frequently resistant against aggregation throughout low pH ranges, although they can readily aggregate in solutions outside of these ranges. The type (positive or negative) and number of charges on the protein are determined by the pH of the solution (which is controlled by the use of pH buffers), which affects electrostatic interactions. Protein cleavage and isomerization may occur at lower pH, but deamidation and oxidation processes may occur at higher pH, both of which increase the aggregation potential.

Stability issues for antibody formulations

1. The oxidation process

In protein medications, methionine and cysteine residues are frequently oxidized, and antibody drugs are no exception. Specific methionine residues within the Fc domain of antibody-based medicines may be vulnerable to oxidation, resulting in the generation of methionine sulfoxide. While cysteines are found as disulfide pairs in the Fc framework, unpaired cysteines in the irregular region may also represent oxidation sites. Oxidation of histidine, tyrosine, tryptophan, and phenylalanine residues can occur in addition to methionine and cysteine residues [1].

2.deamidation

Deamidation appears to be a proclivity for glutamine and asparagine residues [16]. Deamidation of these amino acids can occur in both the light and heavy chains of antibodies [17], and deamidation is one of the principal drivers of charge heterogeneity in monoclonal antibodies[13].

3.aggregation

At low concentrations, liquid formulations of protein therapies frequently show a reduction in concentration due to absorptivity to container walls, while at high concentrations, aggregation events can occur.

Initial antibody drug formulations were given as IV infusions in acute conditions, allowing for medium protein content (1–10 mg/ml) formulations that were not greatly influenced by adherence or aggregation losses.

4.Fragmentation

Antibodies made up of several types of segments with natural flexure sites make up full-length antibodies. At the flexure point between the Fc and Fv domains, a site in particular exists. When evaluating antibody drug stability, cleavage at this hinge region is a typical treatment. To quickly identify fragmentation events in antibody medicines, molecular sizing methods such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion high performance liquid chromatography (SE-HPLC) can be used [9].

Strategies to stabilize antibodyformulations

1.Lyophilization

Although some antibodies have shown a tendency to precipitate when cooled from 37.8 degrees Celsius, antibodies are often resistant to freeze/thaw cycles. Early studies claimed that antibodies were stable for the freeze-drying (lyophilization) process in the absence of excipients that act as cryoprotectants. However, as analytical methodologies advanced, it became clear that antibodies are normally damaged in some way as a result of lyophilization, as evidenced by the formation of large insoluble aggregates.

2.Systems for delivering polymers

Water removal from antibody preparations, either by lyophilization or spray freeze-drying, provides a starting point for consolidation of this dried material into a hydrophobic polymer matrix, such as polylactide-co-glycolic acid (PLGA), which can then be made into microspheres using a solid-in-oil-in-water (S/O/W) encapsulation process [7].

Advantages include: 1) low lot-to-lot variability; and 2) high lot-to-lot consistency.
2) high level of purity
3) The mabs have no effect on healthy cells.
4) Action has a predetermined duration.

1)High expense and complexity associated with the production of a mAb cocktail.
2)Generation is labor-intensive, time-consuming, and technically challenging.
3)It has the potential to be immunogenic.
4) It takes a long time.

Monoclonal antibodies have adverse effects.

Intravenous monoclonal antibodies are administered (injected into a vein). When compared to chemotherapy's adverse effects. While the medicine is being administered, they are more common.

Chills
Diarrhea
Fever
Headache
Blood pressure that is too low
Nausea
Rashes
Weakness in Vomiting

Other antigen-related adverse effects may be experienced by some MBAs.
For example, bevacizumab (Avastin®), a monoclonal antibody that inhibits the formation of cancer blood vessels, can induce high blood pressure, bleeding, poor wound healing, blood clots, and renal damage, among other things.

Applications

1.Diagnostic Applications: Monoclonal antibodies have revolutionized illness diagnosis in the laboratory. For use as diagnostic reagents in biochemical analysis or as instruments for disease diagnosis imaging.
Biochemical Analysis with MAbs:
• Pregnancy: Detection of human chorionic gonadotropin levels in the urine during pregnancy.
• Cancers: The plasma carcinoembryonic antigen in colorectal cancer and prostate specific antigen in prostate cancer are calculated. Estimation of cancer markers is useful for cancer prognosis in addition to diagnosis.
• Hormonal disorders: Thyroid disorders analyzed using thyroxine, triiodothyronine, and thyroid stimulating hormone.

2.Therapeutic Applications:

Monoclonal antibodies have a widely range of therapeutic applications. M-Abs are used in the treatment of cancer.
transplantation of bone marrow and organs.
autoimmune diseases.
cardiovascular diseases.
infectious diseases.

MAbs against antigens on the surface of cancer cells are effective in the therapy of cancer. Antibodies bind to cancer cells and cause them to perish. This is demonstrated by antibody-dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity, and reticuloendothelial system phagocytosis of cancer cells (coated with MAbs).
MAbs are used to treat patients with leukemia, colorectal cancer, lymphoma, and melanoma. A monoclonal antibody that is specific to leukemia cells and is used to eliminate the remaining leukemia cells while causing no harm to other cells. Prior to autologous bone marrow transplantation, MAbs are employed in vitro to detach remaining tumor cells.

Antibodies	**Type**	**Target**	**Medical uses**
Alemtuzumab	Humanized, mAb, IgG1	CD52	B-cell chronic lymphocytic leukemia
Bevacizumab	Human, mAb, IgG2	VEGF	Colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, renal cell carcinoma
Gemtuzumab ozogamicin	Human, ADC, IgG4	CD33	Acute myelogenous leukemia
Trastuzumab-emtansine	Humanized, ADC, IgG1	HER2	Metastatic breast cancer
Brentuximab-vedotin	Chimeric, ADC, IgG1	CD30	Hodgkin's lymphoma
Trastuzumab	Humanized, mAb, IgG1	HER2	HER2-positive breast cancer, gastric/gastroesophageal junction carcinoma
Cetuximab	Chimeric, mAb, IgG1	EGFR	Squamous cell cancer of the head and neck, metastatic EGFR-positive colorectal cancer
Panitumumab	Human, mAb, IgG2	EGFR	EGFR-positive metastatic colorectal carcinoma
Ipilimumab	Human, mAb, IgG1	CTLA-4	Unresectable or metastatic melanoma

Rituximab	Chimeric, mAb, IgG1	CD20	CD20-positive B cell non-Hodgkin lymphoma and chronic lymphocytic leukemia
Ofatumumab	Human, mAb, IgG1	CD20	Refractory chronic lymphocytic leukemia
90Y-Ibritumomab Tiuxetan	Murine, mAb, IgG1	CD20	Relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma

Limitations for direct use of M-Abs in cancer:

1. M-Abs generated in mice and utilized directly for therapeutic purposes may cause hypersensitivity reactions in humans.
2. It's possible that all cancer cells carry the antigen for which M-Ab was developed. As a result, M-Abs may not bind to some cancer cells at all.
3. In the motion, free antigens (of target cells) may bind to M-Abs, preventing them from acting on the target cells.

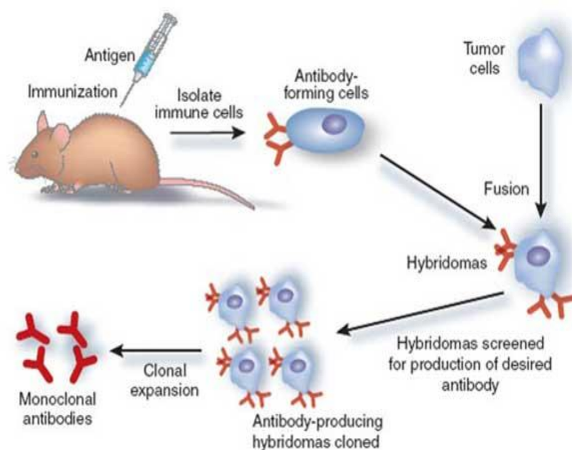
In the treatment of AIDS:

Immunosuppression is the symbol of AIDS. This is due to a decrease in T-lymphocyte CD4 (cluster determinant antigen 4) cells. Using surface membrane glycoproteins, the human immunodeficiency virus (HIV) attaches to particular receptors on CD4 cells (gp120).

In the treatment of autoimmune diseases:

Autoimmune diseases such as rheumatoid arthritis and multiple sclerosis are of great concern. Some successful result has been reported in the clinical trials of rheumatoid arthritis patients by using m-Abs directed against T-lymphocytes and B-lymphocytes.

Antibody	Type	Target	Medical uses
Adalimumab	Human, mAb, IgG1	TNF-α	Rheumatoid arthritis, Crohn's disease, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis
Alemtuzumab	Human, mAb, IgG1	CD52	Multiple sclerosis
Belimumab	Human, mAb, IgG1	BAFF	Systemic lupus erythematosus
Benralizumab	Human, mAb, IgG1	CD125	Asthma
Brodalumab	Human, mAb, IgG2	IL-17	Plaque psoriasis
Canakinumab	Human, mAb, IgG1	IL-1	Cryopyrin-associated periodic syndrome
Certolizumab pegol	Humanized, Fab', IgG1	TNF-α	Crohn's disease, rheumatoid arthritis, axial spondy-loarthritis, psoriatic arthritis
Golimumab	Human, mAb, IgG1	TNF-α	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
Guselkumab	Human, mAb, IgG1	IL23	Psoriasis
Infliximab	Chimeric, mAb, IgG1	TNF-α	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis
Itolizumab	Humanized, mAb, IgG1	CD6	Psoriasis



m-Abs in drug delivery:

In general, when compared to in vitro (in the laboratory), medications are less effective in vivo (in the living body) (in laboratory when tested with cultured cells). This is primarily due to a sufficient amount of the medicine failing to reach the target tissue. Tissue-specific MAbs can be used to alleviate this problem. The medications can be combined with MAb (antibodies directed against a cell surface antigen of the cells, such as a tumor) and tailored to the exact site of action.

.

Conclusion-

Drug delivery using monoclonal antibodies is more effective and safer. Many of these new generation mAbs have received FDA approval for human use. Without a doubt, the future of the mAb will influence the treatment of infectious diseases and malignancies. Whether therapeutic monoclonal antibodies (mAb) will become more widely available and inexpensive in the near future.

REFERENCES

[1] A.L. Daugherty, R.J. Mrsny / Advanced Drug Delivery Reviews 58 (2006) 686–706.

[2] Aliyu Mahmuda1,2, Faruku Bande3, Khalid Jameel Kadhim Al-Zihiry4, Noor Abdulhaleem5, Roslaini Abd Majid1, Rukman Awang Hamat1, Wan Omar Abdullah6 and Zasmy Unyah1 *-Monoclonal antibodies: A review of therapeutic applications and future prospects

[3] Bernett MJ, Karki S, G Moore GL, Leung IWL, Chen H, Pong E, Nguyen DHT, Jacinto J, Zalevsky J, Muchhal US, et al. Engineering Fully Human Monoclonal Antibodies from Murine Variable Regions. J Mol Biol 2010; 396(5):1474–1490.

[4] Chandel P, Harikumar SL. Pharmaceutical monoclonal antibodies: Production, guidelines to cell engineering and applications. Int J Pharm Sci 2013; 5(2):13– 20.

[5] Fukuda J, Iwura T, Yanagihara S, Kano K. Factors to govern soluble and insoluble aggregate-formation in monoclonal antibodies. Anal Sci. 2015;31(12):1233-1240.

[6] Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: Residual immunogenicity resides in the CDR regions. MAbs 2010; 2(3): 256–265.

[7] J. Wang, K.M. Chua, C.H. Wang, Stabilization and encapsulation of human immunoglobulin G into biodegradable microspheres, J. Colloid Interface Sci. 271 (2004) 92 – 101

[8] Lin W, Kurosawa K, Murayama A, Kagaya E, Ohta K. Bcell display-based one-step method to generate chimeric human IgG monoclonal antibodies. Nucleic Acids Res 2011; 39(3): 1–10.

[9] M. Page, C. Ling, P. Dilger, M. Bentley, T. Forsey, C. Longstaff, R. Thorpe, Fragmentation of therapeutic human immunoglobulin preparations, Vox Sang. 69 (1995) 183 – 194

[10] Mahler H-C, Friess W, Grauschopf U, Kiese S. Protein aggregation: pathways, induction factors and analysis. J Pharm Sci. 2009;98(9):2909-2934

[11] O'Brien LM, Goodchild SA, Phillpotts RJ, Perkins SD. A humanised murine monoclonal antibody protects mice from Venezuelan equine encephalitis virus, Everglades virus and Mucambo virus when administered up to 48h after airborne challenge. Virology 2012; 426(2): 100– 105.

[12] Shire SJ. Stability of monoclonal antibodies (mAbs). In: Monoclonal Antibodies. Amsterdam, the Netherlands: Elsevier; 2015:45-92.

https://doi.org/10.1016/ B978-0-08-100296-4.00003-8.

[13] W. Zhang, M.J. Czupryn, Analysis of isoaspartate in a recombinant monoclonal antibody and its charge isoforms, J. Pharm. Biomed. Anal. 30 (2003) 1479 – 1490.

[14] Waliza Ansar1 and Shyamasree Ghosh -Monoclonal Antibodies: a tool in clinical research.

[15] Wang S. Advances in the production of human monoclonal antibodies. Antib Technol J 2011; 1: 1–4

[16] Wang W. Protein aggregation and its inhibition in biopharmaceutics. Int J Pharm. 2005;289(1-2):1-30.

[17] Wei Q, Becherer T, Angioletti-Uberti S, et al. Protein interactions with polymer coatings and biomaterials. Angew Chem Int Ed Engl. 2014;53(31):8004-8031.

[18] Y. Mimura, E.A. Kabat, T. Tanaka, M. Fujimoto, K. Takeo, K. Nakamura, Microheterogeneity of mouse antidextran monoclonal antibodies, Electrophoresis 16 (1995) 116 – 123.