

Monoclonal Antibodies and Their Applications in Cancer

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ABSTRACT

Monoclonal Antibodies, also known as mAbs or mOAbs, are artificial antibodies produced in laboratories. These proteins assist the immune system in recognizing, tagging, and destroying cells in a number of different ways. By tagging antigens as foreign when the body is unable to recognize these cells, they activate the immune system to destroy cells. Monoclonal antibodies are largely preferred over polyclonal antibodies due to their specificity and targeted treatment. This makes them incredibly useful as they can be used to treat any cell-based or pathogen-related disease, including cancer. Monoclonal antibodies can be made to help the body recognize, tag or block receptors of cancerous cells without the side effects of other immunotherapies such as chemotherapy and radiation therapy. There are currently many mAb drugs on the market for cancer, that are either murine, chimeric, humanized or human. We can explore current methods of monoclonal antibodies and their applications, as well as possible treatments centered around immunotherapy. IT may be possible for us to continue expanding the market for monoclonal antibodies by changing the conditions under which they work, change their applications, and reduce their production time to make them more accessible to the public.

Introduction

Antibodies, also known as immunoglobulins, are specific proteins created by the body's immune system in response to the presence of antigens. They attach to foreign substances such as bacteria, fungi, and viruses in an attempt to prevent the spread and function of these antigens. The aim of antibodies is to eliminate these foreign invaders from the body's system. They are created by B-cells in the immune system to immediately recognize pathogens in order to bind to them and destroy them. Antibodies are essential to helping the body fight off infections and are an important member of the active immunity process. However, on occasion the body is unable to produce antibodies for the infectious pathogen. This can be due to several possibilities. The pathogen might replicate and spread faster than the immune system can fight off the infection, or the immune system is unable to create antibodies to fight off the infection accurately and efficiently. This can be detrimental to the body and results in serious cases of infection. In these cases, it is important to interfere with the immune system's natural processes and provide assistance to the immune response. This is where monoclonal antibodies can be used, as these laboratory-based antibodies can assist the immune system by stimulating the same. (Cleveland Clinic. "Monoclonal Antibodies: Definition & How Treatment Works.")

Monoclonal antibodies serve the same function as the body's antibodies. They seek antigens in the body to destroy them. They are designed to recognize and bind to specific antigens by attaching to receptors found on the surface of antigens. They are Y-shaped with two light chains that are epitope specific attached to a larger heavy chain of protein. Monoclonal antibodies work by binding to the intended antigen with their specific antigen-binding site. They then form an antigen-antibody complex. This is shown in Fig.1. The antibody-antigen complex activates the complement system to weaken the antigen further through lysis. Lysis dissolves the cell membrane to make it easier to destroy the cell. During this process, plasma proteins react with

one another, which makes the antigen undergo opsonization and other inflammatory responses. Following opsonization, the bacterial cell or pathogen is ingested by the phagocytes during a process known as phagocytosis. After this, the pathogen is removed from the system.

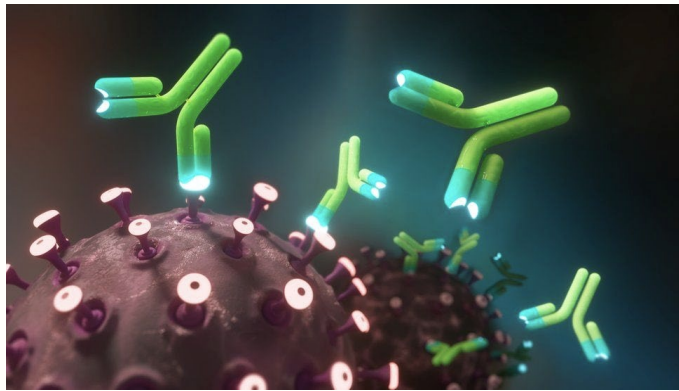


Figure 1. (Source: BBC)

This is just one of the ways that mAb's can ward off antigens. They can also bind to the toxins produced by pathogens, which are antigens in and of themselves. They then inactivate the antigen through a process known as neutralization. Another way that antibodies destroy pathogens is by binding to the receptors of them. This interrupts the function of the receptors. For example, it could prevent viruses from recognizing cells to infect, therefore stopping viral invasion. Otherwise, it could bind to receptors in the pathogen's cell wall that allow nutrition to enter the cell, effectively starving the pathogen. This reduces its pathogenicity. (*How Do Monoclonal Antibodies Work? Rituximab, Infliximab, Adalimumab and Others*, 2019.). This makes antibodies essential to the process of active immunity, in order to tag and destroy pathogens. Monoclonal antibodies are specifically made for each antigen and produced in large quantities of the same type. They are produced from identical B-cells against a specific antigen. mAb's are identical to one another in several different properties as they have similar protein sequences, antigen-binding site region, binding affinity for their targets and identical functional effects. (Sadeghalvad, Mona, and Nima Rezaei. *Introduction on Monoclonal Antibodies. Monoclonal Antibodies*. IntechOpen, 2021.)

Monoclonal antibodies are important as they can be made for various cell-based diseases. They work as a form of immunotherapy and are used to treat a variety of diseases. mAb's are incredibly important and useful to the field of healthcare and have transformed the same. Their current market share is valued at \$55 billion. This is for good reason, as monoclonal antibodies have several applications. They are a targeted and specific form of treatment, and have great versatility as tools for diagnostics, research, therapeutics, and experimentation. Monoclonal antibodies have also been successful in treating a wide variety of diseases including cancer, organ transplant rejection, inflammatory and other autoimmune diseases, infections such as COVID-19, osteoporosis, eye conditions, migraines, and high cholesterol. (Cleveland Clinic. "Monoclonal Antibodies: Definition & How Treatment Works."). It has other uses as well such as pregnancy and diagnostic testing, radio immunodetection and radioimmunotherapy, viral disease and pathogen treatment, and tracing cells and their functions. (WhatisBiotechnology.org. "Monoclonal Antibodies Comprise a Third of All New Drugs.") Their most promising application perhaps, is their possible uses and present work in treating cancer. Monoclonal antibodies have the potential to recognize and destroy cancer cells without the side effects of other forms of therapeutics such as chemotherapy and radiotherapy.

Monoclonal antibodies are the preferred form of therapy over the body's naturally produced polyclonal antibodies. Polyclonal antibodies are different from monoclonal antibodies by their means of production. They can be produced by the body, but are also produced artificially as immunotherapy, in a process different to

monoclonal antibodies. They recognize and bind to multiple epitopes of the same antigen and produce a more general immune response, often resulting in cross-reactivity and cannot be used to treat as many diseases. This makes monoclonal antibodies the better alternative as they are more specific and efficient than polyclonal antibodies and can be used to treat a large range of diseases, due to their process of creation.

Production of Monoclonal Antibodies

Monoclonal antibodies are made from generated mAb producing cells known as hybridomas. Hybridomas are produced by fusing myeloma cells with the specific antigen-producing splenocytes. Splenocytes are the cells that produce the desired antibody, and are also known as B-cells, which are integral for producing the required antibodies with antigen-specific epitopes. mAb are produced from identical clones of the same B-cell, which give them monovalent affinity and the ability to only recognize the one epitope of an antigen, which is what gives them most of their advantages and differentiates them from polyclonal antibodies. Myeloma cells are plasma cells that function as white blood cells and are immortal similar to cancer cells. They produce more antibodies, and as the number of myeloma cells increase, the number of antibodies produced also increases. (“Definition of Myeloma - NCI Dictionary of Cancer Terms - NCI.” February 2, 2011.) After these two cells are fused to produce multiple copies of the desired antibody, the clones are screened and selected based on antigen-specificity and immunoglobulin class. (Molecular Devices. “Monoclonal Antibody Production.”). The lymphocytes that produce the specified antibody are harvested after production *in vitro*, then immortalized so they can continue to undergo cell division. Then these cells are established as clones by the process of limiting dilution, selected, then expanded and preserved. (“What Are Monoclonal Antibodies(MABs) - GenScript.”). The process was first completed in 1975 by Milstein and Köhler

The process begins with the immunization of mice with the specified antigen. After the immunization, their blood is screened for antibodies. Blood collection is done to assess a sufficient number of antibodies using processes such as ELISA. The animal is then sacrificed, and the spleen is isolated. The spleen undergoes tissue digestion from which splenocytes are released. The splenocytes that produce the antibodies are isolated in order to complete hybridoma production *in vitro*. After this, the myeloma cells are prepared for fusion, the result of which will create cells that can produce unlimitedly. This is done by culturing the cells with 8-azaguanine. (Sadeghalvad, Mona, and Nima Rezaei. *Introduction on Monoclonal Antibodies. Monoclonal Antibodies*. IntechOpen, 2021.). The myeloma cells are fused together in the presence of polyethylene glycol (PEG), which causes the cell membranes to fuse. After the fusing, the myeloma-splenocyte fused cells must be separated from the other cells that are unfused or fused incorrectly. This is done through the selective medium hypoxanthine-aminopterin-thymidine, or HAT. In the HAT medium, only cells containing the HGPRT enzyme will be able to survive, which is lacking from unfused myeloma and splenocyte cells. The clones are then screened and selected through ELISA assay which assess antigen-binding ability. After which, the clones undergo dilution using microtiter fluid and are expanded inside bioreactors to produce more antibodies. These are then stored in liquid nitrogen.

The antibodies created from mouse proteins are known as murine and such treatments usually end with the suffix “-omab”. Similarly, antibodies created by both human and mouse proteins end with the suffix “-ximab”. Humanized mAb’s, made from small mouse proteins and attached to human proteins end with the suffix “-zumab”. Finally, fully human proteins that are used to create antibodies have treatments that end with the suffix “-umab”. (“Monoclonal Antibody Side Effects | American Cancer Society.”). These differ from polyclonal antibodies in many ways. Polyclonal antibodies are created in a heterogenous mixture of B-cells and can recognize and bind to different epitopes of antigens. Unlike mAb’s, polyclonal antibodies are extracted directly from serum of the specimen and filtered from the blood. This makes the process much cheaper and quicker to produce, which cannot be said for mAb’s. Monoclonal antibody production is usually very expensive and takes 6-7 months. mAb’s have higher antigen affinity since they can bind to multiple epitopes which is

faster working. They can tolerate small changes in protein structure and can detect smaller and low-quantity proteins. However, the disadvantage of polyclonal is that they are more likely to result in cross-reactivity, where specific antibodies bind to multiple different epitopes of different antigens, producing false positives. (“Polyclonal vs. Monoclonal Antibodies | Labclinics.Com.”). They do not have the homogeneity or the mass-production that mAb’s do, nor the sensitivity. They also cannot treat as many diseases, including cancer.

Monoclonal Antibodies and Cancer

Perhaps monoclonal antibodies' most interesting prospect would be their applications in cancer treatment. Monoclonal antibodies can work as both targeted therapy and immunotherapy and can be taken with other medication or alone as well. They work by assisting the body in the fight against cancer cells in three main ways. (“Monoclonal Antibody Therapy for Cancer: What It Is, Uses & Treatment.”)

Firstly, immunologists make targets for the immune system, by binding to the antigens found on cancer cells so that the immune system is able to recognize and destroy the cells. This is known as antibody-dependent cell-mediated cytotoxicity, or ADCC. This is shown in Fig. 2. Secondly, they are used to carry targeted treatment to the cancerous cells, such as drugs, or radioactive and toxic substances to assist in destroying the cells. Targeted therapy works in two different ways, radiolabeled antibodies and chemolabeled antibodies. Radiolabeled antibodies have radioactive particles attached to them, which, when attached to the antigen, affects the target cell through radiation. Chemolabeled antibodies have powerful chemotherapy drugs attached to them that work to destroy the cell once attached to the antigen. (“Monoclonal Antibody Side Effects | American Cancer Society.”). Lastly, they block cancer cell signals, by blocking certain receptors that signal cancer cells to divide, thus preventing unregulated growth. They can work to cause self-destruct sequences within the cell. An additional way is by reactivating the checkpoint system. CTL’s, which are white blood cells that detect infected or mutated cells secrete toxic molecules that destroy harmful cells. If CTLs are overstimulated, they can sometimes cause damage to the body’s healthy cells, and for this reason the molecule PD1 is used to stop this from occurring. Cancer cells use this to their advantage, by creating an alternative protein called PD-L1 to prevent CTL’s from destroying them. (*Immunology Wars: Monoclonal Antibodies*, 2017.). Monoclonal antibodies can be used to bind to the PD1 or PD-L1 molecule by working against them in order to assure CTLs are working to remove cells efficiently.

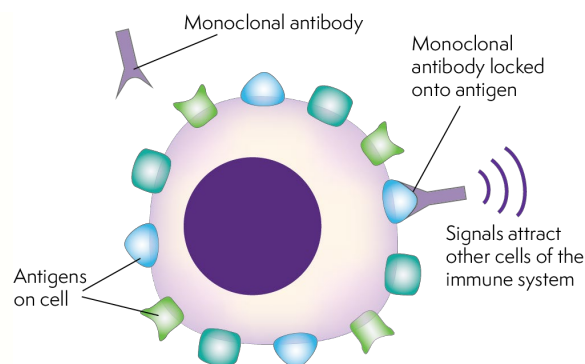


Figure 2. (Source: Lymphoma Australia)

These are some of the ways that monoclonal antibodies can be used to treat cancer, and each therapy uses a different process to treat the disease. For example, Rituximab works to treat chronic lymphocytic leukemia and non-Hodgkin lymphoma by binding to CD20 protein and flagging the cell. Cetuximab is a treatment for bowel, head, and neck cancer. Rituximab and cetuximab are both mAb’s that work by triggering the immune system to attack cancer cells through ADCC. (“Monoclonal Antibodies (MABs) | Immunotherapy | Cancer

Research UK.”). Trastuzumab, used for breast and stomach cancer, binds to a protein called HER2 on the surface of cancerous cells. This prevents the cell from sending signals to grow. Another example would be ipilimumab, nivolumab and pembrolizumab. These treatments work as checkpoint inhibitors, stopping cancer cells from evading the immune system by reactivating the CTL’s. They block such proteins as CTLA-4, PD-1 and PD-L1, therefore ensuring that the immune system works against cancer cells. Bevacizumab, another mAb, blocks a protein known as VEGF, which causes blood vessels to grow. Without VEGF, blood vessels cannot grow and therefore cannot supply tumors with oxygen. This effectively starves the tumors. An example of chemolabeled antibodies include brentuximab vedotin. (*How Monoclonal Antibodies Treat Cancer*, 2020.). This works by delivering a chemotherapy drug directly to the targeted cancer cell. The drug attaches itself to the cancer cell through the mAb, which in turn, kills it.

Many monoclonal antibody therapies have been FDA approved. mAb’s are still being investigated for their properties and possible uses. The current applications of mAb’s still vary, as some are used as standard treatment, while others are still experimental and used only if other treatments have been unsuccessful. This treatment is administered intravenously and is often used with other treatments. This includes hormone therapy and chemotherapy. The details of mAb treatment differ based on cancer type and severity and can also depend on medication the patient is on. Usually, a test is carried beforehand on the patient’s cancer cells to see if monoclonal antibodies would be effective in treating the disease. The main benefit of this treatment is that it does not harm healthy cells. They can also result in less side effects and use the body’s immune system to treat the disease. While monoclonal antibodies seem to be an excellent treatment as they do not harm other cells like chemotherapy and radiotherapy do, they still have their disadvantages and side effects. Some common side effects include allergic reactions, flu-like symptoms, nausea and vomiting, diarrhea, skin rashes and low blood pressure. However, these are very minimal side effects when compared to some possible, life-threatening side effects of this treatment. For example, infusion reactions, which are severe allergic reactions that can very rarely lead to death and are usually prevented with medication beforehand. Heart and lung problems are also common with higher risk of inflammatory disease and changing blood pressure. Skin infections are also common as is internal bleeding. (Mayo Clinic. “Monoclonal Antibody Drugs for Cancer: How They Work.”). Apart from this, the treatment is still not a cure for cancer, rather a treatment that delays the return of cancer, much like present treatments. It is also a very long and expensive process, taking up to 6 months to produce and cannot be used for end-stage cancers. However, is it possible that we could reduce these risks and production costs through scientific innovation and technology? And what other possibilities do we have for monoclonal antibodies?

New Research for Monoclonal Antibodies

As monoclonal antibodies are still a relatively new field of research as compared to current treatments, there is still much to explore and discover. Many treatments are still experimental as of now due to their high cost and lengthy production cost. However, monoclonal antibodies have their fair share of interesting prospects and possibilities. By further engineering monoclonal antibodies, we can treat cancer more effectively. This can be done through bi-specific antibodies and CAR-T cell therapy. We can also find methods to mass produce antibodies in a faster, more industrial manner to be used on a wider scale without the higher production costs. This way, the body can defend against monoclonal antibodies even in later stages of cancer along with other treatments.

Current research in mAb therapy for cancer is CAR-T cell therapy. In CAR-T cell therapy This type of therapy is built off a monoclonal antibody known as the chimeric antigen receptor. T cells, a type of white blood cell, are removed from the patient and are then engineered to produce the CAR. (“CAR T-Cell Therapy and Its Side Effects.”). This receptor binds to specific antigens on the surface of cancer cells, called the CD19. The T cells are then inserted back into the patient. These T cells are then able to bind to the cancer cells and effectively destroy them. This is also a form of cell gene therapy, as the T cells are genetically modified to

produce CAR, and this receptor turns the T cell into an efficient cancer destruction cell. This therapy is likely the most effective as of now, as it only takes a few weeks as compared to the usual 6 months it takes to produce mAb's. These are also more effective than regular mAb treatment as they become a part of active immunity. They can be used to defend against cancer if in case it returns, as the T-cells have learned immunity for the disease. In terms of cost, CAR-T cell therapy is at the same price as normal monoclonal antibody therapy. However, it is more effective and takes less time, as it reduces the chance of the cancer returning. CAR-T cell therapy is currently FDA approved, but still has not found wide-spread use. Studies are still currently underway to investigate whether CAR-T cell therapy can be used for various cancers, as it currently only treats lymphoma, myeloma, and leukemia, but could potentially be used to treat others. If CAR-T cell therapy were to become more widespread, it is likely that costs would reduce, as technology for the same is likely to improve.

The same can be said for monoclonal antibody treatment as a whole. As technology furthers and we find more ways to improve the production of monoclonal antibodies, it is probable that mAb treatment will be faster and less expensive. In terms of antibody production, new research is already being done to produce monoclonal antibodies faster. A team of researchers led by Facundo Batista, from the Francis Crick Institute in London and the Ragon Institute of MGH, MIT, and Harvard, were able to produce specific human antibodies in the laboratory by treating B cells from the patient with tiny nanoparticles coated with both CpG oligonucleotides and the appropriate antigen. This produced antibodies much faster than isolating the B cells from an organism like mice. (ScienceDaily. "New Method to Generate Human Antibodies.")

With more innovations such as this, it is possible for us to create monoclonal antibodies that can be used to treat a variety of cancers in a shorter time and for less money. This would improve the quality of life for the nearly 40% of the population diagnosed with cancer. However, as most causes of cancer are currently unknown, it will be difficult to say whether we can use monoclonal antibodies to prevent all types of cancer. Until we have sufficient knowledge about cancer, promising technology such as monoclonal antibodies are rendered unusable. It should also be noted that monoclonal antibodies are not a cure but a treatment, and unless we discover a method to assist the body in producing its own antibodies against this disease, it is unlikely that monoclonal antibodies can be used as a definitive cure. Another concern is the technology and equipment needed to produce these antibodies, which tend to be expensive. If we perhaps found a way for the body to gain passive immunity to cancer or further CAR-T cell therapy to produce antibodies for any and all types of cancer, we could possibly use monoclonal antibodies as a cure. We still have many answered questions about this possible turning point in the field of immunotherapy. What other biomarkers can we discover that are reliable and predictable when it comes to mAb treatment that provide information on sensitivity and resistance? Would it be possible to use transgenic plants and animals for the production of mAb's on a larger scale at a more affordable cost?

Further research into cancer biology may soon unveil the answers. By developing new ways to produce monoclonal antibodies and their associated treatments, it may also be possible to cure cancer and prevent the return of it to individuals affected once and for all. It must be placed with importance that with sufficient time, technology, resources, and research, this may be in the foreseeable future.

References

1. "Monoclonal Antibodies (MABs) | Immunotherapy | Cancer Research UK." Accessed July 20, 2022. <https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/immunotherapy/types/monoclonal-antibodies>.
2. *How Do Monoclonal Antibodies Work? Rituximab, Infliximab, Adalimumab and Others*, 2019. <https://www.youtube.com/watch?v=h6sGMaf6Yew>.
3. Sadeghalvad, Mona, and Nima Rezaei. *Introduction on Monoclonal Antibodies. Monoclonal Antibodies*. IntechOpen, 2021. <https://doi.org/10.5772/intechopen.98378>.

4. Cleveland Clinic. "Monoclonal Antibodies: Definition & How Treatment Works." Accessed July 20, 2022. <https://my.clevelandclinic.org/health/treatments/22246-mono-clonal-antibodies>.
5. WhatisBiotechnology.org. "Monoclonal Antibodies Comprise a Third of All New Drugs." Accessed July 20, 2022. <https://www.whatisbiotechnology.org/>.
6. "Definition of Myeloma - NCI Dictionary of Cancer Terms - NCI." NciAppModulePage, February 2, 2011. Nciglobal,ncicenterprise. <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/myeloma>.
7. Molecular Devices. "Monoclonal Antibody Production." Accessed July 22, 2022. <https://www.moleculardevices.com/applications/monoclonal-antibody-production>.
8. "What Are Monoclonal Antibodies(MABs) - GenScript." Accessed July 20, 2022. <https://www.genscript.com/how-to-make-mono-clonal-antibodies.html>.
9. Sadeghalvad, Mona, and Nima Rezaei. *Introduction on Monoclonal Antibodies. Monoclonal Antibodies*. IntechOpen, 2021. <https://doi.org/10.5772/intechopen.98378>.
10. "Monoclonal Antibody Side Effects | American Cancer Society." Accessed July 24, 2022. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/monoclonal-antibodies.html>.
11. "Polyclonal vs. Monoclonal Antibodies | Labclinics.Com." Accessed July 21, 2022. <https://www.labclinics.com/2018/09/07/polyclonal-mono-clonal-antibodies/?lang=en>.
12. "Monoclonal Antibody Therapy For Cancer: What It Is, Uses & Treatment." Accessed July 24, 2022. <https://my.clevelandclinic.org/health/treatments/22774-mono-clonal-antibody-therapy>.
13. "Monoclonal Antibody Side Effects | American Cancer Society." Accessed July 24, 2022. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/monoclonal-antibodies.html>.
14. *Immunology Wars: Monoclonal Antibodies*, 2017. <https://www.youtube.com/watch?v=5AXApBbj1ps>.
15. "Monoclonal Antibodies (MABs) | Immunotherapy | Cancer Research UK." Accessed July 20, 2022. <https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/immunotherapy/types/monoclonal-antibodies>.
16. *How Monoclonal Antibodies Treat Cancer*, 2020. <https://www.youtube.com/watch?v=dxnjAc-rqz8>.
17. Mayo Clinic. "Monoclonal Antibody Drugs for Cancer: How They Work." Accessed July 28, 2022. <https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/monoclonal-antibody/art-20047808>.
18. "CAR T-Cell Therapy and Its Side Effects." Accessed July 28, 2022. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/car-t-cell1.html>.
19. ScienceDaily. "New Method to Generate Human Antibodies." Accessed July 28, 2022. <https://www.sciencedaily.com/releases/2017/07/170724105119.htm>.