The Journal of Multidisciplinary Research (TJMDR)

Content Available www.saap.org.in ISSN: 2583-0317

MONOCLONAL ANTIBODIES: FROM HYBRIDOMA TECHNOLOGY TO NEXT – GENERATION THERAPEUTIC INNOVATIONS.

PAIDIMALA BILLGATES*, ABBADASARI RAJU, B.GAYATHRI, CHANDU BABU RAO

Priyadarshini Institute of Pharmaceutical Education and Research, 5th Mile, Pulladigunta, Guntur-522017, Andhra Pradesh, India.

Article History: Received: 26 Feb 2026 Revised: 19 Mar 2026 Accepted: 04 May 2026

Abstract: Immunoglobulin derivatives which are derived from the monoclonal cell line and which offers a wide range of specificity is the monoclonal antibodies. They are specially produced by the hybridoma technology by the fusion of B-cells with the immortal myeloma cells in presence of PEG. Humanized mAbs are considered to be the fastest growing group in clinical trials. After development, these mAbs undergoes analytical evaluation for their efficient characterization. Developed hybridomas can be preserved for long term use through the cryopreservation techniques. Monoclonal antibodies can be delivered for the therapeutic purpose through the various systemic and non-systemic routes. Large groups of the antibodies are found to be very effective through the oral routes and the ophthalmic routes. Besides the therapeutic application for the treatment of various infectious and autoimmune diseases, these groups of therapeutics show different limitations. Monoclonal antibodies after development suffers from the stability issues and using the various techniques, the stability can be increased. With the advancement of science and technology, we can observe various advances in the monoclonal antibody development like brain targeting is possible through the antibody engineering techniques. Variability and control challenges in the serum based acquisitions, consumption of time, difficulty in the development, potential limitation in the sequence and epitope diversity etc. are some of the challenges associated with the monoclonal antibodies

keywords ; Immunoglobulin, Hybridoma Technology, Cryopreservation, Antibody Engineering.

***Corresponding Author**
Paidimala Billgates

Produced and Published by
South Asian Academic Publications

INTRODUCTION

Monoclonal antibodies are immunoglobulin wich are specially derived from monoclonal 2 with the disulfide bridges. They have wide applications in treatment that includes several infectious disease, different malignant conditions and different autoimmune disease condition. Large number of the drugs i.e. more than 250 drugs have been found to undergo clinical trials. Adverse drug reaction is drastically reduced on using the monoclonal antibodies as therapeutic agents. Hybridoma technology is commonly adopted for the production. Such methods significantly reduce the immunogenicity. Drug interaction is highly minimized and protein targeting is specifically achieved through the antibodies therapy. Development of the bio similar antibodies has significantly reduced the higher cost of production. Wide spectrum of the disease can be targeted through the use of these techniques. Antibodies are one of the naturally existing and primary pathways through which the body defends itself against antigens, which may be derived from bacteria, viruses, fungi, parasites, bacterial/virus-infected cells, pollen, or nonliving substances, such as toxins, chemicals, drugs, or foreign particles considered alien to the body as epitopes expressed on cancer cells, etc. Specific binding to their targets is consequenced by either neutralizing and interfering with their pathogenic effect or flagging them for clearance or destruction as one of the Ag-Ab immune complex fates. Antibodies are considered one of the most prominent and promising remarks in the medicinal, pharmaceutical, and even veterinary fields, with a wide range of significant diseases' prophylactic, therapeutic, and diagnostic approaches [1], on the cusp of the mod ern medicine era. The natural development of antibodies within a living creature's body due to active infection are polyclonal antibodies (pAbs), derived from several B cell clones' development due to the nature of our extremely diverse B cell repertoires

[2]. The COVID-19 pandemic and the emergence of antimicrobial-resistant micro- organisms have drawn renewed interest in human mAbs therapies in recent years [3].

1. HISTORICAL BACKGROUND OF MONOCLONAL ANTIBODIES

1.1 Discovery of Antibodies

The historical narrative surrounding the discovery of antibodies is rich and spans significant milestones in immunology and medicine. Antibodies, characterized as Y-shaped proteins synthesized by B cells, are essential in the body's immune response against pathogens such as bacteria and viruses [4]. In 1890, Emil von Behring and Shiba Saburo Kitasato demonstrated the efficacy of serum therapy in treating diphtheria, marking a pivotal moment in antibody research [5]. German researcher Paul Ehrlich coined the term "antibody" in 1897, defining antibodies as branched molecules that bind to toxins, laying the groundwork for understanding their role in immunity [6].

1.2 Milestones in monoclonal Antibody research

Köhler and Milstein introduced hybridoma technology in 1975, a pioneering advancement in biotechnology that has since transformed the landscape of monoclonal antibody production [7]. This innovative technique involves the fusion of antibody-producing B cells with immortal myeloma cells, resulting in hybrid cells termed hybridomas capable of continuous, high-yield production of specific antibodies [8]. Hybridoma technology has revolutionized the field by facilitating the creation of large quantities of identical antibodies, known as monoclonal antibodies. The process commences with immunizing a mammal, typically a mouse, with a specific antigen to trigger an immune response. Subsequently, antibody-producing B cells are extracted from the immunized animal and fused with myeloma cells, generating hybridomas. These hybridomas can be cultured to produce monoclonal antibodies that exhibit chemical identity and high specificity toward the targeted antigen [9].

2. STRUCTURE AND FUNCTIONS OF MONOCLONAL ANTIBODIES

2.1 Molecular Structure

Monoclonal antibodies have a "Y-shaped" structure composed of four polypeptide chains: two identical heavy chains and two identical light chains. The total molecular weight of a monoclonal antibody is around 150 kDa. The two arms of the "Y" are called the Fab (antigen-binding fragment) regions, which contain the variable domains responsible for antigen binding. The stem of the "Y" is called the Fc (fragment crystallizable) region, which determines the class/isotype of the antibody and mediates effector functions. The Fab region contains complementarity-determining regions (CDRs) that allow the antibody to bind to a specific antigen epitope with high affinity. The heavy chain forms the lower part of the "Y" and is the constant region, while the light chain forms the upper arms and is the variable region responsible for antigen binding [10].

2.2 Mechanisms of Action

Through a variety of mechanisms, such as blocking growth-promoting signals, inducing apoptotic signaling through surface antigen cross-linking, and immune-mediated cytotoxicity like complement-mediated cytotoxicity (CMC) and antibody-dependent cellular cytotoxicity (ADCC), monoclonal antibodies (mAbs) have antitumor effects. Additionally, mAbs oppose tumor-associated ligands or receptors to prevent tumor growth and survival, alter the cytokine environment, and function as immune receptor agonists to improve antitumor immunity [11].

2.3 Variability in Specificity and Affinity

The specificity and affinity of monoclonal antibodies (mAbs) can vary significantly due to multiple factors. Research indicates that mAbs may exhibit a spectrum of binding specificities, ranging from highly specific to highly cross-reactive, with some mAbs demonstrating reactivity with multiple normal tissues [12]. Moreover, research underscores the importance of balancing affinity and non-specific binding when optimizing mAbs for therapeutic purposes. Investigations have shown that while most antibodies with high affinity tend to display relatively high non-specific binding, those with substantial reductions in non-specific binding often experience a decrease in affinity [13].

3. CLASSIFICATION OF MONOCLONAL ANTIBODIES

3.1 MURINE ANTIBODIES

Murine antibodies are the "first generation" of monoclonal antibodies, derived entirely from mouse proteins. They are produced by fusing a mouse B cell with a cancerous myeloma cell
(hybridoma technology). While groundbreaking, they often trigger a Human Anti-Mouse Antibody (HAMA) response, where the patient's immune system rejects the drug as a foreign protein [14].

3.2 Chimeric Antibodies

To reduce immune rejection, chimeric antibodies were developed by replacing the mouse "constant" regions with human sequences. They are roughly 65-75% human. The "variable" regions (the parts that actually grab the target) remain mouse-derived. This balance retains the specific targeting of the mouse antibody while being more "human-friendly [15].

3.3 Humanized Antibodies

Humanized antibodies go a step further, being approximately 90-95% human. Only the tiny "fingertips" (Complementarity-Determining Regions or CDRs) that touch the antigen are from the mouse; the entire rest of the molecule is human. This is achieved through a process called CDR grafting [16].

4. PRODUCTION TECHNOLOGIES OF MONOCLONALANTIBODIES

4.1 Hybridoma Technology

Hybridoma technology is a well-established method to produce monoclonal antibodies (mAbs) tailored to specific antigens of interest .This technique entails the fusion of a short-lived antibody-producing B cell with an immortal myeloma cell, culminating in the formation of hybridoma cell lines. Each distinct hybridoma cell line is characterized by the robust expression of a singular specific mAb, thereby facilitating the continuous production of identical antibodies. Originating in 1975, this groundbreaking technology was pioneered by Georges Köhler and César Milstein, who adeptly fused normal B cells from immunized mice with myeloma cells [17].

4.2 Recombinant DNA Technology

Recombinant DNA technology encompasses the intricate process of amalgamating DNA fragments from disparate sources, including distinct species, to generate novel genetic sequences that do not occur naturally [18]. This methodology involves a series of meticulously orchestrated steps: isolation of the desired DNA, precise cleavage at predetermined recognition sites utilizing restriction enzymes, amplification of gene copies via polymerase chain reaction (PCR), ligation of DNA fragments into a vector, and subsequent insertion of the recombinant DNA into a host organism [19]. The advent of recombinant DNA technology facilitates the production of copious amounts of specific proteins, modifying existing genes, and incorporating foreign genes into organisms. Such capabilities find extensive application in diverse realms, spanning medicine, agriculture, and industrial biotechnology [20].

4.3 Transgenic Mice and Phage Display

Two pivotal technologies utilized in the production and engineering of monoclonal antibodies are transgenic mice and phage display. Transgenic mice are genetically modified to express complete human antibody repertoires, facilitating the rapid and efficient generation of human antibodies boasting enhanced affinity and specificity. These mice serve as invaluable platforms for in vivo affinity maturation, culminating in the synthesis of fully human antibodies characterized by reduced immunogenicity and heightened therapeutic efficacy [21].

5. PURIFICATION AND CHARECTERIZATION OF MONOCLONAL ANTIBODIES

Due to their structural complexity and safety requirements, analysis of monoclonal antibodies is crucial after formulation and development. Mass spectrometry is frequently used in conjunction with sophisticated analytical methods like chromatography and electrophoresis to characterize them. Although reverse-phase liquid chromatography is frequently employed, capillary zone electrophoresis is a useful substitute. Adsorption is still a significant problem with both methods, though. Ion-exchange and size-exclusion chromatography are two examples of traditional techniques that are still used despite their drawbacks, which include poor kinetic performance, low resolution, and incompatibility with sophisticated detection systems [22].

6. ANTIBODY ENGINEERING AND ADVANCED FORMATS

6.1.Fundamentals Of mabs' Development and Optimization Technologies

The most common therapeutic antibody modalities are human and humanized mAbs; 51, 34.7, 12.5, and 2.8% of all mAbs in clinical use are for human, humanized, chimeric, and murine antibodies, respectively [23]. The next part covers the standard mAbs' production platforms with a detailed description of their basic and supplementary techniques. They are followed by an illustrative mention of the basis of the optimization and maturation of mAbs binding affinity. Lastly, the wide range of next generation mAbs and the basics of their developing techniques will be discussed while focusing on the structural differences.

6.2.Polyspecific (multispecific) antibodies

Engineered antibodies with two binding sites that can detect different antigens or unique epitopes on the same antigen are known as bispecific antibodies (bsAbs). bsAbs are used extensively in tumor immunotherapy and other conditions and show better therapeutic success than monoclonal antibodies. More than thirty commercial technologies for bsAb generation have been developed as a result of advancements in recombinant DNA and antibody engineering, which have made it possible to establish multiple production platforms. Three bsAbs have been approved for clinical usage, while over 110 are now in various phases of clinical development. With the goal of improving therapeutic approaches and directing future bsAb development, this review addresses bsAb platforms, mechanisms of action, and clinical applications [24].

6.3.Catalytic antibodies (cab's)

Research on catalysts had previously been done, but the development of new catalysts was made possible by catalytic antibodies (antibody enzyme). After decades of research, scientists have found many techniques to produce antibodies with specific properties and catalytic capabilities, as well as natural antibodies that can hydrolyze substrates such as proteins, nucleic acids, and polysaccharides. In the fields of biology, medicine, and chemistry, these antibodies are widely utilized. In the area of infection and immunity, where the onset and development of autoimmune illnesses frequently take a long period, catalytic antibodies can still be involved in the process and even completely prevent it [25].

6.4 Nanobodies (Nbs)

The isolation and successful expression of VHH domains, also known as single-domain Abs or

Nbs, were made possible by the discovery of H chain-only Abs in Trypanosoma evansi-infected camels. These Abs lacked L chains and CH1 domains and consisted of just two H chains, each with a single variable Ag-binding domain (VHH domain) [26]. Nbs can be obtained from a variety of synthetic, naïve, or immune libraries. Camels are immunized as part of the immune library process, after which Nb is isolated, expressed in E. coli systems, and selected using surface plasmon resonance and phage dis play [27].

7. CURRENT THERAPEUTIC APPLICATIONS OF MONOCLONAL ANTIBODIESIN NON COMUNICABLE DISEASES

7.2 Cardiovascular diseases

Because they specifically target proteins involved in lipid metabolism, inflammation, and thrombosis, monoclonal antibodies (mAbs) are becoming increasingly important in the treatment of cardiovascular disease. While anti-IL-1β mAbs decrease inflammation and the likelihood of recurrent cardiovascular events after myocardial infarction, PCSK9-targeting mAbs efficiently cut cholesterol levels. Additionally, some mAbs block platelet glycoprotein receptors, which lowers the likelihood of thrombus formation in high-risk individuals. By inhibiting cholesteryl ester transfer and perhaps lowering PCSK9 levels, anacetrapib, a cholesteryl ester transfer protein (CETP) inhibitor, raises HDL-C and lowers LDL-C. Clinical research shows that both by itself and in conjunction with statins, increased LDL-ApoB catabolism significantly lowers LDL-C, which lowers cardiovascular events. However, the FDA did not approve anacetrapib and its regulatory development was stopped because of its high lipophilicity and buildup in adipose tissue over time

7.3 Respiratory diseases

Monoclonal antibodies (mAbs) target particular immune components in respiratory illnesses such idiopathic pulmonary fibrosis and severe asthma in order to minimize inflammation and regulate the course of the disease. mAbs against IgE or interleukins help manage asthma symptoms, especially in people who don't respond to traditional treatment. The tyrosine kinase inhibitor nintedanib, despite not being a monoclonal antibody, has promise in addressing fibrotic pathways in lungillness. Two well-known monoclonal antibodies for eosinophilic asthma are mepolizumab and benralizumab, Mepolizumab targets interleukin-5 (IL-5), reducing eosinophil development and survival, while benralizumab binds to the IL-5 receptor α (IL-5Rα) on eosinophils.

8. CHALLENGES AND LIMITATIONS

Next-Generation Monoclonal Antibodies

High specificity, quick immunological protection, immune system recruitment, and few side effects make monoclonal antibodies a promising defense against biological warfare agents and new infectious illnesses [28]. They have been shown to be effective against natural diseases and biological threats of military relevance [29]. Now being developed for infectious, autoimmune, and cancer conditions, chimeric, humanized, and fully human mAbs with decreased immunogenicity have been created thanks to advancements in antibody engineering [30]. As evidence of the increasing significance of this therapeutic approach, the worldwide therapeutic mAb market is expected to reach $300 billion by 2025 [31-34].

9. CONCLUSION

Monoclonal antibodies (mAbs) are a major advancement in modern therapeutics, providing high specificity and targeted action with improved clinical outcomes over conventional therapies. They have transformed the treatment of cancer, autoimmune, infectious, and inflammatory diseases. Progress in recombinant DNA technology and antibody engineering has enabled the development of chimeric, humanized, and fully human antibodies with reduced immunogenicity and enhanced efficacy. Although challenges such as high cost and complex manufacturing remain, innovations in biotechnology and biosimilars are improving accessibility. Monoclonal antibodies continue to be central to precision medicine and future therapeutic strategies.

10. AUTHOR CONTRIBUTIONS

All authors are contributed equally.

11. FINANCIAL SUPPORT

None

12. DECLARATION COMPETING INTEREST

The authors have no conflicts of interest to declare.

13. ACKNOWLEDGEMENTS

None

14. REFERENCES

1. Mekala JR, Nalluri HP, Reddy PN, SB S, NS SK, GVSD SK, et al. Emerging trends and therapeutic applications of monoclonal antibodies. Gene. 2024;925:148607. doi:10.1016/j.gene.2024.148607.
2. Pelletier JP, Mukhtar F. Passive monoclonal and polyclonal antibody therapies. In: Fung MK, Eder AF, Spitalnik SL, Westhoff CM, editors. Immunologic Concepts in Transfusion Medicine. Amsterdam: Elsevier; 2020. p. 251-348. doi:10.1016/B978-0-323-67509-3.00016-0.
3. Jahanshahlu L, Rezaei N. Monoclonal antibody as a potential anti-COVID-19. Biomed Pharmacother. 2020;129:110337. doi:10.1016/j.biopha.2020.110337.
4. Shepard HM, Phillips GL, Thanos CD, Feldmann M. Developments in therapy with monoclonal

antibodies and related proteins. Clin Med (Lond). 2017;17(3):220-232. doi:10.7861/clinmedicine.17-3-220.
5. Manso T, Kushwaha A, Abdollahi N, Duroux P, Giudicelli V, Kossida S. Mechanisms of action of monoclonal antibodies in oncology integrated in IMGT/mAb-DB. Front Immunol. 2023;14:1129323. doi:10.3389/fimmu.2023.1129323.
6. Moorthy BS, Xie B, Moussa EM, Iyer LK, Chandrasekhar S, Panchal JP, et al. Structure of monoclonal antibodies. In: Biobetters: Protein Engineering to Approach the Curative. New York: Springer; 2015. p. 81-89.
7. Nap M, Hammarström ML, Börmer O, Hammarström S, Wagener C, Handt S, et al. Specificity and affinity of monoclonal antibodies against carcinoembryonic antigen. Cancer Res. 1992;52(8):2329-2339.
8. Nap M, Hammarström ML, Börmer O, Hammarström S, Wagener C, Handt S, et al. Specificity and affinity of monoclonal antibodies against carcinoembryonic antigen. Cancer Res. 1992;52(8):2329-2339. *(Duplicate of Reference 7; retain only one in final bibliography.)*
9. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256(5517):495-497.
10. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A. 1984;81(21):6851-6855.
11. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature. 1986;321(6069):522-525.
12. Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol. 2010;10(5):317-327.
13. Hamers-Casterman CT, Atarhouch T, Muyldermans SA, Robinson G, Hammers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature. 1993;363(6428):446-448.
14. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Isolating, cloning, and sequencing DNA. In: Molecular Biology of the Cell. 4th ed. New York: Garland Science; 2002.
15. Kothari M, Wanjari A, Acharya S, Karwa V, Chavhan R, Kumar S, et al. A comprehensive review of monoclonal antibodies in modern medicine: tracing the evolution of a revolutionary therapeutic approach. Cureus. 2024;16(6):e62006. *(Verify article number from original source.)*
16. Kothari M, Wanjari A, Acharya S, Karwa V, Chavhan R, Kumar S, et al. A comprehensive review of monoclonal antibodies in modern medicine: tracing the evolution of a revolutionary therapeutic approach. Cureus. 2024;16(6). *(Duplicate of Reference 15; retain only one.)*
17. Yingngam B. Monoclonal antibody. In: Critical Developments in Cancer Immunotherapy. 2024. p. 419.
18. Fekete S, Gassner AL, Rudaz S, Schappler J, Guillarme D. Analytical strategies for the characterization of therapeutic monoclonal antibodies. TrAC Trends Anal Chem. 2013;42:74-83. doi:10.1016/j.trac.2012.09.012.
19. Fan G, Wang Z, Hao M, Li J. Bispecific antibodies and their applications. J Hematol Oncol. 2015;8(1):130.
20. Fan G, Wang Z, Hao M, Li J. Bispecific antibodies and their applications. J Hematol Oncol. 2015;8(1):130. doi:10.1186/s13045-015-0227-0. *(Duplicate of Reference 19.)*
21. Tapia-Galisteo A, Compte M, Álvarez-Vallina L, Sanz L. When three is not a crowd: trispecific antibodies for enhanced cancer immunotherapy. Theranostics. 2023;13(3):1028-1048. doi:10.7150/thno.81494.
22. Zhao D, Chen J, Hu X, Zhang S. Catalytic antibodies: design, expression, and their applications in medicine. Appl Biochem Biotechnol. 2023;195(2):1514-1540. doi:10.1007/s12010-022-04183-1.
23. Hamers-Casterman CT, Atarhouch T, Muyldermans SA, Robinson G, Hammers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature. 1993;363(6428):446-448. doi:10.1038/363446a0. *(Duplicate of Reference 13.)*
24. Ingram JR, Schmidt FI, Ploegh HL. Exploiting nanobodies' singular traits. Annu Rev Immunol. 2018;36:695-715. doi:10.1146/annurev-immunol-042617-053327.
25. Zhang A, Stacey HD, D'Agostino MR, Tugg Y, Marzok A, Miller MS. Beyond neutralization: Fc-dependent antibody effector functions in SARS-CoV-2 infection. Nat Rev Immunol. 2023;23(6):381-396. doi:10.1038/s41577-022-00813-1.
26. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol. 2007;25:21-50. doi:10.1146/annurev.immunol.25.022106.141702.
27. Abdeldaim DT, Schindowski K. Fc-engineered therapeutic antibodies: recent advances and future directions. Pharmaceutics. 2023;15(10):2402. doi:10.3390/pharmaceutics15102402.
28. Rao C. Evaluation of Antiulcer Activity of Picrasma Quassioides Bennett Aqueous Extract in Rodents. Vedic Research International Phytomedicine. 2013 Jan 1.
29. Gindi S, Methra T, Chandu BR, Boyina R, Dasari V. Antiurolithiatic and invitro anti-oxidant activity of leaves of Ageratum conyzoides in rat. World J. Pharm. Pharm. Sci. 2013 Feb 8;2:636-49.

30. Nama S, Chandu BR, Awen BZ, Khagga M. Development and validation of a new RP-HPLC method for the determination of aprepitant in solid dosage forms. Trop J Pharm Res. 2011;10(4):489-494.
31. Kiranmai M, Renuka P, Brahmaiah B, Chandu BR. Vitamin D as a promising anticancer agent. Int J Res Pharm Chem. 2012;2(2):636-649.
32. Kundeti K. A Review on: Pharmacokinetic and Drug Disposition. Journal of Case Studies and Case Reports. 2023 Aug 31:10-5.
33. Dara SR. An Overview of the Use of Natural Indicators in Acid-Base Titrations. UPI Journal of Pharmaceutical, Medical and Health Sciences. 2024 Jul 23:29-35.
34. Adiki SK, Lahari K, Dey B, Khalf AM, Al-Sharif SM, Diaf SR, Katakam P, Chandu BR. Validated UV Method Development for the Simultaneous Estimation of Rabeprazole sodium and Cinitapride in Tablets.