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Engineering the Future of Medicine: The Evolution of Monoclonal Antibody (mAb) Therapeutics

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ABSTRACT

Background: Monoclonal antibodies (mAbs) have become one of the most successful classes of biopharmaceuticals, revolutionizing the treatment of cancer, autoimmune disorders, infectious diseases, and numerous chronic conditions. Since the introduction of hybridoma technology in 1975, continuous advances in antibody engineering have dramatically improved antibody specificity, efficacy, safety, and clinical applicability.

Main Findings: This review summarizes the historical evolution of monoclonal antibody therapeutics, highlighting the major technological advances that have driven the transition from murine antibodies to humanized and fully human antibody platforms. Recent innovations, including Fc (fragment crystallizable) engineering to enhance antibody half-life and immune effector functions, glycoengineering, bispecific antibodies, antibody-drug conjugates (ADCs), nanobodies, and artificial intelligence-assisted antibody discovery, have significantly expanded the therapeutic potential of monoclonal antibodies while addressing many limitations associated with earlier generations. Furthermore, advances in biomanufacturing technologies, computational biology, and precision medicine have accelerated antibody discovery and development, improved production efficiency, and broadened the clinical application of monoclonal antibody therapeutics across a wide range of diseases.

Future Perspectives: Future progress is expected to rely on integrating synthetic biology, machine learning, structural bioinformatics, and next-generation protein engineering to develop safer, more effective, and increasingly personalized antibody therapeutics. Continued improvements in manufacturing efficiency, target selection, and combination treatment strategies are anticipated to further establish monoclonal antibodies as central components of precision medicine and next-generation healthcare.

Keywords: Monoclonal Antibodies; Antibody Engineering; Therapeutic Antibodies; Precision Medicine; Antibody-Drug Conjugates; Immunotherapy

Abbreviations: mAbs: Monoclonal Antibodies; ADC: Antibody-drug conjugates; ADCC: Antibody-Dependent Cellular Cytotoxicity; CDC: Complement-Dependent Cytotoxicity; VH: Variable Domain; VL: One Variable CL: One Constant; FcRn: Fc receptor; CDR: Complementarity-Determining Regions; HAMA: Human Anti-Mouse Antibodies; mAb: Monoclonal Antibody; CHO: Chinese Hamster Ovary; ADA: Anti-Drug Antibodies

Introduction

The remarkable progress in molecular biology, immunology, biotechnology, and genetic engineering has fundamentally transformed modern medicine. Among the most important achievements of this progress is the development of monoclonal antibodies (mAbs), which have become one of the most successful classes of biological therapeutics. Monoclonal antibodies are immunoglobulins derived from a single B-cell clone and recognize specific antigens or epitopes with high affinity and selectivity. This target specificity enables selective modulation of disease-associated pathways and can reduce unwanted effects compared with less targeted therapies. In addition to their therapeutic applications, mAbs are widely used in disease diagnosis, biomarker detection, and biomedical research [1,2]. The scientific basis of antibody therapy originates from the ability of the adaptive immune system to recognize specific molecular structures. B lymphocytes produce antibodies that bind to antigens through their variable regions, whereas the constant region contributes to their biological and immune effector functions. Natural immune responses usually generate polyclonal antibodies, which originate from different B-cell clones and recognize multiple epitopes. Although this diversity is beneficial for host defense, it creates challenges in terms of specificity, reproducibility, and standardized production. A major breakthrough

was achieved in 1975, when Georges Köhler and César Milstein developed hybridoma technology by fusing antibody-producing B cells with immortal myeloma cells. This approach enabled the continuous production of homogeneous antibodies with predefined specificity and established the foundation of modern monoclonal antibody technology [3]. The importance of this discovery was recognized by the 1984 Nobel Prize in Physiology or Medicine, awarded to Köhler, Milstein, and Niels K. Jerne [1]. Early therapeutic mAbs were mainly murine and showed important limitations, particularly immunogenicity and the development of human anti-mouse antibody responses following repeated administration. These limitations encouraged the development of chimeric, humanized, and fully human antibodies, which reduced the amount of non-human antibody sequence and improved clinical tolerability [1,4]. Recombinant DNA technology and antibody engineering subsequently enabled the development of sophisticated discovery platforms, including phage display, transgenic animals, single B-cell cloning, and next-generation sequencing. These approaches facilitate the identification and optimization of antibodies with improved affinity, specificity, stability, pharmacokinetic properties, and manufacturability [1,5]. Structural biology, computational modeling, and artificial intelligence are now further supporting antibody discovery and rational molecular optimization [1]. Monoclonal antibodies have become important treatments for a broad spectrum of diseases, including cancer, autoimmune and inflammatory disorders, infectious diseases, neurological and ophthalmic conditions, cardiovascular and metabolic diseases, and rare disorders [1,2]. In oncology, mAbs can target tumor-associated antigens, growth-factor receptors, angiogenic pathways, or immune-checkpoint molecules, thereby inhibiting tumor growth or enhancing antitumor immune responses. In autoimmune and inflammatory diseases, antibodies can selectively neutralize cytokines or block receptors and signaling pathways involved in pathological inflammation [6]. Their molecular specificity also makes mAbs particularly suitable for precision medicine, in which biomarker-based patient selection can improve therapeutic efficacy and reduce exposure to ineffective treatments [1].

Antibody engineering has expanded the therapeutic potential of mAbs beyond conventional monospecific antibodies. Antibody-drug conjugates (ADCs) combine antibody-mediated targeting with potent cytotoxic payloads, enabling selective delivery of therapeutic agents to target-expressing cells [7]. Bispecific antibodies can simultaneously recognize two different antigens or epitopes and may recruit immune cells, inhibit multiple signaling pathways, or provide other mechanisms that cannot be achieved with conventional antibodies [8]. Fc engineering can also modify effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), while antibody fragments, nanobodies, and multispecific constructs further expand the versatility of antibody-based therapies [1,6]. The COVID-19 pandemic demonstrated the adaptability of monoclonal antibody technology in emerging infectious diseases. Neutralizing antibodies targeting the SARS-CoV-2 spike protein were rapidly developed and evaluated, demonstrating the potential of mAbs as an emergency therapeutic platform. However, viral evolution and mutations in antibody-binding regions also reduced the effectiveness of some antibodies, highlighting the importance of resistance monitoring and broadly neutralizing strategies [9].

Despite their clinical success, mAbs still face challenges including high manufacturing costs, complex production processes, limited tissue penetration, immunogenicity, target heterogeneity, resistance, pharmacokinetic limitations, and accessibility [1,7]. Future advances in artificial intelligence, synthetic biology, protein engineering, continuous bioprocessing, and novel antibody formats are expected to address these limitations and further improve therapeutic efficacy and manufacturability. Overall, monoclonal antibodies have evolved from a pioneering immunological technology into a central platform of modern medicine. Their integration with genomics, structural biology, computational biology, biotechnology, and precision medicine is expected to accelerate the development of increasingly specific, effective, and personalized therapies. As antibody engineering continues to advance, mAbs are likely to remain a cornerstone of next-generation therapeutics and contribute substantially to the diagnosis, prevention, and treatment of a wide range of diseases [1].

Structure and Classification of Monoclonal Antibodies

Monoclonal antibodies (mAbs) are highly specific immunoglobulin molecules produced from a single B-cell clone or its descendants and directed against a defined antigenic determinant. Their ability to selectively recognize molecular targets has made them valuable therapeutic agents in oncology, autoimmune diseases, inflammatory disorders, infectious diseases, and several other clinical fields. [10] The therapeutic potential of mAbs is closely related to their molecular architecture because different structural regions determine antigen recognition, interaction with immune effector systems, pharmacokinetic behavior, and overall biological activity. [11,12] Although antibody engineering has expanded the range of available molecular formats, conventional full-length IgG molecules remain the predominant framework for many therapeutic applications [11]. The development of therapeutic mAbs has progressed substantially since the introduction of hybridoma technology. Early antibodies were predominantly obtained from murine sources and provided an important foundation for antigen-specific targeting. [10] However, their extensive non-human sequence content created immunological and pharmacological limitations when they were administered repeatedly to patients. [13] Subsequent advances in recombinant DNA technology enabled the generation of chimeric, humanized, and fully human antibodies, progressively reducing the proportion of non-human sequences while retaining antigen-binding activity. [13,14] Thus, the classification of therapeutic mAbs according to their species origin also reflects the historical development of antibody engineering and the continuous effort to improve clinical tolerability [13,14].

Antibody Structure

A conventional antibody consists of two identical heavy chains and two identical light chains, which are stabilized by disulfide bonds and non-covalent interactions to form the characteristic Y-shaped structure. In IgG antibodies, the heavy chain contains one variable domain (VH) and three constant domains (CH1, CH2, and CH3), whereas each light chain contains one variable (VL) and one constant (CL) domain. The VH and VL domains form the antigen-binding site, while the constant regions provide structural stability and mediate biological functions. [15] Structurally and functionally, an antibody can be divided into the Fab and Fc regions. The Fab arms contain the variable domains responsible for antigen recognition, whereas the Fc region interacts with immune-system components and contributes to effector functions. Antigen specificity is mainly determined by six complementarity-determining regions (CDRs), three from each variable chain. Although CDRs directly form much of the antigen-binding surface, their structure is also influenced by surrounding framework regions. Therefore, modifications outside the CDRs may affect antibody affinity and specificity, an important consideration in antibody engineering and humanization. [15,16] The hinge region connects the Fab and Fc portions and provides flexibility, allowing the antigen-binding arms to adopt different orientations. The Fc region can interact with Fc receptors and activate complement, thereby enabling mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). [17] In addition, Fc interaction with the neonatal Fc receptor (FcRn) protects IgG from intracellular degradation and contributes to its long circulation time. Consequently, Fc engineering can be used to modify effector activity and pharmacokinetic properties. [18,19] Another important feature is Fc glycosylation. The IgG Fc region contains an N-linked glycosylation site, and variations in glycan composition can influence Fc-receptor interactions and immune effector functions. [20,21] Thus, antibody properties depend not only on amino acid sequence but also on post-translational modifications and higher-order structure. Overall, the modular organization of antibodies enables target-specific recognition to be combined with immune effector functions and pharmacokinetic regulation, making the immunoglobulin framework highly adaptable for therapeutic antibody development [17,19,21].

Types of Monoclonal Antibodies

Therapeutic monoclonal antibodies can be categorized according to the proportion and origin of their human and non-human immunoglobulin sequences. Four major categories are traditionally recognized: murine, chimeric, humanized, and fully human antibodies. [22] This classification represents a gradual transition from antibodies containing predominantly animal-derived sequences toward molecules based entirely on human immunoglobulin repertoires. [22,23] Importantly, these categories should not be interpreted as a simple hierarchy of therapeutic quality. The clinical performance of an individual antibody depends on multiple factors, including target biology, affinity, molecular stability, Fc activity, pharmacokinetics, immunogenicity, and manufacturing characteristics [22,24].

Murine Monoclonal Antibodies: Murine monoclonal antibodies are antibodies whose immunoglobulin sequences are derived predominantly or entirely from mice. They became particularly important after the development of hybridoma technology, which enabled the generation of antibody-producing cell lines with predefined antigen specificity. The hybridoma approach represented a major milestone in antibody research because it provided a reliable method for producing large quantities of monoclonal antibodies with consistent target recognition. [23] Despite their high specificity, murine antibodies have significant limitations when used as therapeutic agents in humans. The extensive presence of mouse-derived protein sequences can cause the human immune system to recognize the administered antibody as foreign. Repeated administration may consequently stimulate the production of human anti-mouse antibodies (HAMAs), which can accelerate drug clearance, reduce exposure, and potentially interfere with therapeutic efficacy. [25,26] In addition, immune reactions against murine antibodies may contribute to adverse reactions in susceptible individuals. [25] The limitations of murine antibodies stimulated the development of molecular engineering approaches designed to preserve antigen specificity while decreasing the amount of foreign protein sequence. Rather than abandoning the antigen-binding properties established in murine antibodies, researchers progressively replaced selected portions of the molecule with human immunoglobulin sequences. This strategy ultimately resulted in the development of chimeric and subsequently humanized antibodies [23,27].

Chimeric Monoclonal Antibodies: Chimeric monoclonal antibodies represent an intermediate stage between fully murine and more extensively human-derived antibodies. In a conventional chimeric antibody, the variable regions of the heavy and light chains are derived from a non-human antibody, most commonly a murine antibody, whereas the constant regions are replaced with corresponding human sequences. This design preserves the antigen-recognition properties of the original antibody while substantially increasing the proportion of human sequence within the molecule. [27,28] The incorporation of human constant regions provides several potential advantages. In particular, the human Fc region can interact more appropriately with human Fc receptors and other components of the immune system. Chimeric antibodies can therefore display improved pharmacological characteristics compared with their fully murine counterparts. [28] Nevertheless, the entire variable region remains non-human, meaning that a substantial source of immunogenicity is retained. As a result, chimeric antibodies can still induce anti-drug antibody responses, particularly following repeated exposure. [22,27] Chimerization was therefore an important technological advance rather than a complete solution to antibody immunogenicity. It demonstrated that antibody structure could be divided into functional modules and that replacing specific regions with human sequences could improve compatibility

with the human host without necessarily eliminating antigen-binding activity. This principle subsequently provided the basis for more extensive humanization strategies [27,28].

Humanized Monoclonal Antibodies: Humanized monoclonal antibodies were developed to further reduce the amount of non-human sequence present in therapeutic antibodies. A commonly used humanization strategy involves transferring the CDRs responsible for antigen recognition from a murine antibody onto a predominantly human variable-region framework. The resulting molecule retains the antigen-binding determinants of the original antibody while replacing most of the surrounding non-human sequence with human counterparts. [29,30] Humanization is considerably more sophisticated than simply replacing large sections of an antibody. The CDRs do not function independently of the framework regions. Their spatial configuration is influenced by neighboring framework residues, and alterations in these supporting regions may change the geometry of the antigen-binding site. Consequently, an inappropriate human framework can result in reduced affinity, altered specificity, or loss of biological activity. [31,32] Successful humanization therefore requires careful selection of the human framework and, in some cases, retention of selected non-human framework residues that are structurally important for maintaining the original binding characteristics. [31,32] The major advantage of humanized antibodies is their substantially increased similarity to human immunoglobulins compared with murine and conventional chimeric molecules.

This reduction in non-human sequence generally decreases the likelihood of clinically relevant immune recognition. [29,33] However, humanization should not be regarded as synonymous with complete absence of immunogenicity. Residual non-human sequences, particularly within antigen-binding regions, can potentially contribute to immune recognition. [33] Furthermore, antibody immunogenicity is influenced by factors beyond species origin, including protein aggregation, formulation, dosing regimen, structural characteristics, and individual patient-related factors [34]. Thus, the principal objective of humanization is not merely to make an antibody appear more human at the sequence level, but to achieve an appropriate balance between maintaining the desired biological activity and minimizing unnecessary foreign sequence. This balance is particularly challenging because the regions that are most important for antigen recognition are also among the regions that may retain non-human sequence information [31-33].

Fully Human Monoclonal Antibodies: Fully human monoclonal antibodies contain human-derived immunoglobulin sequences throughout their variable and constant regions. Their development represents a further step in the effort to minimize sequence-related immunogenicity while maintaining high antigen specificity. Unlike humanized antibodies, in which selected antigen-binding regions may originate from a non-human antibody, fully human antibodies can be generated from human antibody repertoires or technologies designed to reproduce human immunoglobulin diversity. [35,36] Examples of fully human monoclonal antibodies include adalimumab, golimumab, panitumumab, secukinumab, and denosumab (e.g., adalimumab, Humira; golimumab, Simponi; panitumumab, Vectibix; secukinumab, Cosentyx; denosumab, Prolia). [36-40] Several technological platforms have enabled the generation of fully human antibodies. These include phage-display libraries containing human antibody sequences, transgenic animals carrying human immunoglobulin loci, and approaches based on the isolation and characterization of individual human B cells.

Each platform provides access to different aspects of antibody diversity. [35,36] Phage display, for example, permits the selection of antibodies from large recombinant libraries (e.g., adalimumab), whereas transgenic animals can generate antigen-specific antibodies following an immune response within a human immunoglobulin repertoire. [35,36] Single B-cell approaches can directly recover antibodies produced by individual human B cells and have become particularly useful for identifying naturally occurring antigen-specific responses. [41] The fully human format offers an important theoretical advantage because it eliminates the need to introduce murine variable-region sequences into the therapeutic molecule. Nevertheless, the term "fully human" should not be interpreted as meaning that immunogenicity is impossible. Even antibodies composed exclusively of human sequences may induce anti-drug antibody responses. [42,43] This can occur because the therapeutic antibody represents a particular molecular sequence and structural configuration that may not be fully represented in every individual's endogenous immune repertoire. In addition, aggregation, post-translational modifications, formulation, impurities, and other product-related characteristics can influence immunogenicity. [43,44] Therefore, the distinction between humanized and fully human antibodies is useful for describing molecular origin but does not, by itself, predict the complete immunogenicity profile of a therapeutic antibody. Indeed, clinical immunogenicity varies considerably among individual products.

A fully human antibody may still generate anti-drug antibodies, whereas a well-characterized humanized antibody may demonstrate an acceptable safety and efficacy profile over prolonged treatment. [42-44] For example, ustekinumab (Stelara), pembrolizumab (Keytruda), and tocilizumab (Actemra) are humanized antibodies, whereas adalimumab (Humira), golimumab (Simponi), nivolumab (Opdivo), and ustekinumab (Stelara) are fully human antibodies. [39,45-47] Consequently, immunogenicity must be evaluated experimentally rather than inferred solely from the antibody classification. [42-44] The progression from murine to chimeric, humanized, and fully human antibodies illustrates the broader evolution of therapeutic antibody engineering. Early development focused primarily on achieving highly specific antigen recognition, whereas subsequent generations increasingly emphasized compatibility with the human immune system. Modern antibody discovery and engineering now integrate these objectives with additional considerations such as affinity optimiza-

tion, Fc engineering, molecular stability, pharmacokinetics, tissue distribution, and manufacturability [35,44,48].

In summary, the four traditional antibody categories represent different molecular strategies for balancing antigen specificity with host compatibility. Murine antibodies (e.g., muromonab-CD3/OKT3) provided the foundation for monoclonal antibody technology but were limited by their high immunogenic potential in humans. Chimeric antibodies (e.g., rituximab and infliximab) introduced human constant regions while retaining non-human variable regions, thereby improving compatibility but leaving substantial foreign sequence. Humanized antibodies (e.g., pembrolizumab and tocilizumab) further reduced non-human content by incorporating selected antigen-binding regions into human antibody frameworks. Fully human antibodies (e.g., adalimumab, golimumab, panitumumab, secukinumab, and denosumab) extended this approach by employing human-derived sequences throughout the antibody molecule. [35,36,39,45-48] Importantly, the evolution of these formats should be understood as a progression in antibody engineering rather than a rigid ranking of therapeutic performance. The optimal molecular format ultimately depends on the biological mechanism of action and the specific pharmacological and clinical requirements of each therapeutic application [35,44,48].

Production Technologies

The development of monoclonal antibody (mAb) technologies has progressed considerably from conventional hybridoma-based methods to sophisticated platforms that enable rapid antibody discovery, molecular optimization, and large-scale recombinant production. Each technological approach provides distinct advantages in terms of antibody diversity, specificity, production efficiency, and the ability to generate fully human therapeutic candidates. Classical hybridoma technology established the foundation for monoclonal antibody production, whereas recombinant DNA methods subsequently enabled extensive molecular manipulation of antibody molecules. More recently, phage display, transgenic animal platforms, and single B-cell technologies have expanded the available strategies for identifying antibodies with desirable biological and therapeutic properties. [49-51] These approaches can also be combined with high-throughput screening, next-generation sequencing, and computational analysis to improve the efficiency of antibody discovery and development [50,51].

Hybridoma Technology

Hybridoma technology represents one of the most important milestones in the history of monoclonal antibody development. The method is based on combining the antigen-recognition capacity of antibody-producing B lymphocytes with the long-term proliferative characteristics of immortalized myeloma cells. [49,52] In a conventional hybridoma workflow, an experimental animal is first immunized with the antigen of interest. Following the development of an immune response, antibody-producing B cells are isolated, typically from lymphoid tissues such as the spleen, and fused with suitable myeloma cells. The resulting hybrid cells, known as hybridomas, are capable of continuous growth while retaining the ability to produce a specific antibody. [49,52]. Following cell fusion, the resulting population contains a mixture of different cell types and antibody-producing clones. Selective culture conditions are therefore used to enrich hybridoma cells, after which individual clones are screened for the desired antigen-binding activity. Positive clones can subsequently be subcloned to establish monoclonal populations and expanded for antibody production. [49,52] This process provides a relatively well-defined route for obtaining antibodies with a single antigenic specificity [52].

A major advantage of hybridoma technology is its ability to generate stable antibody-producing cell lines. Once a suitable clone has been established, the corresponding antibody can be produced repeatedly under controlled culture conditions. The technology has therefore played a central role in the development of numerous research, diagnostic, and therapeutic antibodies. [49,53] Nevertheless, conventional hybridoma technology has several limitations. Because the initial immune response is generally generated in an animal, the resulting antibodies may contain non-human sequences and can potentially induce unwanted immune responses when administered to humans. In addition, the identification of rare antibody-producing clones can be laborious, and the efficiency of cell fusion and subsequent screening may vary considerably. [49,54] The emergence of recombinant antibody engineering has reduced some of these limitations by allowing antibodies initially obtained through hybridoma technology to be further modified. Variable regions derived from murine antibodies can, for example, be incorporated into human antibody frameworks to generate chimeric or humanized molecules. Consequently, hybridoma technology remains relevant not only as a production method in its own right but also as an important source of antibody sequences for subsequent molecular engineering [50,55].

Recombinant DNA Technology

Recombinant DNA technology has fundamentally changed the production and engineering of monoclonal antibodies by enabling direct manipulation of the genes encoding antibody molecules. Instead of relying exclusively on intact antibody-producing cells, the genetic sequences encoding the heavy and light chains can be isolated, modified, and introduced into appropriate expression systems. This approach provides considerably greater control over antibody structure and allows the production of molecules with tailored characteristics. [56] One of the major applications of recombinant DNA technology is the generation of chimeric and humanized antibodies. In chimeric antibodies, antigen-binding regions originating from a non-human antibody are combined with human constant regions. [27] Humanization can further reduce the non-human component by incorporating selected antibody-binding regions into predominantly human antibody

frameworks. [29,30] These strategies have contributed substantially to reducing the immunogenicity associated with earlier murine antibodies and have facilitated the development of antibodies suitable for repeated administration in humans. [22,33] Recombinant expression systems also provide opportunities to modify antibody characteristics at the molecular level. Antibody sequences can be engineered to alter binding affinity, specificity, stability, Fc-mediated effector functions, and pharmacokinetic behavior. [11,18,19] More complex formats, including antibody fragments, bispecific antibodies, antibody–drug conjugates, and other multispecific molecules, can likewise be generated using recombinant approaches [7,8,56].

For large-scale production, mammalian expression systems are particularly important because they are capable of producing complex proteins with appropriate folding, assembly, and post-translational modifications. [57] Chinese hamster ovary (CHO) cells are among the most widely used production hosts for therapeutic antibodies. Their extensive use is associated with their established manufacturing performance, adaptability to large-scale culture, and ability to support the production of structurally complex glycoproteins. [58,59] Despite these advantages, recombinant antibody production involves several technical challenges. The establishment of stable and highly productive cell lines, control of product heterogeneity, maintenance of appropriate glycosylation patterns, and consistency between production batches are critical considerations. [58,59] Furthermore, optimization of upstream cell culture and downstream purification processes is required to achieve high yields while maintaining product quality. [58] Thus, recombinant DNA technology has evolved beyond simple antibody expression and now represents a central platform for both antibody engineering and industrial manufacturing [56,58].

Transgenic Animal Platforms

Transgenic animal platforms provide an alternative strategy for generating human antibodies by combining the natural processes of antibody diversification with genetically modified immune systems. In these models, human immunoglobulin gene segments are introduced into the animal genome, while endogenous immunoglobulin loci may be modified or functionally suppressed. Following immunization, the animal can therefore generate antibodies that contain human or predominantly human antibody sequences. [36,60] The major rationale for this approach is to obtain antibodies with human molecular characteristics directly through an in vivo immune response. In contrast to conventional murine hybridoma technology, transgenic animals can generate antibody repertoires that are more compatible with subsequent therapeutic use in humans. The antibodies produced through these systems can undergo natural processes such as somatic hypermutation and affinity maturation, potentially resulting in high-affinity interactions with the target antigen. [36,61,62] Transgenic mouse platforms have been particularly important in the development of fully human monoclonal antibodies. These models provide the biological advantages of conventional immunization while reducing the need for extensive conversion of non-human antibody sequences into human-compatible formats.

Following immunization, antibody-producing cells can be isolated and used to identify candidate antibodies, including through hybridoma-based or recombinant approaches. [60,61,63] The use of transgenic animals is not limited to mice. Other animal systems have also been investigated as platforms for generating antibodies with human or human-like sequences. Differences in immune repertoire, antibody diversification, and physiological characteristics among animal species may provide opportunities to address targets that are challenging in conventional models. [62] Despite their potential, transgenic animal platforms require sophisticated genetic engineering and specialized animal production systems. Establishing appropriate immunoglobulin loci and ensuring their effective expression and diversification can be technically demanding. In addition, the generation and maintenance of transgenic animal colonies can be associated with considerable time and financial requirements. Nevertheless, these platforms remain valuable for therapeutic antibody discovery because they can combine the diversity of an in vivo immune response with the molecular characteristics required for human therapeutic applications [60,62,63].

Single B-Cell Technologies

Single B-cell technologies have emerged as powerful approaches for identifying antibodies directly from individual antigen-specific B lymphocytes. Unlike conventional hybridoma methods, these approaches do not necessarily require the immortalization of B cells. Instead, individual B cells are identified and isolated according to their antigen specificity or other phenotypic characteristics, after which the antibody genes expressed by each cell are recovered and characterized. [60,61] A typical single B-cell workflow begins with the identification of an appropriate B-cell population from an immunized animal or a human donor. Antigen-specific cells can be enriched or identified using techniques such as flow cytometry. Individual cells are then isolated, and the variable regions of their immunoglobulin heavy- and light-chain genes are amplified. Importantly, because both chains originate from the same individual B cell, their natural pairing can be retained. The recovered sequences can subsequently be cloned into recombinant expression systems and evaluated for antigen binding, affinity, specificity, and biological activity. [60,61] The preservation of native heavy- and light-chain pairing represents one of the principal advantages of single B-cell technologies. Antibody function depends on the structural interaction between these two chains, and maintaining their natural combination can facilitate the recovery of antibodies that have already undergone biological selection within the immune system. This feature can be particularly valuable for identifying rare antibodies with high affinity or specialized functional properties. [60,62] Single B-cell approaches are also compatible with modern high-throughput technologies.

Advances in single-cell sequencing, microfluidics, multiplexed screening, and bioinformatic analysis have substantially increased the number of individual B cells that can be investigated. Consequently, large portions of an individual's antibody repertoire can potentially be analyzed at high resolution. This has made single B-cell technologies particularly attractive for studying naturally occurring human antibody responses and for discovering antibodies against infectious agents, cancer-associated targets, and other disease-related molecules. [61,62] Nevertheless, several limitations should be considered. The success of the approach depends on the availability and accurate identification of antigen-specific B cells. Rare B-cell populations may be difficult to isolate, and the recovery of complete antibody sequences from individual cells can require highly efficient molecular workflows. In addition, the functional characterization of large numbers of recovered antibodies can become a bottleneck when screening capacity is limited. [60,61] Taken together, single B-cell technologies represent a major transition toward direct interrogation of the natural antibody repertoire. Their combination with single-cell sequencing and high-throughput functional screening has the potential to accelerate the identification of therapeutically relevant antibodies while providing detailed information about antibody diversity and immune responses [61,62].

Comparative Perspective of Antibody Production Technologies

The major antibody production and discovery platforms differ in their biological principles, technical requirements, and potential applications. Hybridoma technology provides a robust and well-established route for generating stable monoclonal antibody-producing clones, whereas recombinant DNA technology offers extensive control over antibody sequence, structure, and production. [51,56] Phage display enables large antibody libraries to be screened entirely in vitro, making it particularly suitable for recombinant antibody discovery and engineering. [5] Transgenic animal platforms exploit physiological immune mechanisms while enabling the generation of human antibody sequences. [36,60] In contrast, single B-cell technologies provide direct access to naturally selected antibody sequences while preserving native heavy- and light-chain pairing. [61] Rather than replacing one another, these technologies increasingly function as complementary components of modern antibody development. For example, antibodies identified through immunization or single B-cell screening can subsequently be engineered using recombinant DNA methods, while phage display can be employed to improve affinity or explore additional sequence variants. [5,56,61] The integration of these platforms with next-generation sequencing, computational antibody design, artificial intelligence, and high-throughput functional screening is expected to further increase the speed and precision of mAb discovery. [63] Consequently, the future of antibody production is likely to rely on integrated workflows in which antibody identification, molecular engineering, functional screening, and scalable manufacturing are closely interconnected [56,63].

Challenges and Limitations

Despite their remarkable clinical success, monoclonal antibodies (mAbs) face several limitations that can influence their efficacy, safety, accessibility, and clinical applicability. These challenges arise at different stages of the therapeutic development process, ranging from antibody discovery and manufacturing to pharmacokinetics and long-term treatment. [64-66] Addressing these limitations is essential for improving the therapeutic potential of current mAbs and facilitating the development of next-generation antibody-based medicines [64,65].

Immunogenicity and Safety

Immunogenicity remains one of the major challenges associated with therapeutic mAbs. Administration of a therapeutic antibody can induce the formation of anti-drug antibodies (ADAs), which may reduce drug exposure, alter pharmacokinetics, decrease therapeutic efficacy, or, in some cases, contribute to adverse reactions. [66,67] Although the transition from murine to chimeric, humanized, and fully human antibodies has substantially reduced sequence-related immunogenicity, human-derived antibodies can also induce immune responses. [33,66] Immunogenicity is influenced not only by antibody sequence but also by structural properties, aggregation, post-translational modifications, formulation, dose, treatment duration, and patient-specific factors. [67,68] Therefore, immunogenicity cannot be predicted solely from whether an antibody is classified as humanized or fully human. [66,67] Safety considerations extend beyond immunogenicity. Depending on their target and mechanism of action, mAbs may interfere with physiological signaling pathways or induce unintended immune activation. Fc-mediated effector functions can also contribute to therapeutic activity as well as treatment-associated toxicity. [11,69] Consequently, the balance between target-specific activity and off-target or excessive immune effects must be carefully evaluated during antibody development [69].

Manufacturing Complexity and Cost

The production of therapeutic mAbs is technically demanding because these molecules require appropriate protein folding, assembly, post-translational modification, and glycosylation. [55,58] Mammalian expression systems, particularly Chinese hamster ovary (CHO) cells, are widely used because they can generate complex antibody molecules with appropriate structural characteristics. [57,58] However, large-scale mammalian cell culture requires sophisticated bioreactors, controlled culture conditions, extensive downstream purification, and rigorous quality control. These requirements contribute substantially to the complexity and cost of mAb manufacturing. [56,70] Product heterogeneity represents an additional manufacturing challenge. Variations in glycosylation, charge, oxidation, aggregation, and other structural characteristics can influence antibody stability, pharmacokinetics, immunogenicity, and biological activity. [58,71] Maintaining consistent critical quality attributes between

production batches is therefore essential for ensuring product safety and efficacy. [72] The high cost associated with development and manufacturing can ultimately affect patient access. As demand for antibody-based therapies continues to increase, improving production efficiency while maintaining product quality has become an important objective. Continuous manufacturing, improved cell-line engineering, process analytical technologies, and optimized purification strategies are being investigated as potential approaches to reduce production time and cost [70,73,74].

Pharmacokinetic and Tissue-Distribution Limitation

The large molecular size of conventional mAbs strongly influences their pharmacokinetic behavior. Although FcRn-mediated recycling contributes to the relatively long systemic half-life of IgG antibodies, their size can restrict diffusion across biological barriers and limit penetration into certain tissues. This is particularly relevant for tumors, dense extracellular matrices, and tissues protected by specialized barriers such as the blood–brain barrier. [74,75] Limited tissue penetration may result in an uneven distribution of the therapeutic antibody within the target tissue. In solid tumors, for example, heterogeneous vascularization, high interstitial pressure, and dense extracellular structures can restrict antibody movement and create concentration gradients. Consequently, high circulating concentrations do not necessarily guarantee equivalent exposure at the site of disease. [76,77] These limitations have encouraged the development of antibody fragments, engineered Fc regions, alternative antibody formats, and targeted delivery systems designed to improve tissue distribution [78].

Resistance and Loss of Therapeutic Response

Resistance to mAb therapy can develop through several mechanisms. Tumor cells or other target cells may acquire mutations that alter the antibody-binding epitope, reduce target expression, or activate alternative signaling pathways. In cancer therapy, biological heterogeneity within a tumor can further contribute to treatment failure because not all malignant cells necessarily depend on the same molecular pathway. As a result, selective pressure exerted by treatment may favor populations that are less sensitive to the antibody. [79,80] Loss of response may also occur through mechanisms that do not involve direct alteration of the antibody target. Changes in downstream signaling, activation of compensatory pathways, altered target internalization, or changes in the tumor microenvironment can reduce therapeutic effectiveness. [79,80] In addition, the development of ADAs may increase drug clearance and reduce effective exposure in some patients. [67] These mechanisms highlight the importance of understanding disease biology and patient heterogeneity when designing long-term mAb treatment strategies.

Stability, Aggregation, and Formulation Challenges

Protein stability is another important consideration during the development and storage of therapeutic mAbs. Antibodies can undergo aggregation, fragmentation, oxidation, deamidation, or other structural changes during production, purification, formulation, transport, and storage. Such changes may decrease biological activity and potentially increase immunogenicity. [68,81,82] High-concentration formulations can be particularly challenging because increased protein concentration may promote self-association and increase viscosity. This can complicate subcutaneous administration, which is increasingly desirable because it can facilitate outpatient treatment and improve patient convenience. Consequently, formulation development must achieve a balance between sufficient antibody concentration, physical stability, acceptable viscosity, and long-term product quality. [83,84] Taken together, these limitations demonstrate that the successful development of a therapeutic mAb requires optimization beyond antigen-binding affinity alone. Immunogenicity, safety, manufacturability, pharmacokinetics, tissue distribution, stability, and resistance must be considered as interconnected aspects of antibody development [63,67,74,80,81].

Emerging Trends and Future Perspectives

The future of monoclonal antibody therapeutics is increasingly characterized by the integration of molecular engineering, advanced screening technologies, computational approaches, and innovative manufacturing strategies. Rather than focusing exclusively on conventional full-length antibodies, current research is expanding toward multifunctional molecules with improved specificity, pharmacokinetics, tissue penetration, and therapeutic activity. [1,5,8,85] One important direction is the development of bispecific and multispecific antibodies. Unlike conventional mAbs, which generally recognize a single target, these engineered molecules can simultaneously interact with two or more antigens or epitopes. This property can be exploited to recruit immune cells, block complementary signaling pathways, or combine different therapeutic mechanisms within a single molecule. Such approaches may be particularly valuable in oncology and diseases characterized by complex or redundant biological pathways. [8] Another rapidly developing area is the use of antibody–drug conjugates (ADCs) and other antibody-based targeted delivery systems. ADCs combine the target specificity of an antibody with a potent therapeutic payload, allowing selective delivery of cytotoxic or pharmacologically active molecules to target cells. Continued advances in linker chemistry, payload design, and antibody engineering are expected to improve the therapeutic index of these platforms.

Similarly, antibody-based delivery systems are being explored for transporting therapeutic molecules to tissues that are difficult to reach with conventional antibodies [7,78]. Fc engineering and glycoengineering represent additional strategies for optimizing therapeutic antibodies. Modification of Fc interactions can be used to enhance or attenuate immune effector functions depending on the intended mechanism of action. Altering Fc glycosylation may also influence interactions with Fc receptors and consequently modify antibody activity. Such approaches provide opportunities to tailor an antibody's

biological function without changing its antigen-binding specificity. [69,86] Advances in artificial intelligence (AI), machine learning (ML), and computational protein design are also transforming antibody discovery. Computational approaches can assist in predicting antigen–antibody interactions, identifying promising antibody sequences, optimizing affinity, assessing developability, and estimating potential immunogenicity. Integration of these approaches with next-generation sequencing and high-throughput experimental screening may substantially reduce the time required to identify and optimize therapeutic candidates.

Recent developments suggest that AI-assisted antibody engineering is increasingly moving from theoretical modeling toward practical applications in candidate selection and molecular optimization. [85,87,88] Another important future direction is the improvement of manufacturing technologies. Continuous processing, advanced process analytical technologies, automated monitoring, and data-driven process control may improve production efficiency and reduce variability. Machine learning-based monitoring of cell culture and manufacturing parameters may further facilitate real-time optimization of production processes. These technologies could contribute to reducing manufacturing costs while maintaining stringent quality requirements. [72,89] Future antibody development is also expected to place greater emphasis on patient-specific treatment strategies. Differences in disease biology, target expression, immune status, pharmacokinetics, and genetic background can influence therapeutic response. Combining biomarkers with antibody-based treatment may therefore improve patient selection and enable more individualized therapeutic approaches. In oncology, for example, molecular characterization of tumors can help identify patients who are most likely to benefit from a particular antibody or antibody-based combination. [90,91] Overall, the next generation of mAb therapeutics is likely to move beyond the concept of a single highly specific antibody toward precisely engineered, multifunctional, and data-guided therapeutic systems. The combination of antibody engineering, computational biology, advanced manufacturing, and biomarker-driven clinical development may allow future antibody therapeutics to overcome several limitations associated with conventional mAbs [1,8,85,89,90].

Conclusion

Monoclonal antibodies have become an important component of modern medicine because of their ability to selectively recognize and modulate disease-associated molecular targets. The development of antibody technology has progressed from early murine antibodies to chimeric, humanized, and fully human molecules, accompanied by major advances in antibody discovery, recombinant production, and molecular engineering. These developments have substantially improved the clinical applicability and versatility of mAb-based therapies. Nevertheless, several challenges continue to limit the broader potential of these therapeutics. Immunogenicity, treatment-related adverse effects, complex and costly manufacturing processes, pharmacokinetic limitations, restricted tissue penetration, molecular instability, and the emergence of resistance remain important considerations. In addition, the increasing complexity of engineered antibody formats introduces new challenges related to product characterization, manufacturing consistency, and regulatory evaluation. Future progress will depend on the ability to integrate advances in antibody engineering with computational technologies, high-throughput screening, novel delivery strategies, and improved manufacturing platforms. Bispecific and multispecific antibodies, antibody–drug conjugates, Fc-engineered molecules, and AI-assisted antibody discovery represent promising approaches for expanding the therapeutic capabilities of antibody-based medicines.

At the same time, improved control of product quality and manufacturing efficiency will be essential for making these therapies more accessible. In conclusion, the evolution of monoclonal antibody technology is moving from the development of highly specific molecules toward the design of multifunctional and increasingly optimized therapeutic systems. Continued integration of molecular biology, protein engineering, computational science, and clinical medicine is expected to further expand the range of diseases that can be targeted by antibody-based therapies. The future success of mAbs will therefore depend not only on discovering new targets, but also on developing safer, more effective, more stable, and economically sustainable antibody platforms.

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