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Therapeutic application of monoclonal antibodies: a review

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ABSTRACT

Introduction. Antibodies are an important class of proteins produced when a foreign entity elicits an immune response in the body. There are 5 major classes of antibodies, IgM, IgG, IgA, IgD, and IgE. The structure and immune function of immunoglobulins differ. An antibody contains two light and two heavy chains, which are linked by multiple disulphide bonds. Variable regions are found on light and heavy chains, known as the fragment antigen-binding (Fab) region, and a constant region, which is also known as the fragment crystallizable (Fc) region. Antibodies are, as a class, broad-spectrum antimicrobial agents with activity against all classes of pathogens. However, individual antibodies are usually pathogen-specific. Monoclonal antibodies, which specifically recognize one epitope of the cognate antigen, can be generated by using antibody engineering techniques such as hybridoma, phage display, and transgenic technologies. Removal of the entire constant region or part of the whole Fc portion generates antibody fragments such as Fab, scFv, and diabodies. Better tissue or tumor penetration characteristic of antibody fragments make them suited for the therapy overusing the whole antibody, hence most suited for therapy. Aim. Nowadays, it is possible to use antibodies for different therapeutic applications by modifying either their structural or functional properties.

Review Article

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INTRODUCTION

The introduction of a trail to treat infectious human diseases using animal antibodies dates back a century [1]. In the 1930s and 1940s antibiotics such as sulfonamides and penicillin replaced serum therapy. The decline of therapeutic horse and rabbit antisera is caused by the associated toxicity of serum sickness [2]. Polyclonal immunoglobulin and human antisera preparation were better tolerated; however, lot-to-lot variation, low content of specific antibodies and infectious agents such as HIV and hepatitis viruses are the limitations [3].

In the late 1970s and early 1980s, the discovery and diffusion of monoclonal antibody (MAb) technology become a new promising area of human therapeutics. MAbs could be selected with exquisite specificity. They function on various components of the immune system such as antibody-dependent cell cytotoxicity (ADCC) and complement, and they showed a high biological half-life in blood and tissues, rendering them effective for prophylactic use. Due to their biological nature, the toxicity of infused MAbs is expected to be low. Which enthusiastically hailed in press accounts at the time as the solution to cancer. This concept was proven by successful clinical results of mouse anti-idiotypic MAbs in the treatment of lymphomas and leukemias [1] and by FDA approval in 1986 of the OKT3 anti-CD3 mouse MAb for acute renal transplant rejection [4].

The adverse clinical and laboratory effects make the dark age of skepticism real. Therapy given by using rodent MAbs leads to the development of human anti-murine antibody (HAMA) response in more than 50% of treated patients. Also, the effector functions of mouse antibodies have proven to be less efficient in the human context. The human immunoglobulins half-life is longer than the mouse, the factor that limits usefulness. Therefore human MAbs are theoretical solutions despite human MAbs from hybridomas and lymphocyte cell lines have proven too difficult to generate easily [5].

In 1994 the spell was broken when the FDA approved the anti-platelet mouse MAbs ReoProÔ (Centocor) to treat the complications of angioplasty [6]. Then between 1997 and 1999, FDA approved another engineered

antibodies such as RituximabÔ, a mouse/human chimeric anti-CD20 antibody (IDEC/ Genentech, San Francisco, CA, USA) to combat follicular non-Hodgkin's B cell lymphoma [7]; ZenapaxÔ, a humanized anti-IL2 TAC receptor antibody develop by Protein Design Labs/Hoffmann- La Roche (Basel, Switzerland) to manage the acute rejection of kid [8], and HerceptinÔ, then Genentech developed the first antibody-based drug for adjuvant therapy of solid tumor (breast cancer) [9].

High target specificity and their organization into distinct structural and functional domains make antibodies preferable therapeutic candidates. When an antibody is designed as a drug, features including immunogenicity, affinity, stability, effector functions, half-life, and tissue penetration and distribution should be considered and optimized accordingly. Antibody engineering uses antibody characteristics domain and a variety of methods to develop a suited particular therapeutic use. Nineteen therapeutic antibodies have been approved by the FDA in clinical use and over 150 antibodies in clinical trials. MAbs and their recombinant derivatives are being evaluated for the prophylaxis, therapy, and control of allergic and autoimmune diseases; complications of angioplasty; sepsis; a variety of inflammatory diseases; many viral and bacterial infections; organ transplant rejection; and solid and hematological tumors [10]. Therefore, this review is organized to give insight on antibody engineering and its therapeutic application areas.

GENERAL FEATURES OF ANTIBODY

Antibody Structure

An antibody contains two light and two heavy chains, which are linked by multiple disulphide bonds (Figure 1). The light and heavy chains contain a variable region, also known as the Fab (fragment antigen binding) region, and a constant region, which is known as the Fc (fragment crystallizable) region. Also, short hypervariable amino acid sequences found in the variable domains of both light and heavy chains are known as antigen-binding complementarity determining regions (CDRs), the majority of the sequence variations associated with antibodies found in CRDs. Among the six CDRs in an IgG molecule, CDR3s have the greatest variability. In IgG, IgA, and IgD antibody isotypes, the Fc region (the tail region of antibody) is composed of two identical protein fragments, derived from the second and third constant domains of the antibody's two heavy chains. The Fc regions in IgM and IgE contain three heavy chain constant domains in each polypeptide chain (Figure 2). The IgG isotype is most commonly used in therapeutic applications [11].

Functions of antibody

Antibodies recognize specific antigens on target pathogens and have protective effects. Some of the protective effects are virus and toxin neutralization, binding of the antibody alone provides sufficient steric interference to disrupt the interaction between the cellular receptor and the antigen, thereby abrogating virus uptake and replication or intoxication. But other protective effects, such as complement activation or antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis activity lies in the bound antibody recruiting other components of the immune system. This occurs through Fc receptors (FcR) on the surface of immune cells to the Fc region of the antibody or the binding of complement proteins in the serum. Direct antifungal and *in vitro* antibacterial effect of antibody [12] and cell-mediated immune and inflammatory response regulative characteristics [13] were found recently (Figure 3).

Classification of antibodies

There are 5 major classes of antibodies, IgM, IgG, IgA, IgD, and IgE. These immunoglobulins differ in both their structure and immune function. IgG antibodies are monomer in structure and are the dominant form of immunoglobulin found in the serum. IgM is pentameric in structure and is clinically significant because they are predominant in early immune responses to most antigens. IgAs are polymeric and are the predominant immunoglobulin in saliva, tears, nasal mucosa, prostatic fluid, and many other bodily fluids. IgD antibodies are monomeric and trace amounts are present in serum and are found on the surface of human B lymphocytes. IgE antibodies are present in serum in a monomeric form and represent only a small fraction of total antibodies in the blood. They are involved in the production and release of vasoactive mediators e.g. histamine and other chemicals that cause an inflammatory reaction. In healthy adults, the four-polypeptide chain IgG monomer constitutes approximately 75% of the total serum immunoglobulins [14] (Figure 2).

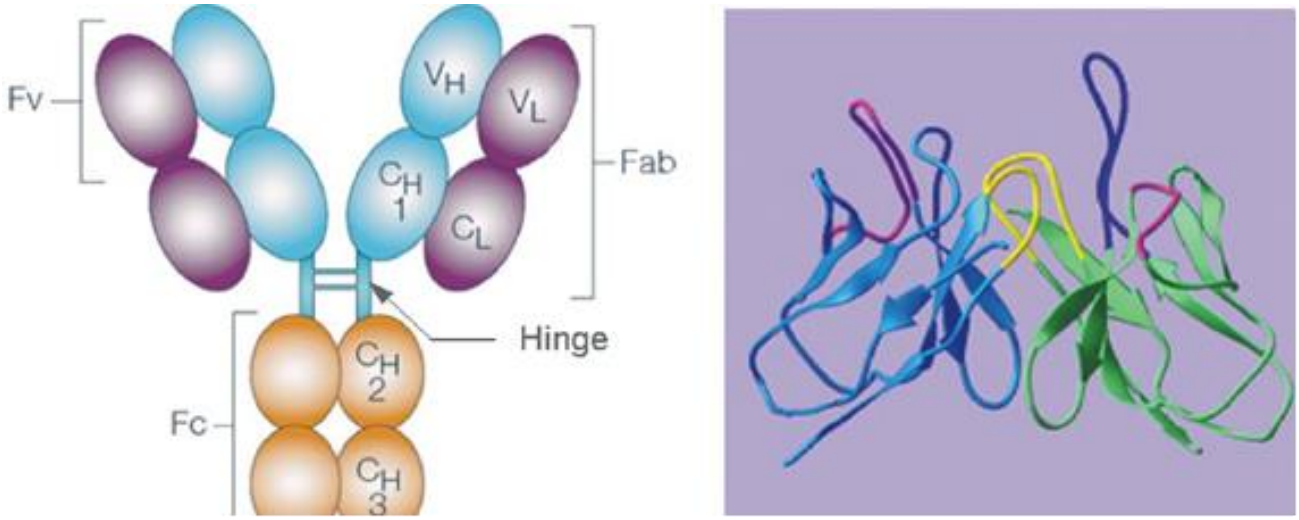

Figure 1. The modular structure of antibody molecule (A) and the CDR1, 2, and 3 within the heavy and light chain variable domains (B); [10].

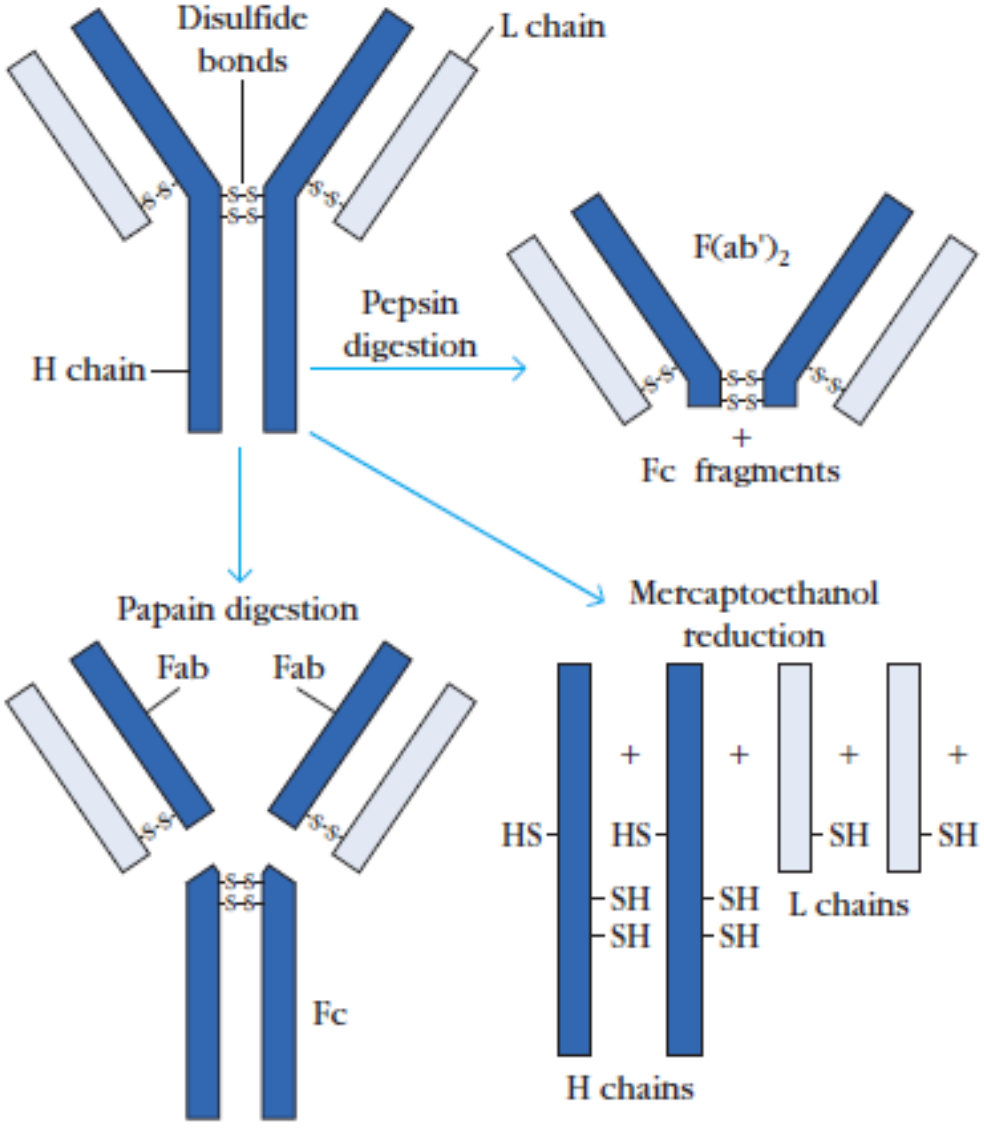

Figure 2. Prototype structure of IgG, showing chain structure, and interchain disulfide bonds. The fragments produced by enzymatic digestion with pepsin or papain or by cleavage of the disulfide bonds with mercaptoethanol are indicated; [15].

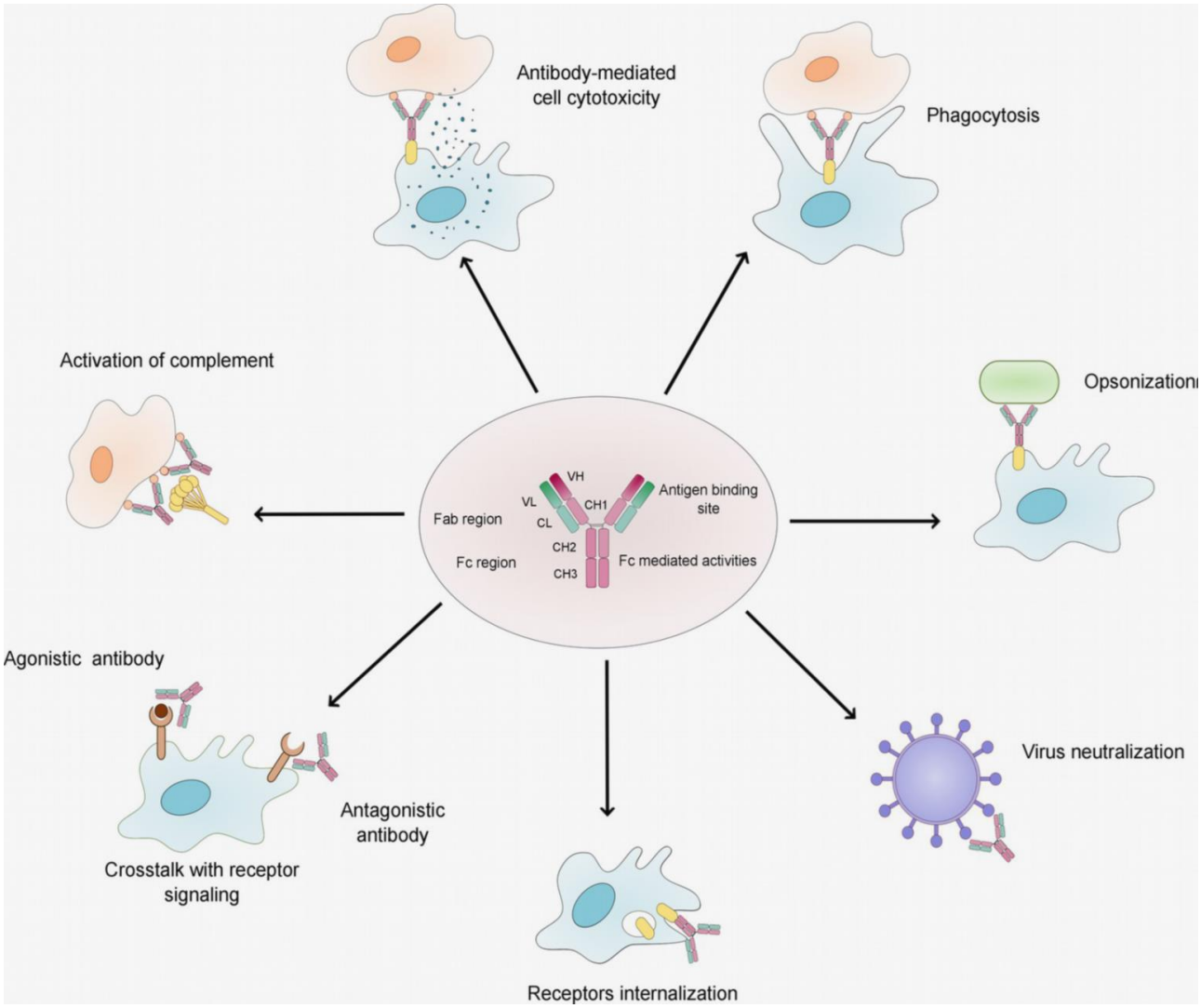

Figure 3. Overview of the natural function of antibodies; [16].

ANTIBODY ENGINEERING

Monoclonal antibodies production by Hybridoma Technology

Over a century ago Paul Ehrlich (19th Century) coined the phrase magic bullets to explain how antibodies might target and interact with their respective antigens. Seventy-five years later, Georges Kohler and Cesar Milstein invented the technology for cloning individual antibodies. Monoclonal antibodies specifically recognize one epitope of the cognate antigen. They can be generated using hybridoma technology which involves fusing lymphocytes from the spleen of an immunized mouse with an immortal cancer cell line (myeloma). The myeloma cell lines are selected so that they do not possess the capability to produce immunoglobulins unless fused to lymphocytes. This fusion is performed using polyethylene glycol (PEG) which is a poly wax solution that enhances adjacent cell fusions and the exchange of nuclei [17].

A mixed population of fused cells, unfused myelomas, and lymphocytes results, and these are incubated for 7 days before screening. The fused hybridoma cells are selected using HAT (Hypoxanthine, Aminopterin, and Thymidine)-containing medium. Myeloma cells lack the HGPRT (hypoxanthine-guanine phosphoribosyltransferase) enzyme and when the de novo synthesis of purine and pyrimidines is blocked by HAT addition the cells die, while lymphocytes which do not grow in this culture media eventually die. Therefore, in the presence of HAT supplemented media only the hybridoma cells will proliferate. Secreted antibodies in conditioned media from each hybridoma are tested against the immunized antigen by ELISA. Positive hybridomas are scaled up and subsequently cloned (by limiting 21 dilutions) to select for a single monoclonal antibody-producing hybridoma cell. The hybridoma cells can be stored indefinitely in liquid nitrogen [18] (Figure 4).

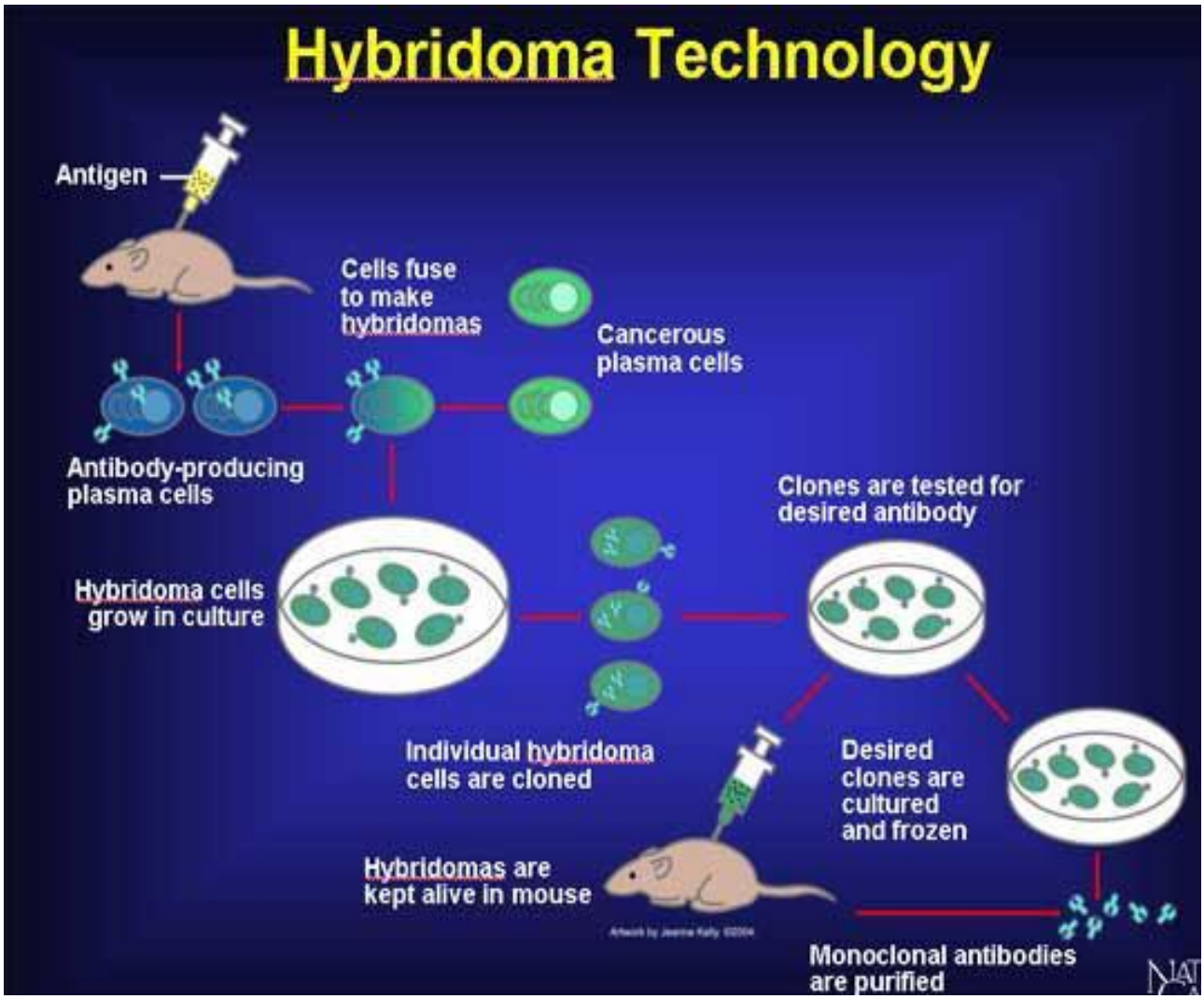

Figure 4. Production of monoclonal antibody by hybridoma technology. The hybridoma technology outline involves the isolation of spleen cells from immunized mice, their fusion with immortal myeloma cells, and the production and further propagation of monoclonal antibodies from the hybrid cells; [18].

Recombinant monoclonal antibodies

Chimerization. Chimeric antibody refers to the replacement of murine constant region with equivalent human regions contributes significantly to the immunogenicity. Also, it allows for better interaction with the complement system and human effector cells (Figure 5). This strategy led to therapeutic successes such as basiliximab (Simulect: IgG1 anti-CD25, Novartis) or cetuximab (Erbitux: IgG1 anti-EGFR, ImClone) [19].

Humanization of murine antibodies. Antibody humanization technology was developed and made possible by the transfer of all xenogeneic CDRs (complementary determining regions) onto the framework of a human Ab. The first humanized Abs was constructed based on human sequences with the known crystal structure, which permits the identification of residues contributing to the antigen-binding. In the "best fit" strategy, the closest human sequence, usually rearranged, is used as a framework to receive the murine CDRs [20] (Figure 5).

Generation of fully human monoclonal antibodies. Human antibodies have been generated by a combined polyethylene glycol/electrofusion method. Recent technologic advances allowed the production of new monoclonal antibodies Moreover; alternative approaches based on transgenic mice or in phage display were developed [21].

Human antibodies by phage display technology. Antibody phage display technology consists of the selection of antibody fragments from combinatorial libraries displayed on the surface of filamentous phage. The principle is to generate antibodies by cloning immunoglobulin variable genes using recombinant DNA technology into the phage genome. This technique allows displaying on the surface of bacteriophages the antibody fragment of interest in an scFv or Fab fragment format [22] (Figure 6).

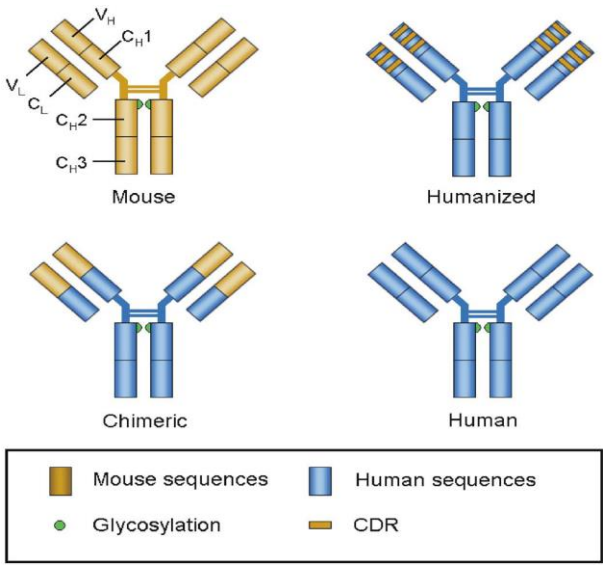

Figure 5. Antibody engineering for humanization; [10].

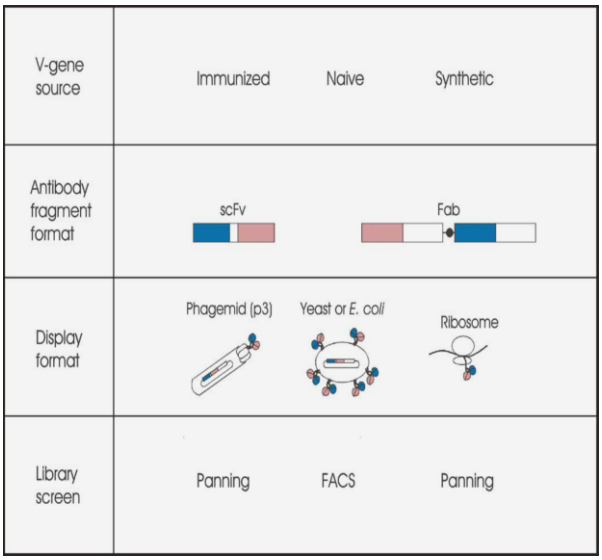

Figure 6. The display and screening system of antibody libraries; [10].

Immune library: Humans exposed to specific desired antigens through diseases or vaccination have high levels of circulating antibodies. Small libraries (~105) from immunize are enough to give rise to specific antibodies. Due to antibody genes *in vitro* experienced affinity maturation, antibodies that do not require further affinity maturation can potentially be isolated. Human antibodies cannot be generated by immunization to give human MAbs because of ethical issues. Immunological tolerance makes it difficult to isolate against self-antigens, many of which are potentially important therapeutic targets, particularly in cancer [10].

Naïve library: stands for the V-gene repertoire created by cloning the antibody genes found in non-immunized individuals. Peripheral lymphocytes, bone marrow, tonsils, and cadaver spleens are sites for mRNA isolation. From the mRNA, IgM and/or IgG variable regions are amplified by PCR using degenerate oligonucleotide primer sets and cloned into vectors suitable for screening. Subsequently, from mRNA IgM repertoires are preferred to IgG because they have not been subjected to antigen selection or tolerance they are more diverse. In contrast, IgG chains can be biased by host immune responses will not be reactive with self-antigens. Once a library has been made, it can be propagated and used repeatedly to isolate antibodies against numerous antigens. Even though, host bacteria toxicity and poor expression are often issues with antibodies isolated from naïve libraries. This condition can be circumvented by using synthetic antibody repertoire libraries [23] (Figure 5). *Synthetic library*: refers to fully synthetic repertoires, germline antibody gene segments, VH, DH, and JH or Vκ/λ and Jκ/λ are cloned and arranged combinatorially *in vitro* to reconstitute genes encoding complete VH and VL chains. These synthetic libraries of 107-1010 clones gave rise to antibodies with specificity to self-antigens [23].

Antibody library screening is the most frequently used technique. It is based on the display of antibodies on the surface of filamentous bacteriophages. The antibody library in the Fab or scFv format is fused to a surface protein of phages, displaying antigen-specific antibodies. This can readily be enriched by selective adsorption onto immobilized antigen, a process known as panning. The bound phage is eluted from the surface and amplified through infection of *E. coli* cells. They are 5-8 rounds of panning, elution, and amplification are sufficient to select for phages displaying specific antibodies, even they are up to 1011 clones large libraries [24].

Cell display antibodies are antibodies that are displayed on the surface of microbial cells such as E. coli and Saccharomyces cerevisiae. A library of cells, each displaying multiple copies of a different antibody variant, is incubated with a fluorescently tagged ligand in a buffer for screening. Cells displaying antibodies become fluorescently labeled after binding with the ligand and isolated by fluorescence-activated cell sorting (FACS). The binding of each clone in the library to a particular ligand is quantified by flow cytometry. Parameters such as ligand concentration or time for the dissociation of antibody ligand complexes can be easily optimized to ensure the isolation of only the highest affinity antibodies [25]. *Ribosome display* stands for the formation of a ternary complex between ribosomes, mRNA, and the polypeptide. From the ribosome-mRNA-protein complexes, the mRNA is reverse transcribed to produce the DNA encoding the antibodies responsible for the binding of the complexes to the immobilized ligand. Another cycle of ternary complex formation and selection was done after transcribing the DNA by RNA polymerase [26].

Human antibodies from transgenic mice. The creation of transgenic mice expressing human immunoglobulin genes required two major genetic manipulations, the inactivation of the mouse antibody production machinery and the introduction of human immunoglobulin loci in their germline configuration. This complex process required the separated generation of mouse strains with inactivated mouse immunoglobulin genes or newly introduced human immunoglobulin loci and their successive crossbreeding. At the end of the process, it was possible to obtain mouse strains with a large and diverse V gene repertoire that, in a full immunocompetent context and upon immunization, can produce high-affinity human IgGk and IgG antibodies [27].

Antibody produced by transgenic technology

Antibodies produced in transgenic plants. In 1989 the first functional antibodies were produced from tobacco plants [28]. Independent cloning of heavy and light chain antibody genes in *Agrobacterium tumefaciens* vectors, the transformation of plant tissue in vitro with the recombinant bacterium, the reconstitution of whole plants expressing individual chains and their sexual cross was used to produce to generate plant antibodies. A fully assembled and functional antibody was recovered from plant tissue in some double transgenic plants found in Mendelian fashion. The progressive improvement of vectors for plantibodies, purification strategies, and the increase in transformable crop species could lead to the nearly limitless availability of inexpensive (even edible forms of) recombinant immunoglobulins for human and animal therapy and industrial applications of antibodies (e.g., catalytic antibodies) [29].

Antibodies produced in transgenic animals. In the field of recombinant immunoglobulins, there are at least three examples of the application of transgenic animal technology. The first application is related to the use of transgenic animals as bioreactors with the production of antibodies in milk. Many human proteins of therapeutic importance found in the milk of transgenic animals have been well known for years, with average yields that amount to grams per liter in different domestic species (e.g., cattle, goats, sheep, and pig) were expressed. Antibody production from mammary tissue-specific promoters with correct assembly and function of the foreign bodies in milk [30]. Creating animals that constitutively produce recombinant antibodies or antibody fragments capable of neutralizing common pathogens of the species was the second application of transgenic animal technology. The other technology was to produce mice that carry human constant and variable gene segments in germline configuration. The animal transgenesis technology uses mice produced rearranged human antibodies in their B cells and produce human antibodies after conventional immunization procedures [31].

Engineering of Antibody fragments

The whole antibody with a molecular weight of about 150 kDa diffuses poorly from the vascular bed into a solid tumor mass and clears slowly from the body. Antibody fragments such as Fab, scFv, diabodies, and mini bodies can be generated by removing the entire constant region or part or whole of the Fc portion (Figure. 7). These antibody fragments are known to have better clearance from the whole body and tissue/tumor penetration characteristics. Antibody fragments are thought to be easy to produce in bacteria in large amounts and are therefore considered to bypass the hurdles associated with mammalian cell-based production of whole antibodies. Thus, antibody fragments are better suited for imaging and/or radiotherapy [32].

The smallest fragment of an antibody that retains the antigen-binding specificity of the whole antibody is the Fv, in which single V domain binding to antigens is possible but commonly the VH and VL domains are noncovalently associated. Because of its instability at low concentrations, the VH and VL domains of Fv are linked by a flexible peptide linker to make a single-chain Fv (scFv) (Figure. 7B). The most common peptide linker is flexible (Gly4Ser). Also, Fv is engineered to form a disulfide bond by introducing two cysteine residues in the framework regions of VH and VL to yield a disulfide stabilized Fv (dsFv) (Figure 7C) [10].

Diabodies are scFvs homodimers are covalently linked by a short peptide linker of four amino acids which forces the V domains to make inter-molecular complexes with their cognate domains (Figure 7D) [33]. In a slightly different format called (dsFv) (Figure 7E) , the VH and VL are engineered to form a dsFv but the VH is expressed twice in tandem separated by a flexible linker. When the VH and VL are co-expressed, a divalent molecule is formed (Figure 7F) [34]. *Minibodies* are scFvs-CH3 fusion protein homodimers (Figure 7G). In a different variant called Flex mini body (Figure 7H), the scFv is fused to the hinge region of IgG1 which in turn is fused via an additional 10 residues to the CH3 domain [35].

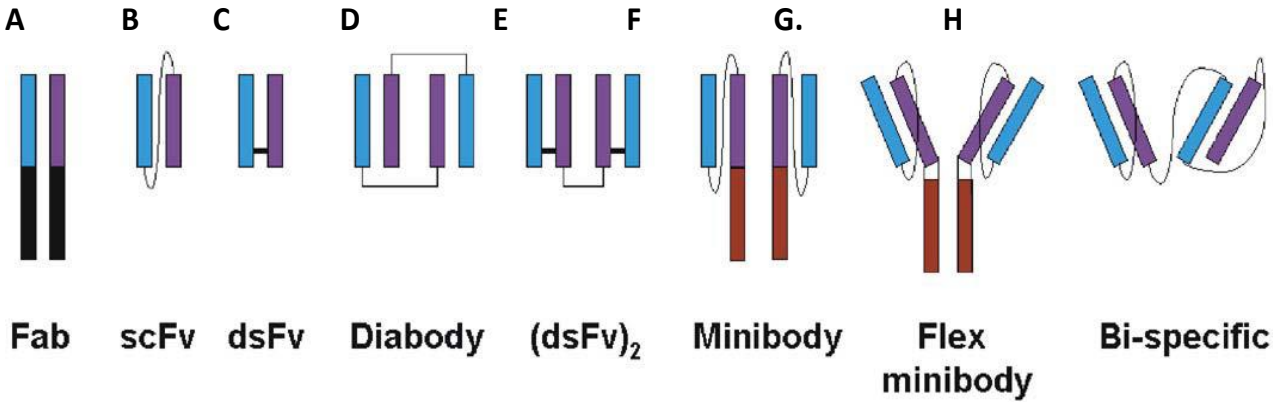

Figure 7. Schematic representation of antibody fragments; [10].

Bispecific antibodies are artificially designed molecules, capable of simultaneously binding two different antigens; hence, they can be applied to redirect effector cells to tumor cells. Technologically, bsAbs can now be produced in various ways in more than 50 formats, including quadromas, chemical conjugates, genetically linked antibody fragments, and engineered constant regions that enable heterophilic association of two heavy chains (so-called "knobs-into-holes" technology) [36], (Figure 8).

A key advantage of using bsAbs for therapy is that they make it possible to block multiple therapeutic targets with a single agent, and the effects are more pronounced when they provide pharmacological effects that a simple combination of each single-specific antibody cannot achieve. Removab (catumaxomab), the first bsAb was approved for the treatment of malignant ascites in 2009. This antibody can simultaneously bind to CD3 and EpCAM, and thus, can activate and redirect T cells to EpCAM-positive tumors [37].

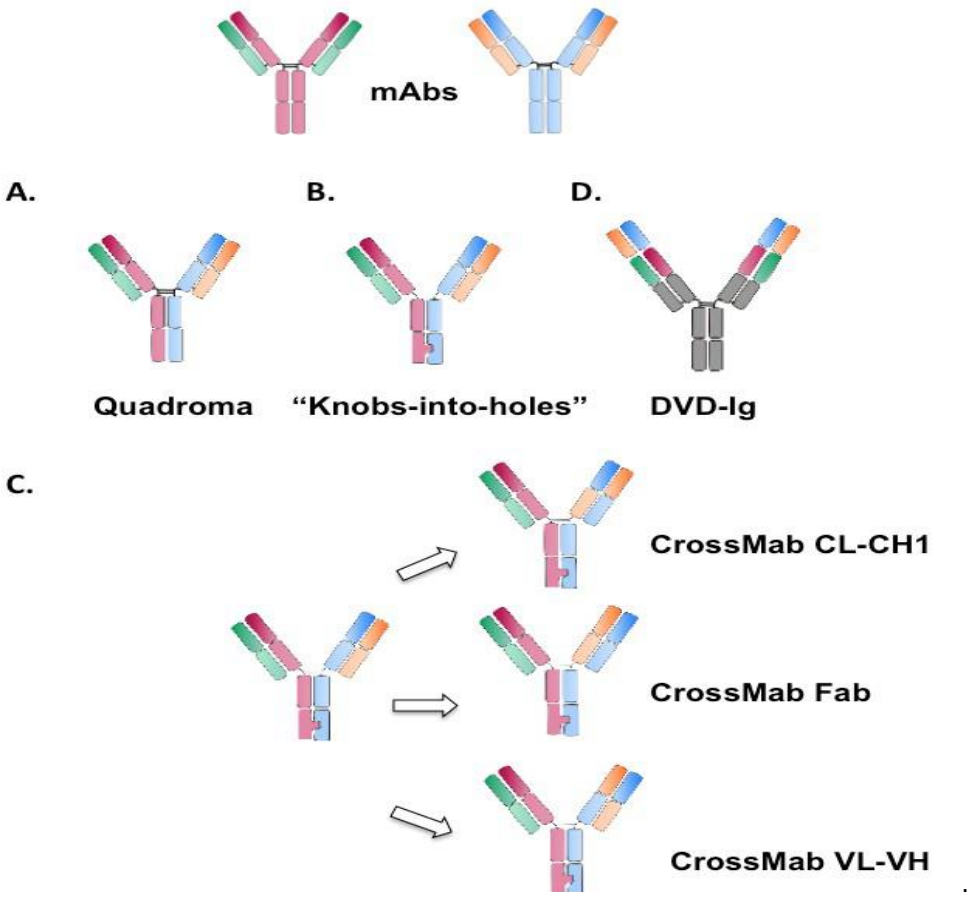

Figure 8. Schematic illustration of different formats of IgG-like bispecific antibodies with (A) Quadroma approach, (B) "Knobs-into-holes approach", (C) Cross MAb approach. (D) Dual-Variable-Domain immunoglobulin (DVD-Ig) bispecific antibody with two variable domains; [16].

Improvement of antigen-binding affinity and effector functions

Improvement of antigen-binding affinity. Improving the antigen-binding activity of antibodies is one of the most promising study areas in antibody engineering. There are two common approaches. Creating a very large library of randomly mutated CDRs or the entire variable domains and selecting higher affinity variants from this large collection of mutants. The large libraries are created by chain shuffling, error-prone, random CDR mutagenesis, and DNA shuffling [38]. The other approach is focused on or hot spot mutagenesis mimicking *in vivo* affinity maturation in small libraries [39].

Improvement of effector functions. To improve effector functions therapeutic antibodies follow two basic mechanisms. The first mechanism is triggering intracellular signals. In this mechanism, the antibodies are largely dependent on their antigen-binding function rather than effector function in apoptosis and blocking ligand interaction. Recruiting immune system components following antigen-binding ability as well as their ability to trigger effector activity is the second mechanism of therapeutic antibodies. Antigen binding ability and engineering antibodies improve antibody binding to FcγRs or the complement factors determine the therapeutic efficacy of the antibodies. Glycoengineering of IgG Fc and mutating the residues that contribute to FcγR binding which improves ADCC activity [40]. The molecules of C1qs to the hinge and CH2 domain of antibodies in which the CDC depends on. Employing domain switching and site-directed mutagenesis of IgG constant domains indicated that the CH2 domain had an important contribution to the CDC [10].

Altering pharmacokinetics

Increased FcRn binding. FcRn receptor binding with a mapped and well-characterized site on IgG determines the plasma half-life of IgG1. Binding to FcRn is increased by mutating the FcRn binding site such that More recently two mutations, T250Q and M428L, have been discovered that caused respectively a 3- and a 7-fold increase in FcRn binding and when combined together caused a 28-fold increase in FcRn binding [41].

PEGylation. An increase in the half-lives of antibody fragments is an important area of research in the development of the methods. PEGylation of proteins and liposomes has been a time tested and successful technique that offered the advantage of reducing immunogenicity, increasing the plasma half-life, increasing solubility, and reducing protease sensitivity. This process is conjugating polyethylene glycol chains to the antibody fragments. Prolongation of the phase, a phase that represents the redistribution of a molecule in the extravascular environment increase in half-life observed with PEGylated antibody fragments. PEGylation slows the redistribution of the molecules from the plasma to the interstitial compartment. Therefore, the science of antibody PEGylation has two focus areas (a) to preserve the antigen-binding activity completely and (b) to link the PEG molecule to the antibody in a stable manner [42].

Other antibody constructions

Antibodies can be produced genetic fusion proteins with toxins, drugs, enzymes, and other functional groups and modified in their constant domains to alter the original effector mechanisms and properties of the antibody molecule [43]. Immunoadhesins are fusion proteins that combine the hinge and Fc regions of an antibody with domains of a ligand-specific cell surface receptor. These molecules are used as laboratory experimental tools and as promising applications in medicine [44]. Antigenized antibodies are made up of peptide epitopes derived from antigens that are different from immunoglobulins in the place of the CDR loops of immunoglobulin by grafting. The conformationally restricted exposure of short foreign peptides using the V region framework and the characteristics of the constant antibody domains create promising combinations for immunoprophylaxis or immunotherapy. They can also be extended to the peptide hormone field and the rational design of new drugs [43].

APPLICATION OF ENGINEERED ANTIBODIES FOR THERAPEUTIC PURPOSES

Antibody engineering as an opportunity for selection and optimization of anti-HIV therapeutic agents

Clinical need for new therapeutic approaches

Human Immunodeficiency Virus type 1 (HIV-1) is the causal agent of the acquired immunodeficiency syndrome (AIDS). The introduction of highly active antiretroviral therapy (HAART), has greatly improved the quality of life of many infected individuals, and mortality for AIDS has dropped dramatically. However, currently available antiretroviral drugs have three major shortfalls: 1) the drugs have relatively high toxicities that cause undesirable side effects, including myocardial infarction; 2) the drug activity of the Reverse

Transcriptase inhibitors is cell-dependent since they are active post-infection, and 3) HIV has a high mutation rate leading to the rapid development of drug-resistant viral variants. For these reasons, many patients treated with HAART regimens still fail to achieve or maintain optimal control of the infection and development of HIV-1 drug resistance due to the failure of available Anti-retroviral drugs. Therefore, other methods of intervention through control viral replication are needed. Drugs that target the cellular receptor complex are promising areas in HIV therapy and are a good area of interest for multi-drug resistant viruses [45].

Anti-HIV antibodies currently in clinical trial. The major characteristics of the antibodies already entered in clinical trials as HIV entry inhibitors are summarized in Table 1. The monoclonal antibody Ibalizumab, formerly named TNX-355 and Hu5A8, is a humanized IgG4 mAb that binds to the second (C2) domain of CD4. In contrast to attachment inhibitors, Ibalizumab decreases the flexibility of CD4, thereby hindering the access of the CD4-gp120 complex to the co-receptors CCR5 and CXCR4 rather than preventing gp120 binding to CD4. This mAb is a potent inhibitor of HIV-1 *in vitro* and shows synergy when combined with other anti-HIV drugs or the fusion inhibitor enfuvirtide [46].

Table 1. Antibodies who have Completed Clinical Trials for HIV

Antibody Name	Target Ag	Origin	Isotype	Trial status	References
Ibalizumab	CD4	Humanized mAb	IgG4	II	[46]
PRO542	Gp120	Human fusion protein	IgG2	II	[47]
PRO140	CCR5	Humanized mAb	IgG4	I	[48]
HGS004	CCR5	Human mAb	IgG4	I	[49]

Antibody therapeutics for cancer therapy

Monoclonal Antibodies (mAbs) comprise a class of therapeutic biologics that have been increasingly used over the last decades. The concept of using antibodies to selectively target tumors was proposed by Paul Ehrlich over a century ago [50].

Mechanism of action of monoclonal antibodies for the treatment of cancer

Altering signal transduction in the downstream intracellular pathways. Various cell surface receptors that activate intracellular pathways are expressed by cancer cells leading to growth. Amongst these, EGFR or ErbB1, ErbB2 or HER-2/Neu, HER-3, and HER-4 are of the same family and are over-expressed in epithelial malignancies originating from the colon, breast, lung, and head and neck resulting in rapidly proliferating disease and increased metastatic potential. Downstream activation of the receptor and increasing receptor internalization inhibited by Anti-EGFR antibodies bind to the receptor domain of the EGFR receptor. The cancer cell cycle inhibited by antibodies and cause apoptosis [51].

Antibody-dependent cell cytotoxicity (ADCC). The cancer cells that are coated by antibodies resulted in immune-mediated destruction. The effector cells in the antibody-dependent cytotoxicity include macrophages, NK cells, and neutrophils. ADCC depends on the Fc portion of the antibody that binds an Fc gamma receptor (FcgR) on the effector cells. ADCC occurs when the Fab and Fc portions of the mAb engage both tumor cell antigen and an activating FcgR, respectively, thus creating a bridge from the tumor cell to the effector cell. Target cell recognition is then coupled to a lytic attack on the target cell mounted by effector cells [52].

Complement-mediated cytotoxicity (CDC). CDC results from a cytolytic cascade mediated by a series of complement proteins, resulting in lysis of the antibody-bound cell [51].

Soluble ligand neutralization. Antibodies can bind to circulating proteins and interfere with their ability to find their targets to help facilitate the growth of the tumors. One important example of this mechanism is bevacizumab which is a fully-humanized monoclonal antibody against VEGF-A. Bevacizumab binds and inactivates the biological activity of VEGF-A, inhibiting angiogenesis, and thus, tumor growth and proliferation [53].

Cytotoxic drug delivery. Tumor-targeted monoclonal antibodies are linked to Cytotoxic agents to deliver them specifically to the tumor cells which is preferable for its limited systemic side effect [54]. Two exciting samples of this technology are trastuzumab-DM1 T-DM1, a HER2 directed antibody-drug conjugate, and brentuximab vedotin, a CD30 directed antibody-drug conjugate [51]. Few antibody therapies are developed for cancer in animal besides human cancer therapy. for instance, Aratana Therapeutics, a pet health company

based in Kansas City, USA is developing antibody therapies for canines. Canine lymphoma monoclonal antibody- AT-005 (against CD52) has been approved in 2012, for the treatment of T-cell lymphoma in dogs [55].

Monoclonal antibodies approved for clinical use in oncology. The major characteristics of monoclonal antibodies approved for oncology therapy are summarized in Table 2.

Table 2. Monoclonal antibodies approved for clinical use in oncology

Antibody Name	Target	Antibody format	Application
Cetuximab	EGFR	Chimeric	Colorectal, breast, and lung cancer
Panitumumab	EGFR	Human	Colorectal cancer
Nimotuzumab	EGFR	Humanized	Head and neck cancer
Rituximab	CD20	Chimeric	Non-Hodgkin lymphoma
Trastuzumab	HER2	Humanized	Breast cancer
Alemtuzumab	CD52	Humanized	Chronic lymphocytic leukemia
Bevacizumab	VEGFA	Humanized	Colorectal and lung cancer
Ofatumumab	CD20	Human	Chronic lymphocytic leukemia
Ipilimumab	CTLA-4	Human	Metastatic melanoma
Pertuzumab	HER2	Humanized	Breast cancer
Denosumab	RANK Ligand	Human	Solid tumor bony metastases
Brentuximab vedotin	CD30	Chimeric	Hodgkin's or systemic anaplastic cell lymphoma
Gemtuzumabozogamicin	CD33	Humanized	Acute myelogenous leukemia
90Y-Ibritumomab tiuxetan	CD20	Mouse	Low grade or transformed B cell Non-Hodgkin's lymphoma
Tositumomaband 131I-tositumomab	CD20	Mouse	Lymphoma

Source: [56]

The use of antibodies in the treatment of infectious diseases

Anti-bacterial antibodies. Antibiotic therapy targets bacterial exotoxins. for instance, B. anthracis produces a potent bioweapon, a tripartite exotoxin consisting of a protective antigen (PA), lethal factor (LF), and edema factor (EF). Post-exposure prophylaxis with mAb against PA protected against a lethal inhalational anthrax challenge in rabbits and monkeys and a mAb against LF protected rats against a challenge with a lethal toxin, a mixture of PA and LF [57]. An anti-PA mAb acts synergistically with the antibiotic ciprofloxacin for cover against inhalational anthrax [58].

Escherichia coli, Shiga toxin-producing significant exotoxin-producing pathogen causes severe gastrointestinal disease. Complications like hemolytic uraemic syndrome, acute kidney failure, and death may result from toxin entry into the bloodstream. Currently, only supportive treatments are available. a human IgG1 mAb generated in transgenic mice against Shiga toxin subunit A prevented fatal systemic complications in piglets following administration after the onset of diarrhea [59]. Examples of other exotoxins against which mAbs are shown to possess some efficacy include *Pseudomonas aeruginosa* exotoxin A, eubacteria *Clostridium perfringens* epsilon toxin, and *Clostridium botulinus* neurotoxin [60]. Even though targeting of exotoxin requires prior knowledge of the pathology of the infectious agent and initial characterization of the exotoxin. Therefore the genetic nature, surface carbohydrates regions that show relatively little variability between bacteria subtypes and are explored as potential target antigens. Antibody targets against shared or invariant epitopes like the core carbohydrate backbone, as many bacterial species often exhibit variability in their carbohydrate side-chain residues. Also, targets preventing septic shock by promoting the clearance of LPS endotoxin within the bloodstream. Carbohydrates targeting mAbs mixed results and MAbs raised against the inner core LPS of varied *Neisseria meningitides* serotypes have shown poor phagocytic activity despite their avidity for whole-cell bacteria and showed poor binding to full-length LPS. MAbs produced against the deacetylated core carbohydrate backbone of the *S. aureus* surface carbohydrate, poly-N-acetylglucosamine (PNAG), conferred protection from a bacterial challenge in mice and performed better than mAbs against a totally acetylated wild-type PNAG [61].

In another study, mAbs raised against *Streptococcus pneumoniae* serotype 6B capsular polysaccharide with strong cross-reactivity for serotype 6A showed avidity-dependent in vitro opsonization and *in vivo* protection against a bacterial challenge with either subtype. Thus, while a generic target for bacteria is attractive, much

work remains needed for it to be applied within the clinical setting [62]. Treatment of antibiotic-resistant bacteria, such as *S. aureus* is another area of interest during which an antibody that has reached the clinical test stage is tefibazumab (Aurexis), a humanized mAb that binds clumping factor A (ClfA), a serious virulence determinant in *S. aureus*. Tefibazumab has been shown to induce phagocytosis of ClfA-coated beads by human polymorphonuclear cells in vitro, protect against an intravenous challenge with *S. aureus* during a rabbit model of infective endocarditis and enhance the efficacy of vancomycin therapy within the rabbit therapeutic model [63].

Antibodies and viral disease. Palivizumab, the sole mAb currently on the marketplace for the treatment of infectious diseases, was developed as a prophylactic treatment against the viral disease Respiratory Syncytial Virus (RSV). Although mAbs are shown to be ready to neutralize many viral pathogens in vitro, the utility of mAb therapy in viral diseases remains a matter of contention because it is unclear to what extent viral clearance depends on antibody-mediated immunity. The clearance of a virus infection is typically related to T cell-mediated adaptive immunity. CD8+ T cells act by killing virus-infected cells, thus preventing viral replication and reducing the viral load. However, in acute infections, neutralizing therapeutic antibodies should be ready to help by suppressing viral replication and viremia, giving the host system time to develop an efficient response for viral clearance. Additionally, antibodies can promote the killing of infected cells expressing viral proteins on their surface through the activation of natural killer (NK) cells that mediate ADCC, additionally to their viral neutralization properties [64].

For viral infections where the host system is unable to completely clear the virus, resulting in chronic infection, the administration of neutralizing antibodies might not be ready to achieve complete clearance. In two separate clinical trials using human mAbs against a hepatitis B virus S antigen to treat patients with chronic hepatitis B infection, the viral DNA and S antigen load in serum were significantly and rapidly reduced after antibody administration. It has been also shown the upkeep of a 90% reduction in S antigen levels 15 days after cessation of therapy in half the patients, and this correlated with the persistence of the administered mAb in serum. However, in both studies, DNA and S antigen levels eventually recovered once antibody levels within the serum declined following the cessation of therapy. Nonetheless, regular administration of therapeutic antibodies should prove useful by preventing disease transmission, the infection of healthy cells, or the event of pathology through the continued suppression of viral levels [65].

LIMITATIONS OF ANTIBODY ENGINEERING

Production costs

Monoclonal antibodies are large (150 kDa) multimeric proteins containing numerous disulphide bonds and post-translational modifications like glycosylation. They have classy eukaryotic machinery to be produced in active form. Moreover, most studies have shown that these molecules need to be injected in large amounts to realize clinical efficacy (e.g. 8-16 doses of 375 mg·m-2, that is, a complete amount of 6-12 g per patient for Rituximab. Consequently, the assembly of therapeutic antibodies necessitates extensive purification steps; under good manufacturing practice conditions, resulting in extremely high production costs and limiting the wide use of those drugs [66].

Pharmacokinetics versus tissue penetration

Monoclonal antibodies against tumor-specific antigens only 20% of the administered dose typically interacts with the tumor cells in murine xenograft models. Largely remain in the blood is the major limitation observed [67]. Favorable pharmacokinetics and efficient penetration and retention in the targeted tissue, and various characteristics of mAbs, such as molecular size, shape, affinity, and valency determine tumor cell antibody uptake. The large size of the monoclonal antibody prevents them from the renal clearance threshold (~70 kDa), preventing them from being eliminated through the kidney glomeruli and the half-life is prolonged. Fc portion of IgG molecules can interact with various receptors expressed at the surface of several cell types, which increase their retention in the circulation. Moreover, the Fc portion of IgG molecules can interact with various receptors expressed at the surface of several cell types, which increases their retention in the circulation. Most importantly, the Fc portion can interact with the neonatal Fc receptor (FcRn) expressed at the surface of several cell types, including vascular endothelium cells, monocytes, and macrophages as well as with barrier sites such as the blood-brain interface, the glomerular filter in the kidneys and the intestinal epithelium [68].

Mode of action and associated limitations

Binding of the antibody to its antigen, thereby interfering with its activity and interaction with binding partners is the simplest way MAbs to function. ADCC plays a major role in the *in vivo* efficacy of mAbs. However, they trigger ADCC by therapeutic antibodies that have several limitations. The first limitation arises from, 80% of the population expresses a low-affinity variant of the receptor as a major issue and the affinity between the Fc and its receptors plays a crucial role. The other one that arises from IgG1 molecules are glycosylated in the CH2 domain (Asn 297) of the Fc region. Modification is extremely important as it modulates the affinity of the Fc for FcγRIIIa, thereby modifying the *in vivo* efficacy of antibodies. The third limitation because of the competition of therapeutic antibodies with a high concentration of patient's IgGs for binding to FcγRIIIa. Even though, 66% of those molecules and the serum concentration of IgG is 8–17 $mg{\cdot}mL^{-1}$ capable of interacting with FcγRIIIa are IgG1. To get the therapeutic effect and to reach a serum concentration between 10 and 100 $\mu g{\cdot}mL^{-1}$ much high doses of an antibody are required. Whereas, 10 $ng{\cdot}mL^{-1}$ *in vitro* is the level of antibodies saturating ADCC, in the absence of competing IgGs [69].

CONCLUSION

Antibodies are an extremely versatile class of antimicrobial proteins that are effective against a variety of diverse microorganisms. Modern biotechnology by using a variety of *in vivo* and *in vitro* screening methods enables researchers to produce fully human antibodies against a specific target. Antibody engineering is applied in the therapeutic industry to improve the current therapeutic nature of antibodies so that they will be effective in the treatment of different diseases. Hybridoma technology, recombinant antibody fragments, Transgenic and phage technology, and improvement of effector functions are some of the technologies that are applied to antibodies to improve their therapeutic capability. Genetic engineering can be used to harness and to reformat individual antibody obtained from hybridoma, transgenic mouse, or phage selection. Currently, diseases that are global issues such as Cancer, HIV-AIDS, and many infectious bacterial and viral livestock diseases are being treated using this new technology. Relatively expensiveness to manufacture, the requirement of systemic administration, and specificity to a particular pathogen or serotype are among the challenges in the application of antibody therapeutics. Besides researches are ongoing to improve the efficacy of antibody, to reduce the cost of production and improving affinity and specificity, with considerable success. Therefore, further investigation is needed on antibody engineering to extend the application beyond therapy. Furthermore, the technology for the antibody production should be adopted in developing countries since it is less labor demanding and used to treat a wide range of infectious agents.

DECLARATIONS

Authors' contributions

All authors contributed equally to this review.

Conflicts of interest

The Authors declare no conflict of interest.

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