Retrospective analysis of the preparation and application of immunotherapy in cancer treatment (Review)

JIACHEN LU[1,2*], JIANING DING[1,2*], ZHAOXIA LIU[1] and TINGTAO CHEN[1,3]

[1]Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330006; [2]Queen Mary School, Nanchang University; [3]National Engineering Research Center for Bioengineering Drugs and Technologies, Institute of Translational Medicine, Nanchang University, Nanchang, Jiangxi 330031, P.R. China

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Abstract. Monoclonal antibody technology plays a vital role in biomedical and immunotherapy, which greatly promotes the study of the structure and function of genes and proteins. To date, monoclonal antibodies have gone through four stages: murine monoclonal antibody, chimeric monoclonal antibody, humanised monoclonal antibody and fully human monoclonal antibody; thousands of monoclonal antibodies have been used in the fields of biology and medicine, playing a special role in the pathogenesis, diagnosis and treatment of disease. In this review, we compare the advantages and disadvantages of hybridoma technology, phage display technology, ribosome display technology, transgenic mouse technology, single B cell monoclonal antibody generation technologies, and forecast the promising applications of these technologies in clinical medicine, disease diagnosis and tumour treatment.

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Correspondence to: Dr Tingtao Chen, National Engineering Research Center for Bioengineering Drugs and Technologies, Institute of Translational Medicine, Nanchang University, 1299 Xuefu Road, Honggu, Nanchang, Jiangxi 330031, P.R. China
E-mail: chentingtao1984@163.com

Professor Zhaoxia Liu, Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Nanchang University, 1 Minde Road, Donghu, Nanchang, Jiangxi 330006, P.R. China
E-mail: lzxia77@163.com

*Contributed equally

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1. Introduction

Tumours are a series of diseases caused by uncontrolled cell proliferation mainly due to a change in genes. A tumour often forms in a local site and invades the surrounding tissues so as to induce metastasis (1). As the most prevalent disease in the world, it arises under the synergistic and sequential effects of multiple oncogenic factors such as chemical carcinogens, ionising radiation, viruses and bacteria, which can induce the transformation of proto-oncogenes to oncogenes and the inactivation of tumour-suppressor genes (2,3). Under the influence of these factors, alterations in apoptosis regulatory genes and DNA repair genes develop, accompanied by abnormal expression levels of cellular proteins (4).

According to the cellular characteristics, tumour morphology, treatment method and degree of harm to the body, tumours can be divided into: i) solid tumours which can be detected by clinical examination such as X-ray, CT scan, ultrasound, or palpation (5,6), and ii) non-solid tumours which are mainly present in the blood circulation and not visible to the naked eye or on imaging (7). In general, non-solid tumours have a wide distribution of tumour cells in the blood and bone marrow, and thus cannot be removed surgically, but only by chemotherapy (8). In contrast, the majority of solid tumours can be treated with a wider range of strategies, such as surgery, chemotherapy, radiotherapy, immunotherapy, tumour biotherapy, oncolytic virotherapy, target treatment, hormone therapy, minimally invasive interventional therapy, microwave therapy, radiofrequency therapy and cryotherapy (9,10). Among these treatments, immunotherapy, characterised as having high specificity, precise targeting capability, powerful antitumour effects and low side effects, relies on activation of the patient's own immune system to kill tumour cells which makes the target different from other treatments (surgeries, chemotherapy, radiotherapy and targeted therapies), and shows bright and unparalleled prospects due to the unusual and miraculous effects (11).

As the most prominent component of immunotherapy, monoclonal antibodies (mAbs) are highly homogeneous antibodies produced by a single B-cell clone and directed only against a specific antigenic epitope (12). While it has the advantages of high purity, high sensitivity, high specificity, low cross-reactivity and low cost of preparation, some disadvantages also exist, such as its production and preparation requiring certain technology (13). However, with the optimisation of preparation techniques and the production of numerous mAb drugs (such as abciximab and rituximab) over the years, the scope of application of mAbs has gradually broadened, and they have been widely used in immune checkpoint therapy, targeted tumour therapy, radioimmunotherapy and near-infrared photoimmunotherapy (NIR-PIT) to date, specifically showing great development prospect in tumour therapy (9,14,15). As mAb research can be applied to many other areas of technical research, it not only drives the research process of full human mAb preparation, but also perfectly demonstrates its unparalleled value in tumour control and treatment research, under the efforts of countless researchers.

In view of these factors, this review will focus on the relatively mature techniques for the preparation of mAbs and the application of mAbs to demonstrate the importance of mAb research. This review reviewed 242 articles published mainly between 2005 and 2021, including the PubMed, Excerpt Medica Database, Medline, OVID and the Cochrane Library databases, by searching the key word monoclonal antibody, immunotherapy or tumour.

2. Immunotherapy and antibodies

Tumour immunotherapy is a therapy used to restore the normal antitumour immune response of the body by restarting and maintaining the tumour immune cycle for tumour control and clearance, which has a major impact on the treatment of metastatic tumours and has altered the standards of care for many types of tumours (16). As its indispensable components, antibodies are specific binding immunoglobulins produced by plasma cells derived from B lymphocytes or memory cells in response to antigen stimulation by the body's immune system (17,18). Its functions refer to combining with antigens and effectively removing foreign bodies such as invading microorganisms and parasites (19).

In general, antibodies can be divided into polyclonal antibodies and mAbs. Polyclonal antibodies are produced from multiple B cell clones after the body is stimulated by a variety of antigenic determinants, which can be regarded as a mixture of multiple mAbs (20). In contrast, mAbs are the antibodies that can target the particular antigen determining cluster, characterised as high specificity, strong binding force, high purity, low cost and mass production (18). As a kind of highly specific and homologous antibody, the mAb was first produced by Köhler and Milstein in 1975 with the use of the hybridoma technique, which used the HAT culture medium to screen for hybridoma cells that could grow steadily, recognise a particular antigenic epitope and produce mAbs (21). In 1982, Levy of the Stanford Medical Centre in the US prepared a unique mAb against B-cell lymphoma; the patient's condition was alleviated and the tumour disappeared after treatment with this unique antibody. This was the first time that mAbs had been used in clinical treatment (22), and showed promise for application as targeted therapies for tumours, inflammation, and cardiovascular, autoimmune and infectious diseases. Due to the great contribution that hybridoma technology has made to the field of life sciences, Milstein and Köhler were awarded the Nobel Prize in Medicine and Physiology in 1984 (14), which indicated the people's recognition of mAbs and how optimistic people are about their prospects to some extent. Soon after this, orthoclone was produced by Ortho Biotech, which was also named muromomab-CD3. This was approved by the food and drug administration (FDA) as the first mAb drug in 1986 and was used to inhibit acute rejection of kidney transplantation and treat human diseases (23), opening a new era of mAb therapy.

To date, mAbs have undergone different stages of optimisation and development, including murine mAbs, humanised mAbs and fully human mAbs (24). The advent of hybridoma technology has made possible the implementation of the large-scale preparation of uniform murine mAbs (25). Compared with the polyclonal antibodies studied in the past, murine mAbs showed a huge difference in terms of specificity and consistency, as even the different batches of polyclonal antibodies prepared with the same antigen cannot guarantee their consistency but perfectly consistent murine mAbs can be produced continuously once the hybridoma is successfully prepared (21,26). Nevertheless, the murine mAbs, as the heterologous protein, may lead to an immune response and the production of the human anti-murine antibody (HAMA) *in vivo* which can in turn clear the murine mAbs, resulting in the emergence of autoimmune diseases and an ultimate reduction in therapeutic effectiveness (27,28).

The humanised mAb refers to the murine mAb reconstructed by gene cloning and DNA recombination (29). The construction forms the constant parts of mAb (the CH and CL regions) or all parts of the mAb encoded by human antibody genes, leading to the basic preservation of the affinity and specificity of the original murine mAb and a reduction of its heterology (30). As the most widely used mAb, the advantage of humanised mAbs is that they can overcome the human anti-murine antibody reaction, preventing the rapid elimination of mAbs as foreign proteins by the immune system, and improving the biological activity of mAbs (31).

As the ideal antibody for treatment, fully human mAbs have the humanised V and C regions (29). With the use of transgenes, the transchromosome technique or some other technique, all of the genes encoding human antibodies can be transferred into genetically engineered animals with their antibody genes deleted, so that the animals can express human antibodies and achieve the goal of full humanisation (32). At present, the human hybridoma technique, the EBV transformation of B lymphocytes, phage display, the transgenic mouse technique and the preparation of a single B cell antibody can all be used to produce fully human mAbs (33-35) (Fig. 1). Among them, mAbs obtained by transgenic mouse technology are relatively complete, and those obtained by phage display technology are generally incomplete (35).

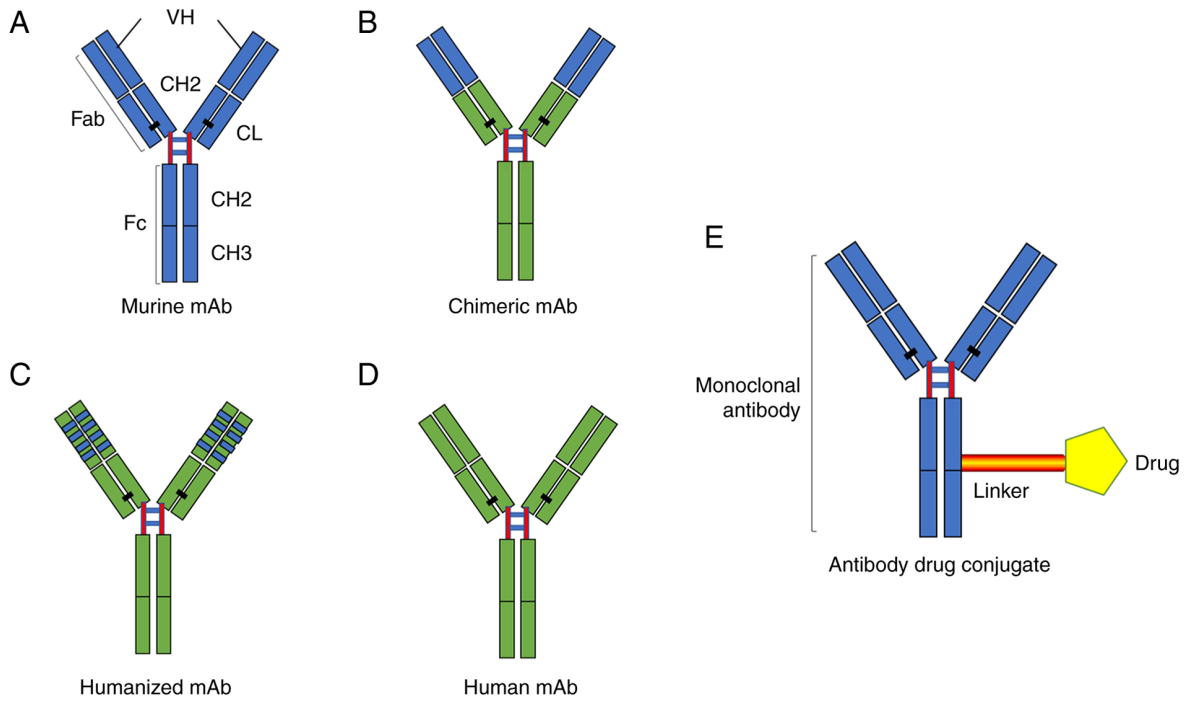

Figure 1. Schematic overview of humanization from murine antibodies (blue domains) to fully human antibodies (green domains) and associated suffixes. (A) The murine monoclonal antibody. (B) The chimeric monoclonal antibody: V regions are of murine origin, and the rest of the chains are of human origin. (C) Humanized monoclonal antibody: only contain the hypervariable segments of murine origin. (D) Human monoclonal. (E) Antibody-drug conjugate. CH, domains of the C region of the heavy chain; CL, C domain of the light chain; VH, V domain of the heavy chain; VL, V domain of the light chain; Fab and Fc, fragments resulting from proteolysis.

3. Monoclonal antibody preparation techniques and their applications

To date, many relatively mature mAb preparation technologies, e.g. the hybridoma technique, the phage display technique, the transgenic mouse technique, the ribosome display technique, and single B cell antibody preparation techniques, can be selected depending on the characteristics of the desired antibody (Fig. 2).

Hybridoma technique

Development history. As the earliest technology used to produce mAbs, the hybridoma technique, also known as the lymphocytic hybridoma technique, is developed from somatic cell fusion technology, which enables the realisation of the large-scale preparation of the uniform murine mAbs (26). It was first invented by Köhler and Milstein in 1975 to produce hybridoma cells through the fusion of mouse myeloma cells and immunised animal spleen cells, which have the ability to reproduce endlessly and secrete highly specific antibodies that can recognise specific antigens-mAbs (21). As they did not choose to patent the hybridoma technique, it is allowed to be used in academia and the pharmaceutical industry, leading to potential future treatments for a range of diseases including tumours (36).

Advantages and disadvantages. Although antibodies produced by the hybridoma technique possess attractive advantages, such as good specificity, high purity and large-scale production (25), some defects of murine-derived antibodies remain unavoidable. On the one hand, the low affinity of murine mAbs to the Fc fragment on the immunocyte surface, can cause light antibody-dependent cell-mediated cytotoxicity (ADCC), resulting in a mild killing effect on tumour cells (37). In addition, the killing effect has a short time in which to take effect because of the short half-life of murine mAbs in the blood (38).

On the other hand, murine mAbs cause immunogenicity (39) and can further produce HAMA (27), which means that the repeated use of murine mAbs can lead to decreased efficiency and harm to humans due to allergic reactions (26). Additionally, it is not uncommon for patients treated with mAbs to produce human anti-murine immunoglobulin responses, possibly due to the immune deficiency associated with certain types of tumours (40). Therefore, some early murine mAbs, such as the E5 murine mAb, not only failed to achieve the desired effect in the treatment, but increased the mortality of patients, leading to a period of downturn in the development of mAb preparation and mAb treatment (41).

Clinical therapeutic applications. Rituximab, as the first lymphoma mAb developed in 1982, was shown to alleviate the condition of tumour patients, which raises great hope for the use of mAbs in tumour treatment (42). In addition, the first mAb drug, anti-cd3 mAb OKT3, was approved by the US FDA to enter the market in 1986; this mAb can alleviate the anti-rejection reaction during organ transplantation (23,34). Overall, the mAb drugs produced at that time were murine mAbs and rabbit mAbs, which have some clinical drawbacks (26). Recently, with the advances in technology, human hybridoma technology has been developed as a new mAb preparation technology on the basis of mouse hybridoma technology and rabbit hybridoma technology, which causes

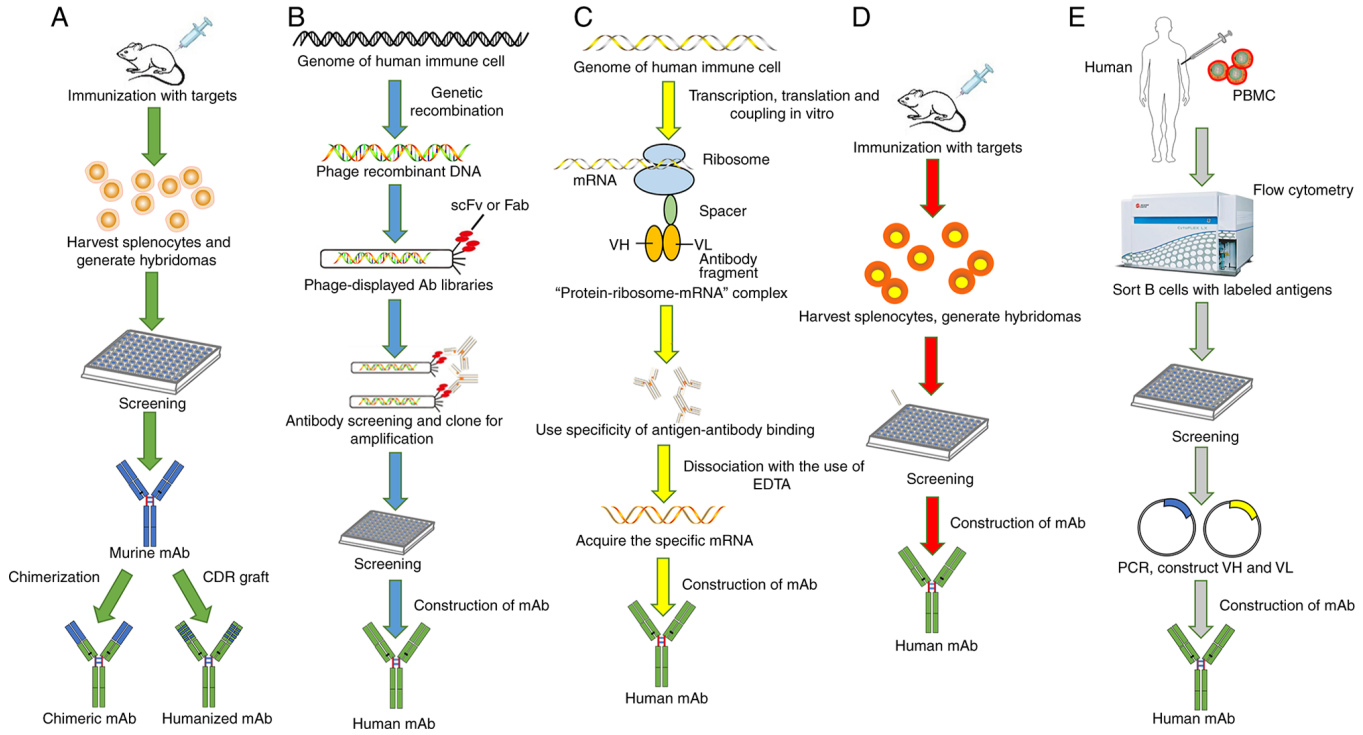

Figure 2. Approaches for the preparation of therapeutic monoclonal antibodies (mAbs). (A) Hybridoma technique. The traditional murine hybridoma technique starts by the immune response of mice triggered with the desired antigens. After that, splenocytes are harvested and fused with myeloma cells to produce hybridoma cells. After the screening, selected hybridoma cells are used to persistently generate chimeric or humanized monoclonal antibodies. (B) Phage display. A human phage-displayed antibody library is used to select the antigens of interest. After immuno-positive phage clones screened by ELISA and DNA sequences, construction of the mAb is made to help express humanized mAb. (C) Ribosome display. 'Protein-ribosome-mRNA' complex is constructed to help establish the Protein library of phage display by using specificity of antigen-antibody binding. Following dissociation with the use of EDTA, the acquired specific mRNA can help establish the DNA library of the specific antibody by RT-PCR, which expressed the specific humanized mAb with high affinity. (D) Transgenic mouse technique. Similar to the mouse hybridoma technique. (E) The single B cell technique. peripheral blood mononuclear cells (PBMCs) are prepared from infected or vaccinated donors so as to isolate suitable B cells by flow cytometry. After that, VH and VL information of each B cell informs the generation of human mAbs by RT-PCR.

the fusion of immunised human B cells and human myeloma cells to produce hybrid cells that can divide indefinitely and secrete antibodies (26). However, the technology has produced a very limited number of multiple myeloma cell lines, has a low cell fusion success rate and easily causes the loss of chromosomes (43).

DNA recombinant antibody technique

Development process. Advances in genetic engineering techniques facilitate the development of chimeric (murine/human) mAbs. With the use of DNA recombination technology, chimeric genes consisting of a combination of the Variable (V) region gene of murine antibodies and the Constant (C) region gene of human antibodies, inserted into the expression vector containing the C region of human antibodies, is used to express chimeric mAbs which possess a humanised C region and heterogeneous V region. The resulting antibody (44) causes reduced immunogenicity of the allogenic antibody while retaining the ability of the parental antibody to specifically bind to the antigen. However, because there is still some residual immunogenicity in the FR of V region, HAMA may be induced (45). In view of this, primate antibodies produced by immunising macaques can be chosen as the heterologous antibody to chimerism with the C region, because the V region of primate antibodies show few differences to the V region of human antigens, thus decreasing the immunogenicity.

Advantages and disadvantages. Compared with murine mAbs prepared by the hybridoma technique which are limited in clinical application due to their ability to cause HAMA reactions *in vivo*, the chimeric mAbs prepared by DNA recombinant antibody technique can significantly alleviate adverse reactions because of an approximately 70% reduction in immunogenicity of the heterologous antibody, thus improving the curative effect (46). For example, infliximab is capable of preventing and reducing inflammation as the chimeric mAb to tumour necrosis factor (TNF)-α in the treatment of rheumatoid arthritis (RA) and Crohn's disease (47). In addition, it also has the effector functions of human antibodies because of the existence of a humanised Fc fraction, which makes the chimeric mAb possess more potent complement-dependent cytotoxicity (CDC) and ADCC (48). On the basis of the mechanism, the rituximab chimeric anti-CD20 mAb was developed to treat relapsed indolent lymphoma because the cell-surface antigen CD20 is expressed on more than 90% of B-cell lymphomas and chronic lymphocytic leukaemias (49). Abciximab, a Fab fragment of a chimeric mAb, functions as a GP IIb/IIIa receptor antagonist to significantly decrease the size of coronary artery aneurysms in children with Kawasaki disease by promoting vascular remodelling, and decrease the risk to go through early stent thrombosis in diabetic patients with ST-segment elevation myocardial infarction (50,51). In addition, basiliximab and cetuximab, serving as chimeric mAb,

can function to prevent early acute or slow rejection reaction after organ or allogeneic hematopoietic stem cell transplantation so as to treat acute graft-vs. -host disease (52,53), and treat different cancers including recurrent and metastatic head and neck squamous cell carcinoma and metastatic colorectal cancer (54,55), respectively. However, chimeric antibodies can partially solve the problem of heterologous protein rejection, but they may still induce an HAMA reaction, interfere with antibody efficacy and induce a hypersensitivity reaction due to the fact that they also contain the murine V region which limits their clinical application to some extent (56,57).

Phage display. Phage display techniques can clone the peptide-coding or protein-coding gene fragment into the appropriate position of the phage shell protein structure (58), so that the foreign polypeptide/protein and shell protein are expressed in the form of a fusion of each other and then displayed on the phage surface as the progeny phage reassembles (59,60). The demonstrated polypeptide or protein can maintain a relatively independent spatial structure and biological activity, which is conducive to the recognition and binding of target molecules, providing a way to screen for single-chain antibodies with high specificity and binding ability (61). In theory, as long as enough of this type of peptide is expressed in the library, one or more phage can bind to these targets.

Development process. Phage display was pioneered by Greg Winter and his colleagues (59). In 1985, G.P. Smith developed phage display technology (62) based on the research of phage biology and molecular biology, which show unique advantages in virus infection, including HIV infection and tumour diagnosis and treatment. Later, in 1987, Geysen *et al* proposed that short peptides containing key amino acid residues can mimic the antigenic determinants of proteins and the interaction between proteins is achieved by the interaction between local peptides (63). In 1988, Parmley and Smith proposed the idea that the construction of a random peptide library could provide insight into the antigen-determining cluster epitopes recognised by antibodies (64). Subsequently, Scott and Smith fused random short peptides to the surface protein PIII of filamentous phage and displayed it on the surface of the phage, creating the first phage random peptide library (65). In the same year, McCafferty *et al* used phage display technology to screen for single-chain antibodies to lysozyme bacteria, propelling phage display technology into an era of widespread application (66). Recently, because of the pioneering work and application in the phage display of peptides and antibodies, Professors George P. Smith and Gregory P. Winter both won a quarter share of the 2018 Nobel Prize in Chemistry (59). Until now, the application scope of phage display technology has been expanding, and the technology has also been constantly improving and developing.

Advantages and disadvantages. The emergence of phage display technology has opened up a simple and fast route for the production of genetically engineered mAbs, which bypasses the technical difficulty of hybridomas (35). It clones and amplifies VH and VL gene fragments in human lymphocyte spectrum by RT-PCR, and randomly combines gene fragments into expression vectors, in order to construct a large-capacity human antibody library (27). In addition, the phage display can simplify the cloning process and acquire a large amount of material to produce peptides or proteins because of the small size of the phage genome and high efficiency of the phage infection (67,68).

The phage display offers the direct physical link between a protein and its genetic material, which helps people to effectively screen the desired cloning again and again, and then amplify it (67). In the process of library screening, specific phage clones are enriched continuously due to their specific affinity for ligands, and relatively rare clones that can bind ligands can be quickly and effectively screened out from a large library (58). Therefore, the biggest advantage of phage display is that once the phage library is established, specific antibodies against the target antigen can be directly screened from the library according to the needs within 23 weeks, which greatly reduces the preparation cycle of mAbs (61). In addition, by specific construction, the filamentous phage may act as a vector, and generate a peptide library of phage display that contains hundreds of millions of unique peptides, which are conducive to their application in antiviral research (69). However, due to the different binding properties of antibodies in bacteria and eukaryotic cells, the applicability of the technology is limited to a certain extent (70). The processes of the phage display refer to bacterial transformation, phage packaging, and even transmembrane secretion processes, which limit the capacity of the phage display library and their molecular diversity. At present, the capacity of the phage display library is usually 10^{11} (27). Also, limited by the expression system, the antibody library is not large enough to support the acquisition of some rare antibodies and not all sequences are well expressed in phages, because the realisation of some protein functions acquire folding, transportation, membrane insertion and complexation, resulting in the need for additional selection pressure during *in vivo* screening (45). It is difficult to obtain antibodies that inhibit the growth or function of phages or the expression host, as the phage display system depends on the expression of intracellular genes, which may make the diversity of the library decrease rapidly (71). Also, a phage display library cannot take on the effective mutation and recombination *in vitro*, which in turn limits the genetic diversity of the molecules in the library (72,73). Nevertheless, these temporary shortcomings cannot obscure the great potential of its applications.

Clinical therapeutic applications. Nowadays, with the establishment of more phage display libraries, the construction of advanced genetic operating system and the development of more efficient phage display systems, phage display technology plays an important role in different fields, especially in protein and antibody-related fields (34). Phage display technology has become an advantageous tool for detecting the protein spatial structure, exploring the binding sites between receptors and ligands, and searching for ligands with high affinity and biological activity (74). It has had a far-reaching impact on research into the mutual recognition of protein molecules, the preparation of phage-functionalised biosensors, the development of new vaccines and tumour therapy (75,76). In addition, Humira, ramucirumab and other mAbs developed by phage display technology have been widely used in clinical practice,

especially Humira, which is widely applied in the treatment of rheumatoid arthritis (RA), psoriatic arthritis, Crohn's disease, ankylosing spondylitis and uveitis (45).

Transgenic animal technology

Development process. Transgenic animal technology is mainly based on the idea of 'why can't mice be more like people'. It transfers human antibody loci into animals including mice, chickens and cows, and rearranges and re-expresses human antibody V region genes in their lymphocytes, so that transgenic animals can produce B lymphocytes which fully express human antibodies; this is essentially the partial humanisation of animals (77-79). Under the stimulation of antigens, these lymphocytes can be cloned and differentiated continuously to form plasma cells that are capable of producing high-affinity human antibodies (80). In addition, transgenic animals carrying human DNA fragments have complete functions, including effective homologous conversion and affinity maturation, which can produce high affinity human antibodies after the animals are immunised by any target antigen (37).

As early as 1985, the production of fully human antibodies using transgenic mice was first proposed by Alt *et al* (81). Later, many difficulties, including the large size of the human Ig loci, were followed but overcome one after another (82). In 1996 and 1997, Medarex and Abgenix successfully established the HuMab-Mouse® (Medarex), which significantly improved the efficiency of full human mAb production (83) and XenoMouse™ (Abgenix) (84,85).

Advantages, disadvantages and clinical therapeutic applications. In the past three decades, transgenic animal technology through genetic engineering has been envisaged to improve food quality, animal production and the production of biological products, to reduce or minimise the environmental impact of animal production and to add value to animal products. Recently, with the advance of the ability for targeted genome engineering via genome editing methods such as TALENs, ZFN and the CRISPR/Cas9 system, this technique has been widely used to obtain a series of human mAbs against the interleukin-6 (IL-6) receptor, TNF receptor and epidermal growth factor receptor (EGFR), which play important roles in the treatment of tumours and other diseases (86,87). mAbs against the human IL-6 receptor can show strong antitumour activity *in vivo* against multiple myeloma cells by inhibiting IL-6 functions (87). H-R3, as a humanised anti-EGFR antibody with antitumour, anti-proliferative, anti-angiogenic and pro-apoptotic properties, can act as an effective EGFR antagonist to inhibit signal transduction, in order to directly or indirectly affect cell proliferation, cell survival and angiogenesis-inducing capacity (88). As an important milestone in validating XenoMouse strains as well as other human immunoglobulin-producing mouse technologies, the first fully human mAb, panitumumab, which was developed from XenoMouse technology and approved by a regulatory agency, has a positive risk-benefit profile in advanced, chemotherapy refractory colorectal tumours and has the potential to increase treatment rates of this disease in earlier lines of therapy (89). Recently, the first transgenic rabbit strain for human antibody production has been created with the discovery that the antibody diversification mechanism at work in rabbits can act on the fragments of the human transgenic immunoglobulin gene (90), which further expands the application of human mAbs in drug development and promises to lead to new treatments for various diseases.

Ribosome display technology. Ribosome display technology is a powerful tool for protein screening using functional protein interactions *in vitro* (91). By associating genotypes with protein phenotypes, it can use specific ligands of target proteins to select target proteins and corresponding gene sequences from the protein display library (92). It combines the correctly folded protein and its mRNA on the ribosome at the same time to form mRNA-ribosome-protein trimer, in order to screen some high-affinity proteins with specific binding to target molecules, including antibodies, peptides and enzymes (93,94). The preparative technique involves different key processes, including specific processing and modification of the DNA that encodes proteins, transcription and translation, affinity screening *in vitro*, the separation of mRNA and molecular orientation evolution *in vitro* (95).

Development process. The ribosomal display technology has undergone a certain period of research from the time it was proposed to the time it was developed and matured. In 1994, Mattheakis *et al* of the Afflymax Institute in the US put the ideas of their predecessors into practice for the first time and established the prototype of ribosome display technology, which mainly used 'polypeptide-polyribosome-mRNA' complex to construct peptide libraries on polyribosomes, thus screening the polypeptide ligands of immobilized mAbs with an affinity constant of 10^9 (Nmol level) from a peptide library with a capacity of 10^{12} (96). Later, Hanes and Plückthun improved the polyribosome display technology and established a new technology, ribosome display technology, in 1997, for the screening of complete functional proteins such as antibodies *in vitro*, on the basis of previous research results (93).

Advantages and disadvantages. Traditional screening techniques have insurmountable drawbacks, mainly related to cell transfection, phage packaging, transmembrane secretion and protein degradation in library construction and screening (97,98). The library capacity and molecular diversity of phage or mRNA display technology are somewhat limited, which reduces the efficiency of library screening (71). In contrast, a ribosome display is a powerful way to screen large libraries and acquire molecular evolution (99,100). It has the advantages of simple library construction, large library capacity, strong molecular diversity, simple screening methods and no need for selection pressure, and can even improve the affinity of target proteins by introducing mutation and recombination technology (93,94). As a system to produce and screen folded proteins entirely *in vitro*, the ribosome display technique is shown to greatly exploit replicability of mRNA, allowing efficient enrichment of target genes, avoiding the step of bacterial transformation and making the technique unconstrained by the efficiency of cellular transformation (101). On the one hand, the technique greatly increases library capacity and screening throughput, and makes it easy to build a very large volume of antibody library (101). On the other hand, while the expressed proteins have the correct spatial fold conformation, the technique can be combined with

some special PCR techniques to improve the protein expression diversity (28,102,103). It also can be used for the screening and research of cytotoxic fractions (94).

However, there are still some technical problems that need to be further advanced. Undoubtedly, maintaining mRNA stability and preventing the degradation of mRNA is the first problem in a ribosome display system (28). Facing the problem, Yamaguchi *et al* reported a novel screening method-cDNA display, which prevents the degradation of mRNA by promoting the binding of mRNA to linkers and the reverse transcriptional synthesis of cDNA, thus converting mRNA-protein fusions to cDNA-protein fusions and avoiding problems due to the stability of mRNA (104). The use of modified nucleotides as substrates for transcription reactions can also stabilise mRNA (105). In addition, how to construct the more stable 'mRNA-ribosome-protein' complex was one of the problems (106), as it only occurs in cases where the complex is complicated but its stability is poor in practice due to ribosomal display. To solve the problem, Roberts and Szostak developed a simpler and more effective display system-mRNA display system, which allows mRNA to bind to its encoded polypeptide in the presence of puromycin to form a stable mRNA-peptide complex that screens for the target peptide (107). The anti-small stable RNA A (anti-SsrA) oligonucleotides were designed by Muranaka *et al* to inhibit the function of SsrA and obviously promote the form of the 'mRNA-ribosome-protein' complex (108). In addition, how to improve the display of large molecular protein in the ribosome is also a problem that needs to be solved.

Clinical therapeutic applications. At present, there are numerous reports on the preparation of human mAbs by ribosomal display technology, and the advantages of this technology represent the developmental direction of mAbs (28). On the one hand, it can be applied widely in antibody engineering, proteomics, epitope mapping, and synthetic enzymes (93,103). On the other hand, it also opens up a new way to screen new therapeutic antibodies and new drugs for diagnosis and treatment in tumours, autoimmune diseases, infectious diseases, and inflammatory disorders (94). Ribosome display technology, as a new cloning display technology, will show a more extensive application space in protein interaction research, new drug development and proteomics (95,109).

A single B-cell monoclonal antibody generation technology

Development process. As early as 2003, Wardemann *et al* prepared autoreactive antibodies with the use of early human B-cell precursors isolated from the bone marrow, to examine the structure, development and silencing of autoreactive B cells (110). In 2004, Traggiai *et al* immortalised the isolated human memory B cell with EBV and screened 35 mAbs that were well neutralised against influenza virus, which makes it possible for memory B cells to produce mAbs (111). Since then, monoclonal B-cell technology for generating mAbs has been gradually applied to various experimental research and has made great contributions to the development of many fields of life science as an important tool for modern life science research.

Advantages and disadvantages. In recent years, single B-cell antibody preparation techniques have begun to spring up and have gradually become widely used, alongside the development of molecular cellular biology (112). This is because mAbs prepared by single B-cell antibody technology have the characteristics of full human origin, high specificity and uniformity, showing unique advantages and good application prospects in the treatment of pathogenic microbial infections, tumours, autoimmune diseases and organ transplantation (113,114).

Compared with other mAb preparation techniques, monoclonal B-cell technology, is a technique for the cloning and expression of B-cell antibodies with single antigenic specificity *in vitro*, which preserves the natural pairing in the V region of the light and heavy chain, and has the advantages of good gene diversity, high efficiency, full humanisation and the small number of cells required (115). Studies have shown that human memory B cells can survive in humans for more than 50 years, providing a historical record of the specific antibodies produced during most of the host's lifetime (116). In contrast, antibodies in the body fluids used in most traditional methods usually decay after macroglobulin clearance, which means that people lose their protective antibody within a few years (117). In addition, it is proposed that memory B cells in the blood of virus-infected patients may store records of early infection with the virus in patients-the genes, which provide a new direction for the research and development of mAbs (118).

Therapeutic application in clinical. Currently, the preparation of memory B-cell antibodies has become a popular method used to prepare the humanised antibody, which also promotes immunological research including antibody affinity maturation, the defence mechanism against vaccine immunity, vaccine development, and the treatment of tumours and autoimmune diseases at the same time (1,119). With the maturity and improvement of B-cell sorting technology, subsequent PCR gene amplification methods and the high-throughput analysis and identification of antibody genes, memory B-cell antibody preparation technology will play an unprecedented important role in the diagnosis, pharmacodynamic and clinical application in the future, leading to a new era of therapeutic antibody research (120-122). As this approach has also been successful in widely isolating neutralizing antibodies against viruses including SARS and H5N1 influenza, it can provide not only neutralizing antibodies for passive serum therapy, but also information for vaccine design, and is expected to accelerate the development of therapeutics in the field of infectious diseases (119,123). Additionally, Wrammert *et al* produced anti-H1N1 antibodies in 2009 by isolating plasma cells from peripheral blood, to analyse the characteristics of antibodies in detail generated from plasma blasts induced by pandemic H1N1 infection (124).

4. Application of monoclonal antibodies in tumour therapy

Mechanisms of monoclonal antibodies as antitumour drugs. Traditionally, mAbs produce cytotoxic effects in tumour cells though ADCC, CDC, changing signal transduction, elimination of the cell-surface antigen, and targeted conveying payloads (125).

ADCC. In general, ADCC is achieved by the specific binding of mAbs and the targeted antigen of tumour cells (126).

Namely, the Fc fraction of mAbs can bind to the receptor of immune effector cells (NK cells, macrophages, neutrophils, granulocytes), and achieve activation of intracellular signals in the next moment, resulting in ADCC (126,127). NK cells activated by antibodies can release cellular cytotoxic granules (perforin and granzyme) to achieve cell apoptosis on the one hand, while they can release cytokines and chemokines to inhibit cell proliferation and angiogenesis on the other (128).

CDC. CDC refers to the cytotoxic effect involving complement; that is, after the binding of specific mAbs and the corresponding antigens on the surface of the cell membrane, the complex activates the classical pathway of complement and forms an attack membrane complex to induce a lysis effect on target cells (127,129). It is worth noting that, although CDC does not directly preside over the antitumour effects of most mAbs, it produces a variety of factors that enhance ADCC (130,131).

Changing signal transduction. Almost every clinically effective unconjugated mAb, directly or indirectly, interferes with the signal transduction that influences the proliferation and survival of targeted cell populations (132). Growth factor receptors are some of the most commonly targeted tumour-associated antigens whose activation under normal conditions induces mitotic reactions and promotes cell survival, are overexpressed in numerous malignancies, leading to promotion of tumour cell growth and insensitivity to chemotherapy drugs (133-135). Therefore, the use of mAbs is likely to normalise the cell growth rate and restore the sensitivity of cells to cytotoxic drugs by reducing the signal that passes through these receptors (136). For example, the pertuzumab block shows receptor heterodimerisation (the dimerisation of HER2 with HER3 and other HER family receptors) that is required for signal transduction to play an antitumour role (130,137).

Application of monoclonal antibodies in molecular-targeted therapy. Targeted antitumour drugs provide a new concept concerning tumour therapy, referring to several tumour-associated signaling pathways and targets. For instance, the most common target antigens in solid tumours refer to epithelial cell adhesion molecule (Ep-CAM; also known as epithelial glycoprotein-2, EGP-2/GA 733-2), carcinoembryonic antigen (CEA), EGFR family including EGFR (also known as c-erbB-1), HER2/neu (c-erbB-2), HER3 (c-erbB-3) and HER4 (c-erbB-4) (135,138,139). Compared with them, the mAbs applied in lymphoma usually target CD52, CD20, CD30, CD22, CD37 and CD79 (135,136), with the easier achievement of a better effect because it is simpler to manage tumour penetration. In contrast to the above, tumour stroma and tumour vasculature offer some unique targets for antibody-based interaction because the new generation of tissue and vasculature show some components that differ from those in the normal situation, leading to the situation whereby fibroblast activation protein (FAP) (140) and tenascin-C (TNC) (141) are regarded as targets in the tumour stroma, Fibronectin ED-B (142) and prostate-specific membrane antigen (PSMA) (143) are regarded as targets in the tumour vasculature. In addition, ligands including vascular endothelial growth factor (VEGF) are thought to target cell-surface receptors expressed on tumour cells or their supporting tissue (140,144).

Application of monoclonal antibodies in immune checkpoint therapy (ICI). The fight between the immune system and tumour cells is a long-term dynamic process, which has both positive and mutual influences (145). In the process whereby a healthy individual's immune cells detect and kill tumour cells via the antitumour immune response, activated T cells cause upregulated expression of several surface receptors, which can bind with relevant ligands expressed highly on the surface of tumour cells, resulting in inhibition of the immune response and downregulation of potent immune response (146). These surface receptors, namely the suppressive regulatory molecules, are essential for maintaining self-tolerance, preventing autoimmune response and minimizing tissue damage by regulating the time and intensity of the immune response; this is called the immune checkpoint (146,147). The immune checkpoint results in the inhibition of cellular function, meaning that the body cannot produce an effective antitumour immune response, ultimately leading to immune escape of the tumour (148).

On the theoretical basis of the immune checkpoint, some mAbs have been developed as immune checkpoint inhibitors to block the interaction between tumour cells expressing immune checkpoints and immune cells, in order to block the inhibitory effect of tumour cells on immune cells (148,149). During the occurrence and development of tumours, immune checkpoint inhibitors can enhance the immune function of the body and restore the recognition ability of T cells, in order to eliminate tumours or slow down the development of tumours (150,151) (Fig. 3). Recently, tumour-related immune checkpoint molecules mainly include programmed death-1 (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), T-cell immunoglobulin mucin 3 (TIM3) and lymphocyte activation gene-3 (LAG3) (145,146,150).

Anti-CTLA-4. CTLA-4 (also known as CD152) is a transmembrane protein expressed on the surface of activated $CD4^+$ T cells, activated $CD8^+$ T cells, and regulatory T cells (Treg cells), which can bind to the CD86(B7-2) and CD80(B7-1) ligand to negatively regulate T-cell activation (152). In addition, there is an intracellular pool of CTLA-4 in recycling endosomes of Treg and memory T cells which can be rapidly cycled to the cell surface upon activation (153). Therefore, the anti-CTLA-4 mAb can decrease Treg cells and activate T cell immune response by terminating the activity of CTLA-4 (145). Ipilimumab is an mAb that blocks CTLA-4, which was approved by the FDA to treat melanoma in 2011 (150,154). The blocking effect of ipilimumab on CTLA-4 is, that Ipilimumab binds to CTLA-4 to further impede the interaction between CTLA-4 receptor and the B7 ligand, thus increasing the activation and proliferation of T cells (145). This means that the antitumour effect of ipilimumab on melanoma is indirect and the action mechanism may be to help the body's immune system recognize, target and attack melanoma cells (155). Hence, it is possible for ipilimumab to be used as a carrier of imaging agent with good targeting properties (156). In addition, it is reported that patients with human CTLA-4 insufficiency develop immune dysregulation,

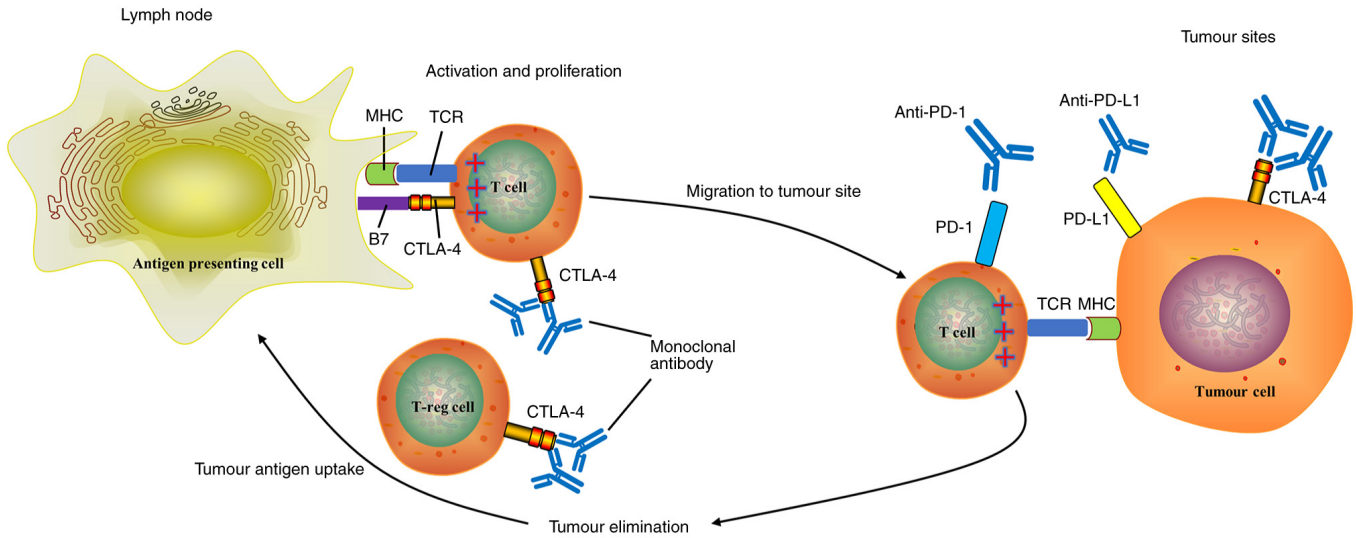

Figure 3. CTLA-4 and PD-1 pathway blockade in immune checkpoint therapy. CTLA-4 pathway blockade allows for activation and proliferation of more T cells, which can reduce Treg-mediated immunosuppression. PD-1 pathway blockade restores the activity of antitumor T cells that have become quiescent. A dual pathway blockade can take a synergistic effect, resulting in a more powerful and more lasting antitumor immune response. CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TCR, T-cell receptor; Treg, regulatory T cell.

lymphadenopathy and hepatosplenomegaly (153,157,158). Therefore, anti-CTLA-4 mAbs should be able to inhibit the inhibitory signalling pathway and maintain the killing function of T cells, thereby killing tumour cells (146).

Although some anti-CTLA-4 mAbs do not cause an adverse reaction similar to cytotoxic drugs, including myelosuppression and alopecia, due to the different actions of cytotoxic drugs, they cause pathological damage to the body while producing an antitumour reaction, namely immune-related adverse events (IRAEs) (146,149). Different from others, the toxicity profile of ipilimumab mainly manifests as symptoms associated with infusion, including rigor, pruritus, fatigue, nausea, dizziness, colitis, less frequently hypophysitis, hepatitis pneumonitis, and even hypotension, angioneurotic oedema, and dyspnoea (159-161). When facing the problems that occur in anti-CTLA-4 immunotherapy, combination therapy is put forward which may thereby provide greater antitumour activity than either agent alone by enhancing antitumour immune responses, and presenting a miraculous, unprecedented therapeutic effect (159). For example, in the treatment of progressive melanoma, the combined blocking of PD-1/PD-L1 and CTLA-4 can further improve efficacy in patients compared with the single blocking of PD-1/PD-L1 or CTLA-4 (162). Based on this, a trial involving patients with advanced melanoma was carried out and revealed that nivolumab plus ipilimumab provides the longer progression-free and overall survival, and better health-related quality of life than ipilimumab alone (159,163). From another perspective, CTLA-4 inhibition can synergize with local chemotherapy, improving applicability and sensitivity to immune-checkpoint inhibition (164).

At present, not only ipilimumab, but also some other anti-CTLA-4 mAbs, have been optimised and developed, and have now attracted more and more attention from the public (148). Another anti-CTLA-4 mAb, tremelimumab, which was exploited by Pfizer, is being investigated in clinical trials (165). However, the research into tremelimumab has made little progress, meaning that ipilimumab is still regarded as the most promising anti-CTLA-4 mAb applied in tumour treatment (148).

Anti-PD-1 and anti-PD-L1. PD-1 (also known as CD279), as a member of the immunoglobulin superfamily which is mainly expressed on the surface of activated T cells, can be used as an immunosuppressive molecule to regulate the immune system and promote self-tolerance by downregulating the response of the immune system to human cells and suppressing the inflammatory activity of T cells, which may prevent the immune system from killing tumour cells (166,167). PD-1 has at least two ligands, PD-L1 (also known as CD274 or B7-H1) and PD-L2 (CD273 or B7-DC) (146). In general, as one of the means by which human tissue protects them, PD-1 can bind with specific ligands on the immunocyte surface to prevent immune cells from activating and killing normal cells (166,168). Additionally, the binding of PD-1 with ligands promotes the programmed death of T cells and reduces the apoptosis of regulatory T cells by suppressing the T cell activation signal primed by the interaction between MHC and TCR, in order to make the tumour cells acquire immune escape (146). Certain types of malignant tumours express a mass of PD-1 on the cell surface; therefore, they evade the attack of immune cells by powerfully suppressing the activation of immune cells (129). PD-1 is also expressed on the surface of activated B cells and macrophages, indicating that PD-1 negatively regulates the immune response more widely than CTLA-4 (145). Therefore, immune regulation targeting of PD-1 plays an important role in antitumour, anti-infection, anti-autoimmune diseases and organ transplantation survival (169). Facing the situation, anti-PD-1 mAbs are manufactured as PD-1 inhibitors to activate the immune system to attack tumours and treat some types (145).

Compared with anti-CTLA-4 mAb therapy, immunotherapy with anti-PD-1 mAbs has a broader antitumour effect and fewer

overall side effects (170,171). The biggest difference between immune drugs and others is that it shows more persistent efficacy which could lead to long-term survival or even a clinical cure for patients with advanced disease (146). Currently, there are various drugs approved by the FDA, including nivolumab (also known as Opdivo) and pembrolizumab (also known as Keytruda) (172). The indications of nivolumab include melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), classical Hodgkin's lymphoma (CHL), squamous cell carcinoma of the head and neck (SCCHN), and urothelial carcinoma (148). In contrast, pembrolizumab is mainly applied in melanoma, NSCLC, and SCCHN (167,172).

At present, the anti-PD-1 mAbs are mostly used in combination therapies, because traditional therapy using anti-PD-1 mAbs alone can lead to various IRAEs, including fatigue, skin rash, colitis, hypophysitis, pneumonitis, myasthenia gravis and interstitial nephritis (171). For tumours that are highly dependent on the immunosuppressive mechanism of PD-1/PD-L1, such as malignant melanoma, Hodgkin's lymphoma, certain types of lung tumour, and colon tumours, it has shown remarkable efficacy (147), but for the vast majority of unselected solid tumours, the efficacy of PD-1 inhibitor alone is not high. In comparison, combination therapy improves the treatment effect and transforms patients who are not suitable for PD-1 inhibitor treatment into those who can benefit from it (173). First, the anti-PD-1 mAb can combine with another immunotherapy drug (174). A combination of the PD-1 and CTLA-4 mAbs has been shown to be more effective in treating several tumours, including malignant melanoma, than either antibody alone (162). In addition, PD-1 can combine with anti-CTLA-4 or VEGF tyrosine kinase inhibitors (TKIs) to adjust first-line therapy for metastatic kidney carcinoma (175). Secondly, PD-1 in combination with chemotherapy is considered a promising treatment strategy (176). Chemotherapy has a profound impact on the antitumour immune by directly regulating immune cellular subsets or indirectly stimulating the immune system through the induction of immunogenic cell death, revealing the existence of synergy between cytotoxic chemotherapy and immune checkpoint inhibition (176). This combination therapy is also approved for the first-line treatment of advanced NSCLC (176). Nevertheless, there are some patients with chemotherapy-refractory metastatic solid tumours; for those patients, PD-1 inhibitor combined with radiotherapy is regarded as a salvage treatment (177,178). This means that the anti-PD-1 may combine with radiotherapy to improve the overall survival (179). In addition, anti-PD-1 can combine with targeted drugs including acitinib, and levatinib (180,181). Recent studies have shown that the association of acitinib with pembrolizumab provides improved clinical benefit in patients with previously untreated advanced renal cell carcinoma, which is well tolerated (182). In addition, the strategy of using anti-PD-1 mAbs in combination with oncolytic virus (OV) to enhance antitumour immunity and therapy has been developed (183). To validate this, research into the effect of IL-15-armed OV in combination with PD-1 inhibitors in mice with colon or ovarian carcinoma processes, has shown some results including tumour regression and the prolongation of overall survival (184). Moreover, a personalised mutanome vaccine can be used in combination therapy with anti-PD-1 mAb as it enhances the persistence of anti-PD-1-mediated effect and extends anti-PD-1 therapies to patients with no preexisting T cell response (185). Finally, the anti-PD-1 mAb can combine with novel tumour-specific immune cells, such as the chimeric antigen receptor T-cell (CAR-T), to produce better therapeutic effects (186). Some research has found that PD-1 blocks CAR-T cell therapy within solid tumours, therefore the anti-PD-1 mAb which prevents the PD-1-related inhibition of CAR-T cell response can increase the levels of cytolysis and cytokine secretion and enhance the *in vivo* antitumour function of CAR-T cells (187,188).

PD-L1, expressed on the surface of tumour cells, can bind with PD-1 on the surface of activated T cells and B cells to conduct inhibitory signals and reduce T cell proliferation (146,189). As promising new agents, there are some anti-PD-L1 mAbs approved by the FDA in clinic, including atezolizumab (also known as Tecentriq) which is used to treat locally advanced or metastatic urothelial carcinoma, durvalumab (also known as Imfinzi) which is used to treat locally advanced or metastatic urothelial carcinoma and NSCLC, and avelumab (also known as Bavencio) which is used to treat meningioma, metastatic Merkel cell carcinoma and carcinoma of the urinary bladder (128,167,190). In theory, compared with anti-PD-1 mAbs which bind to PD-L2, anti-PD-L1 mAbs have specific effects and demonstrate a certain superiority (146). The anti-PD-L1 mAbs can block the co-suppression of B7-1 and PD-1, which is conducive to fully activate the function of T cells and produce cytokines (191,192). Therefore, anti-PD-L1 mAbs may more fully activate the immune system to kill tumours (191). Furthermore, it has been shown that durvalumab as a third-line or later treatment can significantly benefit advanced NSCLC patients with EGFR mutations or ALK rearrangements ($EGFR^+/ALK^+$) with ≥25% of tumour cells expressing PD-L1, although it is unsuitable for patients with $EGFR^+/ALK^+$ to use the immune checkpoint inhibitor because of the low curative effect and subsequent severe adverse reactions (193). In summary, anti-PD-1 mAbs and anti-PD-L1 mAbs each have their own indications and application scope, and the combined utilisation can achieve mutual complementarity in the interest of our common development (146).

Others. In addition, there are various other useful checkpoints, such as the lymphocyte activation gene-3 (LAG-3), B7-H3, B7-H4, T cell immunoglobulin-3 (TIM-3), T cell immunoglobulin and ITIM domain protein (TIGIT), and V-domain immunoglobulin-containing suppressor of T cell activation (VISTA) (194-197), which are either entering the clinic or under active development.

Potential combined therapeutic strategies

Radioimmunotherapy, chemo-immunoconjugate and immunotoxin. In tumour-guided therapy, mAbs against tumour antigens are used in the guidance of chemotherapy drugs or radiotherapy drugs to the target organ, thereby directly killing the tumour cells or producing antibody-directed enzyme prodrug therapy (ADEPT) by specifically activating prodrugs within the tumour (198).

Radioimmunoconjugates. Radiation can act directly on DNA molecules and cause their damage, by ionising water

molecules in living organisms to produce free radicals which break macromolecules and lead to cell damage (199). Based on this theory, mAbs with specific affinity for tumours can be utilised as a carrier of highly active radiopharmaceutical agents, thus forming radioimmunoconjugates that target tumour tissue to kill tumour cells or inhibit their growth while reducing radiation damage to normal tissue, using the ionising radiation effects of radioisotopes (11).

Chemo-immunoconjugate. The cytotoxic agent can conjugate with antitumour mAbs to form chemo-immunoconjugate, which can bind to the surface of antigen-positive tumour cells through the guidance of mAbs, inducing the internalisation of the conjugates (200). After that, these chemical drugs play their cytotoxic effects by binding to DNA molecules, thus killing tumour cells by inhibiting cell DNA and protein synthesis, interfering with cell nucleic acid or protein function, and inhibiting mitosis (201,202). Common conjugates include cisplatin, cyclophosphamide, etoposide, adriamycin, paclitaxel, methotrexate, and vinblastine (203,204).

Immunotoxin. Immunotoxin has a specific affinity for tumour cell surface antigens and can release bacterial or plant protein toxins to tumour cells without harming normal cells (205). Once the toxin enters the cell, it kills the tumour cell by inhibiting protein synthesis and altering signalling transmission (206). The main toxins currently used in reagents are diphtheria toxin, abrin, ricin, gelonin, and *Pseudomonas aeruginosa* endotoxin (198,207,208).

Antibody-drug conjugates (ADCs). Antibody-based immunotherapy has been a major and rational therapeutic strategy in the clinical management of oncology (209,210). In clinical practice, therapeutic mAbs have a limited effectiveness in the treatment of solid tumours due to their large molecular weight, but a high degree of targeting (198). With a few exceptions such as mAbs to HER2, EGFR and CD20, most mAbs can bind with effector molecules by using specific linkers to produce antibody-drug conjugates (ADCs), which expand the scope of medical treatment while possessing highly targeted selection, achieving the complementary advantages of the two therapeutic drugs, which have little antitumour effects after binding the target antigen (15,198). In contrast, small molecule chemicals are highly effective against tumour cells, in spite of the fact that they are less selective and may cause serious side effects, accidentally injuring normal cells due to off-target toxicity (211). Therefore, mAbs can bind with effector molecules by using specific linkers to produce ADCs, which expand the scope of medical treatment while owning highly targeted selection, achieving the complementary advantages of the two therapeutic drugs (17,135). The mAbs can bind with effector molecules by using specific linkers to produce ADCs, which expand the scope of medical treatment while possessing highly targeted selection, achieving the complementary advantages of the two therapeutic drugs that can usually target tumour-associated antigens or specific receptors on the surface of tumour cells and show a selective directing effect on tumour cells (135). The effector molecules that act as payloads which produce a killing effect on tumours include radiopharmaceutical agents, cytotoxic agents and bacterial or plant protein toxins, conjugating respectively with mAbs to form radioimmunoconjugates, chemo-immunoconjugates and immunotoxins used in tumour-guided therapy (198). Currently, ADCs that have been approved by the FDA include brentuximab vedotin, trastuzumab emtansine, gemtuzumab ozogamicin, Inotuzumab ozogamicin, and polatuzumab vedotin (211,212).

Action mechanism. Generally, ADCs are injected intravenously into the blood system to prevent the hydrolysis of mAbs by gastric acid and protease and are distributed into the tumour tissue by exosmosis of the endothelial pores and the endocytosis of endothelial cells (198). After the mAbs specifically direct drugs to the surface of tumour cells expressing tumour-specific antigens, ADCs come into tumour cells by internalisation (211,213). As a general rule, there are three distinguished pathways to internalise, including clathrin-mediated endocytosis, caveolae-mediated endocytosis and pinocytosis (211). Later, with the influence of the acidic environment of the cytoplasm, some ADCs with cleavable linkers release effector molecules which can damage tumour cells, while other ADCs undergo the enzymatic fracture of linkers or mAb degradation with the influence of lysosomal protease (214). Finally, payload and degradation products are released into the cytoplasm of tumour cells, disturbing their cellular action mechanism, affecting the tumour microenvironment and inducing the death of cells (213). As ADCs are formed from maytansinoid drugs which are derivatives of maytansine and huC242, humanised mAbs which bind to the CanAg antigen expressed on colorectal tumours, pancreatic tumours and certain NSCLCs, huC242-maytansinoid conjugates can be disintegrated with the influence of lysosomal acidic conditions in order to release the maytansinoid, which contributes greatly to the antitumour effect of conjugates and the bystander effect (214). If the released payload is permeable, a bystander effect is produced, which means that the internalised payload enters and kills adjacent tumour cells, showing the effect of apoptotic tumour cells on bystander tumour cells (215). Although ADCs cannot directly kill the adjacent antigen-negative tumour cells, they can kill the antigen-positive tumour cells in order to indirectly kill the adjacent tumour cells by the bystander effect (216). Not only do ADCs damage the growing tumour, they can also disrupt the structure supporting tumour growth, such as tumour stromal cells and tumour vessels, in order to enhance the antitumour effect (217). More importantly, it was proposed that the activation of bystander effects by apoptotic tumour cells may be crucial to achieving the permanent eradication of tumours (215).

Basic strategies for selecting monoclonal antibodies. As an important part of ADCs, mAbs used in tumour-guided therapy must have some special characteristics. First, the ideal mAb must effectively bind to antigens on target cells so that the cytotoxic drugs are concentrated at the site of the tumour (212). Secondly, the mAbs should bind selectively to tumour cells and have little cross-reactivity with normal cells (212). If the antibody selectivity is poor or the selected antigen is present in normal tissues, cytotoxic drugs will be delivered to normal cells, resulting in targeted toxicity including allergic reactions, rashes and alopecia (218,219). In addition, the Fc fractions of some mAbs

Table I. Monoclonal antibody drugs approved by FDA from 1986 to November 2021.

Trade name (generic name)	Company	Disease target	Antibody target	FDA approval date	Clinical trials
Othoclone (muromomab)	Ortho Biotech	Allotransplantation	CD3	1986	NCT01932554
Reopro (abciximab)	Centocor	Cardiovascular disease (CVD)	Glycoprotein IIβ/IIIα	12/22/1994	NCT01932554
Panorex (edrecolomab)	Centocor	Tumour	Glycoprotein 17-1A	1995	NCT00002968
Rituxan (rituximab)	IDEC/Genentech	Non-Hodgkin's lymphoma	CD20	11/26/1997	NCT02433522
Zenapax (daclizumab)	PDL	Renal transplantation	CD25	12/10/1997	NCT01051349
Simulect (basiliximab)	Novartis	Renal transplantation	CD25	5/12/1998	NCT00724022
Synagis (palivizumab)	MedImmune	Respiratory tract infection (RTI)	F protein of RSV	6/19/1998	NCT02442427
Remicade (infliximab)	Centocor	Rheumatoid arthritis (RA)	TNF-α	8/24/1998	NCT02096861
Herceptin (trastuzumab)	Genentech	Breast carcinoma	HER-2	9/25/1998	NCT00045032
ENBREL (etanercept)	Amgen/Wyeth	RA and psoriasis	TNFR-Fc	11/2/1998	NCT02376790
Mylotarg (gemtuzumab)	Celltech/Wyeth	Leukaemia	CD33	5/17/2000	NCT00927498
Campath (alemtuzumab)	Ilex/Millennium Pharmaceuticals/Berlex	Lymphoma	CD52	5/7/2001	NCT00530348
Zevalin (ibritumomab)	IDEC	Lymphoma	CD20-Y90	2/19/2002	NCT00220285
Humira (adalimumab)	CAT/Abbott	RA	TNF-α	12/31/2002	NCT02745080
Raptiva (efalizumab)	Xoma/Genentech	Psoriasis	CD11a	10/27/2003	NCT00256139
Bexxar (tositumomab)	Corixa Corp. and GlaxoSmithKline	Non-Hodgkin's lymphoma	CD20	6/27/2003	NCT00022945
Xolair (omalizumab)	Genentech/Tanox/Novartis	Moderate to severe allergic asthma	IgE	6/20/2003	NCT01157117
Avastin (bevacizumab)	Genentech	Colorectal carcinoma	VEGF	2/26/2004	NCT00976911
Erbitux (cetuximab)	ImClone/BMS	Colorectal carcinoma	EGFR	2/12/2004	NCT01228734
Tysabri (natalizumab)	Biogen Idec	Multiple sclerosis	α4-integrin	11/23/2004	NCT02730455
Lucentis (ranibizumab)	Genentech	Neovascular age-related macular degeneration	VEGF-A	6/30/2006	NCT01489189
Vectibix (panitumumab)	Amgen	Colorectal carcinoma	EGFR	9/27/2006	NCT01328171
Soliris (eculizumab)	Alexion Pharmaceuticals	Paroxysmal nocturnal haemoglobinuria (PNH)	Complement protein 5a	3/16/2007	NCT00122330
Cimzia (certolizumab)	UCB	Moderate to severe RA in adults	TNF-α	4/22/2008	NCT01087788
Ilaris (canakunumab)	Novartis Pharmaceutical Corp.	Cryopyrin-associated periodic syndrome	IL-1β	6/17/2009	NCT01327846
Stelara (ustekinumab)	Centocor Orth Biotech Inc.	Psoriasis	IL-12/23	9/25/2009	NCT01369355
Arzerra (ofatumumab)	Genmab and GSK	Chronic granulocytic leukaemia	CD20	10/26/2009	NCT01457924
Actemra (tocilizumab)	Genentech Inc.	Rare childhood arthritis	IL-6 receptor	1/8/2010	NCT01331837
Prolia (denosumab)	Amgen Inc.	Osteoporosis in postmenopausal women	IgG-2	6/1/2010	NCT00523341
Benlysta (belimumab)	HGS and GSK	Systemic lupus erythematosus in adult patients	BLyS	3/9/2011	NCT01639339
Yervoy (ipilimumab)	Bristol Myers Squibb Co.	Metastatic melanoma	CTLA-4	3/25/2011	NCT02899299

Table I. Continued.

Trade name (generic name)	Company	Disease target	Antibody target	FDA approval date	Clinical trials
Adcetris (brentuximab)	Seattle Genetics, Inc.	Hodgkin lymphoma (HL) and recurrent anaplastic large cell lymphoma	CD30	8/19/2011	NCT01100502
Perjeta (pertuzumab)	Genentech Inc.	End-stage breast carcinoma	HER-2	6/9/2012	NCT00545688
Abthrax (raxibacumab)	Human Genome Science Inc.	Inhalational anthrax	Anthracis Toxin	12/17/2012	NCT02339155
Kadcyla (ado-trastuzumab emtansine)	Genentech, Inc.	Breast carcinoma	HER2	2/22/2013	NCT02675829
Simponi Aria (golimumab)	Janssen Biotech, Inc.	RA	TNF	7/23/2013	NCT02846545
Gazyva (obinutuzumab)	Genentech	Chronic lymphocytic leukaemia (CLL)	CD20	11/5/2013	NCT02242942
Sylvany (siltuximab)	Janssen Biotech	Multicentre Castleman disease	IL-6	4/23/2014	NCT01024036
Entyvio (vedolizumab)	Takeda Pharmaceuticals USA	Ulcerative colitis and Crohn's disease	α4β7 integrin	5/20/2014	NCT00783718
Keytruda (permbrolizumab)	Merck Sharp & Dohme	Non-small cell lung carcinoma and head-neck tumours	PD-1	9/4/2014	NCT02775435
Cyramza (ramucirumab)	Eli Lilly and Co.	Advanced stomach cancer, adenocarcinoma of gastroesophageal junction	VEGFR2	11/7/2014	NCT01170663
Cosentyx (ecukinumab)	Novartis	Plaque psoriasis	IL-17a	1/21/2015	NCT02745080
Unituxin (dinutuximab)	United Therapeutics Corp.	Neuroblastoma in children	PD-L1	3/10/2015	NCT01767194
Praluent (alirocumab)	Sanofi	Decrease LDL-C	PCSK9 inhibitor	7/24/2015	NCT01663402
Repatha (evolocumab)	Amgen	Decrease LDL-C	PCSK9 inhibitor	8/27/2015	NCT02392559
Praxbind (idarucizumab)	Boehringer Ingelheim	Anti-coagulating effect therapy	Dabigatran	10/16/2015	NCT02104947
Nucala (mepolizumab)	GlaxoSmithKline	Severe asthma	IL-5	11/4/2015	NCT01000506
Darzalex (daratumumab)	Johnson and Johnson	Multiple myeloma	CD-38	11/16/2015	NCT03277105
Portrazza (necitumumab)	Eli Lilly and Co.	Squamous non-small cell lung cancer	EGFR	11/24/2015	NCT00981058
Empliciti (elotuzumab)	Bristol-Myers Squibb	Multiple myeloma	SLAMF7 protein targeted	11/30/2015	NCT01239797
Anthim (obiltoxaximab)	Elusys Therapeutics Inc.	Inhalational anthrax	/	3/18/2016	NCT01932242
Taltz (ixekizumab)	Eli Lilly and Co.	Moderate-to-severe plaque psoriasis.	IL-17A	3/22/2016	NCT02757352
Cinqair (reslizumab)	Teva Respiratory LLC	Asthma	IL-5	3/23/2016	NCT0250162
Tecentriq (atezolizumab)	Genentech	Non-small cell lung cancer and urothelial carcinoma	PD-L1	5/18/2016	NCT02425891
Zinbryta (daclizumab)	Biogen	Multiple sclerosis	IL-2	5/27/2016	NCT01797965
Lartruvo (olaratumab)	Eli Lilly and Co.	Soft-tissue sarcoma	PDGFR-α	10/19/2016	NCT01185964
Zinplava (bezlotoxumab)	Merck Sharp Dohme	*Clostridium difficile* infection	*Clostridium difficile* toxin A and B	10/21/2016	NCT01513239
Siliq (brodalumab)	Valeant	Plaque psoriasis	IL-17R	2/15/2017	NCT01708629
Bavencio (avelumab)	Pfizer and Merck & Co	Ovarian and gastric cancer	PD-1	3/23/2017	NCT02603432

Table I. Continued.

Trade name (generic name)	Company	Disease target	Antibody target	FDA approval date	Clinical trials
Dupixent (dupilumab)	Sanofi and Regeneron Pharmaceuticals	Atopic dermatitis	IL-4Rα	3/28/2017	NCT02414854
Ocrevus (ocrelizumab)	Roche Holdings	Multiple sclerosis	CD20	3/28/2017	NCT02545868
Imfinzi (durvalumab)	Astrazeneca	Metastatic urothelial carcinoma	PD-L1	5/1/2017	NCT03043872
Kevzara (sarilumab)	Sanofi and Regeneron Pharmaceuticals	RA	IL-6R	5/22/2017	NCT01768572
Tremfya (guselkumab)	Johnson and Johnson	Plaque psoriasis	IL-23	7/13/2017	NCT03162796
Besponsa (inotuzumab ozogamicin)	Pfizer	Acute lymphocytic leukaemia	CD22	8/17/2017	NCT01535989
Fasenra (benralizumab)	Astrazeneca AB	Severe asthma	IL-5	11/14/2017	NCT03557307
Hemlibra (emicizumab)	Roche Group	Type A von Willebrand disease	FIXa-FX	11/16/2017	NCT03020160
Trogarzo (ibalizumab-uiyk)	TaiMed Biologics	HIV	CD4+T cell receptor	3/6/2018	NCT02475629
Ilumya (tildrakizumab)	SUN Pharma	Psoriasis	IL-23	3/20/2018	NCT02980692
Crysvita (burosumab-twza)	Ultragenyx Pharmaceutical	Rhachitis	FGF23	4/17/2018	NCT02915705
Aimovig (erenumab-aooe)	Amgen Inc.	Migraine	CGRP	5/17/2018	NCT02066415
Poteligeo (mogamulizumab-kpkc)	Kyowa Kirin	Granuloma fungoides and Sézary syndrome	CCR4 (cellular chemokine receptor type 4)	8/8/2018	NCT01728805
Takhzyro (lanadelumab)	DYAX Corp.	Types I and II hereditary angioedema	Kallikrein	8/23/2018	NCT02586805
Lumoxiti (moxetumomab pasudotox-tdfk)	AstraZeneca	Hairy cell leukaemia	CD-22	9/13/2018	NCT01829711
Ajovy (fremanezumab)	Teva	Episodic migraine	Calcitonin gene-related peptide (CGRP)	9/14/2018	NCT02621931
Emgality (galcanezumab)	Eli Lilly and Co.	Episodic migraine	CGRP	9/27/2018	NCT02614261
Libtayo (cemiplimab-rwlc)	Sanofi and Regeneron	Cutaneous squamous cell carcinoma	PD-L1	9/28/2018	NCT03836105
Gamifant (emapalumab-lzsg)	Swedish Orphan Biovitrum AB	Haemophagocytic lymphohistiocytosis	IFNγ	11/20/2018	NCT01818492
Ultomiris (ravulizumab)	Alexion Pharm	PNH	Complement (C5 protein)	12/21/2018	NCT02946463
Cablivi (caplacizumab-yhdp)	Ablynx	Acquired thrombotic thrombocytopenic purpura	von Willebrand factor	2/6/2019	NCT02553317
Evenity (romosozumab-aqqg)	Amgen	Osteoporosis	Slerostin	4/9/2019	NCT01631214
Skyrizi (risankizumab-rzaa)	AbbVie	Plaque psoriasis	IL-23	6/10/2019	NCT04433442

Table I. Continued.

Trade name (generic name)	Company	Disease target	Antibody target	FDA approval date	Clinical trials
Polivy (polatuzumab vedotin-piiq)	Genentech and Roche Group	Diffuse large B-cell lymphoma	CD79b	6/10/2019	NCT05006534
Beovu (brolucizumab-dbll)	Novartis	Wet type age-related macular degeneration	VEGF	10/7/2019	NCT04690062
Adakveo (crizanlizumab-tmca)	Novartis	Sickle cell anaemia	P-selectin	11/15/2019	NCT05020873
Padcev (enfortumab vedotin-ejfv)	Astellas	Refractory bladder cancer	Nectin-4	12/18/2019	NCT05014139
Enhertu (fam-trastuzumab deruxtecan-nxki)	Daiichi Sankyo Pharmaceutical	Metastatic HER2-positive breast cancer	HER2	12/20/2019	NCT05113251
Tepezza (teprotumumab-trbw)	Horizon Pharma	Thyroid eye disease	IGF-1R	1/21/2020	NCT05002998
Vyepti (eptinezumab-jjmr)	Lundbeck	Migraine in adults	CGRP	2/21/2020	NCT04921384
Sarclisa (isatuximab)	Sanofi	Multiple myeloma	CD38	3/2/2020	NCT04802031
Trodelvy (sacituzumab govitecan-hziy)	Gilead	Metastatic triple-negative breast cancer	Trop-2	4/22/2020	NCT04559230
Uplizna (inebilizumab-cdon)	Viela Bio	Neuromyelitis optica spectrum disorder	CD19	6/11/2020	NCT02200770
Monjuvi (tafasitamab-cxix)	MorphoSys and Incyte	Relapsed or refractory diffuse large B-cell lymphoma	CD19	7/31/2020	NCT04680052
Blenrep (belantamab mafodotin-blmf)	GlaxoSmithKline	Multiple myeloma	BCMA	8/5/2020	NCT05002816
Enspryng (satralizumab-mwge)	Roche	Neuromyelitis optica spectrum disorder	IL-6R	8/14/2020	NCT04660539
Inmazeb (atoltivimab, maftivimab, and odesivimab-ebgn)	Regeneron Pharmaceuticals	Ebola virus	/	10/14/2020	/
Danyelza (naxitamab-gqgk)	Y-Mabs Therapeutics	High-risk refractory or relapsed neuroblastoma	Gangliosides GD2	11/25/2020	NCT04909515
Margenza (margetuximab)	Macrogenics	HER2-positive breast cancer	HER2	12/16/2020	NCT04425018
Ebanga (ansuvimab-zykl)	Ridgeback Biotherapeutics	Ebola virus	Glycoprotein	12/21/2020	NCT05067166
Evkeeza (evinacumab-dgnb)	Regeneron Pharmaceuticals	Homozygous familial hypercholesterolemia	ANGPTL3	2/11/2021	NCT04863014
Jemperli (dostarlimab-gxly)	Glaxosmithkline	Endometrial cancer	PD-1	4/22/2021	NCT04581824
Zynlonta (loncastuximab tesirine-lpyl)	Adc Therapeutics Sa	Relapsed or refractory large B-cell lymphoma	CD19	4/23/2021	NCT03589469
Aduhelm (amivantamab-vmjw)	Janssen Biotech	Non-small cell lung cancer	EGFR/MET	5/21/2021	NCT04606381

Table I. Continued.

Trade name (generic name)	Company	Disease target	Antibody target	FDA approval date	Clinical trials
Zynlonta (aducanumab-avwa)	Biogen Inc	Alzheimer's disease	Amyloid beta-protein	6/7/2021	NCT02484547
Saphnelo (anifrolumab-fnia)	AstraZeneca	Moderate-to severe systemic lupus erythematousus	IFN receptor	7/30/2021	NCT04877691
Tivdak (tisotumab vedotin-tftv)	Seagen	Recurrent or metastatic cervical cancer with disease progression on or after chemotherapy	Tissue factor	9/20/2021	NCT03657043

should have the affinity to bind with the Fc receptor of immune cells, thereby activating the killer effect of immunocytes (211). Thirdly, mAbs must own the ability to induce the internalisation of tumour cells, resulting in the release of the payload in the cytoplasm (220). In this way, the cytotoxic molecule can be released to extend the extent of damage to tumour cells, while mAbs play a certain antitumour role (212). Fourth, mAbs should be optimised to significantly reduce the non-specific binding of ADC drugs and prolong the half-life of ADCs in the blood (221). The immune interaction of the constant Fc fragment of an ADC is one of the major determinants of its cyclic half-life (222). As a consequence, humanised mAbs and fully human mAbs should be selected and the Fc fraction should be modified to decrease a part of immunogenicity and immunotoxicity and increase the cyclic half-life (223). Fifth, the molecular weight of mAbs should be appropriate. If the molecular weight is too large, ADCs will have difficulty penetrating the capillary endodermis and extracellular spaces (217). If the molecular weight is too small, the half-life will be influenced (224). Finally, the mAbs of ADCs should have some of the function of mAbs, including ADCC and CDC (217), which means that the mAb alone can be seen as an effective drug.

Nowadays, all ADCs in clinical trials use IgG because the biomolecule not only contains multiple natural sites for conjugation, but can also be modified to produce other conjugate sites (225). Due to their high affinity to target antigens and long circulating half-life in the blood, IgG can accumulate in the tumour region (226). Also, compared with others, IgG1 is most often chosen as the antibody part of ADCs (211). Generally, different IgG subtypes have different immune functions including ADCC and CDC (217). Compared with IgG4 and IgG2 subtypes, human IgG1 and IgG3 have stronger ADCC and CDC (217,227). Furthermore, IgG3 antibodies have a short half-life and rapid clearance compared to IgG1, IgG2 and IgG4, making them impossible to use in ADC synthesis (223). As a result, IgG1 is being used more selectively for ADC development.

In the research of tumour treatments, the development of fully human mAbs is very important, as murine mAbs and chimeric mAbs induce the immunogenicity of allogenic antibodies which can cause allergic reactions in humans (212). In early studies, the use of murine mAbs often triggered a severe immune response in humans, and patients produced human anti-murine antibodies which greatly reduced the therapeutic effect (228). Therefore, it is necessary to develop an mAb preparation technique, in order to make it possible to use better humanised or fully human mAbs as an essential component in an ADC in the future.

Near-infrared photoimmunotherapy (NIR-PIT). Traditional tumour treatments, such as surgery, chemotherapy, radiation therapy and photodynamic therapy (PDT), often damage the function of normal cells while killing diseased tissue; this can break the delicate balance between the pathogen tissue and the surrounding healthy cells (229). For example, after the photosensitiser is administered and enriched in the tumour, the PDT uses certain wavelengths of visible light to activate the photosensitiser, generating singlet oxygen to kill the tumour cells, in order to achieve a therapeutic effect (230). In the process, it is inevitable to damage normal tissues or organs because of the accumulation of photosensitisers in normal cells (231). In contrast, NIR-PIT, as a molecularly targeted phototherapy with

Table II. Monoclonal antibody drug of global drug sales TOP100 in 2020.

Ranking	Drug	Sale (billion dollars)	Manufacturer	Adaptation disease
1	Humria® (adalimumab)	19.832	Abbvie	Autoimmune disease
2	Keytruda® (pablizumab)	14.38	MRK	Melanoma and non-small lung cancer (NSCLC)
7	Opdivo® (nivolumab)	7.81	Bristol-Myers Squibb	Melanoma and NSCLC
8	Stelara® (ustekinumab)	7.707	Johnson & Johnson (J&J)	Psoriasis
14	Avastin® (bevacizumab)	5.321	Roche	Cancers including colon cancer
16	Ocrevus® (ocrelizumab)	4.611	Roche	Multiple sclerosis (MS)
18	Darzalex® (daratumumab)	4.19	J&J	Multiple myeloma
19	Perjeta® (pertuzumab)	4.139	Roche	Breast carcinoma
20	Remicade® (infliximab)	4.077	J&J/MSD	Autoimmune disease
21	Soliris® (eculizumab)	4.064	Alexion	Paroxysmal nocturnal haemoglobinuria (PNH)
22	Dupixent® (dupilumab)	4.045	Sanofi	Atopic dermatitis
23	Cosentyx® (secukinumab)	3.995	Novartis	Psoriasis
25	Herceptin® (trastuzumab)	3.978	Roche	Cancers including breast carcinoma
31	Lucentis® (ranibizumab)	3.473	Roche/Novartis	Age-related macular degeneration (ARMD)
32	Rituxan® (rituximab)	3.418	Roche	Leukaemia
35	Xolain® (omalizumab)	3.281	Roche/Novartis	Asthma
37	Entyvio® (vedolizumab)	3.252	Takeda	Ulcerative enteritis and Crohn's disease
42	Actemra® (tocilizumab)	3.05	Roche	Autoimmune disease
46	Tecentriq® (atezolizumab)	2.919	Roche	Metastatic urothelial carcinoma
50	Prolia®/Xgeva® (denosumab)	2.763	Amgen/Daiichi Sankyo	Osteoporosis
61	Hemlibra® (emicizumab)	2.335	Roche	Haemophilia
65	Simponi® (golimumab)	2.243	J&J	Autoimmune disease
69	Cimzia® (certolizumab)	1.887	UCB	Autoimmune disease
72	Imfinzi® (durvalumab)	2.042	AstraZeneca	Lung cancer
78	Tysabri® (natalizumab)	1.946	Biogen	MS
79	Xgeva® (denosumab)	1.935	Amgen	Giant cell tumour
82	Kadcyla® (ado-trastuzumab emtansine)	1.445	Roche	Her2-positive metastatic breast cancer
84	Taltz® (ixekizumab)	1.788	Eli Lilly and Co. (LLY)	Plaque psoriasis
90	Yervoy® (ipilimumab)	1.682	Bristol-Myers Squibb	Melanoma and NSCLC
98	Erbitux® (cetuximab)	1.552	LLY/Merck	Colorectal tumour

the selective killing of diseased tissue developed on the basis of photodynamic therapy and immunotherapy, uses mAbs to direct the near-infrared, water-soluble, silicon-phthalocyanine derivative, IRdye700DX(IR700), to tumour sites, solving the problem of the low selectivity of photodynamic therapy (229,232).

As early as the beginning of the 1980s, Mew *et al* started studying PIT *in vitro* and *in vivo*, indicating that photo-immunoconjugates show higher selectivity to tumour tissues than photosensitisers or mAbs alone (233,234). In subsequent years, although various photosensitisers, cross-linking methods and mAbs were developed and applied to photoimmunotherapy (PIT), the application of photo-immunoconjugate was still limited *in vivo* due to the hydrophobic nature of the photosensitisers (235). Later, a new type of PIT was developed in 2011 by Mitsunaga *et al*, NIR-PIT, which uses a target-specific photosensitiser based on NIR phthalocyanine dye, IR700, in combination with mAb targeting EGFR (236). In this treatment, mAb-IR700 conjugate binds to tumour cells that overexpress antibody targets (235). When irradiated with near-infrared light, IR700 in the conjugate is activated and rapidly destroys the hydrophobic tumour cell membrane, resulting in the death of the cancer cells (229,237). In addition, the conjugate *in vivo* can indirectly activate cytotoxic T cells to kill tumours and inhibit tumour metastasis and recurrence by targeting CD44, CD133 and other tumour stem cell biomarkers (229,232).

In short, mAbs, as a specialised tool for identifying specific proteins on the surface of cancer cells, can act as a carrier to selectively deliver the photosensitisers which have a poor targeting ability to the tumour site, helping photosensitisers to locate and attach to cancer cells (238). More importantly, photoimmunotherapy could be applied to a range of cancers simply by altering mAbs in photo-immunoconjugates which have different targets, such as EGFR, HER2, PSMA, CD25, CEA, mesothelin, GPC3, CD47, CD20 and PD-L1 (139,229,238). Therefore, it is necessary to design and manufacture mAbs with better properties so that the PIT has a greater prospect.

5. Monoclonal antibody drugs and the market value

To date, biotechnology medicines have been developing rapidly, and half of the pharmaceuticals are synthesised by biotechnology companies around the world, especially those drugs with complex molecular structures including multi-specific drugs; this is because biotechnology is simpler than chemical synthesis and can produce higher economic efficiency (239). Therefore, as important components of biotechnology medicines, mAb drugs produced by lymphocyte hybridoma technology or genetic engineering technology, among others, have been widely used in the medical and biological fields as diagnostic and treatment agents in the last 30 years (24,240). From the perspective of the ingredients of mAbs, these drugs can be divided into four generations: a) Murine-derived mAb drugs (-momab); b) human-murine chimeric mAb drugs (-ximab); c) humanised mAb drugs (-zumab); and d) fully human mAb drugs (-mumab) (29).

According to the investigative report of the American Pharmaceutical Research and Producers Association, the antibody drugs currently under development and already on the market are summarised as follows. To date, the FDA has approved 108 mAb drugs, which have made a breakthrough in tumour immunotherapy and greatly improved the survival of patients with certain types of tumours and other diseases (24,39) (Table I). From the perspective of diseases, tumours, and autoimmune, infectious, endocrine, cardiovascular and neurological diseases are the six sectors with the largest market size, all worth billions of dollars (24,58) (Table II). In summary, mAbs have become a new force that cannot be ignored in biological drugs at present, and it will be the main force in the field of biomedicine in the future (239).

6. Prospects

In the past 30 years, from murine-derived mAbs to fully human mAbs, the preparation of mAbs has made great progress. The research into human antibodies has made a breakthrough in the last 10 years, which has played an important role in medicine. Nowadays, mAbs are mainly used in the treatment of tumours, organ transplant rejection, autoimmune diseases and other diseases. Because of their good targeting, mAbs have a quick effect, small side effects and good effects. They are taking an increasing share in the sales of biotechnology drugs and have broad development prospects. However, the mature affinity of antibodies, the stability of antibodies secreted by human hybrid tumour cells and the mass production of antibodies still need to be solved.

With the development of the human genome project, the high specificity of mAbs has meant that they will play an irreplaceable role in the in-depth study of the subtle structure of proteins. Additionally, the emergence of phage display technology and ribosome display technology has greatly shortened the preparation cycle of mAbs and reduced their production costs. In addition, the safety of fully human mAbs has greatly promoted their wide application in the clinical treatment of infectious diseases, tumours, organ transplantation, haematological diseases, toxic diseases, allergic diseases, autoimmune diseases and other aspects. It is believed that with the development of molecular biology technology, especially the murine antibody humanisation technology, antibody library technology and transgenic technology, the clinical application of mAbs will become more extensive.

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Authors' contributions

TC and ZL provided the concepts of this review and designed its framework. JL and JD conducted the research, selected the literature findings and wrote the manuscript. All authors edited the manuscript. All authors read and approved the final manuscript for publication.

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Competing interests

All authors declare that they have no competing interests.

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