Review

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Monoclonal antibody-related drugs for cancer therapy

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ABSTRACT: Much progress has been made during the last few decades in the treatment of malignancies. Many types of cancer cells comprising the tumor mass carry molecular markers that are not expressed or are expressed at much lower levels in normal cells. These findings provide new leads to drug design and development of therapeutic strategies involving monoclonal antibodies (mAbs) or related antibody drugs to treat malignancies. This article reviews recent advances in this targeting approach with a focus on the evolution and current use of prospective antibody drugs as effective ways to treat cancer. Additionally, the development of prospective antibody-drug conjugates will also be briefly described.

Keywords: Antibody, targeted therapy, cancer, antibody-drug conjugate

1. Introduction

In 1986, the US Food and Drug Administration (FDA) approved the first monoclonal antibody drug, muromonab (a murine IgG1 specific for CD3), as a therapy for transplant patients experiencing rejection (*1*). Since then, the pharmaceutical industry has entered a new era of targeted therapy. Dozens of monoclonal antibodies (mAbs), including murine, chimeric, and humanized antibodies, have been developed for use against multiple diseases, including (but not limited to) autoimmune disorders and cancer, in humans (*2*). Early in 2008, engineered antibodies were predicted to account for over 30% of all revenue in the biotechnology market (*3*). The latest global forecast is that the protein engineering market could reach $168 billion by 2017, and the market's growth is primarily attributed to mAbs, which account for more than 50% of revenue (*4*).

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Cancer remains one of the leading causes of mortality worldwide, affecting over 10 million new patients every year. Currently, the clinical treatment options mainly include surgical resection, radiation, and chemotherapy. Although over 90 chemotherapeutic drugs have been approved for clinical use by the FDA, their efficacy has been severely hindered by dose-limiting toxicity and patient morbidity (*5*). The story of targeted therapy for malignant cancer is quite a long one. Targeted cancer therapies can be defined as drugs developed against a specific target based on its important biological function in cancer (*6,7*). Drugs developed for targeted therapy including some small molecules, such as tyrosine kinase inhibitors, and antibody-related drugs. With dramatically improved antitumor action and a substantial reduction in toxicity, mAbs represent a major advance in targeted therapy.

Increased understanding of the molecular events involved in cancer development has led to the identification of a large number of novel targets and, in parallel, to the development of multiple approaches to anticancer therapy (*8*). Given the urgent need for clinical options and the need for a better prognosis for patients with malignant tumors, new therapeutic strategies must be developed with new drugs (*9*). The ability of antibodies to exploit antigenic differences between normal and malignant tissue and to induce a variety of antitumor responses while having minimal effect on normal cells offers significant advantages to conventional forms of therapy (*10*). As a result, pharmacological research on molecular agents, including antibody-related drugs, is making greater progress than ever before. Research on antibody-drug conjugates has also appeared and made significant progress thanks to careful optimization of several parameters, including mAb specificity, drug potency, linker technology, and the stoichiometry and placement of conjugated drugs (*11*).

2. Stages of antibody development

The concept of targeted therapy using mAbs has been put into practice and revised for several years. Recent studies have used tumor-specific antigens that facilitate

targeted oncologic therapy. Targeted therapy focuses on specific signal transduction molecules in malignant cells, including crucial molecules involved in cell invasion, metastasis, apoptosis, cell-cycle control, and tumor-related angiogenesis (*12*). mAbs are developed with a high specificity for antigens expressed on tumor cells; thanks to their specific affinity for a selected target, antibody drugs can affect proximal events in signaling pathways that drive abnormal growth and have relatively low toxicity (*10*). Unlike many small molecules, mAbs offer unique target specificity, offering better efficiency and fewer side effects (*13*). The high specificity and affinity of mAbs suggest a bright future for their development and clinical use.

First-generation mAbs were murine antibodies. Second-generation mAbs include chimeric antibodies, humanized antibodies, and fully humanized antibodies. The main objective of modifying antibodies is to reduce their immunogenicity (*14*). As was just mentioned, the first generation of mAbs typically consisted entirely of mouse cells, so they were viewed as foreign by the human immune system. The human immune system can generate unique and highly specific antibodies to all foreign antigens. This ability is unmatched since the immune system can also vary individual antibody isotypes in order to optimize the body's antibody response. The second generation of mAbs involves "humanized antibodies". This term refers to modifying the protein sequences of antibodies from non-human species to increase their similarity to antibody variants produced naturally in humans (*15,16*).

3. Mechanisms of mAbs in cancer therapy

The mechanisms underlying the therapeutic efficacy of mAb-based immunotherapy have often been investigated (Figure 1). First, mAbs can induce signal arrest and lead to apoptosis in targeted tumor cells by binding with their specific receptor, inducing modulation of the receptor or interfering with ligand binding and/or dimerization of the receptor (*17*). mAbs can display action through Fc-based mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which can be influenced by the nature of glycosylation of the antibodies (*13,18-21*).

In ADCC, mAb binds to the tumor antigen. The Fc region of the mAb is exposed and interacts with the Fc gamma receptors (FcγR) on the surface of effector cells, such as natural killer cells, macrophages, monocytes, and eosinophils. Stimulatory effects are mediated through FcγRI on macrophages, dendritic cells (DCs), and neutrophils and through FcγRIIIa on NK cells, DCs, and macrophages (*22*). The FcγR immunoreceptor tyrosine-based activation motif (ITAM) is then phosphorylated, triggering the activation of effector cells and the secretion of various substances such as cytokines, lytic enzymes, perforin, granzymes, and TNF that mediate the destruction of target cells. In murine models, the cytotoxicity resulting from FcR activation of a NK cell, γδ T cell, or macrophage is responsible for antitumor activity (*23*). Previous studies have found that ADCC plays a key role in the effectiveness

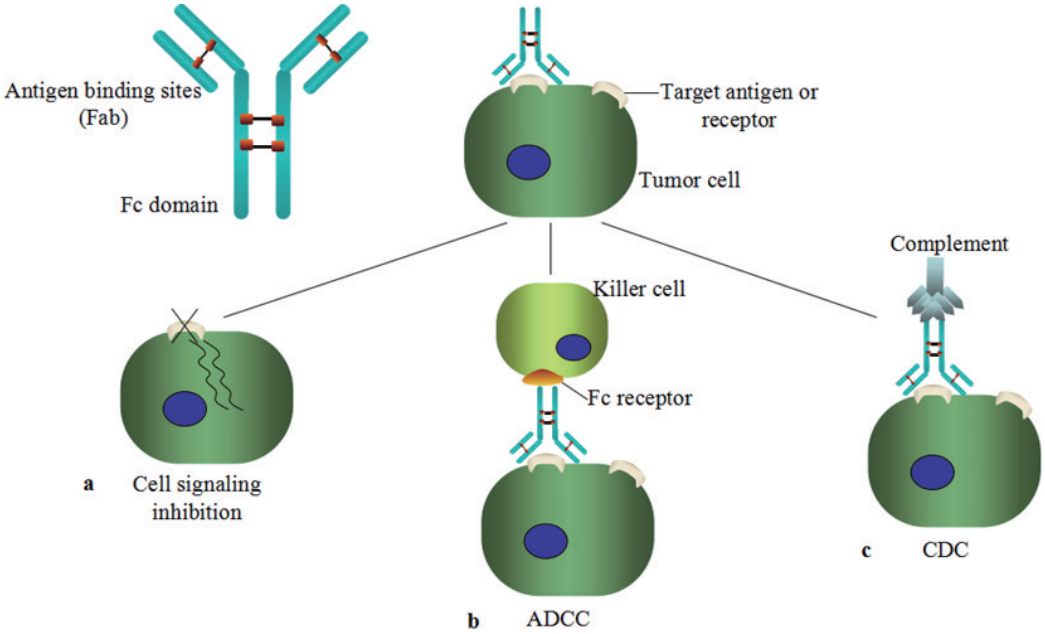

Figure 1. Mechanisms underlying the therapeutic efficacy of mAb-based immunotherapy. (a) mAbs can induce signaling inhibition and lead to apoptosis in targeted tumor cells by binding with their specific receptor, inducing modulation of the receptor or interfering with ligand binding and/or dimerization of the receptor. **(b)** The Fc region of an antibody recognizes the Fc gamma receptors (FcγR) on the surface of immune effector cells, while the Fab domain specifically bounds to a target cell. The FcγR ITAM is then phosphorylated, triggering the activation of the effector cell. **(c)** C1q binds to the antibody, triggering the complement cascade that leads to the formation of the membrane attack complex (MAC) (C5b to C9) on the surface of the target cell as a result of classic pathway complement activation.

of therapeutic antibodies, which require antibodies, antigen-coated target cells, and FcγR-bearing effectors (*24-27*).

In contrast, CDC involves C1q, a bundle of six heterotrimeric subunits consisting of globular heads and collagen-like tails. C1q binds the antibody that triggers the complement cascade, leading to the formation of membrane attack complex (MAC) (C5b to C9) on the surface of the target cell as a result of classic pathway complement activation (*28*).

There are five classes of immunoglobulins – IgA, IgD, IgE, IgG and IgM – that are classified on the basis of the constant region of the heavy chain. Most therapeutic antibodies developed for clinical use are of the human IgG1 isotype, which can induce a stronger ADCC or CDC in comparison to other heavy-chain isotypes of human antibodies (*21*). In addition to its effector mechanisms, IgG1 has a long half-life in blood that is as long as 21 days or so (*29,30*). Previous studies found that IgG1 ≥ IgG3 >> IgG4 ≥ IgG2 in terms of the strength of their ADCC effector action and that IgG3 ≥ IgG1 >> IgG2 ≈ IgG4 in terms of their level of CDC effector action (*20,31*).

4. Targets and typical mAb drugs

About 50% of mAbs in the commercial clinical pipeline have been studied as cancer agents (*32*). MAbs for cancer therapy have greatly helped cancer patients. For researchers, the first challenge in developing an effective mAb-based therapeutic strategy is the identification of the right antigen to attack the surface of target cells (*10*). In order for an antigen to be of use in targeted antibody therapy, the targeted molecule must be expressed at sufficiently elevated levels on tumor cells relative to normal cells. For example, about 20% of breast cancers have increased amounts of the HER2 protein, so HER2 can serve as a target for development of an antibody drug to treat breast cancer patients with elevated HER2 (*33,34*).

There are several existing targets of mAb development that have been successfully used in antibody development, and several mAbs have been approved by the US FDA for treatment of cancer (Table 1). Many more mAbs are still in the clinical or preclinical stages of research. According to sales figures for 2012, Rituximab, which is commonly used to treat a variety of human lymphomas and chronic lymphocytic leukemia, has become the largest-selling biologic drug in clinical oncology.

5. Development of antibody-drug conjugates

Antibodies are modified *via* attachment to protein toxins or highly potent, low-molecular-weight drugs to enable antibodies to function as cytotoxic anticancer agents, and the toxins delivered to the interior of cancer cells act as bullets to cancer cells (*48*). The magic bullet concept put forth by Paul Ehrlich is over 100 years old, while the first credible experiments linking chemotherapeutic agents to antibodies were performed almost 55 years ago (*49-51*). Antibody-drug conjugates, or ADCs, are a new class of highly potent biopharmaceutical drugs designed as a targeted therapy for the treatment of cancer. These conjugates consist of an antibody with high specificity (a whole

Table 1. Representative target antigens and monoclonal antibodies for the treatment of cancer in clinical use

Target	mAbs	Trade name	Molecular type	Main indication	Company	Year	*Ref.*
CD20	Rituximab	Rituxan	Chimeric human/mouse antibody	B-cell lympoma	Roche	1997	*34*
	Ibritumomab*	Zevalin	Murine IgG1	B-cell lympoma	Spectrum pharms	2002	*35*
	Tositumomab	Baxxar	Murine IgG2a	B-cell lymphoma	Smithkline Beecham	2003	*36*
	Ofatumumab	Arzerra	Human IgG1κ	Choronic lymphocytic leukemia	Glaxo Grp Ltd	2009	*37,38*
CD52	Alemtuzumab	Campath	Humanized IgG1	B-cell chronic lyphocytic leukaemia	Ilex pharmaceuticals	2001	*39*
EGFR	Cetuximab	Erbitux	Humanized IgG1	Colon, lung cancer	Imclone/Bristol-MyersSquibb	2004	*40*
	Panitumumab	Vectibix	Fully human IgG2	Colon cancer	Amgen	2006	*41*
HER2	Trastuzumab	Herceptin	Humanized IgG1	Breast cancer	Roche	1998	*33*
	Pertuzumab	Perjeta	Humanized IgG1	Metastatic breast cancer	Genentech	2012	*42,43*
VEGF	Bevacizumab	Avastin	Recombinant humanize IgG	Colon, lung, breast and renal cancer	Roche	2004	*44*
RANKL	Denosumab	Xgeva	Human IgG2	Breast and prostate carcinoma	Amgen	2010	*45*
CTLA-4	Ipilimumab	Yervoy	Human IgG1κ	Melanoma	Bristol-Myers Squibb	2011	*46*

* Coupled with ^{90}Y or ^{111}In.

mAb or an antibody fragment) linked *via* a stable chemical linker with labile bonds to a "small" (300 to 1,000 Da) biologically active toxin or drug with manageable toxicity (Figure 2) (*52,53*). Conjugated mAbs are also referred to as tagged, labeled, or loaded antibodies and are divided into three groups: mAbs with radioactive particles attached, mAbs with chemotherapy drugs attached, and mAbs attached to cell toxins. Thus, ibritumomab tiuxetan (Zevalin®) and tositumomab (Bexxar®) are, strictly speaking, examples of radiolabeled mAbs that fall under ADC drugs.

Denileukin difitox (trade name: Ontak) is the first ADC drug that was approved in 1999 by the US FDA for the treatment of diffuse large B-cell lymphoma. Although it is not an mAb, IL-2 normally attaches to certain cells that contain the CD25 antigen, which makes it useful for delivering the toxin to these cells. Gemtuzumab ozogamicin (trade name: Mylotarg) was later approved for the treatment of acute myelogenous leukemia in 2000 (*54*). However, Pfizer/Wyeth withdrew the drug from the market in June 2010 (*55,56*). Another ADC that was approved in 2011 was Brentuximab vedotin (SGN-35, trade name: Adcetris, marketed by Seattle Genetics and Millennium/Takeda), which is clinically used to treatment relapsed and/or refractory Hodgkin's lymphoma and systemic anaplastic large-cell lymphoma.

With the FDA approval of trastuzumab emtansine (T-DM1, trade name: Kadcyla, marketed by Genentech and Roche) on February 22, 2013, the development of antibody-drug conjugates based on antibodies ushered in a new era of personalized cancer treatment with greater clinical efficacy and manageable toxicity. The success development of T-DM1 for metastatic breast cancer has done away with the view that ADCs are only useful against blood cancers (*57,58*). ADCs are also showing promising efficacy and limited adverse toxicity in the treatment of cancer. According to a previous study, at least 30 ADCs are now in clinical use, accounting for around 15% of the clinical-stage anticancer antibody-based pipeline and outnumbering other modified mAbs (*57,59*).

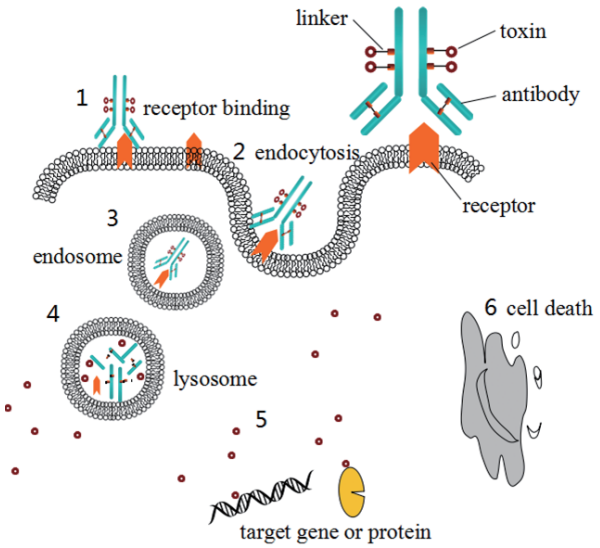

Figure 2. Mechanism of antibody-drug conjugate. An antibody-drug conjugate consists of an antibody (whole mAb or an antibody fragment) linked *via* a stable chemical linker with labile bonds to a biologically active cytotoxic anticancer toxin or drug. After binding to the targeted cell receptor, ADC is internalized and degraded, and the toxin or drug that is released ultimately induces cell death by acting on specific genes or proteins.

6. mAb-related drugs being developed for cancer therapy

Despite all the advances, there are still many questions to answer besides the identification of optimal cellular targets and antibody forms. Personalized therapies now need to identify the optimal dose, schedule, and combinations of agents for specific malignancies for specific patients (*60*). High specificity and high affinity are both important for antibodies developed as cancer therapies to have little impact on normal cells. The right target that is highly expressed on tumor cells but little expressed in normal cells must be chosen. To obtain a high affinity, more work has to be done to design the antigen-binding site. Dozens of MAbs are in various clinical stages, some of which are the later stages of phase II and III, indicating development of promising drugs for FDA approval (*32,61*).

For ADCs, MAbs that cross-react with the corresponding target antigen expressed on cells from rodents and/or non-human primates must be selected whenever possible because most target antigens used in ADCs are tumor-selective rather than tumor-specific (*62,63*). Thanks to the development of linker technology and utilization of sufficiently potent toxins, some promising ADCs are currently undergoing clinical trials, and ADCs to treat hematologic malignancies and solid tumors that are in phase II or III are shown in Table 2. Most ADCs in clinical development utilize humanized or fully human mAbs (*63*).

7. Main problems

Although there has been much progress in the development of antibody drugs with considerable specificity in patients with advanced cancer, problems and limitations still exist. mAbs are large molecules that would be expected to have limited distribution within solid tumor blood vessels, and the limited penetration of full-length antibodies into solid tumors is as an important factor restricting their efficacy (*70,71*). Despite the fairly mild side effects of mAbs, such as fever, chills, weakness, headaches, nausea, vomiting, diarrhea, low blood pressure, and rashes, the major drawback of mAbs is their immunogenicity, which may induce production of anti-drug antibodies that can neutralize a drug's therapeutic activity, provoke

Table 2. Representative emerging antibody-drug conjugates in Phase 2 or Phase 3 clinical studies for cancer therapy

Agent	Company	Target	Toxin	Main indication	Highest stage (phase)	*Ref.*
Inotuzumab ozogamicin (CMC-544)	Pfizer	CD22	ozogamicin	relapsed/refractory acute lymphoblastic leukemia	III	*63*
Glembatumumab vedotin	Celldex Therapeutics	GPNMB*	MMAE	advanced GPNMB-expressing breast cancer	II	*64*
SAR3419	Sanofi-aventis	CD19	Maytansine DM4	Diffuse Large B-cell Lymphoma	II	*60,65*
BT062	Biotest	CD138	Maytansine DM4	Multiple Myeloma	II	*66*
Anti-PSMA ADC	Progenics	PSMA	MMAE	Prostate Cancer	II	*67*
Lorvotuzumab mertansine	ImmunoGen	CD56	Maytansine DM-1	Small Cell Lung Cancer	I/II	*68*

* glycoprotein NMB.

autoimmune symptoms, and affect the pharmacokinetic process (*72-75*). Although sequence humanization was believed to be sufficient to tackle these problems, multiple clinical examples now demonstrate that humanization does not suffice to avoid immune responses (*76-79*). Side effects due to target inhibition have also emerged. For example, bevacizumab, which targets tumor blood vessel growth (HER2), may cause side effects such as high blood pressure, bleeding, poor wound healing, blood clots, and kidney damage (*45,80*). Although a study suggested that brain metastases are not a risk factor for intracranial hemorrhage with bevacizumab treatment (*81*), bevacizumab may potentially increase the risk of bleeding.

In ADCs, the antibodies serve as a carrier to internalize the toxic component of an immunoconjugate, potentially making it more therapeutically active (*82*), so combining the cytotoxicity of highly potent natural or synthetic anti-neoplastic agents with mAbs conjugated by blood-stable optimized linkers is a key strategy for a new generation of ADCs (*56*). The first challenge is to ensure drug potency, which means to make sure that sufficient quantities of the ADC reach the target tumor cells (*11,59*). The second task is to design appropriate linker molecules to couple drugs to the antibody. These molecules must maintain antibody binding capacity following conjugation and also undergo selective, rather than systemic, enzymatic or chemical degradation inside the cell or on the cell surface (*71*).

8. Outlook and prospects for the future

After years of preclinical development, antibody drugs offer vast resources for drug discovery and new drug research. As new targets emerge, new avenues for drug design will open. Investigation of natural compounds will also offer more options for ADC drug research. Modern biological techniques have allowed the rapid production of chimeric antibodies, humanized antibodies, and totally human antibodies. Understanding of the effects and molecular mechanisms of these antibodies has improved with the development of functional genomics, proteomics, and molecular research, and the number of clinical trials using antibody-related drugs to treat cancer has increased markedly. Some studies have found impressive clinical responses, indicating the beginning of a new and exciting phase of cancer treatment. Moreover, bispecific mAb consisting of fragments of two different mAbs have appeared. These mAbs bind to two different types of antigen, pointing the way for antibody drug development. Thus, antibody drugs have a promising future that offers better personalized therapy and combination therapy to treat malignant cancer.

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