Therapeutic Monoclonal Antibodies for Cancer:
The Past, Present, and Future

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Monoclonal antibodies (mAbs) for cancer therapy can be broadly divided into three classes. First are those that target biological features expressed by the tumor cell itself, second are those that target factors produced by the tumor cell or as a host response to the tumor, and third are those that target the immune regulatory networks responsible for inhibiting antitumor immune responses. Of these, tumor-specific therapeutic mAbs are particularly unique because they partially and passively reconstitute the humoral arm of the tumor antigen-specific immune response, as well as provide critical support in establishing the antigen-specific adaptive immune response through cross-priming. This review focuses on these categories and will discuss ongoing clinical trials.

Key words: monoclonal antibody, HER2, immune checkpoint, PD-1, CTLA-4

Introduction

The commercial pipeline of monoclonal antibodies (mAbs) is highly dynamic, with a multitude of transitions occurring during the year as product candidates advance through the clinical phase and onto the market. Rituximab (anti-CD20) and trastuzumab (anti-HER2), the first mAbs were approved for cancer therapy. Then two mAbs were approved in use that target soluble host factors involved in the antitumor response, bevacizumab (anti-VEGF) and denosumab (anti-RANKL). Recently, the application of therapeutic mAbs ipilimumab (anti-CTLA-4) and nivolumab/pembrolizumab (anti-PD-1) that specifically target the nodal checkpoints for T-cell activation and effector function is a new area of increasing preclinical and clinical investigation. The clinical developments of mAbs in various categories are described and summarized in this review (Figure-1).

Monoclonal antibodies target to tumor-specific antigens

1. Trastuzumab and pertuzumab target HER2/neu pathway

Trastuzumab (Herceptin®), a humanized mAb specific for the human epidermal growth factor receptor 2 (HER2/neu), is widely used to treat HER2-overexpressing breast cancers at every stages except ductal carcinomas that are negative for HER2. Trastuzumab was first approved in 1998 and the second-generation mAb pertuzumab (PERJETA®) was approved in 2012 as treatment for metastatic breast cancer. It has also been approved for the treatment of HER2-positive metastatic adenocarcinoma of the stomach and gastroesophageal junction. Trastuzumab has been thought to function primarily by inhibiting signal transduction via the cell surface HER2 receptor, but it can also modulate tumor immunity through multiple mechanisms. It recruits innate immune effector cells such as natural killer cells and...
macrophages to the tumor microenvironment in order to facilitate antibody–dependent cellular cytotoxicity (ADCC). Trastuzumab can also induce the ubiquitination and degradation of internalized HER2 proteins, thereby increasing proteasome-dependent antigen presentation on MHC molecules. Accordingly, it augments the cytotoxic activity of HLA Class I-restricted HER2–specific cytotoxic T lymphocytes (CTLs) against HER2-positive breast cancers. In HER2–transgenic mice study, HER2 mAb therapy alone induced new CD8-positive CTLs that break immune tolerance.

In humans, a single dose of trastuzumab induced apoptosis in primary tumors within 24 hours, and trastuzumab–based neoadjuvant chemotherapy is associated with the development of T-bet-positive lymphoid nodules in patients with locally advanced breast cancer. Importantly, these lymphoid nodules are also associated with enhanced overall survival. Finally, chemotherapy combined with trastuzumab has been associated with the development of HER2–specific CD4-positive T cell responses in patients with both early and metastatic breast cancers.

The combination of low-dose cyclophosphamide, anti–HER2 mAb and HER2–specific peptide vaccination generated the highest numbers of HER2–specific CTLs, and protected up to 70% of neu–N transgenic mice from the outgrowth of established tumors. These observations provide strong support for testing trastuzumab in combination with HER2 targeted vaccination in patients with malignancies dependent on HER2–signaling. Two clinical trial of trastuzumab combined with vaccination have been reported. In one study, a HER2–specific T–helper peptide vaccine was combined with standard trastuzumab therapy regimen in 22 patients with metastatic breast cancer. This study demonstrated the safety of the combination, with about 15% of patients displaying an asymptomatic decline in cardiac ejection fraction. Immune responses against HER2 and other tumor–associated antigens were observed and the magnitude of the immune response was inversely correlated with serum TGF–beta levels, indicating the promotion immune modulating effect by the treatment with trastuzumab. In the second, the combination with trastuzumab, cyclophosphamide and GM–CSF–secreting breast cancer cell–based vaccine achieved an overall survival 40 months compared to the historical overall survival (OS) of 13–24 months in similar patients who received standard trastuzumab alone. These clinical data strongly argues for the establishment of combination immunotherapy incorporating trastuzumab and other immune modulators such as cyclophosphamide or immune checkpoint inhibitors in patients with HER2–positive cancers.

2. Antibody–drug conjugate of trastuzumab

Antibody–drug conjugates (ADCs) are becoming an increasingly important sub-class of antibody–related therapeutics. Trastuzumab emtansine (Kadcyla) were recently approved for marketing.

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**Figure 1** The clinical antibodies in various therapeutic categories
both by the US Food and Drug Administration (FDA), European Medicines Agency (EMA) and the Japanese Ministry of Health, Labour and Welfare and Pharmaceuticals and Medical Devices Agency (MHLW-PMDA) as a treatment for human HER2-positive breast cancer. Kadcyle is an ADC comprising trastuzumab linked to ImmunoGen’s DM1 maytansinoid drug. One of major pharmaceutical company Roche entered into licensing agreements with ImmunoGen giving rights to use ADC technology to develop therapeutics for specific targets. It is currently also undergoing evaluation in various clinical Phase trials comparing efficacies and overall survival to trastuzumab and taxane as standard regimen.

3. Rituximab and other mAbs target CD20

Rituximab (Rituxan®), a chimeric mAb specific for the cell surface molecule CD20 expressed by normal B cells and over 95% of B-cell leukemia and lymphoma, is widely used both as a single agent and combined with chemotherapy and radiation. Although the primary mechanism of action remains unclear, it has been shown to mediate ADCC, promote apoptosis, and enhance cross-priming of the adaptive immune response. Rituximab modulate src signaling in follicular lymphoma cells, leading to lower levels of active STAT-3 and bcl-2 and increased sensitivity to chemotherapy.

Rituximab therapy is known to result in profound B-cell depletion. B cells have been shown to inhibit tumor-specific CTL induction, suggesting that B-cell depletion in the setting of rituximab therapy may not inhibit, and could enhance, vaccine-induced tumor immunity. Based on this idea, several preclinical and clinical trials of rituximab combined with adaptive immunotherapy against B-cell malignancies have been demonstrated. Additionally, rituximab has been indicated for the treatment of rheumatoid arthritis (RA) with another prescription medicine called methotrexate, to reduce the signs and symptoms of moderate to severe active RA in adults, after treatment with at least one other medicine called tumor necrosis factor (TNF) antagonist has been used. Therapeutic use of rituximab for the treatment of RA is approved only by FDA and EMA but not by MHLW-PMDA in Japan so far.

In recent years, new generations of anti-CD20 monoclonal antibodies (mAbs) have been developed for potential benefits over the classical, first-generation mAb rituximab. These second-generation mAbs, which include ofatumumab, veltuzumab, and ocrelizumab, are humanized or fully human to reduce immunogenicity, but with an unmodified Fc region. Ofatumumab is a fully human anti-CD20 IgG1 mAb in clinical development for hematological malignancies and autoimmune diseases. Ofatumumab specifically recognizes an epitope encompassing both the small and large extracellular loops of CD20 molecule, and is more effective than rituximab at CDC induction and killing target cells. Veltuzumab is a humanized anti-CD20 mAb with complementarity-determining regions similar to rituximab. This antibody has enhanced binding avidities and a stronger effect on CDC compared with rituximab. Ocrelizumab is a humanized mAb with the potential for enhanced efficacy in lymphoid malignancies compared with rituximab due to increased binding affinity for the low-affinity variants of the FcγRIIIa receptor. Ocrelizumab is undergoing phase III clinical trials for rheumatoid arthritis and lupus nephritis, however, these clinical trial has been discontinued because of several adverse reactions at 2010.

The third-generation mAbs are also humanized mAbs, but in addition they have an engineered Fc to increase their binding affinity for the FcγRIIIa

Figure-2

Three clinical antibodies to HER2/erbB2
One of anti-CD20 mAb, obinutuzumab, is a fully humanized, type II, IgG1 mAb derived from humanization of the parental B-Ly1 mouse antibody and subsequent glycoengineering using GlycoMab® technology. Obinutuzumab was designed for enhanced ADCC and superior direct cell-killing properties, in comparison with currently available type I antibodies. These kinds of mAbs having an engineered Fc to increase their binding affinity for the FcγRIIIa receptor will be commonly used for the treatment of not only B-cell leukemia/lymphoma but also nonhematological malignancies.

**Monoclonal antibodies target to tumor microenvironment factors**

1. Bevacizumab and Ramucirumab to VEGF/VEGFR interaction

Bevacizumab is a chimeric, partially humanized mAb specific for the vascular endothelial growth factor (VEGF), a cytokine critical for tumor-associated angiogenesis. It is currently approved for treating colorectal cancers, glioblastomas, non-small cell lung carcinomas, and renal cell carcinomas. In addition to promoting neo-vascular formation, VEGF inhibits T cell developments and dendritic cells differentiation, leading to tumor-related immune suppression in cancer patients. Monoclonal antibody specific for VEGF can improve the numbers and function of dendritic cells in tumor-bearing mice, thereby potentiating dendritic cell-based immune therapy. Bevacizumab therapy can increase the B- and T-cell subsets in cancer patients, and augment the ability of dendritic cells derived from cancer patients to stimulate T-cell responses to recall antigens. Importantly, blockades of VEGF/VEGFR receptor (VEGFR) pathways can decrease macrophage and myeloid-derived suppressor cell (MDSC) infiltrating tumor tissues. In addition, the efficacy of adoptive cellular therapy would be synergistically enhanced by VEGF mAb promoting the infiltration of transferred T cells into tumor. To date, one clinical trial combining bevacizumab and a cancer vaccine has been reported. Patients with metastatic prostate cancer in biochemical relapse were treated with a combination of prostatic acid phosphatase (PAP)–pulsed antigen presenting cells (APCs) and bevacizumab. All patients developed immune responses to PAP and almost half displayed some decrease in PSA from baseline, indicating that blocking of VEGF would be useful for the adjuvant immunotherapy.

In order to inhibit VEGF/VEGFR interaction, several monoclonal antibodies specific for VEGF receptors have been developed. Tumor-bearing nontolerant mice treated with a specific for VEGF receptor–2 (VEGFR2) develop HER2–specific T cells, even in the absence of vaccination. In the setting of antigen–specific immune tolerance, sequencing vaccination with cyclophosphamide and doxorubicin in the setting of treatment with VEGFR2 mAb unmasks the T-cell dependent–activity of the VEGFR2 mAb and allows the vaccine to work, resulting in a tumor–free survival rate of about 70%. Based on these findings, ramucirumab (Cyramza®) was approved by FDA in 2014. Ramucirumab that targets human VEGFR2 is indicated for the treatment of advanced gastric cancer or gastro-esophageal junction adenocarcinoma, as a single-agent after prior fluoropyrimidine– or platinum-containing therapy. Currently, ramucirumab is undergoing regulatory review by EMA for treating gastric cancer and hepatocellular carcinoma, and also evaluation in clinical Phase III studies in patients with non–small cell lung carcinoma, colorectal cancer and breast cancer.

2. Denosumab specific for RANKL

Denosumab is a mAb recently approved for the management of malignant bone disease in multiple myeloma or breast cancer; it is also used to prevent fragility fractures in osteoporosis. This mAb is specific for the signaling pathway controlled by the interactions of receptor activator of NF–kappa-B ligand (RANKL) and its antagonist osteoprotegerin. RANKL is expressed at high levels by activated T cells, and its receptor RANK is expressed by monocytes, macrophages and dendritic cells. To date, the FDA approved denosumab to prevent skeletal–related events (SREs) associated with metastatic solid tumors. 

**Monoclonal antibodies target to immune checkpoints**

Recently, it has been reported various molecules contributed to immune checkpoint pathways in lymphocyte activation and differentiation. T cells
that recognize the cognate antigen may also express a number of molecules on their surface, such as CTLA-4, PD-1, LAG-3, and BTLA. These molecules serve as checkpoints, controlling the quality and magnitude of a T-cell response. In normal circumstance, these checkpoints would act to protect the host from an overactive immune reactions and autoimmunity. In case of cancer, blockade therapy of these checkpoints could help break tolerance and could serve as to help maintain a high quality long-lasting T-cell response. The first T-cell immune checkpoint targeted for antibody blockade was CTLA-4 and then PD-1. In this chapter, the preclinical studies and clinical benefits of therapeutic mAbs to CTLA-4, PD-1 and PD-L1 are introduced.

1. CTLA-4 blockade

Cytotoxic T-lymphocyte antigen 4 (CTLA-4; CD152) is a negative regulator of T-cell function, and antibodies have been developed which inhibit this important immunologic checkpoint. In preclinical studies, CTLA-4 blocking antibodies were able to induce potent antitumor immunity and provided rationale for clinical use. Ipilimumab (Yervoy) a IgG1 mAb against CTLA-4 and was the first drug to demonstrate an overall survival benefit for patients with melanoma in clinical Phase III trial. Tremelimumab is an IgG2 antibody with a plasma long half-life of approximately 3 weeks.

The clinical development of ipilimumab was built upon a foundation of basic research into the mechanisms that regulate T-cell activation. The “two signal” model of T-cell activation was the product of fundamental studies performed in the 1970s and 1980s. In this model, antigen-specific T-cell activation requires both T-cell receptor engagement (signal 1) and a co-stimulatory signal (signal 2) for fully function, this hypothesis has been validated and expanded in the 2000s. It is now clear that a diversity of co-stimulatory and co-inhibitory molecules are required to both promote and regulate the complex process of T-cell activation. Especially, CTLA-4 plays a fundamental role as an inhibitory receptor, or checkpoint, in the process of T-cell activation, such as proliferation, cytokine production and differentiation.

As shown previously, CTLA-4 blocking antibody therapy in humans was developed based on the preclinical activity seen in mouse models. Both ipilimumab and tremelimumab, fully human antibodies against CTLA-4, have been most widely tested in patients with metastatic melanoma, where durable clinical responses have been well documented. The promising results seen in Phase II studies led to Phase III evaluation. Several Phase III studies were performed and the survival benefit of ipilimumab was confirmed in a subsequent randomized Phase III trial. OS was significantly longer for patients who received dacarbazine with ipilimumab (11.2 mo.) than patients with dacarbazine with placebo (9.2 mo.). Based on OS in Phase III studies, FDA approved ipilimumab for the treatment of patients with unresectable or metastatic melanoma in 2011. Another Phase III study of tremelimumab was halted after an interim analysis failed to demonstrate a benefit. Expectedly, immune-related adverse effect such as colitis, dermatitis, hepatitis and endocrinopathies were observed in patients treated with ipilimumab. To date, a number of combination strategies of ipilimumab with novel immunotherapies or molecularly targeted therapies are likely to be promising based upon have been explored in clinical trials. At present, ipilimumab is being tested in combination with antibody to PD-1 (Nivolumab) for patients with NSCLC.

2. PD-1 and PD-L1 blockade

Several other checkpoint molecules are up-regulated after the T cell and antigen-presenting cell (T/APC) interaction. These molecules help limit the T-cell response once the cells begin to migrate to tissue. This is believed to be a means of maintaining peripheral tolerance. One of important molecules, programmed cell death-1 (PD-1) is expressed by chronically activated CD4 and CD8 T cells. PD-1 is also significantly up-regulated on T cells present in cancerous tissues in various tumor types, including melanoma, prostate cancer. The PD-1-positive tumor-infiltrating T cells (TILs) exhibit an exhausted phenotype with impaired effector function compared with PD-1-negative TILs and PBLs. When PD-1 binds its ligand PD-L1 (B7-H1) or PD-L2 (B7-H2), cytokine production and T-cell activation are diminished. This inhibitory mechanism is present to help prevent peripheral autoimmunity. In case of tumor toler-
ance, PD-1 antibody blockade may serve as a means to maintain a high level of T-cell response post DC vaccination.

Two blocking monoclonal antibodies to PD-1 were just approved for clinical use in 2014 by FDA. During clinical phase studies, several drastic antitumor effects including primary and metastatic tumor sizes and overall survival were observed. Unexpectedly, the dose escalation trial showed a very low incidence of adverse events such as colitis, with only rare grade III toxicities. These autoimmune adverse events appeared to be significantly less than those noted with ipilimumab (anti-CTLA-4 blockade).

Nivolumab (Opdivo) was first approved to use for the treatment of melanoma in US and has orphan drug designation in Japan. Recently, FDA has granted nivolumab Breakthrough Therapy designation for the treatment of patients with Hodgkin lymphoma (HL) after failure of autologous stem cell transplant and brentuximab. In addition, various clinical settings in Phase III trials are undergoing of patients with non–small cell lung carcinoma, renal cell carcinoma and head and neck squamous carcinomas. Another candidate of PD-1 mAb, pembrolizumab (Keytruda), was also approved in 2014 for unresectable or metastatic melanoma and is undergoing evaluation in a Phase III study of patients with NSCLC.

Additionally, therapeutic mAb specific for the ligand of PD-1 (termed PD-L1 or B7-H1) is undergoing evaluation in Phase III clinical trial as treatment for NSCLC by Genentech/Roche. An Fc-engineering anti-PD-L1 mAb, MPDL3280A, has been compared with docetaxel in patients with locally advanced or metastatic NSCLC who have failed platinum therapy. Another PD-L1 mAb MED14736 contains three mutations in Fc portion that abrogates Fc-mediated effector function. After review and approval, these mAbs will be delivered in clinical setting soon.

3. New candidates of immune checkpoint inhibitor

Base on the success of PD-1 and CTLA-4 mAbs, several new mAb candidates have entered clinical phase studies. One of promising therapeutic mAb, bavrituximab, which recognize phosphatidyl-serine (PS) on tumor cells and endothelial cells in tumor microenvironments. PS is normally expressed inner cell membrane and known as “Eat-me-signal” molecule that is recognized by phagocytes. After the irradiation or treatment of anti-cancer drugs, PS is reversed to express outside of cell membrane of various tumor cells and endothelial cells. Bavrituximab, chimeric IgG1 mAb, was given US Fast Track designation of NSCLC. The clinical Phase III study of bavrituximab plus docetaxel vs. docetaxel alone in patients with late-stage non–squamous NSCLC began recruiting patients in 2013.

Another target molecule related in immune checkpoint is the lymphocyte activating gene-3 (LAG-3). Antibody specific to LAG-3 was designed and generated by Bristol-Myers Squibb. The purpose of this study is to characterize the safety, tolerability, dose limiting toxicities and maximum tolerated dose of BMS-986016 (code number of anti-LAG3) administered to subjects with relapsed or refractory chronic lymphocytic leukemia (CLL), lymphomas and multiple myeloma (MM), and Phase I trial just began to recruit patients with CLL.

Summary

Antibody therapeutics are generally recognized as having higher phase transition rates compared with small molecules drugs, but nonetheless discontinuations do occur. The development of biological drugs such as therapeutic antibodies is a time-consuming, risky business. Canonical therapeutics antibodies such as tumor-targeting antibodies, which are the majority of clinical approved antibodies, thus have less approval success rate of 23% compared with those for antibody–drug conjugates (ADCs) of 35%. The success rate will be increased by novel techniques of the antibody engineering and cultivation of a better understanding of biological functions of both target molecules and therapeutic antibodies in the future.

References


