Commentary

Monoclonal Antibodies as Cancer Therapeutics

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Abstract

Monoclonal antibodies have emerged as key therapeutic modalities for a range of human diseases. Owing to selective targetting of these agents on tumor cells, cancer has become one of the major focuses for development of monoclonal antibody therapeutics. The most significant breakthroughs in the development of monoclonal antibodies as oncology therapeutics have been the introduction and approval of bevacizumab (Avastin), an anti-vascular endothelial growth factor antibody and of trastuzumab (Herceptin), an anti-human epidermal growth factor receptor 2 antibody and of cetuximab (Erbitux), an anti-epidermal growth factor receptor antibody. In current review, advances in antibody engineering, mechanisms of action of anti-cancer antibodies and trends in the clinical development of monoclonal antibodies for cancers are overviewed and future directions of research and development for this class of therapeutics are discussed.

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Chemotherapeutic agents as current cancer therapeutic modalities cause intolerable toxicity owing to factors such as difficulties in differentiating tumor cells from health cells. Monoclonal antibodies (mAbs) represent a novel approach to overcoming this problem as they can selectively target tumor cells and initiate a variety of responses once bound. Monoclonal antibodies can eradicate tumor cells by carrying toxic agents to the target or can destroy tumor cells by either activating immune system components or blocking growth factor receptor activation.

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During last three decades, more than 400 therapeutic mAbs have been entered in clinical development. Approximately half of them are anti-cancer mAbs.[1] To date, 12 of these anti-cancer mAbs have been approved for marketing in at least one country (**Table 1**). Improved understanding of cancer biology has led to more potential new targets, while advances in antibody technology has significantly extended list of mAbs available for study.[2,3] Therefore, that rate at which new oncology mAbs entered into clinical study has more than triple for 2000-2010 in compared to those in 1980s. More approved anti-cancer antibodies are the outcome of years of work in two research areas: development of antibody engineering and production methods, and study of mode of action of antibody.

Antibody Engineering and Production Methods

The initial method for producing mAbs involves use of mouse derived hybridoma technology developed by Kohler and Milstein.[4] This method can provide mAbs capable of specific interactions with their target antigens. However, Studies revealed that murine antibodies often had short circulating half-lives [5] and patients frequently developed antibodies to the mouse-derived proteins, which limited their utility.[6] Due to the sequence and structure difference between murine and human antibody Fc region, only certain murine mAb isotypes have been shown to effectively bind to and activate elements of the human immune system, thereby triggering cytotoxic effector functions.[7] These hurdles were overcome by generation of chimeric and humanized antibody that contained human Fc domains and retain targeting specificity by incorporating portions of the murine variable regions. This can be accomplished by grafting either the entire murine variable regions (chimeric antibodies) or the murine complementarity-determining regions (humanization) into the human IgG framework.[8-10] In addition, human mAbs derived from phage display and transgenic mice became available in the 1990s.[11,12] It should be noted that human mAbs were also made from human hybridomas, but this production method was not commercially viable because the cell lines did not reliably produce sufficient quantities of the desired mAb.

During last decade, many new antibody technologies have already matured for antibody lead identification and optimization such as yeast display, ribosomal display and mRNA display and some new technologies are emerging such as bacterial display, mammalian cell display, antibody array and others.

Table 1. Approved anti-cancer therapeutic monoclonal antibodies.

Generic name	Trade name	Description	Approved indication	Year (country) of first approval
Rituximab	Rituxan	Chimeric, IgG1κ, anti-CD20	Relapsed or refractory low-grade non-Hodgkin's lymphoma	1997 (United States)
Trastuzumab	Herceptin	Humanized, IgG1κ, anti-HER2	HER2 overexpressing metastatic breast cancer	1998 (United States)
Gemtuzumab ozogamicin	Mylotarg	Humanized, IgG4κ, anti-CD33, immunotoxin (calicheamicin)	Relapsed acute myeloid leukemia	2000 (United States)
Alemtuzumab	Campath	Humanized, IgG1κ, anti-CD52	Chronic lymphocytic leukemia	2001 (United States)
Ibritumomab tiuxetan	Zevalin	Murine, IgG1κ, anti-CD20, radiolabelled (Y-90)	Relapsed or refractory low-grade, follicular transformed non-Hodgkin's lymphoma	2002 (United States)
I-131 ch-TNT	N/A	Chimeric, IgG1κ, anti-DNA associated antigens, radiolabelled (I-131)	Advanced lung cancer	2003 (China)
I-131 tositumomab	Bexxar	Murine, IgG2aλ, anti-CD20, radiolabelled (I-131)	Non-Hodgkin's lymphoma in rituximabrefractory patients	2003 (United States)
Cetuximab	Erbitux	Chimeric, IgG1κ, anti-EGF receptor	EGFR-expressing metastatic colorectal cancer	2003 (Switzerland)
Bevacizumab	Avastin	Humanized, IgG1, anti-VEGF	Metastatic colorectal cancer	2004 (United States)
Nimotuzumab	TheraCIM	Humanized, IgG1, anti-EGF receptor	Advanced head/neck epithelial cancer	2005 (China)
Panitumumab	Vectibix	Human, IgG2κ, anti-EGF receptor	Metastatic colorectal cancer	2006 (United States)
Ofatumumab	Arzerra	Human IgG1,anti-CD20	Chronic lymphocytic leuakemia	2009 (United States)

Technological advances have had a significant impact on the trends in mAb category in clinical development in the 1980s, 1990s and last decade. Murine candidates constituted 86% of the total in clinical study in the 1980s. No single category has dominated since the 1990s because of the availability of a variety of mAb categories. The rate at which humanized mAbs entered clinical study increased dramatically between the 1980s and the 1990s. Since 2000, the mAbs entering clinical study were mainly humanized or human mAbs.

Mechanisms of Action

Cancer targeting mAbs need to bind to appropriate tumor cell surface antigens with sufficient quantities and lead to destruction of targeted cells. Mechanisms of action include tumor cell toxicity via antibody-conjugate, modulation of host immune system (such as ADCC/CDC), blockade of ligand binding and signaling perturbation.

Antibody-conjugates

Monoclonal antibodies (mAbs) have been used extensively in clinical trials to target cytotoxic agents to tumor cells.[13] These agents include radioisotopes as well as toxins, drugs, cytokines and enzymes. The two radioisotopes most commonly conjugated to mAbs studied in the clinic were Y-90 (2.7-day half-life) and I-131 (8.0-day half-life). Three radiolabelled mAbs - two murine, Y-90 ibritumomab tiuxetan (Zevalin; Biogen Idec) and I-131 tositumomab (Bexxar; Corixa/GSK), and one chimeric, I-131 ch-TNT (Shanghai Medipharm Biotech)—have been approved for non-Hodgkin's lymphoma or lung cancer (**Table 1**). However, only 14% of anticancer mAbs currently in the clinic study are radio-immunoconjugates due to the limitations of radio-immunoconjugates as cancer therapeutics (for example, mAbs did not deliver sufficient radiation doses, especially to solid tumor sites,[14,15] complicated chemistry was required for conjugation, and potential toxic effects on normal tissues).

Monoclonal antibodies (mAbs) were also conjugated with either protein or small molecule cytotoxins as cancer therapeutics.[16] The protein cytotoxins used include various modified versions of *Pseudomonas* exotoxin, *Staphylococcus* enterotoxin, neocarzinostatin, and the plant-derived molecules ricin (and ricin A chain) and gelonin. Small molecules incorporated in therapeutic antibody-conjugates included vinblastine, methotrexate, doxorubicin, calicheamicin, maytansine derivatives and auristatin. Small molecule drugs are attached to mAbs through chemical linkers, whereas protein toxins can be produced through either chemical conjugation or genetic engineering [17]. Development of antibody-toxin-conjugates has met several challenges. The protein toxins can be immunogenic and the drug toxins could lack potency at the doses delivered to the tumor site.[18] Since internalization of antibody-toxin is required to achieve cytotoxic effect on tumor cells, the choice of target and antibody might be another limitation factor. With chemically conjugated toxin, decay of the linker used to join the antibody to the toxin could alter efficacy and toxicity. Of 44 antibody-toxin-conjugates that entered clinical trials after 1980, only one antibody-toxin-conjugate, Mylotarg, (gemtuzumab ozogamicin, UCB/Wyeth), a humanized anti-CD33 mAb conjugated to calicheamicin for relapsed acute myeloid leukemia, has been approved to date (**Table 1**). 14% of mAbs are now in the clinic studies are antibody-toxin-conjugates. Therefore, for anticancer antibody therapeutic development, the current trend has been toward functional unmodified mAbs. Of the current candidates in clinical study, functional unmodified mAbs outnumber antibody conjugates by more than two to one. But, with improvements in potency and linker stability, the antibody-conjugates as cancer therapeutics might spur new round of study in the future. One recent promising development is that Herceptin-DM1 (trastuzumab-DM1, ImmunoGen/Genentech) has showed a total clinical benefit rate of approximately 53 % on the patients with HER2 positive and Herceptin-resistant metastatic breast cancer in a phase 2 clinical trial.[19]

Modulation of host immune system

Unmodified functional monoclonal antibodies make up the majority of the mAbs in clinical development. One primary mode of action of the unmodified mAbs is the destruction of targeted cells through activation of components in human immune system. After binding to the targeted cells, mAbs can either recruit effector cells such as natural killer cells, to execute antibody-dependent cell cytotoxicity (ADCC) or activate complement to trigger complement-dependent cytotoxicity (CDC).[3] Both ADCC and CDC are mediated through the Fc portion of the mAbs. Due to differential affinities of Fc receptors, human IgG1 and IgG3 and murine IgG2a are potential potent isotypes at inducing ADCC and CDC.[3,20-22] In current clinical trials, of the unmodified mAbs with a known isotype, 95% are IgG and 5% are IgM which can function through CDC. The majority (>80%) of IgG mAbs with human Fc regions are IgG1. Avoidance of IgG3 could be due in large part to the reported short serum half-life of IgG3 relative to other IgG isotypes (7 days versus 21 days).[23] Although mAbs have been designed to function through different modes of action (blockade of growth factor–receptor interaction, and inhibition of signaling, and others), commercial interests have been focused on humanized or human mAbs which might have ADCC and CDC activities. Seven approved unmodified mAbs are IgG1 (**Table 1**), three mAbs—rituximab (Rituxan; Biogen Idec), alemtuzumab (Campath; Genzyme) and ofatumumab (Arzerra: Genmab/GlaxoSmithKline)—use ADCC and/or CDC as the primary mode of action. The other four mAbs function mainly through different modes of action (blockade of growth factor–receptor interaction, receptor down-regulation, and inhibition of signaling). It should be kept in mind that mAbs can function through a variety of modes after binding to the targeted cell. For example, trastuzumab (Herceptin; Genentech) was reported to induce ADCC as a possible alternative or additional mechanism to human

epidermal growth factor receptor 2 (HER2) downmodulation.[24] Similarly, besides eliciting ADCC and CDC mechanisms upon binding to CD20 positive cells, rituximab can also affect intracellular calcium levels and might induce cell death through apoptosis.[25]

Several studies have established the importance of Fc–FcγR interactions for the *in vivo* antitumour effects of certain monoclonal antibodies in murine models and clinical trials. A seminal paper showed that the antitumour activities of trastuzumab and rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec) were lower in FcγR-deficient mice than wild-type mice.[26] The role of FcγR in the antitumour response has been further supported by the finding that polymorphisms in the gene encoding FcγRIII, which lead to higher binding of antibody to FcγRIII, are associated with high response rates to rituximab in patients with follicular non-Hodgkin's lymphoma.[27] Efforts to modify the Fc domain primary structure using computational and high-throughput screening have resulted in Fc domains with higher affinity for FcγRIIIA and an enhancement of ADCC.[28] The new CD20-specific antibodies ocrelizumab (2H7; Genentech/Roche/Biogen Idec) and AME-133 (Applied Molecular Evolution/Eli Lilly) both contain mutated Fc domains and promote enhanced ADCC compared with rituxumab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec) . Modification of Fc domain oligosaccharide content provides another approach for enhancing ADCC. Most of the currently used therapeutic antibodies are highly fucosylated owing to the nature of the cell lines used for manufacturing. However, antibodies with defucosylated oligosaccharides show a significant enhancement in ADCC *in vitro* and enhanced *in vivo* antitumour activity.[29] Phase I trials of non-fucosylated antibodies specific for CC-chemokine receptor 4 (CCR4), which is expressed by some lymphoid neoplasms and is used by TReg cells to facilitate their migration to the tumour microenvironment,[30] have shown promise and early data suggest efficacy at significantly lower doses than conventional therapeutic antibodies.[29]

Optimization of antibody-based complement activities can enhance antitumour activity. For example, the CD20-specific antibody ofatumumab (Arzerra; Genmab/GlaxoSmithKline), which mediates improved CDC, was approved for the treatment of patients with chronic lymphocytic leukaemia (CLL) in 2009. This fully human antibody binds a different epitope than rituximab with improved binding kinetics, and it induces potent tumor cell lysis through improved activation of the classical complement pathway.[31] An initial study in patients with refractory CLL showed a 50% response rate to ofatumumab, suggesting a higher efficacy than rituximab in patients with CLL, although this higher response rate may not be solely due to enhanced CDC.[31] Several studies indicate that both CDC and ADCC can contribute to monoclonal antibody-induced tumor cell lysis. However, the relative clinical importance of each mechanism, and whether these mechanisms are synergistic, additive or antagonistic, remains uncertain. For example, in a mouse model of lymphoma, depletion of complement enhances NK cell activation and ADCC, thus improving the efficacy of the antibody.[32] It should be kept in mind that the enhanced ADCC/CDC is a double-edge sword, while it enhances the killing of targeted tumor cells, it might specifically damage normal cells which express low level of receptors otherwise not be affected by antibodies bearing weak ADCC/CDC activities.

Blockade of ligand binding and signaling perturbation

Unmodified functional mAbs can exert their primary activity on tumor cells by blocking the binding of critical growth factors to the receptors and inhibit proliferation of tumor cells. Three approved unmodified mAbs function primarily by blocking epidermal growth factor receptor (EGFR, also known as HER1) - cetuximab (Erbitux; ImClone/Bristol-Myers Squibb), nimotuzumab (TheraCIM; YM BioSciences) and panitumumab (Vectibix; Amgen) (**Table1**). These mAbs inhibit binding of EGF to and subsequent activation of the EGF receptor.[33,34] The *in vivo* mechanism of action of trastuzumab has not yet been resolved; downmodulation of HER2-mediated signaling and increased tumor cell apoptosis have been proposed in addition to activation of ADCC.[35,36] A new HER3-targeted antibody, MM-121 (Merrimack Pharmaceuticals), is currently being developed and has been shown to specifically bind HER3, inhibit growth of mouse xenograft tumours and block heregulin-dependent signalling through the protein kinase AKT, leading to tumor cell death.[37] Another approved unmodified mAb - bevacizumab (Avastin; Genentech/Roche) - binds to the ligand vascular endothelial growth factor (VEGF) and inhibits subsequent activation of VEGF receptors, resulting in decreased tumor angiogenesis.[38]

mAbs in current clinical studies

Anti-cancer mAbs from 50 company worldwide are currently in clinical study. Most of current mAbs in clinical testing are in early phase of the study, only less than 10% percent are in Phase III studies. Most of those mAbs are humanized or human mAbs. Antibody-conjugates make up less than one-third of the total, and none is currently in Phase III studies. Unmodified mAbs in clinical study act via various modes of action, including ADCC, CDC, receptor blockade, growth factor inhibition, programmed cell death and immunomodulation. Most mAbs studied in the clinic have targeted various glycoproteins, glycolipids and carbohydrates on the surface of cancer cells,[33,39] although a few (5%) have targeted soluble proteins. The 206 anticancer mAbs in the clinical trials were specific for at least 76 targets (note that the targets were unknown for 11 mAbs). Of the 76, 43 (57%) were targets for only one mAb. At least two clinically studied mAbs were specific for each of the remaining 33 targets (**Table 2**). About half of the mAbs directed towards the common "hot" targets. At least four mAbs each target CD22, CD33, EGFR, EpCAM, HER2 and MUC1 — with four mAbs in late stage clinical studies (targeting CD20, CD22, EGFR and EpCAM). One candidate in Phase III

studies, catumaxomab (TRION Pharma GmbH) is unusual because the mAb is bispecific as well as trifunctional.[40] Catumaxomab is designed to bind to both human EpCAM (target on tumor) and human CD3 (target on T cells), bringing cancer cells into proximity with the immune-system cells that can destroy them. Another promising example of a bispecific antibody is blinatumomab (MT103; Micromet/MedImmune), specific for tumor-associated CD19 and effector cell-expressed CD3, which is being investigated in Phase II clinical trials for the therapy of minimal residual disease of B cell-precursor acute lymphoblastic leukaemia indication. Bispecific antibodies directed against two different tumor associated or immunological antigen targets are another strategy that has been investigated, but with only limited success owing partly to the highly heterogeneous mixtures that result from the multiple possibilities of immunoglobulin chain association and also to scale-up and purification issues.[41]

Table 2. Major targets and anticancer monoclonal antibodies in clinical study.

Target	All mAbs	Functional mAbs	Conjugate mAbs
EpCAM	17	16	10
EGFR	12	11	3
CD20	10	9	4
MUC1	10	10	9
CEA	9	8	7
HER2	9	4	2
CD22	6	6	6
CD33	6	6	5
LEWISY	6	5	4
PSMA	6	6	3
TAG-72	5	4	4
CD30	4	2	0
CD19	3	2	2
CD44V6	3	3	3
CD56	3	3	3
GD2	3	3	1
GD3	3	3	0
HLA-DR10	3	3	1
IGF1R	3	3	0
TAL6	3	3	1
TRAILR2	3	3	0
VEGFR2	3	2	0
5T4 oncofetal antigen	2	1	1
Integrin αvß3	2	2	0
CAIX	2	2	1
CD5	2	2	1
CD40	2	2	0
CD55	2	2	1
CTA1	2	2	2
FAP	2	2	1
IL-6	2	2	0
VEGF	2	2	0
Unknown target	11	10	4
Total	**163**	**146**	**79**

Note: The table lists molecules that were the targets of a minimum of two mAbs in clinical study between 1980 and 2007.

Future Journey

Development of oncology therapeutics mAbs have gone through disappointment of failure to successful market products during last twenty-five years. The remarkable achievement is the approval of 12 mAb therapeutics to patients with a variety of cancers (**Tabel 1**). The attributes of target specificity, low toxicity and the ability to activate the immune system suggest the continuing promise of

therapeutic antibodies. The next generation of antibody therapies will ultimately yield many effective new treatments for cancer. These advances will result from the identification and validation of new targets, the more understanding on modes of action of mAbs, and the optimization of antibody structure to promote the various functions of mAbs. Due to the complex nature of cancer, combination of mAbs agents with other target therapeutics will be the best therapeutic approach in clinic.[42,43] Improved conjugation technologies will spur the additional round of development of antibody-conjugates. Multifunctional mAbs will more selectively bind to tumors by targeting pairs of normal antigens that are only present together on a given tumor cell. More precise identification of potentially responsive tumors will lead to improved patient selection.

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