The Current Status and Issues Concerning Monoclonal Antibody Therapy

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Abstract Monoclonal antibodies (mAbs) against growth factor or its receptor have raised the second wave of antibody therapy for solid tumors. Trastuzumab (humanized anti-HER2 mAb) is the first mAb approved for the treatment of a solid tumor, metastatic breast cancer. Large-scale phase III clinical trials are now ongoing to further evaluate the additive effects on chemotherapy and the efficacy as a maintenance monotherapy. Overexpression of HER2 is found only in a small percentage of patients with solid tumor, whereas epidermal growth factor receptor is expressed in a variety of solid tumors with high frequency. More broadly applicable for solid tumors is anti-angiogenesis therapy because it is targeting not tumor cells but tumor vasculature. Cetuximab (chimeric anti-EGFR mAb) and bevacizumab (humanized anti-VEGF mAb) have recently been shown to be clinically of remarkable effect for metastatic colorectal cancer. The points at issue are unexpected serious adverse effects including cardiac dysfunction of trastuzumab and thrombotic events of bevacizumab. In contrast to the mAbs as signal inhibitors, no apparent objective responses were seen in most clinical studies of mAbs against cell surface glycoproteins or adhesion molecules. However, several groups reported the survival benefit of those mAbs, in which anti-idiotypic antibody response may play an important role. In addition, some of their anti-idiotype mAbs showed the survival benefit in patients with solid tumor. Altered self-antigens have recently been attempted to intensify active immunotherapy including dendritic cell therapy. Considering that dendritic cells efficiently cross-present tumor antigens after ingesting them as immune complex, those "modest" mAbs may be of use for dendritic cell therapy as immune complex with altered self-antigens.

Key words: monoclonal antibody, HER2, epidermal growth factor receptor, vascular endothelial growth factor, VEGF receptor, human anti-mouse antibody, epithelial cell adhesion molecule, cytotoxic T lymphocyte-associated antigen 4, dendritic cells

Introduction

Development of monoclonal antibodies (mAbs) against cancer-associated antigens was initiated in the 1970s based on hybridoma technology,[1] when it was expected that a highly specific and effective antibody as the "magic bullet" for cancer would be realized. However, the results obtained from several clinical trials in the early 1980s revealed limited clinical responses and adverse effects mainly due to the xenogenicity of the mAbs. Through numerous subsequent preclinical studies on a variety of mAbs, the efficacy of shutting off receptor-mediated signaling on cell growth and viability was noticed. As a re-

sult, remarkable objective responses were observed during clinical trials using rituximab (chimeric anti-CD20 mAb)[2] or trastuzumab (humanized anti-HER2/neu/ErbB-2 mAb),[3] resulting in a second wave of mAb therapy. A variety of mAbs against human epidermal growth factor receptor 2 (HER2),[3-7] epidermal growth factor receptor (EGFR),[8-13] vascular endothelial growth factor (VEGF),[14,15] and VEGF receptor (VEGFR)[16] are now being evaluated in clinical trials and hopeful therapeutic effects have been revealed (Table 1).

In addition to the mAbs that function as signal inhibitors, there is another group of mAbs against cell-surface glycoproteins or adhesion molecules (Table 1).[17-28] Most mAbs were developed to detect diagnostic markers for solid tumors, and hence the corresponding antigens are expressed at a high frequency in tumors. This is convenient for antibody therapy. However, the clinical effects of these antibodies have been very modest even when used as radioimmunoconjugates,[29,30] although the latter is due to the low radiosensitivity of solid tumors rather than the characteristics of the antibodies themselves. It seems apparent that this low efficacy originates from the fact that the target molecules are not directly involved in the signal transduction pathway for cell growth or survival. On the other hand, the anti-idiotype antibody response against some of these mAbs has been shown to play a possibly important role in their clinical effects.[31] This suggests the possible utilization of these "modest" mAbs as immunogens for active immunotherapy rather than as molecular targeting drugs. This article presents the current status and issues concerning monoclonal antibody therapy especially with regards to solid tumors.

The second wave of mAb therapy

Trastuzumab (Herceptin®) is a humanized mAb, which was approved by the United States Food and Drug Administration in 1998 for the treatment of advanced breast cancer. This was the first approval of mAbs use in solid

Table 1. Monoclonal antibodies evaluated in clinical trials

Antigen	mAb		Reference	Antigen	mAb		Reference
HER2	Trastuzumab	H	3	Ep-CAM	17-1A	murine mAb	17
	CH401	C	4	CEA	T84.66	C	18
	Pertuzumab(2C4)	H	5		hMN-14	H	19
	OKT3 x trastuzumab	B	6	TAG-72	huCC49	H	20
	MDX-H210[1]	B	7	A33	huA33	H	21
EGFR	Cetuximab(C225)	C	8	GD2	ch14.8	C	22
	ICR62	rat mAb	9	L6	chL6	C	23
	ABX-EGF	FH	10	CD44v6	BIWA4	H	24
	h-R3	H	11		chU36	C	24
	EMD72000	H	12	PSMA	Huj591	H	25
	MDX-447[2]	B	13	Lewis-Y	BR96	C	26
VEGF	Bevacizumab	H	14		SGN-10[3]	scFv	27
	HuMV833	H	15	CC242	huC242 + DMI[4]	H	28
VEGFR	IMC-1C11	H	16				

H, humanized; C, mouse-human chimeric; B, bispecific; FH, fully humanized; scFv, single chain antibody; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Ep-CAM, epithelial cell adhesion molecule; CEA, carcinoembryonic antigen; PSMA, prostate specific membrane antigen.
1) Bispecific antibody of anti-Fcγ receptor I (CD64) and anti-HER2 mAbs.
2) Bispecific antibody of anti-Fcγ receptor I (CD64) and anti-EGFR mAbs.
3) BR96 scFv fused with Pseudomonas exotoxin (PE40).
4) Immunoconjugate of maytansine derivative (DM1) and humanized mAb huC242.

tumor therapy. Three years later, it was approved in Japan. The approved use of trastuzumab in HER2-positive metastatic diseases includes first-line treatment in combination with paclitaxel and as a monotherapy in patients who have received one or more chemotherapeutic regimens. HER2 overexpression is observed in 15-30% of breast cancer.[32)33)] The most serious, but unexpected toxicity observed during the pivotal trials was cardiac dysfunctions, including clinically manageable left ventricular systolic dysfunction, and occasionally advanced congestive heart failure in a small percentage of patients.[34)] The incidence and severity of cardiac dysfunction was greatest in patients receiving concomitant trastuzumab and anthracycline plus cyclophosphamide.[35)] Although the pathophysiological mechanisms of cardiac dysfunction associated with trastuzumab are not clearly understood, Crone et al demonstrated that mice with a ventricular restricted deletion of the HER2 gene developed dilated cardiomyopathy,[36)] indicating that HER2 signaling in the cardiomyocytes is essential for the prevention of this disorder. There are currently four major phase III multicenter trials that in total will randomize approximately 12,000 patients to chemotherapeutic regimens with or without trastuzumab.[37)] HER2 expression is examined using approved fluorescence in situ hybridization or immunohistochemistry assays. The duration of trastuzumab maintenance is 1 or 2 years after chemotherapy with or without trastuzumab. HER2 expression is observed in solid tumors other than breast cancer. Although some objective responses have been observed during clinical trials for colorectal and non-small cell lung cancers,[38)39)] the low overexpression rate of HER2, 8.6% in colorectal cancer and 9% in non-small cell lung cancer, limits the potential for further investigation.

EGFR and VEGF/VEGFR are additional possible targets of mAb therapy. In contrast to HER2, EGFR is expressed at a high frequency in a variety of tumors.[40)] Clinical trials have therefore been performed in a number of cancers including head and neck, breast, lung, colorectal, pancreatic, kidney, bladder, ovarian and brain cancers. Although there are a number of mAbs being subjected to clinical trials,

a murine-human chimeric mAb, cetuximab (C 225), has been extensively evaluated and has offered most of the currently available clinical data.[8)] One recent result was obtained from clinical trials for colorectal cancer.[41)] In total, 329 patients with irinotecan-refractory metastatic colorectal cancer were randomized into two trial arms (arm A, cetuximab plus irinotecan; arm B, cetuximab alone). The median times of progression of arms A and B were 4.1 and 1.5 months, respectively, indicating the significant additional effect of cetuximab ($P < 0.0001$). The therapeutic activity of cetuximab alone has also been revealed in chemotherapy-refractory colorectal cancer patients.[42)43)] The adverse characteristic of anti-EGFR-targeted therapy is an acne-like skin rash, which was observed in 77% of 813 patients treated in 21 trials.[44)] Of the 27 responders in one clinical study, 26 had a skin rash, suggesting that it might be useful in identifying patients whose cancers were more likely to respond to cetuximab.

Anti-angiogenesis therapy is unique in targeting tumor vasculature, but not tumor cells themselves, and therefore it is broadly applicable for most solid tumors.[45)] Although numerous growth factors are involved in angiogenesis, VEGF is hypothesized to play a pivotal role in tumor angiogenesis.[14)] Bevacizumab is a humanized anti-VEGF mAb, which has been most extensively investigated in a variety of tumors including non-small cell lung, breast, prostate, renal and colorectal cancers.[14)] The latest notable finding was as a result of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil (5-FU), leucovorin (LV)) as the first line therapy of 925 patients with metastatic colorectal cancer.[46)] Patients were placed randomly in the following three groups: IFL/placebo (n = 412); IFL/bevacizumab (n = 403); 5-FU/LV/bevacizumab (n = 110). The median survival times of the IFL/placebo and IFL/bevacizumab groups were 15.6 and 20.3 months, respectively ($P = 0.00003$). It should be noted that median survival time of the IFL/bevacizumab individuals was equal to that of a recent combined chemotherapy trial, the FOLFOX regimen (5-FU/LV/Irinotecan/Oxaliplatin);[47)] however, in this trial serious adverse effects such as hemorrhaging and

thrombosis were observed. A clinical trial of bevacizumab with or without 5-FU/LV for metastatic colorectal cancer (n = 105) resulted in gastrointestinal bleeding, epistaxis and thrombotic events in 10.3, ~50, and 19.1% of the patients receiving bevacizumab alone, respectively.[14] Thrombosis seems to be more serious than bleeding, and one patient had a fatal pulmonary embolism. Considering that the incidence of thrombosis among cancer patients receiving chemotherapy was estimated at approximately 10%,[48] it is possible that bevacizumab has some causal additional effects. A phase I trial of SU5416, a tyrosine kinase inhibitor for VEGFR, combined with 2 chemotherapeutic drugs for advanced cancer patients resulted in five vascular and four venous thromboembolic events in 8 of the 19 patients studied, leading to termination of the study.[49] This suggests that bleeding and thrombotic complications might be a common adverse side effect of anti-VEGF-targeted therapy. Although the mechanism is unclear, the disruption of tumor endothelium could potentially lead to hemorrhaging and set off a clotting cascade along the denuded vessels.

Advantages and disadvantages of mAbs immunogenicity

Administered murine mAbs evoke a human anti-mouse antibody (HAMA) response, which neutralizes the injected mAb by immune complex formation. This consequently eads to rapid clearance or adverse effects such as allergic (rash, hives, flushing, and so on) and anaphylactic reactions. Circulating HAMA with high titer also restricts the number of times the mAbs are administered. Approximately 50 to 75% of patients with solid tumors develop HAMA after treatment with murine mAbs, whereas it is found in only approximately 30% of patients with relapsed B-cell malignancies probably due to disease-related B-cell dysfunction.[31] In a clinical trial of 17-1A antibody (a murine mAb against the epithelial cell adhesion molecule, Ep-CAM) for colorectal cancer, HAMA was detected in 80% of the treated patients (n = 83).[17] Adverse events were observed in 37% of the patients, but only nine (11%) discontinued antibody therapy. To reduce the immunogenicity of murine mAbs,

their chimerization (replacement of the murine constant region with a human one) or humanization (grafting of a murine complementary determining region (CDR) into human immunoglobulin) has been performed, and mice carrying human Ig transgenes have been available as a source of fully human antibodies.[50] Although human anti-human antibody (HAHA) responses occur at a high frequency even after humanization, adverse events seem to decrease. Phase I and II clinical studies of huA33 (a humanized version of murine mAb A33 that recognizes a cell surface glycoprotein belonging to the immunoglobulin superfamily) in colorectal cancer showed that 63% (26 of 41) of the patients treated with repeated doses of huA33 developed HAHAs against a conformational antigenic determinant located in the VL and VH regions of huA33.[51] In these studies, however, the HAHAs indicative of infusion-related adverse events were detected in only 17% of patients. Thus, it is now generally considered that reduced immunogenicity could be useful in improving the safety of repeated mAb administrations, especially those against growth factor or its receptor.

However, several reports have documented the therapeutic benefit of HAMA. DeNardo et al., for example, suggested that a threshold HAMA titer might be required to obtain a survival benefit.[31] A high serum HAMA titer was associated with increased survival in patients with B-cell lymphoma (n = 51) treated with radiolabeled Lym-1. Patients with a serum HAMA titer > 1000 μg/ml showed a median survival of 244 weeks whereas with less than 1000 μg/ml it was 57 weeks.[52] Miotti et al. also reported a similar finding in ovarian cancer patients.[53] In phase I and II clinical studies of T-cells retargeted with the bispecific (ab')2 OC/TR (genetically prepared using anti-folate receptor-alpha (MOv18) and anti-CD3 mAbs), a significantly longer median survival probability was observed in patients with high HAMA levels compared to those with lower levels. Although the mechanisms concerning HAMA responses and survival remain unknown, anti-idiotype antibodies have been proposed as a possible candidate. A significant association between anti-idiotype antibodies and survival was ob-

served in clinical trials with mAb 17-1A (edrecolomab) for colorectal cancer,[54] bispecific (ab')2 OC/TR for ovarian cancer[53] and 3F8 (anti-GD2 mAb) for neuroblastoma.[55] Frodin et al.[54] detected the anti-idiotype antibodies (Ab2) to 17-1A in 95% (41/43) of the patients studied and the anti-anti-idiotype antibodies (Ab3) in 47% (20/43) after 17-1A therapy. The patients generating Ab3 survived significantly longer than those who did not (80 vs 38 weeks) and a significant correlation was found between the presence of Ab3 and tumor response. Cheung et al.[55] showed that long-term progression-free survival correlated significantly with the Ab3' (anti-GD2) response at 6 months and with the Ab3 response at 6 and 14 months after therapy. These findings suggested the importance of Ab3 generation on favorable outcomes. Moreover, as described above, a high HAMA titer against murine or chimeric antibodies might be associated with prolonged survival. This suggests that the xenogenic antigens of these mAbs might serve as non-specific helpers for driving the anti-idiotype network. There is conflicting evidence concerning 17-1A.[56] Analysis that differentiated between patients who developed recurrences and those who remained tumor-free did not show any difference in the antibody titers between the 2 groups, or between the total HAMA or IgG, IgM, or Ab2 levels. However, in this study Ab2 was detected in 78% (18/23) of patients whereas no Ab3 response was found (0/8). Although further studies with a larger sample size are required to confirm the clinical significance of anti-idotypic responses in murine or chimeric mAb therapy, clinical trials using humanized mAb, to which anti-idiotype antibodies can be generated, might be useful.

Intensified active immunotherapy - the possible application of mAbs

The inadequate immunogenicity of self/tumor antigens based on immunological tolerance is the most important factor with regards to active immunotherapy. A variety of experiments are being attempted to enhance the immunogenicity of tumor antigens. Altered peptide ligands instead of native self-peptides have been shown to improve the reactivity of T cells specific to tumor antigens.[57][58] Fong et al.[57] reported an impressive result during phase 1 clinical trials of altered CEA peptide-pulsed dendritic cell therapy for metastatic or recurrent colorectal or non-small cell lung cancer (n=12). An agonist epitope of $CEA_{605-613}$ has been identified with aspartate substituting asparagine at position 610. This substitution does not increase the class I MHC binding of the peptide, but possesses increased potency in inducing cytotoxic T lymphocytes against CEA. A complete response was observed in 2 colorectal cancer patients, while a mixed response and stable disease was seen in 1 and 2 patients, respectively. Another report documented a phase I trial of altered peptides from a gp100 melanoma-associated antigen combined with a cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade in stage IV melanoma.[58] CTLA-4 (CD152) binds to CD80 and CD86, but with greater affinity than to CD28, and antagonizes T cell activation. Fourteen patients were treated with serial intravenous administrations of a humanized anti-CTLA-4 antibody in conjunction with subcutaneous vaccinations of 2 modified peptides emulsified in incomplete Freund's adjuvant. Two patients experienced complete tumor responses, and one showed a partial response. Serious autoimmune effects (grade III/IV) including enterocolitis, dermatitis, vitiligo, hypophysitis, hepatitis and so on, were observed in 9 patients. This was considered a nonspecific phenomena resulting from the CTLA-4 blockade because the distribution of gp100 expression was inconsistent with the target organs of autoimmunity. Although these adverse effects had to be treated with immunosuppressive drugs, autoimmune reactions were observed in both patients with complete responses, suggesting that nonspecific immunostimulation with the anti-CTLA-4 antibody might enhance specific tumor immunity. In addition to altered peptides, the utilization of xenogeneic proteins as tumor antigens has been attempted to intensify the immune response. A phase I trial using dendritic cells pulsed with recombinant mouse prostatic acid phosphatase as a tumor vaccine was performed on 21 patients with metastatic prostate cancer.[59] Clinical stabilization and the development of a human PAP-

specific T cell response correlated with an increase in progression-free survival was seen in 6 of these 21 patients. These findings suggest that altered self-antigens could improve the clinical efficacy of active immunotherapy.

It has been revealed that immune complexes internalized through the Fcγ receptor on dendritic cells are efficiently presented to cytotoxic T lymphocytes through the MHC class I pathway.[60][61] Regnault et al.[60] suggested that antigen presentation by dendritic cells through Fcγ receptor-mediated uptake can be increased 100-fold over pinocytosis of soluble antigens, and that immune complexes themselves can effectively induce dendritic cell maturation. Very recently, preclinical studies on mice showed that immune complex-loaded dendritic cells had a more efficient *in vivo* anti-tumor effect than the loading antigens alone.[62][63] Some mAbs against cell surface glycoproteins or adhesion molecules could be used for this immune complex-loaded dendritic cell therapy. Corresponding antigens composed of altered peptides or xenogenic proteins might therefore be desired. For example, there is a mouse homologue of the Ep-CAM (17-1 A) gene encoding the epithelial cell adhesion molecule, which shares 82% amino acid identity with human Ep-CAM.[64] Since mAb 17-1 A cross-reacts with rat Ep-CAM,[64] it should recognize the murine antigen. Ep-CAM was shown to be expressed in human myeloma cell lines, but not in normal hematopoietic cells.[64][65] The immune complex of murine mAb 17-1A and the recombinant murine Ep-CAM protein would be a good candidate for the dendritic cell therapy of various epithelial tumors and multiple myeloma. Murine mAb is also expected to offer some non-specific helper epitopes.

Acknowledgements

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