

# Monoclonal antibodies as therapeutic agents in oncology and antibody gene therapy

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Antibodies as therapeutic agents are mostly used in oncology, as illustrated by their applications in lymphoma, breast cancer or colorectal cancer. This review provides a brief historical sketch of the development of monoclonal antibodies for cancer treatment and summarizes the most significant clinical data for the best-established reagents to date. It also discusses strategies to improve the anti-tumor efficacy of antibody therapy, including antibody gene therapy and exploitation of bone marrow derived primary mesenchymal stem cells as the antibody gene transporter.

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## Introduction

The discovery of monoclonal antibodies (mAbs) in 1975 by Kohler and Milstein ushered in cancer treatment to a modern era of targeted therapy [1, 2]. Therapeutic antibodies have become a major strategy in cancer therapy due to their ability to specifically bind to primary and metastatic cancer cells with high affinity. They elicit anti-tumor effects by complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity, or by focused delivery of radiation or cellular toxins (conjugated antibodies) [3-5]. Initially, the overall clinical outcomes were poor due to immunogenicity of the murine antibody used in therapy, modulation of targeted antigens and poor ability of these antibodies to mediate sufficient antibody-dependent effector functions in humans. Technological advances designed to overcome these main limitations have accelerated the development of antibodies as therapeutics. Since 1997, there have been nine anti-cancer therapeutic antibodies approved by the FDA for sale in the USA. A large number

of additional therapeutic antibodies are in early or late stages of clinical development.

## Development of recombinant antibodies as cancer therapeutics

Therapeutic mAbs are from the typical immunoglobulin G class (IgG), containing two heavy and two light chains. The first report of successful cloning of an immunoglobulin gene segment was published in 1977, nearly one decade after the discovery of restriction endonuclease. It took another few years before the first recombinant antibodies, also named as "chimeric" mAbs, were generated by fusing rearranged murine variable V(D)J gene segments of a mouse mAb to human constant domains, or as recombinant Fab fusion proteins by replacing the Fc fragment with an enzyme moiety [6, 7]. Compared with murine mAbs, chimeric antibodies were capable of mediating antibody-dependent cellular cytotoxicity with human effector cells, and/or activating the complement cascade very efficiently, both *in vitro* and in pre-clinical trials. The use of chimeric antibodies significantly reduced the human anti-mouse antibody (HAMA) responses, but did not eliminate them completely.

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In order to further decrease the immunogenicity of murine antibodies, the first mouse mAb was “humanized” in 1986 by grafting the gene segments encoding the six antigen-binding loops onto human framework regions [8-10]. Although this process further reduced or eliminated the HAMA responses, it was still necessary to further redesign the antibody in many cases in order to re-establish the required specificity and affinity of the original murine antibody. Another approach to reduce the immunogenicity of mouse mAbs was to replace the immunogenic epitopes in the murine variable domains with benign amino-acid sequences. The de-immunized variable domains are then genetically linked to human IgG constant domains to yield de-immunized antibodies. In addition, primatial antibodies that featured the human and monkey chimeric antibody structure were developed to further reduce their immunogenicity. As nearly exact copies of human antibodies, they could be administered repeatedly and continuously for the treatment of chronic diseases. Also, fully human antibodies have been developed using murine sources, phage antibody libraries for human Ig genes and transgenic techniques [11].

The approval of nine mAbs by the FDA for clinical tumor therapy signifies important progress in antibody study. They include rituximab (Rituxan<sup>®</sup>), ibritumomab (Zevalin<sup>®</sup>), <sup>131</sup>I-tositumomab (Bexxar<sup>®</sup>), gemtuzumab ozogamicin (Mylotarg<sup>®</sup>), alemtuzumab (Campath<sup>®</sup>), trastuzumab (Herceptin<sup>®</sup>), bevacizumab (Avastin<sup>®</sup>), cetuximab (Erbix<sup>®</sup>) and panitumumab (Vectibix<sup>®</sup>). These mAbs can be divided into two groups according to their targets [12]. The first group is directed against antigens that are either specifically expressed by tumor cells, such as the Her-2/neu protein on breast cancer and other carcinomas, or are shared with normal cells, such as differential antigens (CD20, CD52, CD19, etc.). The aim of using this type of antibodies is to induce tumor death by neutralizing the effect of a growth factor, or by inducing apoptosis, or by activating effector mechanisms of the host. The second group of therapeutic mAbs targets the stroma-tumor interactions. A leading example of this group is an mAb that neutralizes vascular endothelial growth factor (VEGF), a tumor-derived growth factor that increases neo-angiogenesis and thus supports tumor growth. Neutralization of VEGF blocks angiogenesis and leads to tumor starvation. There are also other mAbs being tested, including those targeting VEGF receptors or neutralizing adhesion molecules or proteases used by the tumor cells to invade their environment.

Apart from these two groups of mAbs, there is another type of therapeutic mAb on clinical trial, which aims to potentiate immune reactions against tumors. It is now acknowledged that there are immune reactions in cancer patients against their tumors. It was strongly suggested 35

years ago in acute leukemia and demonstrated in viral-associated tumors, such as Burkitt lymphoma, cervical carcinoma or hepatocellular carcinoma. Recently, a study of about 1 000 patients has established the case in colorectal cancer [13]. However, the immune reaction is inefficient in patients with growing cancers, and the efficacy of therapeutic vaccine is still low. Tumors use several mechanisms to escape the immune system. For example, they induce lymphocytes to inhibit the immune reactions. These immune suppressors, also called regulatory lymphocytes, express specific markers (CD25, CTLA-4) that could serve as targets of mAbs to inhibit or destroy these cells, allowing for a better immune response to the tumor. However, the first therapeutic trial using a human mAb directed against CTLA-4 showed that the therapeutic efficacy on cancer was accompanied by auto-immunity due to the fact that regulatory lymphocytes also control anti-self-reactivity [14]. Other ways by which tumors escape immune attacks include modulation of their surface antigens, inhibition of complement-mediated cytotoxicity and ectopic expression of Fcγ receptors [15].

### Approved antibody therapeutics for malignancies

The earliest and most successful clinical use of antibodies as anti-cancer therapeutics has been for the treatment of hematologic malignancies. Advances in recombinant technologies make it possible to generate more specific and higher affinity mAbs with reduced immunogenicity after humanization or de-immunization, which together with the emerging conjugation capabilities, have enabled antibody therapeutics to become a major weapon in the treatment of leukemia and lymphoma. Since 1997 there have been nine commercially available therapeutic mAbs approved by the FDA, among which five are being used for treatment of hematologic malignancies and the other four for solid tumors (Table 1).

#### Rituximab (Rituxan<sup>®</sup>)

Rituximab has arguably become the commercially most successful anti-cancer drug of all mAb types since the introduction of taxanes. Rituximab sales exceeded \$ 1700 million in the US in 2004. Rituximab, which targets the CD20 surface receptor that is strongly overexpressed in most B-cell lymphomas, is a chimeric, monoclonal IgG1 antibody that induces apoptosis, antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity. Rituximab was originally used in aggressive recurrent or refractory non-Hodgkin’s lymphoma (NHL), and has been incorporated later into numerous chemotherapy regimens with promising results since its approval by the FDA in

**Table 1** FDA-approved mAbs in oncology

Product	Target	Type	Approved year	Indications
Rituximab (Rituxan <sup>®</sup> )	CD20	Chimeric IgG1	1997	B-cell lymphoma
Trastuzumab (Herceptin <sup>®</sup> )	HER2/neu	Humanized IgG1	1998	Breast cancer
Gemtuzumab (Mylotarg <sup>®</sup> )	CD33	Humanized IgG4-toxin-conjugate	2000	Acute myeloid leukemia
Alemtuzumab (MabCampath <sup>®</sup> )	CD52	Humanized IgG1	2001	Chronic lymphatic leukemia
<sup>90</sup> Y-ibritumomab (Zevalin <sup>®</sup> )	CD20	Murine IgG1-radiionuclide-conjugate	2002	B-cell lymphoma
<sup>131</sup> I-tositumomab (Bexxar <sup>®</sup> )	CD20	Murine IgG1-radiionuclide-conjugate	2003	B-cell lymphoma
Bevacizumab (Avastin <sup>®</sup> )	VEGF	Humanized IgG1	2004	Colorectal cancer
Cetuximab (Erbix <sup>®</sup> )	EGFR	Chimeric IgG1	2004	Colorectal cancer
Panitumumab (Vectibix <sup>®</sup> )	EGFR	Entirely human IgG2	2006	Colorectal cancer

1997. Today, more than 540 000 patients globally have received rituximab as part of more than 200 completed, ongoing or planned clinical trials. Rituximab has recently been approved to be administered as either a 4- or an 8-dose weekly regimen in patients who have previously responded to rituximab, and in patients who have bulky tumors (>10 cm). A multicenter, open-label, single-arm study was conducted in 166 patients, with relapsed or refractory low-grade or follicular B-cell NHL, who received 4 weekly doses of 375 mg/m<sup>2</sup> of Rituxan (given as an i.v. infusion). Thirteen patients with tumor masses >10 cm or with >5 000 lymphocytes/μl in the peripheral blood were excluded from the study [16]. The overall response rate (ORR) was 50%, with 6% complete response (CR) and 44% partial response (PR) rates. The median time to onset of response was 50 days and the median duration of response was 11.2 months. Disease-related signs and symptoms were present in 23% (39/166) of the patients at the study entry, and among these 64% (25/39) showed resolution of symptoms. In another study, prolonging the standard treatment from 4 weekly doses to 8 did not lead to a higher response rate but prolonged response duration [17]. Thirty-seven patients with relapsed or refractory, low-grade NHL received 8 weekly doses of 375 mg/m<sup>2</sup> of Rituxan. The ORR was 57% (CR 14%, PR 43%), with a projected median duration of response of 13.4 months.

Based on *in vitro* data suggesting synergistic actions of

rituximab and cytotoxic agents including anthracyclines [18], the combination of six cycles of chemotherapy consisting of CHOP (cyclophosphamide, adriamycin, oncovin and prednisone) with concurrently administered rituximab in 40 patients with predominantly untreated follicular lymphoma greatly increased the response rate (55% CR, 40% PR), suggesting synergistic actions with no significant additional toxicity [19]. Median time to disease progression was recently determined to be 82.3 months [20]. Results are comparable if, instead of parallel administration, CHOP is followed by rituximab [21]. The combination of rituximab with chemotherapy containing purine analogs, such as fludarabine, further increased the CR rate to 80% [22]. The superiority in achieving CR with combinations containing purine analogs is supported by a trial published in abstract form comparing fludarabine/mitoxantrone with rituximab consolidation to CHOP/rituximab showing CR rates of 87% and 76%, respectively [23]. Recently, there has been increasing interest in using rituximab as a first-line therapy. Arguments in favor of this approach include its excellent toxicity profile, the presumed low rate of secondary malignancy and a lack of stem cell toxicity. In a phase II study, 62 patients with indolent NHL, most of whom suffered from follicular lymphoma, were treated with rituximab as the first-line drug at the standard 4 weekly doses of 375 mg/m<sup>2</sup> [24]. Patients achieving an objective remission received maintenance treatment of rituximab every 6 months for a

maximum of 2 years. The ORR was 73% (CR 37%, PR 36%) and the median actuarial progression-free survival was 34 months, which were clearly better than those in pre-treated patients.

The main toxicities associated with rituximab included infusion-related chills, fever, nausea, vomiting and general fatigue. Bone marrow suppression was not a significant side effect in patients treated with rituximab.

### <sup>90</sup>Y-ibritumomab (Zevalin®)

The Zevalin® (ibritumomab tiuxetan) therapeutic regimen is a cancer therapy for patients with relapsed or refractory low-grade, follicular or transformed B-cell NHL, and also for patients with follicular B-cell NHL that is refractory to Rituxan® (rituximab) therapy. Zevalin is the first agent in a new class of targeted cancer treatments called radio-immunotherapies. It is composed of the murine version of the anti-CD20 mAb rituximab that has been covalently linked to the metal chelator, MD-DTPA, permitting stable binding of <sup>111</sup>In when used for radionuclide tumor imaging and <sup>90</sup>Y when used to produce enhanced targeted cytotoxicity. The safety and efficacy of the Zevalin therapeutic regimen was evaluated in two pivotal multicenter clinical trials conducted in the US [25]. ORR was measured based on standardized NHL tumor shrinkage criteria (International Workshop Response Criteria – IWRC). The first efficacy study involved 54 patients with relapsed follicular NHL who failed to respond to Rituxan, the ORR to Zevalin treatment was 74%, with 15% of the patients achieving a complete remission. Many of the patients participating in this trial were heavily pre-treated (with a median of four prior anti-cancer regimens) and had large or “bulky” tumors. For this population of patients who had essentially exhausted other available treatment options, Zevalin was shown to be highly effective. The second pivotal study, a phase III randomized, controlled trial, was conducted among 143 patients with relapsed or refractory low-grade follicular or transformed B-cell NHL [26]. Of them, 73 patients who received the Zevalin therapeutic regimen (which includes Rituxan) showed an 80% ORR, compared to 56% ORR in the remaining 70 patients who received Rituxan alone. Thirty percent of Zevalin-treated patients achieved a complete remission and 4% achieved an unconfirmed complete remission to therapy, compared to 16% complete remission for patients treated with Rituxan only (4% achieved an unconfirmed complete remission), according to the IWRC. In addition to the two pivotal trials, a phase II study of 30 patients was conducted to study the safety and efficacy of a reduced dose of Zevalin in patients with low blood platelet counts (mild thrombocytopenia) [27]. While the risk and incidence of hematologic toxicity were higher among this

already compromised group than in the two other trials, patients responded well to the Zevalin regimen, showing a 67% ORR without an increase in non-hematological adverse events. However, there was a greater incidence of hematologic toxicity than in the two pivotal studies.

<sup>90</sup>Y-ibritumomab received the orphan drug status and was approved by the FDA for the treatment of relapsed/refractory B-cell NHL in 2002, including patients with rituximab-refractory diseases. Treatment with the radiolabeled anti-CD20 antibody seems to produce a somewhat higher response rate compared to treatment with “naked” antibody; however, this does not lead to increased survival. There is considerable toxicity compared with rituximab, and there are also ongoing concerns regarding the long-term safety of radiation exposure.

### <sup>131</sup>I-tositumomab (Bexxar®)

Another radio-immunotherapeutic agent targeting the CD20 antigen is <sup>131</sup>I-labeled tositumomab. Due to the highly variable elimination of <sup>131</sup>I in different patients, it is necessary to calculate each patient’s individual dose, with a target total body radiation dose of 75 cGy. The nature of <sup>131</sup>I as a combined β- and γ-emitter with the γ-radiation penetrating tissues up to 1 m allows dosimetry analysis of the therapeutic drug. The downside of this effect is that the total body dose delivered to the patients by the drug is higher than by its <sup>90</sup>Y-carrying counterpart <sup>90</sup>Y-ibritumomab. Patients receiving the drug should avoid close contact with other people and stay away from the public for 3-4 days due to their emission of radiation. Similar to <sup>90</sup>Y-ibritumomab, <sup>131</sup>I-tositumomab administration is preceded by an infusion of the unlabeled antibody rituximab.

Clinical trials with <sup>131</sup>I-tositumomab preceded those with <sup>90</sup>Y-ibritumomab, and therefore longer follow-up and more reliable information of long-term toxicity are available. Similar numbers of patients have been treated with <sup>131</sup>I-tositumomab, and the results of non-myeloablative radioimmunotherapy (RIT) with the drug are comparable to what is achieved with <sup>90</sup>Y-ibritumomab. An ORR of 71% was achieved in a trial with 59 patients with relapsed or refractory advanced lymphoma. Median survival of responders was 12 months [28]. In a trial enrolling 24 patients with relapsed low-grade NHL, a myeloablative dose of <sup>131</sup>I-tositumomab was administered. Apart from the expected marrow toxicity, effects on other organs were acceptable and considerably lower than in conventional myeloablative therapy. The ORR was 87% with a CR rate of 81% [29]. In a retrospective analysis of 125 patients receiving autologous stem cell transplants after either myeloablative RIT or a combination of total body irradiation and high-dose chemotherapy, the overall 5-year survival

was significantly higher in the RIT group (67% vs 53%) [30]. In 2003, <sup>131</sup>I-tositumomab was approved by the FDA for the non-myeloablative treatment of patients with B-cell NHL refractory to rituximab.

### Gemtuzumab ozogamicin (Mylotarg®)

Gemtuzumab-ozogamicin (GO) is a humanized antibody to CD33 conjugated with calicheamycin, a highly potent cytotoxic antibiotic. CD33 is expressed on leukemic blast cells in most patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). After administration, the antibody portion binds to CD33 and forms a complex that is internalized by the target cells. Following endocytosis, calicheamycin migrates into the nucleus producing double-strand breaks that ultimately result in cell death.

One hundred and forty-two patients with untreated first relapse of AML were subsequently enrolled in three multicentre phase II studies [31]. Patients received two doses of 9 mg/m<sup>2</sup> GO, with the second administration being given 2 weeks after the first treatment. The overall remission rate was 30%, consisting of 16% CR and 14% CR without platelet recovery (CRp). Median time to remission was 60 days, and median length of remission was 7.2 months in patients achieving CR, and 4.4 months in patients achieving CRp. In a recent trial, 51 patients over 65 years with untreated AML or advanced MDS were randomized to receive single agent GO or GO and Interleukin 11 [32]. An 8% CR was achieved in patients who received standard GO alone and 36% in patients who received GO and IL-11. In a phase II trial in 49 patients < 65 years with *de novo* AML, addition of one dose of 6 mg/m<sup>2</sup> GO to the standard induction treatment consisting of idarubicin/cytarabine led to a CR rate of 84% [33]. On the basis of the above data, GO achieved the orphan drug status and was approved by the FDA for the treatment of patients ≥ 60 years with relapsed AML who were not considered to be candidates for standard cytotoxic therapy.

Side effects were more common and pronounced than those usually observed with “naked” antibodies: fever and chills occurred in 80% of patients, and grade 3 or 4 elevation of transaminases was observed in 20% of the patients. Severe thrombocytopenia and neutropenia developed in most of the patients. Hepatic veno-occlusive disease was found in 12% of the patients who received GO [34].

### Alemtuzumab (Campath®)

Campath® (alemtuzumab) is a recombinant DNA-derived humanized mAb binding to CD52, an antigen with unknown function found on > 95% of peripheral blood lymphocytes and monocytes, and to a smaller extent on

granulocytes and in the male genital tract. Most B- and some T-cell lymphomas highly express CD52, whereas the protein is absent on bone marrow progenitor cells.

Ninety-three patients with chronic lymphocytic leukemia (CLL), after prior therapy with an alkylating agent and without response to fludarabine, were treated with increasing doses of Campath-1H (target dose 30 mg, three times weekly for a maximum of 12 weeks) [35]. Overall response was 33% (2% CR, 31% PR) and median time to disease progression was 9.5 months for responders. Despite that infection, prophylaxis was put in force beginning on day 8 and continuing for a minimum of 2 months after treatment, and grade 3 or 4 infections were reported in 27% of the heavily pre-treated patients, including pneumocystis and aspergillus pneumonia, systemic candidiasis, cryptococcal pneumonia and listeria meningitis leading to death in 11 patients. Based on these data and other trials [36, 37], alemtuzumab received the orphan status and was approved by the FDA for the treatment of CLL refractory to fludarabine in 2001.

Combination of alemtuzumab with other mAbs such as rituximab is another approach to enhance response rates. In the largest retrospective analysis including 48 patients with relapsed or refractory NHL, most of whom had CLL, the ORR was 52% in patients who received combined rituximab/alemtuzumab [38].

Apart from CLL, the most promising indication for alemtuzumab is T-prolymphocytic leukemia. A retrospective analysis of 76 T-prolymphocytic leukemia patients treated with alemtuzumab showed an ORR of 51%, with a 40% complete response [39]. However, the median duration of CR was shorter than 8.7 months, and the outcome was unsatisfactory if no other treatment followed. Nevertheless, it opens a window of opportunity for allogeneic stem cell transplantation in patients eligible for this type of treatment.

### Trastuzumab (Herceptin®)

The proto-oncogene human epidermal growth factor receptor (HER2 or c-erbB-2) encodes the HER2 receptor, a transmembrane protein found on numerous epithelial tissues and over-expressed in 20 to 30% of breast cancers. Herceptin® (trastuzumab) is a humanized IgG1 antibody targeting the HER2 protein. An international open-label study included 222 patients with HER2 over-expressing metastatic breast cancers that had progressed after one or two chemotherapy regimens [40]. HER2 status was assessed by immunohistochemistry. The patients were treated with trastuzumab as a single-agent therapy with a loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg. The ORR was 15% (CR 4%, PR 11%) with a median dura-

tion of response of 9.2 months. The results of this trial led to the FDA approval of trastuzumab single agent therapy as a second-line treatment for HER2-positive metastatic breast cancer.

In the largest comparative trial, 469 patients were randomly assigned to receive standard chemotherapy or standard chemotherapy plus trastuzumab [41]. Patients without prior anthracycline therapy were treated with a combination of doxorubicin or epirubicin and cyclophosphamide (AC or EC, 138 women) alone or with trastuzumab (143 women). Patients with a history of anthracycline exposure were treated with paclitaxel alone (96 women) or paclitaxel with trastuzumab (92 women). Patients received trastuzumab weekly until the diseases progressed. Chemotherapy was administered every 3 weeks for at least six cycles. There were 118 responses in the chemotherapy/trastuzumab groups (50%) compared to only 74 responses in the chemotherapy only group (32%). Addition of trastuzumab to chemotherapy prolonged the median survival by 4.8 months, from 20.3 to 25.1 months, a result that is even more remarkable in light of the fact that 72% of patients originally randomized to chemotherapy treatment received trastuzumab after progression of their disease.

Recently, Genentech announced that, as part of a treatment regimen containing doxorubicin, cyclophosphamide and paclitaxel, the US FDA approved trastuzumab for the adjuvant treatment of HER2-positive node-positive breast cancer. Adjuvant therapy was given to women with early-stage (localized) breast cancer, who had initial treatment surgery with or without radiation therapy, with the goal of reducing the risk of cancer recurrence and/or the occurrence of metastatic disease.

Trastuzumab is generally well tolerated. Just like the cases with other mAbs, there were infusion-related reactions, which included fever, rigors, chills, nausea, dyspnoea and hypotension. The frequency of these events was reported to be around 40% in patients receiving the first dose, with a marked reduction to 5% during the following administration. Symptoms usually responded well to interruption of the infusion or symptomatic treatment. An adverse event not anticipated in the pivotal combination chemotherapy trial was cardiac toxicity. Concurrent treatment with anthracycline, cyclophosphamide and trastuzumab significantly increased the risk of cardiac dysfunction, as compared with the treatment with only an anthracycline and cyclophosphamide [41]. For this reason, patients who are candidates for trastuzumab treatment should be carefully evaluated for cardiac function before and throughout the therapy.

### **Bevacizumab (Avastin®)**

Avastin is a recombinant humanized antibody designed

to bind to and inhibit VEGF, a protein that plays a critical role in tumor angiogenesis. Targeting tumor vasculature has become an increasingly attractive approach. Advantages of this approach include its applicability to various tumor types, low toxicity on normal tissues, low likelihood of resistance due to the genetic stability of endothelial cells that are not themselves transformed and good accessibility of the target structure to the antibody.

Bevacizumab received the FDA approval as a first-line treatment for metastasized colorectal cancer in combination with a 5-FU based regimen in 2004. Addition of the antibody to chemotherapy increased the ORR from 35% to 45% and prolonged median survival from 15.6 to 20.3 months in a randomized trial [42]. Bevacizumab administration could result in the development of gastrointestinal perforation and wound dehiscence, and even fatality in individual cases [43]. Gastrointestinal perforation, sometimes associated with intra-abdominal abscess, occurred throughout the treatment with Avastin and was not correlated to duration of exposure. The incidence of gastrointestinal perforation in patients receiving bolus-IFL with Avastin was 2%. The typical presentation was reported as abdominal pain associated with symptoms such as constipation and vomiting. Gastrointestinal perforation should be included in the differential diagnosis of patients on Avastin presenting with abdominal pain. Avastin therapy should be terminated in patients with gastrointestinal perforation or wound dehiscence requiring medical intervention. The appropriate interval between termination of Avastin and subsequent elective surgery required to avoid the risks of impaired wound healing/wound dehiscence has not been determined. Serious or even fatal hemoptysis occurred in patients with non-small-cell lung cancer treated with chemotherapy and Avastin.

### **Cetuximab (Erbix®)**

Cetuximab is a chimeric mAb against the epidermal growth factor receptor (EGFR), which is expressed on a variety of malignant tumors such as non-small-cell lung cancer, head and neck or colon cancer.

A randomized phase II study carried out in Europe and presented at the 2003 American Society of Clinical Oncology conference confirmed earlier phase II evidence for the activity of cetuximab in patients with EGFR positive, irinotecan-refractory metastatic colorectal cancer. Cetuximab was given intravenously at a dose of 400 mg/m<sup>2</sup> on week 1, followed by 250 mg/m<sup>2</sup> once a week thereafter. The investigators reported better results for re-treatment with cetuximab and irinotecan than with cetuximab alone. There were significant differences in response rate (23% in the combination group vs 11% in the group treated

with cetuximab alone), median time to progression (4.1 vs 1.5 months) and median overall survival time (8.6 vs 6.9 months) [44, 45]. On the basis of these findings and those of previous studies, cetuximab received FDA approval in February 2004, for use in combination with irinotecan for patients who are refractory to irinotecan, or as a single agent for patients who are unable to tolerate irinotecan-based chemotherapy. Other indications are currently undergoing testing. One of the most promising cases is non-small-cell lung cancer where the addition of cetuximab to cisplatin/vinorelbine in inoperable patients increased the response rate from 29% to 50% in a small randomized trial [46].

Toxic effects of cetuximab consist of infusion reactions and constitutional symptoms, as well as an acne-like skin rash that occurred in up to 75% of patients and was of grade 3 severity in 16% of patients. Development of this rash after treatment with cetuximab is a predictor of increased survival: the more intense the rash is, the greater the benefit is [47].

### **Panitumumab (Vectibix®)**

Panitumumab is the first fully human monoclonal therapeutic antibody that targets the EGFR, a protein that plays an important role in cancer cell signaling. As a fully human antibody, panitumumab can be administered without any pre-medication and has the promise of decreased infusion reactions. Clinical studies have demonstrated that panitumumab had significant activity as a single agent and improved progression-free survival when compared with best supportive care (BSC). In a large, randomized, non-blind, multicenter phase III study in pre-treated adult patients with metastatic colorectal cancer and EGFR staining in  $\geq 1\%$  tumor cells, treatment with panitumumab at 6 mg/kg every 2 weeks plus BSC was significantly ( $p < 0.0001$ ) more effective in improving progression-free survival than BSC alone; recipients of panitumumab plus BSC had a 46% lower disease progression rate than those receiving BSC alone after a median follow-up of 19 weeks [48]. Panitumumab at 6 mg/kg every 2 weeks or 2.5 mg/kg/week, administered as a mono-therapy, produced partial response rates of 8-13% and stable disease rates of 21-30% in pre-treated patients with metastatic colorectal cancer in three non-comparative, multicenter phase II studies. Preliminary phase II results also suggested a potential role for panitumumab as a first-line therapy in combination with fluorouracil, folinic acid and irinotecan in patients with metastatic colorectal cancer [49]. Panitumumab received the FDA approval in September 2006 on the basis of these promising data at the treatment of patients with EGFR expressing metastatic colorectal cancer after disease progression on or following fluoropyrimidine-, oxaliplatin- and irinotecan-containing

chemotherapy regimens.

Panitumumab was generally well tolerated. Grade 3/4 skin-related toxicities were reported in 14% of patients receiving panitumumab plus BSC in the phase III study (vs 0% of patients receiving BSC alone). An analysis of pooled data found that high-affinity binding antibodies to panitumumab were detected in  $< 1\%$  of patients.

### **Strategies to improve anti-tumor efficacy of mAbs**

Numerous strategies for improving the efficacy of anti-tumor antibodies are now being tested, including:

(1) Enhancing effector functions such as improving antibody-dependent cellular cytotoxicity and/or complement-dependent cytotoxicity by means of site-directed mutation or manipulation of antibody glycosylation [50].

(2) Direct arming of antibodies entails their covalent linkage to killing moieties, such as radionuclides or toxins (e.g. small molecules or proteins). Armed antibodies typically show more potent anti-tumor activity in pre-clinical tumor xenograft studies than their “naked” parents. The humanized anti-CD33 antibody-calicheamicin conjugate Mylotarg was approved for treatment of CD33-positive AML in first-relapse patients  $\geq 60$  years old who are not candidates for cytotoxic chemotherapy. Arming antibodies with radionuclides enables them to kill bystander cells. Alternatively, arming antibodies with cytokines is intended to create high intra-tumor concentrations of cytokines to stimulate the anti-tumor immune response, while avoiding the toxicities associated with the cytokine delivery [51].

(3) Indirect arming of antibodies achieved by attaching engineered antibody fragments to the surface of liposomes loaded with drugs or toxins for tumor-specific delivery. Bi-specific antibodies that bind to two different antigens can be pre-loaded with the cytotoxic agents before administration or alternatively pre-targeted to the tumor before delivery of the cytotoxic payload. Engineered antibody fragments can be attached to the surface of stealth liposomes for selective tumor targeting of large payloads of drugs, toxins or even DNA for gene therapy. Such large payloads offered an important potential advantage over direct antibody arming, in which only one or a few molar equivalents of the payload were attached per antibody to avoid compromising antigen binding, conjugate solubility or promoting aggregation [52, 53].

(4) Pre-targeting strategies aiming for the selective delivery of radionuclides to tumors or selective intra-tumor activation of pro-drugs, thereby diminishing the systemic toxicities of these cytotoxic agents. For pro-drug pre-targeting, an antibody-fragment-enzyme fusion protein is typically allowed to localize to a tumor and be cleared off the system. A pro-drug is then administered and ide-

ally converted to an active drug solely within the tumor. For radionuclide pre-targeting, an antibody-streptavidin conjugate is allowed to accrue within a tumor and is then used to capture the biotin-chelator-radionuclide complex [54, 55].

(5) The combination of anti-angiogenesis mAbs and chemotherapy resulting in synergistic anti-tumor efficacy [56]. Three distinct mechanisms may help to explain the chemosensitizing activity of anti-angiogenic mAbs: normalizing tumor vasculature, preventing rapid increase in tumor cell population and augmenting the antivasculature effects of chemotherapy.

### Antibody gene therapy as a new strategy for cancer treatment

Although nine mAbs have been approved by the FDA, wider application of mAbs is limited due to the following reasons: (1) high dose and purity required for antibody protein treatment increase the production cost; (2) the relatively large molecular weight of full-length antibody and high pressure within the tumor make it difficult for full-length antibody to penetrate into large-volume solid tumors, thus greatly reducing the therapeutic effect. It is expected that resolving these deficiencies would markedly increase the potential of antibody therapy in human cancer treatment. Therefore, antibody *in vivo* gene therapy is proposed as one of the best candidate approaches. The efforts of antibody gene therapy were reported by our lab in 2004 [57, 58] and subsequently by Jooss K's research group in 2005 [59].

In our study, we chose adenovirus as the antibody gene transfer vector for the following reasons [57, 58]: (1) adenovirus has been used in more than 5 000 clinical patients without significant adverse effects and toxicity, exhibiting the conclusive safety and efficacy as an *in vivo* vector [60]; (2) adenoviral manufacture technologies are easy, convenient and simple; (3) adenovirus is the only commercial product approved in the world for gene therapy [61, 62]. We synthesized full-length anti-HER-2 antibody light chain and heavy chain genes [57, 58]. The two genes were linked together with internal ribosome entry site and were under the control of the mCMV promoter. Recombinant anti-HER-2 expressing adenovirus (Ad5-Tab), with the expression cassette inserted in the *E1* region of adenovirus 5, was prepared in HEK293 cells. Administration of Ad5-Tab *in vivo* produced high serum concentrations of the full-length antibody, which has the same bioactivity as commercial trastuzumab. A single injection of Ad5-Tab resulted in effective therapeutic concentrations lasting for 4 weeks and a peak serum level of >160 µg/ml at as early as day 7 after injection. The antibody produced by Ad-Tab *in vivo*

demonstrated anti-tumor efficacy not only in small-volume but also in the large-volume tumors in HER-2<sup>+</sup>/SKOV-3-inoculated nude mice. All these data demonstrated that: (1) this adenovirus-mediated antibody expressing system could be a new approach for antibody therapy; (2) the complicated, expensive and time-consuming antibody protein preparation and purification procedures could be replaced by using adenovirus as an antibody expression tool for its technical simplicity and low cost.

Fang *et al.* [59] also described a mAb delivery system that allows for continuous production of a full-length antibody at high-concentrations *in vivo* after gene transfer. They developed an antibody expression system that uses the foot-and-mouth-disease virus (FMDV)-derived 2A self-processing sequence to express the full-length antibody from a single open reading frame. Using an FMDV 2A sequence adjacent to a furin cleavage site to link the antibody heavy and light chain sequences, they engineered a mAb expression cassette that, in the context of AAV-mediated gene transfer, resulted in high levels of full-length, functional mAbs *in vitro* and *in vivo*. Sustained mAb serum levels of 1 000 µg/ml were achieved in mice with a single administration of rAAV8 vector expressing DC101, an anti-angiogenic mAb targeting the vascular endothelial cell growth factor receptor-2 (VEGFR2 or Flk-1). The rAAV8-mediated gene transfer of DC101 resulted in significant anti-tumor efficacy in two tumor models, demonstrating the generation of functional antibodies *in vivo* using this expression system.

### Strategy for improving antibody's anti-tumor effect by exploiting mesenchymal stem cells

Another big hurdle for cancer antibody therapy is that the large molecule full-length antibody has great difficulty entering solid tumors, due to compacted stroma within the tumors and high intra-tumor pressure produced by lymphatic return barrier. Therefore, the anti-tumor efficacy of conventional antibody therapy is greatly reduced in treating large-volume solid tumors. Since mesenchymal stem cells (MSCs) can recognize the emitting signal from tumors and help to construct matrix or junction tissues for tumor growth, it would be an interesting attempt to use MSCs as a vehicle to deliver full-length antibody to tumors. In our study, the bone marrow derived primary mesenchymal stem cells (mBMSCs) were used as a vehicle to target tumor cells (our unpublished data). As MSCs cannot be effectively infected by commonly used adenovirus type 5, a chimeric adenovirus with better infection efficiency on MSCs was constructed. We reconstructed an adenoviral variant Ad5-F35, which had its fiber knob and shaft from Ad35 and had its fiber tail and penton base from Ad5. The

novel Ad5-F35 chimera demonstrated better infection ability compared with Ad5. The antibody gene expression cassette was released from Ad-Her and introduced into Ad5-F35 to produce Ad5-F35-Her. The MSCs infected with Ad5-F35-Her were administered by tail vein injection to treat pre-established lung metastatic SK-OV-3 tumors. The treatment group showed stronger anti-tumor efficacy compared with the group treated with non-infected MSCs ( $p < 0.001$ ) (our unpublished data). There were no differences in lung weight and volume between the group treated with modified MSCs and the normal mice group without any disease ( $p > 0.05$ ). Few necrosis foci and hemorrhage points were observed on the lung surface of mice treated with the modified MSCs. Some lung lobes were atrophic. Pathological examination showed micro-metastasis in lung tissues in only two out of nine mice in the group treated with the modified MSCs, but in all of the mice (9/9) treated with the commercial antibody Herceptin (our unpublished data). These results demonstrated that targeted therapy, using MSCs carrying a full-length antibody gene, could overcome the disadvantages of low antibody penetration into solid tumors, markedly enhancing the anti-tumor efficacy, and hence it has the potential to be developed into a safe, efficient, low cost and targeting therapeutic system.

### Potential side effects of cancer antibody gene therapy

Although antibody gene therapy is less time consuming and more economical compared with traditional antibody protein therapy, its potential drawbacks are as follows: (1) antibodies from gene therapy are presumably produced in liver and lung cells that are efficiently infected by the viral vector. It is unknown whether some other somatic cells will also express the antibodies; (2) host antibodies against Adenovirus or AAV may decrease the anti-tumor efficacy of virus-mediated full-length antibody gene therapy; and (3) when this approach is applied to humans, the administration routes, the desired antibody gene expression time and possible inflammation response need to be investigated. There is still a long way to go before antibody gene therapy could be put into clinical use.

### Perspectives

Antibodies have started to fulfill their promise as anti-cancer therapeutics with the nine antibodies now marketed as drugs in the US. The recent clinical and commercial success of anticancer antibodies, such as rituximab and trastuzumab, has generated great interest in antibody-based therapeutics for hematopoietic malignancies and solid tumors. Although, currently, there are a large number of agents in both early- and late-stages of clinical develop-

ment, it is expected that only a handful will pass regulatory approval and become successful products. Full-length antibody cancer gene therapy will also prove to be a promising and feasible strategy for shortening the development process from pre-clinical research to clinical trials, avoiding the complicated procedures of antibody protein preparation and purification and reducing the treatment cost. If successfully implemented, it will become a revolutionary progress in antibody therapy for cancer. MSCs can also act as vehicles to bring the full-length antibody into the very inner parts of solid tumors, thus overcoming the poor penetration of solid tumors by the large molecule antibody. Given the likely lower toxicity of antibodies vs small molecules, the potential increase in efficacy by conjugation to radioisotopes and other cellular toxins, and the ability to characterize the target with clinical diagnostics to improve the drug's clinical performance, it is anticipated that current and future antibody therapeutics will find substantial roles either alone or in combination with other strategies for the treatment of cancer.

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