

## Clinical Development of Antibody-based Cancer Therapy

Li Yan and Zhenping Zhu



**About Author:** Li Yan, MD., Ph.D., joined Merck & Co., Inc. after his tenure at Centocor, Inc., a Johnson & Johnson company. He is Associate Director of Clinical Oncology responsible for Ph1 and Ph2 development of both small molecule anti-cancer drug and monoclonal antibody. He joined Centocor in October 2001 as a Senior Scientist in Oncology Discovery, and was promoted to Principal Scientist in 2004. During these 4 years, he has led five discovery programs developing monoclonal antibodies as cancer therapeutics. In 2005, he joined Clinical Hematology and Oncology as an Assistant Director and Clinical Leader of Compound Development Team to lead the clinical development of one of the antibodies he discovered in research. He received the Philip Hoffmann's Scientific Award, the 2<sup>nd</sup> most prestigious award in Johnson & Johnson, for his contribution to novel biologics oncology drug research and development. Dr. Yan received his MD from Medical College of Beijing University and his fellowship training at Beijing Cancer Hospital. He studied tumor biology and received his PhD from University of Kansas Medical Center. His postdoctoral fellowship at Children's Hospital focused on tumor angiogenesis, in the Department of Surgery headed by Dr. Folkman, the pioneer and founder of tumor angiogenesis. He became an instructor (assistant professor) at Harvard Medical School.



**About Author:** Dr. Zhu joined ImClone Systems Incorporated in 1996, and has been serving as Vice President of Research since 2005. He is a member of the company's Research and Development Review Committee (RDRC) and Research Management Committee (RMC). Dr. Zhu received his medical training from Jiangxi Medical College, and passed the two steps of USMLE and secured the ECFMG certification. He received his MSc in Pharmacology from Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, his PhD in Immunology and Pathology from Dalhousie University, and performed postdoctoral work in antibody engineering at Genentech, Inc. Dr. Zhu has been working in the areas of antibody technologies / applications and cancer biotherapeutics for > 20 years and has authored over 160 peer-reviewed scientific publications, including original research papers, invited reviews/commentaries and book chapters. In addition to be a frequently invited speaker at various international meetings, Dr. Zhu has also organized several international conferences in life sciences in China, including the First (September 2000, Tianjin) and the Second (October 2005, Beijing) China International Symposiums on Antibody Engineering and Antibody-based Therapeutics, each was attended by over 200 scientists with 35 to 40 invited speakers who are internationally renowned experts in the antibody field.

### Abstract

*Monoclonal antibodies (mAbs) have emerged as a class of novel cancer therapeutics. The selectivity and specificity, the unique pharmacokinetics, and the ability to engage and activate the host immune system differentiate these biologics from traditional small molecule anticancer drugs. In this review, we focus on the clinical development of five antibodies approved for treating cancer, rituximab (Rituxan<sup>TM</sup>) for lymphoma, trastuzumab (Herceptin<sup>®</sup>) for breast cancer, bevacizumab (Avastin<sup>TM</sup>) for colorectal cancer, non-small cell lung and breast cancer, cetuximab (Erbix<sup>®</sup>) for colorectal cancer and head and neck cancer, and panitumumab (Vectibix<sup>TM</sup>) for colorectal cancer. The anticancer effects of these antibodies derive from blockade of growth factor/receptor interaction and/or down-regulation of oncogenic proteins (e.g., growth factor receptors) on the tumor cell surface, and from*

*the ability to elicit effector mechanisms of the immune system, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC) for some of these antibodies. This review provides perspectives on challenges and opportunities of oncology antibody clinical development including considerations for early phase development, proof-of-concept (POC) strategies, and the use of biomarker and pharmacogenomics to expedite or increase the possibility-of-success (POS) of mAb clinical development. In addition, the mechanism-of-action (MOA) behind each antibody, registration trials for their approved indications, as well as emerging indications are presented. Opportunities and challenges to develop mAb therapeutics in China are also discussed.*

## 1. Introduction

The concept of using antibodies as “magic bullets” to specifically attack malignant tumor cells was originally proposed by Paul Ehrlich at the beginning of the 20<sup>th</sup> century.<sup>1</sup> The invention of somatic hybridization by Kohler and Milstein to generate “hybridoma” cell lines converted this laboratory concept closer to practice in 1976.<sup>2</sup> The development of therapeutic antibodies suffered a brief set-back in the 1980s when a number of murine mAbs failed to show efficacy in clinical trials, mainly due to intrinsic immunogenicity of these rodent-derived mAbs and resulting immune responses and fast clearance. This obstacle was quickly overcome by newly developed molecular antibody engineering methods to produce chimeric, humanized or fully human mAbs with substantially reduced immunogenicity. Accompanied by technological advancement in mammalian cell expression, therapeutic mAb can now be readily developed for specific targets, engineered to customize the size, binding specificity, affinity, and effector function, and produced in large-scale suitable for clinical use. Among 21 marketed therapeutic mAbs, 9 mAbs are approved for cancer treatment (Table 1), and there are over 100 mAbs currently in clinical development. In combination with cytotoxics or radiation therapy, these mAbs have delivered significant clinical improvement in treating lymphoma [rituximab (Rituxan<sup>TM</sup>)], metastatic breast cancer [trastuzumab (Herceptin<sup>®</sup>)], and bevacizumab (Avastin<sup>TM</sup>), colorectal cancer [bevacizumab (Avastin<sup>TM</sup>), cetuximab (Erbix<sup>®</sup>), and panitumumab (Vectibix<sup>TM</sup>)], non-small cell lung cancer [bevacizumab (Avastin<sup>TM</sup>)], and squamous cell cancer of the head and neck [cetuximab (Erbix<sup>®</sup>)], and are expanding into broader indications.

## 2. Anti-CD20 antibodies

CD20, a.k.a., human B-lymphocyte-restricted differentiation antigen Bp35, is a non-glycosylated phosphoprotein of 33-37 kDa.<sup>3</sup> CD20 is expressed on cell surface of normal B lymphocytes and B-cell lymphomas, and forms tetramers that can act as a Ca<sup>2+</sup> channel. CD20 plays a role in B-cell proliferation activation, and differentiation<sup>4,5</sup>.

Rituximab (IDEC-C2B8, Rituxan<sup>®</sup>, MabThera<sup>®</sup>) is a chimeric IgG1 anti-CD20 mAb of ~145 kDa. The mechanisms of action of rituximab include ADCC, CDC, induction of apoptosis, anti-proliferation, and chemosensitizing<sup>6-8</sup>. Rituximab was genetically engineered by fusing the murine variable regions of the anti-CD20 mAb 2B8 with the human IgG1 constant regions<sup>9</sup>. Immunogenicity was observed in 3/237 (2%) patients, developed a detectable HACA reaction after treatment<sup>10</sup>.

## 2.1 Clinical development of rituximab in non-Hodgkin's lymphoma

### 2.1.1. Single agent trials leading to registration approval

The first Phase I study was initiated in November 1993<sup>11</sup>. Fifteen patients with relapsed low-grade B-cell lymphoma were treated with a single dose i.v. of antibody from 10 to 500 mg/m<sup>2</sup>. The overall response rate (ORR) was 2/15 patients (13%) with tumor regressions occurred in 6/15 patients (2 partial and 4 minor responses). A multiple-dose phase I/II study evaluated rituximab administered in weekly infusions times four from 125 to 375 mg/m<sup>2</sup> in twenty patients with relapsed low-grade (n = 15) or intermediate-/high-grade (n = 5) lymphoma<sup>12</sup>. Six of 18 assessable patients (33%) had a partial response (PR), with a median time-to-progression (TTP) of 6.4 months. Minor responses were observed in five patients. The schedule of 375 mg/m<sup>2</sup> weekly for 4 weeks was chosen for subsequent Phase II studies<sup>12</sup>. In the first Phase II study, thirty-seven patients with relapsed low-grade or follicular NHL were treated<sup>13</sup>. All patients had relapsed after chemotherapy (median of 2 prior regimens) and 54% had failed aggressive chemotherapy. Clinical remissions were observed in 17 patients, with 3 complete and 14 partial remissions, with an ORR of 46%. The onset of these tumor responses was as soon as 1 month posttreatment and reached a maximum by 4 months posttreatment. In the 17 responders, the median TTP was 10.2 months, with a median duration of response of 8.2 months. In these early trials, no significant toxicities were observed. Infusional side effects consisting of mild fever, chills, respiratory symptoms, and occasionally hypotension were observed mostly with the initial antibody infusion and were rare with subsequent doses.

The multi-center pivotal non-randomized trial was conducted in 166 patients with relapsed low grade or follicular lymphoma<sup>14</sup>. Rituximab was administered in an outpatient treatment setting at 375 mg/m<sup>2</sup> weekly for four doses. The median number of prior regimens was 3 (range from 1–10), consisting of chemotherapy, radiation therapy, and autologous stem cell transplantation. The ORR in the intent-to-treat group was 48%, with 6% complete response (CR) and 42% PR, and 76% of patients had at least a 20% reduction in tumor size. The median duration of response was 11.2 months, with a TTP of 13.0 months. As observed in earlier trials, the majority of adverse events occurred during the first infusion and were grade 1 or 2; fever and chills were the most common events.

In 1997, rituximab was approved by the FDA as the first mAb for the treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell, non-Hodgkin's lymphoma.

The success of rituximab development, only 4 years from Phase 1 to registration, highlights the unique feature of mAb clinical development. In this case, early POC and selection of a clinical dose achieved as early as in Phase 1, together with relatively short time length to demonstrate treatment benefits, contributed to the short timelines in developing rituximab.

### 2.1.2. Other Rituximab trials

Rituximab also demonstrated clinical activity in 31 patients with bulky (>10 cm lesion) relapsed/refractory low-grade or follicular NHL<sup>15</sup>. Rituximab was also efficacious in re-treatment of patients with low-grade or follicular NHL who relapsed after a response to rituximab therapy<sup>16</sup>.

Besides patients with low-grade or follicular NHL, rituximab has been tested as a single agent in relapsed aggressive NHL<sup>17</sup>, relapsed mantle cell NHL<sup>18,19</sup>, indolent NHL<sup>20,21</sup>, and also in combination with chemotherapy drugs in indolent NHL<sup>22</sup>, and aggressive NHL<sup>23</sup>.

## 2.2 Rituximab clinical efficacy and FcγR polymorphism

The relationship between individual clinical outcomes after rituximab treatment and FcγRs polymorphism also represents an example for the potential influence of patient pharmacogenomics and pharmacogenetics on antibody therapeutic development and clinical use<sup>24</sup>. In patients with NHL, clinical and molecular responses to rituximab were associated with the FcγRIIIa CD16 genotype, determined by a SNP at residue 158<sup>25</sup>. Patients with 158VV genotype showed better clinical responses to rituximab than those 158F carriers. A subsequent study confirmed and also expanded this finding to include FcγRIIa CD32 as well in predicting responses to rituximab treatment<sup>26</sup>. A higher rituximab response rate was found in patients with FcγRIIa 131 H/H (histidine/histidine) than those of arginine (R) carriers, and also independently in patients with FcγRIIIa 158 V/V than those F carriers. Knowledge obtained from the association between FcγR and clinical responses to rituximab treatment has prompted research into modification of antibody dosing to compensate for reduced antibody binding in patients with FcγRIIIa-158F, a lower-binding allotype<sup>27</sup>. However, this association in clinical responses to rituximab treatment and FcγR polymorphism appears

to be only valid in patients with NHL, and no such association was observed in patients with B-cell chronic lymphocytic leukemia treated with rituximab<sup>28</sup>.

## 2.3. Other anti-CD20 antibodies

Two radioisotope conjugated anti-CD20 antibodies include ibritumomab tiuxetan (Zevalin<sup>®</sup>), a <sup>90</sup>Y-labeled anti-CD20 antibody<sup>29</sup>, and tositumomab/<sup>131</sup>I-tositumomab (Bexxar<sup>®</sup>), a <sup>131</sup>I-labeled anti-CD20 antibody for non-Hodgkin's lymphoma<sup>30</sup>. Although these antibodies have demonstrated impressive clinical activity and efficacy, their use has been hindered by the requirements for specialized radiation professionals to administrate the treatment.

There are several follow-on anti-CD20 antibodies in various stages of clinical development, led by HuMax-CD20 (ofatumumab) which was developed by Genmab and was licensed by GSK in 2006.

## 3. Anti-HER2 antibodies

The HER2/neu (erbB2) proto-oncogene product HER2 is a member of the HER (erbB) family of receptor tyrosine kinases. HER2 is overexpressed in ~25% of breast cancer patients with or without HER2/neu gene amplification<sup>31</sup> and its overexpression correlates with disease aggressiveness and patient survival<sup>32</sup>. Numerous signaling pathways are activated by HER2 signaling network, which in turn influence cell proliferation, differentiation, migration, adhesion, resistance to apoptosis, and transformation. Dysregulation of HER2 signaling is associated cell proliferation, differentiation, migration, adhesion, resistance to apoptosis, and transformation.

### 3.1 Trastuzumab (Herceptin<sup>®</sup>)

Trastuzumab is a humanized IgG1 derived from 4D5, one of over 100 murine mAbs generated following immunization of mice with cells overexpressing human HER2. Trastuzumab binds the HER2 extracellular domain with high affinity ( $K_d = 0.10$  nM) to block HER2 homodimer formation and therefore HER2 signaling. The in vitro and in vivo antitumor activity of 4D5, as well as its ability to elicit ADCC is dependent on the level of tumor HER2 expression.

Two pivotal trials investigated trastuzumab in patients with metastatic breast cancer, either as a single agent in previously treated patients<sup>33</sup> or in combination with chemotherapy drugs in the first-line setting.<sup>34</sup> In the single agent trial, H0649g, trastuzumab was given in a loading

dose of 4 mg/kg i.v., followed by weekly maintenance doses at 2 mg/kg. Eight CR and 26 PR were observed in 222 patients enrolled, accounting for an objective RR of 15%, with 26% of patients deriving clinical benefits of stable disease (SD)  $\geq$  6 months. The median duration of response was 9.1 months; the median duration of survival was 13 months. The most common adverse event, infusion-associated fever and/or chills, occurred in  $\sim$ 40% of patients and usually during the first infusion. The most clinically significant adverse event, cardiac dysfunction, occurred in 4.7% of patients. In the combination trial, H0648g, 469 patients with HER2-overexpressing breast cancer [2+ or 3+ immunohistochemistry (IHC) score] with no prior chemotherapy treatment for metastatic disease were randomized to receive chemotherapy alone or in combination with trastuzumab. Patients who received combination treatment experienced significantly improved median TTP (7.4 vs. 4.6 months), overall RR (50% vs. 32%), median duration of response (9.1 vs. 6.1 months), and median survival (25.1 vs. 20.3 months) despite the fact that 65% of patients receiving chemotherapy were allowed to cross-over at disease pro-

gression. The most important adverse event was cardiac dysfunction, which occurred more frequently in patients receiving concurrent trastuzumab and an anthracycline.

Retrospective analysis revealed that patients with HER2 overexpression defined as IHC3+ or gene amplification [fluorescence in-situ hybridization (FISH) (+)], were more likely to benefit from trastuzumab. Herceptin<sup>®</sup> was approved in 1998 for patients with tumors evaluated to overexpress HER2 or to have HER2 gene amplification by HercepTest<sup>®</sup> (IHC test) or PathVysion<sup>®</sup> (FISH assay) respectively. The prospective inclusion of only HER2 overexpressing patients in the trial represents the first case in which a biomarker successfully expedited the clinical development of an antibody therapeutic and in which a companying diagnostic kit approved together with an antibody therapeutic.

Trastuzumab has since been extensively investigated in combinations with different chemotherapy or hormone therapy agents, to refine dosing schedules, in adjuvant and neoadjuvant settings, and with other novel agents

**Table 1.** FDA Approved Monoclonal Antibody Therapeutics for Solid Tumors

Antibody	Target	Antibody Type	Indication	Year Approved	Company
Rituxan	CD20	Chimeric IgG1	NHL	1997	Biogen Idec/Genentech
Herceptin	HER2	Humanized IgG1	Breast Cancer	1998	Genentech/Roche
Mylotarg	CD33	Calicheamicin-labeled humanized IgG4	AML	2000	Wyeth
Campath-1H	CD52	Humanized IgG1	B cell CLL	2001	Millenium/Ilex/Berlex
Zevalin	CD20	<sup>90</sup> Y-labeled mouse IgG1	NHL	2002	Biogen Idec
Bexxar	CD20	<sup>131</sup> I-labeled mouse IgG2a	NHL	2003	Corixa/ GlaxoSmithKline
Erbix	EGFR	Chimeric IgG1	Colorectal Cancer Head & Neck	2004 2006	ImClone Systems /Bristol Myers Squibb
Avastin	VEGF	Humanized IgG1	Colorectal Cancer NSCLC Breast Cancer	2004 2006 2008	Genentech/Roche
Vectibix	EGFR	Human IgG2	mCRC	2006	Amgen

such as bevacizumab, lapatinib, and IL-12. Most recently, Herceptin was approved for the adjuvant treatment of HER2-overexpressing breast cancer either as part of a treatment regimen containing doxorubicin, cyclophosphamide, and paclitaxel<sup>35</sup>, or as a single agent for the adjuvant treatment of HER2-overexpressing node-negative (ER/PR-negative or with one high-risk feature) or node-positive breast cancer, following multi-modality anthracycline-based therapy.<sup>36</sup>

### 3.2 Pertuzumab (Omnitarg™)

Pertuzumab is a humanized IgG1 anti-HER2 antibody that binds to different epitope(s) than that of trastuzumab, and prevents HER2 from both homodimerizing with HER2 and hetero-dimerizing with HER1 and HER3. Unlike trastuzumab, the antitumor activity of pertuzumab is independent of tumor HER2 expression level. Single agent activity has been observed in Phase 1 studies. Phase 2 studies are being conducted in patients with prostate, ovarian, breast, or NSCLC, with evidence of drug activity observed in 26 out of 65 refractory/relapsing ovarian cancer patients. In combination with trastuzumab, pertuzumab delivered 5 confirmed PR (21%) and 12 SD (50%) in patients with HER2+ metastatic breast cancer who progressed during treatment with trastuzumab.

## 4. Anti-Angiogenic Antibodies

Angiogenesis, the formation of new blood vessels, is an essential process required for both tumor growth and metastasis (Folkman, 2001; 2002). The VEGF pathway is well-established as one of the key regulators of this process. Consequently, considerable effort has been invested in generating and testing various approaches to inhibit VEGF or its receptors including mAb therapeutics [for reviews, see <sup>37-39</sup>]. VEGF is a strong inducer of vascular permeability, stimulator of endothelial cell migration and proliferation, and is an important survival factor for newly formed blood vessels. VEGF binds to and mediates its activity mainly through two tyrosine kinase receptors <sup>40</sup>, VEGFR1 (fms-like tyrosine kinase 1 or Flt-1) <sup>41,42</sup> and VEGFR2 (kinase insert domain-containing receptor, or KDR in humans, and fetal liver kinase or flk1 in mice) <sup>43,44</sup>. Numerous studies have shown that over-expression of VEGF and VEGFR2 is strongly associated with invasion and metastasis in human malignant diseases <sup>45-47</sup>. Taken together these data suggest that blockade of VEGF/VEGFR pathway by mAb therapy would be a useful therapeutic strategy for inhibiting both angiogenesis and tumor growth. To this end, antibodies that neutralize

VEGF and its receptors have been developed and have shown potent anti-angiogenic and anti-tumor activities in both laboratory and clinic settings.

### 4.1 Bevacizumab (Avastin™)

Bevacizumab is a humanized IgG1 derived from a murine anti-human VEGF mAb A.4.6.1. Bevacizumab recognizes all isoforms of VEGF with high affinity (Kd, 0.8 nM), and inhibits VEGF-induced proliferation of endothelial cells in vitro and tumor growth in vivo with potency and efficacy similar to the parent murine antibody.

#### 4.1.1 Bevacizumab in colorectal cancer (CRC).

In a pivotal phase III trial<sup>48</sup>, over 800 previously untreated metastatic CRC (mCRC) patients were given bolus IFL (irinotecan, 5-fluorouracil (5-FU) and leucovorin) plus placebo or bevacizumab. The overall RR was 44.8% in the bevacizumab group and 34.8% in the placebo group, with duration of response of 10.4 months and 7.1 months, respectively. The median PFS was 10.55 versus 6.24 months, and median survival was 20.34 versus 15.61 months. The main toxicities were grade 3 hypertension (11% versus 2.3%), proteinuria, and arterial thromboembolic events (4.4% versus 1.9%). These promising outcomes led to the FDA approval of the bevacizumab as first-line therapy in combination with IFL in mCRC patients in February 2004. Recent results from a phase III study<sup>49</sup> showed that bevacizumab plus FOLFOX4 also extended survival in second-line settings with a 25% reduction in risk of death. Median survival was 13.0 versus 10.8 months. In 2006, bevacizumab was approved by the FDA for second-line mCRC in combination with intravenous 5-FU-based chemotherapy.

#### 4.1.2. Bevacizumab in lung cancer.

In a phase III study, ECOG4599, 878 NSCLC patients were randomized to receive paclitaxel and carboplatin with or without bevacizumab, 15 mg/kg every three weeks.<sup>50</sup> Patients with squamous cell tumors, brain metastases, and clinically significant hemoptysis, were excluded from this trial due to serious hemorrhagic events observed in earlier studies, particularly in patients with cancers of squamous type. Median OS and median PFS were 12.3 versus 10.3 months, and 6.4 versus 4.5 months, respectively. The RR was 27% and 10%. Grade 3/4/5 bleeding occurred in 4.4% versus 0.7% of patients, including 5 fatal pulmonary hemorrhage in the bevacizumab plus chemotherapy arm. The most common adverse events were neutropenia, hypertension and thrombotic events. Bevacizumab was approved to use with chemotherapy in first-line NSCLC in 2006. In the

AVAiL trial, combination of bevacizumab with cisplatin and gemcitabine was investigated at two dose levels, 7.5 mg/kg and 15 mg/kg every three weeks. Although improvements in PFS were observed in bevacizumab combination groups, no difference in treatment effects, PFS or OS, was observed between the high and the low dose groups. Furthermore, Avastin, at either dose, failed to prolong OS in this trial (Genentech Press Release April 21, 2008). These results highlight the importance of thoroughly investigating the optimal dose level in the early development for mAb therapeutics.

#### 4.1.3. Bevacizumab in breast cancer.

Two pivotal phase III trials have investigated bevacizumab plus capecitabine in patients with metastatic breast cancer who had prior anthracycline- and taxane-based chemotherapy<sup>51</sup>, and bevacizumab plus paclitaxel in patients with previously untreated metastatic breast cancer.<sup>52</sup> In the first study, no difference was observed in either PFS (4.86 versus 4.17 months) or OS (15.1 versus 14.5 months) although the bevacizumab combination yielded a higher RR (19.8% versus 9.1%). In the second study, E2100, paclitaxel plus bevacizumab significantly increased median PFS (11.8 vs. 5.9 months) and objective RR (36.9% vs. 21.2%). However, median OS was similar in the two groups (26.7 vs. 25.2 months). More frequent Grade 3/4 toxicities were observed in patients receiving paclitaxel plus bevacizumab including hypertension, proteinuria, headache, and cerebrovascular ischemia, as well as infection rates. A separate Phase 3 trial, AVADO (BO17708) study, also demonstrated PFS improvement of bevacizumab in combination with docetaxel chemotherapy in chemo-naïve patients for their locally recurrent or metastatic HER2-negative breast cancer (Genentech press release Feb 12, 2008). On February 22, 2008, the FDA granted accelerated approval of bevacizumab in combination with paclitaxel for first-line treatment of metastatic HER2-negative breast cancer.

#### 4.2 Anti-VEGF Receptor (VEGFR) antibodies

A phase I study was performed using IMC-1C11<sup>53</sup>, a chimeric IgG1 anti-VEGFR2 antibody<sup>54,55</sup>, in patients with liver metastatic colorectal cancer. When IMC-1C11 was infused at 0.2, 0.6, 2.0 and 4.0mg/kg for 4 weeks, no serious toxicities were observed. Five out of total 14 enrolled patients had SD by week 4 and continued on therapy, with one patient maintaining SD for 6 months<sup>56</sup>.

ImClone Systems is currently developing a fully human IgG1 anti-VEGFR2 antibody for the treatment of solid tumors<sup>57-59</sup>. This antibody, IMC-1121B, was generated

from a Fab fragment originally isolated from a large antibody phage display library<sup>58,59</sup>. The antibody specifically binds VEGFR2 with high affinity of 50 pM and blocks VEGF/VEGFR2 interaction with an IC50 value of approximately 1 nM. Phase I clinical trials of IMC-1121B have been completed in patients with various advanced malignancies. In one study, a total of 37 patients (in 7 cohorts) were treated with IMC-1121B at escalating weekly doses of 2 to 16 mg/kg. Partial responses were achieved in 4 patients, and at least nine patients have experienced prolonged stable disease of greater than 6 months. IMC-1121B is currently in multiple phase II trials in patients with renal cell carcinoma, hepatocellular carcinoma and melanoma. Phase III trial in patients with breast cancer is expected to start enrolling patients in 2H08.

### 5. Anti-EGF Receptor (EGFR) Antibodies

EGFR is a rational target in solid tumors. Activation of the EGFR promotes processes responsible for tumor growth and progression, including proliferation and maturation, angiogenesis, invasion, metastasis, and inhibition of apoptosis. In addition, EGFR expression has been detected to varying degrees in a wide range of solid tumors. Although the prognostic significance of EGFR expression remains unclear, as reports on this issue are contradictory, a retrospective review of EGFR studies reported that EGFR expression levels are highly predictive of clinical outcome for patients with head and neck, ovarian, cervical, bladder, and esophageal cancers. They are of moderate prognostic value for gastric, breast, endometrial, and colorectal tumors and of relatively low prognostic value for NSCLC.<sup>60</sup> EGFR gene amplification or mutation and dysregulation of EGFR-mediated signaling pathways have also been detected in various malignancies. In addition to EGFR, KRAS mutations seem to play a potential role in the effectiveness of EGFR-targeted agents<sup>61</sup>. Recent clinical studies have confirmed that the presence of mutant KRAS is a negative predictor of clinical outcome in colorectal cancer patients to anti-EGFR therapies<sup>62</sup>.

#### 5.1 Cetuximab (Erbix®)

Cetuximab (Erbix®) is the most extensively studied anti-EGFR monoclonal antibody. Cetuximab is a chimeric monoclonal G1 (IgG1) antibody that binds to the EGFR with high affinity. The antibody blocks ligand binding and induces receptor internalization and degradation, resulting in downregulation of surface EGFR expression. In a dose-dependent manner, cetuximab inhibits the growth and proliferation of several tumor cell lines and xenograft

tumors. Putative mechanisms include blocking the G1 phase of the cell cycle, promoting programmed cell death, or both, and inhibiting tumor angiogenesis.<sup>63</sup> Cetuximab also has been shown to block the transport of EGFR into the nucleus, preventing activation of an important DNA-repair kinase, DNA-PK, implying that cetuximab could sensitize tumor cells to conventional DNA-damaging chemotherapies and radiation. As an IgG1 isoform of antibody, cetuximab also has the potential to mediate host immune responses such as antibody-dependent cell-mediated cytotoxicity (ADCC).<sup>64</sup>

Cetuximab is currently approved in several countries as monotherapy or in combination with irinotecan for the treatment of patients with irinotecan-refractory mCRC, as monotherapy for metastatic squamous cell cancer of the head and neck (SCCHN), or in combination with radiation therapy for unresectable SCCHN. In the phase II European randomized BOND trial, single-agent cetuximab and cetuximab in combination with irinotecan were examined in patients with irinotecan-resistant colorectal cancers. The overall response (OR) rate for the combination of cetuximab and irinotecan was 22.9% compared with 10.8% for cetuximab monotherapy. The combination therapy resulted in a statistically significant increase in median time to disease progression (TTP) (4.1 months vs 1.5 months observed in the monotherapy group,  $P < 0.001$ ). In another trial (IMCL-0144) reported by Lenz et al., cetuximab as a single agent yielded an 11.6% PR rate in 346 patients refractory to both irinotecan and oxaliplatin, with another 31.8% of patients experiencing SD for at least 6 weeks. Median overall survival was 6.7 months. Recently, a randomized, multicenter, Phase III trial (NCIC CTG CO.17, also known as BMS-025) compared cetuximab plus best supportive care (BSC) to BSC alone in 572 patients with mCRC whose disease was refractory to all available chemotherapy, including irinotecan, oxaliplatin, and fluoropyrimidines. Patients who received cetuximab lived an average of 6.1 months compared to 4.6 months for patients who received BSC alone, representing a 23 percent increase in overall survival ( $P = 0.005$ ). Cetuximab treatment also resulted in PR in 23 patients (8%), compared to 0% in patients who received BSC alone ( $P < 0.0001$ ), and a 32 percent reduction in the risk of disease progression ( $P < 0.0001$ ). Further, SD was seen in an additional 31.4% of patients receiving Cetuximab, and only in 10.9% of patients on BSC. The antibody was generally well tolerated with rash as the most common toxicity. These are the first data of an anticancer therapy to demonstrate overall survival in refractory mCRC patients.

Likewise, in a phase III trial involving 424 patients with locoregionally advanced SCCHN, the addition of cetuximab to high-dose radiation resulted in a median survival of 49 months compared with 29 months with radiation alone and a 26% reduction in the risk of mortality ( $P = 0.03$ ). In another first-line Phase III study (EXTREME study), 442 patients with stage III/IV recurrent and/or metastatic SCCHN were treated with cetuximab in combination with 5-fluorouracil plus either cisplatin or carboplatin, or with 5-fluorouracil plus either cisplatin or carboplatin alone. The addition of cetuximab significantly improved median overall survival and median PFS, and increased response rate compared with chemotherapy alone

In addition to mCRC and SCCHN, cetuximab has also been tested in combination with chemotherapeutic agents in patients with advanced NSCLC. In a large, randomized multi-national, phase III study (FLEX study), cetuximab in combination with platinum-based chemotherapy (vinorelbine plus cisplatin) significantly increased the patient's overall survival compared with chemotherapy alone. Cetuximab thus may provide a new option for NSCLC patients, particularly those who are not eligible for or can not tolerate bevacizumab treatment.

## 5.2 Other anti-EGFR mAbs

Other monoclonal antibodies currently undergoing evaluation in preclinical and clinical trials include panitumumab (Vectibix™), matuzumab (EMD-72000), MDX-447, nimtozumab (h-R3), and mAb806, an antibody directed against a mutant form of EGFR (EGFR vIII) that also recognizes overexpressed wild-type EGFR receptor. Of these, the most well studied is panitumumab, a fully human monoclonal IgG2 antibody that, like cetuximab, competitively inhibits EGFR ligand binding, promotes receptor internalization, and prevents tyrosine kinase phosphorylation. Unlike cetuximab, however, panitumumab does not induce receptor degradation upon internalization, suggesting that the EGFR may be recycled to the cell surface.<sup>65</sup> As an IgG2 isoform of antibody, panitumumab is also unlikely to mediate ADCC responses. In an open-label phase III trial<sup>66</sup>, 463 patients with mCRC who had failed standard chemotherapy, including oxaliplatin and irinotecan, were randomized to receive 6 mg/kg panitumumab plus best supportive care (BSC) ( $n = 231$ ) or BSC alone ( $n = 232$ ). The objective response rate was 8% with panitumumab versus zero with BSC alone, and the median duration of response was 17 weeks. The SD rate was 28% with panitumumab versus 10% with BSC alone. However, an interim analysis revealed that the overall survival between the two

groups was similar. In a recent retrospective analysis of the trial, responses to panitumumab were seen only in those patients with wild-type KRAS, and these patients also had a longer median time to progression from 7.4 to 12.3 weeks. Further, when the patients in panitumumab group and those who crossed over to panitumumab were analyzed together, a longer overall survival was seen in patients with wild-type KRAS than those with KRAS mutations (hazard ratio, 0.67; 95% CI, 0.55 – 0.82)<sup>62</sup>. Panitumumab has been approved as monotherapy in refractory mCRC patients in the US, and recently, in EU as monotherapy for mCRC patients with wild-type KRAS selected using TheraScreen K-RAS kit<sup>67</sup>

## 6. Perspectives in therapeutic oncology antibody development

### 6.1 Pharmacokinetics, Biodistribution and Tumor Penetration<sup>68</sup>

Antibodies are large (150 kD) IgG molecules and are therefore not filtered by the kidney or excreted in urine. The neonatal receptor FcRn, expressed by cells in close contact with serum, binds to the antibody Fc portion, playing a major role in antibody clearance. Antibodies undergo pinocytosis and are transported to endosomes, where they bind to FcRn at low pH and are shuttled back to the cell surface rather than being degraded. This accounts for the long half-lives of IgG antibodies, typically in the range of 1-3 weeks. Therefore, every week to every three week dosing schedules are commonly used for antibody therapeutics. This long t<sub>1/2</sub> can be further altered by introducing Fc mutations to influence the antibody affinity for FcRn.

Interactions with target antigen expressed in normal tissues or tumor also affect antibody clearance. Below a threshold concentration, antibodies are rapidly cleared from the circulation, binding to accessible target antigen. Dosing of antibodies must maintain trough levels above this threshold. Dosing regimens composed of a large amount loading dose followed by low amount of maintenance doses are one of such approaches to saturate antibody binding sites and maintain trough levels.

### 6.2 Clinical development

Clinical development of novel therapeutic antibodies also differs from that of small molecules including safety, dose and schedule, efficacy endpoints, and patient/tumor selection.

Side effects of mAb therapeutics include target-specific cross-reactivity with normal tissues (e.g. skin rashes with anti-EGFR mAbs). More commonly seen mAb side effects however are immunogenicity responses, and infusion reactions. Immunogenicity responses are unique to antibody and other protein therapeutics. The formation of human anti-human antibodies (HACA) can rapidly inactivate the therapeutic antibody and reduce efficacy. The rate of HACA has dropped considerably to approximately 5% with chimeric mAbs and less than 1% with fully human mAbs. The exact mechanism responsible for infusion reactions to mAbs is not known, but like the taxanes, these reactions are unlikely to be true, type 1 IgE-mediated hypersensitivity reactions. Theoretically, infusion reactions to chimeric and humanized monoclonal antibodies may be a result of their ability to elicit HACA or HAHA respectively. These clinical reactions are described as a flu-like syndrome with fever and chills that can appear in upwards of 40% of patients, mainly at the first drug infusion, but can be treated or prevented with antihistamines and corticosteroids

Identifying a dose and schedule may also be different for mAbs. The mAb dosing is generally not limited by toxicity, therefore a maximum tolerated dose (MTD) may not be achieved in Phase 1 studies. Dose finding may require the use of pharmacodynamic endpoints in tumor or accessible surrogate tissues (skin, hair, blood cells). The latter should minimally be validated in preclinical models as reflective of intratumoral pharmacodynamics. The dose range above which pharmacodynamic activity is seen may determine a “biologically effective dose” (BED) below the MTD. BED and MTD/highest tested doses (if found) may need to be compared in a randomized Phase 2 setting. However, this may prove to be challenging as showcased by the difficulties encountered for bevacizumab dose selection in NSCLC.

Since most antibody therapeutics are cytostatic because they generally do not provide shrinkage of tumors (radiological response) unless combined with chemotherapy. Therefore, the efficacy of antibodies is best demonstrated through improvements in PFS and OS in randomized trials of standard chemotherapy alone or in combination with mAb therapeutic.

### 6.3 Pharmacogenetics and pharmacogenomics

An increasing emphasis has been given on improving mAb therapeutic efficacy by applying pharmacogenetics and pharmacogenomics approaches.<sup>24</sup> The use of biomarkers has contributed to the successful clinical

development of rituximab and trastuzumab by testing these two antibodies in patient populations with CD-20-positive hematological malignancies or with HER-2 overexpression/gene amplification breast cancer respectively. More recently, the accompanying K-RAS diagnosis with panitumumab approval for CRC in EU is another case in which biomarkers played a key role in developing antibody therapeutics. Integration of biomarkers into clinical development of new antibodies will expedite the process and increase the possibility of success. Such use in clinical practice will make personalized medicine a reality to not only customize the most suitable mAb therapy for individual cancer patients to ensure treatment efficacy, but also to avoid and reduce exposure to unnecessary and costly treatments.

#### 6.4 Strategies for developing antibody therapeutics in China

##### 6.4.1 Attractiveness

mAb therapeutics can be developed in relatively short timelines. For example, it only took Rituxin 4 years from initiating Phase 1 clinical trials to gaining approval, a much shorter timeline as compared to average 8 years for small molecular drug development. In addition, the 25% success rate for mAb therapeutics is also in general higher than that of traditional anti-cancer drugs of <10%. Furthermore, the demands for infrastructure investment for mAb therapeutics research are relatively less intense. Existing biology and animal laboratories, recombinant engineering, small scale fermentation and protein purification are capable of supporting early stage mAb research and even clinical development. With appropriate support, it is envisioned that numerous early mAb therapeutics candidates will emerge from both academic laboratories and biotechnology companies, with some innovative mAbs entering clinical development.

##### 6.4.2 Challenges

The foremost challenge facing mAb therapeutics development is mAb manufacturing in scale as well as quality. The common requirement of mAb therapeutics is 1-10 mg/kg every 1 to 4 weeks, or 70 mg to 2800 mg every month for a patient of 70 kg. The current manufacturing capacity in China can only support limited number of Phase 2 and Phase 3 studies. Each manufacturing plant with five 20,000-liter reactors demands US \$750 million dollars and 5 year to construct.

The complexity in manufacturing mAb also determines the high cost of these therapeutics, ranging from US \$10,000 to \$100,000 a year. Such high prices and as-

sociated burdens to healthcare systems further limit the development of this new class of therapeutics.

mAb R&D also requires large teams of experts from basic research, safety assessment, eukaryotic cell expression, large protein expression, purification, and analysis, as well as experts in protein pharmacokinetics and pharmacodynamics.

##### 6.4.3 Strategies and path-forward

With current mAb research and manufacturing capacities in China, consolidated resources to support a few carefully selected mAb projects will increase the POS, leading to initial successes and a healthy growth of the field. Because of the high demand for manufacturing capacities in late stage clinical development, contract manufacturing capacities need to be either developed or secured in advance. Decreasing the amount of mAb required for treatment by enhancing potency will also help the field to escape from the high cost restraints. The successful R&D of <sup>131</sup>I-iodine metuximab (Licartin™) which takes only 9 mg/course in treating hepatocellular carcinoma, and its approval in China provides a promising case for such approaches<sup>69</sup>.

## 7. Summary

Monoclonal antibodies are established as standard of care agents in several solid tumor applications. They generally afford a high level of target specificity, relatively low toxicity, and the opportunity for efficacy by both target inhibition and immune-mediated mechanisms. Clinical development of monoclonal antibodies provides unique opportunities and challenges. Increased use of biomarker and pharmacogenomic information, as well as increased application of engineered antibody variants, will contribute to the further realization of antibody therapeutic potential.

## References

1. Ehrlich P. Proc R Soc London, 1900;66:429.
2. Kohler G and Milstein C. Nature 1975;256:495-7.
3. Valentine MA, et al., J Biol Chem 1989;264:11282-7.
4. Clark EA and Shu G. J Immunol 1987;138:720-5.
5. Clark EA et al., Proc Natl Acad Sci U S A 1985;82:1766-70.
6. Shan D et al., Cancer Immunol Immunother 2000;48:673-83.
7. Shan D et al., Clin Cancer Res 2001;7:2490-5.
8. Shan D et al., Blood 1998;91:1644-52.
9. Reff ME et al., Blood 1994;83:435-45.

10. McLaughlin P et al., *Oncology* (Williston Park) 1998;12:1763-9; discussion 9-70, 75-7.
11. Maloney DG et al., *Blood* 1994;84:2457-66.
12. Maloney DG et al., *J Clin Oncol* 1997;15:3266-74.
13. Maloney DG et al., *Blood* 1997;90:2188-95.
14. McLaughlin P et al., *J Clin Oncol* 1998;16:2825-33.
15. Davis TA et al. *J Clin Oncol* 1999;17:1851-7.
16. Davis TA et al., *J Clin Oncol* 2000;18:3135-43.
17. Coiffier B, et al. *Blood* 1998;92:1927-32.
18. Foran JM and Rohatiner AZ, *J Clin Oncol* 2000;18:317-24.
19. Ghilmini M, et al. *Ann Oncol* 2000;11 Suppl 1:123-6.
20. Hainsworth JD. *Semin Oncol* 2000;27:25-9.
21. Colombat P, et al. *Blood* 2001;97:101-6.
22. Czuczman MS. *Semin Oncol* 1999;26:88-96.
23. Vose JM, et al., *J Clin Oncol* 2001;19:389-97.
24. Yan L and Beckman RA. *Biotechniques* 2005;39:565-8.
25. Cartron G, et al. *Blood* 2002;99:754-8.
26. Weng WK and Levy R., *J Clin Oncol* 2003;21:3940-7.
27. Dall'Ozzo S, et al., *Cancer Res* 2004;64:4664-9.
28. Farag SS, et al., *Blood* 2004;103:1472-4.
29. Krasner C, et al., *Curr Pharm Biotechnol* 2001;2:341-9.
30. Cheson B. *Bexxar*, *Science* 1989;244:707-12.
32. Slamon DJ, et al., *Science* 1987;235:177-82.
33. Cobleigh MA, et al., *J Clin Oncol* 1999;17:2639-48.
34. Slamon DJ, et al., *N Engl J Med* 2001;344:783-92.
35. Romond EH, et al., *N Engl J Med* 2005;353:1673-84.
36. Piccart-Gebhart MJ, et al., *N Engl J Med* 2005;353:1659-72.
37. Hicklin DJ, et al., *Drug Discov Today* 2001;6:517-28.
38. Ferrara N. *Endocr Rev* 2004;25:581-611.
39. Paz K and Zhu Z. *Front Biosci* 2005;10:1415-39.
40. Ferrara N, et al., *Nat Med* 2003;9:669-76.
41. Shibuya M, et al., *Oncogene* 1990;5:519-24.
42. de Vries C, et al., *Science* 1992;255:989-91.
43. Terman BI, et al., *Biochem Biophys Res Commun* 1992;187:1579-86.
44. Millauer B, et al., *Cell* 1993;72:835-46.
45. Zhu Z and Witte L. *Invest New Drugs* 1999;17:195-212.
46. Hicklin DJ and Ellis LM. *J Clin Oncol* 2005;23:1011-27.
47. Witte L, et al., *Cancer Metastasis Rev* 1998;17:155-61.
48. Hurwitz H, et al., *N Engl J Med* 2004;350:2335-42.
49. Giantonio BJ, et al., *J Clin Oncol* 2007;25:1539-44.
50. Sandler AB, et al., *Journal of Clinical Oncology*, 2005 ASCO Annual Meeting Proceedings 2005;23:4.
51. Miller KD, et al., *J Clin Oncol* 2005;23:792-9.
52. Miller K, et al., *N Engl J Med* 2007;357:2666-76.
53. Hunt S. *Curr Opin Mol Ther* 2001;3:418-24.
54. Zhu Z, et al., *Cancer Res* 1998;58:3209-14.
55. Zhu Z, et al., *Cancer Lett* 1999;136:203-13.
56. Posey JA, et al., *Clin Cancer Res* 2003;9:1323-32.
57. Zhu Z, et al., *Leukemia* 2003;17:604-11.
58. Lu D, et al., *Int J Cancer* 2002;97:393-9.
59. Lu D, et al., *J Biol Chem* 2003;278:43496-507.
60. Nicholson RI, et al.. *Eur J Cancer* 2001;37 Suppl 4:S9-15.
61. Benvenuti S, et al., *Cancer Res* 2007;67:2643-8.
62. Amado RG, et al., *J Clin Oncol* 2008;26:1626-34.
63. Kim ES, et al., *Curr Opin Oncol* 2001;13:506-13.
64. Mellstedt H. *Drugs Today (Barc)* 2003;39 Suppl C:1-16.
65. Yang XD, *Crit Rev Oncol Hematol* 2001;38:17-23.
66. Gibson TB, *Clin Colorectal Cancer* 2006;6:29-31.
67. Cross J. *Pharmacogenomics* 2008;9:463-7.
68. Beckman RA, *Cancer* 2007;109:170-9.
69. Chen ZN, et al. *Int J Radiat Oncol Biol Phys* 2006;65:435-44.