Monoclonal Antibodies: The Foundation of Therapy for Colorectal Cancer in the 21st Century?

Review Article

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The treatment of colorectal cancer has undergone enormous changes in the past decade. From a disease with a single treatment option (ie, fluorouracil, a modestly effective drug), the treatment options have evolved to include at least five new classes of antineoplastic agents. Among the considerable number of recently approved drugs, two are monoclonal antibodies and are the testing ground for our rapidly emerging knowledge about cancer cell biology. Cetuximab (Erbitux) targets the epidermal growth factor receptor, an important molecule involved with cell cycling, survival, invasion, and metastasis. Bevacizumab (Avastin) neutralizes the vascular endothelial growth factor, blocking its ability to activate its receptor on the endothelial cells. The development of both antibodies resulted from decades of research in molecular and cell biology, as well as preclinical and clinical studies, and signals a new paradigm where the tumor cells' own unique features are exploited in a rational way.

Despite recent advances in its treatment, colorectal cancer remains the number 2 cause of cancer death in the United States.

Background

The therapeutic strategy developed for antibodies is based on the concept of harnessing the immune system to fight cancer. The initial antibodies were polyclonal and very difficult to generate in large quantities. A significant problem was presented by the inability of patients' immune systems to recognize the cancer cells as foreign and consequently to mount a meaningful reaction against them. However, the development of the hybridoma technique by Kohler and others in 1975 created new opportunities and changed the scenario dramatically, leading eventually to a Nobel prize.

In a very simplified model of antibody production, mice are exposed to specific human tumor antigens and mount immunologic reactions. Murine immunoglobulin-producing lymphocytes are selected and fused with immortal, non-immunoglobulinproducing myeloma cells, forming a hybridoma. After a careful selection for cells producing the desired antibody, the cells are grown in special culture mediums and are able to produce vast amounts of specific antibodies. The original antibodies produced were completely murine and were frequently associated with allergic reactions as well as the development of human antimouse antibodies (HAMA).

Subsequent improvements in DNA recombinant technology have allowed for the replacement of most of the structure of the antibody with human IgG, creating a chimeric antibody where the variable regions remain murine or a humanized antibody where only the hypervariable regions remain murine and the vast majority of the antibody is human in origin.

Although the development of human antichimeric antibody (HACA) and human antihuman antibody (HAHA) remains possible, the chimeric and humanized antibodies tend to have a considerably better toxicity profile and a much longer half-life than the original murine antibodies.

The degree of humanization of an antibody can be easily recognized by the suffix that is added to its official name: murine antibodies are identified by "-momab," chimeric by "-ximab," and humanized by "-zumab." Murine antibodies are still being developed, but most clinically relevant antibodies are currently either chimeric or humanized due to the characteristics mentioned above; fully human monoclonal antibodies developed through genetic engineering are currently entering clinical trials. As stated previously, the initial antibodies were developed with the goal of activating the patient's immune system against cancer, mostly through antibody-dependent cell-mediated cytotoxicity (ADCC) or complementdependent cytotoxicity (CDC). Ideally, this would use cell surface antigens as
a kind of homing beacon, allowing the antibody to recognize the cancer cells and engaging the immune system. However, these initial antibodies were not very successful, and none proved effective against colorectal cancer. A new generation of antibodies has been developed with a slightly different and more refined strategic approach. Although most antibodies are still based on an IgG molecule and several could theoretically act by ADCC and CDC, the primary objective is to eliminate a ligand or receptor and thereby to inhibit the activation of specific signal transduction pathways required for cell survival, growth, invasion, and/or metastasis. Specific signaling proteins located at the cell surface or their circulating protein ligands have become the main targets, and cell death is achieved as a result of interrupting critical growth-promoting signals. Although many antibodies have been and are currently being studied against colorectal cancer, only two have received approval by the United States Food and Drug Administration (FDA) for routine clinical use. The first one targets the epidermal growth factor receptor (EGFR) and is known as cetuximab (Erbitux). The second, known as bevacizumab (Avastin), is particularly interesting because it is believed that its target is not located directly in cancer cells. Bevacizumab targets circulating vascular endothelial growth factor A (VEGF). VEGF is a ligand to the VEGF receptor present mostly on endothelial cells and is one of the main proangiogenic factors in humans. Anti-EGFR Monoclonal Antibodies

The HER growth factor receptor family comprises four structurally related receptor tyrosine kinases: HER1 (EGFR, erbB1), HER2 (neu, erbB2), HER3 (erbB3), and HER4 (erbB4). The four receptors are composed of an extracellular region consisting of glycosylated domains, a transmembrane domain containing a single hydrophobic anchor sequence, and an intracellular region containing the catalytic tyrosine kinase domain with the exception of HER3 which lacks tyrosine kinase activity.[11] EGFR was the first HER family member to be described and is one of the best characterized. Most normal cells, particularly those of endothelial origin, and many malignant tissues express EGFR. Known ligands for EGFR include epidermal growth factor (EGF), transforming growth factor-alpha (TGFalpha), amphiregulin, betacellulin, heparin-binding EGF (HB-EGF), and epiregulin. EGFR activation follows three basic steps: ligand binding, receptor dimerization (either EGFR homodimerization or heterodimerization with other HER family members), and activation of the receptor tyrosine kinase via intramolecular phosphorylation. EGFR activation is followed by rapid endocytosis and degradation or recycling of both the receptor and the ligand.[11,12] Dysregulation or increased activity of EGFR-mediated signaling appears to confer a proliferative and/or malignant phenotype, possibly by altering signaling pathways involved in cell cycle progression, proliferation, apoptosis, angiogenesis, and metastasis. Many different solid tumors have been shown to express EGFR, including colorectal cancer. Several monoclonal antibodies directed against EGFR are currently undergoing clinical investigation, including cetuximab, ABX-EGF, EMD 72000, hR3 (the humanized version of ior-egf/r3 [TheraCIM]), and ICR62. These antibodies are similar: they all bind to EGFR and competitively inhibit ligand binding, which in turn prevents activation of the receptor tyrosine kinase. Among these antibodies, cetuximab is at the most advanced state of clinical development, having recently been approved for the treatment of patients with irinotecan-resistant colorectal cancer. Cetuximab is a chimeric antibody with a human IgG1 and a murine variable region against EGFR.[13] It has demonstrated antitumor activity in EGFR-expressing tumor cells, both in vitro and in vivo; numerous preclinical studies provide evidence for cetuximab-mediated inhibition of tumor cell cycle progression and proliferation, promotion of apoptosis, enhancement of antibody-dependent cytotoxicity, and inhibition of angiogenesis. However, the precise mechanism of cetuximab's anticancer activity remains unclear. It is known that the tumor microenvironment is characterized by low pH and pO₂ tension,[14] and that survival signals are necessary for tumor cells to live in this adverse environment. By inhibiting the autocrine or paracrine activation of EGFR, tumor cells that might typically survive in this caustic environment may undergo spontaneous apoptosis. Thus, the term cytostatic therapy, which has frequently been applied to targeted therapeutics due to the cytostatic effects often encountered in vitro, may underestimate the therapeutic potential of targeted therapy in the clinic. This does not imply that anti-EGFR therapy cannot also lead to tumor stabilization, but it suggests that inhibition of critical signaling pathways may induce tumor cell apoptosis and regression in a finite percentage of patients.
Additionally, several preclinical in vivo studies support an antiangiogenic mechanism as a component of the antitumor actions of cetuximab. For example, the efficacy of cetuximab against tumor cells is more pronounced in xenografts than in cell culture— an effect that has been explained, in part, by the antiangiogenic consequences of EGFR blockade. Treatment of a variety of EGFR-expressing tumor cells with cetuximab resulted in downregulation of one or more angiogenic mediators, including VEGF, interleukin-8, and basic fibroblast growth factor (bFGF), both in vitro and in vivo. Recently, Saltz et al, working in the United States, reported the results of a phase II trial in which patients with EGFR-positive colorectal cancer, refractory to irinotecan, received cetuximab alone (see Table 1).[15] The response rate of 9% and median survival of 6.4 months were very similar to the single-agent cetuximab experience in Europe, which was presented at the 2003 annual meeting of the American Society of Clinical Oncology (ASCO).[16] The consistency of the results from these two trials serves as reassurance that these very interesting results are real and indicates that EGFR is a valid target in colorectal cancer.[17] The combination of irinotecan and cetuximab in irinotecan-refractory patients has been more extensively investigated; the overall response rate was approximately 20% in two relatively large phase II trials (Table 1).[16,18] The apparent explanation for this synergistic activity in irinotecan-resistant patients is that signaling through the EGFR regulates a number of other cellular processes in addition to mediating proliferative signals. Activation of the EGFR leads to downstream signaling that activates the mitogenic and survival pathways, such as mitogen-activated protein (MAP) kinases and phosphatidyl-inositol-3 kinase (PI3K)/AKT pathways.[19] By inhibiting those pathways, cetuximab can lead to induction of BAX, activation of caspase-8, and down-regulation of BCL-2 and NFκB.[20,21] The effects are thought to render cancer cells more sensitive to apoptotic stimuli, such as chemotherapy. Most anti-EGFR clinical trials have selected patients with any EGFR-positive staining cells, no matter how weak or how few. Based on preclinical studies, one would assume that greater levels of expression of EGFR correlate with better response to anti-EGFR therapy. However, it is interesting that detailed analyses of recently published trials fail to demonstrate that the level of EGFR expression has any impact on response rates.[15,16] On the same day the FDA approved cetuximab to be used in irinotecan-refractory colorectal cancer, it also approved a new EGFR immunohistochemical kit to be used in conjunction with EGFR-directed therapy. The standardization of EGFR positivity is an important first step toward providing oncologists and pathologists with a common reference by which patients can be properly selected. One intriguing and unanswered question is whether those tumors that are now considered EGFR negative may respond to cetuximab. This is an important issue when one considers that all epithelial cells express at least low levels of EGFR, and that the tumor microenvironment is often rich in the EGFR ligands TGF-alpha and EGF. In addition, some EGFR activity may be mediated through heterodimerization with other HER family members. A number of investigators have hypothesized that one could predict response to anti-EGFR therapy by observing the occurrence of adverse effects that pharmacodynamically support target modification. For example, retrospective studies showed that patients who developed a rash while on therapy were more likely to respond than patients who did not. This suggests that skin rash could be used as a "poor man's test" to optimize anti-EGFR therapy, with dose escalation planned until the desired biologic effect is achieved—in this case a follicular rash, typical of EGFR inhibition in the skin. Although demonstration of inhibition of

### Table 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Arms</th>
<th>N</th>
<th>OR</th>
<th>TTP</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunningham</td>
<td>Irinotecan + cetuximab</td>
<td>218</td>
<td>22.9%</td>
<td>4.1 mo</td>
<td>8.6 mo</td>
</tr>
<tr>
<td></td>
<td>Cetuximab alone</td>
<td>111</td>
<td>10.8%</td>
<td>1.5 mo</td>
<td>6.9 mo</td>
</tr>
<tr>
<td>Saltz[18]</td>
<td>Irinotecan + cetuximab</td>
<td>120</td>
<td>22.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltz[15]</td>
<td>Cetuximab alone</td>
<td>57</td>
<td>9%</td>
<td></td>
<td>6.4 mo</td>
</tr>
</tbody>
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*BOND* trial.

OR = overall response; TTP = time to progression.
activation of the EGFR in normal tissues, such as skin, may not accurately represent the dynamics in the tumor, it suggests that if one does not observe activity in the skin, it is less likely that the EGFR will be inhibited in the tumor. More sophisticated molecular markers, such as phosphorylation of downstream signaling molecules, are also being investigated as predictive markers for therapy. Considering the clinical data available, cetuximab is currently indicated after failure of other irinotecan-based therapies. The usual paradigm of drug development has been to move effective second-and third-line therapies to the front-line treatment of solid tumors, frequently with better results observed in chemotherapy-naive patients. However, at a time when a growing number of active agents are available, the rational sequencing of these agents in the treatment of colorectal cancer seems to be just as important. Therefore, the decision on what role cetuximab will play in combination with chemotherapy in frontline treatment for colorectal cancer will have to wait for the results of the large clinical trials that are ongoing or being planned. **Anti-VEGF Monoclonal Antibodies** The VEGF family currently comprises six glycoproteins, designated VEGF-A, VEGF-B, VEGF-C, VEGFD, VEGF-E, and placenta growth factor (PIGF).[22] It is one of the most important proangiogenic molecules and its best characterized member is VEGF-A, which is commonly referred to as VEGF and was originally described as vascular permeability factor (VPF). It is a homodimeric glycoprotein that undergoes alternative splicing to yield mature proteins of 121, 145, 165, 189, and 206 amino acids,[23,24] with VEGF-165 being the predominant isofrom identified in most tumors. VEGF receptors have been identified on endothelial cells[25,26] and have been cloned; they include a family of specific tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR and the murine homolog Flk-1). These two receptors share 44% homology with each other and possess a characteristic structure consisting of seven extracellular domains, a single transmembrane domain, and a tyrosine kinase domain.[27] Although VEGF-2 was initially shown to be primarily responsible for most of the effects of VEGF, growing evidence suggests that VEGFR-1 mediates distinct effects in response to VEGF stimulation. More recently, several other VEGFRs have been identified, including VEGFR-3, neuropilin-1 (NRP-1), and neuropilin-2 (NRP-2). VEGFR-2 is the primary receptor for VEGF and is believed to mediate the majority of VEGF's functional effects.[28] Studies in various cultured endothelial cell systems have established that VEGF-2 mediates the majority of the downstream effects of VEGF in angiogenesis, including microvascular permeability.[29] VEGFR-2-mediated proliferation of endothelial cells is believed to involve activation of the Ras/MEK/Erk pathway,[30] whereas migration is believed to involve PI3K and focal adhesion kinase.[31] Interestingly, VEGFR-3 is initially expressed throughout the embryonic vasculature, but with maturation its expression is limited to lymphatic endothelial cells.[32] VEGFR-3 is believed to play diverse roles, assisting in cardiovascular development and remodeling of primary vascular networks during embryogenesis and facilitating lymphangiogenesis in adulthood. Nonetheless, some evidence supports a continuing role in the vasculature.[33] The inhibition of VEGFR-3 signaling by the use of a soluble VEGFR-3 has been shown to decrease tumor lymphangiogenesis and lymph node metastasis, implying a role for VEGF-C and VEGF-D in these processes.[34] In 1993, Kim and associates reported preclinical studies with an antibody targeting VEGF-A.[35] This antibody inhibited the growth of human tumor xenografts in mice, and the inhibition of tumor growth correlated with the relative levels of VEGF expressed by the tumor cells. This antibody was then humanized so that it could be studied in clinical trials. In phase I clinical trials with recombinant human monoclonal antibody to VEGF (bevacizumab), there were a few anecdotal reports of tumor stabilization and response.[36,37] Intravenous administration of the antibody was relatively safe, although notable adverse effects included tumor-related asthenia, headache, and nausea. The half-life of the drug was between 17 and 21 days, allowing intravenous infusion every 2 to 3 weeks. Bevacizumab was then moved into phase II studies in combination with several chemotherapy regimens. In a phase II study, patients with previously untreated metastatic colorectal cancer were randomized to one of three treatment arms: 5-FU and leucovorin; 5-FU, leucovorin, and lowdose bevacizumab (5 mg/kg); or 5-FU and leucovorin plus high-dose bevacizumab (10 mg/kg).[5] As this was a small study, the results must be interpreted with caution. But it was quite interesting that the addition of low-dose bevacizumab to 5-FU and leucovorin led to a significant improvement in response rates and time to progression. However, overall survival was not statistically different among the groups. This trial raised the question of optimal dosing of antiangiogenic agents. Because the randomized phase II trial suggested that a lower dose might be more beneficial, it has been difficult to choose the preferred
dose of bevacizumab in subsequent clinical trials. At the 2003 ASCO meeting, results were reported from a phase III randomized trial comparing two treatments: combined irinotecan (Camptosar), 5-FU, and leucovorin (IFL), which was considered the standard chemotherapy, with and without the addition of bevacizumab (see Table 2). [38] In this larger clinical trial, the patients who received chemotherapy plus lowdose bevacizumab were observed to have a significant improvement in overall survival, progression-free survival, and response rate. With the addition of bevacizumab to the chemotherapy, response rate improved from 35% to 45% ($P = .0029$), progression-free survival was extended from 6.2 to 10.6 months ($P = 0.0014$), and, more importantly, overall survival improved from 15.6 to 20.3 months ($P = .00003$). Although one can argue that a 5-month difference is not a major breakthrough, this is the greatest improvement in overall survival seen in any large randomized trial in colorectal cancer. This trial was also the first to demonstrate a true clinical benefit from the use of antiangiogenic therapy in a large clinical trial setting, proving that this strategy is definitively worth pursuing. The adverse events in this trial were similar among the treatment groups, with some notable exceptions. The patients who received bevacizumab had an 11% incidence of grade 3 hyhypertension and, surprisingly, a 1.5% incidence of bowel perforations. Six patients in the bevacizumab arm developed perforations, and one patient eventually died from related complications. No patients in the IFL arm presented with such a problem. There are several theories regarding the cause for the perforations, but since only six patients presented with the problem, no definitive explanation can be determined at this time. Physicians using bevacizumab should be aware of this potentially lethal problem, and abdominal complaints from patients receiving the antibody must be taken seriously. In February 2004, the FDA approved bevacizumab for use in combination with any intravenous 5-FU-based chemotherapy as a firstline treatment for colorectal cancer. Currently, there are no available data regarding its use in second- and thirdline regimens, but ongoing trials should produce some interesting data in the relatively near future. Similarly, although it is certainly expected that the addition of bevacizumab to most colorectal cancer treatment regimens will result in similar improvements in efficacy, few data are available regarding the use of this antibody with oxaliplatin (Eloxatin) combinations. The results from the Eastern Cooperative Oncology Group (ECOG) 3200 trial, which compared second-line FOLFOX (leucovorin/5-FU/oxaliplatin) with the same regimen combined with bevacizumab, are currently maturing and are eagerly awaited. Because response and survival rates of pretreated patients tend to be considerably inferior to those of patients who receive first-line treatments, [39] a negative result in this trial (which targeted a pretreated population) may need to be viewed with caution. Several large phase III trials are currently exploring the use of bevacizumab with the infusional 5-FU-based regimens FOLFIRI (leucovorin/5-FU/ irinotecan) and FOLFOX. Hopefully, the addition of bevacizumab will benefit those regimens, which are clearly better tolerated and potentially more active than the IFL regimen used in the original, pivotal trial. The possible usefulness of adding bevacizumab in the adjuvant setting is also an important issue, and several trials are being planned. However, since there are potentially significant toxicities associated with this antibody, and there are no available data regarding its long-term use, bevacizumab should not be used as an adjuvant therapy outside a clinical trial. **Conclusions** The development of effective monoclonal antibodies against EGFR and VEGF has revolutionized the treatment of colorectal cancer, and it will take time to understand the full impact of these agents on the disease. The viability of
molecular targeted treatment for solid tumors has been confirmed; it will be difficult to consider treatment of advanced colorectal cancer without considering the eventual use of one or both of these agents. Its greatest impact on the treatment of colorectal cancer may still lie ahead of us. Recent experience tells us that the use of adjuvant therapy for resected disease and neoadjuvant therapy for patients who present with relatively limited but inoperable metastasis yields the greatest chances for cure in locoregional and metastatic disease. One of the main problems to be faced by practicing oncologists in the immediate future is the lack of a reliable predictive test that can help select those patients who would truly benefit from the therapy. Treating all patients without this information may not only be very expensive but also counterproductive, as the ideal situation would be for patients to receive individualized chemotherapy and targeted therapy to maximize their response rates and survival. For now, targeted therapy is being used very much the same way chemotherapy has been used for decades—with a shotgun approach. Ultimately, the combined efforts of both basic and clinical researchers will be essential to determine how best to use the wealth of therapeutic options that are now available.

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**References:**


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