The Present and Future of Angiogenesis-Directed Treatments of Colorectal Cancer

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:
1. Discuss the current status of clinical trials targeting angiogenesis in colorectal cancer.
2. Identify the potential different mechanisms whereby antiangiogenic agents may interact with cytotoxic chemotherapy.
3. Define potential mechanisms for resistance and sensitivity to antiangiogenic agents in colorectal cancer.

ABSTRACT
The level of angiogenic activity in colorectal tumors has been shown to be a determinant of survival. Recent trials established that, in both the first- and second-line treatment of metastatic colorectal cancer, the addition of the vascular endothelial growth factor (VEGF)-directed antibody bevacizumab to chemotherapy significantly prolongs survival compared to chemotherapy alone. Those trials provided proof of principle that inhibition of angiogenesis has the potential to enhance the effectiveness of treatment of this disease. Oral agents directed toward VEGF receptor signaling are in advanced development, but none to date has proven beneficial in phase III trials in advanced colorectal cancer. Additional trials are needed to determine if improved pharmacological characteristics of the small molecules can be modified to replicate the activity of the antibody or if mechanistic differences require a more specific approach. Since bevacizumab has minimal activity as a single agent, a key question for future therapeutic development relates to the interaction between antiangiogenic strategies and cytotoxic therapies. We hypothesize that bevacizumab may potentiate the efficacy of cytotoxics not solely by alterations of tumor interstitial pressure but also by promoting sensitivity to proapoptotic signals consequent upon nutrient and oxygen withdrawal. The Oncologist 2006;11:992–998

INTRODUCTION
The seminal studies of Folkman showed the importance of new blood vessel formation in the genesis and propagation of tumors and suggested that inhibition of blood vessel formation could be a therapeutic target in solid tumors [1]. The cloning of vascular endothelial growth factor (VEGF) by Leung et al. [2] and the elucidation of the role of this factor in angiogenesis by this laboratory and others [3, 4] prompted the development of molecules designed to inhibit the relevant pathways. Two major classes of agent have been developed: antibodies or antibody fragments, directed to either the growth factor itself or to its principal...
receptor VEGF-R2, and small molecule inhibitors of receptor signaling that have variable specificity for the pathways of interest and varying potency and pharmacokinetics [5]. Several of these molecules have now undergone early clinical testing and have been shown by various methods to exert antiangiogenic effects. Most recently, an antibody to VEGF, bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA), has been tested in several solid tumors including colorectal cancer (CRC). In this paper, we review some of the recent trials directed toward angiogenesis in CRC and describe ongoing studies to both refine and extend these initial observations. We discuss the major issues that are under investigation to define the mechanisms of sensitivity and resistance to interventions directed to the blood vessels and to determine the basis for the favorable interaction with chemotherapy.

**Bevacizumab**

An early focus in the development of bevacizumab was CRC. In a randomized phase II trial, patients with metastatic CRC who received bevacizumab (at a dose of 5 mg/kg) plus 5-fluorouracil (5-FU) plus leucovorin (LV) had a higher objective response rate (40% vs. 17%), a longer time to disease progression (9.0 months vs. 5.2 months), and a longer median survival time (21.5 months vs. 13.8 months) than a control group who received chemotherapy alone (a concurrent 10 mg/kg group did not have results that were as striking) [6]. The principal safety concerns of the bevacizumab–5-FU–LV combination were hypertension and thrombosis, but they were manageable. Another phase II trial indicated that a combination with irinotecan (Camptosar®; Pfizer Pharmaceuticals, New York) and 5-FU was safe and effective. Based on these two studies, Genentech initiated a phase III trial to test the addition of bevacizumab to irinotecan–5-FU chemotherapy in previously untreated patients with metastatic disease. In that trial a markedly longer survival time was associated with the use of bevacizumab: 20.3 months versus 15.6 months, a strikingly significant difference [7]. Interestingly, and not necessarily anticipated, response rates to the regimen were also significantly greater [7]. Another arm of the trial, discontinued upon demonstration of the safety of the combined regimen, was 5-FU plus bevacizumab, and this regimen was also superior to irinotecan plus 5-FU [8]. This trial provided the first proof of principle that targeting angiogenesis could have therapeutic benefit.

At the time of the initiation of the Genentech phase III trial, the combination of oxaliplatin (Eloxatin®, Sanofi-Synthelabo Inc., New York) and 5-FU was gaining acceptance as a second-line therapy and was in trials to establish its role as the optimal regimen for the first-line treatment of colorectal cancer. In the Eastern Cooperative Oncology Group, we initiated a randomized study (E3200) of oxaliplatin plus 5-FU (FOLFOX) versus the same regimen with bevacizumab; a single-agent bevacizumab control was also included. The results of that 800-patient study were presented in 2005 and show a superior survival time, time to progression, and response rates with the addition of bevacizumab [9]. A median overall survival time of 12.5 months was observed in patients receiving FOLFOX plus the antibody, compared with 10.7 months in the group treated with chemotherapy alone (hazard ratio, 0.74; p = .0024). The bevacizumab-alone arm was discontinued prematurely by the Data Monitoring Committee for inferiority to the chemotherapy-containing arms.

A number of interpretations follow. First, the therapeutic benefit of bevacizumab is expressed in combination with multiple drug combinations, a conclusion further supported by results in breast and non-small cell lung cancers [10, 11]. Second, at least in colon cancer, chemotherapy plus bevacizumab is superior to the antiangiogenic therapy alone. Third, the benefit of bevacizumab is not restricted to the first-line setting of disease treatment. However, it must be emphasized that this does not support the continued use of bevacizumab in patients who have failed a bevacizumab-containing combination in the first line: a randomized trial is planned to address this issue, as discussed below. Finally, the results of that trial may also be logically used to support the use of bevacizumab in combination with oxaliplatin plus 5-FU in the first-line treatment of colon cancer, a strategy already adopted by the majority of treating oncologists.

**Tyrosine Kinase Inhibitors of Angiogenesis Signaling**

The development of small molecule inhibitors of the VEGF pathway occurred in parallel with that of the antibody. Several are in clinical trials: that which was targeted earliest to CRC was PTK 787 (vatalanib), a collaboration of Schering AG (Berlin, Germany) and Novartis Pharmaceuticals Corporation (East Hanover, NJ). This molecule is a potent inhibitor of the VEGF-R2 intracellular tyrosine kinase, and it also inhibits the kinases of other VEGF receptors, as well as those of platelet-derived growth factor and basic fibroblast growth factor [12]. The potent inhibition of VEGF-R2 also held the possibility of an advantage over bevacizumab: since the antibody affects only VEGF-A, one of five members of the VEGF family, albeit the most abundant, an additional increment in activity might result from receptor-targeted therapy. The initial clinical studies showed vatalanib to be well tolerated, and elegant tumor blood flow studies by dynamic enhanced magnetic resonance imaging helped...
to define an appropriate dose for subsequent studies [13]. Following the phase I trials, two phase III trials were conducted by Novartis: Colorectal Oral Novel Therapy for the Inhibition of Angiogenesis and Retarding Metastases (CONFIRM)-1 and CONFIRM-2. Their designs were identical: oxaliplatin plus 5-FU with or without vatalanib. CONFIRM-1 was conducted in patients without prior chemotherapy for metastatic disease, and CONFIRM-2 was conducted in those who had failed front-line therapy with irinotecan and 5-FU. The results of CONFIRM-1 were presented at the American Society of Clinical Oncology annual meeting in 2005: no significant benefit in progression-free survival was demonstrated, though a trend toward such was evident \( (p = .012) \) [14]. In August 2005, as required by Securities and Exchange Commission regulations, the company revealed that the results of CONFIRM-2 were also negative, though no specific data have been published.

How should one interpret these negative trials? Obvious possibilities include: the inhibition of the receptor was insufficient (seems unlikely since the drug is potent), the duration of receptor inhibition was insufficient (once-daily dosing was used, and though the half-life is long, the levels required for activity are undefined; in contrast, bevacizumab has a half-life of 35 days), the dose was too low in a proportion of the patients (possible, since the variability of drug action was not defined in phase II studies), there were compliance issues with an oral medication (will be addressed by a further analysis of the trial), and adverse effects from inhibition of kinases other than VEGF-R2 might have attenuated drug effects (possible, but no currently known biological basis). The first three of these possibilities are essentially pharmacological and will be clarified by future studies with vatalanib or by trials of other inhibitors with different pharmacological characteristics. The fact that the studies were negative makes compliance an issue and illustrates the importance of incorporating some measure of adherence into trials of oral therapies, so that at least one may address it as a consideration in the case of negative results. The last possibility cannot be so resolved and speaks to the need for more detailed analyses of tumor samples from the trials of both bevacizumab and vatalanib; it is not reasonable in this era to perform large studies in CRC patients without having provision for analyses of the available tumors (even if only surgical resection specimens from the primary) to build models for the individualization of cancer therapy.

### Current Data and Their Implications

Importantly, recruitment to the study arm containing bevacizumab alone was stopped by the Data Monitoring Committee prior to the end of the study. This apparent dependence on the combination of the antibody with chemotherapy for optimal efficacy may reflect a wider phenomenon with targeted agents, relating to the need for a proapoptotic signal to be stimulated. It is appropriate to ask whether patients have to be treated with both 5-FU and oxaliplatin: a regimen of chemotherapy less aggressive than we have become used to giving may suffice. However, it is reasonable at least to assume that FOLFOX plus bevacizumab will be active first line.

### Immediate Priorities and Trials to Address Them

Since the results of the E3200 study indicate that the two modalities need to be used together, questions that need to be considered concern both the targeted agents and chemotherapy.

#### Should Bevacizumab Be Used Even in Patients Who Have Failed Chemotherapy/Bevacizumab?

The E3200 study established that treatment with an antiangiogenesis inhibitory approach in the second line is of value to patients. However, before interpreting this to mean that patients should receive this treatment routinely in second-line therapy, it must be appreciated that those entering this trial had not received bevacizumab in the first-line therapy of their disease. The question arises, therefore, whether such patients, upon progressing on first-line chemotherapy/bevacizumab, retain sensitivity to the antibody. The answer is not intuitively obvious: the proximate target of bevacizumab is the endothelial cell, which as a normal cell type is reputedly less likely to acquire resistance. Hence, one can argue that the endothelial cell effects should be manifest at every level of treatment. On the other hand, if the therapeutic effects of inhibiting angiogenesis are exerted indirectly through induction of hypoxia, glucose deprivation, and the like in the tumor cells, resistance mechanisms may well evolve and make such treatment less effective. An example may possibly be provided by the results of a randomized trial of capecitabine (Xeloda®; Hoffmann-La Roche Inc., Nutley, NJ) with or without bevacizumab in third-line breast cancer, in which no apparent advantage accrued from angiogenesis inhibition. To address this issue formally, a trial will soon be activated. The BOND 3 study will address the potential benefit of angiogenic inhibition second line in patients who have already been exposed to bevacizumab plus chemotherapy in the first-line setting. The intention is to randomize patients to either cetuximab
(Erbitux®, ImClone Systems, Inc., New York) plus irinotecan or cetuximab plus irinotecan plus bevacizumab.

**Can Combinations of Targeted Agents Improve Results in Previously Untreated Patients with Metastatic Disease?**

A second important element of strategy is to determine how best to combine targeted agents in the first-line treatment of CRC. Both angiogenesis and the epidermal growth factor receptor (EGFR) have been validated as targets in the treatment of CRC. Can simultaneous inhibition of both pathways further enhance treatment? To address this question, the Cancer and Leukemia Group B/Southwest Oncology Group (CALGB/SWOG) study 80405 will take more than 2,000 patients treated with their physicians’ choice of chemotherapy (FOLFOX or 5-FU, leucovorin, and irinotecan [FOLFIRI]) and randomize them to the addition of bevacizumab, cetuximab, or bevacizumab plus cetuximab (Fig. 1). The study is powered to detect a 25% superiority in outcome.

**Will the Improvement in Survival Extend to the Adjuvant Setting for Both Colon and Rectal Cancer?**

In the adjuvant setting, the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 trial is accruing stage III patients at the rate of 100 per month (toward a target of 2,632). The study is comparing FOLFOX6 with a combination of modified FOLFOX6 plus bevacizumab. The study will be critical in demonstrating whether antiangiogenesis is an effective strategy against the micrometastases assumed to be present, but with only a limited blood supply.

The combination of FOLFOX plus bevacizumab versus FOLFOX alone is also being investigated in the E5204 trial of adjuvant therapy in stage III rectal cancer.

Issues concerning chemotherapy center on the role of the oral fluoropyrimidine capecitabine in combination, particularly with oxaliplatin, and the use of molecular markers to select early-stage patients who may require chemotherapy. In the adjuvant setting, the AVANT trial is randomizing patients to one of three arms: FOLFOX4, FOLFOX4 plus bevacizumab, or capecitabine plus bevacizumab.

**Biological Markers of Outcome Applied to Adjuvant Populations**

Regarding the importance of markers, study E5202 in stage II CRC patients is attempting to divide patients into low- and high-risk groups based on tumor biology. Low-risk patients will be observed. Those judged at high risk (on the basis of 18q and microsatellite instability) will be randomized to receive either FOLFOX or FOLFOX plus bevacizumab. However, the main importance of the study is to determine whether it is feasible to identify a group of patients likely to benefit from 5-FU–containing chemotherapy.

**Individualizing Therapy Based on Materials from Current Trials**

The successful clinical outcomes observed when combining bevacizumab with chemotherapy pose the biological question: what is the basis for the superior response rates and survival when combining cytotoxic and antangiogenic agents? As noted above, the bevacizumab-alone arm of the E3200 study was terminated prematurely based on results inferior to those of the chemotherapy-containing arms. This suggests a dependence on the combination of antibody with chemotherapy for optimal results, supported by the higher response rates observed, not just markers of response duration. These findings prompt consideration of the potential mechanisms of interaction between an effective antiangiogenic agent and chemotherapy.

One characteristic of disordered vasculature in the tumor is an increase in interstitial fluid pressure, which acts as a barrier to blood flow. The prevailing hypothesis is that bevacizumab normalizes the vascular architecture, thus allowing greater penetration of chemotherapy and resulting in further damage to the vasculature and tumor shrinkage (Fig. 2) [15, 16].

Willett and colleagues investigated bevacizumab in human rectal cancer, showing that 12 days’ exposure to the VEGF-specific antibody produces a decrease in blood flow and other markers of tumor perfusion (including number

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**Figure 1.** Combinations of targeted agents: schema of the Cancer and Leukemia Group B/Southwest Oncology Group (CALGB/SWOG) 80405 study. Abbreviations: FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX, oxaliplatin plus 5-fluorouracil; HR, hazard ratio.
of vessels) and also a very striking decrease in interstitial fluid pressure (Fig. 3) [17]. Demonstrating greater penetration of cytotoxic agents is more difficult, but the data of Willet et al. [17] are consistent with this hypothesis. However, increasing delivery of chemotherapy as a means of overcoming resistance was addressed in the days of multidrug resistance and glutathione modulation. That experience did not suggest that outcome could be improved by ensuring that more drug is present in the tumor. The problem seemed to lie more in the fact that tumor cells did not undergo apoptosis because of molecular abnormalities in cell-death pathways that now seem to define many cancers. Second, many years of investigation by radiobiologists and others suggest that rendering tumor cells hypoxic does not sensitize them to chemotherapy.

An alternative hypothesis is that bevacizumab has pro-apoptotic effects resulting from the deprivation of oxygen and nutrients, which add to those induced by the cytotoxic agents [18]. We addressed this hypothesis through the derivation of cell lines with varying susceptibilities to hypoxia-induced apoptosis (Fig. 4) [18]. These lines have been used to identify markers predictive of sensitivity or resistance to hypoxia, and several have been isolated, including macrophage migration inhibition factor, a cytokine, the expression of which is associated with apoptosis [19], and cyclic AMP response element-binding protein, the overexpression of which confers resistance. These and other markers are being evaluated in tissues derived from patients treated in the E3200 study.


Figure 3. Direct evidence that the vascular endothelial growth factor–specific antibody bevacizumab has antivascular effects in human rectal cancer. Abbreviations: CECs, circulating endothelial cells; FDG, fluorodeoxyglucose; IFP, interstitial fluid pressure; PS, permeability–surface area product; α-SMA, alpha smooth muscle actin. Reprinted with permission from Willett et al. Direct evidence that the VEGF–specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004;10:145–147.

These cell lines have further been implanted in mice and recapitulate their in vitro phenotype in that setting: while treatment with bevacizumab results in equivalent
levels of hypoxia in the tumors of the treated animals, tumor shrinkage occurs most markedly in hypoxia-susceptible models. This dissociation of hypoxia induction and apoptosis suggests that, in human tumors also, the response to bevacizumab treatment may depend both on the inhibition of tumor blood flow as well as on the ability of the tumor cells to enter cell-death pathways in response to that stimulus. This prompts the notion that a clinical response may depend on characteristics inherent in the tumor’s biology and not on effects on the vasculature per se.

These two models are not mutually exclusive, but they do have different implications. The model suggesting the central role of vascular changes and increased exposure to cytotoxic agents would focus our attention on oxaliplatin resistance and identifying patients who might benefit on that basis. The alternative model supports a focus on the molecular features that make cells resist entering death pathways in the face of hypoxic stress.

**Figure 4.** Altered susceptibility to hypoxia in conditioned cells. Reprinted with permission from Yao et al. In vitro hypoxia-conditioned colon cancer cell lines derived from HCT116 and HT29 exhibit altered apoptosis susceptibility and a more angiogenic profile in vivo. Br J Cancer 2005;93:1356–1363.

**DISCUSSION**

The situation with novel VEGF inhibitory agents targeted against angiogenesis in some way mirrors that with EGFR agents. There are indications from the first-line setting in CRC that a small molecule (vatalanib) is relatively ineffective, except perhaps in subgroups of patients. In contrast, the VEGF antibody bevacizumab has proven benefit. This difference arises despite the fact that the receptor tyrosine kinase inhibitor is purported to hit a broader range of molecular targets than the antibody.

Whatever the eventual outcome (and several other oral agents are in development), it is safe to assume that bevacizumab will not in the short term be displaced by vatalanib as the partner for first-line FOLFOX.

Given the CONFIRM data cited, it is likely that any investigation of vatalanib in the adjuvant setting will be postponed. In the long term, however, and certainly for adjuvant therapy, an oral agent would have practical advantages. There is likely to be a limit to the number of i.v. infusions that patients will tolerate on frequent schedules, while oral agents can be given for prolonged periods, as has been amply demonstrated in breast cancer. There may also be advantages in toxicity: the profile of vascular toxic effects of vatalanib, and the less well-characterized AZ2171 (sunitinib; Pfizer Pharmaceuticals) and sorafenib (Bayer Pharmaceuticals Corporation, West Haven, CT; Onyx Pharmaceuticals, Emeryville, CA), indicates a lower incidence of venous and arterial thrombotic effects. The limited data available with these small molecules directed against VEGF-R2 suggest also that bleeding events are less frequent than with bevacizumab. It is not clear whether this is a result of lower efficacy or because of greater selectivity in targeting, thus causing less damage to normal endothelium. It is also possible that antibodies with a shorter half-life than bevacizumab may have a better therapeutic index.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

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