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Review

Clinical Experience with Bevacizumab in Colorectal Cancer

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Abstract. *Angiogenesis, the process of generating new capillary blood vessels, is a fundamental requirement for normal physiological processes including embryogenesis, reproductive function and wound healing. Angiogenesis is also implicated in various pathological conditions including age-related retinal macular degeneration, diabetic retinopathy, rheumatoid arthritis, psoriasis and cancer growth and metastasis. Vascular endothelial growth factor (VEGF) is one of the best characterized of the pro-angiogenic growth factors, and multiple strategies have been developed to inhibit this pathway. Bevacizumab, a monoclonal antibody developed against VEGF, has shown initial preclinical and clinical activity. This review discusses the critical role of VEGF and summarizes the available data on the use of bevacizumab in colorectal cancer.*

Thirty years ago, Folkman proposed that new blood vessel formation was important for cancer growth (1). A number of angiogenesis inhibitors are currently in clinical development. These agents include small molecules, peptides, antibodies and complex proteins. The number of potential anti-angiogenesis targets for cancer is large and growing. Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor known to play a central role in tumor angiogenesis and has, therefore, emerged as a promising target for therapeutic intervention. Several potential anti-VEGF strategies are currently under investigation. The best studied of these approaches include inhibition of VEGF and VEGF receptor activity with monoclonal antibodies and inhibition of receptor signaling with tyrosine kinase inhibitors.

By inhibiting the activity of VEGF, bevacizumab, a recombinant humanized monoclonal antibody, prevents intracellular signal transduction and the consequent migration and proliferation of endothelial cells that initiate new blood vessel formation. Preclinical studies have demonstrated that these effects of bevacizumab can cause inhibition of tumor neovascularization, thereby preventing tumor growth.

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Vascular Endothelial Growth Factor Gene Family

The VEGF gene family is comprised of several members including placental growth factor and VEGF-A through VEGF-E. Most of the angiogenic activity is mediated by VEGF-A, which will be referred to as VEGF. VEGF is a basic heparin-binding glycoprotein with a molecular weight of 45 kd (2). Several different isoforms of VEGF are generated through alternative RNA splicing with resulting amino acid sequence lengths of 121, 145, 165, 189 and 206 (3). The 121, 145 and 165 isoforms are freely diffusible or secreted from the cell, while the 189 and 206 isoforms remain associated with the cell surface or are tightly bound to extracellular heparin-containing proteoglycans (4). The VEGF 165 isoform appears to be the most prominent isoform in most systems and has a 50- to 100-fold increased potency in endothelial cell growth assays (5).

VEGF shows significant mitogenic activity for arterial, venous and lymphatic endothelial cells and can induce an angiogenic response in several *in vivo* models (6). It is also able to induce vascular permeability and, in fact, was originally named vascular permeability factor (7). The increase in vascular permeability is probably crucial for angiogenesis, because the leakage of plasma proteins results in extracellular remodeling and the formation of a fibrin gel.

The fibrin gel then serves as a substrate to support endothelial or tumor cell migration and growth (8, 9). VEGF also appears to function as an endothelial cell survival factor. In particular, immature blood vessels are dependent on the presence of VEGF for survival, with the removal of VEGF resulting in vascular regression *via* apoptosis. This dependence decreases as endothelial cells mature and pericyte coverage is completed (10, 11).

VEGF gene expression is a tightly controlled process, with oxygen tension playing a prominent role. Hypoxia causes up-regulation of VEGF gene expression through a promoter sequence (hypoxia inducible factor-1) in a manner similar to erythropoietin regulation (12).

VEGF binds to two high-affinity tyrosine kinase receptors: Flt-1 (fms-like tyrosine kinase 1 or VEGF receptor 1) and KDR (kinase domain region or VEGF receptor 2) (13, 14). Both receptors require dimerization to induce downstream signaling following ligand binding. The angiogenic properties of VEGF are mediated by the Flt-1 and KDR receptors, but the exact role that each receptor plays is not fully known. It is clear that deletion of either receptor in a gene knockout mouse model is embryonically lethal with resultant absent or disorganized vasculogenesis (15, 16). The KDR receptor is generally believed to be responsible for the endothelial cell mitogenic activity of VEGF. In contrast, Flt-1 probably functions in a regulatory manner by providing a ligand-binding receptor without active signal transduction (17). Such a "decoy" receptor can negatively regulate the activity of VEGF by decreasing its availability for binding with the KDR receptor. However, other researchers have noted a primary role for Flt-1 in mediating angiogenesis. Pavco *et al.* (18) reported that targeting Flt-1 through an RNA ribozyme had a greater impact on angiogenesis and tumor growth than a similar ribozyme against KDR. Although KDR and Flt-1 are the primary receptors responsible for the angiogenic properties of VEGF, other receptors including Flt-4 and the newly discovered neuropilin-1 receptor also play roles that are, as yet, uncertain (19).

VEGF is expressed in approximately 50% of colorectal cancers, with minimal to no expression in normal colonic mucosa or adenomas (20). Vessel counts correlate with disease recurrence, metastasis and survival (21). Also, increased VEGF expression is significantly correlated with advanced lymph node status and distant metastasis. Among patients with the highest levels of VEGF expression, survival was significantly worse than in patients with negative or lower levels of VEGF expression. On multivariate analysis, VEGF expression was no longer a significant variable, implying that VEGF expression is closely correlated with other prognostic indicators (20). In a further study, VEGF receptors (VEGF-R-1 and -2) and rRNA for these receptors were found to be highly expressed in human liver metastases from primary colorectal carcinomas (22).

Direct evidence implicating VEGF in tumor growth was provided by preclinical data. Transfection of VEGF into a human colon cancer cell line was shown to enhance angiogenesis, tumor growth and metastasis when the cancer cells were xenografted into nude mice (23). Preoperative serum VEGF levels have also been shown to correlate with advanced tumor stage or nodal status at the time of surgery (24).

From the above evidence, it is apparent that anti-VEGF therapy has great potential in the treatment of cancer. In brief, targeting VEGF prevents angiogenesis induced by all activators upstream of this molecule, and also affects several downstream pathways that can lead to death of a large tumor mass (25).

Clinical Studies

Based on preclinical data, a randomized, open-label, phase II multicenter trial evaluated the efficacy, safety, pharmacokinetics and pharmacodynamics of bevacizumab in combination with 5-fluorouracil/leucovorin (5-FU/LV) as first-line chemotherapy in patients with metastatic colorectal cancer (26). One hundred and four previously untreated patients with measurable metastatic colorectal cancer were randomly assigned to one of the following three treatment groups: 36 to 5-FU (500 mg/m^2)/LV (500 mg/m^2) alone, 35 to 5-FU/LV + low-dose bevacizumab (5 mg/kg every 2 weeks), and 33 to 5-FU/LV + high-dose bevacizumab (10 mg/kg every 2 weeks). 5-FU/LV was given weekly for the first 6 weeks of each 8-week cycle. Patients in the control arm who experienced disease progression were given the option of receiving monotherapy with 10 mg/kg bevacizumab every 2 weeks. These cross-over patients underwent tumor assessments per protocol for the remainder of the treatment period or until disease progression after a minimum of 4 doses of bevacizumab. Table I presents the results for the end-points of response, time to disease progression and survival from that trial. Compared with 5-FU/LV alone, both combination regimens were associated with higher response rates (17% in the control arm *versus* 40% in the low-dose bevacizumab arm and 24% in the high-dose bevacizumab arm).

Combination regimens were also associated with longer median times to disease progression (5.2 *versus* 9.0 months and 7.2 months, respectively). Median survival was 13.8 months in the control arm, 21.5 in the bevacizumab 5-mg/kg arm and 16.1 in the bevacizumab 10-mg/kg arm. At the 18-month time-point, 37% of patients in the 5-mg/kg arm were alive. Twenty-two patients (61%) in the control arm received single-agent bevacizumab at 10 mg/kg as cross-over therapy. For these 22 patients, the median duration of bevacizumab therapy was 2 months, with follow-up ranging from 1 day to 7 months. Two cross-over patients experienced

a partial response and 7 patients had stable disease. The median time to progression for cross-over patients was 2 months, with follow-up ranging from 1 day to 5 months.

The rates of overall response and time to disease progression were significantly higher in the low-dose arm than in the control arm. No significant differences were seen when the high-dose and control arms were compared. This may reflect small patient numbers or imbalances in randomization. However, it is possible that the 10-mg/kg dose of bevacizumab may have caused excessive vascular collapse, limiting delivery of chemotherapy, whereas the 5-mg/kg dose had an antitumor effect and improved the delivery of chemotherapy. Median survival also appeared to be longer in the low-dose bevacizumab arm, but statistical significance has not yet been reported.

Therapy with bevacizumab was generally well tolerated. Fifty deaths (48%) were reported on study. Three patients died from a cause other than disease progression: mucositis/diarrhea/neutropenia (control arm), respiratory distress (5-mg/kg arm) and pulmonary embolism (10-mg/kg arm). More patients in the bevacizumab arms experienced at least one National Cancer Institute common toxicity criteria (version 1) grade 3 or 4 adverse event. The increase in incidence of grade 3 and 4 events seen in the bevacizumab arms compared with the control arm was statistically significant ($p=0.042$). The incidence and severity of adverse events known to be associated with 5-FU/LV (diarrhea, leukopenia and stomatitis) was as expected when bevacizumab was added to the regimen. Bevacizumab therapy was associated with fever, headache, rash, epistaxis and chills; these events were generally mild to moderate in severity. Bleeding, hypertension and thrombosis have been observed in other clinical trials of bevacizumab and occurred at an increased incidence in the bevacizumab arms in this trial; 16 patients required antihypertensive therapy. The most common type of bleeding in this study was transient epistaxis (lasting <5 minutes), reported in 11% of control patients, 46% of 5-mg/kg patients and 53% of 10-mg/kg patients. Three patients in the 10-mg/kg arm had a grade 3 or 4 gastrointestinal hemorrhage; the relationship to therapy was unclear.

Interim safety data from 18 patients have been released from a further phase II trial (the Eastern Cooperative Oncology Group [ECOG] study E2200) (27). All patients were treated with irinotecan (125 mg/m^2), 5-FU (500 mg/m^2) and LV (20 mg/m^2) weekly for 4 of 6 weeks, plus bevacizumab (10 mg/kg) every 2 weeks. The authors of that study concluded that adding bevacizumab to a combination regimen of 5-FU/LV plus irinotecan as first-line therapy did not increase the incidence of known toxicities of the chemotherapy regimen. Grade 1/2 epistaxis or hemoptysis was reported in 4 patients, but no significant bleeding or thrombotic events occurred.

The phase III study of standard bolus irinotecan/5-FU/LV (IFL) plus bevacizumab (5 mg/kg) was recently reported (28). Of 813 patients with previously untreated metastatic colorectal cancer, we randomly assigned 402 to receive irinotecan, bolus 5-FU/LV (IFL) plus bevacizumab (5 mg per kg of body weight every 2 weeks) and 411 to receive IFL plus placebo. The median duration of overall survival, the primary end-point, was significantly longer in the group given IFL plus bevacizumab than in the group given IFL plus placebo (20.3 months *vs.* 15.6 months), which corresponds to a hazard ratio for death of 0.66 ($p<0.001$), or a reduction of 34% in the risk of death in the bevacizumab group. The 1-year survival rate was 74.3% in the group given IFL plus bevacizumab and 63.4% in the group given IFL plus placebo ($p<0.001$). In the subgroup of patients who received second-line treatment with oxaliplatin, the median duration of overall survival was 25.1 months in the group given IFL plus bevacizumab and 22.2 months in the group given IFL plus placebo. The addition of bevacizumab to IFL was associated with increases in the median duration of progression- free survival (10.6 months *vs.* 6.2 months; hazard ratio for progression, 0.54, for the comparison with the group given IFL plus placebo; $p<0.001$); response rate (44.8% *vs.* 34.8%; $p=0.004$); and the median duration of response (10.4 months *vs.* 7.1 months; hazard ratio for progression, 0.62; $p=0.001$). Toxicities were generally mild: although grade 3 hypertension occurred more often during treatment with IFL plus bevacizumab than with IFL plus placebo (11.0% *vs.* 2.3%), it was easily managed. The overall incidence of grades 3 and 4 adverse effects increased by about 10% with bevacizumab, mostly because of hypertension, requiring treatment, diarrhea and leukopenia.

Two large prospective trials evaluated the safety and efficacy of oxaliplatin-based regimens in the treatment of metastatic colorectal cancer; these data were presented at the 2005 Gastrointestinal Cancers Symposium. The randomized, multicenter TREE-2 (A Randomized, Prospective Study Comparing Three Regimens of Eloxatin Plus Fluoropyrimidine and Bevacizumab for Evaluation of Safety and Tolerability in First-Line Treatment of Patients with Advanced Colorectal Cancer) is the first study evaluating the safety and tolerability of bolus, infusional and oral fluoropyrimidine + oxaliplatin-based regimens combined with bevacizumab for the first-line treatment of metastatic colorectal cancer (29). In the TREE-2 study, 213 adults aged 18 or older with metastatic colorectal cancer were treated with 1 of 3 Eloxatin-containing chemotherapy regimens: Eloxatin plus infusional 5-FU/LV (FOLFOX), Eloxatin plus bolus 5-FU and Eloxatin plus capecitabine (CAPEOX), all used in combination with bevacizumab. The preliminary results of TREE-2 assessed the tolerability of the 3 oxaliplatin/fluoropyrimidine regimens with bevacizumab.

There were no unexpected toxicities. The best treatment response rates were seen when bevacizumab was added to FOLFOX or CAPEOX. Full efficacy results are expected to be presented at the 2005 ASCO Annual Meeting in May 2005. The E3200, a phase III randomized study of Eloxatin, fluorouracil and leucovorin calcium with or without bevacizumab *versus* bevacizumab alone in patients with previously-treated advanced or metastatic colorectal adenocarcinoma, was sponsored by the National Cancer Institute (NCI) and conducted by a network of researchers led by the Eastern Cooperative Oncology Group (30). A total of 829 patients were enrolled in the study between October 2001 and April 2003. The study demonstrated a significant 26% reduction in the risk of death for patients receiving Eloxatin-based chemotherapy (FOLFOX4) plus bevacizumab compared to those who received FOLFOX4 alone. Although patients in the E3200 study had previously been treated for advanced or metastatic colorectal cancer, the median overall survival with Eloxatin-based chemotherapy plus bevacizumab was 12.5 months compared to 10.7 months with FOLFOX4 alone. The difference is statistically significant and corresponds to a 17% improvement in median overall survival in this previously-treated patient population.

Table I. *Response rate and survival.*

End-points	5-FU/LV	5-FU/LV+Bevacizumab 5 mg/kg	10 mg/kg
Objective response rate	6 (17)	14(40)[a]	8 (24)
Median time to disease progression (months)	5.2	9.0[b]	7.2
Median overall survival (months)	13.8	21.5[c]	16.1

p values are for comparision with the 5-FU/LV arm:
[a]$p=0.03$
[b]$p=0.005$
[c]$p=0.137$

Conclusion

The clinical data obtained to date indicate that bevacizumab monotherapy is an effective and well-tolerated treatment for patients with colorectal cancer. There are, however, issues that need to be addressed. For example, it remains to be determined which subset of patients benefits most from bevacizumab therapy. Importantly, phase III data suggest that all clinical subgroups benefit from therapy. Evaluation of molecular predictors is ongoing. The optimal dose of bevacizumab is also currently being explored. While the available phase II and phase III data for colorectal cancer have demonstrated a benefit for bevacizumab at a dose of 5 mg/kg every 2 weeks, it should be noted that the current ECOG second-line study of the FOLFOX regimen, alone or in combination with bevacizumab, is using a dose of 10 mg/kg. It is possible that both doses will be active, and further study may be needed to define the optimal dose for this agent in colorectal cancer.

From a safety point of view, the mechanism of bleeding and thrombosis associated with bevacizumab treatment remains to be clarified. VEGF appears to act as a critical survival factor for endothelial cells in newly-formed vessels by inhibiting apoptosis (31). Increased apoptosis of these cells through the blockade of VEGF receptors, leading to exposure of subendothelial cells, may trigger a coagulation cascade. Therefore, thrombosis has been speculated to be a class effect for all anti-angiogenic agents. Importantly, however, the incidence of thrombosis and cardiovascular events was not elevated in the phase III study of bevacizumab plus IFL in metastatic colorectal cancer, suggesting these potential complications are more related to advanced colorectal cancer and systemic chemotherapy than to bevacizumab treatment. Modest elevations in blood pressure occurred occasionally and were easily managed with standard antihypertensive medications.

Lastly, evaluation of bevacizumab in the early stages of disease (adjuvant and neoadjuvant) is warranted. While bevacizumab has, thus far, been remarkably well-tolerated, the effects of long-term therapy may uncover novel mechanisms of tumor resistance or toxicities in the late stages of disease that are not yet appreciated. There is a strong rationale for using anti-angiogenic therapy in early disease when tumor neovascularization is particularly critical. In addition, such therapy may reduce metastasis.

References

1 Folkman J: Tumor angiogenesis: therapeutic implications. N Engl J Med *285*: 1182-1186, 1971.

2 Ferrara N and Henzel W: Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun *161*: 851-858, 1989.

3 Tischer E, Mitchell R, Hartman T *et al*: The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem *266(18)*:11947-11954, 1991.

4 Park J, Keller G and Ferrara N: The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell *4*: 1317-1326, 1993.

5 Keyt B, Berleau L and Nguyen H: The carboxyl-terminal domain (111-165) of vascular endothelial growth factor is critical for its mitogenic potency. J Biol Chem *272*: 7788-7795, 1996.

6 Leung DW, Cachianes G, Kuang WJ, Goeddel DV and Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. Science *246*: 1306-1309, 1989.

7 Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS and Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science *219*: 983-985, 1983.
8 Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J and Dvorak AM: Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. Lab Invest *57*: 673-686, 1987.
9 Dvorak H: Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med *315*: 1650-1659, 1986.
10 Benjamin L, Hemo I and Keshet E: A plasticity window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial networks and is regulated by PDGF-B and VEGF. Development *125*: 1591-1598, 1998.
11 Benjamin LE, Golijanin D, Itin A, Pode D and Keshet E: Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest *103*: 159-165, 1999.
12 Goldberg M and Schneider T: Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. J Biol Chem *269*: 4355-4361, 1994.
13 de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N and Williams LT: The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science *255*: 989-991, 1992.
14 Terman BI, Dougher-Vermazen M, Carrion ME *et al*: Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun *187*: 1579-1586, 1992.
15 Shalaby F, Rossant J, Yamaguchi T *et al*: Failure of blood-island formation and vasculogenesis in Flk-1 deficient mice. Nature *376*: 62-66, 1995.
16 Fong GH, Rossant J, Gertsenstein M and Breitman ML: Role of Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature *376*: 66-70, 1995.
17 Ferrara N: Molecular and biological properties of vascular endothelial growth factor. J Mol Med *77*: 527-543, 1999.
18 Pavco P, Bouhana K, Gallegos A *et al*: Antitumor and antimetastatic activity of ribozymes targeting the messenger RNA of vascular endothelial growth factor receptors. Clin Cancer Res *6*: 2094-2103, 2000.
19 Soker S, Takashima S, Miao HQ, Neufeld G and Klagsbrun M: Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell *92*: 735-745, 1998.
20 Lee JC, Chow NH, Wang ST and Huang SM: Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients. Eur J Cancer *36*: 748-753, 2000.
21 Choi HJ, Hyun MS, Jung GJ, Kim SS and Hong SH: Tumor angiogenesis as a prognostic predictor in colorectal carcinoma with special reference to mode of metastasis and recurrence. Oncology *55*: 575-581, 1998.
22 Warren RS, Yaun H and Matli MR: Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J Clin Invest *95*: 1789-1797, 1995.
23 Kondo Y, Arii S, Mori A, Furutani M, Chiba T and Imamura M: Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into LoVo human colon cancer cell line. Clin Cancer Res *6*: 622-630, 2000.
24 Kumar H, Heer K, Lee PW *et al*: Preoperative serum vascular endothelial growth factor can predict stage in colorectal cancer. Clin Cancer Res *4*: 1279-1285, 1998.
25 Fernando NH and Hurwitz HI: Inhibition of vascular endothelial growth factor in the treatment of colorectal cancer. Semin Oncol *30(6)*: 39-50, 2003.
26 Kabbinavar F, Hurwitz H, Fehrenbacher L *et al*: Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol *21*: 60-65, 2003.
27 Giantonio BJ, Levy D, Catalano PJ, O'Dwyer PJ and Al B Benson III: Incorporating angiogenesis inhibition with bevacizumab (anti-VEGF) into frontline chemotherapy with irinotecan (CPT-11), fluorouracil and leucovorin (FU/LV) for advanced colorectal cancer (AdvCRC): a toxicity analysis of ECOG study E2200. Proc Am Soc Clin Oncol *21*: 126a, 2002.
28 Hurwitz H, Fehrenbacher L, Novotny W *et al*: Bevacizumab plus irinotecan, 5-fluorouracil, and leucovorin for the treatment of metastatic colorectal cancer: results of a randomized phase III trial. N Engl J Med *350*: 2335-2342, 2004.
29 TREE-2: Trials presented at the 2005 Gastrointestinal Cancers Symposium in Hollywood, Florida, U.S.A.
30 [No authors listed]. The addition of bevacizumab to FOLFOX4 prolongs survival in relapsed colorectal cancer: interim data from the ECOG 3200 trial. Clin Colorectal Cancer *4*: 300-301, 2005.
31 Ferrara N: Role of vascular endothelial growth factor in the regulation of angiogenesis. Kidney Int *56*: 794-814, 1999.

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