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Combined Vascular Endothelial Growth Factor–Targeted Therapy and Radiotherapy for Rectal Cancer: Theory and Clinical Practice

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Abstract

Despite the routine use of adjuvant and neoadjuvant chemoradiotherapy, patients with advanced rectal tumors experience significant rates of treatment failure and disease recurrence. Resistance to radiation is a particular problem. Adding a vascular endothelial growth factor (VEGF)–targeted therapy may improve outcomes in these patients. Epidemiologic studies have shown that tumor expression of VEGF predicts disease recurrence and lower overall survival in patients treated with radiation. In tumor xenograft models in mice, VEGF-targeted agents increase the response to radiation, with a greater probability of tumor control and a greater delay in tumor growth. In addition to killing cancer cells indirectly by damaging tumor blood vessels (antivascular effect), VEGF-targeted therapy may sensitize tumors to radiation through two mechanisms: by normalizing the tumor vasculature, leading to greater tumor oxygenation, and thereby increasing the cytotoxicity of radiation to cancer cells, and by increasing the radiosensitivity of tumor-associated endothelial cells. In addition, anti-VEGF agents may inhibit the regrowth of tumors after radiation by decreasing the number of circulating endothelial cells and endothelial progenitor cells. A phase I dose-escalation study has shown the safety of bevacizumab at a dose of 5 mg/kg in combination with 5-fluorouracil and radiation in patients with rectal carcinoma, and has provided evidence of both vascular normalization and antivascular mechanisms. Phase II evaluation of bevacizumab in this setting is under way.

Surgery is the mainstay of treatment in patients with rectal cancer.^{1–3} Survival at 5 years in patients with early stage tumors (confined to the colon or rectal wall without node involvement) is more than 80%,^{4,5} but rates of treatment failure in patients who undergo potentially curative resection for more advanced tumors continue to be high.^{1,2,6} Preoperative or postoperative chemotherapy and radiotherapy are now used to improve outcomes in these patients. Randomized trials in the past 15 years have shown significantly greater local control, freedom from distant metastases, and survival in patients treated with concomitant radiation and 5-fluorouracil (5-FU)–based chemotherapy.^{1,2,7,8} Despite these improvements, however, a large number of tumors do not respond to or recur after treatment with radiotherapy and chemotherapy. Anti-vascular endothelial growth factor (VEGF) therapy is one of the most promising approaches to increase the efficacy of radiotherapy.^{9,10}

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VEGF-Targeted Agents and Radiation in Experimental Tumor Models

Some of the early preclinical studies that used non-VEGF-targeted antiangiogenic agents combined with radiation found that the combination induced greater delays in the growth of tumors than did either modality alone.^{11,12} Other studies showed that adding antiangiogenic agents could compromise the response to radiation.¹³ However, preclinical studies using selective inhibitors of VEGF combined with ionizing radiation have shown good tumor control. For example, the growth of various xenografted tumors (eg, lung carcinoma, squamous cell carcinoma, esophageal carcinoma, glioblastoma) treated with antibodies to VEGF plus local radiation at a total dose of 20 or 40 Gy was suppressed in a synergistic manner.¹⁴ Similarly, adding a monoclonal antibody to VEGF to radiation at a dose of 20 or 30 Gy produced a delay in tumor growth that was additive in colon carcinoma and more than additive in glioblastoma xenografts in mice.¹⁵ Recent experiments with the monoclonal antibody DC101, which blocks mouse VEGFR-2, combined with radiation had similar results.^{16–18}

Almost all of the experimental studies mentioned above examined only the short-term delay in growth. We recently conducted the first experiments to determine the probability of greater tumor control with radiation by adding an antiangiogenic agent at various times throughout the therapy.¹⁶ The use of DC101 before, during, and after local fractionated irradiation of the moderately radiosensitive human lung tumor 54A and the highly radioresistant human U87 glioblastoma decreased the dose of radiation necessary to control 50% of tumors locally by 1.7- and 1.3-fold, respectively (Fig 1A). Blockade with DC101 did not increase the skin radiation reaction.¹⁶

The relative timing of antiangiogenic and radiation therapy has been analyzed in human U87 glioblastoma xenografts implanted orthotopically in the brain of mice.¹⁷ As shown in Fig 1B, radiation (3 daily fractionated doses of 7 Gy each) was given at different times before, during, and after the administration of DC101. The longest delay in tumor growth was achieved when the tumors were irradiated on days 4 to 6 after the initiation of DC101 (RT4 in Fig 1B). Only in this case was the effect of the two modalities synergistic; in the other schedules their effects were additive. By studying the DC101-induced changes in the tumor microenvironment we established that the optimum timing for irradiation coincided with vessel normalization and maturation and decreased hypoxia (measured by pimonidazole staining, a marker of hypoxia). A similar normalization window and its importance for tumor oxygenation and radiosensitivity were reported with another antiangiogenic agent, thalidomide, that downregulates VEGF.¹⁹ Whether other antiangiogenic agents induce normalization of the tumor vasculature and reduce tumor hypoxia requires further study.

Mechanisms of Action and Schedule Optimization of Anti-VEGF Therapy With Radiotherapy

The mechanisms of action by which anti-VEGF agents increase the efficacy of radiotherapy are not completely understood.^{20,21} Three potential mechanisms have been reported.^{9,10,20} First, the inhibition of VEGF-VEGFR-2 signaling can radiosensitize tumor-associated endothelial cells, thereby reducing vascular density and inhibiting the formation of new vessels. Impaired vascular delivery of nutrients and oxygen may result in cancer cell death.²² Second, anti-VEGF agents can normalize the tumor vasculature and microenvironment and, as a result, increase tumor oxygenation and radiosensitivity. Third, anti-VEGF agents can reduce the number of circulating endothelial cells and progenitor cells. This may lead to the inhibition of recurrent tumor growth after irradiation. The effect of the third mechanism in radiotherapy remains to be shown in preclinical and clinical studies. The relative effects of these three mechanisms on the outcomes with radiotherapy may differ with the antiangiogenic agent,

tumor type, site, and host. The mechanism of action of anti-VEGF agents is discussed in detail elsewhere in this supplement.

Multitargeted Agents That Target VEGF Receptors

In addition to antibodies that specifically inhibit VEGF or its main receptor, VEGFR-2, there are also a number of small molecules (receptor tyrosine kinase inhibitors) that inhibit VEGFR-2 in parallel with inhibition of other pathways.²⁰ Some of these agents (eg, SU5416, PTK787/ZK 222584) inhibit primarily VEGF receptors, and others (eg, ZD6474, SU11248) inhibit the activity of several receptor tyrosine kinases in various stromal and cancer cells. Multitargeted inhibitors given as monotherapy appeared to be highly potent anti-VEGF agents in some animal studies (eg, PTK787, SU5416). In addition, they have good efficacy when combined with radiotherapy in preclinical models.⁹ Because of the diversity of their mechanisms of action, however, optimizing combined therapy with them may be more difficult than with specific anti-VEGF agents.

Clinical Trials

Chemoradiotherapy is a standard treatment for patients with locally advanced rectal cancer. To test the hypothesis that adding an anti-VEGF agent to a chemoradiotherapy protocol is safe, we conducted a phase I/II trial of neoadjuvant bevacizumab in combination with 5-FU and radiation therapy in patients with T3 or T4 rectal cancer (Fig 2).^{23,24} Patients were given bevacizumab monotherapy in cycle 1 (2 weeks) and bevacizumab plus 5-FU and radiation therapy in cycles 2 to 4. Bevacizumab was given as a 90-minute infusion on day 1 of each cycle. The dose was escalated in cohorts of six patients (5 mg/kg in the first cohort and 10 mg/kg in the second). 5-FU was infused daily at a fixed dose of 225 mg/m² in each week of treatment. External beam radiation was administered at a total of dose of 50.4 Gy in 28 daily fractions over 5.5 weeks. Surgery was performed 7 to 9 weeks after the completion of the therapy. The primary objective of the study was to determine the maximum tolerated dose of bevacizumab given with 5-FU and external beam radiation therapy. A secondary goal was to gain insight into the effects of bevacizumab alone in patients with locally advanced rectal cancer.

The phase I segment of the study has been completed.^{23,24} The first six patients were given bevacizumab 5 mg/kg plus 5-FU and radiation, with no dose-limiting adverse events. All of these patients subsequently underwent surgery without complications. Enrollment in the second cohort of patients (bevacizumab 10 mg/kg plus 5-FU and radiation) was halted, however, after five patients had been treated, owing to dose-limiting toxicities (grades 3 and 4 diarrhea and colitis) in two patients. Both patients were able to complete the 5-FU and radiation therapy after recovery from these events. All of the patients in the second cohort underwent surgery. One of them had a pulmonary embolus on postoperative day 1, but recovered with anticoagulation therapy. Ileostomy obstruction with stent-related ileal perforation occurred in another patient 10 days after surgery, and laparotomy and ileostomy revisions were required.

At surgery, five of six patients in the 5-mg/kg bevacizumab cohort exhibited only microscopic disease.^{23,24} Pathologic evaluation of surgical specimens revealed two complete pathologic responses in the bevacizumab 10-mg/kg cohort and none in the 5-mg/kg cohort.

The design of the study made it possible for us to evaluate the effect of bevacizumab alone (in cycle 1) on surrogate markers of antiangiogenic therapy in rectal carcinoma before the addition of 5-FU and radiation.^{23, 45} Before treatment and 12 days after the first infusion of bevacizumab, patients underwent flexible sigmoidoscopy with tumor biopsy, tumor interstitial pressure measurement, perfusion computed tomography scan to measure tumor blood volume

and perfusion, [^{18}F]fluorodeoxyglucose positron emission tomography scan, and analysis of blood and urine for a number of angiogenesis markers (these assessments were repeated after the completion of cycle 4, before surgery). We observed several changes consistent with vascular normalization, including reduced tumor interstitial pressure and increased pericyte coverage of the vasculature (Figs 3 and 4). Tumor metabolism (quantified by [^{18}F]fluorodeoxyglucose uptake on positron emission tomography scan) was not significantly changed after the initial dose of bevacizumab, despite the reduced tumor vascular density and reduced tumor blood flow, but decreased significantly after three cycles of combination treatment. Tumor vascular normalization, with subsequent reduction in tumor hypoxia, may potentiate the effects of chemoradiotherapy. Preliminary data suggest that bevacizumab monotherapy can increase both apoptosis and the proliferation of cancer cells. The former support the antivascular and anticancer hypothesis and the latter supports the normalization hypothesis that results in chemoradiation sensitization. While these data are supportive of the hypothesized mechanism of action of bevacizumab, data from correlative studies such as these in large blinded trials will be critical in validating the mechanism of action of bevacizumab and other antiangiogenic agents.

With the phase I segment of our trial completed, the phase II segment continues to evaluate bevacizumab at the maximum tolerated dose of 5 mg/kg in combination with 5-FU and radiation in patients with rectal carcinoma.²⁴ Other phase II studies are evaluating bevacizumab in combination with capecitabine with or without oxaliplatin and radiation in patients with T3 and T4 rectal carcinoma.^{25,26} These trials use bevacizumab in the neoadjuvant setting, in which greater tumor shrinkage could make downgrading and subsequent resection possible in otherwise-inoperable tumors.

Summary

The successful clinical use of anti-VEGF agents, alone (multitargeted tyrosine kinase inhibitors) and in combination with chemotherapy (bevacizumab), raises important questions about their use in combination with radiation. VEGF plays an important role in the resistance of tumors to radiation. In tumor xenograft models, VEGF-targeted agents are known to sensitize tumors to radiation, increasing the delay of tumor growth and the probability of local control. The radiation-enhancing effects of VEGF-targeted therapies may occur through at least three mechanisms: (1) the normalization of tumor vascular and subsequent reoxygenation, directly increasing the radiosensitivity of cancer cells; (2) the killing of endothelial cells in the tumor vasculature, leading to indirect killing of cancer cells; (3) the reduction of the number of blood endothelial and progenitor cells. A dose-escalation trial conducted in patients with rectal carcinoma has shown the safety of bevacizumab 5 mg/kg in combination with 5-FU and radiation therapy. Phase II evaluation of bevacizumab 5 mg/kg in combination with 5-FU and radiation in patients with rectal carcinoma is under way.

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Figure 1.

(A) Probability of tumor control of 8-mm 54A and U87 tumors, by total dose of radiation (RT) alone and combined with DC101 20 or 40 mg/kg given every 3 days $\times$ 6 injections. Radiation was given on 5 consecutive days (days 0 – 4 for RT alone and days 1–5 when combined with DC101). In 54A xenografts the TCD₅₀ (95% confidence intervals) were 66.2 Gy (59.6 – 73.6) with RT alone; 54.8 Gy (45.1 – 66.6) with RT + DC101 20 mg/kg and 39.1 Gy (31.7 – 48.1) with RT + DC101 40 mg/kg. The corresponding values for U87 tumors were 97.8 Gy (85.3 – 112.0) with RT alone; 86.3 Gy (74.6 – 99.8) with RT + DC101 20 mg/kg and 74.8 Gy (63.7 – 87.7) with RT + DC101 40 mg/kg. (Adapted and reprinted with permission from Kozin et al.¹⁶) (B) Delay of tumor growth of orthotopic U87 gliomas in untreated control and with monotherapy with DC101 40 mg/kg every 3 days $\times$ 3 injections, local RT for 3 consecutive days, and 5 different combination schedules in which RT was given before, during, and after DC101 (RT1–RT5; see diagram for schedules). The dashed lines show the range of the expected additive effect of DC101 and RT. * $P < .05$ versus control and versus expected additive effect. (Modified and reprinted with permission from *Cancer Cell*, copyright 2004, from Elsevier.¹⁷)

Figure 2.
Design of phase I/II trial of neoadjuvant bevacizumab in combination with 5-FU and radiation.

Figure 3.

Reduced tumor blood flow but no change in [18F]fluorodeoxyglucose uptake after treatment with bevacizumab 5 mg/kg at day 12. (Left) Significant decreases in both measures after completion of bevacizumab and chemoradiotherapy before surgery. (Right) Functional computed tomography images of tumor blood perfusion and positron emission tomography imaging of [18F]fluorodeoxyglucose uptake before treatment (day 0), after bevacizumab (day 12), and after completion of bevacizumab and chemoradiotherapy. (Reprinted by permission from Macmillan Publishers Ltd: *Nature Medicine*, copyright 2004.23)

Figure 4. Hemodynamic, metabolic, and vascular effects of therapy with bevacizumab 5 mg/kg. (Reprinted by permission from Macmillan Publishers Ltd: *Nature Medicine*, copyright 2004.²³)