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Complete pathological response to bevacizumab and chemoradiation in advanced rectal cancer

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SUMMARY

Background—Localized rectal cancer responds well to 5-fluorouracil and radiation-based regimens. A phase I–II trial is currently testing the efficacy of adding bevacizumab, a VEGF-specific antibody, to standard chemoradiotherapy. The case presented here is a complete pathological response seen in a patient with extensive and locally invasive carcinoma after receiving this combined treatment.

Investigations—Physical examination, rectal ultrasound, PET–CT scan, laboratory tests, proctoscopic examination, chest radiograph, rectal forcep biopsies with immunohistochemistry, and protein and flow cytometric analyses.

Diagnosis—Large, invasive, ultrasound stage T4 carcinoma of the rectum, which was positive for survivin.

Management—One 2-week cycle of bevacizumab alone, followed by 3 cycles of bevacizumab with continuous 5-fluorouracil infusion, and external-beam radiation therapy given 5 days per week to the pelvis, abdominoperineal resection with posterior vaginectomy, hysterectomy and bilateral salpingo-oophorectomy.

Keywords

bevacizumab; chemotherapy; radiation therapy; rectal carcinoma; VEGF

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Competing interests

CG Willett, BG Czito, J Bendell, HI Hurwitz, and RK Jain have declared associations with the following companies: Astra-Zeneca, Dyax, Genentech, Novartis, Pfizer, Roche and Thrombogenics. See the article online for full details of the relationship. The other authors declared they have no competing interests.

THE CASE

A 55-year-old woman was referred for management of a distal rectal adenocarcinoma. She reported a 2-year history of diarrhea ranging from four to eight bowel movements per day but did not receive any treatment before presentation. In the last 2 months before presentation, she reported blood in her stool but no other symptoms. Two weeks before referral, colonoscopy revealed a circumferential, ulcerated and friable mass in the rectum extending from the anal verge to 10 cm proximally. No other masses or polyps were observed in the colon. Forcep biopsies of the lesion confirmed invasive moderately differentiated adenocarcinoma. The patient had a history of Bell's palsy and had undergone appendectomy, a laparotomy for ovarian cyst, tubal ligation, cholecystectomy and repair of a nasal fracture. She had smoked approximately one half packet of cigarettes per day for over 12 years and had no family history of cancer. Physical examination was unremarkable and auscultation of the heart and lungs showed no abnormalities. The abdomen was free of palpable masses. Digital rectal examination (DRE) revealed a circumferential hard mass that started just superior to the dentate line. The mass was large with significant bulk and extended into the rectovaginal septum and immediately adjacent to the vaginal mucosa. On rigid proctoscopic examination, the tumor extended to approximately 10 cm.

Endorectal ultrasonography showed a hypoechoic circumferential mass with well-defined endosonographic borders, measuring up to 14 mm in thickness. There was sono-graphic evidence suggesting invasion into the vagina (ultrasound stage T4). A small lymph node was seen in the mesorectum. The results of laboratory tests, including blood count and electrolyte, blood urea nitrogen, liver function studies, and creatinine were within normal limits (details of these results can be found in a Supplementary table online). Carcinoembryonic antigen (CEA) level was 122.8 ng/ml—a normal reference in smokers is 0.0–5.0 ng/ml. An (^{18}F)-fluorodeoxyglucose (FDG)-PET-CT and CT with oral and intravenous contrast enhancement of the abdomen and pelvis revealed thickening of the rectal wall that was most prominent anteriorly. A 4 mm node in the right perirectal fat region was noted. The maximum standardized uptake value (SUV_{max}) of the rectal mass measured by PET was very high (30.5). Two small ill-defined lesions in the left hepatic lobe were noted and described as indeterminate. A chest radiograph showed no evidence of lung metastases, and the cardiac, mediastinal, pleural, and bony structures were normal. Following clinical and radiographic evaluation, this patient was judged to have a clinical stage T4N1MX distal rectal cancer.

The patient enrolled in a phase II clinical trial (NCI protocol #5642) of neoadjuvant bevacizumab (a VEGF-specific antibody), in combination with 5-fluorouracil (5-FU) and radiation therapy. The patient received a 2-week cycle of 5 mg/kg bevacizumab, followed by three 2-week cycles of 5 mg/kg bevacizumab with a concurrent continuous infusion of 225 mg/m²/day 5-FU for 5.5 weeks. External-beam radiation therapy was administered to a total dose of 50.4 Gy in 28 fractions given 5 days a week over 5.5 weeks (Figure 1). A restaging CT scan of the abdomen and pelvis 3 weeks following treatment showed two small stable liver lesions and interval decrease in circumferential thickening of the rectum, with no change in size of the small perirectal lymph node. Eight weeks following completion of neoadjuvant therapy, the patient underwent abdominoperineal resection with posterior vaginectomy, total abdominal hysterectomy and bilateral salpingo-oophorectomy because of the distal location of the tumor and its adherence to the vagina. In addition, a vaginal and perineal reconstruction with vertical rectus myocutaneous flap was performed. Evaluation of the surgical specimen showed a 6.5 × 6.0 cm ulcer in the distal rectum (Figure 2). Microscopic examination showed no residual carcinoma in the anus, rectum (Figure 3), five sampled regional lymph nodes, vagina, uterus or ovaries. Pathologically, the lesion was staged as ypT0-ypN0. The patient

recovered from surgery without any complications, completed adjuvant 5-FU-based chemotherapy, and remains free of disease 8 months after surgery.

DISCUSSION OF DIAGNOSIS

The design of this study permitted evaluation of the effect of VEGF blockade by bevacizumab alone on surrogate markers of antiangiogenic therapy before the addition of 5-FU and radiation therapy.^{1,2} Imaging, tissue and blood biomarker studies were conducted before, during and after combined treatment at five time points in total (Figure 1). Figure 2 shows an endoscopic view of the tumor before treatment and 12 days following the first bevacizumab infusion. Consistent with our previous findings,^{1,2} tumor metabolism assessed by FDG uptake on PET was not significantly changed after the initial dose = 26 at day 12 versus of bevacizumab ($SUV_{max} = 30.5$ pretreatment), but decreased SUV_{max} significantly after three cycles of combination = 6.2 pre-surgery, Figure 2). treatment (SUV_{max} This decrease in metabolism was consistent with the presence of the ulcer but no residual macroscopic tumor appearance was detected on endoscopy, and a pathologic complete response was seen after surgery (Figures 2 and 3). As seen in all 11 patients studied in the phase I clinical trial,^{1,2} the ulceration of the mucosa at the tumor site in this patient did not subside at 7–9 weeks post-combination therapy. The role of bevacizumab, which has a half-life of approximately 20 days (range of 11–50 days), in this delay in healing of the rectal mucosa is unclear.

Immunohistochemistry for the pericyte marker α -smooth muscle actin and the blood vascular endothelial cell marker CD31 in serial biopsies showed an increase in the number of perivascular cell-covered vessels, from 16.7% before bevacizumab infusion to 44.8% 12 days after treatment. In addition, staining for the cell proliferation marker Ki-67 showed an increased level of epithelial-cell proliferation in biopsies ($61.0\% \pm 12.0\%$) following bevacizumab therapy compared with pretreatment values ($41.6 \pm 11.8\%$). These data are consistent with a 'vascular normalization' effect of bevacizumab.^{1–3} Conversely, epithelial-cell apoptosis evaluated by terminal deoxynucleotidyltransferase biotin-dUTP nick end labeling (TUNEL) did not seem to change with bevacizumab treatment and remained at about 1.7%. Furthermore, this tumor stained positive for survivin ($2.2\% \pm 2.0\%$ nuclear and $11.2\% \pm 5.9\%$ cytoplasmic cancer cell staining), a marker of radioresistance, decreased apoptosis and increased cell proliferation.⁴ Survivin expression in cancer cells was further elevated at day 12 of bevacizumab treatment ($6.7\% \pm 1.7\%$ in the nucleus and $85.9\% \pm 11.5\%$ in the cytoplasm). Nevertheless, this patient's tumor demonstrated a pathologic complete response after combination therapy, suggesting a synergy between VEGF blockade and the cytotoxic regimen.^{1–3}

We evaluated circulating progenitor and endothelial cell numbers by four-color flow cytometry using a published protocol.^{1,2,5} The number of circulating progenitor cells decreased by 50% 3 days after bevacizumab infusion, but then reached baseline levels after 12 days and remained stable at day 33 before surgery (Table 1). Similar to other patients in this trial,² the number of viable circulating endothelial cells (CECs) in this patient were low at baseline, and remained low ($\leq 0.5\%$ of blood mononuclear cells) at all four time points evaluated during treatment (i.e. days 3, 12 and 33 and presurgery). Of interest, Batchelor and colleagues have recently shown that the changes in viable CECs correlated with the changes in tumor contrast-enhanced volume during treatment, and that lower levels of viable CECs during treatment predicted a better response (i.e. prolonged time-to-progression) in patients with recurrent glioblastoma treated with AZD2171 (Recentin®, AstraZeneca PLC, London, UK), a pan-VEGF receptor inhibitor.⁶

To determine the changes in plasma angiogenic protein expression, we used standard enzyme-linked immunosorbent assay and multiplex array techniques.^{2,6} At days 3, 12 and 33 of

bevacizumab treatment the plasma levels of VEGF and placental-derived growth factor increased, and soluble VEGFR2 levels decreased (Table 1). These rapid and sustained changes support the 'pharmacodynamic marker' value suggested by our previous studies for these three biomarkers.^{2,6} Interestingly, all the angiogenic markers analyzed—with the exception of sVEGFR2—had higher than baseline plasma levels before surgery. Plasma CEA dropped substantially from a pretreatment value of 122.8 ng/ml to 63.6 ng/ml 12 days after the first bevacizumab infusion and declined to 4.9 ng/ml 1 month after completion of neoadjuvant therapy before surgery. The prognostic value of all these potential biomarkers is currently being examined in the phase II trial.

TREATMENT AND MANAGEMENT

In 2007, approximately 41,420 patients are anticipated to be diagnosed with rectal cancer in the US.⁷ While some patients have rectal bleeding, a change in bowel habits, or a sense of rectal 'fullness', many patients are asymptomatic at presentation.⁸ Initial DRE and a rigid proctoscopy are essential to the treatment decision-making process. On DRE, fixation of the lesion to the anal sphincter, its relationship to the anorectal ring, and fixation to both the rectal wall, the pelvic wall muscles (levators) and anterior structures (vagina, cervix, or prostate) can be assessed.⁸ Rigid proctoscopy permits accurate measurement of the tumor distance to the anal verge, dentate line and anorectal ring, and can aid clinicians in therapeutic judgment.

Following the initial history, DRE, rigid proctoscopy and additional preoperative staging studies can facilitate selection of the appropriate operative procedure and can help define whether preoperative radiation therapy and chemotherapy should be given. Accurate preoperative staging is gaining importance as combined-modality therapy and sphincter-preserving surgical approaches are considered. Abdominal and pelvic CT has been used extensively and is part of the routine workup of patients. CT seems to be much more useful for identifying enlarged pelvic lymph nodes and metastases outside the pelvis than the extent or stage of the primary tumor.^{9–11} Standard CT does not permit the differentiation of the specific layers of the rectal wall and, therefore, its utility in the staging of small primary cancers is limited.^{9,10,12} The sensitivity of CT is 50–80% with a 30–80% specificity (65–75% accurate for tumor staging, and 45–65% accurate in mesorectal lymph-node staging).^{9,10,13} CT can detect distant metastasis including pelvic and para-aortic lymph nodes to a greater degree than perirectal nodal involvement (75–87% versus 45%).^{9,10,13–15} Detection of lymphadenopathy near the rectum should be considered abnormal.

Endorectal ultrasound (ERUS) techniques are helpful in staging locally advanced rectal cancers. ERUS has been reported to be 80–95% accurate in tumor staging and 70–75% accurate in mesorectal lymph-node staging.^{9,10,16,17} ERUS is very good at distinguishing layers of the rectal wall, especially the mucosa, muscularis mucosa, submucosa and muscularis propria.^{9,10,18,19} ERUS is, therefore, particularly helpful in differentiating T1 versus T2 lesions. In one review, the accuracy of ERUS was greatest (95%) in distinguishing whether a tumor was confined to the rectal wall or invaded through it (i.e. T1/2 versus T3 or greater) compared with the detection of other types of tumor invasion.¹⁷ Despite these data, there is considerable interobserver variability and a significant learning curve with ERUS.⁸ This technique can also identify enlarged perirectal lymph nodes but is not effective in detecting lymph nodes beyond the mesorectum.²⁰ ERUS can be useful in determining extension of disease into the anal canal, which is an area that is poorly visualized on CT, but of critical importance for planning sphincter preserving surgical procedures.²¹ In a recent prospective study, high-resolution MRI was accurate at predicting whether the circumferential resection margin was clear or involved by the tumor.²² This imaging technique should further aid clinicians in identifying high-risk patients.

Tumor markers such as CEA are neither sensitive nor specific enough to screen for the presence of colorectal cancer; however, preoperative CEA levels are of prognostic value.²³ Patients with a preoperative serum CEA greater than 5 ng/ml seem to have a worse prognosis, stage for stage, than those with lower CEA levels. In this patient, the immunohistochemical, plasma protein and circulating-cell data revealed interesting patterns at baseline and changes with treatment. Biomarker studies in the phase I trial have been very promising¹ and should be confirmed in larger patient cohorts. Ultimately, understanding the diagnostic or predictive value of these potential biomarkers might allow individualized therapy for patients using these combination regimens.

CONCLUSIONS

In the US, the standard management of a patient with locally advanced distal rectal cancer is preoperative radiation therapy (45–54 Gy) with 5-FU-based chemotherapy followed by surgery. The response of this patient's tumor to this regimen plus bevacizumab was dramatic with no residual cancer in the surgical specimen. A phase II trial evaluating the feasibility, tolerance and efficacy of bevacizumab with the standard template of radiation therapy and 5-FU is ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Combination treatment schema for patients with rectal cancer enrolled in a clinical trial. This phase I–II trial design allowed for a 2-week cycle of bevacizumab monotherapy. Subsequently, patients underwent three further cycles of bevacizumab and chemoradiotherapy. At 7–9 weeks after treatment cessation, patients underwent surgery. Both before treatment, and 12 days after the first infusion of bevacizumab, the patient underwent flexible sigmoidoscopy with tumor biopsy (for immunohistochemical studies), CT scan, PET scan, and analysis of blood for a number of angiogenesis and tumor markers: CECs, CPCs, plasma angiogenic proteins and plasma carcinoembryonic antigen. The blood analyses were also performed at days 3 and 33 (i.e., day 5 in the second cycle of the combined treatment) and 1 week before surgery. CT and PET scans were repeated 1 week before surgery. Abbreviations: CECs, circulating endothelial cells; CPCs, circulating progenitor cells; 5-FU, 5-fluorouracil.

Figure 2.

Evaluation of tumor response using imaging. PET–CT imaging of the tumor before treatment, 12 days after first bevacizumab infusion, and after completion of the combined treatment. Note the presence of a large, highly metabolically active tumor at baseline and day 12, and the significant response after treatment. The panels on the right show endoscopy imaging at corresponding time points, revealing a large ulcer at the site of the tumor in the surgical specimen.

Figure 3. Microscopic evaluation of tumor response. Low magnification (objective, 20×) image showing the ulcer at the tumor bed site, including the edge of the adjacent colonic mucosa. In the insert, a higher magnification (objective, 100×) image deep in the wall of the fibrosis shows a high number of lymphocytes and fibroblasts scattered throughout the scar tissue.

Table 1
Changes in the patient's blood angiogenic markers and CEA levels^a

Blood marker	Baseline	Day 3	Day 12	Day 33	Presurgery day 96
PlGF (pg/ml)	17.3	30.5	24.4	31.6	22.4
sVEGFR2 (pg/ml)	734	237	37	672	451
VEGF (pg/ml)	168	1,343	943	1,519	305
bFGF (pg/ml)	7.58	4.36	4.41	22.3	43.6
sVEGFR1 (pg/ml)	76.6	63.5	707	40.8	1,476
CEA (ng/ml)	122.8	N/A	63.6	N/A	4.9
Viable CECs (%) ^b	0.24	0.39	0.50	0.41	0.42
CPCs (%) ^b	0.10	0.05	0.09	0.12	0.09

^a All samples were assessed in duplicate.

^b Values were calculated as percentages of blood mononuclear cells. Abbreviations: bFGF, basic fibroblast growth factor; CEA, carcinoembryonic antigen; CECs, circulating endothelial cells; CPCs, circulating progenitor cells; PlGF, placental-derived growth factor; sVEGFR1, soluble vascular endothelial growth factor receptor 1; sVEGFR2, soluble vascular endothelial growth factor receptor 2.