VOLUME 27 · NUMBER 18 · JUNE 20 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Efficacy, Safety, and Biomarkers of Neoadjuvant Bevacizumab, Radiation Therapy, and Fluorouracil in Rectal Cancer: A Multidisciplinary Phase II Study

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Submitted November 21, 2008; accepted January 14, 2009; published online ahead of print at www.jco.org on May 26, 2009.

Supported by the Grants No. R21CA099237 (C.G.W.), P01CA80124, and R01CA115767 from the National Institutes of Health, and a grant from the National Foundation for Cancer Research (R.K.J.).

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2718-3020/$20.00

DOI: 10.1200/JCO.2008.21.1771

ABSTRACT

Purpose
To assess the safety and efficacy of neoadjuvant bevacizumab with standard chemoradiotherapy in locally advanced rectal cancer and explore biomarkers for response.

Patients and Methods
In a phase I/II study, 32 patients received four cycles of therapy consisting of: bevacizumab infusion (5 or 10 mg/kg) on day 1 of each cycle; fluorouracil infusion (225 mg/m^2/24 hours) during cycles 2 to 4; external-beam irradiation (50.4 Gy in 28 fractions over 5.5 weeks); and surgery 7 to 10 weeks after completion of all therapies. We measured molecular, cellular, and physiologic biomarkers before treatment, during bevacizumab monotherapy, and during and after combination therapy.

Results
Tumors regressed from a mass with mean size of 5 cm (range, 3 to 12 cm) to an ulcer/scar with mean size of 2.4 cm (range, 0.7 to 6.0 cm) in all 32 patients. Histologic examination revealed either no cancer or varying numbers of scattered cancer cells in a bed of fibrosis at the primary site. This treatment resulted in an actuarial 5-year local control and overall survival of 100%. Actuarial 5-year disease-free survival was 75% and five patients developed metastases postsurgery. Bevacizumab with chemoradiotherapy showed acceptable toxicity. Bevacizumab decreased tumor interstitial fluid pressure and blood flow. Baseline plasma soluble vascular endothelial growth factor receptor 1 (sVEGFR1), plasma vascular endothelial growth factor (VEGF), placental-derived growth factor (PlGF), and interleukin 6 (IL-6) during treatment, and circulating endothelial cells (CECs) after treatment showed significant correlations with outcome.

Conclusion
Bevacizumab with chemoradiotherapy appears safe and active and yields promising survival results in locally advanced rectal cancer. Plasma VEGF, PlGF, sVEGFR1, and IL-6 and CECs should be further evaluated as candidate biomarkers of response for this regimen.

J Clin Oncol 27:3020-3026. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Antibody blockade of vascular endothelial growth factor (VEGF) with bevacizumab (Avastin; Genentech, South San Francisco, CA) with chemotherapy has been demonstrated efficacy in patients with metastatic colorectal cancer.[1] However, the effect of anti-VEGF therapy in patients with localized disease is not known. Moreover, there are no validated biomarkers to predict the response to anti-VEGF treatment with bevacizumab—or any other anti-VEGF agent—in cancer patients.

To this end, we initiated a National Cancer Institute (NCI) phase I/II trial that integrated bevacizumab into a contemporary treatment program of preoperative radiation therapy and chemotherapy followed by surgery for primary/nonmetastatic rectal cancer patients. Phase I study results have established a feasible dose of bevacizumab combined with radiation therapy and fluorouracil (FU).[2] Correlative studies have demonstrated antivascular and vascular normalizing effect of VEGF blockade on these tumors.[2,3] In addition, they showed that bevacizumab alone increases plasma VEGF and placental-derived growth factor (PlGF), and decreases circulating endothelial cells (CECs) and circulating progenitor cells (CPCs).[2] However, little data exists on the impact of such a therapeutic

approach on clinical outcomes of patients with localized disease and the role of biomarkers in assessing response and outcome of combination therapy. This report describes the complete clinical results of the phase II trial. In addition, we explored potential biomarkers of response by measuring before and after treatment a series of imaging, physiologic, angiogenic, and inflammatory biomarkers that have been previously found to change in response to anti-VEGF therapies in the phase I study and in other translational trials.[2,4,5]

PATIENTS AND METHODS

Patients

This phase I/II trial received approval from the Cancer Therapeutics Evaluation Program of the NCI as well as the internal review boards of participating institutions. Eligibility criteria included: histologically documented adenocarcinoma of the rectum; endorectal ultrasound or surface coil magnetic resonance imaging–staged T3/T4 tumors; no evidence of metastatic systemic disease; age older than 18 years; Karnofsky performance status higher than 70%; and normal hepatic, renal, and bone marrow function. Informed written consent was obtained from all patients. There were 10 female and 22 male patients. Median age was 51 years (range, 35 to 72 years). The targeted accrual was reached from 2002 to 2008. One patient was excluded from analysis due to change in pathological diagnosis with review of the surgical specimen.

Study Treatment

Patients received four cycles of therapy: bevacizumab infusion (5 or 10 mg/kg) on day 1 of each cycle; FU infusion (225 mg/m^2/24 hours) during cycles 2 to 4; external-beam radiation therapy to the pelvis (50.4 Gy in 28 fractions over 5.5 weeks); and surgery 7 to 10 weeks after completion of all neoadjuvant therapy (Fig 1A).

In the phase I study, six patients received 5 mg/kg of bevacizumab without dose-limiting toxicity (DLT). At the 10 mg/kg dose of bevacizumab, two of five patients developed DLTs and the recommended phase II dose level was 5 mg/kg.[2] Subsequently, 21 patients received 5 mg/kg bevacizumab in the phase II study.

Seven to 10 weeks after completion of bevacizumab, radiation therapy, and chemotherapy, all 32 patients underwent surgery (one abdominoperineal resection with posterior exenteration and vaginal resection; seven abdominoperineal resections; and 24 low anterior resection usually with temporary diverting ileostomy and often with J-pouch reconstructions). No patient developed progressive disease or metastases on restaging or at surgery.

After recovery from surgery, patients usually received adjuvant chemotherapy with regimen selection at the discretion of the treating medical oncologist. Thirteen patients received FU, leucovorin, and oxaliplatin, four patients received capecitabine and oxaliplatin, 10 patients received FU and leucovorin, and three patients received capecitabine. No patient received adjuvant bevacizumab. No further radiation therapy was administered postoperatively. Patients were scored for local failure, distant metastases, and survival. Data are reported with a median follow-up of 31 months (range, 4 to 64 months).

Imaging Studies

Functional imaging—dynamic computed tomography (CT) scans and 18-fluorodeoxyglucose positron emission tomography (PET) scans—was conducted before and 12 days after bevacizumab infusion, as well as after completion of all therapies (1 week before surgery). These imaging techniques assess tumor metabolism (PET) and perfusion (dynamic CT). Functional CT was performed on a 16-slice multidetector row CT scanner (Lightspeed 16, GE Healthcare, Piscataway, NJ). After administration of 300 to 500 mL water per rectum, a 2-cm region of tumor was selected on noncontrast CT of pelvis. Subsequently, dynamic CT of this region was performed for 45 seconds at the same table position, immediately after initiation of intravenous infusion of 1 mL/kg of iodinated nonionic contrast media (Isovue 300, Bracco Diagnostics, Princeton, NJ) at the rate of 7 mL/s. Delayed images of the tumor were obtained every 20 seconds for 6 minutes after the initiation of contrast. Regional PET scanning of the pelvis was performed on a GE Discovery STE PET/CT scanner.

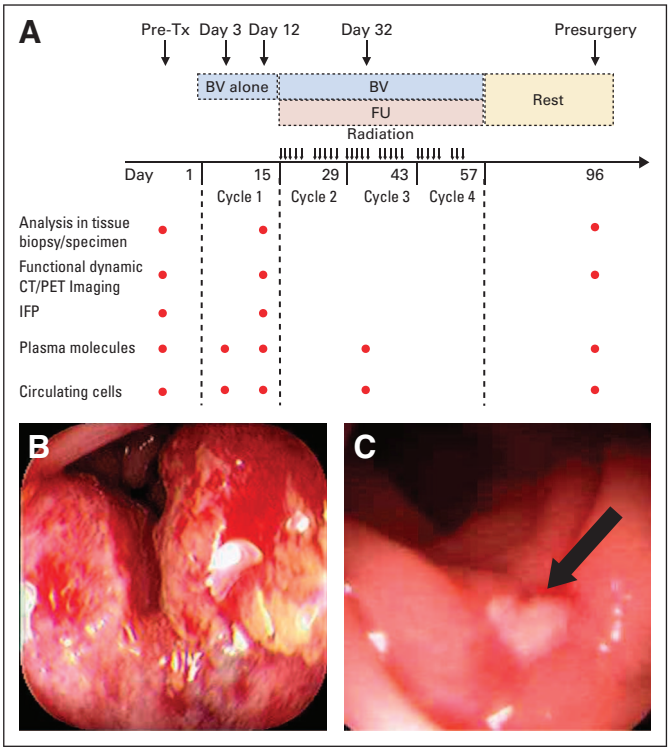

Fig 1. Study design and macroscopic tumor response. (A) Red dots indicate time points of data collection. (B, C) Representative sigmoidoscopy images of a (B) locally advanced rectal tumor before therapy and presurgery, (see arrow in C) after completion of all neoadjuvant therapies. Pre-Tx, pretreatment; BV, bevacizumab; FU, fluorouracil; CT, computed tomography; PET, positron emission tomography; IFP, interstitial fluid pressure.

Data were analyzed on a workstation (Advantage Windows, GE) using commercially available CT perfusion 3.0 software, which implements a deconvolution approach to calculate regional blood flow, blood volume, and permeability-surface area (PS) product. The external iliac artery and vein served as arterial and venous input respectively. Multiple, non-overlapping regions of interest (ROI) were drawn over the tumor for each of four slices and blood perfusion values were obtained. A mean of the values from the individual ROIs was used to calculate mean blood perfusion. In previous studies, similar values of blood perfusion were obtained with functional CT and radioactive microspheres. PET data were analyzed on a GE Xeleris workstation. Circular two-dimensional ROIs were drawn in each axial slice through the tumor to determine the maximum standardized uptake value. We obtained reliable measurements from 24 patients.

Sigmoidoscopy and Interstitial Fluid Pressure Measurements

Before, and then 12 days after the first bevacizumab infusion, flexible sigmoidoscopies were performed on all 32 patients, which permitted tumor visualization and assessment of gross response, and measurement of tumor interstitial fluid pressure (IFP). To measure IFP a 23-gauge needle, with a 2- to 3-mm side-hole at 4 to 5 mm from the tip, was connected to PE-90 tubing that was inserted through a trochar sleeve and the working channel of the endoscope. The IFP was measured in two to five tumor locations. Stable pressure measurements with a good fluid communication between the tumor interstitial space and needle were considered valid. Reliable IFP measurements were obtained for 13 patients pre- and post-treatment.

Measurement of Plasma Biomarkers

Peripheral blood was obtained with informed consent from all patients at baseline (pretreatment), 3 and 12 days after a dose of bevacizumab alone, 32 days after initiation of treatment (during bevacizumab with chemoradiotherapy) and 1 week before surgery (8 to 9 weeks after completion of preoperative

therapy). The number of samples varied because of patient withdrawal from study due to toxicity (n = 3) or to factors unrelated to treatment. Additional samples were obtained with informed consent from four patients who underwent standard chemoradiotherapy therapy at four time points: pretreatment, 3 and 12 days after initiation of treatment, and presurgery. Blood was collected in an EDTA-containing vacutainer, spun down, and plasma was aliquoted and frozen immediately. Plasma analysis was carried out for circulating soluble vascular endothelial growth factor receptor (sVEGFR), plasma vascular endothelial growth factor (VEGF), placental-derived growth factor (PlGF), basic fibroblast growth factor (bFGF), granulocyte-macrophage colony-stimulating factor, interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor-α using multiplex array plates from Meso-Scale Discovery (Gaithersburg, MD) and for sVEGFR2, and stromal-derived growth factor 1 alpha (SDF1α) enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Emeryville, CA). CEA ELISA kits were purchased from Calbiotech, Inc (Spring Valley, CA). All samples were run in duplicate.

Circulating Cell Biomarkers

Blood circulating cells were phenotyped and enumerated by flow cytometric analyses of CD31, CD34, CD45, and CD133 expression using fluorescence-labeled monoclonal antibodies and a standard protocol in fresh samples.[6] The number of samples analyzed varied because of patient withdrawal from study due to toxicity (n = 2) or to factors unrelated to treatment. Percent values were obtained before initiation of the therapy, and then at 3, 12, 32, and 96 days after the first infusion of bevacizumab.

Data and Statistical Analyses

We report median values with interquartile ranges. Comparisons versus pretreatment values were performed for all variables using Wilcoxon exact test for paired data. Comparisons of variables for different subgroups were performed using the Wilcoxon exact two-sample test. Correlations were quantified using Spearman's rho coefficients. In these exploratory, hypothesis-generating studies the concern was to avoid both false positive and false negative results for association with outcome. Since the parameters measured by us were not random but rather mechanism-based biomarkers, we did not adjust for multiple statistical tests.

RESULTS

Patient Characteristics

All 32 patients presented with clinically staged locally advanced rectal cancer: 28 with stage T3 (87.5%), four with stage T4 (12.5%), and lymph node involvement was detected in 23 patients (71.8%; Table 1). Thirty of 32 patients received adjuvant chemotherapy after recovery from surgery.

Table 1. Patients Characteristics

Characteristic	No.	%
No. of patients enrolled	32	
Sex		
Male	22	69
Female	10	31
Median age, years	51	
Range	35 to 72	
Clinical tumor category		
T_3	28	87
T_4	4	12
Clinical nodal category		
N_0	9	28
N_{1-2}	23	71
Mean tumor size, cm	5	
Range	3 to 12	

Efficacy

Sigmoidoscopy at day 12 postbevacizumab alone showed no significant regression of the rectal cancers, despite a dramatic change from a hyperemic and hemorrhagic appearance pretreatment to a pale appearance of the tumors. Nevertheless, after combination therapy, the surgical specimens showed tumor regression from a mass with a mean size of 5 cm (range, 3 to 12 cm) to an ulcer and/or scar with mean size of 2.4 cm (range, 0.7 to 6.0 cm) in all 32 patients (Figs 1B, 1C). Histologic examination of the residual ulcer revealed either no cancer at the primary site, ypT0 or Mandard grade[7] 1 (five of 32; 16%), or varying numbers of cancer cells in a bed of fibrosis, ypT1-3 or Mandard grades 2 and 3 (23 of 32; 72%). All patients underwent an R0 resection with the exception of two patients who had evidence of microscopic tumor at the radial margin.

A decrease in the T stage (T downstaging was considered a post-treatment stage of ypT0-yT2 from initial T3/4) was seen in 50% of patients. Of the 23 patients with imaging detectable lymph node disease at presentation, 13 patients (56.5%) had N downstaging (no lymph node disease) post-treatment. Nineteen (59.3%) of 32 patients had no histologic evidence of lymph node metastasis, and the rest had

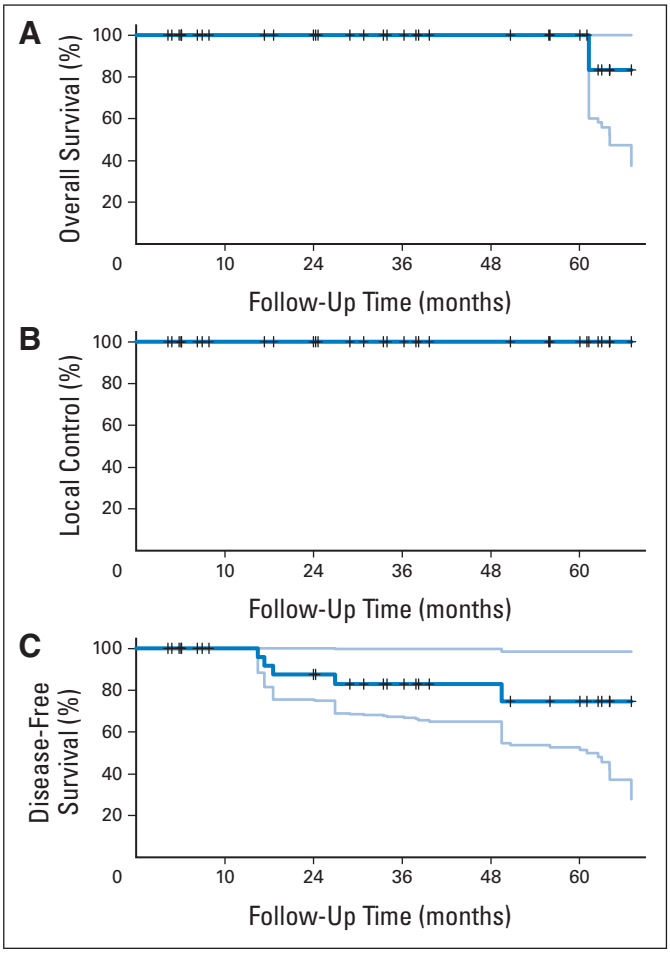

Fig 2. Phase II trial treatment outcome: Kaplan-Meier local control and survival distributions. (A) Overall survival, (B) local control, and (C) disease-free survival in 32 advanced rectal cancer patients receiving bevacizumab with chemoradiotherapy.

Table 2. Adverse Events in 32 Rectal Cancer Patients After Bevacizumab With Chemoradiation (including 5 patients treated with bevacizumab at 10 mg/kg)

Adverse Event	Grade 1	Grade 2	Grade 3
GI			
Anorexia	6	1	1
Constipation	8	1	1
Dehydration	—	2	1
Diarrhea	12	5	7
Mucositis	7	8	1
Perirectal abscess	—	—	1
Proctalgia/proctitis	1	5	—
Colitis	—	—	1
GU			
Frequency/urgency	13	5	—
Hesitancy	2	—	—
Hypertension	7	1	3
Hand foot	3	1	1
Infection	1	8	—
Skin, radiation dermatitis	13	5	2
Neurologic	4	0	1
Wound, separation	—	—	1

NOTE. There were no grade 4 events.
Abbreviation: GU, genitourinary.

microscopic nodal metastases. This combination treatment resulted in an actuarial 5-year local control and overall survival rates of 100% (Figs 2A, 2B). One patient died of metastases after 61 months with nine patients having follow-up of more than 5 years. Actuarial 5-year disease-free survival was 75% (Fig 2C). Five patients (15.6%) have developed metastatic disease: one in the liver, three in the lung, and one in both the liver and the lung.

Safety

Most of the adverse events of this regimen were mild (grade 1/2), but some of the patients experienced grade 3 toxicities (Table 2). There were no grade 4 or 5 toxicities. Postoperative complications (within 90 days of surgery) included: anastostomotic leak with presacral abscess requiring drainage (one patient), vaginal tear with presacral hematoma and abscess requiring drainage (one patient), pelvic hematoma (one patient), delayed healing of perineal incision (two patients), ileus (two patients), neurogenic bladder (one patient), perforated ileostomy–stent related (one patient), pulmonary embolus (one patient), and wound infection (three patients). It is unclear if bevacizumab treatment contributed to any of these complications, and observation of these patients will establish long-term morbidity of this combined modality approach of neoadjuvant therapy, surgery, and adjuvant chemotherapy.

Imaging Analyses and IFP Measurements Mirror Phase I Data

Bevacizumab alone significantly decreased tumor IFP at 12 days after treatment, consistent with the phase I data (Appendix Table A1, online only).[2,3] Functional imaging parameters revealed significant but differential vascular and tumor responses after bevacizumab alone or after completion of bevacizumab, radiation therapy, and chemotherapy (presurgery). Blood flow and permeability-surface area product measured by dynamic CT significantly decreased at day 12 compared with pretreatment ($P < .05$; Appendix Table 1). Moreover, all vascular parameters measured—blood flow, blood volume, median transit time, and permeability-surface area product—were significantly decreased presurgery compared with pretreatment ($P < .01$; Appendix Table A1). This indicates that VEGF blockade alone or with chemoradiotherapy decreases vascular permeability and induces pruning of the rectal cancer vasculature. In contrast, the 18FDG uptake (a measure of tumor metabolic rate) was not changed by bevacizumab alone but significantly decreased by combination therapy compared to pretreatment ($P < .0001$; Appendix Table A1). The changes in these imaging parameters suggest an improvement in tumor vessel function after bevacizumab monotherapy. Neither the baseline measurements, nor the changes in IFP or CT- and PET-derived parameters correlated with the extent of regression at surgery.

Circulating Biomarker Analyses Confirm Phase I Data and Identify Potential Biomarkers of Response

Consistent with phase I results, bevacizumab monotherapy significantly increased plasma PlGF and free VEGF ($P < .05$, Table 3 and Appendix and Appendix Fig A1, online only). These changes appear to be specific to anti-VEGF therapy, as the cytotoxic treatment alone did

Table 3. Circulating Markers That Change Significantly After Bevacizumab Alone, and After Combination Therapy Compared to Pretreatment Values

	Pretreatment					Bevacizumab Monotherapy: Day 3					Bevacizumab Monotherapy: Day 12				
Marker (pg/mL)	Median	IQR	No. of Patients	P*	P†	Median	IQR	No. of Patients	P*	P†	Median	IQR	No. of Patients	P*	P†
Plasma PlGF	19	12-23	31	NA	NA	31	23-38	31	< .0001	< .0001	34	24-41	29	< .0001	< .0001
Plasma VEGF	98	38-165	31	NA	NA	918	354-1,085	31	< .0001	< .0001	943	417-1,143	29	< .0001	< .0001
Plasma sVEGFR1	127	79-179	31	NA	NA	78	61-169	31	.0028	.010	135	58-190	29	.51	.99
Plasma sVEGFR2	15,457	11,464-17,968	22	NA	NA	16,831	12,844-19,671	24	.0005	.0019	16,962	12,686-20,766	26	.12	.23
Plasma IL-6	1.48	0.87-1.91	26	NA	NA	1.47	1.09-2.12	27	.21	.27	2.08	1.19-2.73	26	.031	.059
Plasma IL-8	3.58	2.42-4.73	25	NA	NA	3.43	2.78-4.15	27	.82	.81	3.13	2.21-3.81	26	.34	.43
Serum CEA	4.06	1.67-9.02	25	NA	NA	4.43	1.90-8.86	25	.57	.72	3.10	1.55-10.28	25	.11	.22

(continued on following page)

Table 4. Potential Biomarkers of Tumor Response After Combination Therapy: Evaluated Before Treatment, After Bevacizumab Alone (days 3 and 12), or During Combination With Chemoradiotherapy (day 32)

Analysis	Pretreatment					Bevacizumab Monotherapy (days 3 and 12)					Combination Therapy (day 32)					Post-Treatment (presurgery)				
	No. of Patients	Median	IQR	ρ	P	No. of Patients	Median	IQR	ρ	P	No. of Patients	Median	IQR	ρ	P	No. of Patients	Median	IQR	ρ	P
ypT stage												None						None		
ΔPlGF						30			−.381	.037										
sVEGFR1	31			.415	.020															
ypT_0 *v* $ypT_{1\text{-}3}$												None								
CECs																				.016
ypT_0																16	0.32	0.26-0.37		
$ypT_{1\text{-}3}$																3	1.16	0.65-1.48		
sVEGFR1					.0074															
ypT_0	5	53	52-77																	
$ypT_{1\text{-}3}$	26	145	85-187																	
ΔPlGF										.036										
ypT_0						5	2.5	2.2-2.6												
$ypT_{1\text{-}3}$						25	1.8	1.4-2.2												
Post-treatment N stage			None					None										None		
VEGF											30			−.446	.014					
ΔIL-6											24			.411	.046					

NOTE. Showed significant associations with ypT, or post-treatment N stage (by calculating Spearman's correlation coefficients). For ypT0 *v* ypT1-3 comparison, we used the Wilcoxon test.

Abbreviations: IQR, interquartile range; PlGF, placental-derived growth factor; sVEGFR1, soluble vascular endothelial growth factor receptor; CEC, circulating endothelial cell; ypT, post-treatment T stage; ypT_0, complete response; VEGF, vascular endothelial factor; IL, interleukin.

not seem to change VEGF or PlGF when evaluated in patients receiving standard chemoradiotherapy (Appendix Table A2, online only). Moreover, bevacizumab transiently increased (at day 3 only) sVEGFR2 and decreased sVEGFR1 in plasma ($P < .05$, Table 3). Finally, bevacizumab increased plasma levels of IL-6 only at day 12, but did not change plasma SDF1α, bFGF, IL-1β, IL-8, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor α, serum CEA (a widely used but not yet validated biomarker of disease burden), or CECs and CPCs (Appendix Table A3 online only).

Combined treatment significantly increased VEGF and PlGF and decreased IL-8 at day 32 and presurgery, and increased IL-6 only at day 32, but did not change IL-1β, granulocyte-macrophage colony-stimulating factor, and TNFα (Table 3). Of note, circulating CEA was significantly decreased at presurgery ($P < .05$; Table 3).

The pretreatment level of circulating cytokines showed no association with the degree of tumor regression after combination therapy. This is in line with published reports that failed to detect a predictive marker for bevacizumab with cytotoxic therapy.[8,9] Nevertheless, pretreatment sVEGFR1 significantly correlated with ypT stage and was higher in patients with no T downstaging after combination therapy ($P < .01$; Table 4). Moreover, the patients who experienced greater ($>$ two fold) increases in plasma PlGF after bevacizumab alone ($PlGF_{\max(\text{days pretx, 3, 12})}$ relative to pretreatment) showed minimal or no residual disease at surgery; $P < .05$; Table 4). None of the other plasma biomarkers measured after bevacizumab monotherapy was associated with tumor regression or the presence of lymph node metastases at surgery. Of the parameters measured at day 32, higher plasma VEGF and lesser increases in IL-6 from baseline were seen in patients with

Table 3. Circulating Markers That Change Significantly After Bevacizumab Alone, and After Combination Therapy Compared to Pretreatment Values (continued)

Marker (pg/mL)	Combination Therapy (day 32)					After Combination Therapy (presurgery)				
	Median	IQR	No. of Patients	P*	P†	Median	IQR	No. of Patients	P*	P†
Plasma PlGF	51	39-63	30	$< .0001$	$< .0001$	33	26-41	22	$< .0001$	$< .0001$
Plasma VEGF	1,660	1,330-1,983	30	$< .0001$	$< .0001$	339	205-709	22	$< .0001$	$< .0001$
Plasma sVEGFR1	156	69-199	30	.81	1.0	134	77-198	22	.93	1.0
Plasma sVEGFR2	16,977	13,289-19,820	25	.28	.36	19,229	13,346-22,565	18	.95	1.0
Plasma IL-6	2.26	1.66-2.88	29	.0014	.0055	1.56	1.05-2.32	24	.78	.85
Plasma IL-8	2.19	1.71-3.06	29	.0013	.0050	2.44	1.82-3.20	24	.020	.044
Serum CEA	3.19	1.34-8.74	23	.96	.97	0.44	0.00-1.34	22	.0001	.0003

Abbreviations: IQR, interquartile range; PlGF, placental-derived growth factor; NA, not applicable; VEGF, vascular endothelial growth factor; sVEGFR, soluble vascular endothelial growth factor receptor; IL, interleukin; CEA, carcinoembryonic antigen.

*P values are from the paired exact Wilcoxon test, unadjusted.

†P values are from the paired exact Wilcoxon test, adjusted to control the false discovery rate over time, with weights proportional to the square root of number of the measurements.

minor or no lymph node disease after combination therapy ($P < .05$; Table 4). Presurgery, only the number of CECs was significantly correlated with pathologic complete response ($P < .05$; Table 4).

DISCUSSION

After completion of bevacizumab, radiation therapy, and chemotherapy, the advanced rectal cancers regressed to an ulcer/scar. Histologic examination confirmed this marked response. These data are complementary to post-treatment pathologic staging as it scores the response of the entire tumor independent of histologic findings within or extending out of the rectal wall. The 5-year actuarial local control, disease-free survival, and overall survival were 100%, 75%, and 100%. Although patient numbers are small in this study, these results compare favorably to the overall results of 415 patients randomly assigned to preoperative chemoradiotherapy in the German Rectal Cancer Study Group.[10] Examination of early and postoperative adverse effects of the combined treatment suggested a good safety profile for the addition of bevacizumab to chemotherapy and radiation therapy. A similar safety profile has been reported in another phase II study of neoadjuvant bevacizumab, capecitabine, and radiation.[11]

Consistent with phase I data,[2,3] functional analyses confirmed that bevacizumab monotherapy has significant antivascular and vascular normalizing effects in rectal cancer. In addition, functional imaging parameters revealed significant vascular and tumor responses after completion of bevacizumab, radiation therapy, and chemotherapy (presurgery). Blood flow and PS product measured by dynamic CT significantly decreased at day 12 and presurgery compared to pretreatment. This indicates that VEGF blockade alone and with chemoradiotherapy decreases vascular permeability and induces pruning of the rectal cancer vasculature. In contrast, the 18FDG uptake (a measure of tumor metabolic rate) was not changed by bevacizumab alone but significantly decreased by combination therapy. The changes in these imaging parameters suggest an improvement in tumor vessel function after bevacizumab monotherapy.[12,13] Indeed, with addition of chemoradiotherapy, the therapy appears to have significant activity in rectal cancer. However, since the drop in vascular permeability after bevacizumab and other anti-VEGF agents occurs within 1 day,[5] upfront combination of these agents with cytotoxics might be optimal.

Bevacizumab—both alone and with chemoradiotherapy—increases circulating PlGF and VEGF. The increases in these key mediators of angiogenesis are similar to those seen in the phase I component of this study,[2] and have been reported for VEGF receptor tyrosine kinase inhibitors (TKIs; eg, cediranib or sunitinib) in cancer patients or in preclinical models.[5,14-18] But in contrast to the VEGF receptor TKIs—all of which significantly decrease sVEGFR2 levels[5,14-18]—bevacizumab induced a mild but significant increase in plasma sVEGFR2 at day 3, and these changes were not seen after combination therapy. These results suggest that the kinetics in plasma VEGF or PlGF may serve as generic pharmacodynamic biomarkers for anti-VEGF therapy. The discrepancies in the kinetics of soluble VEGFR2 (an abundant plasma protein of unknown function) suggest potentially differential antitumor and systemic mechanism of action of bevacizumab compared to anti-VEGF receptor TKIs. Finally, combination of bevacizumab and chemoradiotherapy modulated inflammatory biomarkers such as IL-6 and IL-8.

When we correlated the changes in markers with treatment outcome, we found that pretreatment sVEGFR1 inversely correlated with the extent of regression. Moreover, that early kinetics of PlGF after bevacizumab, and VEGF and IL-6 levels during combined treatment may predict both better pharmacodynamics of the drug and an enhanced effect of the combined treatment on the primary tumor or nodal disease, respectively. Finally, an elevated number of CECs was seen in patients with residual disease. The association between elevated plasma IL-6 and CECs and poorer outcome is consistent with our findings in advanced hepatocellular carcinoma patients treated with sunitinib and glioblastoma patients treated with cediranib, respectively.[4,5]

The results of this single-arm study in 32 patients are encouraging and support further evaluation of this treatment strategy. It will be also important to continue observation of these patients to elucidate long-term outcome and morbidity of this combined modality approach of neoadjuvant therapy, surgery, and adjuvant chemotherapy. The local control and survival data suggest that normalization of the tumor vasculature by bevacizumab may have important and positive clinical consequences.[12,13] Finally, the potential biomarker candidates emerging from this study should be further evaluated in larger studies to validate them, with the goal of optimizing the outcome of combination of bevacizumab with FU/radiation or other cytotoxic regimens.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Dan G. Duda, Takeda (C); Rakesh K. Jain, AstraZeneca (C), Millennium Pharmaceuticals (C), Dyax (C), SynDevRx (U) **Stock Ownership:** None **Honoraria:** Christopher G. Willett, Genentech (C); Rakesh K. Jain, Pfizer (C), Roche (C) **Research Funding:** Brian G. Czito, AstraZeneca, Roche; Rakesh K. Jain, AstraZeneca, Dyax **Expert Testimony:** None **Other Remuneration:** None

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