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Phase II Clinical and Pharmacokinetic Study of Aflibercept in Patients with Previously Treated Metastatic Colorectal Cancer

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Abstract

Purpose: Aflibercept is a recombinant fusion protein of the VEGF receptor (VEGFR) 1 and VEGFR2 extracellular domains. We assessed the safety and efficacy of aflibercept in patients with metastatic colorectal cancer (MCRC) who had received at least one prior palliative regimen.

Experimental Design: Seventy-five patients were enrolled onto this two-stage phase II trial in two cohorts, bevacizumab naïve ($n = 24$) and prior bevacizumab ($n = 51$). Aflibercept was administered at 4 mg/kg i.v. in two-week cycles. The primary endpoint was a combination of objective response rate and 16-week progression-free survival (PFS).

Results: In the bevacizumab-naïve cohort ($n = 24$), the best response was stable disease for 16 weeks or more in five of 24 patients. In the prior bevacizumab cohort ($n = 50$), one patient achieved a partial response and six patients had stable disease for 16 weeks or more. The median PFS in the bevacizumab-naïve and prior bevacizumab cohorts was two months [95% confidence interval (CI): 1.7–8.6 months] and 2.4 months (95% CI: 1.9–3.7 months), respectively. Median overall survival (OS) was 10.4 months (95% CI: 7.6–15.5) and 8.5 months (95% CI: 6.2–10.6), respectively. The most common grade 3 or higher treatment-related adverse events were hypertension, proteinuria, fatigue, and headache. Ten patients discontinued study treatment due to toxicity. Mean free to VEGF-bound aflibercept ratio was 1.82, suggesting that free aflibercept was present in sufficient amount to bind endogenous VEGF.

Conclusion: Aflibercept showed limited single-agent activity in patients with pretreated MCRC with moderate toxicity. Further study of aflibercept with chemotherapy is ongoing. *Clin Cancer Res; 18(21); 6023–31. ©2012 AACR.*

Introduction

VEGF is a critical regulator of angiogenesis, which is crucial for tumor growth and metastasis (1). Inhibition of VEGF with antiangiogenic drugs is thought to improve delivery of chemotherapy via vascular normalization and disruption of tumor vasculature with additional systemic effects (2, 3). Bevacizumab, a monoclonal antibody that binds to VEGF, significantly improves outcomes when added to standard chemotherapy for metastatic colorectal cancer (MCRC; refs. 4–8). Acquired resistance to bevacizumab may develop through signaling via alternate compensatory pathways, vascular remodeling, or selection for hypoxia-resistant tumor cells (9).

Increases in circulating placental growth factor (PlGF) levels have been observed in patients with colorectal cancer treated with bevacizumab (10, 11). Blockade of PlGF inhibited tumor growth in a human colon cancer xenograft model (12). Aflibercept (ziv-aflibercept, VEGF Trap; Regeneron Pharmaceuticals and Sanofi-aventis Oncology) is a recombinant humanized fusion protein of the extracellular domains of VEGF receptor (VEGFR) 1 and VEGFR2 with the constant region (Fc) of human immunoglobin (Ig)G1 that binds to VEGF-A, VEGF-B, PlGF1, and PlGF2 and prevents downstream biologic effects (13). Aflibercept has a higher VEGF-A binding affinity than bevacizumab [dissociation constant (K_d) of ~1 pmol/L (13) compared with ~500 pmol/L for bevacizumab (14)]. Preclinically, treatment with aflibercept resulted in tumor growth inhibition in a variety of xenograft models, including human colon cancer, suggesting that the biologic effects of aflibercept correlated with free aflibercept levels in excess of aflibercept–VEGF complexes (13, 15–17). A phase I study of aflibercept in

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Translational Relevance

Aflibercept is a recombinant fusion protein of the VEGF receptor (VEGFR) 1 and VEGFR2 extracellular domains that binds to VEGF-A, VEGF-B, placental growth factor (PlGF) 1 and 2. We assessed the safety and efficacy of aflibercept in patients with metastatic colorectal cancer (MCRC) who had received at least one prior palliative regimen in a two-stage phase II trial. In the bevacizumab-naïve cohort ($n = 24$), the best response was stable disease for 16 weeks or more in five of 24 patients. In the prior bevacizumab cohort ($n = 50$), one patient achieved a partial response, and six patients had stable disease for 16 weeks or more. Mean free to VEGF-bound aflibercept ratio was 1.82, suggesting that free aflibercept was present in sufficient amount to bind endogenous VEGF. This marker can be explored in future aflibercept trials. Aflibercept showed limited single-agent activity in patients with pretreated MCRC with moderate toxicity.

patients with solid tumors showed promising antitumor activity and an acceptable safety profile with common drug-related toxicities of dysphonia and hypertension (18). Hypertension is a mechanism-related adverse event associated with antiangiogenic therapies. Improvement in clinical outcomes has been associated with the development of hypertension in some studies (19–24).

Despite advances in systemic therapy for MCRC, the majority of patients will succumb to this deadly disease, and thus novel therapies are needed. In light of the tolerability of aflibercept and activity of antiangiogenic drugs in MCRC, we sought to determine the clinical activity, as determined by a combined endpoint of response rate and 16-week progression-free survival (PFS), in 2 cohorts of patients with MCRC—1 with prior bevacizumab exposure and 1 without prior bevacizumab treatment. Hypertension and aflibercept pharmacokinetics were explored as possible predictive markers of benefit.

Patients and Methods

Patients

The study population included patients with incurable histologically or cytologically confirmed colorectal adenocarcinoma. Eligible patients had progressed on at least 1 prior line of systemic therapy for metastatic disease, which could have included chemotherapy and/or EGFR inhibitors. Other inclusion criteria included the following: age 18 years or older; Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; measurable disease defined by Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.0 (25); no prior anticancer therapy for at least 4 weeks before study entry; and adequate hematologic, hepatic, and renal function. Patients were excluded if they had uncontrolled hypertension within the past 3 months, as well as for a history of venous thromboembolic event or clinically significant cardiovascular disease within the preceding 6 months. Patients who had received prior angiogenic inhibitors other than bevacizumab and with brain metastases were excluded from the study.

Study design and treatment

This study was an open-label, 2-stage, phase II clinical trial conducted in 7 Canadian centers and 1 American center by the Princess Margaret Hospital Phase II Consortium. Patients were assigned to 1 of 2 cohorts, depending on whether or not they had received prior bevacizumab. The study was approved by the Research Ethics Boards of each participating center, and all patients gave written informed consent before enrollment onto this trial.

Aflibercept was administered at a starting dose of 4 mg/kg i.v. over 1 to 2 hours every 2 weeks, in repeated 2-week cycles. Response was assessed every 8 weeks by investigators using RECIST criteria (25). Patients were considered to have progressive disease if they had symptomatic deterioration related to disease even in the absence of radiographic progression. All objective responses were confirmed by a follow-up scan at least 4 weeks following documentation of the response. Safety was assessed by documentation of adverse events, bloodwork and urinalysis, blood pressure monitoring, and physical examination at regular intervals. Toxicity was graded according to National Cancer Institute Common Terminology for Adverse Events (CTCAE), version 3.0. Doses were reduced to 3 mg/kg and subsequently to 2 mg/kg for hypertension (grade 2 persistent or grade 3), grade 3 proteinuria, or serious grade 3/4 nonhematologic adverse events.

Pharmacokinetics

Plasma samples were analyzed for levels of free aflibercept, and VEGF:aflibercept complex (VEGF-bound aflibercept) at baseline, before each cycle, and 60 days after the last dose of aflibercept using validated ELISA methods with a limit of quantification of 15.6 and 43.9 ng/mL for free and VEGF-bound aflibercept, respectively (18). Free to VEGF-bound aflibercept ratios were calculated, following conversion of VEGF-bound to free aflibercept equivalent. In addition, serum was collected at baseline, every 4 cycles, and 60 days after the last dose to assess antiaflibercept antibodies using an ELISA method (18).

Statistical analysis

The primary endpoint was a composite endpoint of response rate and 16-week PFS, with responses defined by RECIST (25). Up to 40 evaluable patients were planned for each cohort (prior bevacizumab and bevacizumab naïve) with $\alpha = 0.10$. Stage I comprised 22 patients recruited to each cohort. For the prior bevacizumab cohort, if at least 2 or more objective responses were observed or at least 6 patients were progression free at 16 weeks, an additional 18 patients would be enrolled in stage II. Aflibercept would be considered active if at least 4 of 40 patients had an objective response or 13 patients were progression free at 16 weeks.

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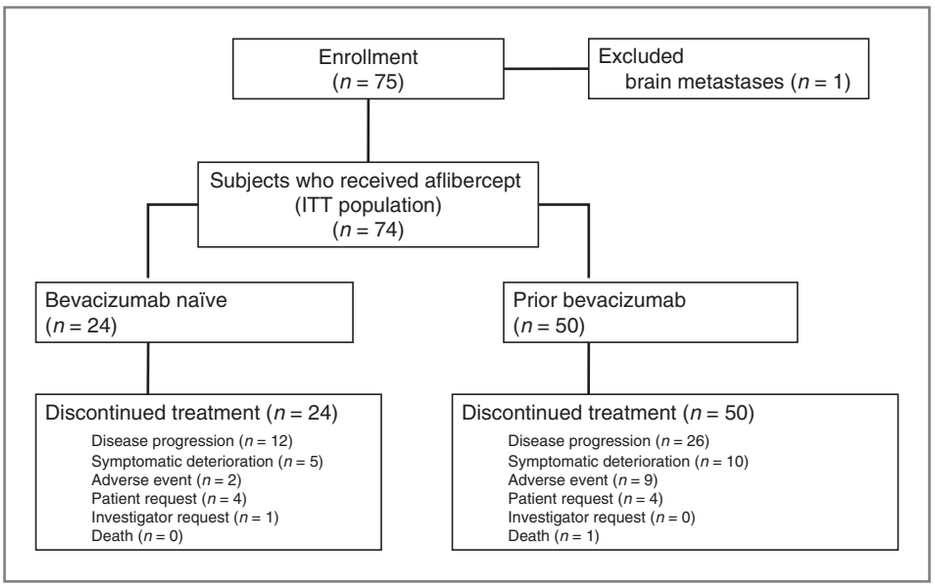

Figure 1. CONSORT diagram. ITT: intent to treat.

This design yielded at least 81% power to detect a true response rate of 15% and at least 85% power to detect a true 16-week PFS of 40%, as opposed to the null hypothesis that response rate was 4% or less and the 16-week PFS was 20% or less. For the bevacizumab-naïve cohort, if at least 2 or more objective responses were observed, or at least 10 patients were progression free at 16 weeks, an additional 18 patients would be enrolled in stage II. This design yielded at least 81% power to detect a true response rate of 15% and at least 79% power to detect a true 16-week PFS rate of 56%, as opposed to the null hypothesis that the response rate was 4% or less and the true 16-week PFS rate was 36% or less.

Table 1. Baseline characteristics

Characteristic	Bevacizumab-naïve cohort ($n = 24$) Number of patients (%)	Prior bevacizumab cohort ($n = 51$) Number of patients (%)	All patients ($n = 75$) Number of patients (%)
Age, y			
Median	60	59	59
Range	42–80	39–77	39–80
Sex			
Male	15 (62.5)	31 (60.8)	46 (61.3)
Female	9 (37.5)	20 (39.2)	29 (38.7)
ECOG performance status			
0	6 (25.0)	24 (47.1)	30 (40.0)
1	17 (70.8)	23 (45.1)	40 (53.3)
2	1 (4.2)	4 (7.8)	5 (6.7)
Metastatic sites			
Lymph node	11 (45.8)	19 (37.3)	30 (40.0)
Liver	18 (75.0)	32 (62.7)	50 (66.7)
Lung	11 (45.8)	28 (54.9)	39 (52.0)
Other	6 (25.0)	16 (31.4)	22 (29.3)
Previous radiotherapy	11 (45.8)	15 (29.4)	26 (34.7)
Previous systemic therapy			
Irinotecan	24 (100)	49 (96.1)	73 (97.3)
Oxaliplatin	18 (75.0)	45 (88.2)	63 (84.0)
Irinotecan and oxaliplatin	18 (75.0)	43 (84.3)	61 (81.3)
Fluoropyrimidine	24 (100)	51 (100)	75 (100)
EGFR inhibitor	7 (29.2)	28 (54.9)	35 (46.7)

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Table 2. Efficacy ($n = 74$)

	Bevacizumab-naïve cohort ($n = 24$)		Prior bevacizumab cohort ($n = 50$)		All patients($n = 74$)	
Variable	**Number of patients**	**%**	**Number of patients**	**%**	**Number of patients**	**%**
Partial response	0	0	1	2.0	1	1.3
Stable disease at 8 weeks	8	33.3	21	42.0	29	39.2
Stable disease $\geq$ 16 weeks	5	20.8	6	12.0	11	14.9

Secondary endpoints included toxicity and overall survival (OS). Response rate was calculated as the sum of patients with confirmed complete or partial responses divided by the number of patients. PFS was defined as the time from first dose to progressive disease or death. PFS and OS were calculated with the Kaplan–Meier method. Analyses of response, toxicity, and survival were carried out on an intent-to-treat population, including all patients without major violations of the eligibility criteria. In an exploratory analysis, χ^2 test was used to determine if hypertension (any grade as well as grade 3 or higher) was predictive for clinical benefit from aflibercept (response or 4 month PFS). In the prior bevacizumab cohort, the relationship between clinical benefit from aflibercept and prior response to bevacizumab as well as time from last dose of bevacizumab was determined with the χ^2 and Wilcoxon's tests respectively.

Results

Patients and treatment

Between November 2006 and May 2008, 75 patients were accrued, 51 of whom had received prior bevacizumab and 24 who were bevacizumab naïve (Fig. 1). One patient was ineligible due to the presence of brain metastases; 74 patients received at least 1 dose of aflibercept and were included in analyses of response, survival, and toxicity. During this time period, bevacizumab was not widely available in Canadian centers. Baseline characteristics are shown in Table 1. Patients were previously treated with a median of 2 prior regimens for metastatic disease (range 1–6) and 34 patients (45.3%) had received prior cetuximab. The majority of patients in both cohorts received prior therapy with a fluoropyrimidine, oxaliplatin, and irinotecan ($n = 61$; 81.3%). The median number of treatment

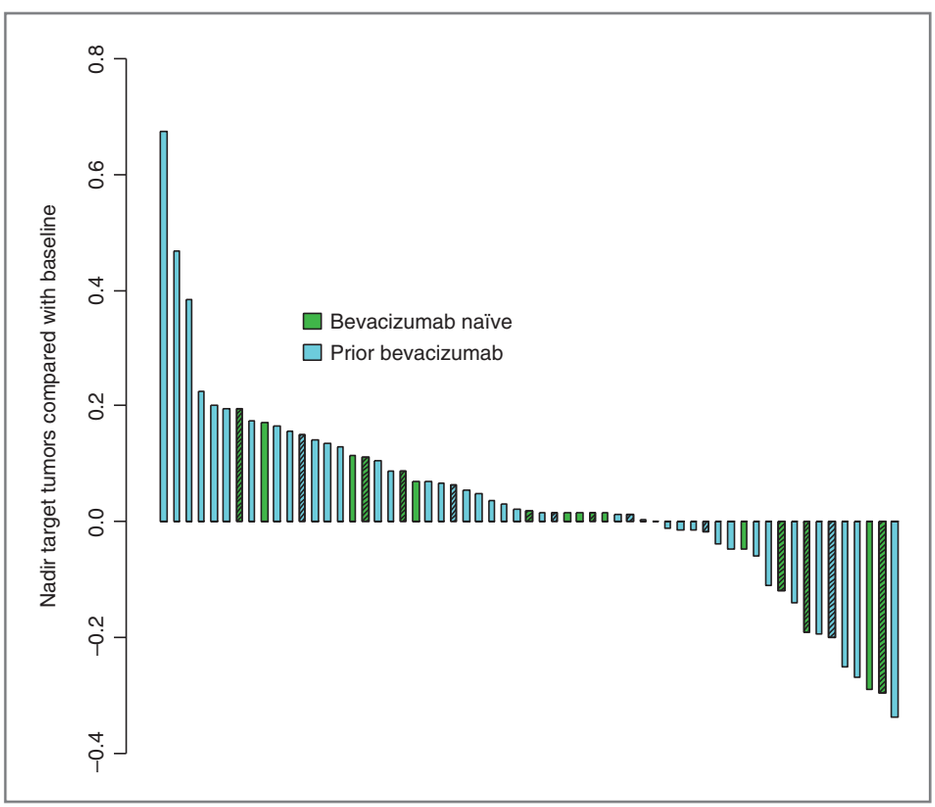

Figure 2. Maximum percentage change in target lesion size by patient during aflibercept treatment ($n = 59$; 1 patient was ineligible and 15 patients withdrew from the study before their first restaging scan). The shaded bars denote patients who had progressive disease and developed new lesion(s).

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cycles was 4 (range 1–16). All patients have stopped study treatment as of the time of this report.

Response and survival

Efficacy data on 74 patients are displayed in Table 2. There were no responses in the bevacizumab-naïve cohort and 5 patients (20.8%) experienced stable disease for at least 16 weeks. One patient in the prior bevacizumab cohort had a confirmed partial response that was sustained for 20 weeks and 6 patients (12.0%) had stable disease for at least 16 weeks. Fifteen patients discontinued the study before the first tumor assessments and had no postbaseline scans. The maximum percentage changes from baseline in target lesions from 59 patients is shown in Fig. 2.

The median PFS in the bevacizumab-naïve and prior bevacizumab cohorts were 2.0 months [95% confidence interval (CI): 1.7–8.6] and 2.4 months (95% CI: 1.9–3.7), respectively (Fig. 3A). The median OS was 10.4 months (95% CI: 7.6–15.5) in the bevacizumab naïve-cohort and 8.5 months (95% CI: 6.2–10.6) in the prior bevacizumab cohort (Fig. 3B).

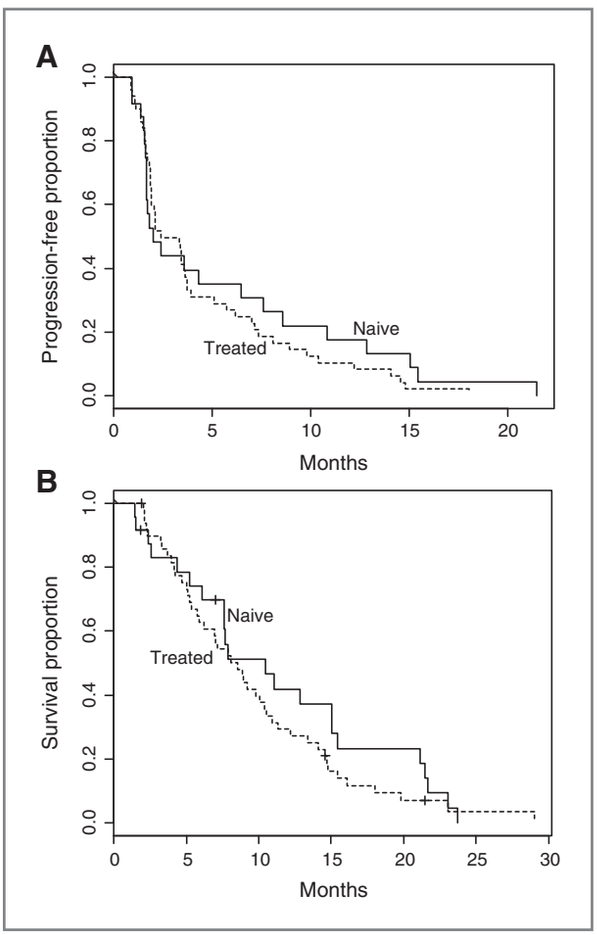

Figure 3. A, Kaplan–Meier curves for PFS in bevacizumab-naïve and prior bevacizumab-treated patients. B, Kaplan–Meier curves for OS in bevacizumab-naïve and prior bevacizumab-treated patients.

Pharmacokinetics

Pharmacokinetic data were available for 49 patients. Mean ± SD [coefficient of variation (CV %)] free aflibercept maximum concentration was 66.4 ± 31.4 (41) μg/mL for 27 patients. Mean free and VEGF-bound aflibercept concentrations observed at predose are graphically presented as function of cycle in Fig. 4. VEGF-bound aflibercept plasma increased regularly with time and reached a plateau by cycle 4. Mean predose (CV%) concentrations observed for free and VEGF-bound aflibercept were 6.73 (79) μg/mL ($n = 28$) and 3.70 (27) μg/mL ($n = 31$), respectively. Free to VEGF-bound aflibercept ratio was below 1 in 18% of patients (8/44) and the mean (CV%) ratio was 1.82 (72). Immunogenicity data were evaluable for 18 patients. One patient developed antiaflibercept antibodies after 4 cycles with aflibercept and came off study due to progressive disease. This immune response was associated with low free aflibercept concentration (0.227 μg/mL).

Adverse events

The dose of aflibercept was reduced from 4 to 3 mg/kg in 12 patients, and subsequently to 2 mg/kg in 1 patient. Dosing was delayed because of toxicity in 20 patients (27.0%). Ten patients (13.5%) discontinued treatment due to adverse events, which included: proteinuria (4 patients), hemorrhage (2 patients), chest pain (1 patient), bowel perforation (1 patient), hypertension (1 patient), and fatigue (1 patient).

The most common treatment-related adverse events included fatigue, hypertension, and proteinuria (Table 3). Treatment related pain (any grade, including the combination of headache, arthralgia, and myalgia) occurred in 74.3% of patients ($n = 55$). The most common grade 3 or 4 treatment-related adverse events were hypertension ($n = 10$; 13.5%), proteinuria ($n = 8$; 10.8%), fatigue ($n = 5$; 6.8%), and headache ($n = 5$; 6.8%). Other serious treatment-related adverse events of note included 1 incidence each of small bowel perforation ($n = 1$), deep vein thrombosis ($n = 1$), and rectal fistula ($n = 1$). We did not observe any arterial thromboembolic events or infusion reactions. One patient died during the study while receiving study treatment due to progressive disease.

Potential predictive markers

No association was found between clinical benefit (response rate or 16-week PFS) from aflibercept and the presence of hypertension (any grade; Supplementary Table S1, or for ≥ grade 3). In the prior bevacizumab cohort, there was neither any association between clinical benefit from aflibercept and time interval from last dose of bevacizumab nor response to prior bevacizumab therapy (data not shown). There was no relationship between free to VEGF-bound aflibercept ratio and clinical benefit (Fig. 4B).

Discussion

VEGF inhibition is a validated target in the treatment of MCRC based on pivotal trials combining bevacizumab with standard chemotherapy in the first and second line setting

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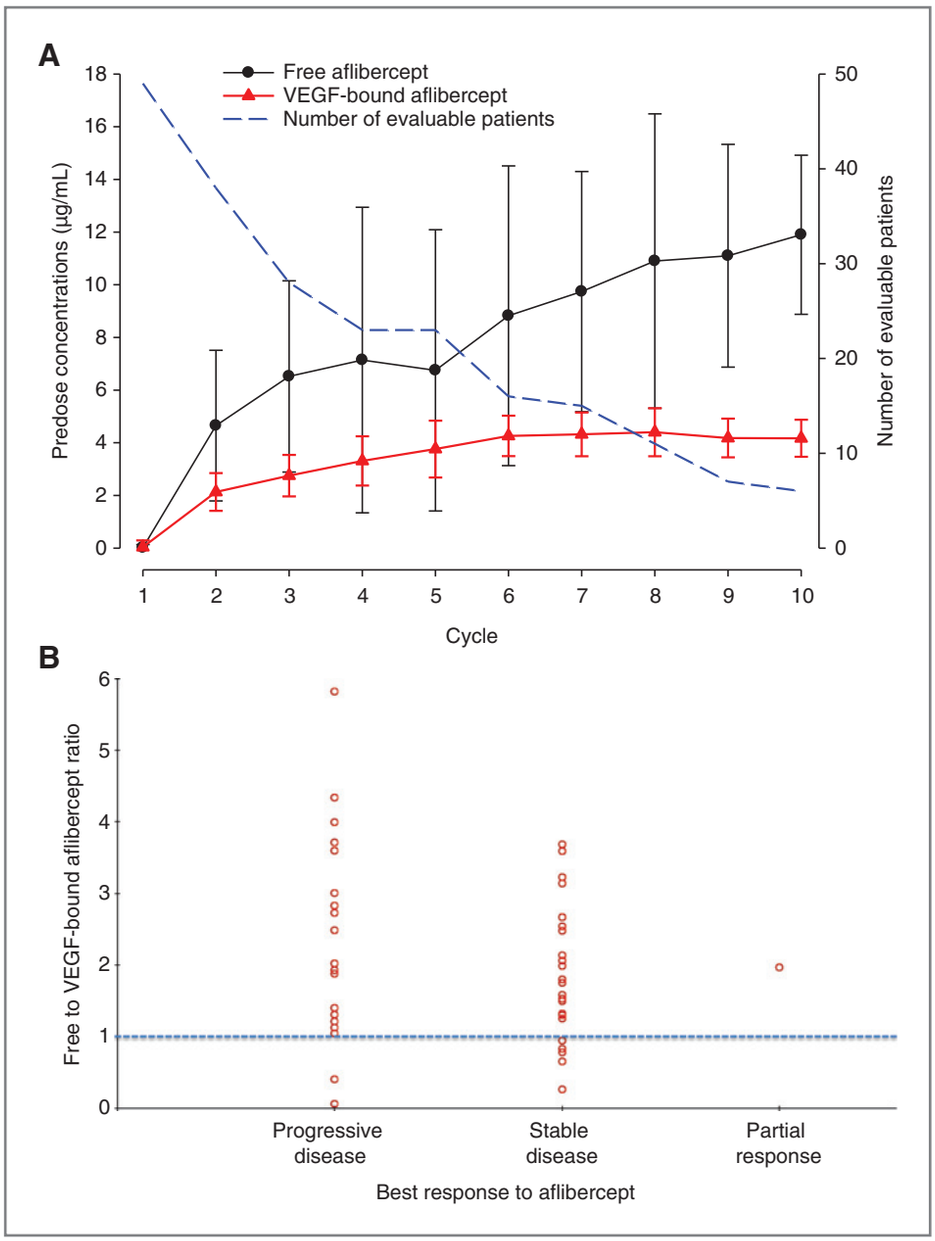

Figure 4. A, mean plasma concentration versus cycle for free and VEGF-bound aflibercept predose concentrations. The SD is indicated by the error bars. B, relationship between response and free to VEGF-bound aflibercept ratio.

(4–8). Prognosis after treatment with fluoropyrimidine, irinotecan, and oxaliplatin in MCRC is poor. The median PFS of the best supportive care arms of phase III trials evaluating cetuximab and panitumumab was just under 2 months (26, 27). Against this backdrop, we evaluated aflibercept in patients with refractory MCRC. PFS was similar in the bevacizumab-naïve and prior bevacizumab cohorts (2.0 and 2.4 months, respectively). A significant number of patients were not included in the waterfall plot because they came off study before the first radiographic assessment, potentially due to factors such as cumulative toxicities in a frail population and clinical progression. Unfortunately, aflibercept did not show promising clinical activity as a single agent and prior bevacizumab therapy did not impact efficacy.

Trials evaluating bevacizumab as a single agent have also been disappointing. Bevacizumab in combination with 5-fluorouracil (5-FU) showed minimal activity in patients previously treated with fluoropyrimidines, irinotecan, and oxaliplatin with a response rate of 1% based on independent review (28). As a single agent in ECOG 3200, treatment with bevacizumab resulted in a PFS of 2.7 months and a response rate of 3.3% in patients previously treated with irinotecan-based therapy (4). Currently, there are no validated biomarkers that predict for benefit from bevacizumab (29, 30).

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Table 3. Most common treatment-related adverse events ($n = 74$)

	All grades		Grade 3/4	
Adverse event	Number of patients	%	Number of patients	%
Fatigue	50	67.6	5	6.8
Hypertension	38	51.4	10	13.5
Proteinuria	36	48.6	8	10.8
Headache	31	41.9	5	6.8
Dysphonia	25	33.8	—	—
Anorexia	16	21.6	—	—
Arthralgia	13	17.6	1	1.4
Myalgia	11	14.9	—	—
Lymphopenia	11	14.9	—	—
Weight loss	9	12.2	—	—
Nausea	9	12.2	—	—
Hypoalbuminemia	9	12.2	—	—
Epistaxis	9	12.2	-	-
Hemorrhage (nonepistaxis)[a]	9	12.2	1	1.4
Diarrhea	8	10.8	—	—

[a]Includes: asymptomatic hematuria detected on urinalysis (3 patients); grade 4 variceal hemorrhage (1 patient).

In the current trial, aflibercept pharmacokinetics were consistent with those reported previously (18). The mean ratio of free to VEGF-bound aflibercept was 1.82 in evaluable patients, suggesting that free aflibercept was present in sufficient amount to bind available endogenous VEGF during treatment, and adequate for tumor activity based on preclinical models (13, 15–17). The free to VEGF-bound aflibercept ratio was not predictive of clinical benefit, however, only 44 patients had evaluable levels. The toxicity of aflibercept was manageable, and was similar to that seen in other studies (18, 31–35).

Ten patients (13.5%) stopped treatment with aflibercept due to adverse events, including 5 patients for severe hypertension and proteinuria. In ECOG 3200, 12.0% of patients experienced grade 3 or greater toxicity leading to cessation of treatment with single-agent bevacizumab (4). The incidence of grade 3 or 4 hypertension in the bevacizumab containing chemotherapy arms of pivotal MCRC trials was 4% to 16% (4, 5, 7, 8), similar to what was observed in this study with aflibercept (13.4%). In light of the high rate of hypertension of any grade (51.4%) and proteinuria (48.6%), close monitoring is recommended in the future. Angiotensin converting enzyme inhibitors or angiotensin II receptor blockers may be considered as first-line therapy for hypertension from aflibercept due to their efficacy at also reducing proteinuria (36). However, this study did not evaluate the efficacy of different classes of antihypertensive medications. Hypertension was not predictive of clinical benefit in this study, possibly related to the relatively small sample size. Although previous studies have shown a relationship between hypertension and clinical benefit from antiangiogenic therapies, different definitions for hypertension were used ranging the presence of grade 3 or higher hypertension according to CTCAE criteria to a diastolic blood pressure more than 90 mmHg (19–24).

Since the completion of this phase II trial, results of the phase III VELOUR trial have been presented (37; VEGF-trap with irinotecan in colorectal cancer after failure of oxaliplatin, clinical trials identifier NCT00561470). The addition of aflibercept to FOLFIRI (5-FU, leucovorin, and irinotecan) significantly improved OS, PFS, and response rate compared with placebo in patients with MCRC previously treated with an oxaliplatin-based regimen. A prespecified subgroup analysis of the VELOUR trial revealed no significant interaction between prior bevacizumab therapy (bevacizumab-naïve patients OS hazard ratio [HR] 0.788; 95.34% CI: 0.669–0.927 vs. prior bevacizumab exposure OS HR 0.862; 95.34% CI: 0.673–1.104; $P = 0.7231$; ref. 38). A phase II, noncomparative trial, randomized chemotherapy naïve patients with MCRC to FOLFOX with or without aflibercept (clinical trials identifier NCT00851084). PFS at 12 months, the primary endpoint, was similar in both arms [FOLFOX aflibercept 25.8% (95% CI: 17.2–34.4) versus FOLFOX 21.2% (95% CI: 12.2–30.3); ref. 39].

Aflibercept did not show meaningful single-agent activity in this heavily pretreated patient population but was associated with prolonged stable disease in a subset of patients. The lack of a randomized phase II design makes interpretation of the activity of aflibercept as a single agent difficult. Given the mechanism of action, future trials should compare bevacizumab with aflibercept in combination with standard chemotherapy regimens and assess quality of life as well as the optimal sequence of antiangiogenic drugs. Further research is required to identify predictive markers for benefit from antiangiogenic therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Development of methodology: P.A. Tang, S.J. Cohen, M.J. Moore
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.A. Tang, S.J. Cohen, C. Kollmannsberger, G. Bjarnason, K. Virik, M.J. MacKenzie, L. Lourenco, M.J. Moore
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.A. Tang, C. Kollmannsberger, L. Lourenco, L. Wang, A. Chen, M.J. Moore
Writing, review, and/or revision of the manuscript: P.A. Tang, S.J. Cohen, C. Kollmannsberger, G. Bjarnason, M.J. MacKenzie, L. Lourenco, L. Wang, A. Chen, M.J. Moore
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.A. Tang, L. Lourenco, M.J. Moore
Study supervision: K. Virik, L. Lourenco, M.J. Moore

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