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## Aflibercept

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### Abstract

Aflibercept, an intravenously administered anti-VEGF and antiplacental growth factor (PIGF) agent, has recently been approved by the U.S. Food and Drug Administration in combination with 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) for the treatment of patients with metastatic colorectal cancer who have previously received an oxaliplatin-containing chemotherapy regimen. In the phase III VELOUR trial, aflibercept plus FOLFIRI statistically significantly prolonged both progression-free survival (PFS; median PFS for the aflibercept plus FOLFIRI arm was 6.90 vs. 4.67 months for the placebo-plus-FOLFIRI arm) and overall survival (median overall survival for the aflibercept-plus-FOLFIRI arm was 13.50 vs. 12.06 months for the placebo plus FOLFIRI arm), but grade 3 or 4 adverse events were more common with the addition of aflibercept. However, the addition of aflibercept to 5-fluorouracil, leucovorin, and oxaliplatin (mFOL-FOX6) in the phase II AFFIRM trial of first-line treatment of mCRC failed to improve PFS or response rate. As a decoy VEGF receptor, aflibercept (VEGF-Trap) has binding affinity for VEGF-A, VEGF-B, PIGF-1, and PIGF-2, and this is a mechanism of significant interest. Optimal strategies for incorporating aflibercept into treatment regimens that include other anti-VEGF and cytotoxic chemotherapeutic agents, as well as development of predictive biomarkers for treatment response, have yet to be defined.

### Introduction

In August 2012, the U.S. Food and Drug Administration (FDA) approved aflibercept (Zaltrap; Sanofi-Aventis) in combination with 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) for the treatment of patients with metastatic colorectal cancer (mCRC) resistant to or progressive on an oxaliplatin-containing chemotherapy regimen. Aflibercept is a fully humanized recombinant fusion protein composed of portions of the extracellular domains of VEGF receptor (VEGFR)-1 and VEGFR-2 fused to the Fc portion of human immunoglobulin G1 (1). As such, it functions as a decoy VEGFR with propensity to bind VEGF-A, VEGF-B, placental growth factor (PIGF)-1, and PIGF-2, thereby preventing these

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Chan

**Study supervision:** J. Berlin

Disclosure of Potential Conflicts of Interest

K.K. Ciombor is a consultant/advisory board member of Bayer. J. Berlin is a consultant/advisory board member of Sanofi-Aventis. E. Chan is a consultant/advisory board member of Amgen, Genentech, Bristol-Myers Squibb, and Bayer.

ligands from binding to and activating their cognate receptors (Fig. 1). Aflibercept may play a potentially significant role in the treatment of cancers dependent on this pathway.

### Targeting Angiogenesis

Angiogenesis, or new blood vessel formation, is an essential and highly regulated process in tumor growth and metastasis. In normal tissues, the production and destruction of proangiogenic and antiangiogenic factors are carefully regulated and balanced. In the setting of malignancy, however, a need for an increased vascular supply leads to overexpression of proangiogenic factors such as those in the VEGF pathway (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF-1, and PlGF-2; ref. 2). Circulating VEGFs bind to their corresponding receptors (VEGFR-1, VEGFR-2, and VEGFR-3) and activate receptor dimerization, ultimately resulting in a cascade of downstream signaling transduction pathways that leads to an increase in vascular permeability, endothelial cell activation, and proliferation, invasion, and migration (2).

Given the importance of these processes to tumors as a whole, a concerted effort has been made to develop treatments that target angiogenesis, largely by targeting VEGF. One of these anti-VEGF therapies, bevacizumab (Avastin; Genentech), has shown clinical efficacy in the treatment of advanced colorectal cancer, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and glioblastoma multiforme, resulting in FDA approval for these indications. Bevacizumab is a humanized monoclonal antibody that binds to VEGF-A and prevents its receptor binding. Another approach to target angiogenesis is via the small-molecule tyrosine kinase inhibitors (TKI), such as sunitinib (Sutent; Pfizer), sorafenib (Nexavar; Onyx Pharmaceuticals, Bayer HealthCare), and pazopanib (Votrient; GlaxoSmithKline). These agents target the VEGFR and other receptors, blocking receptor tyrosine kinase activity. They are FDA approved for the treatment of such tumors as RCC, gastrointestinal stromal tumors, pancreatic neuroendocrine tumors, and hepatocellular carcinoma. Despite their proven efficacy and availability, more anti-VEGF therapies are needed. It has been shown in RCC that patients progressing on one anti-VEGF TKI can still respond to a different anti-VEGF TKI. Therefore, newer anti-VEGF agents are under development to improve VEGF targeting and/or overcome resistance to current anti-VEGF therapies. An example of this is aflibercept, which binds with a higher affinity to VEGF-A than either bevacizumab or VEGFR. In addition, as levels of PlGF increase with exposure to other anti-VEGF agents such as bevacizumab, the ability of aflibercept to additionally target PlGF-1 and -2 might represent a more efficacious way to address angiogenesis of malignancy.

Initial *in vitro* experiments with aflibercept showed inhibition of VEGFR-2 phosphorylation in human umbilical vein endothelial cells as well as inhibition of VEGF-induced fibroblast proliferation (1). Aflibercept significantly inhibits tumor growth and angiogenesis, reduces tumor vessel density, and inhibits metastases in xenografts of various tumor types (3–5). Moreover, decreases in the expression of tumor vascular genes and in the activation of the vascular endothelial signaling pathways in xenografts have been seen (6). When combined in tumor xenografts with other anticancer treatments such as cytotoxic chemotherapy or radiotherapy, aflibercept shows greater inhibition of tumor growth and vasculature than with the individual treatments alone (7, 8). As a result of these and other preclinical data, aflibercept has undergone clinical investigation as monotherapy and in combination with cytotoxic chemotherapeutic agents for the treatment of various malignancies, with the only efficacious data seen in mCRC thus far.

## Early Clinical Development of Aflibercept for mCRC

As reviewed by Gaya and colleagues, many phase I and II studies of aflibercept as a single agent or in combination with cytotoxic chemotherapy established its safety and potential clinical efficacy for the treatment of solid tumors or hematologic malignancies (9). A phase I study of 47 patients with advanced solid tumors who received aflibercept in doses ranging from 0.3 to 7.0 mg/kg i.v. every 2 weeks showed linear pharmacokinetics of free aflibercept from 2.0 to 7.0 mg/kg (10). The half-life of aflibercept was dose dependent and ranged from 1.7 days at 0.3 mg/kg to 5.1 days at 7.0 mg/kg, with steady-state concentrations not reached until at least 3 weeks after the first aflibercept dose. Mean clearance values of free aflibercept were stable between 2.0 and 7.0 mg/kg, indicating binding saturation of endogenously produced VEGF at the 2.0-mg/kg dose. At doses of 2.0 mg/kg or more, maximal VEGF-bound aflibercept complex levels were reached, indicating complete ligand blockade. Free aflibercept levels remained more than bound aflibercept levels throughout the dosing intervals at dose levels of 2.0 mg/kg or more. At doses of 4.0 mg/kg and more, however, the frequency and severity of adverse events increased; therefore the chosen recommended phase II dose of aflibercept was 4 mg/kg i.v. every 2 weeks, despite a half-life considerably less than 2 weeks at this dose. In contrast, the anti-VEGF agent bevacizumab has a half-life of approximately 20 days yet is given every 2 to 3 weeks (11); the anti-VEGF TKI regorafenib has a half-life of 20 to 40 hours and is dosed daily for 21 out of every 28 days (12).

In terms of single-agent efficacy in the phase I aflibercept trial, 1 patient with malignant thymoma treated with the 3.0-mg/kg dose had a confirmed partial response (PR), and 1 patient with ovarian cancer treated with the 4.0-mg/kg dose had an unconfirmed PR. Two patients with ovarian cancer treated with the 7.0-mg/kg dose had confirmed PRs. Interestingly, this study also showed a possible dose–effect correlation of aflibercept on tumor vascularity, permeability, and perfusion when measured by dynamic contrast-enhanced (DCE)-MRI.

A phase I dose escalation and pharmacokinetics trial of aflibercept with irinotecan, 5-fluorouracil, and leucovorin (LV5FU2) was conducted in subjects with advanced solid tumors to determine the recommended dose of aflibercept to be given with irinotecan and LV5FU2 (13). Of the 38 subjects enrolled in this trial, 36 had received prior chemotherapy, 63% having previously received irinotecan. VEGF-bound aflibercept exposure was comparable between the 2- and 6-mg/kg doses, indicating maximal ligand blockade, and free aflibercept concentrations were in excess of VEGF-bound aflibercept concentrations throughout the dosing interval in most patients from 4 mg/kg. On the basis of these pharmacokinetic and safety/toxicity data, aflibercept, 4 mg/kg i.v. every 2 weeks, was selected as the recommended dose to be combined with LV5FU2 and irinotecan. Interestingly, although efficacy was not a primary endpoint in this trial, 9 of 34 evaluable patients had PRs, 5 of which were with the 4 mg/kg aflibercept dose, and 22 patients had stable disease, 5 of which were with the 4 mg/kg aflibercept dose. PRs were seen in synovial sarcoma, ovarian cancer, pancreatic cancer, and 6 colon cancers; 7 of these PRs were seen in patients who had previously received irinotecan.

Following the dose-escalation portion of this phase I study, a double-blind expansion phase was undertaken in which all patients received irinotecan-LV5FU2 and were randomized to either placebo or aflibercept, 4 mg/kg, in cycle 1; subsequently, all patients received irinotecan-LV5FU2 and aflibercept (14). The most frequent grade 3/4 adverse events were neutropenia (37%), fatigue (33%), and hypertension (33%). Four of the 26 evaluable patients had PRs (1 each of anal, colon, ovarian, and liver cancers), and 17 had stable disease on this regimen. In this study, DCE-MRI did not show significant perfusion changes

with aflibercept. These promising safety and efficacy results led to further exploration of this regimen in mCRC.

### Efficacy Studies of Aflibercept in mCRC

Several later-phase clinical trials investigating the efficacy of aflibercept as a single agent or in combination with cytotoxic chemotherapy for colorectal cancer have been conducted. An open-label, 2-stage phase II study of single-agent aflibercept (4 mg/kg i.v. given every 2 weeks) in refractory mCRC was recently reported (15). Two cohorts were studied, those who had received prior bevacizumab ( $n = 51$ ) and those who were bevacizumab naïve ( $n = 24$ ). Only 1 PR (2%) was seen and occurred in the prior bevacizumab cohort. The bevacizumab-naïve cohort had 5 patients (20.8%) with stable disease, and the prior-bevacizumab cohort had 6 patients (11.8%) with stable disease. The median progression-free survival (PFS) durations 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. The median overall survival (OS) durations were 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. Expected grade 3 or higher treatment-related adverse events of hypertension, proteinuria, fatigue, and headache were seen, with 10 patients discontinuing therapy due to toxicity. These results showed little clinical efficacy and moderate toxicity of aflibercept as single-agent therapy in this heavily pretreated mCRC population.

As the phase II trial of single-agent aflibercept in mCRC was accruing, a prospective, multinational, randomized, double-blind phase III study entitled VELOUR (Aflibercept versus Placebo in Combination with Irinotecan and 5-FU in the Treatment of Patients with Metastatic Colorectal Cancer after Failure of an Oxaliplatin Based Regimen) investigated the efficacy and safety of aflibercept plus FOLFIRI versus placebo plus FOLFIRI in patients with mCRC who had progressed on an oxaliplatin-based regimen previously (16). Patients were randomly assigned in a 1:1 ratio to receive either aflibercept, 4 mg/kg, or placebo i.v. on day 1 every 2 weeks, followed immediately by FOLFIRI. Patients were stratified according to prior therapy with bevacizumab (with 30.4% of patients having previously received bevacizumab) as well as Eastern Cooperative Oncology Group (ECOG) performance status, and crossover to aflibercept was not allowed upon disease progression. The primary endpoint of VELOUR was OS assessed by blinded, independent central review.

All 1,226 randomized patients were included in the efficacy analyses. Patients receiving aflibercept plus FOLFIRI had a statistically significant longer OS than patients receiving placebo plus FOLFIRI (median OS, 13.50 vs. 12.06 months,  $P = 0.0032$ ; HR = 0.817; 95.34% CI, 0.713–0.937). The addition of aflibercept to FOLFIRI also increased PFS when compared with placebo (median PFS, 6.90 vs. 4.67 months,  $P < 0.001$ ; HR = 0.758; 95% CI, 0.661–0.869). The response rate (RR) was 19.8% in the aflibercept arm and 11.1% in the control arm ( $P < 0.001$ ). More patients on the aflibercept arm had grade 3 or 4 adverse events (83.5% vs. 62.5%). These events included those expected with anti-VEGF/PIGF agents, such as hypertension, hemorrhage, and arterial and venous thromboembolic events; however, adverse effects associated with FOLFIRI were also seen to a greater degree when the regimen was given with aflibercept compared with placebo. Unfortunately, this trial did not identify biomarkers to predict those patients who would benefit from the addition of aflibercept to FOLFIRI. On the basis of the results of this trial, aflibercept received FDA approval in combination with FOLFIRI for patients with mCRC who had progressed on a prior oxaliplatin-based chemotherapy regimen.

## The Role of Aflibercept for the Treatment of mCRC: When to Use?

Despite the positive VELOUR study results, the optimal role of aflibercept in the treatment of mCRC remains unclear, particularly given the availability of other efficacious anti-VEGF agents. Until relatively recently, bevacizumab was the only FDA-approved agent targeting the VEGF pathway in mCRC. Bevacizumab improves OS in mCRC from 15.6 to 20.3 months when combined with irinotecan, bolus 5-fluorouracil, and leucovorin (IFL), versus IFL alone, in the first-line setting (17). Other studies have shown OS and/or PFS benefit when bevacizumab is combined with cytotoxic chemotherapy in colorectal cancer (18). Given these results, treatment of mCRC with chemotherapy plus bevacizumab has been the first-line standard of care for patients eligible to receive anti-VEGF agents. It was unclear, however, whether the addition of bevacizumab to chemotherapy improved survival after progression on first-line therapy. To answer this question, the ML18147 trial was conducted in Europe (19). This phase III trial studied 820 patients randomly assigned to continuing bevacizumab or not with second-line chemotherapy after progressing on first-line bevacizumab-containing therapy (bevacizumab beyond progression). Continued bevacizumab prolonged median OS (11.2 vs. 9.8 months, respectively; unstratified HR = 0.81;  $P = 0.0062$ ) and PFS (5.7 vs. 4.9 months, respectively; unstratified HR = 0.68;  $P < 0.0001$ ) compared with cytotoxic therapy alone. Because bevacizumab in combination with chemotherapy has now been proved to improve survival in patients with mCRC beyond the first-line setting, the optimal time at which aflibercept should be used is even less clear.

Interestingly, 30.4% of patients in the VELOUR trial had previously received bevacizumab, arguing for additional benefit of aflibercept even in this subset of patients with mCRC. Extrapolating from the ML18147 data, the survival benefit seen with aflibercept treatment may simply be indicative of an incremental but real benefit to continuing anti-VEGF therapy beyond a first-line regimen, as the survival benefit was similar in both studies. Alternatively, perhaps aflibercept has a novel mechanism that may allow it to overcome anti-VEGF resistance developed from bevacizumab treatment, particularly because aflibercept does bind VEGF-B and PlGF in addition to VEGF-A. Further study is needed to discriminate between these possibilities.

To investigate the potential role of aflibercept in the first-line treatment of mCRC with chemotherapy, the phase II AFFIRM trial (Study of Aflibercept and Modified FOLFOX6 as First-Line Treatment in Patients with Metastatic Colorectal Cancer) evaluated the efficacy of aflibercept in combination with 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6; ref. 20). A total of 236 patients were randomized to either aflibercept, 4 mg/kg, plus mFOLFOX6 ( $n = 119$ ) or mFOLFOX6 alone ( $n = 117$ ), with the primary endpoint being PFS rate at 12 months. Notably, the mFOLFOX6-alone arm was included only as a calibrator, and the study was not powered for a statistical comparison. The PFS rate at 12 months was 25.8% (95% CI, 17.2–34.4) for aflibercept plus mFOLFOX6 and 21.2% (95% CI, 12.2–30.3) for mFOLFOX6 alone. The RR was 49.1% (95% CI, 39.7–58.6) in the aflibercept-plus-mFOLFOX6 arm and 45.9% (95% CI, 36.4–55.7) for the mFOLFOX6 arm. The median PFS was also similar in both groups, with 8.48 months (95% CI, 7.89–9.92) for aflibercept plus mFOLFOX6 and 8.77 months (95% CI, 7.62–9.27) for mFOLFOX6 alone. On the basis of these phase II data, there is no apparent role for aflibercept in this setting. These results contrast with those in bevacizumab, for which several first-line trials with positive results have been completed. The reason for this difference remains unclear.

Regorafenib (Stivarga; Bayer), an oral TKI whose targets include VEGFR-1, VEGFR-2, VEGFR-3, and TIE2, among others, also recently received FDA approval for patients with refractory metastatic colon cancer who have already received fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, anti-VEGF therapy and, if applicable, anti-EGF receptor therapy. The phase III CORRECT trial (Regorafenib Monotherapy for

Previously Treated Metastatic Colorectal Cancer) met its OS primary endpoint at a preplanned interim analysis, with median OS of 6.4 months for the regorafenib arm and 5.0 months for the placebo arm (HR = 0.77; 95% CI, 0.64–0.94; 1-sided  $P = 0.0052$ ; ref. 21). Median PFS was 1.9 versus 1.7 months favoring regorafenib (HR = 0.49; 95% CI, 0.42–0.58; 1-sided  $P < 0.000001$ ). Adverse events were slightly different from those with other antiangiogenic agents available in mCRC but were similar to toxicities seen with TKIs and included hand–foot skin reaction, diarrhea, and rash/desquamation. Importantly, predictive biomarkers have not yet been identified for regorafenib (or any other VEGF targeting agent). Although this is another efficacious anti-VEGF therapy for mCRC, its current role is more clearly distinct than that of aflibercept. In contrast to aflibercept, the efficacy of regorafenib as a monotherapy (though limited), its oral administration, and its indication after failure of all other approved agents give it a distinct niche in the landscape of mCRC treatments.

Another important issue that will influence the use of aflibercept for the treatment of mCRC is its cost relative to other therapies. At the time of its FDA approval, it was priced to compete with the higher dose (10 mg/kg every 2 weeks) of bevacizumab originally used in ECOG 3200. Most oncologists in the United States use the lower 5-mg/kg every-2-weeks dose for mCRC approved for first-line therapy, thereby making aflibercept much more expensive than bevacizumab. Sanofi-Aventis has subsequently offered a 50% discount on the purchase price of aflibercept as a result of market and provider pressures, bringing it more in line with the cost of other targeted agents for mCRC such as bevacizumab and regorafenib. Given the trend of increasing numbers of targeted therapies that often provide only incremental benefit to those with advanced cancers, however, the cost of these therapies both to the patient and to the health care system as a whole will continue to be a major factor in deciding how best to treat mCRC.

### Aflibercept in Other Malignancies

Aflibercept has also been studied in other solid tumors, although efficacy data in later phase clinical trials for other tumors in combination with cytotoxic chemotherapy have not been as promising. In the phase III VITAL trial (A Study of Aflibercept versus Placebo in Patients with Second-Line Docetaxel for Locally Advanced or Metastatic Non–Small Cell Lung Cancer), 913 patients with nonsquamous NSCLC were treated with docetaxel, 75 mg/m<sup>2</sup>, and aflibercept, 6 mg/kg i.v., or placebo every 3 weeks (22). The addition of aflibercept to docetaxel did not improve OS (HR = 1.01; 95% CI, 0.87–1.17; stratified log-rank  $P = 0.90$ ), with a median OS of 10.1 months for aflibercept and 10.4 months with placebo. VANILLA, the phase III trial evaluating gemcitabine plus aflibercept or placebo in first-line treatment of metastatic pancreatic cancer, was discontinued after a planned interim efficacy analysis determined that aflibercept would not be able to statistically significantly improve OS when compared with the placebo arm. This was confirmed after final analysis showed an OS of 7.75 months for the aflibercept arm versus 6.54 months for the placebo arm (23). Finally, a study of docetaxel and prednisone plus either aflibercept or placebo in metastatic androgen-independent prostate cancer (VENICE) did not show an improvement in survival for the aflibercept-containing arm, with full results yet to be published (24). As a result of these negative phase III trials in other tumor types, further studies of aflibercept remain focused on colorectal cancer.

### Conclusions and Challenges

Aflibercept has been proved efficacious in combination with FOLFIRI for patients with mCRC who have progressed on an oxaliplatin-based chemotherapy regimen, although the benefit is limited and the financial costs are high. Unanswered questions about aflibercept are many, including its optimal sequence and use when compared with other anti-VEGF

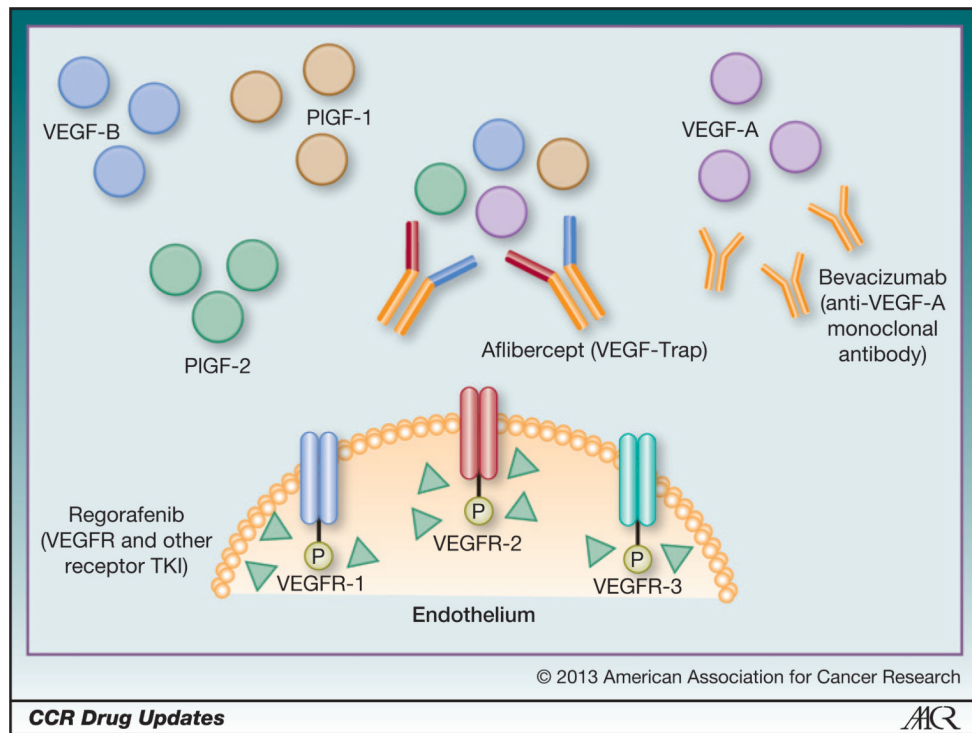
agents, cytotoxic chemotherapeutic agents with which it is best combined, and the development of biomarkers that could predict for treatment response. Perhaps most critical, however, is the unanswered question of what role aflibercept should have in the current management of mCRC, given the toxicity, efficacy, and cost comparison data presented above. While aflibercept is a welcome new tool in the treatment of mCRC, its limitations, in light of the other currently available agents and the data for bevacizumab beyond progression, represent the ongoing challenge to develop safer, more cost effective, and more efficacious targeted therapies against cancer, and highlight the need to develop predictive biomarkers to identify those patients most likely to benefit from therapy with the agent.

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**Figure 1.** Schematic of an endothelial cell depicting VEGFR-1, VEGFR-2, and VEGFR-3 and the mechanisms of action of the antiangiogenic agents aflibercept, bevacizumab, and regorafenib. Note: regorafenib is a multitargeted receptor TKI and therefore inhibits additional receptors (such as PDGFR- $\beta$ , TIE-2, fibroblast growth factor receptor 1, and others) not depicted here.