Update on antiangiogenic therapy in colorectal cancer: aflibercept and regorafenib

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Abstract: Angiogenesis plays an important role in colorectal carcinogenesis and approaches targeting the vascular growth factor receptor (VEGF) signaling such as bevacizumab yielded significant survival improvement for metastatic colorectal cancer patients. Recent evidence demonstrated the benefit of continuing angiogenic suppression after first-progression following bevacizumab-containing cytotoxic regimen though no benefit was observed with the use of bevacizumab in adjuvant setting. Aflibercept, a soluble fusion protein with high affinity for VEGF-A, -B and PlGF, administered in combination with irinotecan-containing regimen improved the survival of metastatic colorectal cancer patients in second-line setting (VELOUR trial). Regorafenib, a small molecule multikinase inhibitor against various pro-angiogenic and –proliferation targets, improved the survival of metastatic colorectal cancer patients who had progressed on all standard therapy. These developments had renewed enthusiasm in the field and the role of aflibercept and regorafenib in other treatment settings will continue to be defined by on-going and future clinical trials. As other anti-angiogenic approaches are being tested clinically, other novel non-angiogenic targets deserve to be evaluated in our effort to improve the outcome of colorectal cancer patients.

Key Words: Antiangiogenic therapy; colorectal cancer; aflibercept; regorafenib; vascular growth factor receptor (VEGF)

Background

Colorectal cancer is a major cause of morbidity and mortality throughout the world. It is the third most common cancer diagnosis worldwide and affects men and women equally (1). In the United States, colorectal cancer accounted for 9% of all cancer mortality in 2012 (2). The survival of patients with metastatic colorectal cancer (mCRC) has markedly improved since the 1990s when 5-fluorouracil (5FU) based chemotherapy achieved an overall survival (OS) of 12 months. The addition of oxaliplatin and Irinotecan increased the OS to approximately 18 months (3-6). The survival was further augmented with anti-angiogenic agents and bevacizumab, in combination with chemotherapy, was the first of the drug class to receive regulatory approval for use in mCRC therapy (7,8). Recently, 2 other anti-angiogenic drugs, aflibercept and regorafenib, were found to improve the survival of mCRC patients in randomized trials which further reiterates the importance of targeting angiogenesis in CRC therapy (9,10).

This article will review the development of aflibercept and regorafenib and their current role in the treatment of colorectal cancer (*Table 1*).

Tumor angiogenesis and VEGF signaling pathway

Angiogenesis refers to a multi-step process leading to the formation of new blood vessels to supply nutrients and oxygen to the tissues (11). The process begins with vasodilatation, increased vessel permeability, stromal degradation and endothelial cell proliferation and migration, resulting in the formation of a new or extended capillary (12). Whilst angiogenesis is ordered and occur only during wound repair, tissue remodeling or inflammation under normal physiologic conditions, the process is chaotic in neoplasms resulting in leaky, tortuous and inefficient vessels (13-15).

The VEGF/VEGFR signaling is a well studied pro-angiogenic pathway and the ligands include VEGF-A,

Table 1 Compare bevacizumab, afibercept and regorafenib

	Bevacizumab	Aflibercept	Regorafenib
Classification	Recombinant humanized Monoclonal antibody	Soluble fusion protein contains domains from VEGFR-1 and VEGFR-2	Small molecule multikinase inhibitor
Targets	VEGF-A	VEGF-A, VEGF-B and PIGF	VEGFR-1, -3, RAF, TIE-2, and mutant oncogenic kinases KIT, RET and BRAF
Molecular weight	149 kD	115 kD	500.83 D
Doses in colorectal cancer	5-10 mg/m^2 IV every 2 weeks in combination with FOLFOX and FOLFIRI	4 mg/kg IV every 2 weeks in combination with FOLFIRI	160 mg oral daily for 21 days of a every 28 days cycle
Common and clinically significant side effects	Hypertension, Proteinuria, Thrombosis, Hemorrahge, delay wound healing, GI perforation(rare)	Hypertension, Proteinuria, GI perforation (rare), delay wound healing, Hemorrhage	Hypertension, Fatigue, Hand-foot syndrome, Hepatotoxicity, GI perforation (rare), Hemorrhage, Reversible Posterior leukoencephlopathy syndrome

VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF) that interact with membrane bound tyrosine kinase receptors VEGFR-1 (FLT-1), VEGFR-2 (FLK-1/KDR) and VEGFR-3 (FLT4); and other co-receptors include neurophilin (NRP)-1 and NRP-2 (16-18). The binding of VEGF-A (or VEGF) to VEGFR-2 had been found to be key mediator of angiogenesis (17). VEGF-A (commonly known as VEGF) is expressed in many human cancers and binding with VEGFR-2 in tumor microenvironment triggers a number of intracellular signaling cascades in endothelial cells leading to formation and enhancement of tumor microvasculature (18,19).

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of VEGF by preventing its binding to VEGFR-1 and VEGFR-2 (*Figure 1*). The therapeutic role of bevacizumab in treating metastatic CRC patients is well established and supported by well-conducted randomized trials (7,8,20-22). These topics had been well reviewed in the literature and we refer readers to those articles (23,24). Recently, the benefit of continuing angiogenetic suppression beyond first disease progression in mCRC patients was confirmed recently by the ML18147 study. In this randomized phase III trial, bevacizumab beyond disease progression while switching the cytotoxic chemotherapy improved the PFS (5.7 *vs*. 4.1 months) and OS (11.2 *vs*. 9.8 months) in the group that continued bevacizumab compared to those who didn't (25).

Despite benefit in metastatic setting, the addition

of bevacizumab had not improved clinical outcome in adjuvant setting in CRC (26,27). The AVANT trial randomized curatively resected stage III or high risk stage II colon cancer to 3 arms: FOLFOX4 for 12 cycles, bevacizumab 5 mg/kg plus FOLFOX4 for 12 cycles or bevacizumab 7.5 mg/kg plus oxaliplatin and capecitabine (XELOX); both bevacizumab arm will receive additional bevacizumab 7.5 mg/kg monotherapy every 3 weeks for eight cycles after completing combination therapy. The hazard ratio (HR) for disease-free survival (DFS) and OS for bevacizumab-FOLFOX4 versus FOLFOX4 were 1.17 (95% CI: 0.98-1.39; P=0.07) and 1.27 (95% CI: 1.03-1.57; P=0.02) respectively; and for bevacizumab-XELOX versus FOLFOX4 was 1.07 (95% CI: 0.9-1.28; P=0.44) and 1.15 (95% CI: 0.93-1.42; P=0.21) respectively (27). In summary, in the AVANT trial, the addition of bevacizumab did not improve DFS including subset analysis according to baseline VEGF-A or VEGFR-1 or 2 levels. Interestingly, the data suggested potential detrimental effect in the bevacizumab-containing arms from more relapses and deaths due to disease progression (27). One hypothesis proposed to explain the failure of bevacizumab in adjuvant setting was that established CRC metastatic tumors were more dependent on angiogenesis than micrometastases, which were more sensitive to cytotoxic chemotherapy (28,29).

Aflibercept

Aflibercept (or VEGF Trap) is a recombinant fusion protein consisting of the extracellular domains of human VEGFR-1 and 2 fused to the Fc portion of human IgG1 (30). The decoy protein binds to VEGF-A, VEGF-B and PIGF and
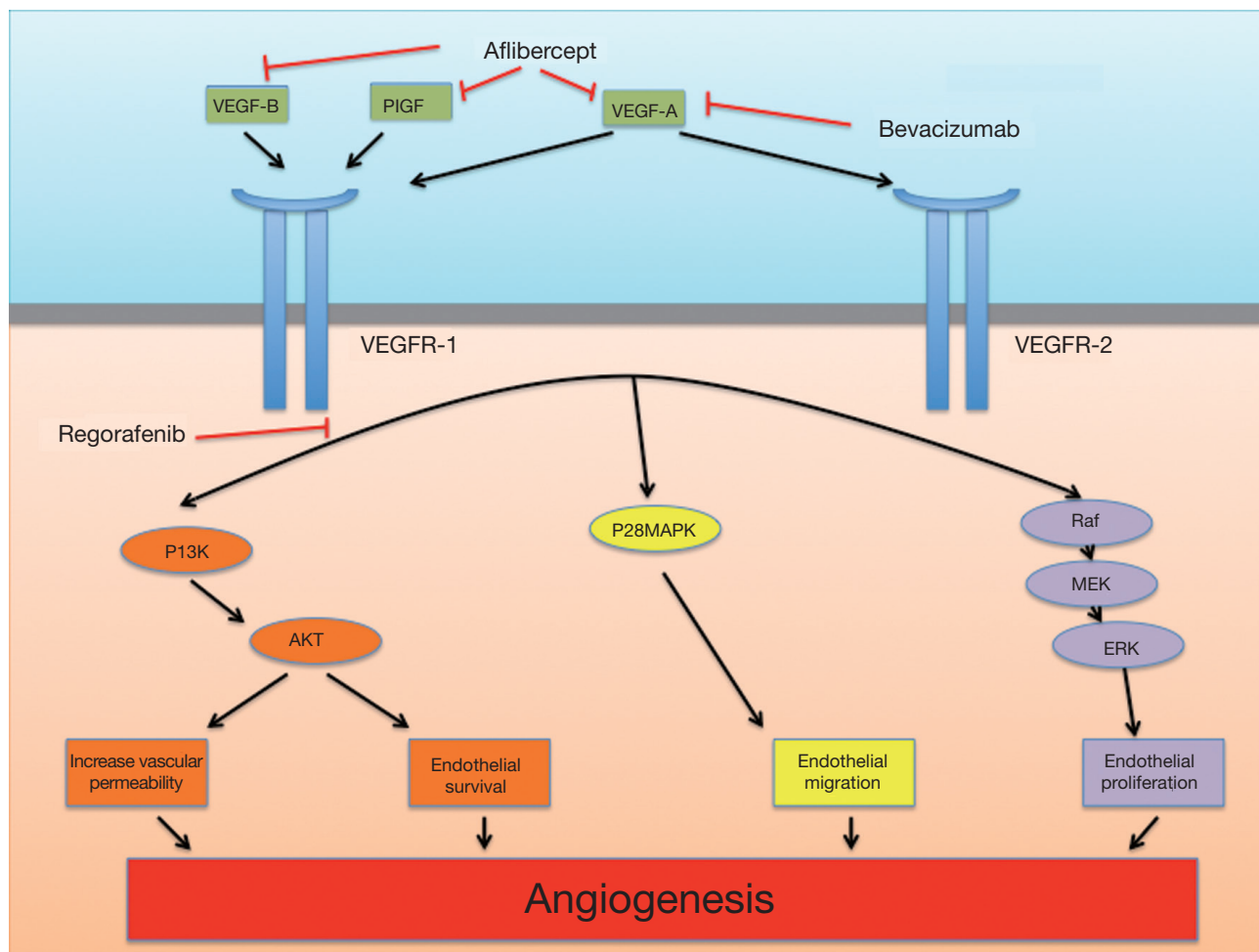
Figure 1 Pro-angiogenic targets of bevacizumab, aflibercept and regorafenib. Bevacizumab binds to VEGF-A and interrupts the interaction with VEGFR-1 and -2. In addition to VEGF-1, aflibercept binds to and interrupts the function of VEGF-B and PlGF. Regorafenib is a small molecule multi-kinase inhibitor which targets include VEGFR-1, -3, RAF, TIE-2, and mutant oncogenic kinases KIT, RET and BRAF

prevents the activation of VEGFR-1 and VEGFR-2 by these ligands, in contrast to bevacizumab in which binds VEGF-A only (*Figure 1*). VEGF-A is a key regulator of tumor angiogenesis and most human malignancies express high VEGF-A level (14,17). PlGF also plays an important role in angiogenesis by enhancing VEGF-A expression (31). Furthermore, patients with metastatic renal cell cancer previously treated with anti-VEGF therapy had increased PlGF level suggesting that PlGF may play a role in resistance to anti-VEGF treatment (32,33). In addition, compared to bevacizumab, aflibercept has a higher affinity for VEGF-A and its native receptor (34). Preclinically, aflibercept inhibited tumor growth, angiogenesis, metastases and improved the survival of tumor-bearing mice for various cancer types including pancreas, ovarian and renal cell carcinoma (30). Aflibercept in combination with cytotoxic drugs (Irinotecan, 5FU, paclitaxel, docetaxel), transtuzumab or radiotherapy exerted greater inhibition

of tumor vasculature and growth than aflibercept alone in tumor xenograft models (35-40).

In the phase I trial, 47 patients with refractory solid tumors or non-Hodgkin's lymphoma were enrolled to receive aflibercept intravenously every 2 weeks at doses ranging from 0.3 to 7.0 mg/kg (41). Dose-limiting toxicities (DLT) were rectal ulceration and proteinuria at 7.0 mg/kg dose. Aflibercept was also evaluated in combination with various chemotherapeutic agents including FOLFOX4 (42,43), irinotecan with 5FU and leucoverin (44), docetaxel (45) alone and with cisplatin (46), and gemcitabine (47) in advanced solid tumors patients. In combination with FOLFOX4, aflibercept doses 2, 4 and 5 mg/kg were explored in patients with advanced solid tumors and no DLT was encountered in the phase I trial (42). Grade 3 or worse toxicities included neutropenia, thrombocytopenia, hypertension, proteinuria, hemorrhagic events (include 1 Grade 5 hemorrhagic stroke at 4 mg/kg), febrile neutropenia and deep vein thrombosis.

In subset of mCRC, partial response was observed.

Aflibercept was also evaluated in combination with irinotecan, 5FU and leucovorin in a dose-escalation study. Aflibercept doses 2, 4, 5 and 6 mg/kg doses every 2 weeks were explored and DLTs observed were Grade 3 proteinuria lasting >2 weeks, acute nephrotic syndrome and thrombotic microangiopathy at 4 mg/kg; Grade 3 stomatitis, esophagitis reflux at 5 mg/kg; and, febrile neutropenia, Grade 3 stomatitis and Grade 3 abdominal pain due to intestinal obstruction at 6 mg/kg (44). As such, aflibercept 4 mg/kg dose level was selected as for further development in combination with irinotecan, 5-FU and leucovorin (41,42,44). The pharmacokinetic studies showed that aflibercept's elimination half-life ranged from less than 1-3 days for free aflibercept and was approximately 18 days for VEGF-bound aflibercept (41,48).

The benefit of aflibercept in combination with FOLFIRI was confirmed in the pivotal phase III VELOUR trial. In the study, patients with metastatic CRC previously treated with oxaliplatin-containing regimen, irregardless of prior bevacizumab treatment, were randomly assigned to received aflibercept 4 mg/kg IV every 2 weeks or placebo combination with FOLFIRI. Overall response rate was 19.8% in the aflibercept arm compared to 11.1% in the placebo (P=0.0001). Compared to the control group, the aflibercept-containing arm had better PFS (6.9 *vs.* 4.67 months; HR 0.758; P<0.0001) and OS (13.5 *vs.* 12.06 months; HR 0.817; P=0.0032). Pre-planned subgroup analysis showed that prior bevacizumab use did not influence aflibercept's effect on PFS and OS though the study was not powered to show a treatment difference between arms (9,18). Toxicities related to aflibercept were consistent with those expected from the anti-VEGF drug class (49). When compared to the bevacizumab-related toxicity profile reported in the phase III trial of IFL with or without bevacizumab, the frequency of grade 3 or 4 proteinuria seemed to be higher for aflibercept than bevacizumab (7.5% *vs.* 0.8%) though risks for Grade 3 or 4 bleeding (2.8% *vs.* 3.1%) and hypertension (11% *vs.* 11%) seemed similar (9,21).

Together with the results from ML18147 study, clinicians now have the option of using aflibercept or bevacizumab with FOLFIRI in mCRC patients who progressed following oxaliplatin containing regimen. The benefit achieved by aflibercept and bevacizumab in second-line setting seemed comparable: in ML18147 study, continuing bevacizumab into second-line while switching the cytotoxic chemotherapy achieved a median OS improvement of 1.4 months (HR 0.81, 95% CI: 0.69-0.94; P=0.0062) (25) whilst the addition of aflibercept to FOLFIRI in the VELOUR trial achieved a comparable median OS survival improvement of 1.4 months

(HR 0.817, 95.34% CI: 0.713-0.937; P=0.0032) (9). The frequency of vascular-related adverse events seemed to be higher with aflibercept than bevacizumab treatment when comparing across trials. Cost is another consideration: aflibercept treatment costs, in average, $11,063 per month, which is more than twice as high as bevacizumab therapy. As such, aflibercept is not recommended routinely in metastatic CRC patients who progressed on oxaliplatin-containing treatment until more evidence available.

Regorafenib

Regorafenib is structurally related to sorafenib and differ from the latter by the presence of a fluorine atom in the center phenyl ring (50,51). The slight structural difference resulted in higher inhibitory potency against various pro-angiogenic receptors than sorafenib including VEGFR2 (IC50 3 *vs.* 90 nM respectively), FGFR1 (202 *vs.* 580 nM) though IC50s for PDGFRβ were similar (52,53). Other receptor kinases inhibited by regorafenib include VEGFR1, -3, RAF, TIE2, and mutant oncogenic kinases KIT, RET and BRAF (52,54). Interestingly, sorafenib did not demonstrate significant anti-tumor activity in CRC. The effect of sorafenib plus 5-FU in colorectal tumor xenograft strudy was not significantly better than treatment using either drugs alone (55). Two of the 66 refractory mCRC patient who received sorafenib in four phase I had best response as stable disease and no objective response was observed (56). In contrast, regorafenib showed significant anti-cancer efficacy in CRC. In preclinical colorectal tumor xenograft studies, regorafenib treatment reduced tumor microvasculature and inhibited tumor growth in a dose-dependent manner (57). N-Oxide (M-2) and N-Oxide/N-desmethyl metabolite (M-5) are 2 active metabolites of regorafenib with potent pharmacologic activities similar to but distinct from regorafenib (57).

In the phase I trial, 53 patients with advanced solid tumor received regorafenib at the dose levels from 10 to 220 mg daily, 21 days on followed by 7 days off in repeating cycle. The most frequent adverse events were voice changes, hand-foot skin reaction, mucositis, diarrhea and hypertension. DLTs at 160 mg were skin toxicity and vomiting; skin toxicity, abdominal pain and asthma at 220 mg. On the basis of these observations, 160 mg once daily orally was determined the maximum tolerated dose (MTD) and the recommended dose for future studies. For efficacy, one mCRC patient had partial response at 220 mg but stopped treatment after 5.3 months for treatment-related side effects (58). Pharmacokinetic studies showed that terminal half-life of regorafenib were 20-40 hours, thus supporting once daily dosing schedule. At the 160 mg

dose, plasma exposure at steady state of M-2 and M-5 were similar to or slightly greater than parent drug. The terminal half-life of M2 was comparable to regorafenib but the elimination of M-5 was slower with an estimated half-life of 51-64 hours (58,59). The unbound plasma concentration of the pharmacologically active species at the 160 mg dose level exceeded the IC50 of many target kinases, therefore, plausible that M-2 and M-5 may contribute to the clinical activity of regorafenib (58).

In an expanded phase I study specific for relapsed or refractory mCRC patients, 38 patients received regorarefnib dose levels ranging from 60-220 mg daily administered on a "21 days on followed by 7 days off" dosing schedule. Enrolled patients had received a median of 4 previous lines of treatment. The most common adverse event leading to dose reduction was hand-foot skin reaction. Other treatment-related adverse events leading to regorafenib discontinuation included hypertension, fatigue, thrombocytopenia and diarrhea. Among 25 patients treated at 160 mg dose level, 6 patients permanently discontinued due to treatment-related adverse events including hand-foot skin reaction, hypertension, fatigue, thrombocytopenia and duodenal ulcer. In efficacy evaluation, 27 evaluable patients achieved 74% disease control rate with partial response in 1 patient (4%) and stable disease in 19 patients (70%). Overall, regorafenib was well tolerated and adverse events were manageable (59).

The multi-national phase III CORRECT trial enrolled mCRC patients who had received all locally-approved standard therapies and had progressed during or within 3 months after the last standard therapy (10). Patients were randomized in a 2:1 ratio to receive regorafenib or placebo. 500 patients received regorafenib at 160 mg orally 21 days on 7 days off and 253 patients received placebo. Median OS was 6.4 months in the regorafenib group versus 5.0 months in the placebo group (HR 0.77; 95% CI: 0.64-0.94; one-sided P=0.0052). Similar clinical benefit was observed in patient with colon cancer and rectal. The most common treatment-related Grade 3 or worse adverse events were hand-foot skin reaction (17%), fatigue (10%), diarrhea (7%), hypertension (7%), and rash or skin desquamation (6%), consistent with that observed in earlier phase trials. These adverse events were mostly manageable with dose reduction or interruption.

Conclusion

Angiogenesis is now a validated therapeutic target in CRC patients with macroscopic metastases. Recent development added 2 new anti-angiogenic drugs to the CRC treatment armamentarium and confirmed the advantage of continuing angiogenic suppression beyond first progression in metastatic CRC patients (60). Evidence so far supports the use of bevacizumab in both first- and second-line treatment of metastatic CRC patients. In comparison, the role of aflibercept in these settings remains unclear given the comparable efficacy but higher cost compared to bevacizumab. Aflibercept targets a broader set of pro-angiogenic growth factors than bevacizumab, and has the theoretical advantage of more effective angiogenic suppression and overcoming bevacizumab resistance. However, these hypotheses are yet to be confirmed in clinical studies. As the chemotherapeutic options and supportive care improve, more metastatic CRC patients nowadays have good performance status by the time they exhausted all standard therapy. For them, regorafenib is a welcomed option in addition to participation in clinical trials. Looking back, the overall survival of patients with metastatic CRC has increased several folds when compared to decades ago even though, it seemed, each drug achieved only incremental improvement individually. However, it is clear more novel treatment approaches are needed to continue this trend.

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