Aflibercept in the treatment of patients with metastatic colorectal cancer: latest findings and interpretations

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Abstract: Inhibition of angiogenesis is an established adjunct in the treatment of metastatic colorectal cancer. Bevacizumab, a monoclonal antibody that binds to vascular endothelial growth factor (VEGF) A, improves clinical outcomes when added to standard chemotherapy for metastatic colorectal cancer. Unfortunately, the development of resistance is inevitable, and novel therapeutic strategies are needed. Aflibercept is an intravenously administered fusion protein of the human vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2 extracellular domains. This antiangiogenic agent binds to VEGF A, VEGF B, and placental growth factor 1 (PlGF1) and PlGF2 with high affinity and inhibits downstream signaling. Common side effects of single agent aflibercept are similar to other antiangiogenic drugs and include hypertension, proteinuria, fatigue, and headache. Recent clinical data regarding the efficacy of aflibercept with standard chemotherapy for metastatic colorectal cancer, associated adverse events, and future areas of research are reviewed.

Keywords: aflibercept, angiogenesis, metastatic colorectal cancer

Introduction

Globally, colorectal cancer is the third most common cancer diagnosed in men, the second most commonly diagnosed cancer in women, and was estimated to lead to 608,000 deaths in 2008 [Ferlay *et al.* 2010]. The majority of patients with metastatic colorectal cancer (MCRC) cannot be cured. The goals of therapy are palliative and focus on prolongation of survival and maintenance of quality of life. Standard cytotoxic chemotherapy for MCRC includes fluoropyrimidine [capecitabine or 5-fluorouracil (5FU)] in combination with irinotecan or oxaliplatin [Tournigand *et al.* 2004; Sanoff *et al.* 2008]. Targeted therapies, that block specific molecules involved with tumor progression, have increased treatment options for MCRC. The regulation of angiogenesis is critical for tumor growth and metastasis [Folkman, 1995]. The advent of antiangiogenic therapy was a major breakthrough in the treatment of MCRC. This review discusses the importance of angiogenesis in MCRC and the clinical efficacy of aflibercept (ziv-aflibercept, Zaltrap, Regeneron Pharmaceuticals, Tarrytown, NY, USA and Sanofi-Aventis Oncology, Bridgewater, NJ, USA), a novel antiangiogenic drug in this devastating disease.

Inhibition of angiogenesis in metastatic colorectal cancer

Physiologic angiogenesis is tightly regulated [Folkman, 2003]. Signaling pathways that have been implicated in the regulation of angiogenesis include vascular endothelial growth factor (VEGF), angiopoietins, Notch, and integrins (reviewed by Carmeliet) [Carmeliet, 2005]. The VEGF family consists of five growth factors: VEGF A, VEGF B, VEGF C, VEGF D, and placental growth factor (PlGF) [Ellis and Hicklin, 2008]. A cascade of events occur when these growth factors bind to the cell surface tyrosine kinase receptors VEGFR1, VEGFR2, or VEGFR3. VEGF A is the most proangiogenic factor, and binding to VEGFR1 or VEGFR2 leads to endothelial cell proliferation, survival, migration, invasion, recruitment of bone marrow

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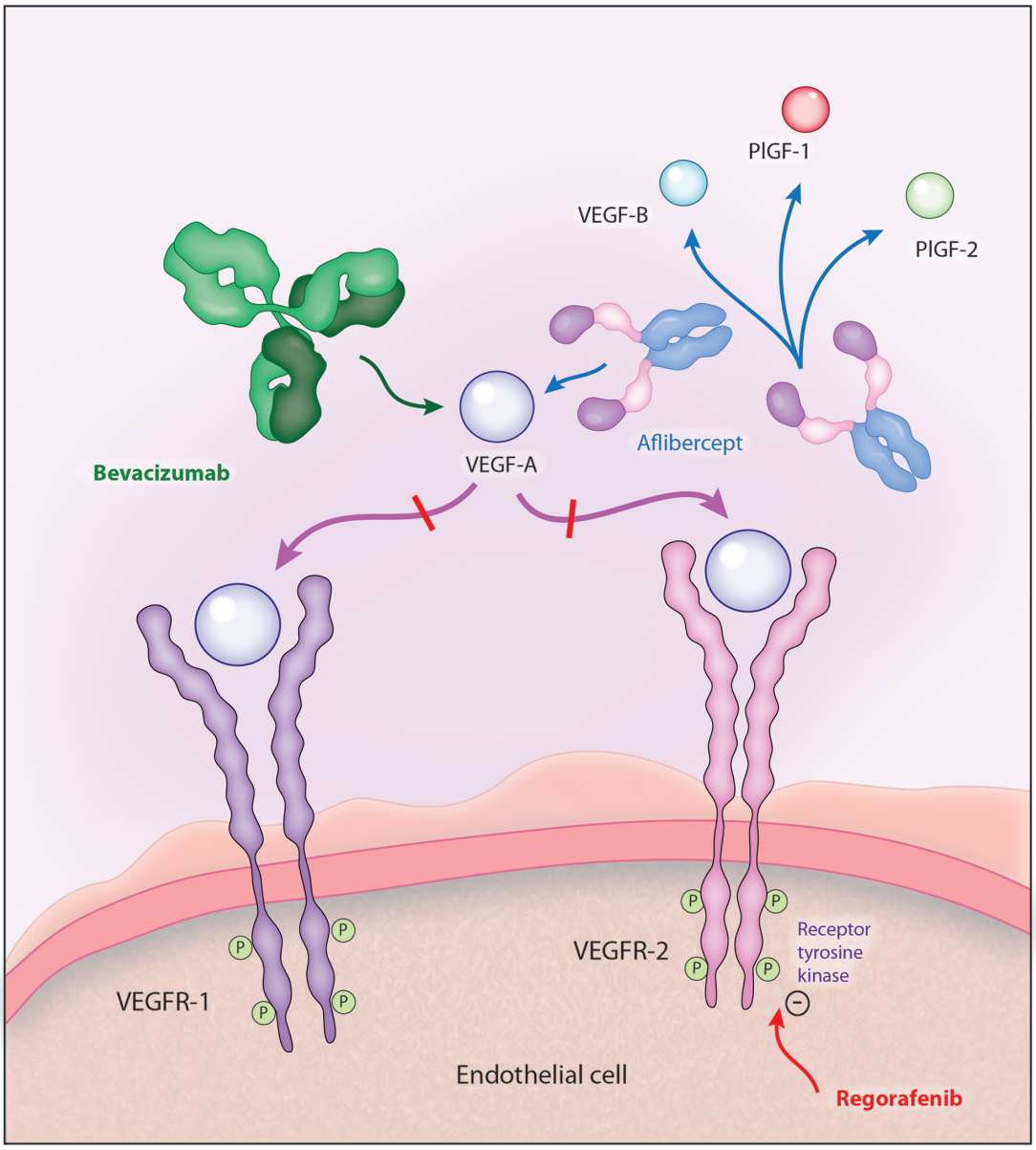

Figure 1. A schema depicting the mechanisms of action of bevacizumab, aflibercept, and regorafenib in relation to an endothelial cell with VEGFR1 and VEGFR2. Note, regorafenib is a multitargeted receptor tyrosine kinase that also inhibits other receptors such as VEGFR3, TIE2, fibroblast growth factor receptor, and others that are not shown here. (Illustration courtesy of Alessandro Baliani. Copyright © 2013. Adapted with permission from Van Cutsem *et al.* [2012].) VEGF, vascular endothelial growth factor; VEGFR1, vascular endothelial growth factor receptor 1; VEGFR2, vascular endothelial growth factor receptor 2; PlGF, placental growth factor.

progenitor cells, and increased vascular permeability and vasodilation [Ellis and Hicklin, 2008]. Other VEGFs, such as VEGF B and PlGF, also contribute to tumor-induced angiogenesis (reviewed by Fischer and colleagues) [Fischer *et al.* 2008]. PlGF binds to VEGFR1 and has four isoforms, PlGF1, PlGF2, PlGF3, and PlGF4 [Yang *et al.* 2003]. The resultant peritumoral vasculature has functional and structural abnormalities, including the absence of normal vascular hierarchy and functional lymphatic vessels, leaking of the endothelial layer, abnormal blood flow, and increased interstitial pressure [Ellis and Hicklin, 2008; Fukumura *et al.* 2010].

VEGF inhibition with antiangiogenic drugs is postulated to block new blood vessel formation, decrease vascular permeability, and lead to capillary regression [Ellis and Hicklin, 2008; O'Connor *et al.* 2009]. Antiangiogenic drugs

Table 1. Randomized phase III trials evaluating bevacizumab with combination chemotherapy in metastatic colorectal cancer.

Study	Treatment arm	*n*	Progression-free survival			Overall survival			Grade 3 or higher adverse events (%)	
			Median (months)	Hazard ratio	*p* value	Median (months)	Hazard ratio	*p* value	Any	Of special interest to antiangiogenic treatment*
First-line therapy										
AVF2107g [Hurwitz *et al.* 2004]	IFL + bevacizumab 5 mg/kg every 2 weeks	402	10.6	0.54	<0.001	20.3	0.66	<0.001	84.9	35.8[$]
	IFL + placebo	411	6.2			15.6			74.0	21.8[$]
N016966 [Saltz *et al.* 2008]	FOLFOX/CAPOX + bevacizumab[‡]	701	9.4	0.83 (97.5% CI 0.72–0.95)	0.0023	21.3	0.89 (97.5% CI 0.76–1.03)	0.0769	80	16
	FOLFOX/CAPOX + placebo	699	8			19.9			75	8
Second-line treatment										
E3200 Previous treatment with FU/OX [Giantonio *et al.* 2007]	FOLFOX + bevacizumab 10 mg/kg every 2 weeks	286	7.3	0.61	<0.0001	12.9	0.75	0.0011	75	14.6
	FOLFOX	291	4.7			10.8			61	5.1
ML18147 After progression on first line chemotherapy with bevacizumab [Arnold *et al.* 2012]	IRI or OX based chemotherapy + bevacizumab 2 mg/kg/week	409	5.7	0.68 (95% CI 0.59–0.78)	<0.0001[§]	11.2	0.81 (95% CI 0.69–0.94)	0.0062[§]	64	12
	IRI or OX based chemotherapy	411	4.1			9.8			58	6

*Proteinuria, hypertension, bleeding, arteriothromboembolic events, perforation, venous thromboembolic events.
[$]Includes all grade arterial and venous thrombotic events, incidence of grade 3 or higher was not available.
[‡]Bevacizumab 5 mg/kg every 2 weeks with FOLFOX or 7.5 mg/kg every 3 weeks with CAPOX.
[§]Unstratified log-rank test.
CAPOX, capecitabine, oxaliplatin; CI, confidence interval; FOLFOX, infusional 5-fluorouracil, leucovorin, oxaliplatin; FU, 5-fluorouracil; IFL: irinotecan, 5-fluorouracil, leucovorin; IRI, irinotecan; OX, oxaliplatin.

may also restore dendritic cell function and sensitize tumor endothelial cells to chemotherapy. In addition, delivery of chemotherapy is improved through normalization of the vasculature and decreased interstitial pressure [Jain, 2005; Jain *et al.* 2009]. Bevacizumab, a monoclonal antibody that binds to VEGF A, changed the landscape of MCRC therapy (Figure 1). The addition of bevacizumab significantly improved outcomes when added to standard chemotherapy for MCRC in the first- and second-line setting [Hurwitz *et al.* 2004, 2005; Kabbinavar *et al.* 2005; Giantonio *et al.* 2007; Saltz *et al.* 2008; Arnold *et al.* 2012] (Table 1).

Inhibition of angiogenesis is associated with specific side effects. Hypertension is a class effect of antiangiogenic drugs which is manageable with standard antihypertensive medication. Grade 3/4 hypertension was observed in 4–16% of patients treated with chemotherapy and bevacizumab in the pivotal trials [Hurwitz *et al.* 2004, 2005; Kabbinavar *et al.* 2005; Giantonio *et al.* 2007; Saltz *et al.* 2008; Arnold *et al.* 2012]. Other severe adverse events associated with antiangiogenic therapies such as bevacizumab occur less frequently (<5%) and include proteinuria (grade 3/4 2%) [Wu *et al.* 2010], bleeding, delayed wound healing, gastrointestinal perforation, arterial thromboembolic events [Hurwitz *et al.* 2004, 2005; Kabbinavar *et al.* 2005; Giantonio *et al.* 2007; Saltz *et al.* 2008; Arnold *et al.* 2012]. Meta-analyses have demonstrated conflicting results regarding the association between bevacizumab and venous thromboembolic disease [Scappaticci *et al.* 2007; Nalluri *et al.* 2008; Hurwitz *et al.* 2011].

Resistance to antiangiogenic therapy

It is difficult to ascertain the relative contributions of resistance to cytotoxic chemotherapy *versus* antiangiogenic therapy to clinical progression that inevitably arises for patients. With respect to inhibition of angiogenesis, two resistance mechanisms have been proposed: intrinsic and adaptive. Intrinsic resistance exists in tumors prior to treatment, while adaptive resistance arises after an initial response to antiangiogenic therapy [Bergers and Hanahan, 2008]. For both modalities, resistance may develop via signaling through alternate compensatory pathways, vascular remodeling, protection of tumor vasculature through recruitment of proangiogenic cells or increasing pericyte coverage, increased ability to coopt normal vasculature, and increased metastatic spread [Bergers and Hanahan, 2008].

PlGF promotes angiogenesis and tumor growth [Fischer *et al.* 2007; Yao *et al.* 2011]. PlGF may mediate resistance to antiangiogenic drugs by promoting proangiogenic signals when VEGF A is blocked [Fischer *et al.* 2007; Yao *et al.* 2011]. Treatment with bevacizumab and combination chemotherapy is associated with an increase in circulating PlGF levels in patients with MCRC [Willett *et al.* 2009; Kopetz *et al.* 2010; Loupakis *et al.* 2011]. PlGF1 and 2 immunohistochemical expression was associated with poor prognosis in a series of colorectal tumors (n = 94) [Escudero-Esparza *et al.* 2009]. PlGF blockade inhibited tumor growth in a human colon cancer xenograft model, demonstrating the therapeutic potential of PlGF inhibition [Fischer *et al.* 2007].

Aflibercept

Aflibercept was designed to block angiogenesis by binding VEGF A, VEGF B, PlGF1 and PlGF2 and prevent downstream biological effects (Figure 1) [Holash *et al.* 2002]. It is a recombinant humanized fusion protein which consists of the extracellular domains of VEGFR1 and VEGFR2 with the constant region (Fc) of human immunoglobin G1 [Holash *et al.* 2002]. Aflibercept has a higher VEGF A binding affinity than bevacizumab [dissociation constant (Kd) of ~1 pM] [Holash *et al.* 2002] compared with around 500 pM for bevacizumab [Ferrara *et al.* 2004]. The ability of aflibercept to bind to VEGF B and PlGF in addition to the high binding affinity for VEGF A may provide more complete blockade of angiogenesis. Preclinically, treatment with aflibercept resulted in tumor growth inhibition in a variety of xenograft models, including human colon cancer [Holash *et al.* 2002; Kim *et al.* 2002; Rudge *et al.* 2007; Gomez-Manzano *et al.* 2008]. Aflibercept demonstrated synergistic activity with 5FU and with irinotecan in animal models [Chiron *et al.* 2007]. The level of VEGF-bound aflibercept is considered to reflect the amount of endogenous VEGF in normal and tumor tissues. Free aflibercept can bind to newly secreted VEGF. *In vivo*, the biological effects of aflibercept correlated with the presence of free aflibercept in excess of VEGF-bound aflibercept [Rudge *et al.* 2007]. The efficacy and safety of aflibercept alone or in combination with chemotherapy has been explored in several clinical trials.

Early phase clinical studies with aflibercept

Phase I

A phase I study evaluated intravenous aflibercept at doses ranging from 0.3 to 7.0 mg/kg intravenously every 2 weeks in 47 patients with advanced solid tumors [Lockhart *et al.* 2010]. Dose-limiting toxicities included rectal ulceration and proteinuria at the 7 mg/kg dose. Common drug-related toxicities included dysphonia and hypertension [Lockhart *et al.* 2010]. Three objective responses were observed. Aflibercept is cleared through binding to VEGF and subsequent proteolysis of the inactive VEGF–aflibercept complex through Fc or pinocytotic mediated pathways [Dixon *et al.* 2009]. Aflibercept has a dose-dependent half life which ranges 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 dose [Lockhart *et al.* 2010]. Despite the relatively short half life, free aflibercept levels were in excess of VEGF-bound aflibercept at aflibercept doses of at least 2 mg/kg or greater (Table 2) [Lockhart *et al.* 2010]. This was interpreted to represent binding of all available VEGF.

The utility of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) as a surrogate for tumor vasculature has been evaluated as a predictive biomarker for antiangiogenic drugs, including aflibercept. DCE-MRI parameters include area under the contrast agent time curve 90 s post injection (AUC90) and volume transfer constant (K^{trans}). These are mixed measures influenced by blood flow, vessel surface area and permeability [Tofts *et al.* 1999]. DCE-MRI was assessed at baseline, at 24 h, and at 8 weeks to evaluate the impact of aflibercept on tumor vascularity and permeability (n = 22) [Lockhart *et al.* 2010]. K^{trans} was significantly decreased in patients treated at all aflibercept doses except for the 0.3 mg/kg and 4 mg/kg cohorts. There was a statistically significant increase in AUC90 between baseline and 24 h in the 0.3 mg and 7 mg/kg dose levels. This change was suggestive of an increase in tumor perfusion with aflibercept treatment, although it did not appear to be dose related. K^{trans} levels were not significantly different between patients with objective responses (n = 3) and nonresponders, however the number of evaluable patients was small. The recommended phase II dose (RPTD) of aflibercept was 4 mg/kg every 2 weeks based on pharmacokinetics and drug-related toxicities.

Two phase I trials evaluated escalating doses of aflibercept in combination with infusional 5FU, folinic acid, and irinotecan (FOLFIRI) [Van Cutsem *et al.* 2011; Yoshino *et al.* 2012] (Table 2). The primary endpoint was to determine the RPTD of aflibercept in combination with FOLFIRI. Van Cutsem and colleagues evaluated the regimen in patients with advanced solid tumors [Van Cutsem *et al.* 2011]; the response rate (RR) was 26% across all aflibercept dose levels. The RR was lower (8.3% at 4 mg/kg of aflibercept) in the parallel trial of Japanese patients with MCRC who had received at least one prior chemotherapy regimen [Yoshino *et al.* 2012]. The most common severe (grade 3/4) adverse events were neutropenia, hypertension, and diarrhea. The pharmacokinetic profile of aflibercept was not affected by concurrent therapy with FOLFIRI [Lockhart *et al.* 2010; Van Cutsem *et al.* 2011; Yoshino *et al.* 2012]. At doses of 4 mg/kg, free aflibercept was present in excess of VEGF-bound aflibercept for most patients, suggesting maximal VEGF blockade [Van Cutsem *et al.* 2011; Yoshino *et al.* 2012]. The recommended phase II dose of aflibercept was 4 mg/kg in combination with FOLFIRI based on the pharmacokinetics, safety profile, and preliminary evidence of antitumor activity [Van Cutsem *et al.* 2011; Yoshino *et al.* 2012].

This regimen was further evaluated in an expansion cohort that randomized patients with advanced solid tumors to FOLFIRI plus placebo or aflibercept at 4 mg/kg for cycle 1, followed by aflibercept plus FOLFIRI for all subsequent cycles (Table 2) [Khayat *et al.* 2013]. The adverse event profile was similar to the prior aflibercept trials [Lockhart *et al.* 2010; Van Cutsem *et al.* 2011; Yoshino *et al.* 2012]. Four patients had a partial response (15%), and 54% had stable disease for over 3 months [Khayat *et al.* 2013]. DCE-MRI was performed at baseline, cycle 1, and cycle 2. No significant perfusion changes were observed in response to aflibercept and there were no predictive baseline imaging characteristics (n = 21). This is in contrast to the DCE-MRI changes noted in the phase I aflibercept monotherapy study [Lockhart *et al.* 2010]. The validity and methodology of DCE-MRI as a biomarker for assessing antiangiogenic therapy is the subject of ongoing research. Preliminary data suggest that DCE-MRI is not a promising predictive marker for clinical benefit from aflibercept.

A phase I study evaluated infusional 5FU, folinic acid, and oxaliplatin (FOLFOX) with escalating

Table 2. Summary of key phase I and II clinical trials evaluating aflibercept in colorectal cancer.

Patient population	Treatment	Results	Major adverse events	Correlative studies
Phase I				
Advanced solid tumors (n = 38) including MCRC (n = 23) ECOG 0–2 Prior therapies:* irinotecan 63% bevacizumab 13% [Van Cutsem *et al.* 2011]	Aflibercept (2–6 mg/kg) + FOLFIRI	RPTD: Aflibercept 4 mg/kg with FOLFIRI Results across all dose levels (n = 34 evaluable): RR 26% SD 65%	DLT (1 patient each) 4 mg/kg: proteinuria, nephrotic syndrome 5 mg/kg: grade 3 stomatitis, grade 3 esphageal reflux 6 mg/kg: febrile neutropenia, grade 3 stomatitis, grade 3 abdominal pain	$AUC_{0\text{-}2\ weeks}$ for aflibercept 2–6 mg/kg: 12.5–20.8 μg*day/ml on cycle 1 and 31.6–38.0 μg*day/ml on cycle 2 At 4 mg/kg, free aflibercept in excess of VEGF-bound aflibercept No antiaflibercept AB
Japanese patients with pretreated MCRC ECOG 0–1 Prior therapies$: irinotecan 18.8% oxaliplatin 93.8% bevacizumab 62.5% [Yoshino *et al.* 2012]	Aflibercept (2 or 4 mg/kg + FOLFIRI	RPTD: Aflibercept 4 mg/kg with FOLFIRI 4 mg/kg results (n = 12): RR 8.3% DCR 75.0% PFS 7.59 mo	Aflibercept 4 mg/kg Grade 3/4 Neutropenia 76.9% Leukopenia 61.5% Hypertension 30.8%	Free aflibercept in excess of VEGF-bound aflibercept No antiaflibercept AB
Advanced solid tumors (n = 27) including MCRC (n = 19) ECOG 0–2 [Khayat *et al.* 2013] Prior therapies*: irinotecan 78% bevacizumab 19%	FOLFIRI + placebo or aflibercept 4 mg/kg cycle 1, then all patients received FOLFIRI + aflibercept 4 mg/kg	All evaluable patients (n = 26) RR 15% SD 65% SD over 3 months 54%	All patients, grade 3/4 Neutropenia 37% Fatigue 30% Hypertension 30%	Free aflibercept in excess of VEGF-bound aflibercept No changes in perfusion with DCE MRI No antiaflibercept AB
Advanced solid tumors (n = 32) including MCRC (n = 1) ECOG 0–2 [Limentani *et al.* 2008]	Aflibercept (2–5 mg/kg) + FOLFOX	RPTD: aflibercept 4 mg/kg with FOLFOX Results across all dose levels RR 16% SD 31%	No DLT related to aflibercept observed Grade 3/4 Hypertension 13% Proteinuria 13% Hemorrhage 9%	Administration of FOLFOX did affect aflibercept concentrations At 4 mg/kg, free aflibercept in excess of VEGF-bound aflibercept
Phase II				
Pretreated MCRC ECOG 0–2‡ Bevacizumab naïve (n = 24) [Tang *et al.* 2012]	Aflibercept 4 mg/kg	RR 0% SD ≥ 16 weeks 20.8% mPFS 2.0 months (95% CI 1.7–8.6)	Grade 3/4 Hypertension 13.5% Proteinuria 10.8% Fatigue 6.8% Headache 6.8%	Free aflibercept in excess of VEGF-bound aflibercept No correlation between clinical benefit and free *versus* bound aflibercept levels One patient developed anti-aflibercept AB
Prior bevacizumab (n = 50)		RR 2.0% SD ≥ 16 weeks 12.0% mPFS 2.4 months (95% CI 1.9–3.7)		

Table 2. (Continued)

Patient population	Treatment	Results	Major adverse events	Correlative studies
AFFIRM No prior systemic therapy for metastatic disease ECOG 0-2 [Pericay *et al.* 2012]	FOLFOX Aflibercept 4 mg/kg (n = 119)	RR 49.1% (95% CI 39.7–58.6) mPFS 8.48 months PFS at 12 months 25.8% (95% CI 17.2–34.4)	Grade 3–4 events that were > 5% higher in aflibercept arm: Hypertension Proteinuria Neutropenia Diarrhea Infections	Free aflibercept in excess of VEGF-bound aflibercept
	FOLFOX (n = 117)	RR 45.9% (95% CI 36.4–55.7) mPFS 8.77 months PFS at 12 months 21.2% (95% CI 12.2–30.3)		

FOLFIRI, irinotecan 180 mg/m^2 over 1 h on day 1, then leucovorin 200 mg/m^2 and 5-fluorouracil (400 mg/m^2 intravenous bolus then 600 mg/m^2 intravenous infusion over 22 h on days 1 and 2), day 1 and 2 [Van Cutsem *et al.* 2011; Khayat *et al.* 2013]; irinotecan 150 mg/m^2 day 1, 5-fluorouracil (400 mg/m^2 intravenous bolus then 2400 mg/m^2 intravenous infusion over 46 h), leucovorin 200 mg/m^2 day 1 [Yoshino *et al.* 2012].

FOLFOX, oxaliplatin, infusional 5-fluorouracil, leucovorin. Oxaliplatin 85 mg/m^2 and leucovorin 200 mg/m^2 over 2 h day 1, 5-fluorouracil 400 mg/m^2 intravenous bolus then 600 mg/m^2 intravenous infusion over 22 h on days 1 and 2 [Limentani *et al.* 2008]; oxaliplatin 100 mg/m^2 and leucovorin 400 mg/m^2 over 2 h day 1, 5-fluorouracil 400 mg/m^2 intravenous bolus then 2400 mg/m^2 intravenous infusion over 46 h [Pericay *et al.* 2012].

*Median three prior lines of chemotherapy.

$Median one prior line of chemotherapy.

‡Median two prior lines of chemotherapy.

AB, antibodies; AUC, area under the curve; CI, confidence interval; DCE MRI, dynamic contrast enhanced magnetic resonance imaging; DCR, disease control rate; DLT, dose limiting toxicity; ECOF, Eastern Cooperative Oncology Group; MCRC, metastatic renal cell carcinoma; mPFS, median progression-free survival; PFS, progression-free survival; RPTD, recommended phase II dose; RR, response rate; SD, stable disease; VEGF, vascular endothelial growth factor.

doses of aflibercept (2–5 mg/kg) in patients with advanced solid tumors (Table 2) [Limentani *et al.* 2008]. No dose-limiting toxicities were observed and the pharmacokinetic profile of aflibercept was not affected by FOLFOX chemotherapy. Objective responses were observed in 16% of patients. The recommended phase II dose of aflibercept was 4 mg/kg in combination with FOLFOX. Given the efficacy of bevacizumab in MCRC, and the potent blockade of angiogenesis induced by aflibercept, it was evaluated in MCRC.

Phase II

The Princess Margaret Phase II Consortium conducted a phase II study of aflibercept in patients with MCRC who had received at least one prior systemic therapy (Table 2) [Tang *et al.* 2012]. Patients were enrolled in two cohorts: bevacizumab naïve (n = 24) and prior bevacizumab (n = 51). The majority of patients (84%) had received prior irinotecan- and oxaliplatin-based chemotherapy and 46.7% of patients had been treated with an epidermal growth factor receptor (EGFR) inhibitor. The primary endpoint was a composite of RR and progression-free survival (PFS) at 16 weeks. In the bevacizumab-naïve cohort, no responses were observed, 20.8% of patients were progression free at 16 weeks, and median PFS was 2.0 months (Table 2). In the prior bevacizumab cohort, one patient had an objective response (2.0%), PFS at 16 weeks was 12.0%, and median PFS was 2.4 months. The most common serious adverse events were consistent with prior studies of aflibercept and antiangiogenic therapy in general: hypertension (13.5%) and proteinuria (6.8%). Pain attributed to aflibercept therapy (any grade, including the combination of headache, arthralgia, and myalgia) was observed in 74.3% of patients. Treatment-related toxicity led to dose reductions (16.2%), dose delays (27.0%), and discontinuation of treatment (13.5%).

There was no association between time interval from the last dose of bevacizumab or best response to prior treatment in the prior bevacizumab cohort. The mean ratio of free to VEGF-bound aflibercept was 1.82 (coefficient of variance 72%), and the ratio was below one in 18% of patients (8/44). There was no relationship between free to VEGF-bound aflibercept ratio and clinical benefit. One patient developed antiaflibercept antibodies but did not have any clinical sequelae. In contrast, antiaflibercept antibodies were not detected in the preceding phase I trials of aflibercept [Van Cutsem *et al.* 2011; Yoshino *et al.* 2012; Lockhart *et al.* 2010; Khayat *et al.* 2013]. Hypertension is a mechanism-related adverse event associated with antiangiogenic therapies. No association was found between clinical benefit (RR or 16-week PFS) from aflibercept and the presence of hypertension. This is consistent with results from an analysis of seven phase III trials of bevacizumab; early treatment-related blood pressure increases did not predict for benefit from bevacizumab based on PFS or overall survival [Hurwitz *et al.* 2013].

Single agent aflibercept had limited activity in pretreated patients with MCRC. Similarly, monotherapy with bevacizumab after progression on irinotecan-based chemotherapy resulted in a PFS of 2.7 months and a RR of 3.3% [Giantonio *et al.* 2007]. Given the mechanism of action, randomized trials evaluating the efficacy of aflibercept with combination chemotherapy in MCRC were conducted [Pericay *et al.* 2012; Van Cutsem *et al.* 2012].

The AFFIRM trial (Study of Aflibercept and Modified FOLFOX6 as First-Line Treatment in Patients with Metastatic Colorectal Cancer) randomized 236 chemotherapy-naïve patients with MCRC to FOLFOX with or without aflibercept given at 4 mg/kg [ClinicalTrials.gov identifier: NCT00851084] (Table 2) [Pericay *et al.* 2012]. The primary endpoint of this noncomparative phase II trial was PFS at 12 months, as assessed by an independent review committee (IRC). Patients were treated until progression and preliminary data were presented in abstract form. Baseline patient characteristics were similar in both arms, 61% of patients were of good performance status [Eastern Cooperative Oncology Group (ECOG) status 0–1], and 97.5% had prior adjuvant therapy. PFS at 12 months was similar in both arms (FOLFOX aflibercept 25.8% *versus* FOLFOX 21.2%). RR was also similar in both arms (FOLFOX aflibercept 49.1% *versus* FOLFOX 45.9%). Serious adverse events that were more common in the aflibercept arm included hypertension, proteinuria, neutropenia, diarrhea, and infections. Biomarker data were collected and the final results have not yet been published.

AFFIRM was a noncomparative phase II trial conducted in chemotherapy-naïve patients with MCRC. The efficacy of bevacizumab was evaluated in the same patient population in a phase III placebo-controlled trial in combination with oxaliplatin and a fluoropyrimidine in MCRC

Table 3. Results of VELOUR, the phase III clinical trial of aflibercept in metastatic colorectal cancer [Van Cutsem *et al.* 2012].

Result	FOLFIRI Aflibercept 4 mg/kg (n = 612)	FOLFIRI Placebo (n = 614)	Hazard ratio (95% confidence interval)	p value
Response rate	19.8%	11.1%	N/A	<0.001
Progression-free survival	6.90 months (median)	4.67 months (median)	0.758 (0.661–0.869)	<0.0001
Overall survival	13.50	12.06	0.817 (0.713–0.937)	0.0032
Reason for discontinuing treatment			N/A	N/A
Disease progression	49.8%	71.2%		
Adverse events	26.6%	12.1%		
Patient request	13.6%	7.3%		

FOLFIRI, irinotecan, infusional 5-fluorouracil, leucovorin; N/A, not applicable; NR, not reported.

(N016966, Table 1) [Saltz *et al.* 2008]. Median PFS as assessed by the investigators, the primary endpoint, improved from 8.0 months in the placebo group to 9.4 months with bevacizumab [hazard ratio (HR) 0.83; 97.5% confidence interval (CI) 0.72–0.95, $p = 0.0023$]. Bevacizumab did not demonstrate a statistically significant improvement in RRs as assessed by the IRC (38% bevacizumab, 38% placebo) or overall survival compared with patients treated with placebo [Saltz *et al.* 2008]. The optimal antiangiogenic partner for FOLFOX appears to be bevacizumab.

Evaluation of aflibercept in the phase III setting

The pivotal phase III VELOUR trial (VEGF-trap with irinotecan in colorectal cancer after failure of oxaliplatin) [ClinicalTrials.gov identifier: NCT00561470] established the role of aflibercept in MCRC [Van Cutsem *et al.* 2012]. The VELOUR trial randomized 1226 patients to FOLFIRI plus placebo or FOLFIRI plus aflibercept (Table 3). Inclusion criteria included the presence of MCRC not amenable to potentially curative treatment, an ECOG performance status of 0–2, no prior therapy with irinotecan, and progression while on or after completion of a prior oxaliplatin-containing regimen. Patients who relapsed within 6 months of finishing an oxaliplatin-based adjuvant therapy were eligible, however this comprised only 10% of the total patients accrued, and the remaining patients had received prior chemotherapy for metastatic disease. Approximately 98% of patients had good performance status (ECOG 0–1) and 30% had received prior treatment with bevacizumab. The addition of aflibercept to FOLFIRI significantly improved overall survival compared with placebo plus FOLFIRI (median survival 13.50 *versus* 12.06 months, HR 0.817; 95.34% CI 0.713–0.937; $p = 0.0032$). RR, assessed by an IRC, was significantly improved with the addition of aflibercept to FOLFIRI compared with placebo plus FOLFIRI (19.8% *versus* 11.1%, $p < 0.001$). The rate of surgery for metastatic disease was similar in both arms (2.0% aflibercept *versus* 1.6% placebo). A higher incidence of grade 3 and 4 adverse events associated with antiangiogenic therapy were observed in the aflibercept arm compared with placebo (Table 4), particularly hypertension (19.3% *versus* 1.5%). A higher incidence of grade 3 and 4 adverse events associated with chemotherapy was observed in the aflibercept arm, including diarrhea (19.3% *versus* 7.8%), asthenia, stomatitis, infections, neutropenia, and complicated neutropenia. The most common reason for discontinuing chemotherapy was progression in both arms. More patients stopped chemotherapy due to adverse events in the aflibercept arm (26.6%) compared with placebo (12.1%). A prespecified subgroup analysis of the VELOUR trial revealed no significant interaction between prior bevacizumab therapy (bevacizumab-naïve patients overall survival HR 0.788, 95.34% CI 0.669–0.927 *versus* prior bevacizumab exposure overall survival HR 0.862, 95.34% CI 0.673–1.104; p value for interaction = 0.5668). This was a subgroup analysis based on a prespecified stratification factor and not the primary endpoint of the trial. Aflibercept is the first biological therapy added

Table 4. Summary of the most frequent adverse events (incidence ≥20% or ≥5% higher in aflibercept arm) in AFFIRM trial [Van Cutsem *et al.* 2012]. Other anti-VEGF-associated events, and most frequent biologic abnormalities: safety population.

Adverse event*	Placebo/FOLFIRI (*n* = 605)			Aflibercept/FOLFIRI (*n* = 611)		
	All grades (%)	Grade 3 (%)	Grade 4 (%)	All grades (%)	Grade 3 (%)	Grade 4 (%)
Any	97.9	45.1	17.4	99.2	62.0	21.4
Diarrhea (PT)	56.5	7.6	0.2	69.2	19.0	0.3
Asthenic conditions (HLT)	50.2	10.4	0.2	60.4	16.0	0.8
Stomatitis and ulceration (HLT)	34.9	5.0	–	54.8	13.6	0.2
Nausea (PT)	54	3.0	–	53.4	1.8	–
Infections and infestations (SOC)	32.7	6.1	0.8	46.2	11.0	1.3
Hypertension	10.7	1.5	–	41.4	19.1	0.2
Hemorrhage	19	1.7	–	37.8	2.8	0.2
Epistaxis	7.4	–	–	27.7	0.2	–
GI and abdominal pains (HLT)	29.1	3.1	0.2	34	5.1	0.3
Vomiting (PT)	33.4	3.5	–	32.9	2.6	0.2
Decreased appetite (PT)	23.8	1.7	0.2	31.9	3.4	–
Weight decreased	14.4	0.8	–	31.9	2.6	–
Alopecia (PT)	30.1	–	–	26.8	–	–
Dysphonia (PT)	3.3	–	–	25.4	0.5	–
Constipation (PT)	24.6	1.0	–	22.4	0.8	–
Headache (PT)	8.8	0.3	–	22.3	1.6	–
Palmar-plantar erythrodysesthesia syndrome	4.3	0.5	–	11.0	2.8	–
Other anti-VEGF-associated events						
Arterial thromboembolic event	1.5	0.5	–	2.6	0.8	1.0
Venous thromboembolic event	7.3	2.6	3.6	9.3	3.1	4.7
Fistula from GI origin	0.3	0.2	–	1.1	0.3	–
Fistula from other than GI origin	0.2	–	–	0.3	–	–
GI perforation	0.5	0.2	0.2	0.5	0.2	0.3
Biologic abnormalities						
Hematologic						
Anemia	91.1	3.5	0.8	82.3	3.3	0.5
Neutropenia	56.3	19.1	10.4	67.8	23.1	13.6
Neutropenic complications	3.0	1.7	1.2	6.5	4.4	1.3
Thrombocytopenia	33.8	0.8	0.8	47.4	1.7	1.7
Nonhematologic						
Proteinuria	40.7	1.2	–	62.2	7.5	0.3
ALT increased	37.1	2.2	–	47.3	2.5	0.2

*Grades were determined according to National Cancer Institute Common Terminology Criteria of Adverse Events, version 3.0. Adapted with permission.
ALT, alanine aminotransferase; FOLFIRI, infusional fluorouracil, leucovorin, and irinotecan; GI, gastrointestinal; HLT, high-level term; PT, preferred term; SOC, system organ class; VEGF, vascular endothelial growth factor.

to FOLFIRI to demonstrate a statistically significant improvement in survival after prior treatment with an oxaliplatin-based regimen.

The role of aflibercept in context

The benefit of aflibercept is consistent with trials evaluating the efficacy of bevacizumab in the second-line setting [Giantonio *et al.* 2007; Arnold *et al.* 2012] (Table 1). In ECOG 3200, the addition of bevacizumab to FOLFOX after progression on irinotecan-based chemotherapy significantly improved outcomes in patients without prior antiangiogenic therapy [Giantonio *et al.* 2007]. The ML18147 trial demonstrated the utility of continuing bevacizumab in combination with standard

second-line combination chemotherapy in patients who had received bevacizumab with their first-line combination chemotherapy regimen (Table 1) [Arnold *et al.* 2012]. Patients who had progressed within 3 months of stopping first-line chemotherapy with bevacizumab were randomized to continuing bevacizumab or not in conjunction with fluoropyrimidine-based combination chemotherapy. Choice of oxaliplatin or irinotecan for second-line treatment was dependent on the regimen used in first-line treatment and was included as a stratification variable. The study met its primary endpoint and demonstrated a statistically significant improvement in median survival with the addition of bevacizumab to second-line chemotherapy (11.2 months of bevacizumab plus chemotherapy and 9.8 months of chemotherapy, HR = 0.81, 95% CI 0.69–0.94; unstratified log-rank test, $p = 0.0062$) (Table 1). However, the RR was not significantly different (5.4% for bevacizumab plus chemotherapy *versus* 3.9% for chemotherapy, unstratified χ^2 test, $p = 0.3113$). Serious (grade 3–5) adverse events were higher in patients with the addition of bevacizumab compared with chemotherapy alone (64% bevacizumab plus chemotherapy *versus* 58% chemotherapy). More patients stopped treatment due to adverse events in the bevacizumab arm (16% bevacizumab *versus* 9%), however the absolute difference was small. There was a small increase in grade 3–5 adverse events related to antiangiogenic drugs (12% bevacizumab *versus* 6%). This pattern was consistent with previously reported toxicities in phase III trials evaluating bevacizumab and suggested that these events were not increased when continuing bevacizumab in the second-line setting.

The inclusion criteria in the ML18147 and VELOUR trial were different which may have influenced the outcomes [Arnold *et al.* 2012; Van Cutsem *et al.* 2012]. The VELOUR trial included patients regardless of the timing of progression on oxaliplatin-based therapy [Van Cutsem *et al.* 2012]. In contrast, the ML18147 trial excluded patients who received less than 3 months of bevacizumab in the first-line setting or who developed progression more than 3 months after the last treatment with bevacizumab [Arnold *et al.* 2012]. In both trials, the majority of patients had good performance status (over 95% were ECOG 0–1) [Arnold *et al.* 2012; Van Cutsem *et al.* 2012], and thus may not be reflective of the general patient population. While it appears that the toxicity profile of bevacizumab is better than aflibercept in the second-line setting, with similar efficacy, a definitive comparison would require a phase III trial with quality of life endpoints and a prospective cost utility analysis. RRs were not significantly increased with the addition of bevacizumab to second-line chemotherapy in MCRC [Arnold *et al.* 2012]. The increase in RR with FOLFIRI plus aflibercept compared with FOLFIRI (19.8% aflibercept *versus* 11.1% placebo, $p < 0.001$) [Van Cutsem *et al.* 2012] is interesting and metastectomy rates should be evaluated in any prospective trials that compare bevacizumab with aflibercept plus chemotherapy.

A cost-effectiveness analysis compared treatment with bevacizumab with aflibercept in combination with chemotherapy as second-line treatment in patients with MCRC who had progressed on first-line treatment containing bevacizumab [Morlock *et al.* 2013]. An adjusted indirect comparison was conducted using the Bucher method using hazard ratios from ML18147 [Arnold *et al.* 2012] and VELOUR [Van Cutsem *et al.* 2012]. Direct patient costs were estimated from wholesale drug acquisition costs and costs of treating toxicities were based on Medicare. Results were presented in abstract form and suggested that the addition of bevacizumab was less costly compared with aflibercept plus chemotherapy (US $39,104 less per treated patient). Similar effectiveness was noted between bevacizumab compared with aflibercept plus chemotherapy [median overall survival 13.3 *versus* 12.5 months; HR 0.94 (95% CI 0.70–1.26); 0.498 *versus* 0.479 quality adjusted life years]. Adverse event rates and costs were higher for aflibercept compared with bevacizumab plus chemotherapy, which were the primary drivers of the model. The exact assumptions used for generating the model are not available due to the preliminary nature of the results. The initial cost of aflibercept was priced to compete with the higher dose of bevacizumab (10 mg/kg every 2 weeks) used in the ECOG 3200 trial. Most oncologists use bevacizumab at 5 mg/kg every 2 weeks, similar to what was used in the ML18147 trial [Arnold *et al.* 2012]. Sanofi-Aventis subsequently decreased the price of aflibercept by 50% [Ciombor *et al.* 2013]. Thus the validity of this economic analysis is unclear. The health technology appraisal from the National Institute for Health and Clinical Excellence for aflibercept in combination with FOLFIRI should provide an unbiased and current analysis on this topic.

Based on the results from the VELOUR trial, the US Food and Drug Administration and the European Commission approved aflibercept for use in combination with FOLFIRI for patients with MCRC whose condition has progressed

following treatment with an oxaliplatin-containing regimen. The clinical activity of aflibercept in the subset of patients who have received prior bevacizumab with oxaliplatin-based chemotherapy, in addition to the ML18147 trial results, suggest that ongoing inhibition of angiogenesis provides clinical benefit [Arnold *et al.* 2012; Van Cutsem *et al.* 2012]. Another promising antiangiogenic agent is regorafenib, an oral tyrosine kinase inhibitor of VEGFR1, VEGFR2, VEGFR3, fibroblast growth factor receptor, platelet-derived growth factor receptor ß, TIE2, KIT, RET, RAF1, and BRAF (Figure 1). Regorafenib was evaluated in patients with MCRC who had already been treated with fluoropyrimidine, oxaliplatin, and irinotecan-based chemotherapy, antiangiogenic therapy, and if applicable, EGFR inhibitors. Treatment with regorafenib significantly improved overall survival compared with placebo (median survival 6.4 months for regorafenib *versus* 5.0 months for placebo, HR 0.77, 95% CI 0.64–0.94, one sided p = 0.0052) in the phase III CORRECT trial (Regorafenib Monotherapy for Previously Treated Metastatic Colorectal Cancer) [Grothey *et al.* 2013]. Quality of life was similar between the two arms. The optimal sequencing of antiangiogenic agents in MCRC is unclear and warrants further study. Until data from randomized controlled trials are available, oncologists will make treatment recommendations by balancing patient preferences with cost-effectiveness data and the toxicity profiles of these antiangiogenic agents. In primarily publically funded healthcare systems such as Canada, cost-effectiveness data will play a major role in the decision to reimburse regorafenib, aflibercept, and bevacizumab after progression on first-line chemotherapy with bevacizumab. The best strategy is even more uncertain for patients with Kras wild-type tumors, who also derive benefit from EGFR inhibitors.

Unfortunately, there are no validated biomarkers for benefit from antiangiogenic drugs (reviewed by Lambrechts and colleagues) [Lambrechts *et al.* 2013]. Circulating levels of short VEGF A isoforms, expression of neuropilin 1 and VEGFR1 in tumors or plasma, and genetic variants in VEGF A or its receptors are promising candidates for predicting benefit from bevacizumab and should be evaluated in patients treated with aflibercept. Biomarkers to identify patients who benefit from antiangiogenic therapy are desperately needed, especially in view of the small incremental improvements in clinical outcomes observed in these recent trials.

Further trials with aflibercept are planned or ongoing in MCRC. A phase III trial conducted in Asia will evaluate the efficacy of aflibercept compared with placebo with FOLFIRI in patients whose condition has progressed on oxaliplatin-containing chemotherapy [ClinicalTrials.gov identifier: NCT01661270]. Aflibercept is also being assessed in combination with capecitabine in a phase I/II study [ClinicalTrials.gov identifier: NCT01661972]. A multicentre, open-label trial of FOLFIRI plus aflibercept will assess safety and quality of life of this regimen [ClinicalTrials.gov identifier: NCT01571284]. Quality of life evaluation from this trial will provide invaluable context to the results of the VELOUR trial [Van Cutsem *et al.* 2012], but will be challenging to interpret in the absence of a control arm.

Conclusion

The combination of aflibercept with FOLFIRI leads to a statistically significant improvement in overall survival, PFS and RR in patients with MCRC previously treated with an oxaliplatin-based regimen [Van Cutsem *et al.* 2012]. Biomarker evaluation from archival tumor specimens from patients who participated in the VELOUR study is planned [ClinicalTrials.gov identifier: NCT01754272] and the results are eagerly anticipated. Aflibercept is a valuable new treatment option in combination with FOLFIRI for patients with MCRC. Given the expanding armentarium of agents for MCRC, future trials should include cost utility, quality of life, and biomarker analyses to guide treatment decision making.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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