Antiangiogenesis therapy in the treatment of metastatic colorectal cancer

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Ther Adv Med Oncol

[2012] 4(6) 301–319

DOI: 10.1177/
1758834012454464

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Abstract: The process of new blood vessel formation, or angiogenesis, has become an important target for therapeutic intervention in many cancers, including metastatic colorectal cancer (mCRC). The growth and metastasis of primary tumors is dependent upon their ability to acquire and maintain an adequate blood supply; however, angiogenesis in tumors is an irregular process leading to chaotic and hyperpermeable vessels that may result in increased intratumoral pressure and poor exchange of macromolecules and oxygen. It has been hypothesized that inhibition of angiogenesis in tumors can both impair the formation of new tumor blood vessels and possibly ‘normalize’ the existing tumor vasculature, causing a more efficient delivery of cytotoxic chemotherapies (CTs). Over the last decade, therapies that target vascular endothelial growth factor (VEGF) have become a component of treatment for several cancers. In particular, the combination of bevacizumab with established chemotherapeutic regimens for mCRC has been shown to improve overall and progression-free survival, as well as response rates, over CT alone. Agents that target various members of the VEGF family, as well as signaling by the VEGF receptors and their tyrosine kinase components, are currently under development and evaluation in clinical trials. Integration of these new therapies into the treatment of mCRC will ultimately increase the available therapeutic options for patients. Still, many challenges remain, including identifying and validating relevant biomarkers to guide the optimal use of antiangiogenesis agents.

Keywords: Aflibercept, antiangiogenesis, bevacizumab, biomarker, clinical trials, metastatic colorectal cancer, regorafenib

Introduction

The process of angiogenesis, or new blood vessel formation, has become widely recognized as an important therapeutic target in many different types of cancer, including metastatic colorectal cancer (mCRC). If the blood supply of a primary tumor is limited, its potential for continued growth and metastasis, the primary causes of cancer-related death, is greatly impeded [Folkman, 2002]. The ability of a primary tumor to acquire this capacity is dependent upon the triggering of an ‘angiogenic switch’, whereby the local balance between proangiogenic and antiangiogenic factors is disturbed [Folkman, 2002]. Unlike the normal vasculature, blood vessel formation in tumors is characterized by dysregulated expression of angiogenic and antiangiogenic factors, as well as structural and functional abnormalities [Baluk

et al. 2005]. Tumor blood vessels are chaotic, irregular, and hyperpermeable, which result in a condition of increased interstitial fluid pressure, impaired fluid circulation, and poor exchange of oxygen and macromolecules in the interior of the tumor [Gerber and Ferrara, 2005; Jain, 2005]. The tumor microenvironment contains many targets for angiogenesis inhibitors [Baluk *et al.* 2005]. At present, although a number of agents are in development, the only biological antiangiogenic therapy approved for the treatment of mCRC is the humanized monoclonal antibody, bevacizumab. Highlighting the importance of an antiangiogenesis approach in mCRC, two additional antiangiogenic agents, aflibercept and regorafenib, have recently demonstrated efficacy in this disease. Cetuximab and panitumumab are monoclonal antibodies also approved for mCRC

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Figure 1. The VEGF pathway and its targets for inhibition. The interaction of VEGF-A with VEGFR-2 is considered the most relevant interaction for angiogenesis. VEGF-A, however, also interacts with VEGFR-1, as do VEGF-B and PlGF; the contribution of the latter two to the angiogenesis pathway and escape from angiogenesis inhibition is not yet fully understood. Targets for angiogenesis inhibition include VEGF ligands [aflibercept and bevacizumab], VEGF receptors [e.g. IMC-1121B], and tyrosine kinase signaling (TKIs) [Chu, 2009]. PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

treatment, but they target the epidermal growth factor receptor (EGFR) as opposed to angiogenesis directly, and are not further discussed in this review. Although important challenges remain, including increasing the efficacy and safety of treatments identifying and validating relevant biomarkers to guide the optimal use of antiangiogenesis therapy, antiangiogenesis strategies have shown great promise in cancer therapeutics [Fischer *et al.* 2008]. In this review, we discuss therapeutic targets in antiangiogenic therapy, the current use of bevacizumab, and antiangiogenic agents that are currently in development for the treatment of mCRC.

Therapeutic targets in antiangiogenic therapy

The vascular endothelial growth factor (VEGF) pathway offers a number of important targets for cancer therapies. Figure 1 shows the key components of the VEGF family of growth factors and their receptors [Chu, 2009]. VEGF, and in particular VEGF-A, is recognized as a key contributor to the process of angiogenesis [Dvorak *et al.* 1995; Ferrara *et al.* 2003, 2005; Fischer *et al.* 2008]. Although VEGF-A has been the most investigated in terms of its role in angiogenesis,

other factors in the VEGF family such as VEGF-B and placental growth factor (PlGF) may play a role in tumor angiogenesis and/or escape from angiogenesis inhibition [Fischer *et al.* 2008]. VEGF-B is closely related to VEGF-A and, like VEGF-A, interacts with and activates VEGF receptor-1 (VEGFR-1) (Figure 1) [Olofsson *et al.* 1998]. Although VEGFR-2 may be the primary modulator of angiogenic effects, VEGFR-1 has been shown to be important in the epithelial-mesenchymal transition, which could play a role in tumor progression and metastasis [Bates *et al.* 2003]. Loss of function of VEGFR-1 tyrosine kinase (TK) activity has been shown to inhibit tumor angiogenesis; in mice deficient in the VEGFR-1 TK, tumor growth was approximately threefold inhibited compared with wild-type (WT) mice; this inhibition correlated with reduced tumor angiogenesis [Hiratsuka *et al.* 2001, 2002]. The induction of matrix metalloproteinase 9 (MMP9) in lung endothelial cells and macrophages has also demonstrated dependence on VEGFR-1 TK activity. This was an important step in the development of lung metastases by primary tumors [Hiratsuka *et al.* 2002]. Both VEGF-B and VEGFR-1 have been found to be upregulated in a number of different tumor types; in some cases, they were associated with poor

prognosis, metastasis, and recurrence [Fischer *et al.* 2008].

PlGF interacts with and activates VEGFR-1 (Figure 1), and this specific interaction has been shown to be important in the recruitment of monocytes, which may be an important source of angiogenic cytokines and a means of tumor escape from angiogenesis inhibition [Barleon *et al.* 1996; Pipp *et al.* 2003]. In addition, PlGF-2 (but not PlGF-1) binds the receptor neuropilin-1 (NRP1) [Migdal *et al.* 1998]. The expression of NRP1 has been shown to be a negative prognostic indicator in some tumors [Fischer *et al.* 2008]. PlGF has also been shown to potentiate the angiogenic response to VEGF-A, which was proposed to be a contributor to the ‘angiogenic switch’ in tumors [Carmeliet *et al.* 2001].

It is important to note, however, that our knowledge of the contribution to tumor angiogenesis of VEGF receptor (VEGFR) ligands other than VEGF-A is still evolving, and that the relevance of inhibiting these factors as part of antitumor therapy is not clearly established. Another important caveat is that we do not yet fully understand the mechanisms by which antiangiogenic therapies work in cancer, and why, despite meaningful short-term improvements in progression-free survival (PFS), the benefits in overall survival (OS) have been more modest [Loges *et al.* 2009]. Indeed, there is some preclinical evidence that demonstrates that while antiangiogenic therapies work to prolong PFS, they may simultaneously promote increased invasiveness and metastatic potential which reduces survival [Loges *et al.* 2009; Ebos *et al.* 2009; Pàez-Ribes *et al.* 2009]. These findings have raised the possibility of an adaptive–evasive response induced by antiangiogenic therapies, which may have important implications for the ways in which we apply such therapies in the future [Ebos *et al.* 2009; Pàez-Ribes *et al.* 2009].

Inhibitors of VEGF ligands

As noted earlier, potential targets in the angiogenesis pathway include VEGF-A, VEGF-B, and the structurally related PlGF, whereas VEGF-C and VEGF-D, which bind to VEGFR-3, are thought to play a more important role in lymphangiogenesis [Chu, 2009; Maglione *et al.* 1993; Sawano *et al.* 1996]. Biologic agents that target VEGF ligands include bevacizumab, a recombinant humanized monoclonal antibody (mAb) to VEGF-A [Ferrara

et al. 2005; Ferrara and Kerbel, 2005; Grothey and Galanis, 2009], as well as aflibercept, a recombinant fusion protein that binds VEGF-A, VEGF-B, and PlGF (Figure 1) [Holash *et al.* 2002; Van Cutsem *et al.* 2011a; Grothey and Galanis, 2009].

Inhibitors of VEGFRs

The key receptors in the VEGF pathway are VEGFR-1, which binds VEGF-A, VEGF-B, and PlGF [Fischer *et al.* 2008]; and VEGFR-2, which binds VEGF-A and is believed to be the major mediator of its angiogenic effects [Fischer *et al.* 2008; Ferrara and Kerbel, 2005]. Antagonistic antibodies to VEGFR are an additional means of targeting angiogenesis in cancer, and agents currently in development include IMC-18F1, which targets VEGFR-1 [Grothey and Galanis, 2009] and IMC-1121B (CT-322 or ramucirumab), which targets VEGFR-2 [Grothey and Galanis, 2009].

Receptor TK inhibitors

A third means of targeting the VEGF pathway in cancer is the use of small-molecule inhibitors that target the receptor TK domain of VEGFR. These molecules vary in specificity, targeting the signaling function of VEGFR (Table 1). Examples of TKIs include sunitinib, a multikinase inhibitor of platelet-derived and vascular endothelial growth factor receptors (PDGFR and VEGFR), as well as c-kit and Ret; brivanib, which primarily inhibits VEGFR-2 and basic fibroblast growth factor receptor (bFGFR); cediranib, a pan-VEGFR, PDGFR, and c-kit kinase inhibitor; regorafenib, a multikinase inhibitor including VEGFR-2 and Tie2, and sorafenib, a pan-VEGFR, PDGFR, and Raf kinase inhibitor [Bhargava and Robinson, 2011; Wilhelm *et al.* 2011].

Use of bevacizumab in the treatment of mCRC

The results of preclinical studies of bevacizumab provided a framework for its use in mCRC. These studies demonstrated antitumor activity in both primary and metastatic tumor models alone and in combination with chemotherapy (CT) [Gerber and Ferrara, 2005].

Initial therapy of mCRC

Bevacizumab has been used as initial treatment for mCRC in combination with established CT agents, including the fluoropyrimidines 5-fluorouracil

Table 1. Antiangiogenic therapies currently in development for metastatic colorectal cancer.

Antiangiogenic agent	Mechanism of action	Latest clinical development phase in mCRC	Key combination therapies under investigation (most advanced phase) for mCRC
Large-molecule inhibitors			
Aflibercept	Decoy receptor/VEGF-A, VEGF-B, PlGF Trap	3	• Aflibercept + Irinotecan + 5FU [ClinicalTrials.gov identifier: NCT00561470]
Ramucirumab	Anti-VEGFR-2 antibody	3	• Ramucirumab or placebo + FOLFIRI (second line) [ClinicalTrials.gov identifier: NCT01183780]
Small-molecule kinase inhibitors			
Axitinib	TKI/pan-VEGFR, PDGFR, cKit inhibitor	2	• Axitinib or BEV with FOLFOX or FOLFIRI [ClinicalTrials.gov identifier: NCT00615056]
BIBF 1120	TKI/pan-VEGFR, PDGFR, FGFR inhibitor	2	• BIBF 1120 or BEV with mFOLFOX6 [ClinicalTrials.gov identifier: NCT00904839]
Brivanib	TKI/VEGFR-2, FGFR inhibitor	3	• CET ± Brivanib for WT <i>KRAS</i> mCRC [ClinicalTrials.gov identifier: NCT00640471]
Cediranib	TKI/pan-VEGFR, PDGFR, cKit inhibitor	3	• Cediranib + CT in untreated mCRC [ClinicalTrials.gov identifier: NCT00399035] • Cediranib or BEV + FOLFOX in mCRC [ClinicalTrials.gov identifier: NCT00384176]
Linifanib	TKI/VEGFR-2/3, PDGFR, cKit, Flt-3 inhibitor	2	• Linifanib or BEV with mFOLFOX6 [ClinicalTrials.gov identifier: NCT00707889]
Regorafenib	TKI/VEGFR-2, Tie2 inhibitor	3	• Regorafenib or placebo in mCRC [ClinicalTrials.gov identifier: NCT01103323]
Sorafenib	TKI/pan-VEGFR, PDGFR, Raf inhibitor	2	• Sorafenib + CET [ClinicalTrials.gov identifier: NCT00326495] • Sorafenib + Capecitabine [ClinicalTrials.gov identifier: NCT01290926] • Sorafenib + CT, second line [ClinicalTrials.gov identifier: NCT00889343] • Sorafenib, CET, Irinotecan [ClinicalTrials.gov identifier: NCT00134069] • Sorafenib + BEV [ClinicalTrials.gov identifier: NCT00826540] • mFOLFOX6 ± Sorafenib [ClinicalTrials.gov identifier: NCT00865709] • Sorafenib + FOLFIRI [ClinicalTrials.gov identifier: NCT00839111]
Sunitinib	TKI/pan-VEGFR, PDGFR, cKit, Flt-3, Ret inhibitor	3	• Sunitinib or placebo + FOLFIRI in mCRC [ClinicalTrials.gov identifier: NCT00457691]
Vatalanib	TKI/pan-VEGFR, PDGFR, c-kit inhibitor	3	• Vatalanib or placebo + Oxaliplatin/5FU/LV [ClinicalTrials.gov identifier: NCT00056446]
Vandetanib	TKI/VEGFR, EGFR inhibitor	2	• Vandetanib or placebo + FOLFIRI [ClinicalTrials.gov identifier: NCT00454116] • Vandetanib or placebo + FOLFOX [ClinicalTrials.gov identifier: NCT00500292]
5FU, 5-fluorouracil; BEV, bevacizumab; CET, cetuximab; CT, chemotherapy; EGFR, endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; Flt-3, Fms-like tyrosine kinase; FOLFIRI, leucovorin, fluorouracil, irinotecan; FOLFOX, leucovorin, fluorouracil, oxaliplatin; mCRC, metastatic colorectal cancer; PDGFR, platelet-derived growth factor receptor; PlGF, placental growth factor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WT <i>KRAS</i> , wild-type <i>KRAS</i>			

(5FU) and capecitabine, irinotecan and oxaliplatin, as well as combinations of these agents [Van Cutsem *et al.* 2009]. In 2004, results were reported from a study comparing irinotecan, bolus fluorouracil, and leucovorin (IFL) with bevacizumab plus IFL in patients with mCRC ($n = 813$), and these results formed the basis for the use of bevacizumab as initial therapy in mCRC [Hurwitz *et al.* 2004]. Results showed that patients in the bevacizumab plus IFL group had longer duration of survival (20.3 months) compared with those treated with IFL alone (15.6 months; hazard ratio [HR] = 0.66; $p < 0.001$); there was also significantly higher 1-year survival (74.3% versus 63.4% of patients; $p < 0.001$), PFS (10.6 months versus 6.2 months; HR = 0.54; $p < 0.001$), response rate (RR; 44.8% versus 34.8%; 0.004), and duration of response compared with IFL [Hurwitz *et al.* 2004]. The results of this trial demonstrated the safety of bevacizumab in this treatment setting [Hurwitz *et al.* 2004]. The incidence of grade 3/4 adverse events (AEs) was higher in the bevacizumab plus IFL group (84.9% versus 74.0%), primarily due to increased grade 3 hypertension (the only AE significantly increased with the combination of IFL plus bevacizumab; $p < 0.01$) [Hurwitz *et al.* 2004]. In a subsequent analysis of these results according to WT or mutant (MT) *KRAS* status, *KRAS* status was not predictive of clinical benefit when adding bevacizumab to IFL in the first-line setting of mCRC, although patients with WT *KRAS* appeared to have a greater benefit in RR with bevacizumab [Hurwitz *et al.* 2009].

In 2004, following the approval of bevacizumab, the BICC-C trial was amended to include a third arm consisting of leucovorin, fluorouracil, irinotecan (FOLFIRI) plus bevacizumab in patients with previously untreated mCRC [Fuchs *et al.* 2007]. Although the trial was not designed to prospectively compare FOLFIRI with FOLFIRI plus bevacizumab, at a median follow up of 22.6 months, the PFS was 11.2 months, the median OS had not yet been reached, and 1-year survival was 87% in the FOLFIRI plus bevacizumab arm [Fuchs *et al.* 2007]. Results of an updated analysis at 34.4 months showed a median of 28-month OS in the FOLFIRI plus bevacizumab arm [Fuchs *et al.* 2008]. OS in this arm was significantly greater when compared with irinotecan plus bolus fluorouracil/leucovorin [Fuchs *et al.* 2008]. Bevacizumab has been used in combination with oxaliplatin-based CT (capecitabine plus oxaliplatin [XELOX] or oxaliplatin, leucovorin, and 5-fluorouracil [FOLFOX4]) as initial therapy in a phase III study of patients with mCRC ($n = 1401$)

[Saltz *et al.* 2008]. Median PFS was 9.4 and 8.0 months in the bevacizumab and placebo groups, respectively (HR = 0.83; $p = 0.023$), and RR was similar, as evaluated by independent response review committee assessment (38% versus 38%; odds ratio [OR] = 1.0; $p = 0.99$) [Saltz *et al.* 2008]. Median OS was 21.3 and 19.9 months in the bevacizumab and placebo groups, respectively (HR = 0.89; $p = 0.077$) [Saltz *et al.* 2008]. Analysis of treatment discontinuation showed that only 29% and 47% of patients in the bevacizumab and placebo groups, respectively, were treated to progression [Saltz *et al.* 2008]. A preplanned analysis of so-called 'on-treatment PFS' (effectively censoring patients who discontinued therapy before progression) demonstrated a larger benefit for patients in the bevacizumab arm. As OS and RR did not differ significantly, it was suggested that treatment to disease progression may be needed to optimize the contribution of bevacizumab [Saltz *et al.* 2008]. In the Bevacizumab Expanded Access Trial (BEAT), the safety and efficacy of first-line bevacizumab with FOLFOX, FOLFIRI, XELOX, or fluoropyrimidines was examined in the context of a broader (i.e. general oncology practice) mCRC patient population [Van Cutsem *et al.* 2009]. Efficacy results showed a median PFS of 10.8 months, ranging from 8.6 months for patients on single-agent CT to 11.6 months for patients on FOLFIRI; median OS was 22.7 months and was higher for patients receiving dual CT regimens (23.7, 25.9, and 23.0 for FOLFIRI, FOLFOX, and XELOX regimens, respectively, versus 18.0 months with single-agent CT) [Van Cutsem *et al.* 2009]. Grade 3/4 AEs of interest for bevacizumab in the overall BEAT population ($n = 1914$) included bleeding (3%), gastrointestinal (GI) perforation (2%), arterial thromboembolism (1%, no fatal events), hypertension (5%), proteinuria (1%, no fatal events), and wound-healing problems (1%, no fatal events) [Van Cutsem *et al.* 2009]. In a similar nonrandomized observational cohort study conducted in the United States, the Bevacizumab Regimens: Investigation of Treatment Effects and Safety (BRiTE) study showed that the addition of bevacizumab to various chemotherapeutic regimens resulted in an OS of 25.1 months and a median PFS of 10 months [Grothey *et al.* 2008b]. The safety and efficacy of adding bevacizumab to several oxaliplatin-containing regimens as initial therapy for patients with mCRC was examined in the Three Regimens of Eloxatin Evaluation (TREE) study [Hochster *et al.* 2008]. Of the 233 patients randomly assigned to the three treatment arms containing

bevacizumab, the OS for all patients was 23.7 months, with a range of 20.4 months for the bolus 5-fluorouracil regimen to 26.1 months for the mFOLFOX6 regimen. Reported in 2010, a small, single-center phase III study of 222 patients with advanced colorectal cancer (CRC) demonstrated no significant benefit of adding bevacizumab to chemotherapy [Stathopoulos *et al.* 2010]. Unfortunately, the report does not provide any details on post-progression therapy which may have greatly influenced OS. In addition, no data on PFS are mentioned, and the study was insufficiently powered to demonstrate a survival difference between the arms.

As second-line therapy

When used in combination with FOLFOX4 in previously treated patients with mCRC (ECOG E3200 study; all patients were oxaliplatin and bevacizumab naïve), bevacizumab prolonged survival compared with FOLFOX4 alone (12.9 *versus* 10.8 months; HR = 0.75; $p = 0.0011$) [Giantonio *et al.* 2007]. Improved PFS (7.3 *versus* 4.7 months; HR = 0.61; $p < 0.0001$) and confirmed RR (using RECIST criteria, 22.7% *versus* 8.6%; $p < 0.0001$) were also observed with bevacizumab compared with FOLFOX4 alone [Giantonio *et al.* 2007]. The incidence of grade 3/4 AEs was higher with the bevacizumab combination therapy *versus* FOLFOX4 alone (75% *versus* 61%, respectively), with higher rates of grade 3/4 neuropathy (16% *versus* 9%), hypertension (6% *versus* 2%), bleeding (3% *versus* 0.4%), and vomiting (10% *versus* 3%) [Giantonio *et al.* 2007].

Use of bevacizumab beyond progression

The results of a large, nonrandomized observational cohort study, BRiTE, suggested that treatment with bevacizumab beyond disease progression might improve OS in patients with mCRC [Grothey *et al.* 2008b]. As noted above, in the overall BRiTE population, median OS was 25.1 months; in terms of post-progression treatment, the median OS was 12.6, 19.9, and 31.8 months for no treatment (with anticancer therapy of any kind) post-disease progression ($n = 253$), no bevacizumab treatment post-disease progression ($n = 531$), and bevacizumab post-disease progression ($n = 642$), respectively [Grothey *et al.* 2008b]. Compared with the no bevacizumab group, treatment with bevacizumab post-disease progression was strongly and independently associated with improved survival in a multivariate analysis (HR =

0.49; $p < 0.001$). These nonrandomized data suggest a benefit to the continuation of bevacizumab beyond progression, but require a definitive randomized study before general adoption in clinical practice. A prospective, randomized, phase III trial investigating the benefit of bevacizumab beyond disease progression completed accrual in May 2010. In this study, 820 patients who had been treated with bevacizumab in first-line therapy with an irinotecan- or oxaliplatin-based regimen were crossed over to a different chemotherapy and either did or did not continue bevacizumab beyond progression (BBP). The primary endpoint of the study was met with a median difference in overall survival of 1.4 months and a HR of 0.81, meaning a 19% reduction of death events with the use of BBP ($p = 0.0062$). This effect was maintained in all prespecified subgroups. In addition, PFS was significantly improved (5.7 *versus* 4.1 months, HR 0.68, $p < 0.0001$). Interestingly, response rates in both second-line treatment groups were low, around 5%, although the overall disease control rate (response rate plus stable disease) was significantly improved in the BBP group (68% *versus* 54%, $p < 0.0001$) indicating that the strength of continuation of bevacizumab into second-line therapy is delaying tumor progression rather than inducing a response by RECIST criteria. No unexpected side-effects were noted with the prolonged use of bevacizumab. While the magnitude of the survival benefit observed with the concept of VEGF-inhibition beyond progression might have disappointed some believers in the concept of BBP, the results establish a new treatment option in second-line CRC, especially for patients with KRAS mutated cancers. Furthermore, the findings provide new insights into tumor biology in general and our understanding of treatment resistance in particular [Arnold *et al.* 2012].

Use of bevacizumab in combination with epidermal growth factor receptor inhibitors

Some studies have examined the use of bevacizumab in combination with epidermal growth factor receptor (EGFR) inhibitors, such as cetuximab and panitumumab. There have been some notable failures of this combination approach. In 2009, results from CAIRO2 showed significantly poorer median PFS for patients receiving a capecitabine, oxaliplatin, and bevacizumab regimen in combination with cetuximab (CBC; 9.4 months), as compared with patients receiving the same regimen without cetuximab (CB, 10.7 months; $p = 0.01$),

whereas OS and RR were not significantly different [Tol *et al.* 2009]. There were also more grade 3/4 AEs in the CBC group as compared with the CB group, mainly due to cetuximab-related cutaneous AEs (81.7% *versus* 73.2%; $p = 0.006$) [Tol *et al.* 2009]. Similarly, the PACCE trial investigating the use of bevacizumab in combination with another EGFR inhibitor, panitumumab, and oxaliplatin- or irinotecan-based chemotherapy was discontinued after interim efficacy analyses demonstrated significantly poorer PFS and OS in the group receiving panitumumab [Amgen, 2007; Goldberg and Carrato, 2008]. As in CAIRO2, more grade 3 or greater AEs were observed in the group receiving panitumumab [Amgen, 2007; Goldberg and Carrato, 2008]. These results have served to illustrate the importance of properly combining antiangiogenic therapies and the additional studies needed to identify optimal combinations of biologics/CTs, as well as groups of patients and/or tumor types that may benefit from such combinations.

Antiangiogenic agents in phase II and III development for mCRC treatment: an overview

The success of treatments such as bevacizumab in mCRC has provided a foundation for further research, and identification of additional VEGF pathway inhibitors that can be exploited for cancer therapy.

Aflibercept

Aflibercept (VEGF Trap) is a recombinant fusion protein derived from the binding domains of VEGFR-1 and VEGFR-2, as well as the Fc region of the human IgG1 antibody [Holash *et al.* 2002]. In line with the binding profile of VEGFR-1 (Figure 1), aflibercept is a multiple angiogenic factor trap designed to block the angiogenesis network by binding VEGF-A, VEGF-B, and PlGF [Van Cutsem *et al.* 2011a]. In preclinical studies, aflibercept has demonstrated a broad range of antitumor activity, both alone and in combination with various cytotoxic agents [Chiron *et al.* 2008], and reflected by tumor regression and regression of metastases [Huang *et al.* 2003], a reduction in tumor burden, decreased ascites, and tumor vessel remodeling [Byrne *et al.* 2003]. Animal studies show that aflibercept-VEGF complexes do not have platelet-activating potential, suggesting that antiangiogenic therapy with aflibercept may be less likely to induce prothrombotic side effects

[Meyer *et al.* 2010]. In phase I studies, aflibercept appeared to be well tolerated at a dose of 4 mg/kg every 2 weeks, with pharmacokinetic (PK) and pharmacodynamic (PD) markers indicative of effective VEGF blockade [Lockhart *et al.* 2010]. PK parameters in the presence of FOLFOX were comparable with single-agent aflibercept studies; the safety profile was consistent with that of the VEGF class of inhibitors, and included hypertension and proteinuria [Limentani *et al.* 2008]. As a single agent, aflibercept demonstrated activity when administered subcutaneously in heavily pretreated patients, with stable disease observed in 53% (9/17) of patients completing the study at or below the 400 $\mu\text{g/kg}$ dose, and 82% (9/11) of patients at the 800 $\mu\text{g/kg}$ dose [Tew *et al.* 2010]. In a phase II study, aflibercept was administered to previously treated patients with mCRC ($n = 51$) in both bevacizumab-naïve ($n = 24$) and previously bevacizumab-treated patients ($n = 27$), with a median PFS of 2 and 3.4 months, respectively [Tang *et al.* 2008]. The most common grade 3 or greater AEs in this study were hypertension (7.8%), proteinuria (7.8%), fatigue (5.9%), and headache (5.9%) [Tang *et al.* 2008].

Recently, in a multinational phase III study (VELOUR; $N = 1226$), aflibercept in combination with FOLFIRI demonstrated statistically significant improvements in median OS (13.5 *versus* 12.1 months; HR = 0.82; $p = 0.0032$), PFS (6.90 *versus* 4.67 months; HR = 0.76; $p = 0.00007$), and overall RR (19.8% *versus* 11.1%; $p = 0.0001$) versus placebo/FOLFIRI in patients with mCRC after treatment with an oxaliplatin-containing regimen [Van Cutsem *et al.* 2011c]. In VELOUR, the benefits associated with the use of aflibercept were apparent without regard to baseline PS, prior exposure to bevacizumab, age, sex, region of clinical trial center, blood pressure, number of metastatic sites, or location of primary tumor [Tabernero *et al.* 2011]. Approximately 30% of patients had received prior bevacizumab in this study, and results from a subanalysis of VELOUR showed that there was no significant impact of prior treatment with bevacizumab on OS (HR = 0.86) or PFS (HR = 0.66) [Tabernero *et al.* 2011]. Grade 3/4 AEs in the aflibercept arm occurring with 2% or greater increased incidence *versus* placebo included diarrhea (19.3 *versus* 7.8), asthenia/fatigue (16.9 *versus* 10.6), stomatitis/ulceration (13.7 *versus* 5.0), infections (12.3 *versus* 6.9), hypertension (19.3 *versus* 1.5), GI/abdominal pains (5.4 *versus* 3.3), neutropenia (36.7 *versus* 29.5) or neutropenic complications (5.7 *versus* 2.8), and

proteinuria (7.9 *versus* 1.2) [Allegra *et al.* 2012]. Discontinuations due to asthenia/fatigue, infections, diarrhea, hypertension, and venous thromboembolic events occurred in 26.6% of patients in the aflibercept arm *versus* 12.1% in the placebo arm [Allegra *et al.* 2012]. Results from VELOUR support aflibercept/FOLFIRI as an option for patients with prior oxaliplatin treatment.

Targeting VEGFR

Ramucirumab

Ramucirumab is a fully humanized mAb directed against the extracellular domain of VEGFR-2, and blocks ligand binding [Grothey and Galanis, 2009]. In a phase I study, the maximum tolerated dose (MTD), safety, anticancer activity, PK, and PDs of ramucirumab were examined in patients with solid tumors ($n = 37$) [Spratlin, 2011]. A dose of 13 mg/kg was considered the MTD since one patient developed hypertension at 16 mg/kg; other dose-limiting toxicities (DLTs) included grade 3 proteinuria and vomiting [Spratlin, 2011]. A total of 15% of patients with measurable disease had a partial response and 30% of patients had either partial response or stable disease lasting at least 6 months [Spratlin, 2011]. Serum VEGF-A levels increased between 1.5 and 3.5 times the pre-treatment values, and tumor perfusion and vascularity was reduced in 69% of patients [Spratlin, 2011]. Ramucirumab added to FOLFIRI is currently being evaluated in a placebo-controlled, phase III registration trial in mCRC patients who have failed prior oxaliplatin- and bevacizumab-containing initial therapy [ClinicalTrials.gov identifier: NCT01183780].

Kinase inhibitors

Some TK inhibitors (TKIs) such as sunitinib and sorafenib (currently FDA approved for other cancers such as renal cell carcinoma) also exhibit inhibitory effects on the VEGF pathway and are under investigation for mCRC (Table 1) [Bhargava and Robinson, 2011]. In addition, second-generation VEGFR TKIs, such as axitinib, have exhibited lower potential for 'off-target' toxicity in clinical trials. These toxicities may arise from inhibition of other TKs, such as those of the platelet-derived growth factor receptor (PDGFR), cKit, RET, c-Raf, and others [Bhargava and Robinson, 2011]. These small-molecule kinase inhibitors have undergone testing in various settings in

mCRC in combination with multiple standard CT backbones or as single agents (Table 1).

Vatalanib

Vatalanib (PTK/ZK) is a small-molecule TKI active against all known VEGF receptors [Thomas *et al.* 2007]. In a phase I study, the efficacy and safety of vatalanib was examined in combination with FOLFOX4 in patients previously untreated with advanced CRC [Thomas *et al.* 2007]. PTK/ZK was well tolerated at a dose of 1250 mg daily in combination with FOLFOX4, with no PK interactions observed [Thomas *et al.* 2007]. In a phase III clinical trial, vatalanib was compared with placebo in combination with FOLFOX4 in patients with previously untreated mCRC ($n = 1168$) [Hecht *et al.* 2011]. PFS (7.7 *versus* 7.6 months; HR = 0.88; $p = 0.118$), OS (21.4 *versus* 20.5 months; HR = 1.08; $p = 0.260$), and overall response rate (ORR) were not significantly improved when vatalanib was added to FOLFOX4 as compared with FOLFOX4 alone; however, in an exploratory *post hoc* analysis, PFS was significantly improved with vatalanib in patients with high serum lactate dehydrogenase (LDH), a marker of hypoxia (7.7 *versus* 5.8 months; HR = 0.67; $p = 0.009$) [Hecht *et al.* 2011]. The latter results suggest that a subgroup of patients with advanced CRC may benefit from the addition of vatalanib to FOLFOX4. Similar findings were reported separately in another phase III study of vatalanib ($n = 855$) in a second-line setting in combination with FOLFOX4 (median OS = 13.1 *versus* 11.9 months with and without vatalanib, respectively, HR = 1.00; $p = 0.957$), although an improvement in PFS was seen in this study (5.6 *versus* 4.2 months; HR = 0.83; $p = 0.013$), with improved PFS also observed in patients with high LDH (HR = 0.63; $p < 0.001$) [Van Cutsem *et al.* 2011a]. In view of the negative data of two phase III trials, development of vatalanib in mCRC is no longer being pursued.

BIBF 1120

BIBF 1120 is an oral inhibitor of all three VEGFRs, PDGFR- α and PDGFR- β , and FGFRs 1–3 [Hilberg *et al.* 2008]; this agent has been compared with bevacizumab in combination with mFOLFOX6 in 88 mCRC patients with no prior CT [Van Cutsem *et al.* 2011b]. Compared with bevacizumab, BIBF 1120 did not impact exposure and intensity of mFOLFOX6, and the 9-month PFS (Kaplan–Meier) rate was 63% and

69% in the BIBF 1120 and bevacizumab groups, respectively [Van Cutsem *et al.* 2011b]. Median PFS was 10.6 months (both groups); confirmed ORR was 61% and 54%, respectively; and resection rate was 14% and 20%, respectively [Van Cutsem *et al.* 2011b]. Of note, serious AEs in the study (34% *versus* 54%) were lower with BIBF 1120 compared with bevacizumab in this trial, as were the AEs leading to discontinuation of BIBF 1120 compared with bevacizumab (25% *versus* 32%), with analysis of serious AEs ongoing [Van Cutsem *et al.* 2011b].

Brivanib

Brivanib is a dual-activity TKI showing selective inhibitory effects on VEGFR-1, as well as the FGFR [Diaz-Padilla and Siu, 2011]. As a single agent, brivanib appears to be tolerable at a dose of 800 mg once daily and has shown promising activity in combination with cetuximab in patients with CRC, as well as a single agent in patients with hepatocellular carcinoma [Diaz-Padilla and Siu, 2011]. In a phase I dose-escalation study, brivanib was combined with cetuximab in patients with GI malignancies who had failed prior therapy [Garrett *et al.* 2011]. The most frequently reported treatment-related grade 3/4 AEs were fatigue (12.9%) and an increase in hepatic transaminases (8.1–9.7%). Toxicities were manageable, and overall RR was 10% with a median PFS of 3.9 months [Garrett *et al.* 2011]. The brivanib/cetuximab combination is currently under investigation in a phase III trial of previously treated patients with WT, *KRAS*-advanced mCRC [Garrett *et al.* 2011; ClinicalTrials.gov identifier: NCT00640471]. Results of this trial were recently reported and showed a benefit of the combination of brivanib/cetuximab *versus* placebo/cetuximab on PFS (HR = 0.72; $p < 0.0001$) and RR (partial response, 13.6% *versus* 7.2%; $p = 0.004$), but no significant impact on OS (HR = 0.88; $p = 0.12$) [Siu *et al.* 2012].

Cediranib

Cediranib is a TKI that has inhibitory activity against all three VEGFRs, as well as a number of other receptors, such as PDGFR- β and FGFR [Lindsay *et al.* 2009]. In a phase I study of patients with previously untreated advanced CRC ($n = 16$), cediranib was combined with the mFOLFOX6 CT regimen [Chen *et al.* 2009]. No grade 4 toxicities relating to cediranib were observed, and common grade 3 toxicities (among 9 patients

at a 30 mg dose) were hypertension ($n = 3$), diarrhea ($n = 4$), fatigue ($n = 3$), and anorexia ($n = 1$) [Chen *et al.* 2009]. There were no PK interactions observed between cediranib and 5-FU or oxaliplatin, and of 14 evaluable patients, there were 6 partial responses (RR = 42%) and 6 with stable disease (median duration 4.8 months) observed, with a median PFS of 9.3 months [Chen *et al.* 2009]. The combination therapy used in this trial is currently undergoing evaluation in two global phase III studies, HORIZON II and HORIZON III, as well as a phase II study in second-line treatment (HORIZON I) [Robertson *et al.* 2009]. Results from HORIZON II presented at the American Society for Clinical Oncology (ASCO) 2011 Annual Meeting reported that while the overall proportion of patients treated to progression was high with both cediranib (72%) and placebo (81%) when used in combination with either FOLFOX or XELOX CT, AEs may have impacted the ability to maintain cediranib to progression, thus impacting the outcome [Fielding *et al.* 2011].

Linifanib

Linifanib is another orally administered multitargeted TKI under development for various cancers, including non-small cell lung, breast, liver, and CRC, and is designed to inhibit both VEGFRs and PDGFRs [Adis, 2010]. Linifanib is under investigation as a second-line treatment (in comparison with bevacizumab) in combination with mFOLFOX6 in a phase II study of advanced CRC patients (Table 1) [Adis, 2010].

Sorafenib

Sorafenib is a first-generation TKI with inhibitory activity at multiple receptors, including those of all three VEGFRs, as well as PDGFR- β , cKit, and RET. The drug has shown activity over placebo in a phase III study of metastatic renal cell carcinoma patients who had failed previous therapy [Escudier *et al.* 2007]; however, it is also associated with ‘off-target’ AEs, including grade 3/4 hand-foot syndrome, fatigue, diarrhea, and rash [Bhargava and Robinson, 2011]. Recently, results of a randomized phase II trial were reported in which sorafenib was added to standard FOLFOX as first-line therapy for patients with mCRC [Tabernero *et al.* 2011]. In the 198-patient trial, the addition of sorafenib to FOLFOX did not demonstrate any improvement, but a trend toward inferior outcome was noted, potentially due to a

shorter duration of therapy in view of treatment-related side effects [Tabernero *et al.* 2011].

Regorafenib (BAY 73-4506)

Regorafenib is also an inhibitor of all three VEGFRs, as well as other TKs including those of cKit, PDGF, Tie2, and FGF receptors [Bhargava and Robinson, 2011; Wilhem *et al.* 2011]. In a phase Ib study of regorafenib administered with FOLFOX or FOLFIRI as first- or second-line treatment for mCRC ($n = 45$), the most common grade 3/4 AEs with regorafenib/FOLFOX included neutropenia, mucositis, and leucopenia; with regorafenib/FOLFIRI, included neutropenia, leucopenia, hand-foot syndrome, and diarrhea [Schultheis *et al.* 2011]. Regorafenib is currently under evaluation *versus* placebo (2:1 randomization) in a phase III study (CORRECT) of 760 patients with mCRC after failure of standard therapy (Table 1) [ClinicalTrials.gov identifier: NCT01103323]. Preliminary results from a preplanned interim analysis have been recently reported, and show an HR for OS of 0.773 (one-sided $p = 0.0052$) and for PFS, 0.49 (one-sided $p < 0.000001$) in favor of regorafenib *versus* placebo; ORR was 1.6% and 0.4% in the respective groups with a higher disease control rate (partial response + stable disease) for regorafenib of 44.8 *versus* 15.3% ($p < 0.000001$) [Grothey *et al.* 2012]. In the regorafenib arm, the most frequently reported grade 3 or higher AEs in this trial included hand-foot syndrome (17%) and fatigue (15%). Results of this trial showed an expected safety profile and a significant benefit of regorafenib in both OS and PFS in patients with mCRC after failure of standard therapies [Grothey *et al.* 2012].

Integrating antiangiogenic therapies into clinical practice

It has been proposed that the combination of antiangiogenic therapies with CT may be beneficial, possibly due to a 'normalization' effect on tumor blood vessels [Gerber and Ferrera, 2005; Jain, 2005], and clinical data with bevacizumab in combination with regimens such as FOLFOX4 [Giantonio *et al.* 2007] and IFL [Hurwitz *et al.* 2004], as well as newer agents such as aflibercept in combination with FOLFIRI [Van Cutsem *et al.* 2011a], appear to support this hypothesis, with significant improvements in OS, PFS, and/or ORR observed with the antiangiogenic combination therapy compared with patients receiving CT

alone ($p \leq 0.0032$). The availability of a range of antiangiogenic therapies with differing mechanisms of action will help expand treatment options in the second- and third-line settings (Table 1).

As noted earlier, the results from some trials suggest that benefits in OS can only be achieved if patients are able to tolerate and continue treatment to disease progression [Saltz *et al.* 2008]. This has led to trial designs that allow for a more optimal use of CT regimens using schedules that reduce key toxicities (e.g. neurotoxicity associated with oxaliplatin) as a backbone for biologic agents such as bevacizumab [Saltz *et al.* 2008]. Notable trials in this regard include CONceptPT, comparing the use of intermittent *versus* continuous oxaliplatin and calcium/magnesium salts to reduce neurotoxicity [ClinicalTrials.gov identifier: NCT00129870; Grothey *et al.* 2008a]. Other trials, such as MACRO [Díaz-Rubio *et al.* 2012] and CAIRO-3 [ClinicalTrials.gov identifier: NCT00442637], are also examining the optimal schedules and durations of CT in combination with biologic therapy in order to allow for continuation of CRC therapies for as long as possible. In MACRO, it was recently reported that there were no differences in PFS, OS, or RR when comparing patients with mCRC who received single-agent maintenance bevacizumab following a bevacizumab-induction chemotherapy regimen; the results showed single-agent bevacizumab to be noninferior to continuing XELOX/bevacizumab [Díaz-Rubio *et al.* 2012]. The ongoing phase III Treatment Across Multiple Lines (TML) trial is assessing the value of bevacizumab beyond progression in patients with mCRC who have experienced disease progression with first-line bevacizumab plus standard CT. Patients in stratum 1 received CT (AIO-IRI, FOLFIRI, CAPIRI, or XELIRI) alone or in combination with bevacizumab, and those in stratum 2 received CT (FUFOX, FOLFOX, CAPOX, or XELOX) alone or in combination with bevacizumab [ClinicalTrials.gov identifier: NCT00700102]. Preliminary data from this trial has been reported as showing a significant OS advantage with continuation of bevacizumab following progression. More definitive reporting is expected during 2012.

Importance of molecular biomarkers in mCRC and antiangiogenic therapy

Biomarkers are increasingly important in the design of cancer therapy, as they can be used to identify patients with poor prognoses at high risk

for an unfavorable clinical course and therefore require more aggressive treatments. Biomarkers may also be utilized to identify patients who are most likely to benefit from a given therapy or combination of therapies, and determine when efficacy of a given agent no longer outweighs its potential toxicity (i.e. when to discontinue therapy). It has been increasingly recognized that biomarker assessments need to be integrated into the design and study endpoints for preclinical and clinical trials of antiangiogenic therapies. This is in view of the fact that unlike cytotoxic CT, reduction in tumor mass with more cytostatic angiogenic therapies may be less amenable to assessment using RECIST criteria [Gerger *et al.* 2011; Chun *et al.* 2009]. Thus, the use of biomarkers may allow for a more precise identification of optimal doses and schedules (e.g. determining what dose of an anti-VEGF agent allows for optimal inhibition of VEGF in a given patient or tumor). Unfortunately, although a number of candidate biomarkers have been identified (Table 2), none has yet been rigorously validated and no such marker is ready for use in clinical practice.

VEGF-A isoforms as a predictive marker

A recent study has investigated the utility of short isoforms of plasma VEGF-A as predictive markers in clinical trials of mCRC (AVF2107g), non-small cell lung cancer (AVAIL), and renal cell cancer (AVOREN) [Jayson *et al.* 2011]. These investigators utilized a novel assay that favored shorter forms of VEGF-A, VEGF-A₁₂₁, and VEGF-A₁₁₀, which, unlike standard VEGF-A assays, had displayed predictive value in breast, pancreatic, and gastric cancer such that higher plasma VEGF-A levels at baseline predicted better PFS and/or OS with bevacizumab treatment. They found that whereas the prognostic value of plasma VEGF-A could be confirmed in all three trials, the potential predictive value using this novel assay could not be replicated in mCRC, non-small cell lung cancer, or renal cell cancer [Jayson *et al.* 2011]. The authors speculate that the difference in predictive value could be due to tumor-specific factors, as the assay favors shorter VEGF-A isoforms; further study will be needed to resolve this issue in different tumor types [Jayson *et al.* 2011].

Table 2. Candidate biomarkers in antiangiogenic therapy.

Biomarker	Rationale for use as a biomarker in CRC/antiangiogenesis therapy	Patient population and setting	Reference
VEGF	Elevated serum VEGF associated with poorer prognosis; VEGF correlated with tumor size and volume; VEGF had low sensitivity as a diagnostic tumor marker	Patients with mCRC; VEGF levels were assessed in serum samples	[Broll <i>et al.</i> 2001]
	VEGF mRNA and protein levels in plasma were higher in patients with CRC <i>versus</i> controls; VEGF mRNA overexpressed in tumors <i>versus</i> normal tissues; high VEGF mRNA tended to be associated with vascular invasion	Patients with CRC; VEGF levels were assessed in serum samples and tumor tissues	[Garcia <i>et al.</i> 2008]
	Preoperative serum VEGF levels did not correlate with CEA levels or provide relevant prognostic information in patients with CRC	Patients with CRC; VEGF levels were assessed preoperatively in serum samples	[Roumen <i>et al.</i> 2005]
PlGF	Serum PlGF, but not VEGF, increased in patients with CRC <i>versus</i> healthy controls; higher preoperative PlGF levels were associated with a greater recurrence risk	PlGF levels were assessed in serum in patients with CRC	[Wei <i>et al.</i> 2009]
	Upregulated prior to progression in patients on FOLFIRI + Bevacizumab	Patients with mCRC; samples assessed in serum at baseline, after bevacizumab, and at time of progression	[Kopetz <i>et al.</i> 2010]
CECs and CEPs	Anti-VEGF therapy with bevacizumab reduces CECs	CEC levels assessed in plasma in patients with adenocarcinoma of the rectum	[Willet <i>et al.</i> 2004]

(Continued)

Table 2. (Continued)

Biomarker	Rationale for use as a biomarker in CRC/ antiangiogenesis therapy	Patient population and setting	Reference
CEA	Correlation between VEGF-induced angiogenesis and CEC number; CECs or CEPs could represent surrogate biomarkers to optimize antiangiogenesis therapy	Evidence from <i>in vitro</i> studies and mouse lung cancer model	[Gupta, 2007]
	Lower CEP levels were associated with longer PFS ($p < 0.001$) and OS ($p = 0.002$) in patients treated with bevacizumab and CT	Patients with mCRC and commencement of a new systemic therapy; plasma samples were assessed for CEP before and after therapy	[Matsusaka <i>et al.</i> 2011]
	Increases sensitivity of VEGF as a preoperative diagnostic marker	Patients with mCRC; VEGF and CEA levels were assessed in serum samples	[Broll <i>et al.</i> 2001]
CA19.9	CEA was not a predictive biomarker for FOLFIRI + Bevacizumab	Patients with mCRC; CEA levels were assessed in serum	[Formica <i>et al.</i> 2009]
	Baseline levels were significantly associated with PFS ($p = 0.02$); patients with abnormal baseline CA19.9 benefitted significantly from bevacizumab treatment	Patients with mCRC; CA19.9 levels were assessed in serum	[Formica <i>et al.</i> 2009]
ERCC-1	ERCC-1 associated with OS in first- or second-line therapy with FOLFOX4; correlation observed between high ERCC-1 and benefit from PTK/ZK + FOLFOX4 treatment; baseline TS and ERCC-1 may determine benefit from FOLFOX4 therapy	Patients with mCRC; ERCC-1 gene expression determined by mRNA levels in tissue samples	[Grimminger <i>et al.</i> 2011]
TS	TS was associated with OS in first-line, but not second-line therapy; baseline TS and ERCC-1 may determine benefit from FOLFOX4 therapy	Patients with mCRC; TS gene expression determined by mRNA levels in tissue samples	[Grimminger <i>et al.</i> 2011]
CD133 (marker for bone marrow-derived CECs)	High CD133 levels were associated with a greater recurrence risk in CRC (OR = 22.2; $p = 0.02$); CD133 levels correlated with CEA levels and a trend towards decreased OS	Patients with CRC; CD133 levels in PBMCs determined by mRNA expression	[Lin <i>et al.</i> 2007]
Stromal cell derived factor (SDF1- α)	Anti-VEGF therapy with bevacizumab upregulates SDF1 in rectal cancer; higher circulating SDF1 levels after bevacizumab are observed in patients who progress more rapidly to metastatic disease	Patients with rectal carcinoma; SDF1- α levels determined in plasma	[Xu <i>et al.</i> 2009]
Chemokine receptor CXCR4	Proportion of CXCR4-positive CECs was associated with longer PFS ($p < 0.001$) and OS ($p = 0.002$) in patients treated with bevacizumab and CT	Patients with mCRC and commencement of a new systemic therapy; plasma samples were assessed for CXCR4-positive CECs before and after therapy	[Matsusaka <i>et al.</i> 2011]
	Bevacizumab treatment increased levels of SDF1 and its receptor CXCR4 in patients with rectal cancer; SDF1/CXCR4 pathway could be a resistance mechanism for antiangiogenesis therapy with bevacizumab	Patients with rectal carcinoma; CXCR4 levels determined by immunohistochemistry in biopsy tissues	[Xu <i>et al.</i> 2009]

CEA, carcinoembryonic antigen; CEC, circulating endothelial cell; CEP, circulating endothelial progenitor; CRC, colorectal cancer; CT, chemotherapy; ERCC-1, excision repair cross-complementing; mCRC, metastatic colorectal cancer; OR, odds ratio; OS, overall survival; PBMC, peripheral blood mononuclear cell; PFS, progression-free survival; TS, thymidylate synthase; VEGF, vascular endothelial growth factor.

Predictive markers for response

D-dimer is a fibrin degradation product associated with the process of angiogenesis, and elevated levels of D-dimer have been found in patients with CRC [Blackwell *et al.* 2004]. In a phase II study comparing bevacizumab plus 5FU/LV with 5FU/LV alone, D-dimer levels were a strong prognostic indicator of survival in both univariate ($p = 0.008$) and multivariate ($p = 0.03$) analysis [Blackwell *et al.* 2004]. In this study, bevacizumab treatment and baseline D-dimer levels were the only independent predictors of OS. As noted earlier, serum LDH may also identify a subgroup of patients that benefit from the addition of vatalanib to CT [Hecht *et al.* 2011; Van Cutsem *et al.* 2011c], although these results could not be replicated in other trials with antiangiogenic agents.

Markers for anti-VEGF resistance

The rapid identification of patients who develop resistance to VEGF-directed therapy is important in considering alternative treatment, and also for halting ineffective therapies and their associated toxicities. One study identified high LDH-5 levels as strongly associated with poor survival in patients with operable CRC ($p = 0.0003$; HR = 15.1) [Koukourakis *et al.* 2006]. The predicted 5-year survival was 52% versus 96% for patients with high and low LDH-5, respectively [Koukourakis *et al.* 2006]; notably, markers of angiogenesis (e.g. VEGF) and tumor vascular density were also related to poor prognosis in this study [Koukourakis *et al.* 2006]. In another phase I/II study of neoadjuvant bevacizumab in combination with CT in rectal cancer, bevacizumab either alone or in combination was found to significantly increase circulating PlGF and VEGF levels ($p < 0.05$); these changes were specific to the anti-VEGF therapy, as they did not occur in patients receiving the chemoradiation therapy alone [Willett *et al.* 2009]. The authors suggest that plasma VEGF and PlGF could be useful as PD biomarkers for anti-VEGF therapy with further validation [Willett *et al.* 2009]. Recent findings suggest that VEGF-D may be a predictive marker of resistance to bevacizumab in patients receiving capecitabine alone or in combination with mitomycin [Weickhardt *et al.* 2011]. These authors found that a lower expression of VEGF-D was associated with a greater benefit of bevacizumab treatment in terms of both OS and PFS, and higher expression of VEGF-D was predictive of resistance to bevacizumab, most significantly with respect to PFS.

There is evidence from preclinical models for an adaptive response to bevacizumab exposure in CRC cells, such that CRC cells exposed to bevacizumab exhibited significantly increased migration and invasive capacity after 1 week of therapy, while proliferation was unaffected [Fan *et al.* 2011]. Notably, increased expression and activation of VEGFR-1, and the ligands for VEGFR-1, PlGF, and VEGF-B were also observed in cells chronically exposed to bevacizumab [Fan *et al.* 2011]. Similar findings have also emerged from clinical studies. In a phase II study of FOLFIRI plus bevacizumab, additional plasma cytokines and angiogenic factors (CAFs) may have mediated resistance to bevacizumab [Kopetz *et al.* 2010]. Results of the study showed a median PFS of 12.8 months, median survival of 31.3 months, and a RR of 65% in patients assigned to FOLFIRI plus bevacizumab [Kopetz *et al.* 2010]. Elevated baseline plasma interleukin-8 levels were significantly associated with poorer PFS (11 versus 15.1 months; $p = 0.03$) and importantly, prior to progression, several CAFs were significantly elevated compared with baseline, most notably bFGF ($p = 0.047$), hepatocyte growth factor ($p = 0.046$), and PlGF ($p < 0.001$) [Kopetz *et al.* 2010]. A recent study identified molecular markers associated with response to bevacizumab in patients receiving GONO-FOLFOXIRI for mCRC [Loupakis *et al.* 2011]; treatment was associated with a decrease in plasma-free, biologically active VEGF concentration; however, VEGF levels remained low even at the time of disease progression [Loupakis *et al.* 2011]. Conversely, compared with baseline, levels of PlGF, soluble VEGFR-2, and thrombospondin-1 increased at the time of disease progression, suggesting a possible involvement of these factors in tumor resistance [Loupakis *et al.* 2011]. Further study of CAFs may help identify other key molecular targets for inhibition to overcome resistance in patients treated with antiangiogenic therapy.

Lastly, it is of interest to consider some findings from preclinical studies on the 'rebound' effect associated with the discontinuation of antiangiogenic therapies such as bevacizumab [Bagri *et al.* 2010]. There is some evidence from tumor xenograft models that discontinuation of antiangiogenic therapy with anti-VEGF neutralizing antibodies resulted in no apparent rebound effect following discontinuation, and growth following chemotherapy exposure could be largely prevented with anti-VEGF cotreatment; hence, making the case for the benefit of continued

antiangiogenic maintenance therapy despite progression [Bagri *et al.* 2010]. By comparison, studies of an experimental prostate cancer model which metastasizes to brain and bone demonstrated that the multi-targeted TKI cediranib was effective in inhibiting tumor growth and increasing survival, while at the same time increasing the invasive and metastatic potential of the tumor [Yin *et al.* 2010]. Interestingly, withdrawal of cediranib led to a rebound/regrowth of brain metastases, whereas bone metastases continued to be inhibited [Yin *et al.* 2010]. Whether these findings relate to a broader inhibition of signaling with TKIs as opposed to more targeted therapies such as bevacizumab, or whether they relate to other tumor and/or host characteristics, is an area worthy of further investigation.

Conclusions

The need for continued revascularization and angiogenesis in solid tumors provides a rational basis for antiangiogenic therapies. Angiogenesis inhibition through manipulation of the VEGF pathway has demonstrated potential, particularly as combination therapy with CT in mCRC, leading to meaningful improvements in PFS, RR, and survival [Giantonio *et al.* 2007; Hurwitz *et al.* 2004]. The success of targeted antiangiogenesis therapies, such as bevacizumab, provides a rationale to further expand treatment options that target angiogenesis pathways. The recombinant fusion protein, aflibercept, binds multiple ligands in the angiogenesis network and could potentially allow for a more efficient inhibition of tumor angiogenesis [Van Cutsem *et al.* 2011a]. Similarly, that the multikinase inhibitor, regorafenib, demonstrated efficacy as single-agent salvage therapy could indicate that in this setting, a promiscuous, multitargeted agent may inhibit various proangiogenic pathways activated by tumor cells during the course of prior anticancer therapy. Importantly, the optimization of antiangiogenic therapy will require a careful assessment of the best combinations, doses, and treatment schedules to allow for effective treatment that is tolerable, does not result in treatment resistance, and does not impair quality of life for patients, a goal in line with the concept of 'continuum of care' [Goldberg *et al.* 2007]. Attainment of the aggressive goal of individualized therapy will also require a reassessment of current efficacy measures in clinical trials, with incorporation of validated angiogenesis biomarkers, once available, into trial designs to identify patients most likely to benefit

from a given therapy, and/or those requiring the most aggressive treatments.

Funding

Medical editorial assistance was provided by Samantha Taylor, PhD, of Phase Five Communications Inc., and supported by sanofi-aventis U.S. LLC., in collaboration with Regeneron Pharmaceuticals.

Conflict of interest statement

Dr Axel Grothey discloses receipt of funding from Sanofi for work on the CONCEPT trial. Dr Carmen Allegra discloses receipt of funding from Sanofi for work on the VELOUR trial. The authors retained full editorial control over the content of the manuscript and received no compensation from any party for their work.

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