Markers of Response to Antiangiogenic Therapies in Colorectal Cancer: Where Are We Now and What Should Be Next?

E. Una Cidon[1], P. Alonso[2] and B. Masters[3]

[1]Department of Medical Oncology, Royal Bournemouth Hospital NHS Foundation Trust, Bournemouth, UK. [2]Department of Clinical Oncology, Clinical University Hospital, Valladolid, Spain. [3]Department of Oncology, Nottingham City Hospital, Nottingham, UK.

Supplementary Issue: Key Difficulties Associated with Cancer Biology

ABSTRACT: Despite advances in the treatment of colorectal cancer (CRC), it remains the second most common cause of cancer-related death in the Western world. Angiogenesis is a complex process that involves the formation of new blood vessels from preexisting vessels. It is essential for promoting cancer survival, growth, and dissemination. The inhibition of angiogenesis has been shown to prevent tumor progression experimentally, and several chemotherapeutic targets of tumor angiogenesis have been identified. These include anti-vascular endothelial growth factor (VEGF) treatments, such as bevacizumab (a VEGF-specific binding antibody) and anti-VEGF receptor tyrosine kinase inhibitors, although antiangiogenic therapy has been shown to be effective in the treatment of several cancers, including CRC. However, it is also associated with its own side effects and financial costs. Therefore, the identification of biomarkers that are able to identify patients who are more likely to benefit from antiangiogenic treatment is very important. This article intends to be a concise summary of the potential biomarkers that can predict or prognosticate the benefit of antiangiogenic treatments in CRC, and also what we can expect in the near future.

KEYWORDS: antiangiogenesis, VEGF, colorectal cancer, predictive markers

SUPPLEMENT: Key Difficulties Associated with Cancer Biology

CITATION: Cidon et al. Markers of Response to Antiangiogenic Therapies in Colorectal Cancer: Where Are We Now and What Should Be Next? *Clinical Medicine Insights: Oncology* 2016:10(S1) 41–55 doi: 10.4137/CMO.S34542.

TYPE: Review

RECEIVED: January 06, 2016. **RESUBMITTED:** March 13, 2016. **ACCEPTED FOR PUBLICATION:** March 15, 2016.

ACADEMIC EDITOR: William Chi-shing Cho, Editor in Chief

PEER REVIEW: Five peer reviewers contributed to the peer review report. Reviewers' reports totaled 878 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: aunacid@hotmail.com

Introduction

Colorectal cancer (CRC) is a major health concern worldwide due to its high prevalence and mortality rate. In developed countries, it is the third most common malignancy and the second most common cause of cancer-related death. Although advances in the treatment of CRC have made a major impact on its management, many patients with advanced disease will eventually die as a result of their cancer. Further research is, therefore, essential to further improve the outcomes from CRC.[1]

Genetic analysis of tumor DNA has shown that many cancers contain a large number of genetic mutations. The mutations often encode important cellular proteins. Several genetic alterations in CRC have been identified, and these relate to specific cellular sgnaling pathways. This has led to the development of several new therapeutic targets.[1]

One of these targeted agents, anti-epidermal growth factor receptor (EGFR) monoclonal antibodies, has been shown to produce improved outcomes in patients with metastatic CRC (mCRC) when combined with chemotherapy.[2,3] *KRAS* and *BRAF* mutations have been shown to predict response to anti-EGFR treatment. Mutations in the phosphatidylinositol 3-kinase (*PI3K*) pathway may also play a role in predicting the response to this targeted treatment.[4,5]

Tumor angiogenesis is a key factor in the growth, metastatic spread, and recurrence of CRC.[6] Anti-vascular endothelial growth factor (VEGF) therapy has also demonstrated improved results in CRC when given in combination with chemotherapy.

Angiogenesis is a complex process, which involves the formation of new vessels from the preexisting blood vessels. These new vessels supply nutrients to the tumor, promoting cancer survival, growth, and dissemination. This process involves numerous factors, but VEGF and its signaling are considered as one of the most important.[7]

The hypothesis that tumor progression can be prevented by the inhibition of angiogenesis has been confirmed experimentally. The addition of bevacizumab (a VEGF-specific blocking antibody) or aflibercept to standard chemotherapy, as well as the use of anti-VEGF receptor tyrosine kinase inhibitors (TKIs), such as regorafenib, has shown efficacy in the treatment of some cancers, including mCRC.[8–10] However, a substantial number of tumors are thought to become

insensitive to antiangiogenic inhibitors that target VEGFA signaling, such as bevacizumab, through therapy-induced injury, metabolic changes, inflammation, and expansion of myeloid-derived suppressor cells (MDSCs).[10] Aflibercept, however, targets placenta growth factor (PlGF) and consequently reduces the source of compensatory upregulation of angiogenic factors by inhibiting the immune cell recruitment and preventing the release of angiogenic factors by the tumor and vascular endothelial (VE) cells. Regorafenib has also been approved for the treatment of CRC. It is a multikinase inhibitor that inhibits the selected tyrosine kinase-mediated signal transduction through the VEGF receptor (VEGFR) 2–3/RAF/MEK/ERK pathway and has been shown to prolong the overall survival (OS).[10]

Current antiangiogenic therapies act by one of the two major mechanisms: they are either able to inhibit the action of proangiogenic factors or its receptors or able to block the tyrosine kinase receptor signaling intracellularly.

The process of angiogenesis. The process of angiogenesis is activated after malignant cells are exposed to certain stimuli. Hypoxia is thought to be the most important, and tumor cells respond by modulating the hypoxia-inducible factor (HIF) 1α, which dimerizes with HIF1β.[1,6–8]

This complex is located within the nucleus and starts the transcription of several growth factors, including VEGF, platelet-derived growth factor (PDGF)-β, basic fibroblast growth factor (bFGF), erythropoietin, angiopoietins, and PlGF.[11] These factors stimulate new vessel formation and the release of several other growth factors, which continue to promote angiogenesis.[11,12]

Regulators of angiogenesis. *VEGF family and VEGF receptors.* The VEGF family of growth factors also includes PlGF1 and -2. These growth factors exert their effect by binding to VEGFRs whose activity depends on their dimerization potential. Dimerizations between VEGFR1 and -2 and VEGFR2 and -3 are involved in some of the physiological effects of the VEGF family members. The activation of VEGFR1 leads to a *decoy effect* as a VEGF-trap. This performs *fine tuning* of VEGF signaling to induce the formation of new vessels. Deletion or blockage of VEGFR1 significantly reduces endothelial cell proliferation and induces premature senescence. The activation of VEGFR2 leads to proliferation, migration, survival, and angiogenesis, while its deletion impairs endothelial cell survival. VEGFR3 has a similar action to VEGFR2 but instead promotes the growth of lymphatic vessels rather than blood vessels.[11]

VEGF-resistant tumors have been shown to respond to treatments with monoclonal antibodies targeting PlGF, even though this is a VEGF family member. Several studies have shown that PlGF binds to VEGFR2 and neuropilin-1 receptor.[12–17]

PDGF. PDGF is a dimeric polypeptide, composed of one of the following four homodimers: A, B, C and D. Its activity is mediated by binding to the dimeric PDGF receptors.

PDGF-B is significantly involved in resistance to anti-VEGF therapy. It is able to recruit mural endothelial cells and stabilize blood vessels, therefore increasing the tumor survival. This has consequently led to the development of new antiangiogenic treatments aimed to target both VEGF and PDGF. These include sorafenib, pazopanib, axitinib, and sunitinib.[18–23]

FGF and FGF receptors. FGFs exert their effects through one of the four FGF receptors 1–4, which have intracellular tyrosine kinase domains. Their activation leads to angiogenesis and maturation of established blood vessels. These factors are also potential targets in VEGF-resistant cancers.

Integrins. Integrins are transmembrane receptors that are able to bind to extracellular matrix proteins and to other adhesion receptors on neighboring cells. Integrins can interact with growth factor receptors to regulate angiogenesis. During tumor angiogenesis, tumor-associated endothelial cells have been shown to overexpress integrin αvβ3 to facilitate the growth and survival of newly forming vessels.[24]

Inhibiting the action of integrins can produce an antiangiogenic effect. The potential benefit of integrin antagonists has already been shown in CRC.[25]

Biomarkers of response to antiangiogenic therapy. *Blood pressure.* Hypertension has been observed in patients treated with anti-VEGF antibodies and TKIs. Several randomized studies have shown that bevacizumab (anti-VEGF antibody) improves both progression-free survival (PFS) and OS.[26] In all these studies, hypertension was found to be a common side effect associated with bevacizumab.

Not all patients, however, benefit from treatment with anti-VEGF antibodies. Currently, there are no definitive biomarkers that are able to predict which patients will benefit from antiangiogenic therapies. However, hypertension is thought to be a possible predictor of response.

Inhibition of the VEGF pathway prevents continued endothelial cell survival signaling, which leads to apoptosis. It also reduces endothelial cell-derived nitric oxide production. This leads to vascular muscle constriction, with subsequent increased vascular resistance and elevation in blood pressure.[27]

Hypertension has been suggested to predict treatment efficacy in patients with metastatic renal cancer treated with bevacizumab or sunitinib.[28,29] In mCRC, Osterlund et al carried out a study to investigate whether treatment-related hypertension was associated with outcome and safety following treatment with bevacizumab-containing chemotherapy. The study showed that early hypertension (within the first three months of treatment) was predictive for an improved OS.[30] Another study has shown that hypertension within one month of commencing bevacizumab therapy for lung cancer was also predictive for survival.[31]

Schneider et al also showed an association between VEGF genotype and the development of clinically significant hypertension. Patients with VEGF-1498TT and VEGF-634CC

genotypes were found to be less likely to develop grade 3/4 hypertension and had poorer survival outcomes.[31]

It has also been observed that mean systolic and diastolic blood pressures of patients treated with bevacizumab increase while receiving treatment and returns to baseline following the treatment completion; although to use this fact as a predictive biomarker, additional data will be needed.[32]

Circulating VEGF. VEGF has been the most widely studied biomarker in predicting response to antiangiogenic treatment. Associations between the efficacy of antiangiogenic treatments and circulating VEGF levels have been reported in several phase II studies. These have shown that the elevated levels of VEGF have been associated with a poor prognosis but do not predict response to antiangiogenic treatments, such as bevacizumab.[33–36] A phase II study of bevacizumab combined with chemoradiation in rectal cancer showed no correlation between VEGF levels and the outcome of therapy.[37]

Low baseline plasma VEGF levels have been directly associated with PFS in patients with advanced non-small-cell lung cancer.[35] However, a retrospective analysis of patients with renal cancer showed that high basal VEGF levels (>131 pg/mL) were associated with a worse prognosis but with an improved PFS when treated with sorafenib.[38] However, another retrospective analysis from a phase II trial, involving bevacizumab patients with refractory renal cell cancer, suggested that patients responding to sunitinib actually had lower basal circulating levels of VEGF and VEGFR3 than nonresponding patients.[39] Similarly, in a phase II/III trial studying bevacizumab and chemotherapy in patients with metastatic NSCLC showed that high baseline circulating plasma VEGF levels did not predict PFS or OS, despite a correlation with an improved overall response rate.[40]

In addition, circulating levels of VEGF have been shown to be significantly elevated after the introduction of antiangiogenic treatment.[41] This was thought to be related to induced tumor hypoxia[42,43] and decreases after treatment is discontinued. Circulating plasma VEGF levels have also been shown to increase following the therapy with anti-VEGFR TKIs.[38,44]

VEGF levels have also been evaluated as a predictive biomarker for oral TKIs.[45] Baseline levels correlate inversely with PFS and OS, and patients with renal cell carcinoma with high baseline levels of VEGF received greater benefit from treatment with sorafenib.[46,47]

These inconsistencies emphasize the need for developing biomarkers that can be used prior to and during antiangiogenic treatment. The practical utility of monitoring drug-induced changes in circulating factor levels as surrogate biomarkers is still unclear. However, currently, VEGF levels have not been shown to be a reliable predictive biomarker of clinical response to bevacizumab.

Tumor expression of VEGF. In the pivotal phase III trial of bevacizumab with chemotherapy in patients with mCRC, VEGF expression in primary tumor tissue was not found to be predictive of outcome.[48]

Another phase III trial, which evaluated bevacizumab in combination with capecitabine in breast cancer, showed that response rates were not increased in patients with tumors that overexpressed VEGF. Overexpression of VEGF was detected by in situ hybridization.[49] In a study of patients with CRC who were randomly assigned to chemotherapy with irinotecan, 5-FU, and leucovorin plus bevacizumab or irinotecan, 5-FU, and leucovorin plus placebo, VEGF expression and thrombospondin levels were measured by in situ hybridization. The addition of bevacizumab improved survival regardless of the level of expression of VEGF or thrombospondin.[35]

Single-nucleotide polymorphisms (SNPs) in VEGF and VEGFR2 have shown promise as the predictors of response to treatment and toxicity. The VEGF-2578AA genotype has been associated with an improved median OS in patients who received paclitaxel and bevacizumab treatments in comparison to other genotypes.[50]

PIGF and soluble VEGF receptors. Circulating levels of PIGF have been shown to increase in response to anti-VEGF treatment. Consequently, plasma PIGF dynamics is now being considered as a potential biomarker.[37,51] In addition, targeting PIGF is being considered as a new approach to prevent tumor resistance to anti-VEGF therapy.[52] Interestingly, the level of increase in PIGF levels in plasma has been associated with an improved outcome in patients with rectal cancer treated with bevacizumab.[37]

However, these studies have been unable to distinguish between predictive and prognostic biomarkers. Therefore, the role of PIGF as a potential biomarker needs to be further explored.

Other possible biomarkers of angiogenesis tend to be treatment specific. For example, circulating levels of soluble VEGFR2 and VEGFR3 have been shown to be decreased by TKIs that directly target these receptors[46,50–53]; however, they are unaffected by bevacizumab.[37]

Unfortunately, the mechanisms by which these changes occur, their biological significance, and predictive biomarker value are currently not well understood.

Other proteins as biomarkers. Exploration of other potential biomarkers is very important, given their known involvement in tumor angiogenesis and vessel maturation. However, in patients with mCRC treated with bevacizumab and chemotherapy, pretreatment evaluation of biomarkers, such as microvascular density, tumor tissue expression of *TSP2*, *P53*, and *KRAS* mutations, has not been predictive of efficacy.[35,54]

In previously untreated mCRC, tumor response to vatalanib plus chemotherapy was correlated directly with tissue messenger RNA levels of VEGFR1, lactate dehydrogenase (LDH) A and Glut1 in tumor tissue and inversely correlated with HIF1α.[55]

Patients with high baseline serum lactate dehydrogenase levels have been shown to have a longer PFS and OS after treatment with vatalanib and chemotherapy.[56] Certain

inflammatory cytokines, such as IL-1β, IL-6, and IL-8, may have proangiogenic properties, and a phase II study suggested that the IL-8A-251T polymorphism might be a molecular predictor of response to bevacizumab-based chemotherapy in ovarian cancer.[57] Another phase II study showed that increased inflammatory cytokines, such as IL-6, in plasma during treatment was associated with an inferior result in rectal and ovarian cancers after treatment with bevacizumab and chemoradiation and in advanced hepatocellular carcinoma (HCC) after treatment with sunitinib.[37,57]

High serum levels of EGF and macrophage-derived chemokine and low levels of IL-10, IL-6, and IL-8 were associated with a higher likelihood of response to treatment.[58,59] IL-8 has also been reported to mediate angiogenesis by stimulating the endothelial cell proliferation in response to hypoxia,[60] and resistance to antiangiogenic therapy has been associated with an increased secretion of IL-8.[61]

The potential predictive role of the low baseline levels of IL-8 and a bevacizumab-induced decrease in IL-8 levels correlate with recently reported clinical data.[62] In a clinical trial with mCRC, neither epithelial nor stromal VEGF expression was found to predict the potential benefit of the addition of bevacizumab to fluorouracil-based therapy.[35]

Circulating cells. Increased numbers of circulating endothelial cells and bone marrow-derived circulating endothelial cell progenitors have been seen in patients with cancer.[35,63,64] Circulating endothelial cell progenitors mobilized by VEGF have been found to promote angiogenesis in mice. High levels correlate with angiogenesis and return to normal following antiangiogenic treatments.[23,35,65]

Blood-circulating cells have been studied as potential biomarkers of antiangiogenic therapy. Willet et al found that bevacizumab reduced the number of viable circulating endothelial cells and bone marrow-derived circulating endothelial cell progenitors in patients with rectal cancer.[41] However, other studies found no difference in the levels of these circulating cells.[61]

However, the number of circulating endothelial progenitor cells and monocytes was reduced in response to sunitinib in HCC and gastrointestinal stromal tumors, respectively.[51,66] In patients with metastatic gastrointestinal stromal tumors, those demonstrating a clinical benefit had the increased levels of circulating endothelial cells. Sunitinib was also found to increase the circulating endothelial cells in patients with gastrointestinal stromal tumors and has been associated with clinical benefits in comparison to patients with progressive disease.[35]

ZD6474 (VEGFR2/EGFR TKI) has been shown to produce an increase in mature circulating endothelial cells but not in circulating endothelial progenitor cells. This could reflect an induced endothelial cell detachment from tumor vessels.[35,67]

Microvessel density and endothelial signaling events. Microvessel density (MVD) at the regions of intense angiogenesis (ie, *hot spots*) has prognostic but not predictive value in many cancers.[35] It does not provide information on the functionality (perfusion) of the tumor vessels. MVD of primary tumors in patients with mCRC did not predict response to bevacizumab[68] either.

The ERK phosphorylation status and AKT phosphorylation status in tumor endothelial cells have been explored as the biomarkers of antiangiogenic therapy. Their phosphorylation has been found in angiogenic vessels, and it has been attenuated by treatment with SU6668.[69]

Tissue-based biomarkers are highly valuable but impractical as biomarkers for routine clinical use. They offer information at the microscopic level, but further studies are needed to demonstrate their usefulness in the clinical setting.

MicroRNAs. MicroRNAs are small noncoding RNA molecules involved in the regulation of gene expression. Many microRNAs are abnormally expressed in cancer and influence tumor progression. Several studies suggest that they may have an active role in CRC cell dissemination, invasion, colonization, angiogenesis, and epithelial–mesenchymal transition (EMT).[70]

Colorectal oral novel therapy for the inhibition of angiogenesis and retarding of metastases (CONFIRM) 1 and 2 phase III trials showed that patients with mCRC with elevated serum LDH had an improved prognosis when vatalanib (a VEGFR inhibitor) was added to FOLFOX4. Authors researched the role of high intratumoral expression of genes regulated by HIF1α, such as LDHA, glucose transporter-1 (GLUT-1), VEGFA, VEGFR1, and VEGFR2, in predicting the outcome in CONFIRM1.[71]

In univariate and multivariate analyses, elevated LDH and VEGFR1 mRNA levels were associated with an improved PFS in patients treated with FOLFOX4/vatalanib. Increased HIF1α and VEGFR2 mRNA levels were also associated with a reduced survival in patients treated with FOLFOX/placebo when compared to FOLFOX4/vatalanib. This data suggest that the intratumoral mRNA expression of genes involved in angiogenesis/HIF pathway may predict outcome to VEGFR inhibitors.[71]

Another study assessed the predictive role of vascular density (VD) in patients treated in the CONFIRM trials.[72] The authors used paraffin-embedded samples from 141 patients and analyzed the expression of the CD31 with immunohistochemistry. Patients with tumors with high VD were found to have an improved PFS when treated with vatalanib (with chemotherapy). A similar effect was noted in patients with high $CD31^+$ VD, and OS was also marginally improved in these patients. Therefore, this suggested that a subgroup of patients with tumors with an increased VD may have an increased benefit from antiangiogenic treatment.[72]

Epigenetic regulation through the action of microRNAs has been considered to be one of the major regulatory mechanisms for tumor neovascularization.[73] MicroRNA-107 has been shown to function as a suppresser of HIF1 and VEGF expressions, and MicroRNA-145 is a regulator of HIF1 in

CRC as it is able to posttranscriptionally target p70S6K1.[74,75] Thrombospondin (TSP1) prevents neovascularization in tumors; however, MicroRNA-17-92 and microRNA-194 are able to repress TSP1 and, therefore, promote angiogenesis in CRC.[76] Overexpression of the microRNA-17-92 cluster has been observed in several tumors, including CRC, and it has been shown to be able to coordinate angiogenesis and proliferation and inhibit cellular differentiation.[77]

Mismatch repair proteins. The National Surgical Adjuvant Breast and Bowel Project protocol C-08 assessed the benefit of the addition of bevacizumab (for one year) to oxaliplatin-based standard adjuvant chemotherapy in patients with stage II and III colon cancers.[78] Overall, there was no significant difference in disease-free survival or OS between the groups. However, a further study performed as a post hoc analysis of data from the trial found that patients with tumors with defective mismatch repair (MMR) were shown to benefit from the addition of bevacizumab to FOLFOX chemotherapy. This suggests that a subgroup of patients with colon cancer may benefit from antiangiogenic treatment.[78]

The infiltration of tumor-associated macrophages (TAMs) has been associated with extensive angiogenesis and poor prognosis in breast cancer. However, antiangiogenic therapy with VEGF-specific monotherapy has been unsuccessful in the treatment of breast cancer.[79] TAMs associated with CRC have been shown to secrete VEGF, thereby promoting angiogenesis and metastasis.[80] CCL18, a chemokine produced by TAMs, has been shown to stimulate angiogenesis in breast cancer.[79] The study found that CCL18-postive TAM infiltration was associated with an increased microvascular density in breast tumors, which was associated with an increased tumor metastasis and poor prognoses. It demonstrated that CCL18 and VEGF synergistically promote endothelial cell migration and angiogenesis, by inhibiting either molecule prevented the migratory effect of TAMs. The authors, therefore, concluded that CCL18 produced by TAMs promotes angiogenesis and tumor progression in breast cancer. Therefore, CCL18 may be a new target for antiangiogenic therapy.[79]

Graver et al.[81] have shown that CCL18 is a potential marker for bevacizumab treatment in tumor cells deficient in MMR. Bevacizumab treatment has also been shown to prolong OS in a subset of patients with MMR-deficient tumors in adjuvant CRC.[78] Gene expression changes in macrophages induced by tumor-conditioned media have also showed that CCL18 is a gene regulated by bevacizumab.[81] An increase in the phagocytic activity of macrophages, in the presence of bevacizumab, has been shown to be significantly more apparent in MMR-deficient cells and may be attributed to CCL18.[81] Also, DNA damage in MMR-deficient cells treated with bevacizumab have been shown to release a cytokine mix that reduces monocyte migration in a bevacizumab-dependent manner, therefore exhibiting a functional response to the combination of MMR deficiency and bevacizumab.[81]

Immunogenicity/cadherin. CRC has previously been considered as a poorly immunogenic tumor. However, there is some evidence that suggests that there is a significant host response associated with an improved prognosis, suggesting that it may alter the natural history of the disease.

The mobilization of immune cells, such as MDSCs and TAMs, has been thought to contribute to drug resistance. Recent studies have shown that VEGFA signaling through VEGFR2 is involved in MDSCs' recruitment to metastases, and once within the tumor, these can mature into tumor-promoting macrophages. Other angiogenic factors, such as PlGF, directly or indirectly stimulate angiogenesis by affecting a wide range of different cell types or by attracting MDSCs and macrophages within the tumor microenvironment. PlGF also promotes inflammation and angiogenesis by interacting with alternative pathways via VEGFR1 signaling.[10]

MMR-deficient (vs MMR-proficient) CRC shows an enhanced immunogenicity with an increased numbers of intraepithelial lymphocytes that may be associated with a favorable clinical outcome.[82]

Microsatellites are short repetitive DNA nucleotide sequences prone to frame shift mutations and base-pair substitutions during replication in the setting of a defective DNA MMR system. Microsatellite instability (MSI) is seen in approximately 15% of sporadic CRC. There is evidence that high MSI cancers may follow a different pathway to angiogenesis. VEGF expression has been found lower among high MSI cancers, and this could partially explain why these cancers are less aggressive, with a better overall prognosis.[82]

Cadherin 12 (CDH12) may play an important role in the invasion and metastasis of salivary adenoid cystic carcinoma, and its role in CRC has been studied by Zhao et al.[83] They found that CDH12 promotes proliferation, migration, invasion, adhesion, and angiogenesis in CRC. These results suggested that CDH12 might actually be an oncogene. Consequently, CDH12 is expected to become a new diagnostic and prognostic marker, as well as a potential new target of treatment for CRC.

The expression of CDH12 and its role in the prognosis of patients with CRC have also been investigated in the study by Ma et al.[84] The authors concluded that CDH12 might act as a predictor of prognosis in patients with CRC and an oncogene promoting the CRC cell proliferation and migration. It may also influence CRC cell progression by promoting the EMT.

It has also been demonstrated that the endothelial cells express the following two dependent intercellular adhesion molecules: VE-cadherin, which is specific for endothelial cells, and N-cadherin, which is present in other cells. There are several studies suggesting that both adhesion molecules play a role in promoting the angiogenesis.[85]

Leucine-rich-alpha-2-glycoprotein 1 (LRG1) has been associated with several tumors and shown to be overexpressed in CRC and, in particular, in more aggressive cancers. It induces the process of EMT and promotes CRC cell

migration and invasion. It also promotes VEGFA expression in CRC cells and, therefore, contributes to tumor angiogenesis. HIF1α can also be induced by LRG1 and is thought to be the mechanism by which LRG1 induces VEGFA expression and EMT.[86] The authors of the study concluded that LRG1 may play an important role in the progression of CRC as it is able to regulate HIF1α expression, and therefore, it may be a potential therapeutic target in treating CRC.[86]

Functional imaging. Monitoring the effects of antiangiogenic therapy is very important and is essential in the implementation of new therapies and for assessing the therapeutic effects. The aim of noninvasive imaging is to visualize, characterize, and quantify the tumor growth, angiogenesis, and metastases at the micromorphological, functional, and molecular levels.[87,88]

Normalization of the tumor vasculature increases drug delivery to the tumor and may also reduce the shedding of cancer cells into the circulation. The normalization of tumor vasculature tends to be transient and occurs after approximately 6 days.

Tumor vasculature and blood flow (BF) are widely known to be heterogeneous. The BF distribution within the tumor determines the distribution of drugs or oxygen to cancer cells. Improved imaging techniques are, therefore, needed to establish the effects of antiangiogenic therapies on the vascular structure within tumors.

Functional imaging relies on the intravenous injection of a contrast agent that enhances the vascular structures, combined with sequential imaging of the tumor prior to, during and following the injection of the contrast.

The time concentration curve of the tracer allows the semiquantitative calculation of several parameters including the relative blood volume (rBV) in the tissue studied, the mean transit time (MTT) of the contrast passing from the arterial to the venous circulation, and the relative BF (rBF) within the tumor. When the contrast agent diffuses from the intravascular into the extravascular space, a plateau phase is observed, which can be used to estimate the vascular permeability, Ktrans.

Magnetic resonance imaging. Magnetic resonance imaging (MRI) is a very useful imaging technique that benefits from high spatial resolution; excellent soft tissue contrast and the ability to functionally characterize tissues by using noncontrast- and contrast-enhanced techniques. These techniques include spectroscopy (to investigate the cell metabolism), diffusion-weighted imaging (to measure the cellularity), time-of-flight (TOF) angiography (to visualize the vessels), arterial spin labeling (ASL) (to monitor the perfusion), and also blood oxygenation level imaging (to assess the vessel oxygenation, responsiveness, and maturity).

Other functional MRI techniques include dynamic contrast-enhanced (DCE) MRI to assess rBV, perfusion, permeability, and vessel size imaging (to quantify the mean vessel diameter).[89] MR angiography in combination with gadolinium-based blood pool contrast agents can also visualize the vessels of 30–100 μm in diameter.[89,90]

DCE MRI in combination with quantitative pharmacokinetic analyses can provide information on BF, rBV, regional distribution volume of the contrast agent, tissue perfusion, and changes in vessel permeability. DCE MRI is useful for estimating angiogenesis. Tumor enhancement is due to the increased VD, vessel diameters and vascular wall permeability. It uses gadolinium chelate agents that cause a decrease in T1 (time of the realignment of hydrogen nuclei in the magnetic field) at low concentrations and a decrease in T2 (time of loss of transverse magnetism) at high concentrations. As this is noninvasive and relatively quick imaging technique, it can be used to monitor the response to treatment in patients who have not undergone surgical removal of their tumors.

Tumors with neovascularization show earlier enhancement than tissues without neovascularization. DCE MRI also shows an increased vascularity and a high permeability of tumor capillaries. Cancers show a pattern of rapid *wash-in and washout*, which is associated with a high vascular volume or a high vascular permeability due to angiogenesis. Neovascularization is an early process in the adenoma–carcinoma sequence by the upregulation of VEGF1 and -2. Tissue VEGF expression has been correlated with immunohistochemical MVD measurements. MVD is a surrogate marker of tumor angiogenesis, and it has been proposed to be a potential predictive marker for patients with the high risk of cancer recurrence. MVD measurements are the most common technique in quantifying the intratumoral angiogenesis. It correlates with CRC tumor grade and stage. Tumors with the high levels of MVD have been shown to have an increased incidence of lymphatic and hematogenous metastases and also an increased rate of local recurrence. However, as a method to assess angiogenesis, it is a relatively limited test as it is a static measure of tumor vascularity.

Consequently, DCE MRI has been used in several studies as it is able to detect changes in tumor vascularity over time due to the effects of antiangiogenic drugs.[91–93] T2 imaging is used early during the arterial input to calculate MTT, rBV and rBF, while T1 imaging is used at later time points to estimate Ktrans. The area under the curve and Ktrans are used to monitor the treatment-induced changes in vascularity and permeability.[94]

Ktrans measurements detect the biological activity within the tumor but have not been shown to predict the clinical response to treatment. Ktrans is dependent on tissue perfusion and decreases during treatment with antiangiogenic therapy, which is thought to be due to tumor blood vessel regression. rBV correlates with the quantity of functional blood vessels within the tumor. MTT, rBF, rBV, and the area under the curve (signal intensity as a function of time) decrease during treatment with antiangiogenic therapy due to vessel regression.

Vascular permeability depends on the contrast agent, the vessel surface area, the size of the interstitial distribution

space, and vessel leakiness. High levels of vascular permeability have been found in tumors with immature blood vessels, and permeability levels have been shown to reduce in antiangiogenic therapy.[95]

Kiessling et al carried out a study with DCE MRI to investigate the early changes in tumor vascularization during antiangiogenic therapy with the VEGFR2 antibody (DC101). Heterotransplants of human skin squamous cell carcinomas in nude mice were treated with DC101. Animals were examined prior to and during two weeks of this treatment. Results achieved by MRI were validated by histological sections immunostained for blood vessels (CD31). A decrease in tumor vascularization was observed two days after the first DC101 application and prior to detecting a reduction in tumor volume. The difference between treated tumors and controls was noticeable after four days. By day 7, the mean tumor volumes of treated and control animals were found to be significantly different. After two weeks of treatment with DC101, treated tumors showed further growth reduction, whereas untreated tumors showed a continued growth. The authors concluded that DCE MRI was a valuable tool for early detection of the antiangiogenic treatment effects prior to the detection of changes in tumor volume.[96,97] This technique has also been shown to work reliably for monitoring the orthotopic tumors and metastases.

Marzola et al carried out a study to compare two DCE MRI techniques in assessing the early antiangiogenic effect of SU11248, a multitargeted TKI. They used a subcutaneous tumor model of HT29 human colonic carcinoma in athymic mice. Early antiangiogenic activity of SU11248 was detected *in vivo* by using macromolecular or low molecular weight contrast agents (Gd-DTPA). With the macromolecular agents, the effect was detected as a 42% reduction in vascular permeability measured in the tumor rim, and a 31% reduction was detected using the low-molecular weight techniques. Pathological slices showed a difference in mean vessel density in treated tumors compared to controls. This study, therefore, suggests that low molecular weight contrast agents may be useful in clinical trials in the future.[98,99]

Lee et al tested PTK/ZK, a new oral angiogenesis inhibitor. In early clinical trials, it demonstrated a dose-dependent reduction in tumor vascular parameters as measured by DCE MRI and an acute increase in plasma VEGF levels. The reduction in tumor vascularity was significantly correlated with an improved clinical outcome in patients with advanced CRC and liver metastases.[99]

This drug has also been studied by Morgan et al to evaluate its pharmacodynamic effects. This was done by assessing changes in contrast-enhanced parameters of metastatic liver lesions using DCE MRI in patients with advanced CRC treated in two ongoing, dose-escalating phase I studies. Tumor permeability and vascularity were evaluated by calculating the bidirectional transfer constant (Ki). Patients with stable disease had a significantly greater reduction in Ki at both day 2 and the end of cycle 1 compared to patients with progressive disease. These results suggest that DCE MRI may be a useful biomarker for defining the pharmacological response and the dose of angiogenesis inhibitors for future clinical trials.[100]

Vessel size imaging MRI technique obtains anatomical information of blood vessels. It is based on the measurement of T2 and T2* relaxation times before and after the intravenous injection of a superparamagnetic contrast agent. T2 relaxation time is dependent on water diffusion and, therefore, on the size and number of vessels per voxel. The changes in the T2* relaxation time are dependent on the amount of contrast agent per voxel. Using both T2 and T2*, it is possible to calculate the mean vessel diameter of tissues.[100,101]

Troprès et al carried out a study of vessel size imaging for brain tumor characterization. The vessel size index for MRI was correlated with the one obtained from histology. The quantitative analysis showed a good correlation between vessel size found histologically and vessel size detecting using MRI. The results support that imaging techniques can, therefore, be used as a quantitative method for tumor vasculature characterization.[101]

Zwick et al assessed vascular remodeling in tumors treated with two antiangiogenic therapies, using DCE MRI and vessel size imaging. They evaluated the vessel size index as a biomarker of antiangiogenic therapy response. Nude mice with human skin squamous cell carcinoma xenografts were treated with bevacizumab or a multitargeted TKI (SU11248). They showed that both methods were the reliable biomarkers of antiangiogenic therapy response, which was confirmed using histology. However, they also concluded that as vascular remodeling is complex, a uniform response cannot be expected in different cancers and as a consequence of different treatments.[102]

Blood oxygenation-level MRI analyzes the antiangiogenic therapy effects on tumors that are dependent on vessel maturation. This technique uses different relaxation characteristics of oxygenated and deoxygenated hemoglobin in the blood. The inhalation of oxygen can be used to induce a signal change in the blood and to characterize tissue vascularization. Using this technique, details on vascular maturity are obtained as only mature vessels respond to the elevated levels of CO_2.[103]

Another method to monitor the tumor angiogenesis includes the use of several MRI probes. The most frequently used are antibody- or peptide-coated ultrasmall superparamagnetic iron oxide nanoparticles (USPIOs)[104,105] and Gd-loaded paramagnetic liposomes.[106]

Magnetic liposomes are phospholipid vesicles that encapsulate magnetic and/or paramagnetic nanoparticles to be applied as contrast agents for MRI. Although it has been shown that using RGD-coated USPIO different levels of integrin, expression on tumor blood vessels can be distinguished in squamous cell carcinomas, antiangiogenic therapy effects can also be monitored with paramagnetic liposomes; however,

there is still debate on the reliability of this technique and its usefulness for biological and medical research.

To assess the angiogenic profile of tumors, alpha(v) beta(3) integrin-targeted USPIOs have been designed. They are coated with 3-aminopropyltrimethoxysilane and conjugated with Arg-Gly-Asp (RGD) peptides. They are recruited by endothelial cells. Authors assessed the ability of RGD–USPIO to distinguish the tumors with the high and low fractions of alpha(v)beta(3) integrin-positive vessels and concluded that this technique worked efficiently in distinguishing the alpha(v)beta(3) integrin expression and tumor angiogenic profile.[105,107]

ASL MRI is a technique that allows the quantitative imaging of BF and tissue perfusion, which are the biomarkers of tissue function associated with tumor angiogenesis and hypoxia. ASL is based on the radiofrequency pulses, which excite the nuclear spin of inflowing arterial protons, and results in magnetic labeling of the inflowing blood. A study carried out by Rajendran et al.[108] in mouse xenograft tumors showed that tumor perfusion may reflect certain aspects of angiogenesis; however, they concluded that further studies are required as the test shows low sensitivity and high heterogeneity.

Another study evaluated antiangiogenic treatment response *in vivo* by using ASL MRI to measure the tumor perfusion quantitatively. The authors used DCE MRI, and tumor vessels were also detected using the CD34 staining. Responses to bevacizumab were assessed in an A498 xenograft mouse model. The study showed that tumor perfusion, vessel density, and size decreased with chronic treatment.[109]

DCE MRI also detected a significant change between treated and control groups in chronic and acute treatments, although only vessel size was found to be reduced 24 hours after the acute treatment.

These results indicate that tumor perfusion measured by MRI can detect early vascular responses to antiangiogenic treatment. However, some authors have concluded that ASL MRI could be valuable for longitudinal assessment of tumor perfusion and its application to human studies.[109]

TOF angiography visualizes vessels by the signal change that occurs when magnetized protons in the blood leave the image voxel during the measurement.[110] Although the visualization of tumor vessels by TOF and ASL improves by using MRI scanners with higher field strength, vessels with very slow blood velocities cannot be reliably depicted.[110] Since these vessels in particular respond to antiangiogenic therapies, contrast-enhanced T1-weighted angiography and DCE MRI are preferred for monitoring therapy.

In a study carried out by Radbruch et al, TOF angiography at 7 T MRI showed the ability to characterize and quantify the internal vascular morphology of glioblastoma. The authors concluded that this may be a potential technique used to detect the therapy response in future studies, as tumor vessels were clearly visible in all patients participating in this study.[111]

DCE CT. DCE CT imaging obtains a baseline image without contrast followed by a series of images over time after an intravenous bolus of a contrast agent. Perfusion CT can quantify the degree of angiogenesis of solid tumors *in vivo*. Dighe et al.[112] carried out a study in patients with colon cancer to assess the technical limitations and its usefulness. Perfusion CT in tumors susceptible to motion during imaging acquisition makes accurate data more difficult to obtain, and that is the reason why motion correction software is essential if perfusion CT is to be used routinely in CRC.

Previous studies in colon cancer have shown that this technique could be applied to cancers either by quantifying the angiogenesis or by differentiating between benign and malignant lesions.[113,114]

If perfusion CT is to be clinically effective in assessing the angiogenesis in clinical setting, the technique needs to be reproducible and standardized.[115] However, this technique is thought to have 1 potential due to the development of safe neoadjuvant treatment strategies with antiangiogenesis drugs, which act by constricting tumor growth and prevent propagation of metastases.[116]

There is also a possibility that perfusion CT could be able to screen patients demonstrating high angiogenic activity and, consequently, would be susceptible to the effects of antiangiogenic treatments.

Dighe et al.[117] carried out a prospective study to investigate the ability of perfusion CT to quantify the degree of angiogenesis in CRC. The perfusion parameters calculated were correlated with the measurement of MVD obtained from immunohistochemical staining of resected surgical specimens.

Perfusion CT is also able to integrate anatomical detail with the assessment of vascular physiology. Pharmacokinetic modeling after tumor enhancement achieved by contrast administration allows the physiologically based quantitative vascular parameters, such as BF, blood volume, MTT, and permeability surface area.[99,118]

Perfusion CT may reflect angiogenesis in CRC; however, not all studies have been able to correlate BF with MVD.[102,119] Perfusion measurements have also been shown to be robust enough to be useful for therapeutic assessment in CRC.[120]

Positron emission tomography. Fluorodeoxyglucose (FDG) positron emission tomography (PET) studies have also shown that the FDG delivery and uptake by rectal cancers do not reduce after bevacizumab monotherapy despite a reduction in MVD and BF. This provides additional evidence supporting the theory that vascular normalization is induced by VEGF blockade.[37,41,121]

FDG PET gives data about tumor cell viability after treatment. It shows metabolic changes in response to treatment even prior to detectable change in the tumor size or structure.[122]

FDG PET is thought to be an important marker in rectal cancer, as sequential imaging after neoadjuvant chemoradiation can predict response to treatment. It is also an independent predictor of disease-free survival and OS.[123,124]

Although FDG PET is a biomarker of response to imatinib in gastrointestinal stromal tumors, early reductions in standardized uptake value have not been seen in patients with rectal cancer treated with bevacizumab monotherapy. However, it may provide the detection of early stages of response to EGFR-targeted TKIs in colon cancer.[125]

FDG is the most common radiotracer used. Tumor cells that overexpress the cell surface GLUT-1 allow the entry of this analog with the attached positron-emitting radionuclide 18F into the cell. Once inside the cells, it is phosphorylated into glucose-6-phosphate and trapped within the tumor cell. Intracellular trapping occurs preferentially in malignant cells because they have higher glycolytic activity with an increased expression of GLUT-1.

Semiquantitative analysis using a standardized uptake value represents the metabolic activity of the tumor compared to that of surrounding tissue, corrected for injected dose and patient weight.[126]

H2 15O PET. Quantification of tumor perfusion using the radioactive water (H2 15O) and PET is a promising method for monitoring the treatment with antiangiogenic agents. H2 15O PET is considered as the reference technique for the quantification of rBF and vascular permeability.[87]

This technique offers the ability to monitor the direct targeting of antiangiogenic treatment. It is increasingly being studied in trials evaluating the effects of these drugs. Also by monitoring the direct targets of anticancer therapy may be superior to indirect tumor vessel size measurements.[127]

In locally advanced breast cancer, initial results with dynamic 15O-H2O PET have been promising. It showed that tumor BF decreased in the responder group after chemotherapy, whereas it increased in the nonresponder group.[128]

Contrast-enhanced ultrasound. This technique is increasingly being used in the clinic for the assessment of tissue vascularity and perfusion. The contrast agent consists of phospholipid-based microbubbles that encapsulate an inert gas. When exposed to an ultrasound pulse, the microbubbles generate nonlinear resonances that allow an enhanced representation of the vasculature.[129]

This is a low cost, nonirradiant imaging technique with good tolerance of contrast media. It is, therefore, a particularly attractive method that can be used for serial monitoring of antiangiogenic response.[130,131]

Ultrasound Doppler technique is the most sensitive imaging to assess blood vessels; however, it cannot provide the assessment of vessels smaller than 200 μm in diameter.[132] Contrast-enhanced ultrasound overcomes this limitation and enables the functional evaluation of tumoral neovascularization.

Ultrasound contrast agents consist of microbubbles with a mean diameter of 0.5–10 μm. Unlike many contrast agents used in CT and MRI, ultrasound contrast microbubbles remain strictly following the intravascular injection. These techniques that have been applied in a clinical setting to monitor the antiangiogenic therapy are based on the assessment of the percent area of contrast enhancement during the passage of an injected bolus of contrast agent.[131,132]

This technique has also been used to predict response in patients receiving bevacizumab-based chemotherapy for mCRC. Thirty consecutive patients with mCRC underwent a contrast-enhanced ultrasound before cycles 1, 2, and 4 of bevacizumab-based chemotherapy. Three parameters (peak, time to peak [TTP], and rise rate) were correlated with radiological response.

There was a significant correlation in TTP between the metastases of responders and nonresponders. In this setting, TTP was also significantly different between responders and nonresponders. In contrast, peak and rise rate did not show any significant difference between responders and nonresponders. This technique may, therefore, serve as a surrogate marker to predict treatment response in patients with mCRC who receive antiangiogenic therapy.[133]

The study by Marybeth et al tried to test a real-time motion compensation algorithm for contrast-enhanced ultrasound imaging of tumor angiogenesis. The authors concluded that this technique was feasible for accurate and reliable quantification of tumor angiogenesis in a human colon cancer xenograft model exposed to motion.

In *in vivo* analysis, the percent contrast areas correlated well with the extent of tumor angiogenesis compared to ex vivo analysis during the antivascular therapy. This algorithm may facilitate the establishment of contrast-enhanced ultrasound imaging as an accurate tool for the real-time quantification of tumor angiogenesis in preclinical and clinical situations.[134]

Other authors have investigated the relationship between contrast-enhanced ultrasound, bFGF, endothelin-1, and HCC recurrence after ablation. Authors found that the levels of tumor rise time, tumor TTP, tumor peak intensity, and tumor parenchymal peak intensity in the recurrence group were significantly lower than those in the nonrecurrence group. They concluded that this technique is a noninvasive and effective method for evaluating the angiogenesis of HCC and predicting its recurrence and prognosis.[135]

Contrast-enhanced ultrasound has several advantages over DCE MRI and CT. These include the fact that the patient is not exposed to ionizing radiation and that the technique is accessible and less expensive than other techniques. It is also able to measure diffusion through the intravascular compartment and is not confounded by extravascular diffusion.[135]

Pysz et al carried out a study to evaluate the effect of different contrast administration on the *in vivo* ultrasound signal in tumor-bearing mice using a maximum intensity persistence algorithm. Authors concluded that a contrast-enhanced ultrasound maximum intensity persistence is reliable in evaluating the tumor vascularity and monitoring the antiangiogenic therapy *in vivo*, provided that a constant microbubble dose is administered.[136]

The accuracy of contrast-enhanced ultrasound for microvascular perfusion measurement and perfusion changes following therapy has been documented both in experimental models and in patients with cancer.[137–139]

Single-nucleotide polymorphism. Different SNPs of VEGF/VEGFR pathways have been investigated in several retrospective studies to detect their potential impact on the clinical outcome of patients with mCRC receiving bevacizumab.

Loupakis et al have reported an association between VEGFA rs833061 C/T variants and PFS in mCRC treated with the first-line FOLFIRI plus bevacizumab. However, a prospective study failed to validate the hypothesized predictive impact of VEGFA rs833061 variants. The authors found that only VEGFR2 rs12505758 variants correlated with PFS. They concluded that angiogenesis is a highly complex process and it is unlikely that a single SNP might be a good predictor of benefit from bevacizumab.[140]

AVITA and AVOREN. Two randomized clinical trials have been used to assess whether genetic variants in the VEGF pathway could be used as biomarkers for bevacizumab treatment outcomes. The AViTA trial randomized patients with metastatic pancreatic adenocarcinoma to receive gemcitabine and erlotinib plus either bevacizumab or placebo. The AVOREN trial randomized patients with metastatic renal cell carcinoma to receive interferon alfa-2a plus either bevacizumab or placebo.

The studies assessed the correlation between 138 SNPs in the VEGF pathway with PFS and OS in a subpopulation of patients from AViTA. Significant findings were confirmed in a subpopulation of patients from AVOREN, and these patients were studied functionally at the molecular level.

DNA of 154 patients from AViTA (77 received bevacizumab) and 110 from AVOREN (59 received bevacizumab) were studied. A SNP in VEGFR1, rs9582036, was significantly associated with OS and PFS in the bevacizumab group of AViTA.

The authors concluded that the VEGFR1 locus containing this SNP serves as a predictive marker for bevacizumab treatment outcome in AViTA. Experiments of fine-mapping of this locus identified rs7993418, which is an SNP affecting the tyrosine 1213 in the VEGFR1 tyrosine kinase domain. It has been identified as the functional variant underlying this association. This SNP causes a shift in codon usage, leading to an increased VEGFR1 expression and downstream VEGFR1 signaling. This VEGFR1 locus correlated significantly with PFS but not with OS in the bevacizumab group in AVOREN.

The final conclusion of these two studies showed that a locus in VEGFR1 correlated with an increased VEGFR1 expression and a poor outcome of bevacizumab treatment. However, prospective assessment is needed to validate the predictive value of this association.[141]

Two similar phase III studies (the Hellenic Cooperative Oncology Group and E2100 studies) provided strong evidence that SNPs in VEGFA have a predictive value as biomarkers for response to bevacizumab. In the AVADO trial, docetaxel alone or with bevacizumab was assessed as the first-line treatment for metastatic breast cancer.[142]

In the carriers of the VEGFA-2578A allele, PFS was improved in those who received docetaxel plus 7.5 mg/kg bevacizumab but not in those who received docetaxel alone or with 15.0 mg/kg bevacizumab.

Improved PFS was also found in patients with the VEGFA-634CC genotype who received docetaxel alone but not in patients who received bevacizumab, which suggested a prognostic effect. No correlation was seen between OS and any of the SNPs tested.

The Hellenic Cooperative Oncology Group did a correlative study on their phase III trial findings for bevacizumab with either FOLFIRI (leucovorin, fluorouracil, and irinotecan) or XELIRI (irinotecan and capecitabine).[143]

The genotypes VEGFA-2578CC and -1154GG were associated with a reduced OS.[144] These findings are similar to that in the E2100 trial where the alternate genotypes (VEGFA-2578AA and -1154AA) were associated with an improved OS. A marginal improvement in PFS was also associated with the VEGFA-1154AA genotype. This greater effect on OS than on PFS supports the findings of the E2100 trial.[145,146]

A small cohort study evaluating FOLFIRI/bevacizumab as first-line in mCRC investigated the associations between median PFS and VEGFA SNPs. VEGFA-1154AA genotype showed an improvement in PFS.[147]

A correlation was also shown between the VEGFA-634GG genotype and an improved response rate. Despite all these findings, the level of evidence for the use of VEGFA SNPs in the clinical setting is inadequate, and thus, more studies are warranted.[148]

Conclusions and Hopes for the Future

Inhibition of angiogenesis for the treatment of cancer has been successfully translated into clinical use. The key issue with this therapy is that patients who receive antiangiogenic drugs experience relatively few clinical benefits and antiangiogenic therapy is associated with adverse events and high financial costs. Therefore, the identification of biomarkers that are able to identify patients who are more likely to benefit from their use is important (See Table 1).

Analyses of tumor samples or blood circulating cells with the intent of finding biomarkers that can help with future drug development have shown some promise. Imaging, molecular, or cellular biomarkers are relevant in phase II and III studies to demonstrate antitumor activity or resistance to the treatment. Specific gene expression signatures in endothelial cells and blood lymphocytes have been reported in response to antiangiogenic drugs.[52,149] These signatures could be used to monitor tumor angiogenesis and antiangiogenic treatment activity. Proteomics approaches have been used to identify specific proteins that are expressed in tumor

Table 1. Biomarkers in angiogenesis in colorectal cancer and their role as predictive or prognostic factors.

BIOMARKERS	TYPES	COMMENTS
Blood pressure	Predictive	– Hypertension has been observed in patients treated with anti-VEGF antibodies and TKIs. However currently the data is very limited.
Circulating VEGF	Prognostic	– Elevated levels have been found to be indicative of a poor prognosis but do not predict response to antiangiogenic drugs.
Tumour expression of VEGF	Predictive	– The VEGF-2578 AA genotype has been associated with improved median overall survival in patients who received paclitaxel and bevacizumab when compared to other genotypes.[50]
PIGF and soluble VEGF receptors	Predictive and prognostic	– Circulating levels of PIGF increase in response to anti-VEGF treatment. – Circulating levels of soluble VEGFR2 and VEGFR3 are decreased by TKIs that directly target these receptors, but are not affected by bevacizumab.
Other proteins as biomarkers	Predictive	– In patients with mCRC treated with bevacizumab and chemotherapy, pre-treatment evaluation of biomarkers such as microvascular density, tumour tissue expression of TSP2, P53 and KRAS mutations have not been predictive of response to treatment.[35,54]
Circulating cells	Prognostic	– Circulating endothelial cell progenitors mobilized by VEGF, have been found to have pro-angiogenic activities in mice. High levels correlate with angiogenesis and normalise following treatment with anti-angiogenic therapies.
Microvessel Density and Endothelial signalling Events	Prognostic	– Microvessel density (MVD) at regions of intense angiogenesis has a prognostic but not predictive value in many cancers.
MicroRNAs	Predictive	– The role of increased intratumoral expression of genes regulated by hypoxia-inducible factor-1 alpha (HIF1α), such as LDHA, glucose transporter-1 (GLUT-1), VEGFA, VEGFR1, and VEGFR2, in predicting the outcome in the treatment of CRC has been identified.
Mismatch repair proteins	Predictive	– Patients diagnosed with mismatch repair defective (dMMR) tumours had a statistically significant benefit in survival from the addition of bevacizumab in contrast with no benefit in patients diagnosed with mismatch repair proficient tumours.
Immunogenicity/cadherin	Prognostic	– The mobilization of immune cells, such as MDSCs and TAMs has been considered as a contributor to drug resistance. Recent studies have shown that VEGF-A signalling through VEGFR-2 is involved in MDSCs recruitment to metastases and, once within the tumour, these can mature into tumour-promoting macrophages.
Functional imaging – Magnetic Resonance Imaging – Dynamic contrast enhanced (DCE) CT – Positron emission tomography (PET) – Contrast enhanced ultrasound	Prognostic	– Magnetic resonance imaging (MRI) has high spatial resolution; excellent soft tissue contrast and the ability of functionally characterise tissues by using non-contrast and contrast-enhanced techniques. – Perfusion CT can quantify the degree of angiogenesis of solid tumours *in vivo*. – FDG-PET is thought to be an important marker in rectal cancer as sequential imaging after neoadjuvant chemoradiation can predict response to the treatment and this is an independent predictor of disease free survival and overall survival.
Single nucleotide polymorphism (SNP)	Predictive	– Different SNPs of VEGF/VEGFR pathways have been investigated in several retrospective studies, to identify the potential impact on clinical outcome in mCRC patients receiving bevacizumab.

vessels[150] and tumor interstitial fluid.[151] These analyses are considered as a promising technique to identify the biomarkers of angiogenesis.[152,153]

Many potential biomarkers of angiogenesis have already been tested in preclinical and clinical studies. However, currently there are no definitive methods that can be used in the clinical setting to monitor or to predict the response to antiangiogenic medications (See Table 1). Validated surrogate markers as a method for detecting drug activity, predicting response, defining optimum biological dose, planning better combination therapies, or identifying resistances to antiangiogenic therapies would be of great value. Imaging biomarkers, such as changes in DCE MRI- and CT-based tissue vascular measures, such as BF and permeability, have been detected after treatment with bevacizumab or anti-VEGFR TKIs in clinical studies. Water self-diffusion is also sensitive to detect changes in tumors following the treatment[154] and might be a predictive marker.[155]

New drugs are currently being developed, and hopefully, the size and effect of our anticancer arsenal will be increased in the near future. A phase III study of nintedanib/placebo plus best supportive care in patients with CRC refractory to standard therapies is ongoing (though not recruiting). It will evaluate the efficacy of nintedanib in patients with mCRC after the failure of previous treatment with standard chemotherapy and biological agents. Ramucirumab, a fully

monoclonal antibody against the VEGFR2, is being tested in mCRC progressive after treatment with bevacizumab-containing chemotherapy.

Combination chemotherapy with or without regorafenib in patients with mCRC is another ongoing randomized phase II trial. Regorafenib is thought to inhibit the growth of tumor cells by blocking several enzymes required for cell growth. However, it is not yet known whether chemotherapy will be more effective with or without regorafenib.

The results of these studies are currently awaited, and further research is needed to draw definitive conclusions. Also the need to tailor therapies to individual patients is becoming more and more important. Unfortunately, currently there is insufficient data supporting the use of any single biomarker as a reliable parameter to guide patient selection for treatment with anti-VEGF monoclonal antibodies. Identification of reliable and easily measurable biomarkers is, therefore, needed, and further randomized trials are required to be able to identify patients who will benefit from antiangiogenic therapy in CRC.

Acknowledgments

The authors thank Drs. Emilio Esteban and Francisco Lopez-Lara for their invaluable support and enriching comments.

Author Contributions

Conceived and designed the experiments: EUC, PA. Analyzed the data: EUC, PA. Wrote the first draft of the manuscript: EUC, PA. Contributed to the writing of the manuscript: BM. Agree with manuscript results and conclusions: EUC, PA, BM. Jointly developed the structure and arguments for the paper: EUC, PA, BM. Made critical revisions and approved final version: EUC. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Suzanne H, Maria CMO, Brendan D. Targeted therapies in colorectal cancer an integrative view by PPPM. *EPMA J*. 2013;4:3.
2. Fakih MG. Metastatic colorectal cancer: current state and future directions. *J Clin Oncol*. 2015;33(16):1809–24.
3. Saletti P, Molinari F, De Dosso S, Frattini M. EGFR signaling in colorectal cancer: a clinical perspective. *Gastrointest Cancer*. 2015;5:21–38.
4. Nelson R. Anti-EGFR Therapy Worsens Survival in Patients with RAS Mutations. Medscape Medical News; 2013. Available at: http://www.medscape.com/viewarticle/810817.
5. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 2008;26(35):5705–12.
6. Rmali KA, Puntis MC, Jiang WG. Tumour-associated angiogenesis in human colorectal cancer. *Colorectal Dis*. 2007;9(1):3–14.
7. Al-Husein B, Abdalla M, Trepte M, Deremer DL, Somanath PR. Anti-angiogenic therapy for cancer: an update. *Pharmacotherapy*. 2012;32(12):1095–111.
8. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182–6.
9. Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013;381(9863):303–12.
10. Giordano G, Febbraro A, Venditti M, et al. Targeting angiogenesis and tumor microenvironment in metastatic colorectal cancer: role of aflibercept. *Gastroenterol Res Pract*. 2014;2014:526178.
11. Nishi J, Minamino T, Miyauchi H, et al. Vascular endothelial growth factor receptor-1 regulates postnatal angiogenesis through inhibition of the excessive activation of Akt. *Circ Res*. 2008;103:261–8.
12. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*. 1983;219:983–5.
13. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun*. 1989;161:851–8.
14. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol*. 2005;23:1011–27.
15. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol*. 2009;21:154–65.
16. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9:669–76.
17. Wang Y, Nakayama M, Pitulescu ME, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature*. 2010;465:483–6.
18. Neufeld G, Cohen T, Shraga N, Lange T, Kessler O, Herzog Y. The neuropilins: multifunctional semaphorin and VEGF receptors that modulate axon guidance and angiogenesis. *Trends Cardiovasc Med*. 2002;12:13–9.
19. Lu C, Sood A. Role of pericytes in angiogenesis. In: Teicher BA, Ellis LM, eds. *Anti-angiogenic Agents in Cancer Therapy*. 2. Totawa, NJ: Humana Press; 2008:117–32.
20. Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science*. 1997;277:242–5.
21. Hellstrom M, Gerhardt H, Kalen M, et al. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol*. 2001;153:543–53.
22. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev*. 1994;8:1875–87.
23. Carmeliet P. Angiogenesis in life, disease and medicine. *Nature*. 2005;438:932–6.
24. Teicher BA. Antiangiogenic agents and targets: a perspective. *Biochem Pharmacol*. 2011;81:6–12.
25. Weis SM, Cheresh DA. αv integrins in angiogenesis and cancer. *Cold Spring Harb Perspect Med*. 2011;1(1):a006478.
26. Reinmuth N, Liu W, Ahmad SA, et al. Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice. *Cancer Res*. 2003;63:2079–87.
27. Ranpura V, Pulipati B, Chu D, Zhu X, Wu S. Increased risk of high-grade hypertension with bevacizumab in cancer patients: a metaanalysis. *Am J Hypertens*. 2010;23(5):460–8.
28. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol*. 2002;20(21):4368–80.
29. Bono P, Elfving H, Utriainen T, et al. Hypertension and clinical benefit of bevacizumab in the treatment of advanced renal cell carcinoma. *Ann Oncol*. 2009;20(2):393–4.
30. Rini BI, Cohen DP, Lu D, et al. Hypertension (HTN) as a biomarker of efficacy in patients (pts) with metastatic renal cell carcinoma (mRCC) treated with sunitinib. In: Proc Am Soc Clin Oncol Genitourinary Cancer Symposium. 2010: Abstract 312.
31. Osterlund P, Soveri L-M, Isoniemi H, Poussa T, Alanko T, Bono P. Hypertension and overall survival in metastatic colorectal cancer patients treated with bevacizumab-containing chemotherapy. *Br J Cancer*. 2011;104:599–604.
32. Dahlberg SE, Sandler AB, Brahmer JR, Schiller JH, Johnson DH. Clinical course of advanced non-small-cell lung cancer patients experiencing hypertension during treatment with bevacizumab in combination with carboplatin and paclitaxel on ECOG 4599. *J Clin Oncol*. 2010;28(6):949–54.
33. Maitland ML, Kasza KE, Karrison T, et al. Ambulatory monitoring detects sorafenib-induced blood pressure elevations on the first day of treatment. *Clin Cancer Res*. 2009;15(19):6250–7.
34. Mass RD, Sarkar S, Holden SN, Hurwitz H. Clinical benefit from bevacizumab (BV) in responding (R) and nonresponding (NR) patients (pts) with metastatic colorectal cancer (mCRC). *J Clin Oncol*. 2005;23:S249–249.
35. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol*. 2001;19:1207–25.
36. Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. *J Clin Oncol*. 2006;24:217–27.
37. Willett CG, Duda DG, di Tomaso E, et al. Efficacy, safety and biomarkers of neoadjuvant bevacizumab, radiation therapy and 5-Fluorouracil in rectal cancer: a multidisciplinary phase II study. *J Clin Oncol*. 2009;27(18):3020–6.
38. Bukowski RM, Eisen T, Szczylik C, et al. Final results of the randomized phase III trial of sorafenib in advanced renal cell carcinoma: survival and biomarker analysis [abstract #5023]. *J Clin Oncol*. 2007;25(suppl):S18.
39. George DJ, Michaelson MD, Rosenberg JE, et al. Phase II trial of sunitinib in bevacizumab refractory metastatic renal cell carcinoma (mRCC): updated results and analysis of circulating biomarkers [abstract #5053]. *J Clin Oncol*. 2007;25(suppl):S18.

40. Dowlati A, Gray R, Sandler AB, Schiller JH, Johnson DH. Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab – an Eastern Cooperative Oncology Group Study. *Clin Cancer Res*. 2008;14:1407–12.
41. Willett CG, Boucher Y, Duda DG, et al. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. *J Clin Oncol*. 2005;23:8136–9.
42. Bocci G, Man S, Green SK, et al. Increased plasma vascular endothelial growth factor (VEGF) as a surrogate marker for optimal therapeutic dosing of VEGF receptor-2 monoclonal antibodies. *Cancer Res*. 2004;64:6616–25.
43. Zaman K, Driscoll R, Hahn D, et al. Monitoring multiple angiogenesisrelated molecules in the blood of cancer patients shows a correlation between VEGFA and MMP9 levels before treatment and divergent changes after surgical vs conservative therapy. *Int J Cancer*. 2006;118:755–64.
44. Saltz LB, Rosen LS, Marshall JL, et al. Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy. *J Clin Oncol*. 2007;25:4793–9.
45. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol*. 2009;27:3312–8.
46. Deprimo SE, Bello CL, Smeraglia J, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. *J Transl Med*. 2007;5:32.
47. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2006;24:16–24.
48. Heymach JV, Ryan AJ, Mann H, et al. Baseline VEGF as a potential predictive biomarker of vandetanib clinical benefit in patients with advanced NSCLC. *Clin Cancer Res*. 2008;26:8009. [ASCO Meeting Abstracts].
49. Hillan KJ, Koeppen KW, Tobin P, Pham T. The role of VEGF expression in response to bevacizumab plus capecitabine in metastatic breast cancer (MBC). *Proc Am Soc Clin Oncol*. 2003;22:766.
50. Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. *J Clin Oncol*. 2008;26:4672–8.
51. Zhu AX, Sahani DV, Duda DG, et al. Efficacy, safety and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol*. 2009;27(18):3027–35.
52. Fischer C, Jonckx B, Mazzone M, et al. Anti-PIGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell*. 2007;131:463–75.
53. Rini BI, Michaelson MD, Rosenberg JE, et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab refractory metastatic renal cell carcinoma. *J Clin Oncol*. 2008;26:3743–8.
54. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J Natl Cancer Inst*. 2005;97:981–9.
55. Wilson PM, Yang D, Shi MM, et al. Use of intratumoral mRNA expression of genes involved in angiogenesis and HIF1 pathway to predict outcome to VEGFR tyrosine kinase inhibitor in patients enrolled in CONFIRM1 and CONFIRM2. *J Clin Oncol*. 2008;26:4002. [ASCO Meeting Abstracts].
56. Kohne C, Bajetta E, Lin E, et al. Final results of CONFIRM 2: a multinational, randomized, double-blind, phase III study in 2nd line patients with metastatic colorectal cancer receiving FOLFOX4 and PTK787/ZK 222584 (PTK/ZK) or placebo. *J Clin Oncol*. 2007;25:4033. [ASCO Meeting Abstracts].
57. Schultheis AM, Lurje G, Rhodes KE, et al. Polymorphisms and clinical outcome in recurrent ovarian cancer treated with cyclophosphamide and bevacizumab. *Clin Cancer Res*. 2008;14:7554–63.
58. Bunger S, Haug U, Kelly FM, et al. Toward standardized high-throughput serum diagnostics: multiplex-protein array identifies IL-8 and VEGF as serum markers for colon cancer. *J Biomol Screen*. 2011;16:1018–26.
59. Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J Immunol*. 2003;170:3369–76.
60. Koch AE, Polverini PJ, Kunkel SL, et al. Interleukin-8as a macrophage-derived mediator of angiogenesis. *Science*. 1992;258:1798–801.
61. Huang D, Ding Y, Zhou M, et al. Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. *Cancer Res*. 2010;70:1063–71.
62. Kopetz S, Hoff PM, Morris JS, et al. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. *J Clin Oncol*. 2010;28:453–9.
63. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. *Nat Rev Cancer*. 2006;6:835–45.
64. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood*. 2001;97:3658–61.
65. Shaked Y, Emmenegger U, Man S, et al. Optimal biologic dose of metronomic chemotherapy regimens is associated with maximum antiangiogenic activity. *Blood*. 2005;106:3058–61.
66. Norden-Zfoni A, Desai J, Manola J, et al. Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. *Clin. Cancer Res*. 2007;13:2643–50.
67. Beaudry P, Force J, Naumov GN, et al. Differential effects of vascular endothelial growth factor receptor 2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. *Clin Cancer Res*. 2005;11:3514–22.
68. Hlatky L, Hahnfeldt P, Folkman J. Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us. *J Natl Cancer Inst*. 2002;94:883–93.
69. Solorzano CC, Jung YD, Bucana CD, et al. *In vivo* intracellular signaling as a marker of antiangiogenic activity. *Cancer Res*. 2001;61:7048–51.
70. Felekkis K, Touvana E, Stefanou CH, Deltas C. microRNAs: a newly described class of encoded molecules that play a role in health and disease. *Hippokratia*. 2010;14(4):236–40.
71. Sobrero AF. Vatalanib in advanced colorectal cancer: two studies with identical results. *J Clin Oncol*. 2011;29:1938–41.
72. Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Vascular density analysis in colorectal cancer patients treated with vatalanib (PTK787/ZK222584) in the randomised CONFIRM trials. *Br J Cancer*. 2012;107(7):1044–50.
73. Heusschen R, van Gink M, Griffioen AW, Thijssen VL. MicroRNAs in the tumor endothelium: novel controls on the angioregulatory switchboard. *Biochim Biophys Acta*. 2010;1805:87–96.
74. Sundaram P, Hultine S, Smith LM, et al. p53-responsive miR-194 inhibits thrombospondin-1 and promotes angiogenesis in colon cancers. *Cancer Res*. 2011;71:7490–501.
75. Xu Q, Liu LZ, Qian X, et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. *Nucleic Acids Res*. 2012;40:761–74.
76. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet*. 2006;38:1060–5.
77. Olive V, Jiang I, He L. mir-17–92, a cluster of miRNAs in the midst of the cancer network. *Int J Biochem Cell Biol*. 2010;42:1348–54.
78. Pogue-Geile K, Yothers G, Taniyama Y, et al. Defective mismatch repair and benefit from bevacizumab for colon cancer: findings from NSABP C-08. *J Natl Cancer Inst*. 2013;105(13):989–92.
79. Lin L, Chen YS, Yao YD, et al. CCL18 from tumour-associated macrophages promotes angiogenesis in breast cancer. *Oncotarget*. 2015;6(33):34758–73.
80. Barbera-Guillem E, Nyhus JK, Wolford CC, Friece CR, Sampsel JW. Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. *Cancer Res*. 2002;62:7042–9.
81. Graver S, Stremitzer S, Sunakawa Y, et al. Immune response triggered by a novel molecular crosstalk of major hallmarks of cancer: angiogenesis, mismatch repair, and immune pathways. *J Clin Oncol*. 2015;33(suppl):abstr11054.
82. Sinicrope FA, Sargent DJ. Clinical implications of microsatellite instability in sporadic colon cancers. *Curr Opin Oncol*. 2009;4:369–73.
83. Zhao J, Li P, Feng H, et al. Cadherin-12 contributes to tumorigenicity in colorectal cancer by promoting migration, invasion, adhersion and angiogenesis. *J Transl Med*. 2013;15(11):288.
84. Ma J, Zhao J, Lu J, et al. Cadherin-12 enhances proliferation in colorectal cancer cells and increases progression by promoting EMT. *Tumour Biol*. 2016.
85. Derycke L, Morbidelli L, Ziche M, De Wever O, Bracke M, Van Aken E. Soluble N-cadherin fragment promotes angiogenesis. *Clin Exp Metastasis*. 2006;23(3–4):187–201.
86. Zhang J, Zhu L, Fang J, Ge Z, Li X. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. *J Exp Clin Cancer Res*. 2016;35:29.
87. Miller JC, Pien HH, Sahani D, Sorensen AG, Thrall JH. Imaging angiogenesis: applications and potential for drug development. *J Natl Cancer Inst*. 2005;97:172–87.
88. Lederle W, Palmowski M, Kiessling F. Imaging in the age of molecular medicine: monitoring of anti-angiogenic treatments. *Curr Pharm Biotechnol*. 2012;13:595–608.
89. Kiessling F, Jugold M, Woenne EC, Brix G. Non-invasive assessment of vessel morphology and function in tumors by magnetic resonance imaging. *Eur Radiol*. 2007;17(8):2136–48.
90. Doblas S, He T, Saunders D, et al. Glioma morphology and tumor-induced vascular alterations revealed in seven rodent glioma models by *in vivo* magnetic resonance imaging and angiography. *J Magn Reson Imaging*. 2010;32:267–75.
91. Des Guetz G, Uzzan B, Nicolas P, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. *Br J Cancer*. 2006;94(12):1823–32.

92. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med*. 1991;324:1–8.
93. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. *Lancet*. 2005;365:153–65.
94. Ehling J, Lammers T, Kiessling F. Non-invasive imaging for studying anti-angiogenic therapy effects. *Thromb Haemost*. 2013;109(3):375–90.
95. Kiessling F, Farhan N, Lichy MP, et al. Dynamic contrast-enhanced magnetic resonance imaging rapidly indicates vessel regression in human squamous cell carcinomas grown in nude mice caused by VEGF receptor 2 blockade with DC101. *Neoplasia*. 2004;6:213–23.
96. Luo Y, Jiang F, Cole TB, et al. A novel multi-targeted tyrosine kinase inhibitor, linifanib (ABT-869), produces functional and structural changes in tumor vasculature in an orthotopic rat glioma model. *Cancer Chemother Pharmacol*. 2012;69:911–21.
97. Dafni H, Kim SJ, Bankson JA, Sankaranarayanapillai M, Ronen SM. Macromolecular dynamic contrast-enhanced (DCE)-MRI detects reduced vascular permeability in a prostate cancer bone metastasis model following anti-platelet-derived growth factor receptor (PDGFR) therapy, indicating a drop in vascular endothelial growth factor receptor (VEGFR) activation. *Magn Reson Med*. 2008;60:822–33.
98. Marzola P, Degrassi A, Calderan L, et al. Early antiangiogenic activity of SU11248 evaluated *in vivo* by dynamic contrast-enhanced magnetic resonance imaging in an experimental model of colon carcinoma. *Clin Cancer Res*. 2005;11(16):5827–32.
99. Lee L, Sharma S, Morgan B, et al. Biomarkers for assessment of pharmacologic activity for a vascular endothelial growth factor (VEGF) receptor inhibitor, PTK787/ZK 222584 (PTK/ZK): translation of biological activity in a mouse melanoma metastasis model to phase I studies in patients with advanced colorectal cancer with liver metastases. *Cancer Chemother Pharmacol*. 2006;57(6):761–71.
100. Morgan B, Thomas AL, Drevs J, et al. Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: results from two phase I studies. *J Clin Oncol*. 2003;21(21):3955–64.
101. Troprès I, Lamalle L, Péoc'h M, et al. *In vivo* assessment of tumoral angiogenesis. *Magn Reson Med*. 2004;51(3):533–41.
102. Zwick S, Strecker R, Kiselev V, et al. Assessment of vascular remodeling under antiangiogenic therapy using DCE-MRI and vessel size imaging. *J Magn Reson Imaging*. 2009;29:1125–33.
103. Gross S, Gilead A, Scherz A, Neeman M, Salomon Y. Monitoring photodynamic therapy of solid tumors online by BOLD-contrast MRI. *Nat Med*. 2003;9:1327–31.
104. Kiessling F, Huppert J, Zhang C, et al. RGD-labeled USPIO inhibits adhesion and endocytotic activity of alpha v beta3-integrin-expressing glioma cells and only accumulates in the vascular tumor compartment. *Radiology*. 2009;253:462–9.
105. Frascione D, Diwoky C, Almer G, et al. Ultrasmall superparamagnetic iron oxide (USPIO)-based liposomes as magnetic resonance imaging probes. *Int J Nanomedicine*. 2012;7:2349–59.
106. Mulder WJ, van der Schaft DW, Hautvast PA, et al. Early *in vivo* assessment of angiostatic therapy efficacy by molecular MRI. *FASEB J*. 2007;21:378–83.
107. Zhang C, Jugold M, Woenne EC, et al. Specific targeting of tumor angiogenesis by RGDconjugated ultrasmall superparamagnetic iron oxide particles using a clinical 1.5-T magnetic resonance scanner. *Cancer Res*. 2007;67:1555–62.
108. Rajendran R, Liang J, Tang MY, Henry B, Chuang KH. Optimization of arterial spin labeling MRI for quantitative tumor perfusion in a mouse xenograft model. *NMR Biomed*. 2015;28(8):988–97.
109. Rajendran R, Huang W, Tang AM, et al. Early detection of antiangiogenic treatment responses in a mouse xenograft tumor model using quantitative perfusion MRI. *Cancer Med*. 2014;3(1):47–60.
110. Wheaton AJ, Miyazaki M. Non-contrast enhanced MR angiography: physical principles. *J Magn Reson Imaging*. 2012;36:286–304.
111. Radbruch A, Eidel O, Wiestler B, et al. Quantification of tumour vessels in glioblastoma patients using time-of-flight angiography at 7 Tesla: a feasibility study. *PLoS One*. 2014;9(11):e110727.
112. Dighe S, Blake H, Jeyadevan N, et al. Perfusion CT vascular parameters do not correlate with immunohistochemically derived microvessel density count in colorectal tumors. *Radiology*. 2013;268(2):400–10.
113. Goh V, Halligan S, Daley F, Wellsted DM, Guenther T, Bartram CI. Colorectal tumor vascularity: quantitative assessment with multidetector CT – do tumor perfusion measurements reflect angiogenesis? *Radiology*. 2008;249:510–7.
114. Goh V, Halligan S, Taylor SA, Burling D, Bassett P, Bartram CI. Differentiation between diverticulitis and colorectal cancer: quantitative CT perfusion measurements versus morphologic criteria – initial experience. *Radiology*. 2007;242:456–62.
115. Goh V, Halligan S, Hugill JA, Bassett P, Bartram CI. Quantitative assessment of colorectal cancer perfusion using MDCT: inter- and intraobserver agreement. *AJR Am J Roentgenol*. 2005;185:225–31.
116. Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther*. 2006;28:1779–802.
117. Dighe S, Castellano E, Blake H, et al. Perfusion CT to assess angiogenesis in colon cancer: technical limitations and practical challenges. *Br J Radiol*. 2012;85(1018):e814–25.
118. Troprès I, Lamalle L, Péoc'h M, et al. *In vivo* assessment of tumoral angiogenesis. *Magn Reson Med*. 2004;51:533–41.
119. Walker-Samuel S, Boult JK, McPhail LD, Box G, Eccles SA, Robinson SP. Non-invasive *in vivo* imaging of vessel calibre inorthotopic prostate tumour xenografts. *Int J Cancer*. 2012;130:1284–93.
120. Gilad AA, Israely T, Dafni H, Meir G, Cohen B, Neeman M. Functional and molecular mapping of uncoupling between vascular permeability and loss of vascular maturation in ovarian carcinoma xenografts: the role of stroma cells in tumor angiogenesis. *Int J Cancer*. 2005;117:202–11.
121. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med*. 2004;10:145–7.
122. Mousa L, Salem ME, Mikhail S. Biomarkers of angiogenesis in colorectal cancer. *Biomark Cancer*. 2015;7(S1):13–9.
123. Capirci C, Rampin L, Erba PA, et al. Sequential FDG-PET/CT reliably predicts response of locally advanced rectal cancer to neo-adjuvant chemoradiation therapy. *Eur J Nucl Med Mol Imaging*. 2007;34:1583–93.
124. Kalff V, Duong C, Drummond EG, Matthews JP, Hicks RJ. Findings on 18F-FDG PET scans after neoadjuvant chemoradiation provides prognostic stratification in patients with locally advanced rectal carcinoma subsequently treated by radical surgery. *J Nucl Med*. 2006;47:14–22.
125. Manning HC, Merchant NB, Foutch AC, et al. Molecular imaging of therapeutic response to epidermal growth factor receptor blockade in colorectal cancer. *Clin Cancer Res*. 2008;14:7413–22.
126. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med*. 2009;50(suppl 1):122S-50.
127. de Langen AJ, van den Boogaart VE, Marcus JT, Lubberink M. Use of H2 15O-PET and DCE-MRI to measure tumor blood flow. *Oncologist*. 2008;13:631–44.
128. Tseng J, Dunnwald LK, Schubert EK, et al. 18F-FDG kinetics in locally advanced breast cancer: correlation with tumor blood flow and changes in response to neoadjuvant chemotherapy. *J Nucl Med*. 2004;45(11):1829–37.
129. Zhang N, Fang Z, Contag PR, Purchio AF, West DB. Tracking angiogenesis induced by skin wounding and contact hypersensitivity using a Vegfr2-luciferase transgenic mouse. *Blood*. 2004;103:617–26.
130. De Giorgi U, Aliberti C, Benea G, Conti M, Marangolo M. Effect of angiosonography to monitor response during imatinib treatment in patients with metastatic gastrointestinal stromal tumors. *Clin Cancer Res*. 2005;11(17):6171–6.
131. Lamuraglia M, Escudier B, Chami L, et al. To predict progression free survival and overall survival in metastatic renal cancer treated with sorafenib: pilot study using dynamic contrast-enhanced Doppler ultrasound. *Eur J Cancer*. 2006;42(15):2472–9.
132. Lassau N, Lamuraglia M, Chami L, et al. Gastrointestinal stromal tumors treated with imatinib: monitoring response with contrast enhanced sonography. *AJR Am J Roentgenol*. 2006;187(5):1267–73.
133. Schirin-Sokhan R, Winograd R, Roderburg C, et al. Response evaluation of chemotherapy in metastatic colorectal cancer by contrast enhanced ultrasound. *World J Gastroenterol*. 2012;18(6):541–5.
134. Pysz MA, Guracar I, Foygel K, Tian L, Willmann JK. Quantitative assessment of tumor angiogenesis using real-time motion-compensated contrast-enhanced ultrasound imaging. *Angiogenesis*. 2012;15(3):433–42.
135. Gao Y, Zheng DY, Cui Z, Ma Y, Liu YZ, Zhang W. Predictive value of quantitative contrast-enhanced ultrasound in hepatocellular carcinoma recurrence after ablation. *World J Gastroenterol*. 2015;21(36):10418–26.
136. Pysz MA, Foygel K, Panje CM, Needles A, Tian L, Willmann JK. Assessment and monitoring tumor vascularity with contrast-enhanced ultrasound maximum intensity persistence imaging. *Invest Radiol*. 2011;46(3):187–95.
137. Wang Y, Iyer M, Annala A, Wu L, Carey M, Gambhir SS. Noninvasive indirect imaging of vascular endothelial growth factor gene expression using bioluminescence imaging in living transgenic mice. *Physiol Genomics*. 2006;24:173–80.
138. Wang LV, Hu S. Photoacoustic tomography: *in vivo* imaging from organelles to organs. *Science*. 2012;335:1458–62.
139. Laufer J, Zhang E, Raivich G, Beard P. Three-dimensional noninvasive imaging of the vasculature in the mouse brain using a high resolution photoacoustic scanner. *Appl Opt*. 2009;48:D299–306.
140. Loupakis F, Cremolini C, Yang D, et al. Prospective validation of candidate SNPs of VEGF/VEGFR pathway in metastatic colorectal cancer patients treated with first-line FOLFIRI plus bevacizumab. *PLoS One*. 2013;8(7):e66774.
141. Lambrechts D, Claes B, Delmar P, et al. VEGF pathway genetic variants as biomarkers of treatment outcome with bevacizumab: an analysis of data from the AViTA and AVOREN randomised trials. *Lancet Oncol*. 2012;13(7):724–33.
142. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol*. 2010;28:3239–47.

143. Pectasides DG, Xanthakis I, Makatsoris T, et al. Irinotecan/capecitabine (XELIRI) plus bevacizumab versus irinotecan/fluorouracil/leucovorin (FOLFIRI) plus bevacizumab as first-line treatment in patients with metastatic colorectal cancer: a randomized phase III trial of the Hellenic Cooperative Oncology Group (HeCOG). *Proc Am Soc Clin Oncol*. 2010;28(suppl):abstr3541.
144. Koutras AK, Antonacopoulou AG, Eleftheraki AG, et al. Vascular endothelial growth factor polymorphisms and clinical outcome in colorectal cancer patients treated with irinotecan-based chemotherapy and bevacizumab. *Pharmacogenomics J*. 2012;12(6):468–75.
145. Ebos JML, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. *Proc Natl Acacd Sci*. 2007;104:17069–74.
146. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell*. 2009;15:220–31.
147. Formica V, Palmirotta R, Del Monte G, et al. Predictive value of VEGF gene polymorphisms for metastatic colorectal cancer patients receiving first-line treatment including fluorouracil, irinotecan, and bevacizumab. *Int J Colorectal Dis*. 2011;26:143–51.
148. Schneider BP, Shen F, Miller KD. Pharmacogenetic biomarkers for the prediction of response to antiangiogenic treatment. *Lancet Oncol*. 2012;13(10):e427-36.
149. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell*. 2009;15:232–9.
150. Phase III C-08 study of Avastin in early-stage colon cancer does not meet primary endpoint [online]; 2009. Available at: http://www.roche.com/media_releases/med-cor-009-4-22.htm.
151. Horowitz NS, Penson R, Boucher Y, et al. A multidisciplinary phase II study of bevacizumab combined with oxaliplatin, gemcitabine in women with recurrent mullerian carcinoma. *Cancer Res*. 2008;68:4484. [AACR Annual Abstracts].
152. Loges S, Mazzone M, Hohensinner P, Carmeliet P. Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. *Cancer Cell*. 2009;15:167–70.
153. Pàez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell*. 2009;15:220–31.
154. Patterson DM, Padhani AR, Collins DJ. Technology insight: water diffusion MRI – a potential new biomarker of response to cancer therapy. *Nat. Clin. Pract. Oncol*. 2008;5:220–33.
155. Hamstra DA, Galbán CJ, Meyer CR, et al. Functional diffusion map as an early imaging biomarker for high-grade glioma: correlation with conventional radiologic response and overall survival. *J Clin Oncol*. 2008;26:3387–94.
156. Holden SN, Ryan E, Kearns A, Holmgren E, Hurwitz H. Benefit from bevacizumab (BV) is independent of pretreatment plasma vascular endothelial growth factor-A (pl-VEGF) in patients (pts) with metastatic colorectal cancer (mCRC). *J Clin Oncol*. 2005;23:3555.
157. Drevs J, Siegert P, Medinger M, et al. Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. *J Clin Oncol*. 2007;25:3045–54.
158. Duda DG, Cohen KS, Ancukiewicz M, et al. A comparative study of circulating endothelial cells (CECs) and circulating progenitor cells (CPCs) kinetics in four multidisciplinary phase 2 studies of antiangiogenic agents. *J Clin Oncol*. 2008;26:3544. [ASCO Meeting Abstracts].