Surrogate biomarkers in evaluating response to anti-angiogenic agents: focus on sunitinib

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Conventional methods to assess the clinical activity of new agents that target specific biological pathways involved in tumour pathology may not provide correlation with clinically relevant outcomes such as patient survival or progression-free disease, and new and alternative methods should be explored. Biomarkers can assist in evaluation, and once validated, serve as a surrogate for clinical activity. Angiogenesis, a process well known to be involved in tumour growth and metastasis, is the target of several agents available today in the treatment of cancer. Laboratory assays used to detect proteins involved in angiogenesis and emerging imaging approaches have provided the bulk of the biomarker data to date in this area, and have already corroborated aspects of the biochemical basis of anti-angiogenic strategy. This symposium article will provide a brief overview of biomarker data in several different tumour types and discuss the effect that sunitinib and other anti-angiogenic agents have on these biomarkers. Surrogate biomarkers discussed include soluble proteins found in the blood or urine, circulating endothelial cells and their progenitors, and non-invasive imaging techniques.

Key words: biomarkers, anti-angiogenesis, surrogate, sunitinib, VEGF

introduction

Continuing advances in molecular biology have opened up new potential methods to help determine the prognosis and prediction of response in many solid tumour types, including those in breast, kidney and prostate cancers. Traditionally, stage of disease at clinical presentation and patient characteristics (i.e. performance status, symptom severity, age and tumour size) were the major determinants of disease prognosis and treatment strategy. While these traditional factors continue to play important roles in treatment choice and outcomes, significant strides have been made in the evaluation and correlation of biomarkers as potential tools in diagnosis, prognosis, treatment strategy and treatment evaluation. This symposium article will provide a brief overview of the role of biomarkers involved in angiogenesis inhibition and of their value in clinical research (with a focus on sunitinib clinical studies), and examine how some of these biomarkers may eventually influence routine clinical practice.

role of biomarkers as surrogate endpoints and their clinical value

A surrogate endpoint is an outcome measure that is a correlative indicator of clinical response or lack of response.

When the surrogate endpoint is a biomarker, the outcome measure can be derived from a laboratory test (e.g. reduction of a serum protein, which may indicate a positive response to therapy). To be valid, a biomarker surrogate endpoint must meet two criteria: the biomarker must correlate with the clinical outcome of interest, and it must accurately capture the effect of the intervention on that outcome [1]. The usefulness of a marker is weighed by several factors including the feasibility of its assessment in patients, the convenience of obtaining samples or readings, and of course its ability to track the disease or the effect of therapy on an actual clinical outcome. Surrogate endpoints have the potential to yield information more rapidly and with smaller sample sizes than traditional endpoints in large studies, which may require participants to be followed for years.

Surrogate markers have a legitimate role in drug development. As the costs of therapies in oncology continue to increase, there is a compelling need to monitor biological activity, select and stratify patients who are most likely to benefit from treatment, and identify optimal biological dose [1]. In addition, when used as outcomes in phase II screening trials, they can provide guidance about biological pathways of interest in larger phase III trials. Surrogate endpoints can also provide corroborating evidence when they are used in conjunction with true clinical outcomes in phase III trials. Finally, if validated, biomarkers can be utilized to help measure therapeutic response to new and existing agents.
b biomarkers of angiogenesis and their value as a response to therapy

Angiogenesis is essential for the growth of large tumours, and vascular endothelial growth factors (VEGFs; VEGF-A is the predominant factor in angiogenesis regulation) and their receptors are known to be among the principal proteins involved in pro-angiogenic signalling pathways [2, 3]. Unfortunately, because anti-angiogenic agents are likely to be most efficacious early during the tumorigenic process, surrogate outcomes such as objective tumour response may no longer be valid in some situations, and alternative or new criteria are necessary to judge the efficacy of these therapies [4]. Currently, there are no well-established biomarkers of efficacy with anti-VEGF therapy. Such biomarkers are needed to help validate the mechanistic hypotheses of action, identify responsive patients and optimal doses and predict outcomes of regimens that include anti-VEGF agents.

Clinical trials for traditional cytotoxic drugs are often designed to show an improvement in the objective response rate (e.g. the disappearance of all known disease or a decrease in total tumour size of ≥50% for at least 4 weeks, without the appearance of new lesions) [5]. However, anti-angiogenic drugs are often cytostatic in action, and tumour shrinkage or regression may not be a realistic estimate of efficacy [5]. For example, increased tumour responses may not be observed, but another clinically relevant endpoint—such as increased overall survival (OS)—may be, as was the case with bevacizumab (a recombinant humanized anti-VEGF-A antibody; Avastin®, Genentech/Roche) in combination with traditional cytotoxic chemotherapy in patients with metastatic colorectal cancer [6]. Other functional and molecular changes have been observed in tumours in response to VEGF blockade, but without a significant reduction in tumour volume [7] (described further below). How these changes during treatment relate to other clinical endpoints, such as progression-free survival (PFS) or OS, has yet to be determined. Strategies that discontinue anti-angiogenic drugs in patients without an objective tumour response can compromise clinical benefit; therefore, to appropriately characterize the treatment effect of anti-angiogenic agents, new measures of objective vascular response are needed [5].

Significant advances have been made in identifying candidate biomarkers that measure response to anti-angiogenic agents. While no one marker has been widely agreed upon, promising results from several different disciplines have been reported [8]. One of the most promising approaches to date is in the measurement of growth factors or soluble growth factor receptors in the blood (serum or plasma) or urine. Two other distinct classes of biomarker approaches that have shown promise in this area are measurements of circulating endothelial cells (CECs) and/or progenitors (CEPs), and vascular imaging techniques [5]. Each of these types of biomarkers is discussed in the following sections.

It is hoped that anti-angiogenic biomarkers will not only provide guidance in terms of response to therapy, but, by facilitating optimal patient selection, may also eventually help determine the best choice of drug(s) from a growing list of agents that target this process. Biomarkers may also be used to assist dose prediction and ongoing benefit analysis. Due to the desired frequency of sample measurements, convenient and minimally invasive methods of biomarker measurement will need to be established. Factors that predict or detect resistance to initial therapy may also be identified in ongoing biomarker studies; such resistance markers may allow the decision to switch to more effective second-line therapies to be made at an earlier point in time.

candidate biomarkers: soluble proteins in blood or urine

Angiogenic growth factors such as VEGF-A, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), placenta growth factor (PlGF), hepatocyte growth factor (HGF) and interleukin 8 (IL-8) are examples of candidate biomarkers that can be detected in blood serum and that may possibly predict survival or response to therapy [7, 9, 10]. VEGF is vital to the growth of tumour blood vessels, and as such has long been regarded as a potential surrogate marker of cancer growth and anti-angiogenesis drug efficacy. Figure 1 shows the key role VEGF plays in signalling pathways that lead to angiogenesis. Most anti-angiogenic drugs currently used in clinical practice target the VEGF pathway. Preclinical studies in mice show that blood plasma levels of VEGF are significantly increased by VEGFR-2 blockade, and are therefore proposed as a surrogate biomarker for VEGFR-2 inhibition. Inhibition of VEGFR-2 is also found to be associated with survival benefit as well as tumour response [9].

Soluble forms of pro-angiogenic growth factors such as VEGF-A, PlGF, stem cell factor (SCF) and their receptors can be measured in the serum and plasma of patients by ELISA. In various clinical phase I trials with anti-angiogenic compounds, VEGF and other soluble markers were used to assess appropriate dose levels for biological activity in patients [10]. Some earlier studies indicate that VEGF-A levels correlate with disease activity and prognosis, as well as vascular changes as reflected by imaging techniques. However, there are many other studies of VEGF as a surrogate marker that have produced inconclusive evidence of its reliability or predictability. It is uncertain if VEGF status alone is adequate as a diagnostic or prognostic marker, because it is only one of many pro-angiogenic factors involved in cancer growth and may be more significant to certain cancers at certain stages of tumour growth. More research is necessary to validate the value of VEGF and other soluble growth factors as biomarkers, especially as surrogates for response to therapy. The majority of existing biomarker data on anti-angiogenic therapies involves VEGF-A or its receptors.

candidate biomarkers: circulating endothelial cells

Recent research has recognized new populations of non-haematopoietic cells in the blood. One of these, CECs and their progenitors (CEPs), is rarely found in the blood of healthy subjects, but elevated amounts are found in neoplastic
disease [11]. CECs and CEPs are currently being assessed as potential biomarkers of anti-angiogenic therapy. Prior studies have shown that anti-angiogenic agents suppress the mobilization and serum levels of CECs and CEPs, and that treatment with a VEGFR-2 antibody causes a dose-dependent reduction in viable CEPs that correlates to its antitumour activity [12, 13]. Therefore, CEC and CEP measurements have promising potential as a surrogate marker for monitoring anti-angiogenic drug activity. Although the exact mechanism of how CEPs contribute to tumour angiogenesis is uncertain, a recent study has corroborated their involvement by showing that treatment of tumour-bearing mice with vascular disrupting agents (VDAs) leads to an acute mobilization of CEPs [14]. CECs and CEPs have been measured in several preclinical and clinical studies as potential biomarkers of response to anti-angiogenic activity [15], and research to further assess their utility is ongoing.

**candidate biomarkers: vascular imaging**

Imaging techniques are non-invasive and have the potential to measure vascular function as a surrogate marker for therapy, regardless of tumour type or location [16]. There are many imaging modalities available for investigating tumour vasculature, including dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), computed tomography (CT) and positron emission tomography (PET), which are being evaluated in clinical trials [17–19]. One study, utilizing serial PET images, showed that patients with advanced malignancies treated with sunitinib had ≥20% reductions in standard uptake values of radiolabeled glucose and water as early as the second week of treatment, indicative of decreased blood flow in metastases [20]. Another imaging technique that has shown promise in a recent study is contrast-enhanced ultrasound: targeted microbubbles (incorporated with avidin and conjugated to biotinylated antibodies that target VEGFR-2- or VEGF-activated blood vessels) can be tracked and used to monitor vascular effects [21]. Evaluation of tumour vascularity has been shown to be feasible with this technique, and one study showed correlation with levels of protein expression of the targets in preclinical models [22]. Intravenously administered microbubbles can also be used as a surrogate for blood flow, with disruptions monitored by contrast ultrasound. Successful preliminary results in patients with advanced hepatocellular carcinoma have been reported using this technique but further studies in larger groups are needed to confirm its value in other patient populations [23].

DCE-MRI involves several scans over a set volume during the injection of a contrast agent (such as gadolinium-DTPA) to measure parameters that are surrogates for blood flow, capillary permeability, capillary surface area and/or

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**Figure 1.** VEGF and its downstream signalling pathways (from http://www.biocarta.com, date last accessed 20 March 2007).
The effects of anti-angiogenic agents have been evaluated with this imaging technique in phase I and II clinical trials of several anti-angiogenesis agents, including AG-013736 (an inhibitor of the VEGFRs, PDGFR-β and KIT; Pfizer) [19], bevacizumab [24], vatalanib (an inhibitor of VEGFR-1 and -2, PDGFR and KIT; Schering) [17], and BAY 57-9352 (an inhibitor of VEGFR-2 and PDGFR-β; Bayer) [25]. Although a standardized method for DCE-MRI has not been agreed upon and the measured constants are not fully defined, the general outcomes of these studies are similar. All agents showed an early (within hours or days) dose-response effect on DCE-MRI measures of vascularity, which indicates that this method has the potential to function as a marker of early treatment benefit [17, 24]. Vascular imaging shows promise as a surrogate measure of objective vascular response, but further evidence is required to determine whether such response is informative in terms of patient survival or other clinically relevant outcomes.

**biomarker data in specific tumour types**

The focus in this section is on data from studies with sunitinib; data from studies with other anti-angiogenic agents are included where available.

**biomarker data in renal cell carcinoma**

Patients with renal cell carcinoma (RCC) traditionally have had a poor prognosis, but sunitinib malate (Sutent®, Pfizer), an oral inhibitor of multiple tyrosine kinases including VEGF receptors, has recently demonstrated antitumour activity in patients with metastatic RCC. A phase III trial in the first-line setting has shown that patients on sunitinib (administered in 6-week cycles of daily oral therapy for 4 weeks followed by 2 weeks off therapy [4/2 schedule]) experienced improved PFS and response rates compared with those on IFN-α therapy [26]. In an earlier phase II trial, patients who had progressed or were intolerant of cytokine therapy were treated with sunitinib (4/2 schedule), and the majority had a reduction in measurable disease per RECIST: 40% of patients achieved partial responses, with an additional 27% demonstrating stable disease lasting ≥3 months [27]. Plasma levels of soluble biomarkers VEGF-A, PIGF (a ligand specific for VEGFR-1) and soluble VEGF receptor 2 (sVEGFR-2) were serially measured via ELISA in patients from this phase II trial. Both VEGF-A and PIGF levels increased and sVEGFR-2 levels decreased by the end of each dosing cycle. VEGF levels are known to increase in response to hypoxia and pharmacologic inhibition of angiogenesis. After 2 weeks off sunitinib, the levels of all three biomarkers returned to near baseline levels. The differences between baseline and end-of-cycle biomarker levels were statistically significant through cycle 8 (P ≤ 0.002), as shown in Figure 2.

Although the mechanism for the consistent decreases in sVEGFR-2 and increases in VEGF ligands is not currently entirely understood, the consistency and high degree of correlation of sVEGFR-2, VEGF and PIGF serum level changes in response to sunitinib treatment is promising. Based upon the emerging data, these biomarkers may provide a simple yet powerful tool to assess the biological activity of VEGFR antagonists and may help evaluate responses to anti-angiogenic therapy.

**biomarker data in metastatic breast cancer**

Positive biomarker results for anti-VEGF agents in the breast cancer setting are limited at present but research in this setting is rapidly expanding. Sunitinib has been evaluated in a phase II trial of 64 patients with refractory metastatic breast cancer (mBC). Of 51 patients with preliminary data, seven (14%) had partial responses and one demonstrated stable disease at ≥6 months, resulting in a clinical benefit rate of 16% [28]. Patients received sunitinib on the 4/2 schedule. Pre-dose plasma samples from 62 patients were obtained on days 1, 14 and 28 of the first cycle and on days 1 and 28 of subsequent cycles. Plasma levels of VEGF, sVEGFR-2, soluble KIT (sKIT) and a novel biomarker, sVEGFR-3 (a potential indicator of lymphangiogenesis and metastatic potential), were measured via ELISA [29].

 Plasma levels of each protein were modulated in most patients during the course of treatment. At the end of the first cycle, VEGF levels increased more than 3-fold relative to baseline in 73% of cases, while sVEGFR-2 levels decreased by at least 30% in 88% of cases, and by >20% in all but four cases. In addition, levels of sVEGFR-3 decreased by >30% in 82% of cases during the first cycle. For each of these markers, levels tended to return to near-baseline after 2 weeks off treatment [29], similar to the pattern in the RCC study. Longitudinal decreases in sKIT were also observed, without a return to baseline during the 2-week break. Clinical benefit, as defined by a reduction in baseline tumour size, time to progression (TTP) and/or OS, was associated with the following trends in exploratory analysis: higher baseline sVEGFR-2 levels and greater decreases in sVEGFR-2 levels during treatment were observed in cases with tumour size reduction; decreases in sVEGFR-3 of ≥20% at the start of cycle 2 (or at the start of the last treatment cycle) were associated with longer OS; and sKIT decreases of >50% by the end of cycle 2 correlated with longer TTP (P < 0.001) and OS (P = 0.02) [29]. This group of circulating proteins may have utility as pharmacodynamic biomarkers of sunitinib activity in patients with mBC. sVEGFR-3 may be a novel biomarker of the biological activity of sunitinib, while sKIT may be correlated with clinical response. Further analysis of these and other biomarkers in larger studies of sunitinib in breast cancer may be warranted.

In a phase II trial of another anti-VEGF agent (bevacizumab), 27 patients with mBC received bevacizumab on days 1 and 15 in combination with docetaxel on days 1, 8 and 15 of a 28-day cycle [30]. The overall response rate was 52% (95% confidence interval [CI] 32–71%), with a median PFS of 7.5 months (95% CI 6.2–8.3 months). Plasma endothelial and adhesion cell markers, including E-selectin, P-selectin, immune cell adhesion molecule-1 (ICAM-1), PDGF, fibroblast-derived growth factor (FGF) and the matrix metalloproteinases (MMPs) MMP-2 and MMP-9 were evaluated at baseline and after one cycle of treatment. In a univariate analysis, during cycle 1 of treatment,
E-selectin and ICAM-1 were the only biomarkers that were significantly associated with response (Table 1). E-selectin maintained significance after controlling for other risk factors, such as performance status, age, oestrogen receptor status and disease-free interval, whereas ICAM-1 did not.

In a retrospective subset analysis of another randomized bevacizumab trial, VEGF expression measured by in situ hybridization in primary breast cancer tumour tissue samples did not correlate with response to bevacizumab [31], and so does not appear to be useful to monitor response with this agent in breast cancer. Thus, although limited by small sample size and lack of correlation to PFS, these results warrant further investigation of E-selectin (an endothelial cell-specific membrane glycoprotein implicated in angiogenesis and tumour metastases [32, 33]) as a biomarker of response to bevacizumab and other VEGF-inhibiting therapies.

**Biomarker data in neuroendocrine tumours**

Sunitinib was evaluated in a phase II study of patients with advanced unresectable neuroendocrine tumour (NET; \( N = 107 \)), a highly vascularized solid tumour. Patients with both carcinoid and pancreatic islet cell NET subtypes were included in the study. Single-agent use of sunitinib was well tolerated and associated with objective tumour responses and a high rate of stable disease in NET patients [34]. Significant changes \( (P < 0.0001) \) in the mean plasma levels for VEGF,

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**Figure 2.** VEGF-A, sVEGFR-2 and PlGF levels during treatment with sunitinib: (A) VEGF-A, (B) sVEGFR-2, and (C) PlGF levels in plasma measured on days 1 (D1) and 28 (D28) of each cycle. The number of cases available for each timepoint is listed in parentheses below each timepoint. Black bars denote mean values for each cycle. The differences between baseline and end-of-cycle biomarker levels were statistically significant through cycle 8 \( (P \leq 0.002) \) [27].
sVEGFR-2 and sVEGFR-3 were observed within the first dosing cycle; changes in IL-8 were also significant ($P = 0.003$). Baseline VEGF and sVEGFR-3 levels tended to be higher in cases with longer PFS and with tumour size reduction or stabilization, while the average decrease in sVEGFR-2 and sVEGFR-3 during cycle 1 also tended to be greater in these groups. Baseline IL-8 levels were lower in cases with improved PFS and with tumour size reduction or stabilization [35]. This trend was also significant ($P = 0.009$) when patients with stable disease for more than 6 months (mean IL-8 22.5 pg/mL; $n = 60$) were compared with those with stable disease for less than 6 months (mean IL-8 42.0 pg/mL; $n = 24$). Greater increases in IL-8 also showed a trend towards association with longer PFS, particularly in the carcinoid tumour subgroup. Clinical benefit, as defined by partial response, disease stabilization and/or improved PFS, was associated with the following trends: higher baseline sVEGFR-3, sVEGFR-2 and VEGF levels, lower baseline IL-8 levels, greater decrease in sVEGFR-3 and sVEGFR-2 levels during treatment and greater increases in IL-8 levels during treatment [35]. Analysis of these and other circulating biomarkers is ongoing in several different trials of sunitinib; if found applicable, they may eventually serve as correlates of treatment efficacy or other parameters in various disease settings.

### Biomarker Data in Gastrointestinal Stromal Tumours

Circulating biomarkers were investigated in blood samples obtained from gastrointestinal stromal tumour (GIST) patients enrolled in a phase II/II study evaluating different dosing schedules of sunitinib after failure of imatinib therapy due to either resistance or intolerance [36]. Sunitinib was administered orally once daily in cycles consisting of 2 weeks on treatment followed by 2 weeks off treatment (2/2 schedule), 4 weeks on then 2 weeks off (4/2 schedule), or 2 weeks on then 1 week off (2/1 schedule). Complete blood counts and differential white blood cell counts were performed both before and after treatment in 73 study patients, and four-color flow cytometry was used to assess serially CECs in 16 of these patients. Cells were stained either using a biotinylated primary antibody plus an avidin-conjugated fluorochrome or using a directly conjugated primary antibody. Mature CECs were defined as CD45+/CD31+/CD146+ (P1H12+)/CD133− cells; under the conditions used, monocytes were defined as CD14+ cells. Plasma levels of VEGF and sVEGFR-2 were also measured.

Changes in mature CECs and monocytes were correlated with clinical outcome, classified as either clinical benefit (CB: progression-free survival >6 months) or progressive disease (PD). The changes in CEC count were significant early after initiation of sunitinib treatment (first follow-up: 6–20 days; $P = 0.03$), but not at subsequent timepoints; CEC counts tended to increase from baseline in the CB group but not in the PD group, perhaps reflecting an increase in apoptotic endothelial cells in the group with greater CB.

The decrease in monocyte count after 2 weeks of sunitinib treatment was inversely correlated with clinical outcome. At baseline, there were no differences between monocyte counts for patients with PD or CB. However, after 2 weeks of sunitinib treatment, the mean monocyte count decreased by 59% in the PD group, but by only 48% in the CB group ($P = 0.007$).

In patients receiving the 4/2 schedule, mean plasma VEGF levels increased by an average of 2.9-fold after 4 weeks on treatment in each cycle, and fell to near-baseline levels during the off-treatment periods. Mean sVEGFR-2 levels decreased by an average of 1.7-fold after 4 weeks on treatment in each treatment cycle, and rebounded to near-baseline levels during the off-treatment periods. Changes in sVEGFR-2 exhibited a moderate correlation with trough plasma levels of sunitinib ($R^2 = 0.33$).

In summary, levels of all four potential biomarkers in this study changed upon administration of sunitinib, and changes in three of the four reversed during the off-treatment periods. These results merit further investigation in future studies of drugs targeting the VEGF pathway.

Another biomarker that appears to have promise as a correlate of response in GIST is sKIT. Plasma sKIT levels decline over time in GIST patients treated with sunitinib, but the decline appears to be greater in patients experiencing clinical benefit as opposed to those with rapid progression [37, 38]. It can be hypothesized that the decline in sKIT may represent, at least in part, a decrease in the number of viable tumour cells in GIST patients, as GISTs are known to be KIT-positive in most cases. Whether sKIT can be considered a biomarker of anti-angiogenic agents per se may be in question, as the modulation of this factor may be linked more with the KIT-inhibitory activity of sunitinib rather than its anti-VEGF activity.
In another substudy in the same GIST trial, levels of phosphorylated PDGFR-β were significantly decreased (average decrease 15.0%) in tumour biopsies from GIST patients treated with sunitinib who had achieved clinical benefit (defined as either partial response by RECIST or stable disease for >6 months). In contrast, those with progressive disease had elevated levels of phosphorylated PDGFR-β (average increase of 11.0%) [39]. These results provide indirect evidence of this agent’s basis for anticancer efficacy, and highlight activated PDGFR-β as a potential biomarker for clinical benefit. The feasibility of collecting serial biopsy samples for exploratory analysis is limited in many patient populations; hence the need for less invasive biomarker approaches. However, results from analyses such as this one in GIST patients are useful in assessing target modulation and biological activity of anti-angiogenic agents in diseased tissue.

**biomarker data in colorectal cancer**

In a phase I trial in rectal cancer patients, bevacizumab increased plasma levels of VEGF and PlGF in all patients analysed, while substantially reducing the percentage of viable CECs in the majority of patients [7]. While the amounts of free versus bevacizumab-bound VEGF have yet to be determined, the kinetics of CECs in circulation in combination with levels of VEGF protein could be potential surrogate biomarkers for efficacy of anti-VEGF therapies in humans. Treatment of these patients with bevacizumab is also associated with a significant decrease in microvascular density, suggesting an effect of tumour vasculature normalization [40].

In two phase I/II dose escalation trials, 63 patients with advanced colorectal cancer were evaluated to assess biological activity of vatalanib [41]. Plasma levels of VEGF-A and bFGF were found to increase in a dose-dependent manner in the first cycle of treatment. Furthermore, a mean change of ≥150% from baseline plasma VEGF-A levels within the first cycle of treatment correlated with a clinical outcome of non-progressive disease by logistic regression analysis ($P = 0.0027$). Other serum biomarkers assessed were activated endothelial cells, E-selectin and a transmembrane tyrosine kinase, TIE-2 [41]. Data from measurements of these biomarkers did not show strong correlations with clinical outcome, precluding a conclusion about their potential utility.

**prognostic value of biomarkers**

The value of biomarkers as prognostic or predictive factors in anti-angiogenic therapy has generally been less evident thus far than their value as indicators of pharmacological and biological effects. For example, in a pivotal trial of 813 patients with metastatic colorectal cancer treated with irinotecan, 5-fluorouracil (5-FU) and leucovorin (IFL) plus bevacizumab or placebo, a retrospective subset analysis revealed that pre-treatment epithelial and stromal levels of VEGF protein did not predict benefit from the addition of bevacizumab [42]. Levels of thrombospondin-2 and microvessel density were also analysed and were similarly found not to be significant prognostic factors. In a study of 52 patients with advanced pancreatic cancer who were treated with bevacizumab and gemcitabine, pre-treatment levels of VEGF were also obtained. A confirmed partial response was seen in 21% of patients, and 46% had stable disease. However, for the 42 patients for whom pre-treatment VEGF plasma levels were obtained, no significant difference in VEGF levels was observed in patients with progressive versus stable disease. In addition, there were no differences noted in OS or PFS between those patients whose VEGF levels were above or below the median [43].

In a phase III randomized study of paclitaxel and carboplatin either with or without the addition of bevacizumab in non-small–cell lung cancer (NSCLC) patients, plasma ICAM levels were reported to be the only one of a group of four circulating angiogenic factors that displayed a significant treatment interaction effect for PFS. The data suggested a benefit from bevacizumab (33% reduction in the PFS hazard rate) in the group with low baseline ICAM levels compared with no benefit for patients with high levels [44]. This exploratory result suggests that baseline ICAM levels may be of use in predicting response to bevacizumab and chemotherapy in NSCLC patients; whether this finding will apply to other anti-angiogenic agents and in other contexts remains to be established.

Results from a small clinical study of the pan-VEGF inhibitor AZD2171 (AstraZeneca) in patients with glioblastoma suggested that tumour progression was associated with increases in plasma levels of bFGF and stromal cell-derived factor-1 (SDF1) alpha, and decreases in PlGF [45]. These factors are potential correlates of glioblastoma relapse during anti-VEGFR treatment, which in the case of bFGF and SDF1 alpha may represent activation of alternative angiogenic pathways, and may be worthy of further assessment in other tumour types and with other agents. Increases in viable CECs and CEPs also correlated with relapse in this study, although the authors point out some of the technical questions remaining in terms of general consensus in the field as to the most appropriate surface markers to identify these cells [46]. Further clinical and laboratory studies to address the identification and characterization of the most useful baseline response predictors, as well as indicators of resistance or relapse, will be an important component of the anti-angiogenesis field. However, emerging results from studies with sunitinib and other agents suggest that pharmacodynamic biomarkers may also be of utility as predictive markers.

**conclusions**

The evidence to date of certain circulating soluble proteins as markers of treatment effect and disease progression is very promising. They have been observed to be useful as indicators of biological activity of anti-angiogenic agents in patients with primary tumours and metastatic cancer, although the data supporting their clinical use are still too premature for routine clinical application. CECs/CEPs and imaging techniques such as DCE-MRI have also shown promise as potential biomarkers of response to therapy. The data are currently more limited in terms of supporting the utility of these three categories of biomarkers as prognostic or predictive factors (rather than dynamic indicators).
Much of the existing clinical work appears to support the use of circulating levels of soluble proteins such as VEGF, sVEGFR-2 and -3, PIGF, KIT, ICAM and E-selectin, as well as CECs/CEPs and imaging techniques, but it is still uncertain whether these biomarkers can reliably monitor clinical response. Preclinical studies indicate that many of the discussed candidate biomarkers and others are promising and are likely to be valid indicators of target expression or drug mechanisms of action [47, 48]. However, the translation of this information and understanding from animal models to human studies has not yet been fully established at this point in time. Ultimately, the choice of biomarkers will probably vary by treatment, tumour type and patient. Current studies are often not sufficiently powered or designed with a sufficient range of doses to elicit dose response, and/or patients are not followed up for long enough to make definitive conclusive statements about anti-angiogenic biomarkers. Although extremely promising, more prospective studies are required to validate their clinical relevance and also to test additional hypotheses and identify additional candidate biomarkers.

disclosures

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