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Molecular Biomarkers of Response to Antiangiogenic Therapy for Cancer

Dan G. Duda
Steele Laboratory for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

Abstract

Antiangiogenic therapy for cancer has gone from an intriguing hypothesis in the 1970s to an accepted treatment approach for many cancer types. It has also become a standard of care for certain eye diseases. Yet, despite the use of molecularly targeted drugs with well defined targets, to date there are no biomarkers to guide the use of antiangiogenic therapy in patients. The mechanisms of action of these drugs are also being debated. This paper discusses some of the emerging biomarker candidates for this type of cancer therapy, which have provided mechanistic insight and might be useful in the future for optimizing cancer treatment.

1. Antiangiogenic Therapy

Approval of an anti-vascular endothelial growth factor (VEGF) blocking antibody (bevacizumab or Avastin, Genentech, South San Francisco, CA, USA) in combination with chemotherapy for metastatic colorectal cancer in 2004 represented a paradigm shift in cancer therapy. For the first time, an agent targeting the tumor stroma (i.e., the vasculature), as opposed to directly targeting the malignant cells proved to be a viable anticancer treatment option.

Over the last decade, the United States Food and Drug Administration has approved eight anti-angiogenic agents for cancer treatment, and three anti-angiogenic agents for wet age-related macula degeneration therapy (Table 1). A large number of other anti-angiogenic agents are in late phases of clinical development (phase III clinical trials). All the approved anti-angiogenic drugs target VEGF signaling. Some are blocking the ligand, VEGF, for example, bevacizumab, aflibercept (Zaltrap/Eylea, Sanofi-Aventis, Paris, France, and Regeneron Pharmaceuticals, Tarrytown, NY, USA), ranibizumab (Lucentis, Genentech, South San Francisco, CA, USA), and pegaptanib (Macugen, OSI Pharmaceuticals, Long Island, NY, USA). Others are inhibiting the activity of the VEGF tyrosine kinase receptors (VEGFR1, VEGFR2), for example, sorafenib and regorafenib (Nexavar and Stivarga, Bayer Healthcare Pharmaceuticals, Leverkusen, Germany, and Onyx Pharmaceuticals, South San Francisco, CA, USA), sunitinib (Sutent) and axitinib (Inlyta) (Pfizer Inc.,New York,NY, USA), pazopanib (Votrient, GlaxoSmith-Kline, Brentford, Middlesex, UK), and vandetanib (Zactima, Astra Zeneca Pharmaceuticals, Alderley Park, Cheshire, UK). Anti-VEGF therapy has become a standard of care for metastatic colo-rectal cancer (in first, second, and third

Correspondence should be addressed to Dan G. Duda, duda@steele.mgh.harvard.edu.

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line of treatment), advanced non-small cell lung cancer, renal cell carcinoma, hepatocellular carcinomas, glioblastoma, gastrointestinal stromal tumor (GIST), pancreatic neuroendocrine tumor, and medullary thyroid cancer [1– 13] (Table 1). Given these developments, anti-angiogenic therapy represents one of the most exciting areas in cancer research and clinical oncology [14–23]. But beyond the proof-of-the-principle efficacy for anti-angiogenic therapy in these advanced cancers, this success brought critical challenges. First, this therapy has not shown the same benefit in other advanced cancers (e.g., breast cancer, ovarian cancer, prostate cancer, and pancreatic ductal adenocarcinoma) [24]. Second, in the patients who respond to anti-angiogenic therapy, the benefit is transient (i.e., the tumors acquire resistance to anti-angiogenic treatment). Other patients do not respond at all (i.e., the tumors are inherently resistant to anti-angiogenic treatment). Third, similar to most other anticancer drugs, anti-angiogenic agents may induce significant side effects [25]. And finally, treatment with current anti-angiogenic agents is extremely costly [26, 27]. How do we tackle these problems? One potential solution for optimizing treatment with current anti-angiogenic agents is to identify useful biomarkers (see Box 1 for definitions) in order to (1) select (or exclude) patients for treatment with a specific anti-angiogenic drug; and (2) detect early the escape from anti-VEGF therapy.

The former would allow a “more personalized” treatment (for patients more likely to benefit) and/or a sparing of a fraction of patients (those unlikely to respond) from the side effects and the high cost of these treatments. The latter would allow us to devise better combinatorial treatment strategies to extend the benefit of anti-angiogenic agents. Biomarkers have been used for “molecularly targeted” agents, but so far only for those that directly attack the cancer cells, for example, human epidermal growth factor receptor 2 (HER2) expression or amplification for anti-HER2 therapy in breast cancer, *RAS* mutation for anti-epidermal growth factor receptor (EGFR) therapy, and $BRAF^{V600}$ mutation for BRAF inhibitors in melanoma [57–59]. But despite agreement that biomarker discovery and validation are a major priority in the tumor angiogenesis field and the widespread use of anti-angiogenic drugs in the clinic, there are currently no validated biomarkers for use in the clinic for treatment of patient with any type of cancer or for the treatment for wet age-related macula degeneration. Among the many reasons for this situation the most notable are (1) tumor heterogeneity, (2) the incomplete understanding of the mechanism(s) of action that lead to benefit of anti-VEGF therapy in some but not all advanced cancers, and (3) the differential targeting of VEGF pathway and off-target effects by the current anti-angiogenic drugs and by the agents in clinical development. I will summarize here the current progress on our mechanistic understanding of anti-VEGF therapy and on the current status molecular and cellular biomarker discovery and will provide a perspective on the future directions in this field.

2. The Angiogenic Balance

Over four decades of research on angiogenesis have unraveled many of the underpinnings of tumor angiogenesis in general and VEGF pathway in particular [60–64]. VEGF is a key proangiogenic molecule in developmental neovascularization as well as in physiological and pathological angiogenesis [62, 65–67]. VEGF exerts its effect by binding the two tyrosine kinase (TK) receptors VEGFR-1 (FLT1) and VEGFR-2 (KDR) as well as the non-TK receptors neuropilin 1 (NRP-1) and NRP-2 [60]. Of these, VEGF interaction with VEGFR2 is thought to convey most of the critical pro-angiogenic signals [18]. However, VEGF interaction with VEGFR-1 and NRP-1 in cancer cells or in nonendothelial stromal cells (e.g., in myeloid cells such as macrophages) may be critical for the growth of tumors that depend on this pathway for survival and, through indirect mechanisms, to angiogenesis in tumors [60]. During development and in physiological conditions, the effects of VEGF are finely tuned and counterbalanced by anti-angiogenic molecules such as the soluble form of

VEGFR-1 (sVEGFR-1/sFLT-1) or thrombospondins (TSP-1 and -2), which ensures stabilization and maturation of the vasculature [68]. In tumors, oncogene or hypoxia-driven VEGF overexpression leads to dysregulated angiogenesis and an abnormal vasculature [60]. Here, the balance is tipped toward pro-angiogenesis. In genetic models in mice, overexpression of VEGFR-1 and sVEGFR-1 led to a "normalization" of the tumor vasculature [69]. Conversely, overexpression of sVEGFR1 may lead to hypertension (e.g., preeclampsia) or defects in developmental angiogenesis [70–72]. Beyond VEGF, other VEGF family members can bind the VEGFRs and participate in angiogenesis: PlGF, VEGF-B, VEGF-C, and VEGF-D. The role of VEGFR-1 and its more selective ligand placental growth factor (PlGF) is currently unclear, but may be particularly important in certain malignancies [60]. Similarly, VEGF-C and VEGF-D might play a role during new blood vessel formation [73]. First, they can bind to VEGFR2, and second, their cognate receptor VEGFR3 is expressed on "tip" cells (specialized endothelial cells responsible for vessel sprouting) [60, 73]. In addition, other angiogenesis modulators (positive or negative) may affect the angiogenic balance, for example, the pro-angiogenic molecules basic fibroblast growth factor (bFGF or FGF-2), angiopoietin 1 (Ang-1), Ang-2, and endoglin or the endogenous angiogenesis inhibitors TSP-1 and TSP-2 [60]. These angiogenic molecules are produced by the cancer cells and by the stromal cells alike. The latter include activated tumor-activated fibroblasts and bone marrow derived cells recruited by the tumor—most notably tumor infiltrating macrophages, neutrophils, and myeloid derived suppressor cells [35, 74, 75].

Could we exploit all this knowledge for biomarker discovery? The answer is likely yes, provided that biomarker studies will be biology driven and prospectively validated [28–31, 76]. The systemic and imaging biomarkers may also play a crucial role in discovery of biomarkers for anti-angiogenic therapy and are discussed in detail elsewhere [30, 77, 78]. Here, I will discuss in the next three sections the candidate biomarkers belonging to VEGF family and to other angiogenic pathways, and the cellular biomarkers.

3. Molecular and Cellular Biomarker Candidates for Antiangiogenic Therapy

Tissue-based biomarkers are ideal because they reflect the changes occurring in a tumor during treatment, but obtaining biopsies is difficult due to the invasive nature of the procedure. Circulating molecular and cellular biomarkers found in blood are a minimally invasive alternative that can be used repeatedly over the course of treatment with an anti-angiogenic agent. Whereas changes in blood circulation may reflect the systemic effects of anti-VEGF therapy, the impact of these changes on tumor response or escape remains unclear and will need to be established in mechanistic studies in preclinical models [31].

3.1. VEGF Family Members as Circulating Biomarkers

VEGF expression is usually elevated both in the tumors as well as in the circulation of cancer patients and is often an indicator of poor prognosis. All the anti-angiogenic drugs that have received or are pending approval from the US Food and Drug Administration target VEGF signaling—either by blocking the ligand (bevacizumab, aflibercept) or by inhibiting the tyrosine kinase receptors (sorafenib, sunitinib, vandetanib, pazopanib, axitinib, and regorafenib). Thus, the natural choice for a biomarker has been VEGF itself. However, to date the results have been highly inconsistent [30]. High VEGF levels are almost invariably associated with poor outcomes in correlative studies [56], which is indicative of its prognostic biomarker value. In some cancers (e.g., breast cancers or HCC) the levels of circulating VEGF in plasma correlated with outcome of anti-VEGF therapy [30, 56, 79]. However, in other cancers neither the intra-tumoral nor the circulating VEGF associated with outcome of bevacizumab treatment [80, 81]. For example, a recent metaanalysis across four randomized phase III trials of bevacizumab with chemotherapy or immunotherapy in

metastatic colorectal cancer, advanced non-small cell lung cancer, and advanced renal cell carcinoma showed that higher baseline levels of circulating VEGF were associated with shortened progression free survival and overall survival regardless of bevacizumab treatment [82]. This indicates that circulating VEGF levels may be prognostic but not predictive biomarkers for bevacizumab containing regimens. Moreover, the authors did not find a good correlation between blood circulating VEGF concentration and intratumor expression of VEGF [82]. On the other hand, more recent studies have measured shorter isoforms of VEGF (e.g., $VEGF_{121}$), which do not bind to the extracellular matrix components (i.e., heparin), and have found intriguing correlations with outcome [83]. However, other studies failed to detect a significant correlation for short isoforms of VEGF [84]. Thus, the clinical significance of circulating or tissue VEGF levels remains to be clarified, as most of the efforts to use VEGF itself as a predictive biomarker have thus far been disappointing. Current ongoing efforts to measure distinct VEGF isoforms or VEGF fragments may yield additional insight and resurrect interest in research on VEGF as predictive biomarker.

3.1.1. Other VEGF Family Members—In addition to VEGF-A (or VEGF), the VEGF family includes VEGF-B, VEGF-C, VEGF-D, and PlGF. These VEGF family members may play a role in tumor angiogenesis [60]. Currently available antiangiogenic drugs affect these factors in a differential manner (i.e., they are not affected by bevacizumab but may be blocked by aflibercept or receptor tyrosine kinase inhibitors) [60]. Of interest, some of these factors have been shown to be upregulated in response to anti-VEGF therapy both in patients and in preclinical models [30]. The most consistent change has been the increase in circulating levels of plasma PlGF, which has been reported essentially for all anti-VEGF drugs and experimental agents, irrespective of their mechanism of VEGF inhibition [30, 85]. This has led to the hypotheses that (1) PlGF change may have pharmacodynamic biomarker value and (2) that PlGF increase may mediate resistance to anti-VEGF agents that do not block this molecule (e.g., bevacizumab). Both of these hypotheses need to be further validated prospectively. Of interest, the increase in PlGF may be due to systemic effects, as tumor-derived PlGF may actually be decreased after bevacizumab treatment [86]. Similarly, VEGF-C and VEGF-D have been proposed as escape biomarkers for bevacizumab in metastatic colorectal cancer patients in other exploratory studies [87].

3.1.2. Soluble VEGF Receptors—As discussed above, there are 3 VEGF tyrosine kinase receptors in the plasma membrane, known as VEGFR-1 (FLT-1), VEGFR-2 (KDR), and VEGFR-3 (FLT-4). In addition to the plasma membrane receptors, soluble receptors are present in blood circulation—as a result of alternative splicing or possibly due to plasma membrane receptor shedding [30].

Of these soluble receptors, sVEGFR-1 has clear biological activity. This has led our group to conduct extensive studies of circulating sVEGFR-1—an endogenous blocker of VEGF and PlGF and a factor linked with “vascular normalization”—as biomarker or response to anti-VEGF agents [88]. Our hypothesis has been that circulating plasma sVEGFR-1 is a “negative” biomarker that could be used to predict response to anti-VEGF therapies in cancer. Specifically, we propose that cancer patients with preexisting high levels of circulating sVEGFR-1 (i.e., in whom VEGF pathway is endogenously suppressed) are resistant to bevacizumab and other anti-VEGF treatments. Indeed, we have shown in exploratory studies that patients with higher plasma levels of sVEGFR-1 have a poor outcome after treatment with bevacizumab, sunitinib, vandetanib, and cediranib [88–94]. Collectively, these results suggest that anti-VEGF therapy may not have a beneficial effect in patients with high sVEGFR-1 levels. In further support of this, we also found that patients with higher sVEGFR-1 levels in circulation experienced fewer side effects from anti-VEGF treatments [88, 92, 93]. Finally, polymorphisms in the *FLT1* gene that are associated with

higher VEGFR1 expression have also been associated with poor outcome of bevacizumab containing regimens in phase III studies (see below) [95]. If confirmed in larger studies, plasma sVEGFR1 may potentially allow stratification of cancer patients to regimens that include anti-VEGF therapy.

Soluble VEGFR2, which is an abundant protein in human plasma, has also been extensively studied. Multiple studies have shown that anti-VEGFR tyrosine kinase inhibitors but not bevacizumab induce a significant decrease in plasma sVEGFR-2 levels (summarized in [30]). The same result has been reported for circulating sVEGFR-3 (i.e., a decrease in plasma sVEGFR3 after treatment with tyrosine kinase inhibitors that block VEGFR-3). The presence of this signature has been associated with improved outcomes in some studies, but its value as a predictive or pharmacodynamic biomarkers is currently unknown [29–31].

3.2. Other Soluble Plasma Biomarker Candidates

3.2.1. Soluble Basement Membrane Components—Collagen IV is one of the main constituents of vascular basement membranes. In glioblastomas, there is an excessive deposition of basement membranes, which more than doubles the thickness tumor blood vessels compared to normal brain blood vessels [96, 97]. Vascular normalization after anti-VEGF therapy results in normalization of the vascular basement membrane—that is, a reduction in thickness—as seen in mice and in patients [96–98]. Thus, we tested the hypothesis that proteolytic degradation of these membranes could release soluble collagen IV in blood circulation and that this biomarker could be used as ameasure of therapeutic efficacy. Indeed, we found that recurrent glioblastoma patients who had an increased in plasma collagen IV levels after anti-VEGF therapy had an increase in progression-free survival [99]. If validated, either alone or in combination with imaging biomarkers of vascular normalization, the change in soluble collagen IV may potentially allow an early assessment of drug activity and stratification of glioblastoma patients to anti-VEGF therapies [99].

3.2.2. Inflammatory Factors—In addition to VEGF family members, many biomarker studies have focused on inflammatory cytokines and chemokines because they may exert pro-angiogenic effects either directly or indirectly (via modulation of bone marrow derived cell recruitment in circulation and infiltration in tumors) (Box 2).

A comprehensive study was conducted in patients with advanced non-small cell lung cancer who were treated with vandetanib plus chemotherapy, vandetanib alone, or chemotherapy alone. Interestingly, the patterns of changes in soluble biomarkers in each of the three study arms were distinct [100]. Specifically, an increased risk of disease progression was associated with increases in a different marker in each arm, increased plasma VEGF levels for vandetanib monotherapy versus increase in plasma Interleukin (IL)-8 concentration for combination therapy. IL-8 may act as a VEGF-independent pro-angiogenic pathway [51] and has been associated with poor prognosis in hepatocellular carcinoma patients treated with sunitinib [94]. Other notable candidates for biomarkers of tumor evasion from anti-VEGF therapy are the stromal-cell-derived factor 1 alpha (SDF1α, also referred to as CXCL12) and IL-6. We have found associations between increased plasma SDF1α after treatment and poor outcome in studies of anti-VEGF agents in recurrent glioblastoma (cediranib), sarcoma (sorafenib), and breast cancer (bevacizumab) patients [89, 92, 101–103]. Moreover, increased plasma SDF1α and plasma IL-6 have been associated with poor outcomes in locally advanced rectal cancer and advanced hepatocellular carcinoma patients treated with bevacizumab, chemoradiation, and sunitinib, respectively [94, 104]. These potential resistance biomarkers may drive the design of trials anti-VEGF agents.

3.2.3. Other Circulating Factors or Soluble Receptors—Finally, recent studies have reported significant changes or associations with outcome for other circulating factors and/or their soluble receptors. Some of the findings have been more consistent, for example, the transient decrease in plasma Ang-2 after anti-VEGF therapy [89, 90, 92]. Others appeared to be more agent/disease specific, for example, changes and correlations between circulating bFGF, platelet derived growth factor (PDGF)-BB, soluble (s)Tie2, soluble intercellular adhesion molecule 1 (sICAM-1), and matrix metalloproteinase (MMP)-2, MMP-9, and MMP-10 [87, 89, 90, 92, 94, 100, 105]. All of these biomarkers will require additional study and prospective validation.

3.3. Tissue Based Biomarkers

Whenever available—for example, when serial biopsies can be performed or when tissues are obtained at surgery or autopsy—tumor specimens have been invaluable for conducting correlative studies and gaining mechanistic insights into the effects of anti-VEGF therapies. These studies have been quite limited because of the invasive and costly nature of these procedures and the difficulty in standardizing immunohistochemical procedures.

As mentioned previously, intratumoral levels of VEGF have not been so far shown to predict survival outcome of anti-VEGF therapy [81, 82], although correlations with response rates have been reported [106, 107]. Given the disappointing data reported so far, and considering the limitations of tissue VEGF evaluation, this biomarker does not appear promising.

However, these intriguing results raised critical questions. If neither circulating nor tissue VEGF correlate with outcome of anti-VEGF agents, then what is the mechanism of action that leads to a benefit after treatment with these drugs? While multiple groups are actively exploring various mechanisms involving the vasculature, stroma, immune system, or cancer cells themselves, several emerging data are standing out. Tumor microvascular density has been often evaluated both as a predictive biomarker and as a pharmacodynamic marker of anti-angiogenic therapy with anti-VEGF agents. Indeed, two studies found a decrease in vascular density after bevacizumab treatment in rectal and breast cancer [92, 104, 108]. But other studies did not find a significant change [109]. This effect was associated with increased apoptotic rate in cancer cells but interestingly, did not change the proliferation rate of cancer cells [108, 109]. One explanation for this paradoxical finding is that the remaining vasculature after anti-VEGF therapy is more “normal” structurally and functionally [110–113]. The association between microvascular density and survival remains unclear, with most studies reporting a lack of correlation [81].

In a study of serial biopsies from rectal cancers, our group has reported that while bevacizumab did no change VEGF or VEGFR expression in the cancer cells, this anti-VEGF treatment decreased PlGF and increased SDF1α and its receptor (CXCR4) expression in the rectal cancer cells [86]. Of interest, increased plasma SDF1α levels during treatment in these patients correlated with distant disease progression pointing toward SDF1α/CXCR4 axis as a potential escape mechanism from anti-VEGF therapy [86, 102].

While enticing, these hypotheses on the mechanism of action of anti-VEGF agents remain to be further confirmed in patients, as our understanding of the dynamics of VEGFR regulation and the interactions between receptor subtypes in tumor tissue is not well enough advanced to allow the use of these levels as biomarkers of therapeutic efficacy.

Finally, genetic studies of tumor samples have also generated mixed results. While establishing the mutational status in various cancers has made a crucial impact on the development and use of anti-cancer agents, for example, *KRAS* mutation for cetuximab

treatment in metastatic colorectal cancer and *BRAF* mutation for vemurafenib treatment in melanoma, it has failed so far to impact the development or the use of anti-VEGF drugs. For example, *P53*, *KRAS*, or *BRAF* mutations in metastatic colorectal cancer did not associate with bevacizumab-chemotherapy treatment outcome in metastatic colorectal cancer [114]. Many studies have focused on single nucleotide polymorphisms (SNPs) in VEGF family genes as well as other genes [115–118]. Some reports found significant correlations between certain VEGF and VEGFR2 genes with survival or risk of developing hypertension after bevacizumab treatment in metastatic breast and colorectal cancer [117, 119]. However, these findings have not been yet reproduced by other studies. More recently, SNPs in VEGFR1 were shown to associate with survival after treatment with bevacizumab based regimens in 2 phase III studies in advanced pancreatic adenocarcinoma and metastatic renal cell carcinoma [95]. These SNPs were associated with higher VEGFR1 expression [95]. These VEGFR1 SNPs correlated with a poor outcome, which is in line with the finding that high circulating sVEGFR1 is associated with poor outcome after anti-VEGF therapy (see above) [88–94]. Also, a consistent finding appears to be the association between SNPs in *CXCR2* and *IL8* genes and outcome after anti-VEGF therapies [115, 116, 118, 120]. Once again, this suggests an important role that inflammatory cytokines and their receptors may play in the outcome of anti-VEGF therapy. These data strongly suggest that SNP evaluation could be used in the future to predict outcome of anti-VEGF therapy. Moreover, the evaluations of gene polymorphisms have the great advantage of being more feasible as they are minimally invasive and less expensive and do not necessarily require tumor tissue. However, only more extensive investigation and validation of the current lead candidates could potentially provide a biomarker for anti-VEGF therapy.

4. Challenges, Conclusions, and Future Perspective

One major challenge for the interpretation of molecular biomarker studies in general is that a vast amount of data was generated in single arm studies, that is, in which all patients received the same therapy. This makes the distinction between prognostic and predictive biomarkers impossible. Another challenge is that while bevacizumab and aflibercept are specific inhibitors of VEGF pathways, all the anti-angiogenic tyrosine kinase inhibitors are promiscuous, inhibiting multiple, and non-angiogenic tyrosine kinases as well as angiogenic ones [121, 122]. Therefore, it can be difficult to know whether a given biochemical or physiological effect is the result of anti-angiogenic activity or due to effects on other oncogenic targets (e.g., c-KIT inhibition by sunitinib in gastrointestinal stromal tumors or EGFR and RET inhibition by vandetanib in advanced medullary thyroid cancer). Even for bevacizumab/aflibercept studies, the interpretation is confounded by the fact that most studies included concurrent chemotherapeutic drugs, making it difficult to tease out the effects of each type of therapy.

In summary, identifying and validating predictive biomarkers of response and gaining the ability to stratify cancer patients to currently approved anti-angiogenic drugs remain major priorities in oncology. A number of potential biomarkers have emerged from correlative clinical studies that warrant further study in large randomized trials. Some such trials are now underway and their results will be critical for advancement of this field, not only for biomarker discovery but also for further elucidation of the specific mechanisms of action of these important new therapies.

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Box 1: Defining biomarkers

According to current US Food and Drug Administration draft guidance, a biological marker, or biomarker, is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or biological responses to a therapeutic intervention [28]. Such characteristics may include genetic differences, either inherited by the individual in the germline or residing in the tumor, or both; changes in RNA, protein, or metabolite levels as a consequence of the disease or of the therapeutic process; changes in physiologic or systemic parameters, such as blood pressure; or anatomical parameters, such as tumor growth, stasis, or shrinkage [28].

When considering biomarker research, it is important to be aware of the different types of biomarkers that can be identified and the limitations posed by certain types of studies. Biomarkers that can be used before treatment include *prognostic* markers, which predict patient outcome regardless of treatment, and *predictive* markers, which provide information about the effect of a specific therapeutic intervention, usually compared to another. The majority of clinical studies of anti-angiogenic agents to date have identified mainly potential prognostic rather than predictive biomarkers because the studies were either too small to show a statistical difference between treatment arms with respect to the biomarker, or because they were early stage trials that included only one treatment arm [29]. This situation is changing as many larger trials of anti-angiogenic cancer therapies are now incorporating preplanned biomarker analyses. *Pharmacodynamic* biomarkers are used during treatment to monitor its course, and/or to detect resistance or drug toxicity. Ideally, both predictive and pharmacodynamic biomarkers should reflect modulation of an identified biological target of the therapy in question. While this requirement is more straightforward for agents that target oncogenic pathways in cancer cells, it may be difficult to attain for biomarkers of anti-angiogenic agents, since their exact mechanisms of action are not yet well defined [30].

In addition to their functional characteristics, biomarkers should be robust, reliable, reproducible, feasible for use in a clinical setting, and carefully validated as to specificity and sensitivity. The US Food and Drug Administration is currently taking an active role in setting standards for biomarker development and pharmacogenomic biomarkers have been incorporated into drug labels for multiple oncologic therapies, both targeted and untargeted. Such guidance will prove increasingly important as cancer treatment becomes more personalized and as new therapies are developed that are designed to hit ever more specific targets in tumorigenic, angiogenic, and genetic pathways.

Reproduced from Duda, [31].

Box 2: Inflammatory molecules and their potential role in liver cancer angiogenesis. Abbreviations: AP-1, activator protein 1; C/EPB, CAAT/enhancer binding protein; CXCR, C-X-C-chemokine receptor; STAT, signal transducers and activators of transcription. Reproduced from [56]

Chronic inflammation is a potential precursor and promoter of carcinogenesis in many cancers [32–35]. In many cancers, nuclear factor kappa B (NF-κB) is involved in tumor initiation and progression mediated via STAT3 activation [36–38]. Inflammatory cytokines induced by NF-κB pathway activation might affect angiogenesis directly via endothelial cells, or indirectly by cancer cells or recruitment and/or activation of inflammatory cells [39–46]. Interleukin (IL)-1α has a critical role by recruitment of inflammatory cells [47, 48]. Tumor necrosis factor (TNF)-α can also promote tumor progression by different pathways: direct effect on tumor cells, induction of CXCR4, and stimulation of epithelial-mesenchymal transition [49]. TNF-α promotes cell survival and angiogenesis or induce endothelial cell apoptosis, vascular disruption, and increased permeability. IL-6 is also induced by activation of NF-κB and other transcription factors (C/EPBb and AP-1) and modulates inflammation via IL-6R and gp130. Vascular smooth muscle cells, T lymphocytes, and macrophages secrete IL-6 to stimulate immune responses and promote inflammation. IL-6 may also have anti-inflammatory effects by inhibition of TNF-α and IL-1, and activation of IL-1Ra and IL-10. The proliferative and survival effects of IL-6 are mediated by STAT3 [34]. Moreover, IL-8 may have a role in cancer cell invasion [50, 51]. IL-8 can promote tumorigenesis and angiogenesis through CXCR1 and CXCR2, and the Duffy antigen receptor for cytokines, which has no defined intracellular signaling capabilities [52]. Overexpression of VEGF induces the expression of the CXCR4 ligand—stromal cell derived factor 1 alpha (SDF1α) or CXCL12— and SDF1α and CXCR4 may drive cell migration and angiogenesis by VEGF-independent mechanisms [53, 54]. Stem Cell Factor (also known as SCF or KIT-ligand) is a cytokine that binds to the c-KIT receptor (CD117), primarily expressed by early hematopoietic precursors. While c-KIT expression is rarely detectable in the cancer cells, both SCF and c-KIT could be expressed during carcinogenesis, for example, in cholangiocarcinomas [55].

Table 1

Anti-angiogenic drugs approved by the United States Food and Drug Administration (2004–2012)

Anti-VEGF drug	Approved indication
Bevacizumab	Metastatic colorectal cancer (with chemotherapy)
	Metastatic nonsquamous non-small cell lung cancer (with chemotherapy)
	Metastatic breast cancer (with chemotherapy)
	Recurrent glioblastoma (monotherapy)
	Metastatic renal cell carcinoma (with IFNα)
	Metastatic renal cell carcinoma (monotherapy)
Sunitinib	Gastrointestinal stromal tumors (monotherapy)
	Pancreatic neuroendocrine tumors (monotherapy)
Sorafenib	Metastatic renal cell carcinoma
	Unresectable hepatocellular carcinoma
Pazopanib	Metastatic renal cell carcinoma
	Advanced soft tissue sarcoma
Vandetanib	Advanced medullary thyroid cancer
Axitinib	Advanced renal cell carcinoma
Regoranfenib	Metastatic colorectal cancer
Aflibercept	Metastatic colorectal cancer (with chemotherapy)
	Wet age-related macula degeneration
Pegaptanib	Wet age-related macula degeneration
Ranibizumab	Wet age-related macula degeneration