Abstract. The process of neo-vascularisation from pre-existing blood vessels (angiogenesis) plays a critical role in both tumour growth and dissemination in multiple cancer types. Tumour angiogenesis is an attractive target for cancer treatment, and the VEGF/VEGF-R and FGF/FGF-R systems have been identified as key factors for neo-angiogenesis. Several active compounds have been developed so far and some of them are already widely used in clinical protocols. However, currently, only very few drugs have been shown to act synergistically with VEGF. Brivanib (BMS-582664) is a novel, orally available and selective receptor tyrosine kinase inhibitor that targets the key angiogenesis receptors VEGF-R2 and FGF-R1 and -2. The drug is currently under clinical evaluation and published data as well as data on biomarker studies with brivanib are reviewed and discussed.

Angiogenesis is a fundamental mechanism in biology that describes the multistep process of new blood vessel formation from existing vasculature (1). The role of angiogenesis in normal biology and pathology is now firmly established. Angiogenesis occurs during normal tissue turnover and organogenesis, including vertebrate embryonic development, menstruation and wound repair (2, 3). Conversely, aberrant angiogenesis may contribute to the pathogenesis of a variety of both non-neoplastic (e.g. diabetic retinopathy) and neoplastic disorders. In cancer, early angiogenesis facilitates tumour cell growth through the delivery of nutrients and the removal of metabolic waste products from the tumour environment. Initially, in the course of tumour growth and expansion, tumour cells surround the microvasculature, and the resulting capillary ‘cuff’ facilitates their growth. Subsequently, an ‘angiogenic switch’, characterized by the expression of multiple pro-angiogenic factors (4), is thought to push cells out of a state of relative dormancy and into one characterised by the invasive phenotype, a hallmark of cancer pathogenesis. Among the many contributors to this process is the vascular endothelial growth factor (VEGF) family of ligands and receptors (Tables I and II).

Since tumour angiogenesis is an attractive target for cancer treatment, several active compounds have been developed so far and some of them are already widely used in clinical protocols. Amongst them brivanib (a novel VEGF-R2 and FGF-R1 and -2 inhibitor) is currently under clinical evaluation and therefore it was the objective of this paper to review and discuss the potential of this new compound in treatment strategies for cancer.

VEGF System

VEGF is the prototype of a large family of angiogenic and lymphangiogenic growth factors, which includes six structurally homologous, secreted glycoproteins called VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (5). VEGF-A (commonly referred to as VEGF) was the first such molecule to be identified by virtue of its ability to induce vascular permeability (6). The VEGF ligands trigger biological effects on their interaction with specific cell-surface receptors. The diversity of these receptors also adds to the biological complexity of angiogenesis and lymphangiogenesis. Two receptors were originally identified on vascular endothelial cells: VEGF-R-1, which is a 180-kDa transmembrane protein, also called fibromyalgia-syndrome-like tyrosine kinase-1 (Flt-1), and VEGF-R2, which is a 200-kDa transmembrane protein, also called kinase domain receptor (KDR). A third structurally related tyrosine kinase receptor is the 180-kDa VEGF-R3 (also called Flt-4), which is expressed broadly on endothelial cells during early embryogenesis (7) (Figure 1). VEGF-R2 is expressed in most, if not all, adult vascular endothelial cells as well as on circulating endothelial progenitor cells. Interestingly, both epithelial and mesenchymal tumour cells express VEGF-R1 more often than VEGF-R2 (8). However, in several experimental tumour models, tumour cell-specific VEGF-R2 expression has been shown to be the critical driver...
in the pathogenesis of tumours (5, 9). VEGF binding induces conformational changes within VEGF-R2 followed by receptor dimerisation and autophosphorylation of tyrosine residues in the intracellular kinase domain. These tyrosine residues (Tyr951, Tyr996, Tyr1054, and Tyr1059) serve as high-affinity docking for a variety of signalling proteins, including phospholipase Cγ, ras-GAP, focal adhesion kinase, src family of tyrosine kinases, PI3K, Akt, PK-C, Raf-1 and MAPs. The interaction of one or more of these molecules with VEGF-R2 may lead to alterations in cell proliferation, migration, differentiation, tube formation, increase in vascular permeability and vascular integrity (9) (Figure 2).

Intrinsic and acquired resistance to anti-angiogenetic drugs are clinically significant problems. Preclinical studies have begun to elucidate the mechanisms of such resistance and, to date, four mechanisms of resistance have been identified: (i) up-regulation of the basic fibroblast growth factor (bFGF), (ii) overexpression of matrix metalloproteinase 9 (MMP-9), (iii) increased levels of the stromal cell-derived factor SDF-1α and (iv) hypoxia-inducible factor (HIF)-1α-induced recruitment of bone marrow-derived CD45⁺ myeloid cells (reviewed in (10)).

**FGF System**

The FGF family comprises 23 distinct, structurally-related proteins that exert biologic effects on different cells and organ systems, including tumour growth and angiogenesis (11). FGFs are heparin-binding proteins, which interact with low-affinity heparan sulfate proteoglycans (HSPGs). HSPGs are ubiquitous cell surface and extracellular matrix (ECM) proteins, which have been shown to protect FGFs from thermal denaturation and proteolysis as well as to increase FGF-receptor affinity and facilitate FGF binding to cell surface receptor. In addition, ECM-associated HSPGs modulate FGF bioavailability by generating a local reservoir for the growth factor and allowing a sustained stimulation of endothelial cells (12). Mobilisation of FGFs from the ECM storage, and in particular of FGF-1 and FGF-2, occurs via HSPG digestion by heparanases or glycosaminoglycan-degrading enzymes. FGFs act through high-affinity binding sites that mediate biological activity via a group of tyrosine kinase membrane receptors that form the FGF-R family. Within the FGF-R family, four members have been identified: FGF-R1, FGF-R2, FGF-R3 and FGF-R4. Structural features shared by the FGF-R family include three glycosylated immunoglobulin-like loops of the extracellular domain and an internal conserved tyrosine kinase domain split by a short insert (13). It has been shown that the four members of the FGF-R family bind both FGF-1 and FGF-4. FGF-2 is able to bind FGF-R1, FGF-R2, and FGF-R3, whereas FGF5, FGF6 and FGF7 act through FGF-R3, FGF-R4 and FGF-R2, respectively (12).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF cytokine family</td>
<td>Angiogenesis, neo-vascularisation, vessel permeability, leukocyte adhesion</td>
</tr>
<tr>
<td>VEGF-R, Neuropilin-1,2</td>
<td>Signal transduction (pro-angiogenetic signals)</td>
</tr>
<tr>
<td>Angiopoietin-1, Tie-2</td>
<td>Vessel stabilisation</td>
</tr>
<tr>
<td>FGF and FGF-R</td>
<td>Endothelial proliferation, synergism with VEGF</td>
</tr>
<tr>
<td>Angiopoietin-2</td>
<td>Augmentation of VEGF, angiopoietin-1 antagonist</td>
</tr>
<tr>
<td>PDGF and PDGF-R</td>
<td>Smooth muscle cell recruitment</td>
</tr>
<tr>
<td>TGF-β, Endoglin</td>
<td>Stimulation of extracellular matrix</td>
</tr>
<tr>
<td>Integrins</td>
<td>Adhesion of endothelial cells to the extracellular matrix</td>
</tr>
<tr>
<td>VE-Cadherin (CD144)</td>
<td>Endothelial cell-cell adhesion</td>
</tr>
<tr>
<td>Ephrines</td>
<td>Regulation of neo-vascularisation</td>
</tr>
<tr>
<td>Matrix metalloproteinases (MMPs), plasminogen activator (PA)</td>
<td>Destabilising and remodelling of extracellular matrix</td>
</tr>
</tbody>
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**Table I. Pro-angiogenetic molecules.**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
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<tbody>
<tr>
<td>Soluble VEGF-R1, soluble neuropilin-1</td>
<td>Regulation of VEGF available</td>
</tr>
<tr>
<td>Thrombospondin-1 and -2</td>
<td>Inhibition of endothelial migration, adhesion and survival</td>
</tr>
<tr>
<td>Endostatin (collagen XIII fragment)</td>
<td>Inhibition of endothelial migration and survival</td>
</tr>
<tr>
<td>Tumstatin (collagen IV fragment)</td>
<td>Inhibition of endothelial protein synthesis</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>Inhibition of bFGF and VEGF binding</td>
</tr>
<tr>
<td>TIMPs</td>
<td>Suppression of tumour angiogenesis</td>
</tr>
<tr>
<td>Interferons (α, β, γ), interleukins (IL-4, -12, -18)</td>
<td>Inhibition of endothelial migration; down-regulation of bFGF</td>
</tr>
<tr>
<td>Mapsins</td>
<td>Protease inhibitor</td>
</tr>
</tbody>
</table>

**Table II. Anti-angiogenetic molecules.**
Transcriptional regulation of VEGF is critically dependent on HIF-1. However, not only hypoxia, but also selected growth factors may induce HIF-1 (14). Results from several studies have provided compelling evidence that hypoxia-triggered up-regulation of other pro-angiogenic factors (e.g. FGF family and PDGF-BB), in the presence of anti-VEGF agents, may re-stimulate tumour angiogenesis in a VEGF-independent fashion, thereby contributing to resistance to VEGF-blocking agents (15-17) (Figure 3).

In terms of the underlying molecular mechanisms, it has been demonstrated that hypoxia induces the expression of HIF-1α (a key protein for tumour angiogenesis) and the
release of bFGF further augments these hypoxic inductions. The PI3K pathway has been shown to be required for these processes as demonstrated by application of the PI3K inhibitor LY294002 (17). In addition, under hypoxic conditions, bFGF activates the MEK1/ERK pathways, and PD98059 (a MEK1/2 inhibitor) suppresses the bFGF-induced HIF-1 transactivity, suggesting that the ras signalling cascade may also be involved in the resistance to anti-VEGF agents.

Furthermore, it has been shown in experimental systems that adding a bFGF inhibitor (brivanib, BMS-582664) to tumours expressing resistance to bevacizumab, SU6668 and ZD6474 can significantly restart re-initiation of angiogenesis and tumour progression (15, Dr. Mark Ayers, PRI Princeton, personal communication). Currently, only few drugs are available to target the FGF receptor (CHIR258, PD173074, BIBF-1120, BMS-582664) (Table IV). Amongst them, brivanib (BMS-582664) is a novel, orally available and elective tyrosine kinase inhibitor that targets the key angiogenesis receptors VEGF-R2 and FGF-R2 (18). Since resistance to VEGF blockade involves vascular re-growth in a VEGF-independent, second wave of angiogenesis (mediated in part by pro-angiogenic ligands of the FGF family), counteracting such mechanisms by multi-targeting alternative pro-angiogenic signalling circuits may improve efficacy of anti-angiogenic therapies.

Brivanib: A Dual Tyrosine Kinase Inhibitor

Brivanib (BMS-58266; Bristol-Myers Squibb, New York, USA, Figure 4) is a small molecule that has shown potent inhibition of VEGFR-2 as well as inhibition of FGFR-1 and -2, another receptor protein tyrosine kinase underlying the angiogenic pathway (Table V). The compound is a prodrug of BMS-540215 and undergoes rapid intestinal resorption after oral administration. It is mainly metabolised to the active metabolite BMS-540215 by the cytochrome P450 system (CYP3A4). Brivanib is no longer detectable in the plasma after approximately one hour and the peak drug concentration is found after 1-2 hours. Following a single oral dose of 800 mg brivanib, the peak plasma concentration was found to be 15 μM (20). Moreover, in animal systems, BMS-540215 has demonstrated a high plasma protein binding ratio (52-97%). In several tumour xenograft models, brivanib induced significant tumour growth inhibition when administered orally once daily. However, no major tumour regression was observed and, with the termination of dosing, tumour xenografts resumed growth (18, 19). Consequently, the anticipated effects of this therapeutic approach predict tumour stasis rather than regression and, therefore, anti-angiogenic agents are expected to be used either in conjunction with standard therapy or in an adjuvant setting.

Phase I data. In the meantime, clinical activity of brivanib has been evaluated in a series of phase I studies (20, 21). The drug demonstrated moderate and manageable side effects (Table VI) with a maximal tolerated dose of 800 mg/day for further testing in phase II trials. However, it is important to note that, in some patients, thromboembolic events and bleeding episodes occurred. This effect, however, has also been reported for other VEGF(-R) inhibitors (e.g. bevacizumab) (22) and is regarded as a class-related toxicity of anti-angiogenetic drugs. It is probably due to the VEGF-R inhibitor-induced obliteration of tumour microvessels. During its phase I programme, antitumour activity of brivanib has also been evaluated in combination with standard chemotherapy or monoclonal antibodies. In a recently published study, 18 patients (among them 15 patients with colorectal cancer) were treated with brivanib (320-800 mg/day) in combination with cetuximab (loading dose: 400 mg/m² followed by 250 mg/m² weekly); medium treatment duration was eight weeks (range: 1-20 weeks). This combination did not enhance cetuximab toxicity and the progression-free survival was longer in patients expressing FGF than in those with no FGF expression (21). Due to these encouraging results a phase III study (brivanib/cetuximab versus cetuximab) in metastatic colorectal carcinoma patients has been designed and is open for recruitment (21). Another combination study (irinotecan/ cetuximab plus placebo versus brivanib plus irinotecan/ cetuximab) is ongoing (23).
Phase II data. Several phase II studies with brivanib are currently open for enrolment (hepatocellular carcinoma, colorectal carcinoma and other solid tumours). For colorectal carcinoma patients, a phase I/II study with folinic acid, 5-FU and oxaliplatin (FOLFOX) has been activated (N=40 patients, FOLFOX plus brivanib versus FOLFOX plus placebo); however, this study was closed after recruitment of 17 patients. In addition, brivanib is being evaluated in one phase II study in patients with hepatocellular carcinoma (HCC). In the first cohort (open since 2006, N=100 patients) clinical efficacy of brivanib is investigated in patients with locally advanced or metastatic HCC (inclusion criterion: no prior systemic chemotherapy). The primary endpoint is progression-free survival after six months (23). In parallel, a second cohort has been activated for HCC patients who have received no more than one prior anti-VEGF therapy. Both studies are ongoing. Preliminary data from these studies have been published at the 2008 meeting of the International Liver Cancer Association in Chicago (24). The results presented clearly demonstrated that brivanib has significant antitumour activity in patients with HCC and is generally well tolerated. This study is continuing for patients who have failed one anti-angiogenetic therapy.

The combination of brivanib with cytostatic drugs, as tested in the aforementioned studies, is critical for the future clinical development of brivanib, since, in xenograft models,
only tumour inhibition but not tumour regression were observed during brivanib therapy. Further evidence of this approach came from an earlier study. Using the matrigel mouse model, Dupont et al. (25) showed that inhibition of the FGF/FGF-R system was able to enhance cisplatin-induced cytotoxicity, suggesting that resistance to cisplatin is mediated, at least in part, by FGF-R.

**Biomarker for brivanib treatment.** The identification of a suitable and easily measured marker of antitumour activity in the clinical setting would clearly facilitate the rapid clinical development of brivanib and probably other anti-VEGF-R agents. Currently, there exists a need to identify biomarkers that will be able to indicate biological activity and predict efficacy at the molecular level for angiogenesis drugs, which are anticipated to result in tumour stasis rather than regression. In an attempt to identify and validate suitable biomarkers that may be used as surrogate end points for the anti-angiogenetic activity of brivanib in clinical trials, research efforts have focused on collagen IV (4A1). Collagen IV (as part of the basal membrane in tumours) has been suggested to play an important role in angiogenesis and tumour progression. Furthermore, collagen IV is co-expressed with VEGF-R2 and may be depleted by VEGF-R2 inhibitors (26). Using a murine HCT116 xenograft model, Ayers et al. (27) have provided evidence that the mRNA expression of collagen IV correlates with the tumour response, following brivanib exposure. A similar decrease of collagen IV levels has also been detected immunohistochemically. These results add weight to the proposal that collagen IV may play a significant role as a surrogate marker for this treatment. Collagen IV is present in the vessels and in the blood, and decreases on commencement of treatment. Therefore, it may be used as a surrogate marker for brivanib treatment and should be investigated in great detail in the ongoing brivanib trials.

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**References**


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