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## Subject Review

# The Role of VEGF and EGFR Inhibition: Implications for Combining Anti-VEGF and Anti-EGFR Agents

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### Abstract

**Multiple cellular pathways influence the growth and metastatic potential of tumors. This creates heterogeneity, redundancy, and the potential for tumors to bypass signaling pathway blockade, resulting in primary or acquired resistance. Combining therapies that inhibit different signaling pathways has the potential to be more effective than inhibition of a single pathway and to overcome tumor resistance. Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors have become key therapies in several tumor types. Close relationships between these factors exist: VEGF signaling is up-regulated by EGFR expression and, conversely, VEGF up-regulation independent of EGFR signaling seems to contribute to resistance to EGFR inhibition. Therefore, inhibition of both pathways could improve antitumor efficacy and overcome resistance to EGFR inhibition. Preclinical studies have shown that VEGF and EGFR inhibitors can have additive effects and that combined inhibition is effective in EGFR inhibitor-resistant cell lines. Clinical trials have also produced promising data: combining the anti-VEGF monoclonal antibody bevacizumab with the anti-EGFR antibody cetuximab or the EGFR tyrosine kinase inhibitor erlotinib increases benefit compared with either of these anti-EGFR agents alone or combined with chemotherapy. The potential of this novel approach to anticancer therapy will be elucidated by large, ongoing clinical trials. (Mol Cancer Res 2007;5(3):203–20)**

### Introduction

The development of targeted therapies designed to inhibit or block key cellular pathways involved in tumor growth and dissemination to metastatic sites has brought with it an increased appreciation of the heterogeneity of tumors and the ability of some tumors to bypass signaling pathway blockade. Thus, some tumors may be primarily resistant, or could become resistant, to therapies targeting a specific pathway. A multi-

faceted approach involving targeted inhibition of multiple signaling pathways may be more effective than inhibition of a single target and may help overcome tumor resistance by blocking potential “escape routes.”

Two key elements in the growth and dissemination of tumors are the vascular endothelial growth factor (VEGF) and the epidermal growth factor (EGF) receptor (EGFR). The VEGF and EGFR pathways are closely related, sharing common downstream signaling pathways (1). Furthermore, EGF, a key EGFR ligand, is one of the many growth factors that drive VEGF expression (2). VEGF and EGFR play important roles in tumor growth and progression through the exertion of both indirect and direct effects on tumor cells (1). Biological agents targeting the VEGF and EGFR pathways have shown clinical benefit in several human cancers, either alone or in combination with standard cytotoxic therapies. Inhibition of VEGF-related pathways is thought to contribute to the mechanism of action of agents targeting the EGFR (3). Conversely, (over)activation of VEGF expression independent of EGFR signaling is thought to be one way that tumors become resistant to anti-EGFR therapy (4). Specific ongoing point mutations in the *EGFR* gene are also thought to convey resistance to anti-EGFR tyrosine kinase inhibitors (5). The possibility that combined VEGF and EGFR pathway blockade could further enhance antitumor efficacy and help prevent resistance to therapy is currently being evaluated in clinical trials (1).

### VEGF and EGFR Signaling Pathways: Independent but Interrelated

#### VEGF

Angiogenesis, the formation of new blood vessels from existing vasculature, is essential for both physiologic and pathologic processes. The formation of new vessels begins with hemangioblasts (precursors of vascular endothelial cells) and ends with mature vasculature (6). It is a complex process that is regulated tightly by proangiogenic and antiangiogenic factors and involves autocrine and paracrine signaling (3). VEGF (VEGF-A) is the predominant stimulator of angiogenesis (7, 8), and mediation of VEGF expression is one of the main mechanisms by which tissue vasculature is controlled under normal physiologic conditions (9). Under pathologic conditions, VEGF is secreted by tumor cells in the majority of cancers and acts on endothelial cells of existing blood vessels to promote new blood vessel formation. VEGF release by tumor cells initiates the angiogenic process by activating endothelial cells and promoting their migration and, thus, induces the angiogenic

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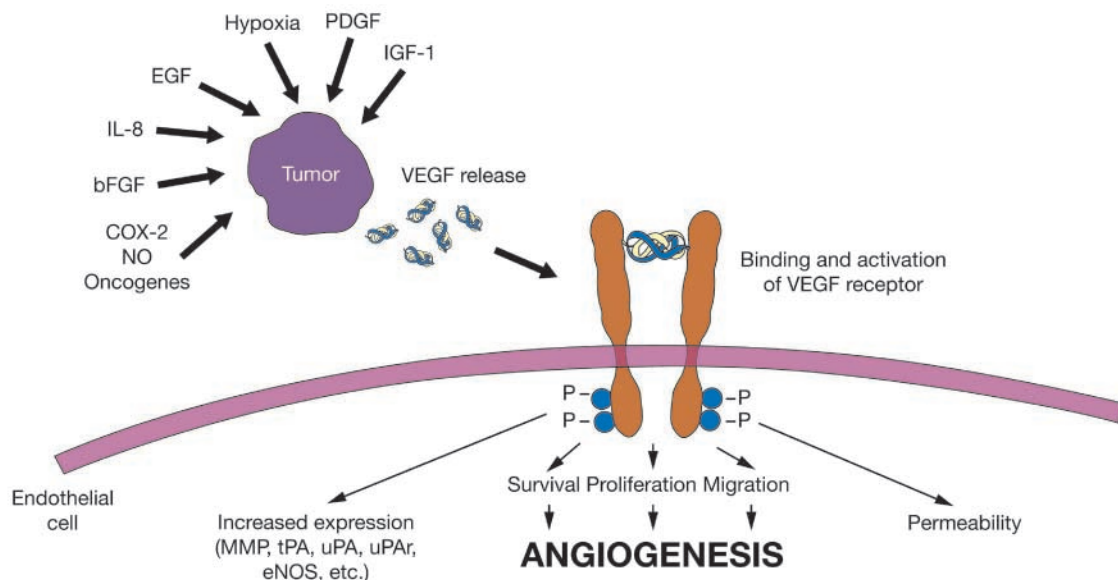
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switch (3). Pathologic angiogenesis is critical to the growth (by providing oxygen and other nutrients) and malignant dissemination (providing a route for metastases) of solid tumors (10-13). Angiogenesis also contributes to the development of hematologic malignancies, particularly multiple myeloma, leukemia, and lymphoma, although the role of angiogenesis has not been as clearly defined in hematologic malignancies and might vary in lymphoma subtypes (14-16). VEGF binds primarily to receptors on endothelial cells but may also act on tumor (3), hematopoietic (17), and neural cells (18). Free VEGF binds two related receptors (9), VEGF receptor 1 (Flt-1) and VEGF receptor 2 (Flk-1 or KDR). Both VEGF receptors 1 and 2 have an extracellular domain composed of seven immunoglobulin-like regions that bind VEGF, a single transmembrane region, and an intracellular tyrosine kinase domain.

VEGF receptor 1 was the first identified VEGF receptor, although its precise function is still under debate (19). It was initially suggested that VEGF receptor 1 may act as a “decoy receptor,” reducing the number of unbound, circulating VEGF molecules available to bind to VEGF receptor 2. However, recent studies suggest that VEGF binding to receptor 1 is also able to induce a mitogenic signal and may be involved in the recruitment of endothelial progenitor cells (9). Although VEGF binds to VEGF receptor 2 with a lower affinity than to VEGF receptor 1 (20), the key role of VEGF receptor 2 in stimulating angiogenesis and hematopoiesis has been clearly defined. Selective activation of VEGF receptors 1 and 2 has shown that VEGF receptor 2 is the primary receptor transmitting VEGF signals in endothelial cells and is capable of inducing endothelial cell proliferation (21). Evidence suggests that VEGF receptor 2 is the major mediator of the mitogenic, angiogenic, and vascular permeability-enhancing effects of VEGF (9). Binding of VEGF to VEGF receptor 2 results in receptor dimerization and ligand-dependent receptor tyrosine kinase phosphorylation, thereby activating intracellular sig-

naling pathways involved in endothelial cell proliferation, migration, survival, sprouting, and tube formation, as well as up-regulation of molecules involved in degradation of the extracellular matrix (9, 22). Endothelial cell activation results in the secretion of matrix metalloproteinases and urokinase plasminogen activator/activator receptor, which degrade the extracellular matrix (3). Degradation of the extracellular matrix allows proliferating cells to migrate towards the growth factor source (tumor cells). This process is interdependent on complex interactions of cellular adhesion molecules such as integrins and cadherins (3).

VEGF expression is driven by many factors that are characteristic of tumors, including oncogene expression [e.g., ras, src, erbB2/human epidermal growth factor receptor 2 (HER2), EGFR] and hypoxia (Fig. 1). VEGF also mediates the effects of other angiogenic molecules and therefore plays a central role in the control of tumor angiogenesis. Oxygen tension is critical to the biology of pathologic angiogenesis in tumor growth (23). VEGF mRNA expression is rapidly and reversibly induced in cells exposed to low oxygen tension (hypoxia), which occurs in a range of pathophysiologic conditions, including within poorly vascularized tumors (20). Hypoxia is possibly the most important regulator of VEGF mRNA expression (9, 19). The hypoxic response pathway is mediated by hypoxia-inducible factors (HIF), which promote the transcription of a large number of angiogenic genes including VEGF (6). HIF-1 is a heterodimer that binds to hypoxia-response elements, activating VEGF. Under normal oxygen tension, HIF expression is down-regulated via proteosomal degradation (9, 24). Under hypoxic conditions, constitutive degradation of HIF-1 via the proteosome pathway is blocked (20). HIF-1 is phosphorylated and stabilized through oncogenic signaling, involving src, ras, protein kinase C, and phosphatidylinositol-3-OH kinase (PI3K). In the nucleus, HIF-1 complexes with the VEGF promoter region of the *VEGF* gene and thus activates transcription of the *VEGF* gene (8, 25).



**FIGURE 1.** Tumor characteristics and environment promote VEGF expression.

Many external factors are involved in stimulating tumor angiogenesis and may also stimulate angiogenesis indirectly by inducing VEGF mRNA expression (11, 26). Some of the principal proangiogenic regulators include growth factors (EGF, transforming growth factor [TGF]- $\alpha$  and  $\beta$  [TGF- $\beta$ ], tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ], keratinocyte growth factor, insulin-like growth factor I (IGF-I), fibroblast growth factor (FGF), platelet-derived growth factor [PDGF], and cytokines [interleukin (IL)-1 $\alpha$  and IL-6]) and transforming events (oncogenic mutations or amplifications of ras or raf; ref. 9). Factors that are involved in down-regulating angiogenesis include angiostatin, endostatin, thrombospondin-1, angiopoietin-2, IFN- $\alpha$ , and IL-12. Although the exact role of each of these factors is not clear, it is well documented that VEGF is the key mediator of vasculogenesis, angiogenic remodeling, and angiogenic sprouting (11). Angiopoietin and ephrin-B2 are also important in the remodeling and maturation of new vessels (11): ephrin-B2 is specifically expressed on arterial vessels and may play a role in the differentiation of blood vessels into arteries (27); angiopoietin is involved in the maturation of new vessels and maintaining vessel stability (28).

#### EGFR

EGFR is a member of the HER/*erbB* family of receptor tyrosine kinases (refs. 29, 30), which include HER1 (EGFR/*erbB1*), HER2 (*neu*, *erbB2*), HER3 (*erbB3*), and HER4 (*erbB4*). The structure of this membrane-spanning glycoprotein includes an important extracellular ligand binding domain, a hydrophobic transmembrane region, and a cytoplasmic domain, which contains the tyrosine kinase domain (30). Overexpression of EGFR and dysregulation or increased activity of EGFR signaling pathways are suggested mechanisms whereby the presence of EGFR may confer or promote a malignant phenotype (31). It is postulated that increased EGFR-mediated signaling may contribute to a cell moving into a state of continuous, unregulated cell proliferation, thereby expanding the population of malignant cells and rapidly increasing tumor mass. In support of this, EGFR expression (or overexpression) has been observed in numerous solid tumor types (31).

This pathway is triggered by EGFR dimerization promoted through the binding of a specific set of ligands (EGF, TGF- $\alpha$ , amphiregulin, betacellulin, heparin-binding EGF, or epiregulin) to the extracellular domain of the receptor (3, 29, 30). This causes the receptors to pair together to form a dimer. Dimerization of the EGFR receptor, either with itself or another member of the HER family, triggers the activation of intracellular signaling pathways. This pairing of receptors activates the cytoplasmic tyrosine kinase enzyme, causing autophosphorylation of intracellular tyrosine residues. Phosphorylated tyrosines serve as docking sites for a number of signal transducers and adaptor molecules that initiate further signaling pathways. Proteins are recruited from the cytoplasm by the activated EGFR to form a linked complex; interaction between these proteins in turn activates the ras protein. Activation of this protein triggers a phosphorylation cascade, which activates mitogen-activated protein kinase. Mitogen-activated protein kinase is involved in the transduction of the signal from the cytoplasm to the nucleus, ultimately triggering the accumulation of cyclin D. In mammals, the progression

of the cell cycle into active cell division is catalyzed by cyclin D binding to cyclin-dependent kinase receptor 4 or cyclin-dependent kinase receptor 6. The effect of signaling through EGFR is dependent on the receptor partner for EGFR, the identity of the activating ligand, and the cellular context (30).

Activation of EGFR pathways has been linked to many processes crucial to tumor progression, including metastasis and cell survival, proliferation, adhesion, differentiation, migration, transformation, and motility (29). Many different solid tumors express EGFR, including breast, head and neck, colon, ovarian, non-small-cell lung cancer (NSCLC), pancreatic, bladder, and glioblastoma (3).

#### Relationship between VEGF and EGFR Signaling Pathways

In solid tumors, the VEGF and EGFR pathways seem to be linked, particularly with respect to angiogenesis. EGF and TGF- $\alpha$  both induce VEGF expression via activation of EGFR in cell culture models and have proangiogenic properties (3, 32). Evidence has shown that tumor-associated endothelial cells express EGFR (33) and that aberrant EGFR expression correlates with poor prognosis (3). It is likely that the EGFR pathway modulates angiogenesis by up-regulating VEGF or other key mediators in the angiogenic process (32). In preclinical models, EGFR blockade with the monoclonal antibody cetuximab resulted in down-regulation of proangiogenic mediators, including VEGF, IL-8, and basic FGF, accompanied by reductions in microvessel density and metastases (3). Data from *in vitro* and *in vivo* studies reviewed by Ellis suggest that at least part of the antitumor effect of cetuximab is mediated by inhibition of angiogenesis by way of interruption of upstream angiogenesis signaling pathways (3). Similar results have also been reported for small-molecule tyrosine kinase inhibitors of the EGFR (e.g., gefitinib; ref. 3). However, EGFR inhibition does not block VEGF, thereby allowing tumor angiogenesis, and therefore tumor growth, to continue.

#### Overview of the Activity of VEGF and EGFR Inhibitors

Of the numerous inhibitors of VEGF and EGFR in development, several have now been approved for clinical use. Some of these are antibodies, which inhibit VEGF receptors or VEGF itself (bevacizumab). Other small molecules inhibiting VEGF receptors or EGFR work by inhibiting the activity of specific receptor tyrosine kinases [vatalanib (PTK787/ZK) or erlotinib (OSI-774) and gefitinib (ZD1839), respectively] that are integral to the VEGF and EGFR signaling cascades. EGFR inhibition through the binding of an antibody directed to the extracellular domain of the EGFR has also been evaluated in clinical trials [cetuximab (IMC-225), matuzumab (EMD72000), panitumumab (ABX-EGF), and nimotuzumab (h-R3)].

#### VEGF Inhibition

Preclinical models have provided consistent information about inhibition of the VEGF pathway. The most exhaustive data are related to the activity of anti-VEGF monoclonal

antibodies of murine or human origin (34-38). Treatment with a murine anti-VEGF monoclonal antibody has been shown to inhibit the growth of several human xenografts, with no effect on the growth rate of the same tumor cells *in vitro* (34). Studies have also shown that maximal inhibition of tumor growth can be achieved by completely blocking circulating VEGF (35). Similarly, preclinical studies have reported decreased angiogenesis and decreased primary and metastatic growth when the angiogenic signaling pathway is inhibited with anti-VEGFR monoclonal antibodies and tyrosine kinase inhibitors of the VEGFR (39-45). Furthermore, there is an emerging body of preclinical data that show improved antitumor activity when VEGF pathway inhibitors are combined with conventional cytotoxic agents (38, 44-51).

**Bevacizumab.** Bevacizumab is a recombinant humanized immunoglobulin G1 monoclonal antibody that binds to VEGF and inhibits its activity, thereby inhibiting angiogenesis and potentially halting tumor growth (36, 52). Bevacizumab is widely approved for use in combination with 5-fluorouracil (5-FU)– and irinotecan-based chemotherapy regimens for the first-line treatment of metastatic colorectal cancer based on the results of several randomized, controlled clinical trials showing survival benefits over chemotherapy alone in this setting (53-55). When administered in combination with irinotecan, 5-FU, and leucovorin (IFL) for metastatic colorectal cancer, bevacizumab significantly improved patient outcomes (55). Median duration of survival in patients receiving bevacizumab plus IFL was 20.3 months, compared with 15.6 months in patients receiving placebo plus IFL ( $P < 0.001$ ; ref. 55). Similarly, median duration of progression-free survival was 10.6 and 6.2 months, respectively ( $P < 0.001$ ), with corresponding response rates of 44.8% and 34.8% ( $P = 0.004$ ; ref. 55). Combined analysis also shows that the addition of bevacizumab to 5-FU/leucovorin improves survival by 3.3 months (14.6 versus 17.9 months;  $P = 0.008$ ), progression-free survival by 3.2 months (5.6 versus 8.8 months;  $P < 0.001$ ), and response rate (25% versus 34%). Bevacizumab has also shown survival benefits when added to oxaliplatin-containing chemotherapy as second-line treatment for advanced colorectal cancer (56). In this instance, the addition of bevacizumab significantly increased median progression-free survival by 2.4 months and median overall survival by 2.1 months in patients with advanced colorectal cancer previously treated with irinotecan and a fluoropyrimidine when compared with those receiving chemotherapy alone (56).

Evaluation of bevacizumab in combination with chemotherapy in metastatic breast cancer has also shown improvements in response rates and survival. A phase I/II dose escalation trial of bevacizumab in previously treated metastatic breast cancer reported median survival of 10.2 months, ranging from 7.6 to 14.0 months across the three treatment arms with an overall response rate of 9.3% (confirmed response rate, 6.7%; ref. 57). A randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer found that the combination therapy significantly increased response rates (19.8% versus 9.1%;  $P = 0.001$ ; ref. 58). However, progression-free survival, overall survival, and time to deterioration in quality of life were comparable between the treatment groups, suggesting

that the optimal time to intervene with an antiangiogenic agent may be earlier in the course of the disease. As breast cancer progresses, angiogenic pathways become numerous and redundant (59); therefore, it is unlikely that inhibition of a single factor would produce a sustained clinical effect in patients with previously treated, highly refractory disease (59). Recent findings from a phase III trial of paclitaxel versus paclitaxel plus bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer have provided evidence that early intervention with an antiangiogenic agent confers better patient outcomes (60). In this study, combination therapy significantly increased response rates in all patients compared with paclitaxel monotherapy (28.2% and 14.2%, respectively;  $P < 0.0001$ ; ref. 60). Paclitaxel plus bevacizumab combination therapy also increased progression-free survival by 4.86 months ( $P < 0.001$ , versus paclitaxel alone; ref. 60). Evaluation of overall survival data for the paclitaxel plus bevacizumab combination is ongoing.

In a randomized phase II trial, first-line treatment of NSCLC with carboplatin and paclitaxel plus bevacizumab resulted in a higher response rate (31.5% versus 18.8%), longer median time to progression (7.4 versus 4.2 months), and an increase in survival (17.7 versus 14.9 months) compared with chemotherapy alone (61). The results of a phase III study in patients with advanced NSCLC with adenocarcinoma histology (E4599) showed that the addition of bevacizumab to carboplatin and paclitaxel significantly prolonged survival (62, 63). A second planned interim analysis for this study reported a higher response rate (10% versus 27%;  $P < 0.0001$ ) and improved median progression-free survival (4.5 versus 6.4 months;  $P < 0.0001$ ) and median overall survival (10.2 versus 12.5 months;  $P = 0.0075$ ) with the addition of bevacizumab to the treatment regimen (63). To date, this is the first trial in lung cancer to show a survival advantage for a triplet over a doublet regimen first-line (62).

Clear-cell renal cell carcinoma is the most common form of cancer of the kidney, accounting for 3% of all human malignancies and 95,000 deaths per year worldwide (64). A large proportion of renal cell carcinomas are characterized by elevated levels of VEGF due to the deletion of the von Hippel-Lindau (*VHL*) tumor suppressor gene (65). Inactivation of the *VHL* gene is the most frequent genetic event both in hereditary and sporadic renal cell carcinoma. Evaluation of bevacizumab in the treatment of relapsed metastatic renal cell carcinoma showed prolonged time to disease progression in patients treated with bevacizumab compared with those receiving placebo (4.8 and 2.5 months, respectively;  $P < 0.001$ ; ref. 66). Given that bevacizumab has shown clinical activity in the treatment of first-line breast cancer, colorectal cancer, and NSCLC (53, 55, 63), it is expected that the clinical activity of bevacizumab will be optimized when used in the first-line treatment of renal cell carcinoma. Several phase II and III trials are ongoing to assess the efficacy of bevacizumab monotherapy or bevacizumab in combination with IFN- $\alpha$  and erlotinib in the first-line setting.

It is clear that combining the antiangiogenic agent bevacizumab with a variety of chemotherapies in different indications improves survival, and it is important to note that this is not at the expense of significantly increased chemotherapy-related

toxicity. Bevacizumab does have its own specific safety profile including common and easily manageable adverse events such as hypertension, proteinuria, and mild bleeding and also less common but more serious events such as arterial thromboembolic events, wound healing complications, and serious bleeding events (55-67). Rare cases of gastrointestinal perforation have been observed (55). These adverse events are postulated to relate to VEGF inhibition and might be expected to occur in any agent targeting the vasculature.

**Sunitinib Malate.** Sunitinib malate (SU11248) is an orally active inhibitor of protein tyrosine kinases, which has activity against VEGF receptors 1, 2, and 3; PDGF receptors  $\alpha$  and  $\beta$ ; c-Kit; and Flt-3 tyrosine kinase receptors. Following positive phase III trial data, sunitinib malate has been launched in the United States for treatment of refractory or relapsed gastrointestinal stromal tumors and advanced renal cell carcinoma. Preclinical data suggest that part of the antitumor activity of sunitinib malate occurs through targeting tumor vasculature. Recent evidence suggests that this multitargeted tyrosine kinase inhibitor is metabolized by the enzyme CYP3A4 to an equipotent metabolite SU12662, which is in turn further metabolized by CYP3A4 (68). This would suggest that exposure to SU11248 could decrease if it was administered concomitantly with other agents that induce CYP3A4.

In a phase II study, 63 patients with advanced renal cell carcinoma who had progressed on first-line cytokine therapy received second-line sunitinib malate daily for 4 of every 6 weeks; the response rate was 40% and 28% of patients had stable disease for at least 3 months (69). In addition, preliminary results from an ongoing phase II study in 106 patients have shown an objective response rate of 39% and stable disease for at least 3 months in 23% of patients (70). Having completed accrual, this second study seeks to validate the efficacy observed in the earlier reported study.

A phase III trial examining the efficacy of sunitinib malate in patients with imatinib-resistant gastrointestinal stromal tumors was halted following positive interim data to allow those randomized to placebo to switch to the active compound. Sunitinib malate delayed tumor growth, with a median time to progression of 6.3 months versus 1.5 months in patients receiving placebo (71). Risk of death also decreased by ~50% versus placebo (71).

Preliminary data from a multicenter phase II trial in previously treated NSCLC have reported thus far a partial response rate of 9.5% and stable disease in an additional 19% of patients (72). Phase II clinical trials of sunitinib malate are also ongoing in patients with breast cancer, colorectal cancer, and neuroendocrine tumors.

Adverse events associated with sunitinib malate are those common to tyrosine kinase inhibitors and include fatigue, diarrhea, nausea, and stomatitis. Fatigue is the most frequently reported grade 3 treatment-related adverse event. Grade 3 diarrhea, nausea, and hand-foot skin syndrome are also commonly associated with the use of sunitinib malate (69, 70).

**Vatalanib.** Vatalanib is a receptor tyrosine kinase inhibitor with activity against VEGF receptors 1, 2, and 3; PDGF receptor; c-Kit; and c-Fms (73). Despite encouraging results in combination with chemotherapy in phase I/II studies as first-line therapy for advanced or metastatic colorectal cancer

(74, 75), a central review of data from the phase III CONFIRM-1 trial and survival data from CONFIRM-2 was less positive (76-78). The central radiology review found no statistically significant response or progression-free survival benefit with the addition of vatalanib to FOLFOX4 (infusional 5-FU/leucovorin/oxaliplatin) versus FOLFOX4 alone in previously untreated metastatic colorectal cancer (77). Additionally, planned interim analysis of CONFIRM-2 data indicates a low probability of showing overall survival benefit with vatalanib in the second-line treatment of metastatic colorectal cancer (78).

Commonly reported grade 1/2 adverse events associated with vatalanib include increased incidence of nausea, fatigue, vomiting, diarrhea, hypertension, dizziness, and thromboembolic events (74, 78). In some instances, grade 3 fatigue, hypertension, ataxia, neutropenia, thrombocytopenia, dizziness, and dysphasia have been reported (74, 75). In a phase I/II trial of vatalanib plus FOLFOX4, of the 35 patients evaluable for toxicity, 11% ( $n = 4$ ) experienced grade 3 fatigue, 26% ( $n = 9$ ) presented with grade 3 neutropenia, and 17% ( $n = 6$ ) reported grade 3 dizziness (75). In the CONFIRM-1 study, the addition of vatalanib to the FOLFOX regimen increased the incidence of grade 4 pulmonary embolism by 4.6% ( $n = 29$ ) versus placebo (77).

**Sorafenib.** Sorafenib (BAY 43-9006) is a pan-kinase inhibitor of raf kinase, VEGF receptor 2, and PDGF receptor  $\beta$  and is proposed to have a dual mechanism of action through targeting of tumor vasculature. Sorafenib monotherapy has shown activity in tumors expressing wild-type and mutant ras, as well as mutant B-raf. However, biomarker expression has not been required for enrollment into clinical trials to date. Sorafenib has shown single-agent activity in phase I and II studies in patients with various solid tumors. A large phase III trial in previously treated advanced renal cell cancer reported that sorafenib increases progression-free survival (6 months for sorafenib versus 3 months for placebo; hazard ratio = 0.44;  $P < 0.001$ ; ref. 79). Despite a low response rate (2%), disease control was also significantly improved with sorafenib compared with placebo, at 80% (2% response rate) versus 55% (0% response rate), respectively (79).

A recently reported uncontrolled, phase II trial evaluated the efficacy of sorafenib in patients with relapsed or refractory NSCLC. Of the 51 patients evaluable for efficacy, no partial responses were reported. However, stable disease was observed in 59% of patients and 29% of patients had tumor shrinkage. For all evaluable patients, median overall survival was >7 months and progression-free survival was nearly 3 months (80).

A phase III trial of sorafenib in combination with carboplatin and paclitaxel in patients with advanced melanoma is ongoing, as is a phase III trial in patients with previously untreated advanced hepatocellular carcinoma. Phase II trials in colorectal cancer and breast cancer are also ongoing.

Commonly reported treatment-related toxicities include rash, diarrhea, hand-foot skin syndrome, fatigue, and hypertension. In a large phase III trial, incidence of any grade treatment-related toxicities included rash (34% versus 13% with placebo), diarrhea (33% versus 10% with placebo), hand-foot skin syndrome (27% versus 5%), fatigue (26% versus 23%), and hypertension (11% versus 1%; ref. 79).

### EGFR Inhibition

**Cetuximab.** Cetuximab is a recombinant chimeric human: murine immunoglobulin G1 antibody that binds to the extracellular domain of the EGFR (81), promoting receptor internalization and degradation without receptor phosphorylation and activation (82-84). Several studies have shown that cetuximab is capable of inhibiting growth of EGFR-expressing tumor cells *in vitro*, and treatment with cetuximab results in marked inhibition of tumor growth in nude mice bearing xenografts of human cancer cell lines. Additionally, treatment with cetuximab in combination with chemotherapeutic drugs or radiotherapy is effective in eradicating well-established tumors in nude mice (81, 85, 86) and may even be able to reverse the resistance to some cytotoxic agents in these xenografts (86).

Cetuximab is approved for use in patients with EGFR-expressing metastatic colorectal cancer refractory to irinotecan-based chemotherapy, either in combination with irinotecan (for irinotecan-refractory patients) or as monotherapy (for irinotecan-intolerant patients). Approval was based on data from phase II and randomized phase II studies (87-89) showing that cetuximab monotherapy produced objective responses, and this activity was clearly improved in those patients that received cetuximab combined with irinotecan. However, data based on extensive phase III studies are still lacking in this setting. The results of a recently and prematurely closed phase III study in the second-line setting in patients refractory to irinotecan-based chemotherapy, evaluating FOLFOX with or without cetuximab, did not show any improvement in efficacy outcomes versus FOLFOX (90). Two different phase III studies evaluating the role of cetuximab in combination with standard chemotherapy schedules in the first-line and second-line settings have recently completed recruitment. These studies will assess whether cetuximab in combination with chemotherapy produces improved clinical activity when used early in the treatment of advanced disease rather than in the refractory setting.

Evaluation of the effect of combining cetuximab with high-dose radiation on locoregional disease control and survival in patients with locally advanced head and neck squamous cell carcinoma reported significant benefit associated with cetuximab (91). In one study, median survival in patients receiving cetuximab plus radiotherapy increased by nearly 20 months and 3-year survival improved by 10% compared with radiotherapy alone (91). Similarly, activity has been reported for cetuximab monotherapy or in combination with a platinum in patients with platinum-refractory recurrent or metastatic head and neck squamous cell carcinoma (median time to progression of 2.8 months and median overall survival of 5.8-6.0 months; refs. 92, 93). These data have led to the recent approval of cetuximab in the United States and in Europe for use in combination with radiotherapy to treat patients with unresectable head and neck squamous cell carcinoma. Cetuximab is also approved in the United States as monotherapy for the treatment of patients whose head and neck cancer has metastasized despite the use of standard chemotherapy.

Several fairly small trials have also evaluated cetuximab alone or in combination in the treatment of advanced NSCLC. The addition of cetuximab to standard cisplatin and vinorelbine chemotherapy in the first-line treatment of NSCLC reported modest improvements in time to progression (0.5 months) and

response rates (7%) compared with chemotherapy alone in a randomized phase II study (94). In second-line treatment, administration of cetuximab in combination with docetaxel produced a 25% response rate with median time to progression of 2.9 months, suggestive of some clinical activity (95, 96). Based on this clinical activity, further study in patients with advanced NSCLC is planned (95). A phase III study is evaluating the role of cetuximab in combination with chemotherapy in the first-line setting.

Cetuximab has also shown promising activity in combination with gemcitabine in patients with pancreatic cancer. Median time to disease progression was 3.8 months and median overall survival was 7.1 months (97), suggesting that further clinical investigation in this setting is warranted. However, in the treatment of renal cell carcinoma, cetuximab monotherapy produced no significant clinical response (98). Consequently, no further studies of cetuximab monotherapy are planned in the treatment of renal cell carcinoma (98).

Commonly reported side effects associated with cetuximab monotherapy include acneiform rash (overall incidence, 80-86%; grade 3 or 4 in 5.2-16%) and allergic reactions (grade 3 or 4 in 3.5-5%; ref. 99). Incidence and severity of rash have been shown to correlate with response and survival (93). Different hypotheses have been proposed to explain the appearance of the acneiform rash. It is possible that development of rash corresponds with saturation of the EGFR receptors because rash was not reported in patients who received doses of cetuximab <100 mg/m<sup>2</sup> (99). It may also be feasible that an EGFR polymorphism that exists in responsive tumors is also found in the skin (99). Incidence of rash is common to all EGFR-targeted agents and further evaluations are required to test these hypotheses. In general, the tolerability of cetuximab makes it an attractive option for combination with radiation therapy and chemotherapy (95).

**Panitumumab.** Panitumumab (formerly known as ABX-EGF) is a high-affinity fully human immunoglobulin G2 anti-EGFR monoclonal antibody. Panitumumab binds to the ectodomain of the EGFR with high affinity, preventing it from binding with its natural ligands. In *in vitro* models, panitumumab has shown inhibition of EGFR tyrosine phosphorylation and cell proliferation. *In vivo*, panitumumab completely prevented the formation of human epidermoid carcinoma A431 xenografts in athymic mice, resulting in complete eradication of established tumors. This effect has also been observed in human pancreatic, renal, breast and prostate tumor xenografts (100-102). Panitumumab is being extensively evaluated in metastatic colorectal cancer. The final results of a phase II study in 148 patients with advanced colorectal cancer who had previously failed standard chemotherapy were presented at the American Society of Clinical Oncology 2005 annual meeting (103), showing a response rate of 10%, a median time to progression of 2.5 months, and a median overall survival of 9.4 months. These encouraging results prompted a large phase III study in patients refractory to at least two chemotherapy regimens comparing panitumumab with best supportive care. The preliminary results of this phase III study were recently presented at the American Association for Cancer Research 2006 annual meeting (104) showing that patients in the panitumumab arm had a statistically significant increase in

overall response rate compared with patients receiving best supportive care (8% versus 0%;  $P < 0.0001$ ). Progression-free survival was also improved in the panitumumab arm (hazard ratio, 0.54; 95% confidence interval, 0.44-0.66;  $P < 1 \times 10^{-9}$ ). Panitumumab has also been evaluated in combination with irinotecan-based chemotherapy. At the 2006 American Society of Clinical Oncology gastrointestinal meeting, Hecht et al. (105) presented the results of a phase II study of panitumumab in combination with two different irinotecan-based regimens: bolus IFL and infusional FOLFIRI (5-FU/leucovorin + irinotecan). Patients receiving panitumumab plus IFL had a response rate of 47%, a stable disease rate of 32%, and a median progression-free and overall survival of 5.6 and 16.8 months, respectively. Patients treated with panitumumab combined with FOLFIRI had a response rate of 33%, with stable disease in 46% of the patients and a median progression-free survival of 10.9 months. Overall survival for this cohort of patients was not available at the time of this analysis. Other phase III studies with panitumumab are ongoing in the first-line and second-line settings, which will further define the role of this fully human antibody.

**Erlotinib.** Erlotinib is a small-molecule quinazolinamine that reversibly inhibits the EGFR tyrosine kinase and prevents receptor autophosphorylation (106-108). Erlotinib is approved in the United States, Europe, and several other countries as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen. Approval of erlotinib was based on results from a large phase III trial in patients with advanced NSCLC following failure of prior chemotherapy in which single-agent erlotinib showed a significant survival benefit over placebo (109). However, results from two earlier phase III trials showed no benefit from the addition of erlotinib to platinum-based chemotherapy as first-line treatment for advanced or metastatic NSCLC (110, 111). In patients who had never smoked, however, there was a substantial survival benefit (112). Evidence from some investigators has suggested that EGFR mutations may confer additional sensitivity to erlotinib and gefitinib in terms of response (113-115), and thus may account for the limited efficacy observed in some clinical trials (110, 111). However, although the presence of an EGFR mutation may increase responsiveness to erlotinib, it is not indicative of survival benefit. Phase II trials for erlotinib and gefitinib have reported correlations between responsiveness to EGFR inhibition and patient characteristics such as gender, histologic type, race or ethnic origin, and smoking status (109, 116-119).

Erlotinib is also approved in the United States in combination with gemcitabine chemotherapy for the treatment of advanced pancreatic cancer in patients who have not received previous chemotherapy. This label extension was based on the results of a recent phase III trial in which erlotinib significantly improved survival when added to gemcitabine in patients with advanced pancreatic cancer (120). Disease control rates were reported to be 58% and 49% for the erlotinib and placebo arms, respectively (120). The recorded hazard ratio for survival (0.81;  $P = 0.028$ ) equates to a 19% reduction in the risk of death. Patients treated with erlotinib also had a 1-year survival rate of 23.8% versus 19.34% for patients receiving

placebo. Thus, erlotinib is the first targeted agent to show a survival benefit, although modest, in pancreatic cancer and the first agent after nearly a decade of trials to be shown to add to the survival benefit of gemcitabine.

Erlotinib monotherapy has shown modest activity in patients with many solid tumors including refractory colorectal cancer with a response rate of 8% in one study; although results have been variable (121, 122). Preliminary results from phase I/II studies of erlotinib plus FOLFOX or XELOX (Xeloda plus oxaliplatin) in advanced refractory colorectal cancer suggest that the addition of erlotinib to standard chemotherapy has some beneficial effects (123-125). Erlotinib has shown modest efficacy in patients with esophageal or gastroesophageal junction cancer, with response rates of 9% to 15%, but has shown no activity in patients with gastric cancer (126, 127). A phase II trial of erlotinib in patients with unresectable hepatocellular carcinoma showed that 25% of patients remained progression-free at 4 months (128). This indicates that erlotinib provides prolonged stable disease and potentially improved survival in patients with previously untreated advanced hepatocellular carcinoma (128).

A recent multicenter phase II study of erlotinib in patients with recurrent or metastatic head and neck squamous cell carcinoma reported an objective response rate of 4.3%. Although this was lower than that previously achieved with conventional anticancer therapy, it indicates that erlotinib monotherapy has some activity in this patient population (129). Furthermore, the median overall survival of 6 months and the 1-year survival rate of 20% are comparable to those achieved in previous trials using conventional anticancer therapy (129). These results are even more profound given that the patients enrolled into this trial were heavily pretreated, suggesting that further investigation is warranted. Preliminary results from a further "pilot" clinical trial examining neoadjuvant treatment of patients with head and neck squamous cell carcinoma with erlotinib showed a superior response rate, with 32% of evaluable patients achieving 30% to 80% decrease in tumor size (130).

Side effects associated with erlotinib are common to EGFR-targeted agents; the most frequently reported drug-related toxicities were rash and diarrhea (110, 111, 129), although in most patients these were mild to moderate. As highlighted previously, the pathogenesis of the cutaneous toxicity resulting in rash remains unclear. Gastrointestinal toxicities such as diarrhea have only been reported with orally available EGFR tyrosine kinase inhibitors.

**Gefitinib.** Gefitinib is a small-molecule anilinoquinazoline that reversibly inhibits EGFR tyrosine kinase autophosphorylation and inhibits downstream signaling (131-135). In 2003, gefitinib was approved in the United States, Japan, and some other non-European countries as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC who have previously received chemotherapy or who are not suitable for chemotherapy. Approval was based on phase II trial results showing tumor responses in up to 19% of patients with previously treated advanced or metastatic NSCLC (116, 117). However, a large phase III study recently failed to show any survival benefit of gefitinib over placebo in this patient population, despite an 8% objective response rate (136).

Following the publication of these data, approval was effectively withdrawn in the United States in June 2005. In addition, gefitinib did not show a survival benefit when added to platinum-based chemotherapy as first-line treatment for patients with advanced NSCLC (137, 138).

Gefitinib monotherapy has shown encouraging activity in a phase II trial in recurrent or metastatic head and neck squamous cell carcinoma (139) and modest results in patients with glioblastoma (140). However, gefitinib mono-

therapy reported only minimal activity in the treatment of hormone-refractory prostate cancer (141) and had limited antitumor activity in patients with advanced renal cell carcinoma (142).

Gefitinib monotherapy also failed to show evidence of clinical activity, as defined by objective tumor response, in two different phase II trials in patients with metastatic colorectal cancer (143, 144). However, preliminary results from two phase II studies of gefitinib plus FOLFOX in metastatic colorectal cancer

**TABLE 1. Activity of Currently Approved Agents Targeting the VEGF or EGFR**

Agent	Description	Treatment	Advanced/Metastatic CRC	Advanced/Metastatic NSCLC
Bevacizumab	Anti-VEGF monoclonal antibody	Monotherapy		
		Combined with chemotherapy	Survival advantage vs chemotherapy alone (irinotecan, 5-FU/leucovorin and 5-FU/leucovorin) as first-line treatment (53-55) Survival advantage vs chemotherapy alone (FOLFOX4) as second-line treatment (56)	Survival advantage vs chemotherapy alone (paclitaxel/carboplatin) as first-line treatment (63)
Sunitinib malate	VEGF receptor tyrosine kinase inhibitor	Monotherapy	Primary study end point (objective tumor response rate) not reached in a phase II study in second-line (no significant clinical activity; ref. 185)	Provocative single-agent activity in previously treated patients with recurrent or advanced NSCLC (72)
Sorafenib	VEGF receptor tyrosine kinase inhibitor	Monotherapy		Promising efficacy in patients with relapsed or refractory advanced NSCLC (80)
		Combined with chemotherapy	Preliminary antitumor activity in combination with oxaliplatin in patients with oxaliplatin-refractory CRC (191)	
Cetuximab	Anti-EGFR monoclonal antibody	Monotherapy	Clinical activity as second- and third-line treatment (192, 193)	
		Combined with chemotherapy	No increase in response rate and progression-free survival vs FOLFOX4 alone as second-line treatment (90) Progression-free survival advantage in combination with irinotecan as second/third-line treatment (88)	Suggested time to progression advantage with the addition of cetuximab to cisplatin/vinorelbine as first-line treatment (94) Clinical activity as second-line treatment (95)
Panitumumab	Anti-EGFR monoclonal antibody	Monotherapy	Clinical activity as third-line treatment (104) Encouraging survival data in patients who have failed multiple lines of standard chemotherapy (103)	
		Combined with chemotherapy	First-line panitumumab in combination with irinotecan-based chemotherapy has shown clinical activity (105)	
Erlotinib	Small molecule EGFR tyrosine kinase inhibitor	Monotherapy	Clinical activity as second/third-line treatment (121, 122)	Survival advantage vs placebo as second-line treatment (109)
		Combined with chemotherapy	Suggested increased activity combined with chemotherapy (FOLFIRI and FOLFOX) as second-line treatment (125, 195)	No benefit vs chemotherapy alone (cisplatin/gemcitabine and cisplatin/paclitaxel) as first-line treatment (110, 111)
Gefitinib	Small molecule EGFR tyrosine kinase inhibitor	Monotherapy	No clinical activity (143, 144)	Tumor responses but no survival advantage vs placebo as second/third-line treatment or first-line chemotherapy-intolerant patients (116, 117, 196)
		Combined with chemotherapy	Suggested increased activity combined with chemotherapy (FOLFOX) as first- or second-line treatment (123, 145)	No survival benefit vs chemotherapy alone (gemcitabine/cisplatin and carboplatin/paclitaxel) as first-line treatment (137, 138)



suggest the addition of gefitinib to standard chemotherapy has some beneficial effects (123, 145). The response rate and median time to progression were 74% and 9.5 months in patients with no prior treatment for metastatic disease and 23% and 5.2 months in patients who had received prior treatment, respectively. Gefitinib in combination with docetaxel has also shown promising antitumor activity in patients with advanced breast cancer (146), with those patients receiving gefitinib plus docetaxel

experiencing a response rate of 64% with a median duration of response of 5.5 months and time to progression of 7.6 months (146). In contrast to the promising activity of gefitinib/docetaxel combination therapy, gefitinib monotherapy has failed to show meaningful activity in patients with advanced refractory breast cancer (147). Furthermore, gefitinib monotherapy has not produced significant clinical activity in patients with advanced refractory gastric cancer (148). However, pharmacodynamic data

**TABLE 1. Activity of Currently Approved Agents Targeting the VEGF or EGFR (Cont'd)**

Advanced Renal Cell Cancer	Metastatic Breast Cancer	Prostate Cancer	Pancreatic Cancer	Recurrent/Metastatic HNSCC
Prolonged time to progression of disease (66)	Progression-free survival advantage vs chemotherapy alone (paclitaxel) as first-line treatment (60)		Suggested increased activity in combination with gemcitabine in advanced pancreatic cancer (184)	
Antitumor activity in second-line. Objective response, survival and time to progression advantage (69)	Improved objective response rates in second-line (186)			
Progression-free survival and overall survival benefit vs placebo (79, 187)	Minimal clinical activity in heavily pretreated patients (188)	Minimal clinical activity in first-line (189)		No clear clinical activity in pretreated patients however, increased disease stabilization suggests benefit (190)
No significant clinical activity (98)			Suggested increased activity in combination with gemcitabine as first-line treatment (97)	Clinical activity as second-line treatment (92)
				Clinical activity in combination with platinum as second-line treatment (93)
				Increase in response rate, suggested survival advantage with the addition of cetuximab to cisplatin as first-line treatment (194)
				Clinical activity as first-line treatment (130)
			Survival advantage vs chemotherapy alone (gemcitabine) in first-line treatment (120)	
Minimal clinical activity (142)	Minimal clinical activity (147)	Minimal clinical activity (141)		Clinical activity reported (139)
	Suggested increased activity combined with chemotherapy (docetaxel) as first-line treatment (146)			

from this study showed that gefitinib was biologically active in its inhibition of the EGFR signal transduction pathway, although this did not translate into clinical activity (149). Three phase II studies have shown promising efficacy of gefitinib monotherapy in patients with advanced esophageal or gastroesophageal junction cancer (150-152).

Side effects associated with gefitinib are those common to all EGFR-targeted agents: rash and diarrhea (139). Evidence suggests that development of rash (skin toxicity) or diarrhea may be associated with statistically significant improvements in response, overall survival, and patient outcomes (139, 140). Nausea has also been reported in some patients receiving gefitinib (143).

In conclusion, EGFR is becoming an increasingly important target in anticancer therapy. However, data have shown that results vary considerably depending on the agent used and the tumor type. This suggests that factors other than inhibition of EGFR influence whether a tumor is responsive to therapy and that there may be a need for inhibition of multiple signaling pathways to achieve optimal patient outcomes. Additive effects between agents, tumor sensitization, or potentiation of the effects of other agents may improve patient survival.

### Combined VEGF and EGFR Inhibition: Why Join Forces?

Currently approved therapeutic agents seem to have limited monotherapy activity in many indications, but benefits are observed when they are combined with chemotherapy (summarized in Table 1). Combining different targeted agents offers potential for improved efficacy over monotherapy while maintaining a favorable safety and tolerability profile compared with chemotherapy combinations. Results from preclinical studies indicate that complete (versus partial) blockade of VEGF is necessary for maximal inhibition of tumor growth (153). Therefore, targeting VEGF seems to be the most effective strategy in inhibiting angiogenesis. Extensive preclinical and clinical data support this theory and the use of bevacizumab to inhibit VEGF, the key mediator of angiogenesis.

Dependence of tumor vasculature on VEGF may decrease as disease progresses due to increasing redundancy of proangiogenic pathways (59, 154). In the presence of VEGF inhibition, some tumors may show VEGF resistance via a PDGF receptor  $\beta$ -driven escape mechanism. The PDGF receptor drives pericyte recruitment activation as opposed to endothelial cells that are dependent on VEGF. It has been suggested that through blocking EGFR signaling, it may be possible to "sensitize" cells to antiangiogenic therapy by lowering the tumor cell survival threshold (i.e., tumor cells would be less likely to survive without surrounding vasculature, more dependent on angiogenesis, and thus more vulnerable to antiangiogenic therapy; refs. 154-156).

Although inhibition of angiogenesis, via down-regulation of VEGF and/or other proangiogenic molecules, is postulated to contribute to the mechanism of action of agents targeting EGFR (3), inhibition of EGFR alone cannot completely block VEGF production because its expression is regulated by many other factors. Furthermore, EGFR inhibition alone is unlikely to sufficiently inhibit stromal VEGF release, which contributes to

tumor angiogenesis. Additionally, tumor cells can become resistant to EGFR inhibition. Many mechanisms have been proposed to account for this phenomenon (155, 157, 158) and there is evidence to suggest that increased expression of VEGF plays a role in resistance to anti-EGFR therapy (4). In one study, acquired resistance of tumor cells to anti-EGFR monoclonal antibodies was associated with increased levels of VEGF, which were accompanied by an increase in angiogenic potential *in vitro* and tumor angiogenesis *in vivo* (159). In another study, chronic administration of EGFR inhibitors to mice with colon cancer xenografts resulted in the development of resistant colon cancer cell lines with increased VEGF expression (160). This overexpression of VEGF rendered tumor cells significantly resistant to EGFR inhibitors (159). It has been postulated that "overactivation" of pathways driving VEGF expression independently of EGFR might result in resistance to EGFR inhibitors due to the inability of these agents to down-regulate VEGF to "critical" nonangiogenic levels, thus reducing the antiangiogenic potential of these antagonists (155). This could potentially be overcome by combining EGFR inhibition with an agent targeting VEGF.

Combined targeting of angiogenic pathways (both directly via VEGF inhibition and indirectly via EGFR inhibition) and tumor growth (directly via EGFR inhibition and potentially via VEGF inhibition) thus offers the potential for improved efficacy versus targeting either pathway alone (Fig. 2). The authors of a preclinical study of a murine model of gastric cancer using dual blockade of the VEGF and EGFR pathways (DC101, an anti-VEGF receptor monoclonal antibody, and cetuximab, respectively) reported that combined blockade of both pathways produced a greater inhibition of tumor growth than either agent alone (41). DC101 monotherapy decreased tumor vascularity and increased endothelial cell apoptosis, whereas cetuximab alone did not affect angiogenesis but inhibited tumor cell proliferation. Similar results were reported for a study conducted in a model of colon cancer carcinomatosis, in which the combination of DC101 and cetuximab produced a greater decrease in angiogenesis and ascites formation than either agent alone. Combined therapy also induced a greater increase in endothelial cell and tumor cell apoptosis than DC101 treatment alone (161). Cetuximab monotherapy did not change any of the measurable variables in this model (161). In a mouse model using human GEO colon cancer cells, combination of a VEGF receptor-2 antisense oligonucleotide plus cetuximab prolonged time to progression of the tumor and significantly improved survival compared with either treatment alone (162). Additionally, significant potentiation of inhibition of VEGF expression and few or no microvessels were observed in the GEO tumors after combined treatment with the two agents (162).

Evaluation of the VEGF receptor-1 and VEGF receptor-2 tyrosine kinase inhibitor vatalanib combined with PKI-166, an inhibitor of EGFR and HER2 tyrosine kinases (163), has been carried out in a mouse model for pancreatic cancer (164). Combination therapy inhibited the growth of pancreatic cancer xenografts. Therapeutic efficacy was reported to directly correlate with a decrease in the circulating proangiogenic molecules VEGF and IL-8, a decrease in microvessel density, a decrease in cell proliferation, and an increase in apoptosis

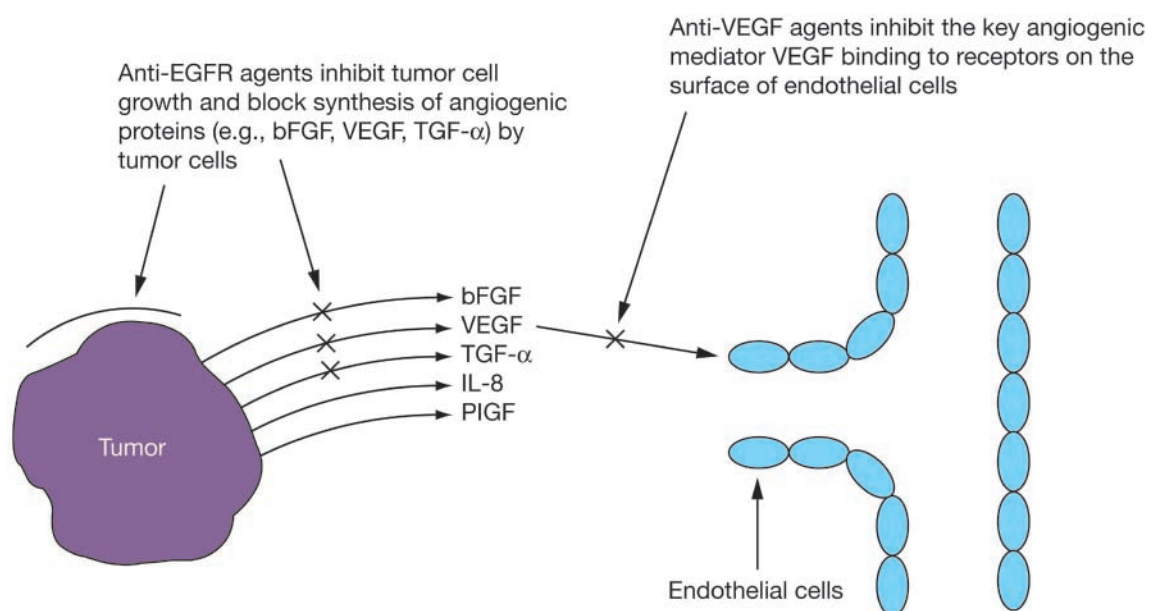
of endothelial and tumor cells (164). It is likely that the decrease in circulating VEGF and IL-8 was attributable to the inhibitory effect of PKI-166 on angiogenic factor induction (164). Similarly, preclinical evaluation of the VEGF inhibitor bevacizumab combined with the HER1/EGFR tyrosine kinase inhibitor erlotinib in a xenograft model for metastatic colorectal cancer showed that the combined regimen produced greater growth inhibition than either agent alone (165).

An anilinoquinazoline tyrosine kinase inhibitor, vandetanib (ZD6474), with activity against VEGF receptors 1, 2, and 3 and EGFR (166, 167), has been evaluated in various murine models (166, 168). In a rat model, p.o. administered vandetanib inhibited VEGF signaling and angiogenesis, tumor-induced neovascularization, and tumor growth (166). Vandetanib has also been evaluated in EGFR inhibitor-sensitive and -resistant cell lines (160, 169). Taguchi et al. (169) examined the antitumor activity of vandetanib in the gefitinib-sensitive lung adenocarcinoma cell line PC-9 and a gefitinib-resistant variant, PC-9/ZD. Treatment with vandetanib resulted in a dose-dependent decrease in the proportion of proliferating cells in the gefitinib-sensitive tumors but not in the gefitinib-resistant xenografts (169). No significant increases in apoptosis were observed in either tumor type (169). In a similar approach, the efficacy of vandetanib has been evaluated in human GEO colon cancer xenografts with acquired resistance to EGFR inhibitors (160). Results from this study suggest that growth of EGFR inhibitor-resistant tumors can be inhibited by vandetanib, probably due to the inhibitory effect of vandetanib on VEGF signaling in cells (160). Vandetanib has been evaluated in combination with the anti-EGFR monoclonal antibody cetuximab in preclinical colon

cancer and NSCLC models, showing a synergistic effect both *in vitro* and *in vivo*, and thus providing an additional rationale for clinical evaluation of the dual blockade of EGFR and VEGF receptor signaling (170).

The molecule AEE788 preferentially targets EGFR, HER2, and VEGF receptor-2 tyrosine kinases (171-173). In the NCI-H596 adenocarcinoma lung carcinoma xenograft model, AEE788 produced a dose-dependent inhibition of squamous cell tumor growth; the effect was similar to that obtained with the reference vatalanib/PKI-166 combination regimen used (171). AEE788 activity was also comparable to that of a vatalanib/PKI-166 combination regimen in the DU145 prostate carcinoma model (171). In a subsequent study, Park et al. (173) also assessed the effects of AEE788 on human cutaneous squamous cell carcinoma xenografts in nude mice; treatment with AEE788 led to dose-dependent inhibition of VEGF receptor 2 and EGFR phosphorylation and growth inhibition. In addition to inhibiting cutaneous cancer growth by blocking VEGF and EGFR signaling pathways *in vitro*, AEE788 also induced tumor and endothelial cell apoptosis (173). Finally, inhibition of VEGF receptor 2 and EGFR phosphorylation, inhibition of cell growth, and induced apoptosis were observed in a murine model for human head and neck cancer following treatment with AEE788 (172). AEE788 effectively inhibited tumor vascularization and growth and prolonged mice survival.

These data indicate that dual VEGF and EGFR tyrosine kinase receptor blockade should be considered for the treatment of solid tumors. Preclinical evidence suggests activity in animal models for gastric, colon, pancreatic, lung, prostate, breast, and head and neck cancers. Simultaneous blockade of the VEGF



**FIGURE 2.** Dual blockade of key signaling pathways provides complementary antitumor effects.

**TABLE 2. Efficacy of Combined VEGF and EGFR Inhibition in Clinical Trials**

Indication	Study	VEGF Inhibitor	EGFR Inhibitor	Chemotherapy	Phase	Assessable Patients (n)	Response Rate (%)	Median Progression-Free Survival (mo)	Median overall Survival (mo)
CRC	Saltz et al. (174)	Bevacizumab	Cetuximab	Irinotecan	II	41	37	7.9 (median time to progression)	Not reported
	Saltz et al. (174)	Bevacizumab	Cetuximab	N/A	II	40	20	5.6 (median time to progression)	Not reported
NSCLC	Herbst et al. (1)	Bevacizumab	Erlotinib	N/A	II	40	20	7.0	12.6
	Natale et al. (181)	Vandetanib	N/A	N/A	II	83	7	2.6	5.3
	Heymach et al. (182)	Vandetanib	100/300 mg	Docetaxel	I	15	6	3.5/4.3	Not reported
	Heymach et al. (183)	Vandetanib	100 mg	Docetaxel	II	42	Not reported	4.4	Not reported
		Vandetanib	300 mg			44		4.0	
		Placebo			41		2.8		
Breast cancer	Dickler et al. (178)	Bevacizumab	Erlotinib	N/A	II	18	6	4.0 (median time to progression)	Not reported
Renal cell carcinoma	Hainsworth et al. (176)	Bevacizumab	Erlotinib	N/A	II	59	25	11	Not reported
	Bukowski et al. (177)	Bevacizumab	Erlotinib	N/A	II	66	14	9.9	Not reported
		Bevacizumab	Placebo			61	13	8.5	
HNSCC	Vokes et al. (180)	Bevacizumab	Erlotinib	N/A	I	51	11	Not reported	Not reported

Abbreviations: CRC, colorectal cancer; HNSCC, head and neck squamous cell carcinoma; N/A, not applicable.

and EGFR signaling pathways has been shown to inhibit tumor growth through both direct antitumor effects and antiangiogenic effects. This supports the investigation of combined VEGF and EGFR inhibition strategies through controlled clinical trials.

### Combined VEGF and EGFR Inhibition in Clinical Trials

As discussed, preclinical studies have shown that anti-VEGF and anti-EGFR treatments are additive in preclinical tumor xenograft models. Encouraging antitumor activity has also been observed in clinical trials with combined VEGF and EGFR blockade. Many of these studies have focused on combining bevacizumab with anti-EGFR agents due to the fact that bevacizumab has proven activity in many tumor types (summarized in Table 2).

In metastatic colorectal cancer for which both bevacizumab and cetuximab are approved for use, a randomized phase II trial (BOND II) examined the efficacy and safety of concurrent administration of bevacizumab plus cetuximab, with and without irinotecan, in irinotecan-refractory disease (174). Adding bevacizumab to cetuximab or cetuximab/irinotecan improved outcomes compared with historical data for these agents. A previous study (BOND I) evaluating treatment of metastatic colorectal cancer with cetuximab/irinotecan reported a 23% response rate and time to progression of 4 months (88). In BOND II, Saltz et al. (174) showed that the addition of bevacizumab to the treatment regimen produced a 37% response rate with median time to progression of 7.9 months. The response rate when patients were treated with cetuximab alone was 11% and time to progression was 1.5 months (88). The addition of bevacizumab to cetuximab produced a partial response in 20% of patients and increased median time to progression to 5.6 months (174). It is important to note that ~60% of patients randomized into the BOND I study had received previous treatment with oxaliplatin-based chemotherapy. More patients (89%) in the BOND II study had received prior treatment with oxaliplatin-based chemotherapy, indicating that the BOND II study population was more refractory. Toxicities observed were as predicted, with no indication of synergistic toxicity. Antibody-related grade 3 toxicities were rash, paronychia cracking, allergic reaction, and headache (first

cetuximab dose only). Among 74 treated patients in BOND II, there were four serious gastrointestinal adverse events and three arterial thrombotic events (174). This trial provided further evidence that bevacizumab adds a consistent benefit when combined with other regimens for metastatic colorectal cancer. Further to this study, the BOND III trial will compare bevacizumab/cetuximab/irinotecan with bevacizumab/cetuximab in bevacizumab-refractory metastatic colorectal cancer.

Other trials have examined the use of bevacizumab with EGFR tyrosine kinase inhibitors, particularly erlotinib, which has shown activity in NSCLC and pancreatic cancer. A phase I/II trial evaluating bevacizumab plus erlotinib in advanced NSCLC reported encouraging efficacy and safety results (1). Of 40 patients enrolled, 20% achieved partial response and 65% recorded stable disease as their best response (1). Median overall survival for the 34 patients treated with the phase II dose level was 12.6 months, with progression-free survival of 6.2 months (1). This is at least comparable to results observed with chemotherapy combinations in similar patients. A phase II trial evaluating the efficacy and safety of bevacizumab in combination with either chemotherapy (docetaxel or pemetrexed) or erlotinib compared with chemotherapy alone for the treatment of recurrent or refractory NSCLC was recently reported (175). The addition of bevacizumab to both erlotinib and chemotherapy was superior to chemotherapy alone. Median progression-free survival was 3.0 months in the chemotherapy alone arm, versus 4.8 months in the bevacizumab plus chemotherapy arm, and 4.4 months in the bevacizumab plus erlotinib arm; the objective response rates were 12.2%, 12.5%, and 17.9%, respectively (175). These data suggest that the combination of bevacizumab and erlotinib may be an alternative to chemotherapy in this setting. No new or unexpected safety signals were noted in either study and the most common adverse events reported by patients were mild to moderate rash, diarrhea, and proteinuria.

Treatment for advanced renal cell carcinoma is historically poor, with few agents showing any antitumor activity (176). However, sorafenib, an oral inhibitor of cytosolic raf kinase and VEGF and PDGF receptors, has recently been launched in the United States as monotherapy for the treatment of renal cell carcinoma refractory to prior immunotherapy. Positive phase II data for sunitinib, an orally active inhibitor of VEGF receptor 2, PDGF receptor  $\beta$ , KIT, and Flt-3 tyrosine kinase receptors,

suggest that approval of this compound will shortly follow. Additionally, substantial clinical activity has been shown in a phase II trial of bevacizumab plus erlotinib for metastatic renal cell carcinoma (176). Of 59 assessable patients, 2% experienced complete response, 23% achieved partial response, 22% achieved minor response, and 61% had stable disease. The median progression-free survival was 11 months and the progression-free survival rates at 12 and 18 months were 43% and 26%, respectively. The median overall survival for this study has not been reached; 12 and 18 months survival values were 78% and 60%, respectively (176). However, the preliminary results of a randomized phase II study did not support an additional benefit with erlotinib combined with bevacizumab over bevacizumab alone in patients with advanced renal cell carcinoma (177). Patients treated with bevacizumab plus erlotinib showed a response rate of 14% and a median progression-free survival of 9.9 months compared with 13% and 8.5 months, respectively, in the bevacizumab arm. In both studies, treatment with bevacizumab and erlotinib was well tolerated; the most common grade 1 or 2 adverse events were rash, diarrhea, proteinuria, bleeding, pruritus, nausea/vomiting, and hypertension. The most common grade 3 adverse events (in  $\geq 5\%$  of patients) were rash, diarrhea, nausea/vomiting, hypertension, bleeding, and proteinuria (176, 177).

Encouraging results have been seen for bevacizumab plus erlotinib in metastatic breast cancer and recurrent or metastatic head and neck squamous cell carcinoma (178-180). Preliminary data for a phase II trial in metastatic breast cancer reported a partial response in 6% of patients; 39% of patients had stable disease as their best response. Median time to progression was 4 months and duration of response was 8.8 months. Bevacizumab/erlotinib combination therapy was well tolerated and associated with acceptable toxicities that were generally mild to moderate; the most common adverse events were rash, diarrhea, fatigue, stomatitis, nausea, vomiting, hypertension, epistaxis, and thrombosis (178, 179). Grade 3 or 4 hypertension, rash, diarrhea, nausea, vomiting, and thrombosis were reported in a small number of patients (178, 179).

Given the apparent efficacy of combining anti-VEGF and anti-EGFR agents, it is of interest to assess whether agents that inhibit both pathways are effective. With these so-called "dual inhibition" agents, achieving complete VEGF and EGFR inhibition is more difficult due to differing affinities for the receptors and the potential for toxicity due to relative overdosing to achieve inhibition. A recently reported trial compared the effects of vandetanib monotherapy with gefitinib monotherapy in patients with NSCLC (181). Patients receiving vandetanib had a significant prolongation of progression-free survival compared with patients receiving gefitinib (2.6 months compared with 1.9 months, respectively;  $P = 0.025$ ; ref. 181). Equally, in a two-part phase II trial of vandetanib plus docetaxel in the treatment of advanced or metastatic NSCLC after failure of first-line platinum-based chemotherapy, the safety, efficacy, and possible pharmacokinetic interaction of the combination were assessed (182). Of 14 assessable patients, partial response was recorded for 14% and stable disease lasting  $>1.5$  months was achieved in 35% of patients (183). Reported toxicities were manageable and reversible by dose interruption followed by

dose reduction. The most commonly reported adverse events were rash (grade 3), gastrointestinal events (nausea/vomiting), and laboratory abnormalities (grade 1 or 2; ref. 182). Part one of this two-part study confirmed that the combination of vandetanib and docetaxel is not associated with significant changes in exposure to either drug. The initial analysis of the second part of this study was recently presented at the American Society of Clinical Oncology 2006 annual meeting. A total of 127 patients were randomized to the double-blind part of this study comparing docetaxel/placebo with docetaxel/vandetanib using two different doses of vandetanib (100 and 300 mg). The combination of docetaxel/vandetanib produced a prolongation of median progression-free survival compared with docetaxel/placebo: 4.4 months with vandetanib 100 mg ( $P = 0.074$ ), 4.0 months with vandetanib 300 mg ( $P = 0.461$ ), and 2.8 months with placebo (183). With reference to the poor prognosis associated with lung cancer, these data are very encouraging and vandetanib is the first of this type of novel agents to show both activity as monotherapy and in combination with standard chemotherapy in the second-line treatment of NSCLC.

Evidence from these trials supports further development of combined VEGF and EGFR inhibition therapy for patients with solid tumors. In some indications, regimens combining VEGF and EGFR inhibitors have produced results comparable to or better than those observed with chemotherapy, indicating the potential of combining targeted therapies. Ongoing studies in which VEGF and EGFR inhibitors are being used in combination show that this is an active area of research and that data are likely to be produced over the coming years to indicate the true benefit of this approach. Studies include those examining combinations of bevacizumab with cetuximab or erlotinib in indications such as pancreatic cancer and colorectal cancer, as well as those evaluating the role of vandetanib in combination with chemotherapy in NSCLC.

## Conclusions

VEGF primarily binds to receptors on endothelial cells but may also act on tumor, hematopoietic, and neural cells. Circulating VEGF binds two related receptors, VEGF receptor 1 and VEGF receptor 2. Although VEGF receptor 1 was the first identified VEGF receptor, its precise function remains unclear. Evidence suggests that VEGF receptor 2 is the major mediator of the mitogenic, angiogenic, and vascular permeability-enhancing effects of VEGF. The EGFR signaling pathway is triggered by EGFR dimerization that is promoted through the binding of a specific set of ligands (e.g., EGF, TGF- $\alpha$ , and amphiregulin) to the extracellular domain of the receptor. The effect of signaling through EGFR is dependent on the receptor partner for EGFR, the identity of the activating ligand, and the cellular context. However, the exact role of these receptors is still an active area of research with further exploration warranted.

VEGF and EGFR signaling pathways are related. VEGF is the key mediator of angiogenesis and VEGF overexpression promotes EGFR resistance. However, EGFR inhibition does not substantially inhibit angiogenesis. Therefore, an anti-VEGF agent is required to inhibit angiogenesis. If treatment requires the use of an anti-EGFR agent, then it should be combined with

an anti-VEGF agent. Several agents targeting VEGF or EGFR have received regulatory approval for cancer therapy, usually in combination with standard chemotherapy regimens. Evidence suggests that combined inhibition of VEGF and EGFR may provide improved efficacy versus inhibition of either pathway alone. Furthermore, combination therapy with VEGF and EGFR inhibitors may help overcome tumor resistance mechanisms. As the tumor progresses, angiogenic pathways become more robust and redundant. It has been suggested that the use of EGFR inhibitors may restore tumor cell sensitivity to VEGF after this resistance has developed. Additionally, resistance to EGFR inhibition is common and correlates with increased VEGF expression and poor outcomes. Concomitantly targeting the VEGF receptor and EGFR signaling pathways may circumvent the problem of acquired resistance to EGFR inhibitors. Therefore, combining VEGF and EGFR inhibition may contribute to better therapeutic outcomes. Results from preclinical and clinical studies show that this therapeutic approach is feasible and shows promising efficacy in a range of solid tumors. Ongoing studies will confirm the efficacy of this approach.

Clinical trials have shown that the efficacy of monotherapy or combination therapy with anti-VEGF or anti-EGFR agents varies greatly between tumor types. However, monotherapy with either type of agent is generally not associated with dramatic improvements in survival, suggesting that their use as part of combination regimens, either with each other or with chemotherapy, will be a focus. This is certainly the case for the antiangiogenic bevacizumab, which has been shown to improve survival in three different indications with a number of different chemotherapy regimens. Anti-EGFR monoclonal antibodies have shown improved response rates and progression-free survival in patients with advanced colorectal cancer without a statistically significant survival benefit. It is possible that the limited sample size of the studies reported thus far has contributed to this. However, it cannot be ruled out that incomplete inhibition of angiogenesis by anti-EGFR agents is responsible for these inferior survival benefits. Several ongoing studies aim to address the effect on survival of the combination of anti-EGFR monoclonal antibodies with standard chemotherapy schedules in different tumor types. However, a survival benefit has been observed in patients with locally advanced head and neck cancer treated with radiotherapy and cetuximab. Furthermore, EGFR tyrosine kinase inhibitors have improved survival in patients with NSCLC and pancreatic cancer. Thus, based on preclinical and clinical evidence, targeting VEGF and EGFR using combination regimens is a rational approach. The extensive data that we have to date favors bevacizumab as the anti-VEGF agent of choice for these combination regimens. The choice of which anti-EGFR agent to use in a combination strategy is likely to depend on the tumor type being treated. Finally, preliminary emerging data have shown encouraging results for dual inhibitors of the EGFR and VEGF pathways, such as vandetanib. Ongoing studies will help to define the role of these dual inhibitors in the treatment of these malignancies.

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