Published in final edited form as:

Pharmacotherapy. 2012 December ; 32(12): 1095–1111. doi:10.1002/phar.1147.

Anti-angiogenic therapy for cancer: An update

Belal Al-Husein, MS¹, Maha Abdalla, PharmD¹, Morgan Trepte¹, David L. DeRemer, PharmD¹, and Payaningal R. Somanath, PhD^{1,2,3,*}

¹Clinical and Experimental Therapeutics, College of Pharmacy, University of Georgia

²Department of Medicine, Georgia Health Sciences University, Augusta, GA

³Charlie Norwood VA Medical Center, Augusta, GA

Abstract

The idea of anti-angiogenic therapy was the brain child of Dr. Judah Folkman in the early 1970s. He proposed that by cutting the blood supply off, cancer cells can be deprived of nutrients and hence treated. His efforts were paid off when Bevacizumab (Avastin®), a monoclonal antibody against the vascular endothelial growth factor (VEGF) was first approved for anti-angiogenic therapy in 2004 for the treatment of breast cancer. Since then, an array of anti-angiogenic inhibitors were developed, used in clinical trials and many got approved for use for the treatment of multiple cancers, alone or in combination with other cytotoxic/chemotherapy drugs. Despite this important breakthrough, anti-angiogenic therapy for cancer met with a number of hurdles on its way to be one of the options for cancer therapy. Herein, we summarize the latest update on the current knowledge on the mechanisms of tumor angiogenesis, pro- and anti-angiogenic factors, potential targets and their mechanisms of action, experimental evidences and the most recent data on the clinical trials on anti-angiogenic agents for cancer therapy.

Introduction

Traditional chemotherapeutic agents are limited by their narrow therapeutic index due to their lack of specificity, resulting in damage to both cancerous and normal cells, severe side effects, variable dosing regimens, and the development of drug resistance with subsequent disease relapse. In the 1970s, Judah Folkman made the observation that in the absence of neovascularization, tumors cannot grow more than 2 to 3 millimeters¹; this gave rise to the field of angiogenesis and laid the foundation for antiangiogenic cancer therapy^{2–3}. In 1971, Folkman proposed the concept of “anti-angiogenesis” as a modality in cancer therapy due to “the prevention of new vessel sprouts from penetrating into early tumor implant”¹. It was not until 2004 that the first antiangiogenic drug, bevacizumab was approved by the first FDA for the management of advanced colon cancer⁴. In the current review, we will discuss pathways regulating tumor angiogenesis, potential therapeutic targets for anti-angiogenic cancer therapy, and provide an update on the various clinical trials on anti-angiogenic cancer therapy.

Tumor angiogenesis

Angiogenesis is the process of forming blood vessels from pre-existing ones, unlike neovascularization, which implies the formation of entirely new blood vessels⁵. Under normal physiologic conditions, there is balance between pro- and anti- angiogenic

*Address for correspondence: Payaningal R. Somanath, PhD, Assistant Professor, College of Pharmacy, University of Georgia, HM1200, Georgia Health Sciences University, Augusta, GA 30912, sshenoy@georgiahealth.edu, Tele: 706-721-4250, Fax: 706-721-3994.

mediators and this balance is shifted towards either of them to promote physiological processes or as part of a pathological condition. Angiogenesis involves various mediators, but a universal agreement puts vascular endothelial growth factor (VEGF) and its signaling as the rate-limiting step of this process. The development of new blood vessels not only serves to supply the tumor tissue with nutrients, but they can also serve as a means for cancer cells to metastasize. However, the complexity of the interaction between tumor and vasculature is not fully understood⁶.

A considerable number of cancers have been reported to be dependent on angiogenesis and respond well to anti-angiogenic therapies. These include cancers of the colon, breast, lung, and bladder as well as renal cell carcinoma and non-small cell lung cancer (NSCLC). Additionally, some of these cancers require VEGF for their survival⁷. Anti-angiogenic therapies target angiogenesis by two major mechanisms: blocking the receptor tyrosine kinases intracellularly or neutralizing angiogenic factors such as VEGF or its receptors.

Events in angiogenesis

Angiogenesis is activated upon exposure of cancer cells to certain stimuli, mainly hypoxia which ensues as the tumor grows more than 2 mm in thickness and core cells become distant from blood supply. Cancer cells respond to hypoxia by modulating hypoxia-inducible factor (HIF)-1 α . In normoxic conditions, HIF-1 α is marked for degradation after ubiquitination by Von Hippel-Lindau (VHL) E3 ubiquitin ligase. However, under hypoxic conditions, HIF1 α dissociates from HSP90 and then dimerizes with HIF1 β ⁸. This complex is localized within the nucleus to initiate the transcription of certain growth factors such as VEGF, VEGF-C, Endothelin-1, Platelet-Derived Growth Factor (PDGF)- β , basic Fibroblast Growth Factor (bFGF), Erythropoietin⁹, angiopoietins, interleukin-8, and Placenta Growth Factor (PlGF)¹⁰. The aforementioned growth factors stimulate endothelial cells to form new blood vessels, and to secrete many other factors. A diagrammatic sketch of how different growth factors affect the tumor microenvironment by stimulating the growth of cancer cells or by recruiting other cells is depicted in Figure 1.

Circulating bone marrow derived cells are recruited to the site of angiogenesis. There is no consensus on the role of these cells in angiogenesis as some consider their contribution to angiogenesis to be negligible while others detect higher incorporation of these cells into new blood vessels. Variations in the role of these cells depend on the markers used to detect the progenitor cells⁷. A major contributing factor that determines the incorporation of the progenitor cells is their prior mobilization from bone marrow by high dose chemotherapy. Therefore, there is a trend shifting towards the use of metronomic therapy, low dose chemotherapy given over long periods of time, instead of high dose chemotherapy.

Modulators of angiogenesis

VEGFs and VEGF receptors

VEGF, first known as vascular permeability factor (VPF), was discovered by Senger et al as a part of tumor secreted factors and inducing leakage of skin blood vessels¹¹. Later in 1989, Ferrara et al isolated VPF, which was then renamed as VEGF¹². VEGFs are a family of secreted dimeric glycoproteins that include VEGF-A (commonly referred to as VEGF), VEGF-B, VEGF-C, VEGF-D in mammals, and VEGF-E and VEGF-F found in other species such as viruses and snake venom, respectively. The VEGF family also includes PlGF- 1 and 2. The effects of these factors are mediated through binding to their receptors (VEGFR-1 “fms-like-tyrosine kinase (Flt)-1”, VEGFR-2 “fetal liver kinase (FLK)-1/kinase domain region (KDR)”, and VEGFR-3 “FLT-4”). VEGFR-1 and -2 interacts with neuropilin (NRP)-1 while VEGFR-3 only associates with NRP-2¹³.

Diversity on the effects of VEGF receptors arises from receptor dimerization potentials¹⁴. Dimerization between VEGFRs 1 and 2 as well as VEGFRs 2 and 3 have been shown to mediate some of the physiological effects of VEGF superfamily members. Furthermore, VEGFRs isoforms each lead to discrete effects compared to their counterparts in ECs¹⁵: VEGFR-1 activation leads to a “decoy effect” as a VEGF sequesterant or VEGF-trap; while VEGFR-2 binding to its ligand leads to proliferation, migration, survival, and angiogenesis. Similar to VEGFR-2, VEGFR-3 mediates these cellular processes but primarily in lymphatic blood vessels.

Other proteins shown to work in concert with VEGF binding and activation include Ephrins and Semaphorin/Neuropilin-1 (known as axon-guidance molecules). Ephrin-B2 mediate the internalization of VEGFR-3¹⁶ allowing ephrin-B2 to activate other pathways (Akt, MAPK/ERK, Rac1) while it is localized intracellularly. Effects of Semaphorins/Neuropilin-1¹⁷, however, involve a more complicated process and have been shown to function as pro – angiogenesis stimuli (VEGF-A₁₆₅ binding to NRP-1/VEGFR-2 and Plexin-A1 or 2) or facilitate migration (Np-1/Np-2/VEGFR-1/Plexin-A1/2 when bound by VEGF-A (165, 145, 121), VEGF-B and PlGF-2).

Although a member of the VEGF family, VEGF-resistant tumors have shown to respond to treatments with monoclonal antibodies targeting PlGF¹⁸. This evidence suggests PlGF surpasses the inhibitory mechanisms of anti-VEGF therapy and therefore, works by other mechanisms. Studies have shown that PlGF binds to VEGFR2 and NRP-1 receptor; however, underlying mechanisms mediating its effects in angiogenesis are not well understood.

Platelet-derived growth factor

PDGF-BB is a major player in resistance to anti-VEGF therapy. PDGF is a dimeric polypeptide, composed of one of four homodimers: A, B, C and D. Effects of PDGF are mediated by binding to the dimeric PDGF-Receptors. PDGFR are composed of one of two homodimers α and β , thus giving rise to three receptors PDGFR $\alpha\alpha$, PDGFR $\alpha\beta$ and PDGFR $\beta\beta$ ¹⁹. Studies showed the development of vascular abnormalities with no pericytes recruitment in PDGF-B knockout mice models, indicating their importance as mediators of mural cells recruitment^{20–22}. PDGF, particularly PDGF-BB, seems to mobilize mural cells and recruit them around endothelial tubes. These actions lead to maturation and stabilization of blood vessels²³. PDGF effects are more pronounced in established blood vessels and leads to their survival independent VEGF stimuli. Hence, most of the new anti-angiogenesis therapies, such as sunitinib, sorafenib, pazopanib, axitinib, intedanib, dovitinib, and linifanib, target VEGF and PDGF simultaneously²⁴.

Fibroblast growth factors (FGFs) and FGF receptors

The seminal observation that agents capable of inducing EC activation and angiogenesis have an affinity for heparin led to further investigations and the discovery of both acidic (aFGF or FGF-1) and basic (bFGF or FGF-2) Fibroblast growth factors¹⁹. Although approximately 23 FGF members identified, only FGF-1 and FGF-2 have been extensively studied. FGFs mediate their effects through one of four FGF receptors (FGFRs 1–4) with intracellular tyrosine kinase domains. bFGF effects are pleiotropic and diverse²⁵. bFGF mediates mural cell recruitment, matrix deposition, both cadherin stabilization and downregulation, enhancement of barrier function, survival integrins modulation and basal lamina degradation, collectively leading to angiogenesis and maturation of established blood vessels. Such dual effects on both immature and mature blood vessels render bFGF a potential target in VEGF resistant cancers.

Endothelins and endothelin receptors

Endothelins (ETs) are a group of polypeptides secreted in an immature form and converted by endothelin-converting enzymes (ECEs) to one of three mediators, namely ET-1, 2 and 3. Their binding to ET receptors A and B (ETAR and ETBR, respectively) results in opposite effects²⁶. Stimulation of the G-protein coupled receptor ETAR leads to invasion, proliferation, angiogenesis and antiapoptosis in tumor tissue and resistance to chemotherapy²⁷. Activation of such a system was reported in multiple cancers²⁸.

Erythropoietin and erythropoietin receptor

Erythropoietin (EPO), is a hematologic agent that mediates the growth stimulation of erythrocytes, is commonly utilized to manage anemia. Interestingly, it has pleiotropic effects that are necessary for tumor growth, mainly antiapoptosis and angiogenesis. Activation of EPO receptor has been reported in many cancers²⁹. Although EPO was found to disrupt HIF1 α induced apoptosis in breast cancer³⁰, it was shown to have antiangiogenic effects in ovarian cancer by reducing VEGF levels³¹.

Angiopoietins and Tie2 receptor

Angiopoietins bind to their cognitive receptors tie-1 and -2. While Ang-1 and -4 seem to work as agonists to the tie-2 receptor, Ang-2 and -3 function as both agonists and antagonists under different conditions^{32–33}. Interactions between angiopoietin receptors have not been extensively studied, however, recent evidence suggest cooperation between tie-1 and tie-2 in regulating angiogenic processes in response to Ang-1³³.

Slits and the ‘roundabout’ receptors

Slits are axon guidance molecules which are chemo-repellents, but they also affect blood vessels by leading to their “patterning”. Roundabout receptors (Robo) function as targets for Slits and seemingly, the proteoglycan molecule ‘Syndecan’ enhances that binding³⁴. Binding recruits paxilin and ArfGAP, leading to the inactivation of Arf; Active Arf induces the internalization of VE-Cadherin³⁵. Inactivation of Arf subsequently leads to the enhancement of VE-cadherin expression on EC surface, stabilizes barrier function and inhibits angiogenesis³⁶. Although many cancers such as cervical, breast, kidney and prostate have been reported to be associated with Robo/Slit pathway inactivation, others were not, while a few had elevated levels of Robo expression³⁷.

Netrin and associated receptors: Unc5, and DCC

Netrins are a group of axon guidance molecules related to laminin that are over-expressed in many cancers³⁸. In prostate cancer, however, studies have shown reduced expression of both netrins and DCC receptors in tumors when compared with control tissue³⁷. They bind to two different receptors; deleted in colorectal cancer (DCC) receptor and uncoordinated-5-homolog (UNC5H, 4 subtypes) receptor³⁹. In the unoccupied state, the receptors mediate proapoptotic effects through the activation of DAPk (Death-associated protein kinase 1) via recruitment of PR65 β /PP2A complex that maintains DAPk in its un-phosphorylated activate form. However, binding of netrin induces dimerization of its receptors, recruitment of CIP2A (Cancerous inhibitor of PP2A) and, consequently, inhibition of receptor interaction with PR65 β /PP2A complex.

Notch signaling and DLL-4

Notch pathway activation has been shown to play a role in many cancers⁴⁰. The role of notch signaling in tumor angiogenesis is not well understood⁷. Interestingly, blocking this signaling pathway results in the formation of imperfect and leaky blood vessels and can ultimately lead to hypoxia. DLL4 is not exclusive to ECs⁴¹, it is also reported on bone

marrow-derived endothelial progenitor cells⁴², where its expression is enhanced by SDF-1 and VEGF and affects local EC activation and stabilization through increased production of fibronectin and ICAM-2.

Integrins

Endothelial cells in general express integrin dimers such as $\alpha 5\beta 1$, $\alpha \nu\beta 3$ and $\alpha \nu\beta 5$ ⁴³. During tumor angiogenesis, tumor-associated ECs start to over-express integrin $\alpha \nu\beta 3$ ^{44–46} and was correlated with tumor grade. Both “inside-out” and “outside-in” signaling regulate integrin activation and, consequently, enhanced binding to ECM components. Integrins are known to interact with multiple growth factor receptors in the regulation of angiogenesis⁴⁷. Another study⁴⁶ that engrafted human skin/human breast cancer cells into SCID (Severe Combined Immuno-Deficiency) mice, showed that treatment of such mice with the anti- $\alpha \nu\beta 3$ monoclonal antibody LM609 have reduced tumor growth and number of blood vessels within such xenografts. Potential benefit of integrin antagonists was also observed in colon cancer⁴⁸, prostate cancer⁴⁹ and many others. RGD peptides, as they resemble $\alpha \nu\beta 3$ binding site in ECM, were used first as targets for such integrin, but later was replaced by monoclonal antibodies.

Potential targets and respective anti-angiogenic agents

According to their mechanism of action, anti-angiogenic agents are classified into “(1) endothelial growth factors inhibitors, (2) EC signal transduction inhibitors, (3) inhibitors of EC proliferation, (4) inhibitors of matrix MMPs, (5) inhibitors of EC survival, and (6) inhibitors of endothelial bone marrow precursor cells”. According to their targets, they are categorized as direct, indirect, or mixed antiangiogenic agents. In addition, depending upon whether they were designed to block angiogenesis as the sole mechanism of action, or were introduced as anticancer agents, which later were found to target angiogenesis also, these agents can also be classified as inclusive and exclusive agents (E.g. thalidomide and bortezomib).

The NCI included a mechanistic classification of angiogenesis inhibitors in their fact sheets. They are classified into (1) agents that directly inhibit endothelial cells (integrin antagonists are included) (2) or those capable of interfering with signaling cascades and finally (3) agents that inhibit the ability of ECs to breakdown ECM⁵⁰. Some modify the classification to include a “miscellaneous” group and put integrin inhibitors in a different category⁵¹.

Antiangiogenic Agents in Cancer Therapy

Three major classes of agents which target VEGF have been developed: monoclonal antibodies, VEGF decoy receptor, and small molecule tyrosine kinase inhibitors (TKIs). These agents are currently in clinical practice or investigation as monotherapy or in combination with cytotoxic chemotherapy or radiation.

Anti-VEGF Monoclonal Antibodies

Bevacizumab

Bevacizumab (BEV), a recombinant humanized monoclonal antibody that binds all VEGF-A isoforms, emerged as the first effective antiangiogenic approach in the treatment of certain types of cancers. Based on the results from a Phase III trial, BEV (5mg/kg every 14 days) was approved in combination with 5-fluoruracil (5-FU) based chemotherapy as frontline therapy for metastatic colorectal cancer (mCRC). Hurwitz et al. randomized 923 patients with previously untreated mCRC to bolus IFL regimen (irinotecan, 5-FU, and leucovorin (LV)) plus BEV (n=402) or bolus IFL plus placebo (n=411), or 5-FU, LV, and BEV (n=110)⁵². The primary comparison groups, IFL +/- BEV, showed that the addition of

BEV significantly improved OS (20.3 vs. 15.6 months; $P<0.001$), PFS (10.6 vs. 6.2 months, $P<0.001$), RR (44.8% vs. 34.8%; $P=0.004$), and duration of response (10.4 vs. 7.1 months; $P=0.001$). This study provided initial evidence that therapeutic targeting of angiogenesis could provide clinical efficacy.

An additional open label Phase III trial (E3200), randomized 829 patients with mCRC refractory to 5-FU and irinotecan-based regimens and were BEV- and oxaliplatin- naïve to BEV (10 mg/kg every 14 days) plus FOLFOX4 ($n=289$), FOLFOX4 alone ($n=290$), or BEV alone ($n=243$)⁵³. The primary endpoint was OS. The E3200 trial demonstrated a median duration of OS treated with FOLFOX4 and BEV was 12.9 months compared with 10.8 months for the group treated with FOLFOX4 alone (HR death=0.75; $P=0.0011$), and 10.2 months for those treated with BEV alone. The BEV alone arm was discontinued early by investigators due to inferiority. There was a significant improvement in median PFS with BEV plus FOLFOX4 compared to FOLFOX4 or BEV alone (7.2, 4.8, 2.7 months, respectively; $P<0.0001$). Overall response rates (ORR) was significantly higher in BEV plus FOLFOX4 compared to FOLFOX4 alone (21.8% vs. 9.2%, $P<0.0001$). Important concepts derived from these results were that BEV in combination with multiagent cytotoxic chemotherapy could provide benefit as well as the clinical limitation of treating with an antiangiogenic as agent as monotherapy.

Given the positive data in the metastatic setting, several Phase III studies were initiated for adjuvant BEV in patients with high risk Stage II or Stage III colon cancer. The National Surgical Adjuvant Breast and Bowel (NSABP) C-08 trial combined BEV with a modified FOLFOX6 regimen⁵⁴. This trial did not meet its primary end point of prolonging disease free survival (DFS) at 3 years. Interestingly, during the first 15 months of the study, BEV improved DFS (HR 0.61; 95% CI 0.48–0.78%, $P<0.001$). This suggests that upon BEV therapy completion in the study, potential benefit was progressively lost. In a preclinical animal model, tumor angiogenesis has been shown to rebound following the removal of VEGF inhibition⁵⁵. An additional randomized Phase III study (AVANT) evaluated the use of BEV in combination with FOLFOX4 or capecitabine in 3541 patients (2867 stage III)⁵⁶. The addition of BEV did not improve DFS or OS. Many questions remain in the use of antiangiogenic agents in the adjuvant setting. Specifically, what are the precise mechanisms of angiogenesis in primary tumors in metastatic vs. non-metastatic disease? How do this effect drug therapy? Despite, early negative adjuvant clinical trials, the use of anti-VEGF therapies continues to be investigated. Future considerations given should include identifying patients who would benefit from long term adjuvant BEV treatment given the cost and adverse events associated with this agent.

Following FDA approval in first and second line treatment of colorectal cancer, BEV was granted FDA approval in non-small cell lung cancer (NSCLC) in 2006 from the E4599 trial. E4599 was a phase III, open-label, multicenter, randomized, active-controlled trial that evaluated the additive effect of BEV (10 mg/kg every 2 weeks) plus paclitaxel and carboplatin compared to chemotherapy alone in 878 treatment naïve patients with advanced non-squamous NSCLC. Plasma VEGF levels were assessed at baseline in the initial 166 patients. Compared to patients receiving chemotherapy alone, the BEV group had a significantly improved median OS (12.5 vs. 10.2 months; $P=0.007$), progression free survival (PFS) (6.4 vs. 4.5 months; $P<0.0001$), and response rate (RR) (27.2% vs. 10%; $P<0.0001$). Baseline plasma levels did not correlate with overall survival ($P=0.15$)⁵⁷. An additional Phase III study, the AVAIL (Avastin in Lung, or B017704) also demonstrated the addition of BEV to chemotherapy improved RR and PFS⁵⁸. However, a follow-up of this study reported a lack of OS improvement in BEV containing arms⁵⁹.

In May 2009, BEV received accelerated approval as monotherapy in second line treatment of glioblastoma. Gliomas have been associated with high expression of VEGF and prognosis⁶⁰. The AVF3708g trial was an open label multicenter study which randomized previously treated patients to BEV (10 mg/kg every other week) alone or in combination with irinotecan until disease progression. Primary endpoints were 6 month PFS and OR rates. Objective response was defined as a complete response or partial response determined on two consecutive assessments 4 weeks apart. Also responding patients were to have decreasing or stable corticosteroid use. All study patients were treated for 104 weeks or until disease progression. The median PFS in the BEV arm was 4.2 months (95% CI, 2.9–5.8) and 5.6 months (95% CI, 4.4–6.2). OS improvement was observed in the BEV arm (9.2 months, 95% CI, 8.2–10.7) compared to combination (8.7 months, 95% CI, 7.8–10.9). The BEV arm reported an OR in 28% of patients (95% CI, 18.5–40.3) with a median duration of response of 5.6 months (95% CI, 3–5.8)⁶¹. These results were encouraging when comparing to previous studies with irinotecan in this setting⁶². An additional BEV single arm study NCI 06-C-0064E also reported a OR of 19.6% (95% CI, 10.9–31.3) and median duration of response of 3.9 months (95% CI, 2.4–17.4) in previously treated glioma patients⁶³.

In July 2009, the FDA granted approval for BEV in combination with interferon alfa (IFN) for the treatment of renal cell carcinoma. The Phase III AVOREN trial compared BEV + IFN (n=327) versus IFN + placebo (n=322)⁶⁴. A recent update of this study revealed that investigators did not meet their primary endpoint of improved OS: BEV + IFN- 23.3 months versus IFN+ placebo 21.3 (HR 0.91, 95% CI, 0.76–1.1, P=0.333). However, in a post hoc analysis it was noted that patients that received TKI based therapies following BEV+IFN increased survival to beyond 3 years. Similarly, the CALBG 90206 study which compared BEV+ IFN to IFN monotherapy which did not show an improvement in OS but in PFS (8.5 vs 5.2 months, P<0.0001)⁶⁵. Given the success of recent oral agents which target VEGF, mTOR, c-KIT, PDGF, or RAF, future prospective studies should evaluate differing sequences which provide best clinical outcomes.

In the metastatic HER-2 negative breast cancer setting, the use of BEV has created a major controversy. The ECOG 2100 trial evaluated the safety and efficacy of BEV as a first line therapy in metastatic breast cancer. Patients were randomized to receive either BEV+ paclitaxel (n=347) or paclitaxel alone (n=326). The primary endpoint was to measure PFS. Secondary endpoints evaluated included OR, OS, and quality of life. Patients that received BEV had a significant improvement in PFS (11.8 vs. 5.9, p<0.001). Also, the addition of BEV improved objective OR rates (36.9% vs. 21.2%, P<0.001) with no differences in quality of life assessments⁶⁶. On February 22, 2008, the FDA granted accelerated approval for BEV in combination with paclitaxel in this setting based upon an improvement in PFS and nearly a 2 fold increase in response rate. In order for conversion to final FDA approval, post-marketing studies were performed to confirm benefit. Which surrogate endpoint for approval was a major concern? Although the ECOG 2100 trial did not show superiority in OS, the study was underpowered to address this. Per meeting minutes between the manufacturer and the FDA–“FDA confirmed that the basis for conversion to full approval will be demonstrated improvement in PFS and evidence that survival is not impaired”⁶⁷.

Both the AVADO⁶⁸ and RIBBON-1⁶⁹ studies were designed to evaluate PFS as a primary endpoint. The AVADO study was a Phase III, three-arm, placebo controlled trial which evaluated BEV (7.5 and 15 mg/kg) with docetaxel in HER2 negative locally recurrent metastatic breast cancer. RIBBON-1 was a Phase III, four arm study, which evaluated BEV (15 mg/kg) in combination with capecitabine or placebo and taxane+anthracycline with placebo. Both studies demonstrated statistically significant improvement in PFS⁶⁷. However, neither demonstrated an improvement in OS. An additional Phase III study (RIBBON-2) evaluated BEV in second line treatment of metastatic breast cancer.

Combination with BEV led to improvements in PFS for patients in taxane (HR 0.64), gemcitabine (HR 0.90), and capecitabine (HR 0.73) cohorts. Interestingly, this improvement was not seen in a vinorelbine cohort. Investigators noted this may have been due to size of cohort (n=76) and having a greater percentage of poorer outcome groups (triple-negative disease)⁷⁰. With the completion of three Phase III studies in front line and one Phase III in second line therapy, BEV is not currently approved in the treatment of metastatic breast cancer. On November 18, 2011, the FDA revoked the accelerated approval of BEV since “these studies did not verify clinical benefit, and that available evidence indicated that the drug was not shown to be safe and effective”²⁵. Also noted was the lack of a subset of patients that would have greater benefit. This decision has sparked a huge debate amongst the oncology community as well as patient advocacy groups. Some believe this decision is partly due to economics, although the FDA does not consider cost in their decision. The estimated cost of BEV in breast cancer in quality adjusted life years (QALY) is \$496,000⁷¹. But given that survival data looks similar in lung and colorectal cancers with associated substantial cost (QALY; lung- \$253,260, colorectal- \$283, 595) as well as the same adverse event profile, many are curious about the future fate of BEV in other malignancies. Recent Phase III data in ovarian (GOG-0218 and ICON-7), gastric (AVAGAST), and prostate (CLAGB 90401) have demonstrated PFS improvement without survival advantage^{72–75}. Identification of subsets of patients with potential biomarkers which predict greater clinical efficacy and the avoidance of unwarranted adverse events in other patients will be essential.

VEGF Decoy Receptor

Aflibercept, also known as VEGF-Trap, is a fusion protein that comprises of the binding domains of VEGFR-1 and -2 fused to the Fc portion of human immunoglobulin G. Aflibercept has a higher affinity to VEGF-A than does BEV and inhibits VEGF-A, VEGF-B, and PlGF2. Phase I–II studies supported further evaluation of aflibercept in combination with chemotherapy in multiple malignancies. The VALOUR trial was a Phase III study which randomized patients with advanced colorectal cancer after failure of an oxaliplatin-based regimen to either FOLFIRI + placebo (n=612) or FOLFIRI + aflibercept 4 mg/kg (n=614). Of note, approximately one-third of that patients received BEV prior to entry in the study. The primary endpoint of this study was OS. Patients that received aflibercept had significant improvements in OS (13.5 months vs. 12.06, HR=0.82, 95% CI, 0.71–0.93; P=0.0032) and PFS (6.9 months vs. 4.7 months, HR=0.74, 95% CI, 0.57–0.99, P=0.0007). Toxicities included diarrhea, asthenia, hypertension, proteinuria, infections, and neutropenia⁷⁶. Aflibercept is also being evaluated in a Phase II (AFFIRM study) front line setting for colorectal cancer in combination with FOLFOX. The VITAL study was a phase III, randomized, double blind trial evaluating the efficacy of combination therapy of aflibercept 6mg/kg and docetaxel versus docetaxel alone in 913 patients with locally advanced or metastatic NSCLC refractory to platinum based therapy. This study failed to meet its primary endpoint of OS (HR 1.01, 95%CI, 0.87–1.17). However, the addition of aflibercept improved efficacy as measured by secondary endpoints, PFS (HR=0.82, 95% CI 0.716–0.937) and overall objective response rate (23.3% vs. 8.9%, respectively)⁷⁷. The results from the VENICE trial which evaluated aflibercept as first line treatment for metastatic prostate cancer with docetaxel and prednisone are anticipated to be released this year.

Small Molecule Inhibitors

Due to the fact that angiogenesis encompasses multiple signaling pathways, the effects of anti-angiogenic agents targeting only a single pathway can be overcome. Mechanisms that can lead to rendering these anti-angiogenic agents ineffective include altering the expression levels of other pro-and anti-angiogenic signals, drug efflux systems, or mutations in tumor cells^{2,3,78}. Therefore, another approach was investigated that led to the development of

TKIs. Several TK receptors, such as VEGFR, FGFR, and PDGFR, play an important role in angiogenesis and are involved in multiple signaling pathways. TKIs bind to the ATP binding site and inhibit the activation of more than one receptor, making the development of resistance unlikely³.

Imatinib

Imatinib, developed in the 1990s, is considered the prototypical TKI that proved to be a major step toward targeted therapy. Imatinib is a selective inhibitor of Bcr/Abl, and it is approved for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST), and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. The success observed with imatinib therapy led to the investigation of multi-targeted TKIs.

Sorafenib

Sorafenib targets VEGFR-2 and -3, PDGFR-b, Flt-3, and c-Kit⁷⁹. The FDA approval of this agent was granted based on the results from a phase III, international, randomized, double-blind, placebo-controlled study in 602 patients with unresectable hepatocellular carcinoma. Treatment with sorafenib significantly prolonged the median OS compared to placebo (10.7 months vs. 7.9 months, respectively; $P=0.001$). Due to the significant improvement observed, the trial was closed early⁸⁰. Sorafenib was also recently approved as a second line therapy for metastatic RCC. In a phase III, randomized, placebo-controlled study of 903 patients with RCC refractory to conventional therapy, sorafenib therapy prolonged median PFS compared to placebo (5.5 months vs. 2.8 months, respectively; $P<0.01$). However, the overall survival failed to reach significance in this trial. Common AEs included diarrhea (43%), rash (40%), and nausea (23%). Serious AEs leading to hospitalization or death occurred in 34% of patients receiving sorafenib; they included cardiac ischemia or infarction⁸¹.

Trials that evaluated sorafenib therapy as first line in the patient population demonstrated that its use resulted in the following: significant increases in PFS compared to placebo⁸², similar PFS when compared to IFN-alfa-2a therapy⁸³, and no significant difference in ORR when compared to IFN-alfa-2b therapy⁸⁴. Sorafenib monotherapy showed minimal clinical activity in patients with prostate cancer² or NSCLC⁸⁵. Moreover, combination therapy of sorafenib with paclitaxel and carboplatin showed no significant benefit in chemotherapy naïve patients with advanced NSCLC. The combination was also associated with serious AEs including rash and infections⁸⁶.

Sunitinib

Sunitinib malate is an oral, multi-TKI that targets VEGFR-1–3, PDGFR, Flt-3 and c-Kit⁷⁹. It was first FDA approved for the management of GIST refractory to treatment with imatinib, and was later approved for metastatic RCC. Based on results from multiple phase II and III trials, utilizing sunitinib for metastatic breast cancer, and advanced or metastatic NSCLC is still under investigation. A multicenter, phase II trial in 64 patients with a history of metastatic breast cancer evaluated the efficacy of sunitinib 50 mg given once daily for 4 weeks. The overall RR, the trial's primary endpoint, was 11% (95% CI, 4–21); the median OS time was 38 weeks, and the median time to tumor progression (TTP) was 10 weeks. The most common grade 3 AEs reported were fatigue (14%), nausea (8%), and diarrhea (6%). Notably, grade 3 neutropenia occurred in 32.8% of patients⁸⁷. Another study of 63 patients with advanced NSCLC evaluated their response to sunitinib monotherapy. Although none of the patients had a complete response, 11.1% of them experienced partial response to sunitinib monotherapy. The median PFS and OS were 12 and 23.4 weeks, respectively, with a 20.2% 1-year survival rate. The most common grade 3/4 AEs included fatigue/asthenia

(29%), lymphopenia (25%), myalgia (17%), and thrombocytopenia (5%). Additionally, 4 patients died as a result of treatment related fatal AEs (pulmonary and cerebral hemorrhage and disseminated intravascular coagulation)⁸⁸. A phase III study in 750 patients with metastatic RCC demonstrated that patients receiving sunitinib had longer OS rates compared with the IFN-alpha group (26.4 vs. 21.8 months; $P = 0.051$). Additionally, the median PFS in the sunitinib group was 11 months compared to 5 months in the IFN-alpha group ($P < 0.001$). The most common grade 3 AEs reported in the sunitinib group was hypertension (12%), fatigue (11%), and diarrhea (9%). A total of 23 patients in the sunitinib group and 20 patients in the IFN-alpha group died while receiving treatment; the causes of death included disease progression, renal failure, and respiratory failure⁸⁹.

Currently, the additive effect of sunitinib in combination with different chemotherapeutic agents is being evaluated in several ongoing trials. For example, a phase I/II trial assessing the combination of sunitinib with paclitaxel and carboplatin in advanced breast cancer is currently underway [NCT00887575]. Also, a phase II randomized trial in sunitinib-refractory patients with metastatic RCC is evaluating the safety and efficacy of bevacizumab alone or in combination with sunitinib [NCT00556205].

Pazopanib

A recent approval has been granted to pazopanib hydrochloride for the treatment of advanced RCC. Sternberg et al conducted an international, randomized, double-blind, placebo-controlled, phase III trial evaluating the safety and efficacy of pazopanib monotherapy in 435 patients with locally advanced or mRCC previously untreated or treated with a cytokine-based regimen⁹⁰. Patients were randomized to receive pazopanib monotherapy ($n=290$) or placebo ($n=145$). The study showed a significant increase in overall median PFS in the pazopanib group compared to placebo (9.2 vs. 4.2 months; HR: 0.46; $P < 0.001$). The ORR was 30% in the pazopanib group compared to 3% in the placebo group. The most common AEs associated with pazopanib were diarrhea (52%), hypertension (40%), changes in hair color (38%), nausea (26%), and anorexia (22%). Additionally, myocardial infarction/ischemia occurred in 2% of the patients receiving pazopanib. Fatal AEs associated with pazopanib occurred in 4% of patients; these AEs included ischemic stroke, rectal hemorrhage, abnormal hepatic function, and bowel perforation. The authors concluded that, in treatment-naïve and cytokine-pretreated patients with advanced and/or metastatic RCC, pazopanib monotherapy demonstrated significant improvement in PFS compared to placebo.

Axitinib

Axitinib is a potent second generation inhibitor of VEGF-1, 2, and 3. Unlike first generation inhibitors, axitinib offers more selective specificity for VEGF, and does not block off-targets such as PDGF, b-RAF, FLT-3, and KIT⁹¹. In January 2012, axitinib received FDA approval in the treatment of renal cell carcinoma after failure of a prior systemic therapy. This approval was based upon the results of one phase III open-label study (AXIS trial). AXIS randomized 723 patients to receive axitinib 5 mg orally twice daily or sorafenib 400 mg orally twice daily. Patients could have their axitinib dose escalated at physician discretion unless patient's blood pressure was greater than 150/80 mm Hg or patient received antihypertensive therapy. The primary endpoint of the study was PFS. Secondary endpoints were OS, objective response rate, duration of response, and disease progression. Investigators met their primary endpoint. The median PFS was 6.7 months with axitinib therapy and 4.7 months with sorafenib (HR=0.665, 95%CI 0.544–0.8112). In patients who received previous cytokine therapy, the media PFS was 12.1 months with axitinib and 6.5 months with sorafenib (HR=0.464, 95%CI 0.318–0.676). Interestingly PFS data favored the sorafenib cohort in those patients who received prior bevacizumab, however, this did not

meet statistical significance. Improvements in objective response rates favored axitinib treated patients (19% vs 9%, $p=0.0001$) with a median duration of response of 11 months. At the time of publication, OS data was not available. The most common adverse events associated with axitinib therapy was diarrhea, hypertension, fatigue, nausea, and dysphonia⁹².

Other multi-TKIs that target VEGFR, among other receptors, and are pending approval include motesanib, cediranib, and vatalanib (Table 1).^{3, 79,93–97}

Biomarkers and Predicting Response

Given the cost and adverse events associated with anti-VEGF therapies, the identification of biomarkers or genetic polymorphisms to identify patients which would best respond to drug therapies is desired. Currently available data suggests that VEGF is not a predictive biomarker for clinical response to BEV. Following a phase III study which evaluated BEV in combination with capecitabine, primary tumor samples from paraffin blocks were placed in tissue microarrays and evaluated for VEGF expression by *in situ* hybridization (ISH). In this small analysis, response rates were not higher in BEV receiving patients who had VEGF overexpression.⁹⁸ Similarly, 312 tissue samples were collected from the 813 colorectal cancer patients that randomly received IFL (irinotecan, 5-FU, leucovorin) plus BEV or placebo. Epithelial and stromal VEGF expression was assessed by ISH and immunohistochemistry on tissue microarrays and whole sections. Thrombospondin (THBS) was also examined by ISH. In this analysis, the addition of BEV improved survival regardless of VEGF and THBS expression.⁹⁹ Similarly others have demonstrated that pretreatment serum levels of VEGF did not correlate with clinical outcome.^{100, 101}

VEGF levels have also been evaluated as a predictive biomarker for oral TKIs. Escudier et al. assessed VEGF levels from 712 patients in the TARGET trial.¹⁰² This study was a randomized, double-blind, placebo controlled study of sorafenib in advanced clear cell renal cell carcinoma. Baseline VEGF levels were categorized as high or low based upon a median value of 131 pg/mL. In a univariate analysis, VEGF levels correlated inversely with PFS ($P=0.013$) and OS ($P=0.0009$). Patients in the high-VEGF group trended to receive more benefit from sorafenib (HR=0.48; 95CI, 0.38–0.62) than the low-VEGF group (HR=0.64; 95CI, 0.49–0.83, $P=0.96$).

Single nucleotide polymorphisms (SNPs) that reside with regions of VEGF and VEGFR2 have shown promise as predictors of response or toxicity. A retrospective trial was performed on paraffin-embedded tumor blocks from breast cancer patients that participated in the E2100 study.¹⁰³ Investigators sought the association of VEGF and VEGF2 protein expression assessed by immunohistochemistry (IHC) with clinical outcomes. Three hundred seventy-seven eligible patients were eligible for VEGF IHC analysis, whereas 341 patients were available for VEGFR-2 IHC. The VEGF-2578 AA genotype was associated with improved median overall survival in patients who received paclitaxel + BEV when compared to alternative genotypes (HR=0.58; 95 CI, 0.36–0.93, $P=0.023$). Also, the VEGF-1154 A allele demonstrated an improved median OS with an additive effect of each active allele in the combination arm (HR 0.62; 95CI, 0.46–0.83, $p=0.001$). Another important finding of this investigation was the association of VEGF genotype with the development of clinically significant hypertension. Patients with VEGF-1498 TT and VEGF-634 CC genotypes were less likely to experience Grade 3/4 hypertension. Some have proposed could the development of hypertension act as a biological surrogate marker for anti-VEGF therapy as does the development of skin rash for EGFR based therapies. In this investigation, patients that developed Grade 3/4 hypertension had a superior median OS compared to no hypertension (38.7 months vs. 25.3 months, $P=0.002$). In metastatic clear

cell carcinoma patients treated with sunitinib, VEGF SNP -634 was the only SNP to be an independent predictor of the duration of HTN ($P=0.02$) whereas patients with VEGF SNP 936 combined with VEGFR2 SNP 889 genotypes were associated with OS.¹⁰⁴ In conclusion, early retrospective investigations suggest that SNPs within VEGF can impact prognosis, clinical efficacy, and toxicity. Further prospective studies are needed to address which patients might derive additional benefit from anti-VEGF based therapies.

Emergence of Tumor Resistance

Resistance to antiangiogenic therapy is a challenging issue that is associated with poor prognosis in some cancers. Multiple mechanisms have been proposed in the development of tumor resistance to anti-angiogenic therapy. One of the major factors contributing to tumor resistance is that the growth requirements for cancer cells at different stages of development are varied and promiscuous. Some evidence show that tumors start secreting their own growth factors, which changes their dependence on the stromal factors. For example, alternate growth stimulators (PIGF, bFGF, PDGF)⁷ have been reported in anti-VEGF-therapy resistant patients.

Recruitment of CD11b+ Granulocyte-differentiation antigen (Gr1+) myeloid suppression cells was also reported as a mechanism of resistance. These cells were found to protect tumors through modifications of immune responses to tumors¹⁰⁵ via secretion of certain angiogenesis modulators and through their ability to shift their phenotype to become ECs (vascular mimicry). The presence of cancer stem cells is a core component of tumor tissue and a precursor to resistance as they are suggested to be resistant to chemotherapy and can induce angiogenesis.¹⁰⁶ Bone marrow mobilization and incorporation of this cell population seems to be higher following treatment with high doses of chemotherapy, suggesting the need for less mobilizing approaches (metronomic dosing) as a way to evade resistance.

The population of cells comprising tumors is not homogenous; rather it is composed of a heterogeneous set of cells with different profiles of expressions, which contributes to the variation in responses seen from tumors to chemotherapy. Administration of chemotherapy does not eliminate all of the cancerous cells as it spares the innately resistant ones⁷.

Conclusions

The anti-angiogenic agents have long been proposed for the treatment of cancers. Recent advances in technology, knowledge on various anti-angiogenic agents, identification of potential targets and understanding on the molecular mechanisms by which these agents elicit their responses in multiple cells have made it possible to use anti-angiogenic therapy as an effective approach for the treatment of many cancers. While a number of anti-angiogenic agents have currently been approved for multiple cancers, many more are in the different phases of clinical trials for cancer treatment where anti-angiogenic agents are either used alone or in combinations with other cytotoxic and/or chemotherapeutic drugs. However, despite this fantastic progress, a few major concerns remain in the anti-angiogenic approach for cancer treatment. These include tumor resistance due to over compensation from the parallel signaling pathways and side effects these agents can cause on many organs because of its effects on the normal vasculature. Additional laboratory research and well-designed clinical studies are necessary to improve on the existing agents, approaches and combinations of drugs for the use of anti-angiogenic agents for cancer treatment.

Acknowledgments

Studies in the laboratory are funded by the University of Georgia Research Foundation, Wilson Pharmacy Foundation of the UGA College of Pharmacy and the Department of Clinical and Administrative Pharmacy through

intramural grants to PRS. We also acknowledge the funding from the National Institutes of Health grant (R01HL103952) and American Heart Association Scientist Development Grant (0830326N) to PRS.

Abbreviations

FDA	Food and Drug Administration
VEGF	vascular endothelial growth factor
NSCLC	non-small cell lung cancer
HIF-1α	hypoxia-inducible factor-1 α
HSP90	heat shock protein 90
PDGF	Platelet-Derived Growth Factor
a/bFGF	acidic/basic Fibroblast Growth Factor
PIGF	Placental Growth Factor
EC(s)	endothelial cell(s)
SDF-1	Stromal Cell-Derived Factor-1
CXCR4	Chemokine Receptor 4
VPF	vascular permeability factor
Flt-1	fms-like-tyrosine kinase-1
FLK-1/4	fetal liver kinase-1/4
KDR	kinase domain region
NRP-1/2	neuropilin-1/2
PI3K	Phosphoinositide 3-kinase
MAPK	Mitogen-Activated Protein Kinase
FAK	Focal Adhesion Kinase
MMP	Matrix Metalloproteinase
uPA(-r)	urokinase-type plasminogen activator Urokinase (receptor)
TTPAI-1	tissue-type plasminogen activator inhibitor-1
ERK	Extracellular Signal-Regulated Kinase
JNK	c-Jun N-Terminal Kinase
SAPK	Stress-Activated Protein Kinase
PKC	Protein Kinase C
Shc	Src Homology-2 Domain-Containing Protein
PLC-γ	Phospholipase C- γ
Cbl	Casitas B-Lineage Lymphoma Protein
IRS1	Insulin Receptor Substrate1
SLP76	Src Homology-2 domain containing leukocyte protein of 76kDa
ETs	Endothelins
ECEs	Endothelin-Converting Enzyme

ETAR/ETBR	ET receptors A/B
EPO	Epoietin
Tie-2	Tyrosine Kinase with Immunoglobulin-like and EGF-like domains-1
Ang-1/2/3/4	Angiopoietin-1/2/3/4
eNOS	endothelial Nitric Oxide Synthase
PAK	p21-Activated Kinase
Robo	Roundabout receptors
ArfGAP	Accessory Recombination Function-GTPase Activating Protein
DCC	deleted in colorectal cancer receptor
UNC5H	uncoordinated-5-homolog receptor
DAPk	Death-associated protein kinase 1
PR65β	Protein Phosphatase 2A 65kDa Regulatory Subunit A beta isoform
DLL4	Delta like Ligand-4
ECM	extracellular matrix
CSL	CBF1 (CMP-Binding Factor), Suppressor of Hairless, Lag-1
CoA	coactivators
ICAM-2	Intracellular Adhesion Molecule-2
SCID	Severe Combined Immunodeficiency
CAF	Carcinoma-associated fibroblasts
TKI	tyrosine kinase inhibitors
CSF-1R	Colony Stimulating Factor-1 Receptor
EGFR	Epidermal Growth Factor Receptor
ErbB2	erythroblastic leukemia viral oncogene homolog 2 protein
HER2	human epidermal growth factor receptor 2
CDK	Cyclin-Dependent Kinase
IL-6	Interleukin-6
TNFβ	Tumor Necrosis Factor- β
BV8	Bombina variegata 8kDa protein
5-FU	5-Fluorouracil
mCRC	metastatic colorectal cancer
ORR	overall response rate
OS	overall survival
PFS	progression free survival
RR	response rate
LV	leucovorin
XELOX	Xeloda® (Capecitabine) plus Oxaliplatin

FOLFOX	Folinic Acid (Leucovorin) plus 5-FU Plus Oxaliplatin
AE	Adverse Events
RECIST	Response Evaluation Criteria in Solid Tumors
HR	Hazard ratio
VEGF-AS	VEGF-antisense
IFN	Interferon
Bcr/Abl	Breakpoint Cluster Region/Abelsome fusion gene
CML	chronic myelogenous leukemia
GIST	gastrointestinal stromal tumor
Ph+	Philadelphia chromosome-positive
TTP	time to tumor progression
RCC	Renal Cell Carcinoma

References

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285:1182–6. [PubMed: 4938153]
2. Hwang C, Heath EI. Angiogenesis inhibitors in the treatment of prostate cancer. *J Hematol Oncol.* 2010; 3:26. [PubMed: 20678204]
3. Wu HC, Huang CT, Chang DK. Anti-Angiogenic Therapeutic Drugs for Treatment of Human Cancer. *J Cancer Mol.* 2008; 4:37–45.
4. FDA. Avastin (bevacizumab) Information. May 25th. 2011 <http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm193900.htm>
5. Medical Subject Headings - National Library of Medicine - National Institute of Health. [Last Accessed: April 11th, 2012] Available from: <http://www.ncbi.nlm.nih.gov/mesh/68018919>
6. Holland, JF. Cancer medicine. 4. Baltimore: Williams & Wilkins; 1997.
7. Kerbel RS. Tumor angiogenesis. *The New England journal of medicine.* 2008; 358:2039–49. [PubMed: 18463380]
8. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A.* 1995; 92:5510–4. [PubMed: 7539918]
9. Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. Transactivation and inhibitory domains of hypoxia-inducible factor 1alpha. Modulation of transcriptional activity by oxygen tension. *J Biol Chem.* 1997; 272:19253–60. [PubMed: 9235919]
10. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature.* 2005; 438:967–74. [PubMed: 16355214]
11. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science.* 1983; 219:983–5. [PubMed: 6823562]
12. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun.* 1989; 161:851–8. [PubMed: 2735925]
13. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol.* 2005; 23:1011–27. [PubMed: 15585754]
14. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol.* 2009; 21:154–65. [PubMed: 19230644]

15. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003; 9:669–76. [PubMed: 12778165]
16. Wang Y, Nakayama M, Pitulescu ME, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature.* 2010; 465:483–6. [PubMed: 20445537]
17. Neufeld G, Cohen T, Shraga N, Lange T, Kessler O, Herzog Y. The neuropilins: multifunctional semaphorin and VEGF receptors that modulate axon guidance and angiogenesis. *Trends Cardiovasc Med.* 2002; 12:13–9. [PubMed: 11796239]
18. Fischer C, Jonckx B, Mazzone M, et al. Anti-PIGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell.* 2007; 131:463–75. [PubMed: 17981115]
19. Lu, C.; Sood, A. Role of Pericytes in Angiogenesis. In: Teicher, BA.; Ellis, LM., editors. *Antiangiogenic Agents in Cancer Therapy.* 2. Totawa, NJ: Humana Press; 2008. p. 117–32.
20. Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science.* 1997; 277:242–5. [PubMed: 9211853]
21. Hellstrom M, Gerhardt H, Kalen M, et al. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol.* 2001; 153:543–53. [PubMed: 11331305]
22. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev.* 1994; 8:1875–87. [PubMed: 7958863]
23. Carmeliet P. Angiogenesis in life, disease and medicine. *Nature.* 2005; 438:932–6. [PubMed: 16355210]
24. Teicher BA. Antiangiogenic agents and targets: A perspective. *Biochem Pharmacol.* 2011; 81:6–12. [PubMed: 20920481]
25. Rusnati, M.; Presta, M. Targeting Fibroblast Growth Factor/Fibroblast Growth Factor Receptor System in Angiogenesis. In: Davis, DW.; Herbst, R.; Abbruzzese, JL., editors. *Antiangiogenic cancer therapy.* Boca Raton: CRC Press; 2008. p. 208–23.
26. Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T. Endothelin-induced apoptosis of A375 human melanoma cells. *J Biol Chem.* 1998; 273:12584–92. [PubMed: 9575219]
27. Smollich M, Wulfig P. Targeting the endothelin system: novel therapeutic options in gynecological, urological and breast cancers. *Expert Rev Anticancer Ther.* 2008; 8:1481–93. [PubMed: 18759699]
28. Wang R, Dashwood RH. Endothelins and their receptors in cancer: identification of therapeutic targets. *Pharmacological research: the official journal of the Italian Pharmacological Society.* 2011; 63:519–24. [PubMed: 21251982]
29. Aapro M, Jelkmann W, Constantinescu SN, Leyland-Jones B. Effects of erythropoietin receptors and erythropoiesis-stimulating agents on disease progression in cancer. *British journal of cancer.* 2012; 106:1249–58. [PubMed: 22395661]
30. Acs G, Chen M, Xu X, Acs P, Verma A, Koch CJ. Autocrine erythropoietin signaling inhibits hypoxia-induced apoptosis in human breast carcinoma cells. *Cancer Lett.* 2004; 214:243–51. [PubMed: 15363551]
31. Hale SA, Wong C, Lounsbury KM. Erythropoietin disrupts hypoxia-inducible factor signaling in ovarian cancer cells. *Gynecol Oncol.* 2006; 100:14–9. [PubMed: 16226302]
32. Wu X, Liu N. The role of Ang/Tie signaling in lymphangiogenesis. *Lymphology.* 2010; 43:59–72. [PubMed: 20848993]
33. Seegar TC, Eller B, Tzvetkova-Robev D, et al. Tie1–Tie2 interactions mediate functional differences between angiopoietin ligands. *Mol Cell.* 2010; 37:643–55. [PubMed: 20227369]
34. Steigemann P, Molitor A, Fellert S, Jackle H, Vorbruggen G. Heparan sulfate proteoglycan syndecan promotes axonal and myotube guidance by slit/robo signaling. *Curr Biol.* 2004; 14:225–30. [PubMed: 14761655]
35. Palacios F, Price L, Schweitzer J, Collard JG, D'Souza-Schorey C. An essential role for ARF6-regulated membrane traffic in adherens junction turnover and epithelial cell migration. *Embo J.* 2001; 20:4973–86. [PubMed: 11532961]
36. London NR, Li DY. Robo4-dependent Slit signaling stabilizes the vasculature during pathologic angiogenesis and cytokine storm. *Curr Opin Hematol.* 2011; 18:186–90. [PubMed: 21423011]

37. Latil A, Chene L, Cochant-Priollet B, et al. Quantification of expression of netrins, slits and their receptors in human prostate tumors. *Int J Cancer*. 2003; 103:306–15. [PubMed: 12471613]
38. Mehlen P, Guenebeaud C. Netrin-1 and its dependence receptors as original targets for cancer therapy. *Current opinion in oncology*. 2010; 22:46–54. [PubMed: 19934758]
39. Delloye-Bourgeois C, Brambilla E, Coissieux MM, et al. Interference with netrin-1 and tumor cell death in non-small cell lung cancer. *J Natl Cancer Inst*. 2009; 101:237–47. [PubMed: 19211441]
40. Sethi N, Kang Y. Notch signalling in cancer progression and bone metastasis. *British journal of cancer*. 2011; 105:1805–10. [PubMed: 22075946]
41. Uyttendaele H, Marazzi G, Wu G, Yan Q, Sassoon D, Kitajewski J. Notch4/int-3, a mammary proto-oncogene, is an endothelial cell-specific mammalian Notch gene. *Development*. 1996; 122:2251–9. [PubMed: 8681805]
42. Real C, Remedio L, Caiado F, et al. Bone marrow-derived endothelial progenitors expressing delta-like 4 (dll4) regulate tumor angiogenesis. *PLoS One*. 2011; 6:e18323. [PubMed: 21483741]
43. Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand binding to integrins. *J Biol Chem*. 2000; 275:21785–8. [PubMed: 10801897]
44. Gladson CL. Expression of integrin alpha v beta 3 in small blood vessels of glioblastoma tumors. *J Neuropathol Exp Neurol*. 1996; 55:1143–9. [PubMed: 8939197]
45. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science*. 1994; 264:569–71. [PubMed: 7512751]
46. Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest*. 1995; 96:1815–22. [PubMed: 7560073]
47. Somanath PR, Ciocea A, Byzova TV. Integrin and growth factor receptor alliance in angiogenesis. *Cell biochemistry and biophysics*. 2009; 53:53–64. [PubMed: 19048411]
48. Reinmuth N, Liu W, Ahmad SA, et al. Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice. *Cancer Res*. 2003; 63:2079–87. [PubMed: 12727823]
49. Romanov VI, Goligorsky MS. RGD-recognizing integrins mediate interactions of human prostate carcinoma cells with endothelial cells in vitro. *Prostate*. 1999; 39:108–18. [PubMed: 10221566]
50. National Cancer Institute. [Accessed September 15, 2011] Angiogenesis Inhibitors in the Treatment of Human Cancer. (Accessed at <http://www.cancer.gov/cancertopics/understandingcancer/angiogenesis/page20>.)
51. Li, W.; Li, V.; Tsakayannis, D. Angiogenesis Therapies: Concepts, Clinical Trials, and Considerations for New Drug Development. In: Fan, T-PDKEC., editor. *The New Angiotherapy*. 2002. p. 547-71.
52. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004; 350:2335–42. [PubMed: 15175435]
53. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol*. 2007; 25:1539–44. [PubMed: 17442997]
54. Allegra CJ, Yothers G, O'Connell MJ, et al. Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: results of NSABP protocol C-08. *J Clin Oncol*. 2011; 29:11–6. [PubMed: 20940184]
55. Mancuso MR, Davis R, Norberg SM, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest*. 2006; 116:2610–21. [PubMed: 17016557]
56. DeGramont A, Van Cutsem E, Tabernero M, Moore MJ. AVANT: Results from a randomized, three-arm multinational phase III study to investigate bevacizumab with either XELOX or FOLFOX4 versus FOLFOX4 alone as adjuvant treatment for colon cancer. *J Clin Oncol*. 2011; 29:362. [PubMed: 21149658]
57. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med*. 2006; 355:2542–50. [PubMed: 17167137]

58. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAiL. *J Clin Oncol.* 2009; 27:1227–34. [PubMed: 19188680]
59. Reck M, von Pawel J, Zatloukal P, et al. Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann Oncol.* 2010; 21:1804–9. [PubMed: 20150572]
60. Godard S, Getz G, Delorenzi M, et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer Res.* 2003; 63:6613–25. [PubMed: 14583454]
61. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol.* 2009; 27:4733–40. [PubMed: 19720927]
62. Cloughesy TF, Filka E, Kuhn J, et al. Two studies evaluating irinotecan treatment for recurrent malignant glioma using an every-3-week regimen. *Cancer.* 2003; 97:2381–6. [PubMed: 12712460]
63. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol.* 2009; 27:740–5. [PubMed: 19114704]
64. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet.* 2007; 370:2103–11. [PubMed: 18156031]
65. Rini BI, Halabi S, Rosenberg JE, et al. Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. *J Clin Oncol.* 2010; 28:2137–43. [PubMed: 20368558]
66. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med.* 2007; 357:2666–76. [PubMed: 18160686]
67. Horning, S. [Assessed March 15, 2012] <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM219979.pdf>
68. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol.* 2010; 28:3239–47. [PubMed: 20498403]
69. Robert NJ, Dieras V, Glaspy J, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. *J Clin Oncol.* 2011; 29:1252–60. [PubMed: 21383283]
70. Brufsky AM, Hurvitz S, Perez E, et al. RIBBON-2: a randomized, double-blind, placebo-controlled, phase III trial evaluating the efficacy and safety of bevacizumab in combination with chemotherapy for second-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol.* 2011; 29:4286–93. [PubMed: 21990397]
71. Fojo T, Parkinson DR. Biologically targeted cancer therapy and marginal benefits: are we making too much of too little or are we achieving too little by giving too much? *Clin Cancer Res.* 2010; 16:5972–80. [PubMed: 21169250]
72. Kelly WK, Halabi S, Carducci MA, George DJ. A randomized, double-blind, placebo-controlled phase III trial comparing docetaxel, prednisone, and placebo with docetaxel, prednisone, and bevacizumab in men with metastatic castration-resistant prostate cancer (mCRPC): Survival results of CALGB 90401. *J Clin Oncol.* 2010; 28s:4511.
73. Ohtsu A, Shah MA, Van Cutsem E, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol.* 2011; 29:3968–76. [PubMed: 21844504]
74. Perren TJ, Swart AM, Pfisterer J, et al. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med.* 2011; 365:2484–96. [PubMed: 22204725]
75. Burger RA, Brady MF, Bookman MA, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med.* 2011; 365:2473–83. [PubMed: 22204724]
76. Tabernero, J.; Van Cutsem, E.; Lakomy, R., et al. Results from VELOUR, a phase 3 study of aflibercept versus placebo in combination with FOLFIRI for the treatment of patients with

- previously treated metastatic colorectal cancer. European Multidisciplinary Congress. Ab.6LBA; 2011.
77. Novello S, Ramlay R, Borbunova VA, et al. Aflibercept in combination with docetaxel for second-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC): Final results of a multinational placebo-controlled phase III trial (EFC10261-VITAL) Proceedings from the interational association for the study of lung cancer:0.43.06.
 78. Ma J, Waxman DJ. Combination of antiangiogenesis with chemotherapy for more effective cancer treatment. *Mol Cancer Ther.* 2008; 7:3670–84. [PubMed: 19074844]
 79. Kelly RJ, Darnell C, Rixe O. Target inhibition in antiangiogenic therapy a wide spectrum of selectivity and specificity. *Cancer J.* 2010; 16:635–42. [PubMed: 21131797]
 80. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med.* 2008; 359:378–90. [PubMed: 18650514]
 81. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med.* 2007; 356:125–34. [PubMed: 17215530]
 82. Ratain MJ, Eisen T, Stadler WM, et al. Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol.* 2006; 24:2505–12. [PubMed: 16636341]
 83. Escudier B, Szczylik C, Hutson TE, et al. Randomized phase II trial of first-line treatment with sorafenib versus interferon Alfa-2a in patients with metastatic renal cell carcinoma. *J Clin Oncol.* 2009; 27:1280–9. [PubMed: 19171708]
 84. Jonasch E, Corn P, Pagliaro LC, et al. Upfront, randomized, phase 2 trial of sorafenib versus sorafenib and low-dose interferon alfa in patients with advanced renal cell carcinoma: clinical and biomarker analysis. *Cancer.* 2010; 116:57–65. [PubMed: 19862815]
 85. Blumenschein GR Jr, Gatzemeier U, Fossella F, et al. Phase II, multicenter, uncontrolled trial of single-agent sorafenib in patients with relapsed or refractory, advanced non-small-cell lung cancer. *J Clin Oncol.* 2009; 27:4274–80. [PubMed: 19652055]
 86. Scagliotti G, Novello S, von Pawel J, et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small-cell lung cancer. *J Clin Oncol.* 2010; 28:1835–42. [PubMed: 20212250]
 87. Burstein HJ, Elias AD, Rugo HS, et al. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. *J Clin Oncol.* 2008; 26:1810–6. [PubMed: 18347007]
 88. Socinski MA, Novello S, Brahmer JR, et al. Multicenter, phase II trial of sunitinib in previously treated, advanced non-small-cell lung cancer. *J Clin Oncol.* 2008; 26:650–6. [PubMed: 18235126]
 89. Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol.* 2009; 27:3584–90. [PubMed: 19487381]
 90. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol.* 2010; 28:1061–8. [PubMed: 20100962]
 91. Hu-Lowe DD, Zou HY, Grazzini ML, et al. Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. *Clin Cancer Res.* 2008; 14:7272–83. [PubMed: 19010843]
 92. Rini BI, Escudier B, Tomczak P, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. *Lancet.* 2011; 378:1931–9. [PubMed: 22056247]
 93. Leigh NB, Raez LE, Besse B, et al. A multicenter, phase 2 study of vascular endothelial growth factor trap (Aflibercept) in platinum- and erlotinib-resistant adenocarcinoma of the lung. *J Thorac Oncol.* 2010; 5:1054–9. [PubMed: 20593550]
 94. Goss GD, Arnold A, Shepherd FA, et al. Randomized, double-blind trial of carboplatin and paclitaxel with either daily oral cediranib or placebo in advanced non-small-cell lung cancer: NCI clinical trials group BR24 study. *J Clin Oncol.* 2010; 28:49–55. [PubMed: 19917841]
 95. Blumenschein GR Jr, Kabbinar F, Menon H, et al. A phase II, multicenter, open-label randomized study of motesanib or bevacizumab in combination with paclitaxel and carboplatin for

- advanced nonsquamous non-small-cell lung cancer. *Ann Oncol.* 2011; 22:2057–67. [PubMed: 21321086]
96. Herbst RS, Sun Y, Eberhardt WE, et al. Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer (ZODIAC): a double-blind, randomised, phase 3 trial. *Lancet Oncol.* 2010; 11:619–26. [PubMed: 20570559]
97. Wang J, Sun Y, Liu Y, et al. Results of randomized, multicenter, double-blind phase III trial of rh-endostatin (YH-16) in treatment of advanced non-small cell lung cancer patients. *Zhongguo Fei Ai Za Zhi.* 2005; 8:283–90. [PubMed: 21108883]
98. Hillan KJ, Koeppen KW, Tobin P, Pham T. The role of VEGF expression in response to bevacizumab plus capecitabine in metastatic breast cancer (MBC). *Proc Am Soc Clin Oncol.* 2003; 22:766.
99. Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. *J Clin Oncol.* 2006; 24:217–27. [PubMed: 16365183]
100. Kindler HL, Friberg G, Singh DA, et al. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. *J Clin Oncol.* 2005; 23:8033–40. [PubMed: 16258101]
101. Holden SN, Ryan E, Kearns A, Holmgren E, Hurwitz H. Benefit from bevacizumab (BV) is independent of pretreatment plasma vascular endothelial growth factor-A (pl-VEGF) in patients (pts) with metastatic colorectal cancer (mCRC). *J Clin Oncol.* 2005; 23:3555.
102. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol.* 2009; 27:3312–8. [PubMed: 19451442]
103. Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. *J Clin Oncol.* 2008; 26:4672–8. [PubMed: 18824714]
104. Kim JJ, Vaziri SA, Rini BI, et al. Association of VEGF and VEGFR2 single nucleotide polymorphisms with hypertension and clinical outcome in metastatic clear cell renal cell carcinoma patients treated with sunitinib. *Cancer.* 2012; 118:1946–54. [PubMed: 21882181]
105. Agaoglu S, Carosella ED, Rouas-Freiss N. Role of HLA-G in tumor escape through expansion of myeloid-derived suppressor cells and cytokine balance favoring Th2 vs Th1/Th17. *Blood.* 2011
106. Marx J. Cancer research. Mutant stem cells may seed cancer. *Science.* 2003; 301:1308–10. [PubMed: 12958339]

Figure 1. Main events in Angiogenesis

Tumors cells, exposed to hypoxia within the tumor core, induce the expression of many factors that in turn recruit endothelial cells to the core of the tumor and promote the formation of new blood vessels. Interactions of both cell types result in many events and leads to the recruitment of many other cell lines. This recruitment of other cell lines completes a vicious cycle that further stabilizes the newly formed blood vessels and enhances tumor growth and angiogenesis.

\$watermark-text

\$watermark-text

\$watermark-text

Clinical Trials (Most recent published data–Breast/Prostate/NSCLC/CRC/RCC)

Table 1

Drug	Class/MOA	Molecular Target	Current Status	Trial Design	N	Cancer Type	Investigational Drug Schedule	Key Findings-Endpoints	Most Frequent AEs	Most Frequent Grade 3 AEs	Treatment-Related Deaths	Authors' Conclusion	Ref.
Aflibercept	VEGF-Trap	VEGF-A and PlGF	Phase III	Phase II, open label, single arm, multicenter trial	98	NSCLC refractory to platinum and erlotinib therapy	4 mg/kg IV every 2 weeks	ORR: 2%; Median: PFS: 2.7 months; Median OS: at 6- and 12-months, 54% and 29%, respectively	Fatigue (42%), hypertension (40%), dyspnea (40%), headache (39%), constipation (28%).	Hypertension (23%), dyspnea (21%), proteinuria (10%), fatigue (7%)	Grade 5 hemoptysis occurred in 2 patients	Although well tolerated, aflibercept monotherapy has little activity in patients with heavily pretreated lung adenocarcinoma	[102]
Cediranib	TKI	VEGFR1–3, c-Kit	Phase III	Phase II/III, double-blind, placebo-controlled	296	Stage IIIB/IV NSCLC	cediranib 30 mg PO once daily +/- paclitaxel and carboplatin	Phase II: adjusted PFS was 0.77 (95% CI, 0.56 to 1.08); significantly higher RR in cediranib compared to placebo (38% vs. 16%, respectively; P<0.0001). Phase III: study did not proceed to that phase	Fatigue (88%), diarrhea (79%), dyspnea (75%), sensory neuropathy (63%)	Neutropenia (49%), fatigue (29%), hypertension (19%), diarrhea (15%), dyspnea (10%),	Death occurred in nine patients due to febrile neutropenia, hemoptysis, disseminated intravascular coagulation, or hepato-renal failure	Although combination of cediranib with paclitaxol/carboplatin improved PFS, cediranib 30 mg was not tolerable.	[103]
Motesanib	TKI	VEGFR1–3, c-Kit, PDGFR	Phase III	Phase II, randomized trial	186	Advanced non-squamous NSCLC- Chemotherapy naïve	Paclitaxel and carboplatin + A) M125 mg daily, B) M75 mg BID 5 days on and 2 days off, OR C) Bev 15 mg/kg every 3 weeks	ORR was as follows: A) 30% (95% CI, 18 to 43%) B) 23% (95% CI, 13 to 36%) C) 37% (95% CI, 25 to 50%); Median PFS and OS were as follows: A) 7.7 and 14 months B) 5.8 and 12.8 months C) 8.3 and 14 months	Patients in group A experienced higher incidence of AEs than groups B and C	More grade 5 AEs occurred in group B than in A and C.		Efficacy of motesanib 125 mg daily os comparable to bevacizumab; both in combination with paclitaxol and carboplatin. Motesanib is associated with higher incidence of manageable toxicities	[104]
Vandetanib	TKI	VEGFR-2, EGFR	Phase III	ZODIAC was a multinational, randomized, double-blind, phase III trial	1391	Locally advanced or metastatic (stage IIIB–IV) NSCLC refractory to chemotherapy	Docetaxol +/- vandetanib 100 mg/day	Median PFS was 4 months in vandetanib group compared to 3.2 months in the placebo group (P<0.0001). ORR significantly improved in the vandetanib group compared to placebo (17% vs. 10%, respectively; P=0.0001)	Diarrhea (42%), rash (42%), alopecia (33%), neutropenia (32%), fatigue (30%), anorexia (29%), leukopenia (18%)	Neutropenia (29%), leukopenia (14%), rash (9%)	In the vandetanib group, fatal adverse events occurred in 42 patients, these included febrile neutropenia, pneumonia, respiratory failure, interstitial lung disease, and stevens-johnson syndrome	Combination therapy of vandetanib and docetaxol significantly improved PFS in patients with advanced, refractory NSCLC	[105]
th-Endostatin	Recombinant human endostatin	VEGFR, c-myc, cyclin-D1, Id1 and -3, HIF1-α, Ephrin B1 and B2, MMP	Phase III	Double-blind, placebo-controlled, randomized, phase III trial	493	Stage IIIB and IV NSCLC	Vinorelbine and cisplatin + Endostar 7.5 mg/m2 on days 1 to 14 or placebo	In the Endostar group compared to placebo, ORR were 35.4% and 19.5%, respectively (P=0.0003), median TTP were 6.3 and 3.6 months, respectively (P<.001), and clinical benefit rates were 73.3% and 64%, respectively (P=0.035)		Neutropenia (28.5%), anemia (3.4%), nausea/vomiting (8%)	Two treatment-related deaths occurred in the Endostar group	The adjunct therapy of Endostar to vinorelbine and cisplatin showed significant improvement in ORR, TTP and clinical benefit rates compared to chemotherapy alone. Endostar has a favorable toxicity profile.	[106]

MBC: metastatic breast cancer; HCC: hepatocellular carcinoma; MCC: Metastatic Colorectal Cancer; mRCC: Metastatic Renal Cell Carcinoma; ORR: objective response rate; DR: duration of objective tumor response; TTP: time to tumor progression; OS: overall survival; PRO: patient reported outcomes; DR: duration of response; TTP: time to progression