LEADING ARTICLE

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# Antiangiogenic Therapy for Metastatic Breast Cancer

## Current Status and Future Directions

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## Abstract

The use of chemotherapy and endocrine therapy have led to objective tumour shrinkage and improved survival in women with metastatic breast cancer. Despite the availability of many chemotherapeutic drugs, these agents do not act specifically on the various growth signalling pathways that drive tumour growth and progression. This lack of specificity is likely to explain the inconsistent responses seen across the population of breast cancer patients and contributes to the undesirable adverse effects. The expanding knowledge of the important molecular pathways involved in tumourogenesis and tumour progression has led to the exciting development of several classes of targeted agents. The potential advantage of such treatment is to improve cancer cell kill with less damage to healthy tissues. Hormonal agents were the first to utilize the specific estrogen receptor-related growth pathways for therapeutic efficacy. Agents directed to the human epidermal growth factor receptor (HER)-2/neu growth signalling pathway exemplify the effectiveness of the new generation of targeted biological agents, but are limited to the 20–25% of breast cancers that overexpress the receptor. However, angiogenesis is a critical component of tumour development that is necessary for all tumour growth and is not limited to a subset of breast cancers. Therefore, agents that can diminish or prevent tumour angiogenesis are likely to have a far broader application and benefit to women with breast cancer.

Several anti-angiogenic agents have been evaluated in phase I, II and III trials for patients with metastatic breast cancer. These trials have demonstrated efficacy of anti-angiogenic agents when used in combination with chemotherapy and the toxicity profile has been better defined. Issues regarding the mechanisms of resistance, identifying combination regimens that result in the greatest clinical benefits and minimizing the adverse effects are areas that require further research.

Clinical research over the past two decades has established the important role of angiogenesis in the ongoing growth and dissemination of malignant cells. Vascular endothelial growth factor (VEGF) [also known as VEGFA] belongs to a family of growth regulators involved in new vessel growth and maturation with direct involvement in malignant cell proliferation. Earlier studies have demonstrated the presence of increased levels of VEGF in a number of malignancies and

its role in enabling malignant cell survival. This factor has also been shown to have a prognostic role in women with early breast cancer. Moreover, the angiogenic pathway can be targeted by several therapeutic agents currently in development.[1,2]

This review provides a brief background to the role of neovascularization in breast tumour development and the pivotal role of the VEGF family in this process. The agents that have demonstrated pre-clinical activity and have entered clinical evaluation for treatment of metastatic breast cancer (bevacizumab, sunitinib, sorafenib, pazopanib, cediranib, motasenib and aflibercept) are discussed.

## 1. Antiangiogenic Therapy

Neovascularization is a crucial component of cancer cell growth and progression. A number of proangiogenic factors have been identified that occur in higher levels in patients with breast cancer and are responsible for the perpetuation of tumour blood vessel development. Of these factors, the VEGF protein family is considered the most important, with the VEGFA isoform (commonly known as VEGF) being the most important member.

*In vitro* studies have confirmed that VEGF acts to: (i) promote endothelial cell growth via the presence of VEGF receptors present on arteries, veins and lymphatic channels; (ii) enhance endothelial cell survival via VEGF interacting with coactivators, such as phosphatidylinositol 3-kinase/Akt, Bcl-2 and survivin;[3] (iii) increase vascular permeability, which allows protein leakage and endothelial cell migration; (iv) stimulate vessel dilatation with subsequent blood flow and blood pressure effects; and (v) serve as a chemoattractant for bone marrow-derived progenitor cells.[4-6]

VEGF mRNA upregulation has been demonstrated in a number of malignancies including gastrointestinal tract, kidney, lung, bladder, intracranial and breast.[7] Levels of VEGF have been shown to increase during malignant transformation from adenomas to carcinomas, again suggesting a pivotal role in malignant progression.[8]

### 1.1 Anti-Vascular Endothelial Growth Factor Therapy

One of the earliest therapeutic agents developed to inhibit VEGF was the murine anti-human VEGF monoclonal antibody A4.6.1. Angiogenesis was potently suppressed in a number of human tumour cell lines that were transplanted into nude mice with resultant inhibition of neovascularization.[9] The humanization of an anti-VEGF monoclonal antibody (bevacizumab) was subsequently shown to decrease microvascular density, tumour perfusion, interstitial fluid pressure, and the number of viable endothelial and progenitor cells in colorectal carcinoma.[10] Several other agents have been developed with a goal to abrogate VEGF activity and these include vatalanib (PTK787, ZK 222584),[11] ZD4190,[12] monoclonal antibody DC101,[13] vandetanib (ZD6474),[14] cediranib (AZD2171),[15] motesanib (AMG706), pazopanib (GW786034)[16] and aflibercept (VEGF-Trap).[17] The mechanism of action of these agents varies, and includes specific anti-VEGF activity (VEGFA, VEGFB, VEGFD, placental growth factor [PIGF]), inhibition of VEGF receptors (VEGFRs) [VEGFR1, VEGFR2, VEGFR3, platelet derived growth factor receptor (PDGFR)-β, c-KIT (CD117), fibroblast growth factor (FGFR)1, FGFR3, VEGF-VEGFR binding], tyrosine kinase or raf kinase inhibition with or without VEGFR inhibition (fms-like tyrosine kinase [FLT]-3, epidermal growth factor receptor, RET).

Although several cytotoxic agents have been shown to have anti-angiogenic properties, this review focuses on targeted biological agents that primarily act as anti-VEGF agents. The mechanism of action of these agents can be categorized into those that inhibit VEGF at the protein level (directly and indirectly), inhibit angiogenic pathways at the receptor level, or interfere with downstream growth signalling pathways.

#### *1.1.1 Monotherapy*

Although monotherapy with anti-VEGF agents results in pruning of tumour blood vessels, normalization of tumour vasculature, reduction

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in the proliferative fraction of cancer cells, and reduction in circulating endothelial and progenitor cells; clinical studies with single anti-VEGF agents have not led to improved survival in patients with solid tumours.[5]

There are several possible mechanisms that may explain the 'resistance' to anti-VEGF agents, such that monotherapy is inadequate for tumour control. Endothelial cell heterogeneity may exist, which can be seen in the variability in function of endothelial cells in healthy tissues (e.g. brain endothelial cells exhibiting higher levels of the multidrug resistance protein responsible for the 'blood-brain barrier').

Tumour cell heterogeneity, with respect to the level and number of proangiogenic factors produced, can lead to a variable response to anti-angiogenic agents that specifically target only one or a few of these factors. In addition, the different 'clonality' of malignant cells in the primary and metastatic tumour will impact on the degree of response to any given single agent.

Tumour progression or regrowth by angiogenesis-independent pathways, such as vessel co-option,[18] intussusceptive microvascular growth, vascular mimicry and vasculogenesis, may contribute to the resistance.[19] The optimal scheduling and duration of therapy with anti-angiogenic compounds has not yet been established and pharmacokinetic factors may account for inadequate delivery of therapy to all tumour sites. Finally, alterations in the tumour microenvironment and compensatory response to therapy leading to a secondary increase in VEGF levels may also contribute to *de novo* or acquired resistance to anti-angiogenic agents given as monotherapy.[20]

#### *1.1.2 Combination Therapy*

Preclinical studies have demonstrated a greater degree of tumour regression with the administration of anti-angiogenic therapy in combination with conventional cytotoxic therapy.[21] It is theorized that simultaneous inhibition of proliferating tumour cells together with blunting of the proangiogenic pathways would result in a greater likelihood of improved patient outcome.

An alternate theory for the benefits of combining conventional cytotoxic drugs with anti-angiogenic agents has been proposed by Folkman and others. These investigators have shown that cytotoxic agents can result in inhibition of tumour angiogenesis in *in vivo* models as a result of interference with mitogen-activated host vasculature (e.g. vinca alkaloids) or a direct effect on tumour endothelial cells.[22,23] Furthermore, it has been demonstrated that the combination of a conventional cytotoxic agent and an anti-angiogenic agent resulted in restoration of response to a previously resistant cell line to the cytotoxic agent or to greater *in vivo* tumour cell control than with either therapy alone.[24,25]

Table I provides a current list of clinical trials that are evaluating anti-angiogenic agents alone or in combination with cytotoxic agents, endocrine therapy or other biological agents.

## 2. Clinical Application in Breast Cancer

### 2.1 Bevacizumab

Bevacizumab is a humanized monoclonal antibody directed against VEGF that prevents its binding to VEGR-1 and -2.[9] It has undergone extensive evaluation in the treatment of several solid tumour types, including colorectal, lung, renal and breast carcinomas. In these tumours, randomized trials have shown superiority in progression-free survival (PFS) or overall survival (OS) in favour of the anti-VEGF combination therapy compared with chemotherapy alone.[27-29]

An open-label trial was conducted in patients with measurable and non-measurable metastatic breast cancer who had received a minimum of one chemotherapy regimen in the advanced setting.[30] The dosage administered commenced at 3 mg/kg every 2 weeks, increasing to 10 mg/kg and then to a maximal dose of 20 mg/kg. A total of 75 patients were entered, with 82% having received two or more prior chemotherapy regimens and 85% of the 21 patients with human epidermal growth factor receptor (HER)-2/neu-positive disease having received trastuzumab. Five patients (6.7%) achieved an objective response, with a total of 12 patients (16%) having responsive or

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**Table I.** List of trial evaluating anti-angiogenic agents in breast cancer[26]

| Protocol identification | Phase | Cancer stage | Agent(s) being evaluated | HER2/neu status | Single arm (S)/ randomized (R) |
|---|---|---|---|---|---|
| SWS-SAKK-22/99 | III | Metastatic[a] | Trastuzumab followed by trastuzumab + paclitaxel vs trastuzumab + paclitaxel | Positive | R |
| AVF3693g | III | Metastatic[b] | Bevacizumab + chemotherapy | Negative/unknown | S |
| A6181094 | III | Metastatic[c] | Bevacizumab + paclitaxel vs sunitinib + paclitaxel | Positive or negative | R |
| BO20231 | III | Metastatic[a] | Trastuzumab + docetaxel vs bevacizumab + trastuzumab + docetaxel | Positive | R |
| A6181114 | III | Metastatic[b] | Sunitinib continuation after prior sunitinib[f] | Inclusion criteria of initial trial | S |
| A6181099 | III | Locally advanced/metastatic[b] | Capecitabine vs capecitabine + sunitinib | Not specified | R |
| MO19391 | III | Locally recurrent/metastatic[a] | Bevacizumab + paclitaxel or docetaxel | Positive or negative | S |
| ECOG-E1105 | III | Metastatic[a] | Trastuzumab + chemotherapy vs trastuzumab + chemotherapy + bevacizumab | Positive | R |
| GEICAM/2006-11 | III | Metastatic[a,d] | Letrozole vs letrozole + bevacizumab | Negative | R |
| CALGB-40503 | III | Inoperable IIIb/metastatic[a] | Tamoxifen or letrozole vs tamoxifen or letrozole + bevacizumab | Not specified | R |
| VEG108838 | II | Inflammatory breast cancer | Pazopanib + lapatinib vs lapatinib | Positive | R |
| CECOG/BC1.3.005 | III | Metastatic[a] | Paclitaxel + bevacizumab vs capecitabine + paclitaxel | Negative | S |
| GUMC-2004-251 | I/II | Metastatic[b] | Sorafenib + anastrozole | Not specified | S |
| 2004-0758 | I/II | Metastatic[b] | Everolimus + docetaxel | Not specified | S |
| 2005-0471 | I/II | Metastatic[e] | Everolimus + trastuzumab | Positive | S |
| AECM-NCI-7703 | I/II | Inoperable locally recurrent/metastatic[b] | Vorinostat + paclitaxel + bevacizumab | Not specified | S |
| 05-030 | I/II | Metastatic[b] | Capecitabine + bevacizumab | Positive or negative | S |
| 40706 | I/II | IIIa/IIIb/metastatic[a] | Sorafenib + letrozole | Not specified | S |
| CT/08.02 | I/II | Metastatic[a] | Paclitaxel + bevacizumab + carboplatin | Triple negative | S |
| CT/07.21 | I/II | Metastatic[a] | Docetaxel + bevacizumab + epirubicin | Negative | S |
| GRN163L CP14A010 | I/II | Metastatic[b] | Paclitaxel + bevacizumab + GRN163L | Positive or negative | S |
| 0903-882 | II | II (N2)/III/metastatic[b] in CR | Tetrathiomolybdate | Not specified | S |
| F050103001 | II | Metastatic[b] progressive on endocrine therapy | Endocrine agent + bevacizumab | Not specified | S |
| EudraCT 2005-004587-23 | II | Metastatic[b] | Nil vs sunitinib | Negative | R |

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**Table I.** Contd

| Protocol identification | Phase | Cancer stage | Agent(s) being evaluated | HER2/neu status | Single arm (S)/ randomized (R) |
|---|---|---|---|---|---|
| VEG20007 | II | Locally advanced/metastatic[a] | Pazopanib + lapatinib vs lapatinib | Positive | R |
| UMN-2006LS016 | II | Metastatic[b] | Naltrexone | Not specified | S |
| DOCET_L_00712 | II | Metastatic[a] | Docetaxel + bevacizumab ± trastuzumab | Positive or negative | Parallel S |
| BRSMTS0007 | II | Metastatic[a] | Paclitaxel + bevacizumab + gemcitabine | Negative | S |
| OSU-06027 | II | Metastatic[a] | Docetaxel + bevacizumab + trastuzumab | Positive | S |
| 10536 | II | Locally recurrent/metastatic[b] | Enzastaurin + capecitabine | Not specified | S |
| CT 4002 | II | Metastatic[b] | Endocrine TAG-1 | Triple negative | S |
| GBG 41 | II | Metastatic[b] | Everolimus | Negative | S |
| JO19901 | II | Metastatic[a] | Paclitaxel + bevacizumab | Negative | S |
| A6181068 | II | Metastatic[b] | Sunitinib | Not specified | S |
| UNC-LCCC-0418 | II | Locally recurrent/metastatic[b] | Multiepitope autologous dendritic cell vaccine + trastuzumab + vinorelbine | Positive | S |
| UPCC 02106 | II | Metastatic[b] | Bevacizumab + Nab paclitaxel | Triple negative | S |
| NU-07B1 | II | Inoperable locally recurrent/metastatic[b] | Paclitaxel vs paclitaxel + sorafenib | Negative | S |
| DCRO_BR_2006_01 | II | Metastatic[a] or first-line chemotherapy | Bevacizumab + Nab paclitaxel + carboplatin | Triple negative | S |
| 9597-07-4R0 | II | Metastatic with brain disease | Bevacizumab + chemotherapy | Not specified | S |
| 20050225 | II | Locally recurrent/metastatic[a] | Paclitaxel + motesanib vs paclitaxel + bevacizumab | Negative | Not defined |
| PMH-PHL-057 | II | Locally recurrent/metastatic[b] | Pazopanib | Positive or negative | S |
| SCCC-2006081 | II | Metastatic[a] | Nab paclitaxel + bevacizumab + gemcitabine | Negative | S |
| 20060341 | II | Inoperable locally recurrent/metastatic[b] | Paclitaxel + motesanib ± bevacizumab | Negative | R |
| 2006-0873 | II | Metastatic[c] | Allogeneic hematopoietic stem cell transplantation + bevacizumab | Not specified | S |
| MAYO-RC0731 | II | Metastatic[b] | Anastrozole/letrozole/exemestane + sorafenib | Negative | S |
| 07-BRE-43-NP | II | Metastatic[a] | Everolimus + fulvestrant | Not specified | S |
| SCCC-112007-035 | II | Metastatic[b] | Paclitaxel + sorafenib | Negative | S |
| IOSI-RO0402 | II | Metastatic[c] with brain disease | RT ± indinavir + ritonavir | Not specified | R |

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**Table I.** Contd

| Protocol identification | Phase | Cancer stage | Agent(s) being evaluated | HER2/neu status | Single arm (S)/ randomized (R) |
|---|---|---|---|---|---|
| I06-025 | II | Locally recurrent/metastatic[a] | Nab paclitaxel + sorafenib | Negative | S |
| BRE06-109 | II | Metastatic[b] | Bevacizumab + sorafenib | Positive or negative | S |
| LUMC-LU200027 | II | Locally recurrent/metastatic[b] | Nab paclitaxel + carboplatin + bevacizumab | Positive or negative | S |
| NCCTG-N0735 | II | Metastatic[a] | Nab paclitaxel + gemcitabine + bevacizumab | Positive or negative | S |
| CRUK-CR0207-22 | II | Metastatic | Exemestane ± ATN224 | Negative | S |
| 07-214 | II | Metastatic[a,b] | Trastuzumab + vinorelbine + bevacizumab | Positive | S |
| SNDX-275-0301 | II | Metastatic | Exemestane ± entinostat (SNDX-275) | Not specified | S |
| CT/08.03 | II | Metastatic[b] | Vinorelbine (oral) + bevacizumab | Not specified | S |
| OHSU-4318 | II | Locally recurrent/metastatic[a] | Sorafenib + fulvestrant | Negative | S |
| FHCRC-6628 | II | Metastatic[a] | Nab paclitaxel + bevacizumab followed by bevacizumab + erlotinib | Triple negative | S |
| NCI-05-C-0022 | I | Metastatic[c] | Sorafenib + bevacizumab | Not specified | S |
| A6181113 | I | Locally recurrent/metastatic[a] | Docetaxel + trastuzumab + sunitinib | Positive | S |
| CRAD001J2102 | I | Metastatic[b] | Everolimus + vinorelbine + trastuzumab | Positive | S |
| CRAD001J2101 | I | Metastatic[b] | Everolimus + paclitaxel + trastuzumab | Positive | S |
| BRSMTS0010 | I | Metastatic[b] | Everolimus + capecitabine | Not specified | S |
| 20050200 | Ib | Locally recurrent/metastatic[b] | Motesanib + paclitaxel or docetaxel | Not specified | S |
| 06-402 | I | Metastatic[b] | Vandetanib + cyclophosphamide + methotrexate | Not specified | S |
| PTC299-ONC-003-BRC | I | Metastatic[b] | PTC299 | Not specified | S |
| UMN-2006LS040 | I | Metastatic[c] | Bortezomib + gemcitabine | Not specified | S |
| UMN-2007LS086 | I | Metastatic[c] | Sorafenib + pemetrexed + cisplatin | Not specified | S |
| 07-149 | Not specified | Metastatic[b] | bevacizumab + paclitaxel or vinorelbine | Not specified | S |
| V0801 | Not specified | Early breast cancer | Aspirin | Not specified | S |

a First-line chemotherapy for metastatic disease.

b Second- or subsequent-line chemotherapy for metastatic disease.

c Advanced malignancy.

d First-line endocrine therapy for metastatic disease.

e Prior trastuzumab therapy.

f Patients previously in A6181107, A6181108, A6181110, A6181111, A6181112.

**CR** = complete remission; **HER** = human epidermal growth factor receptor; **Nab** = nanoparticle albumin-bound; **RT** = radiation therapy; **TAG** = cationic liposome-encapsulated preparation of paclitaxel.



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stable disease at the final protocol-determined efficacy evaluation at 22 weeks. Clinical benefit was seen at all dose levels, with a median response duration of 5.5 months. Fifteen serious adverse events occurred with 11 episodes (73%) being attributed to bevacizumab. These included hypertension (five patients), venous thromboembolism (two), proteinuria (two) and severe headache (two), the latter occurring at the highest dose level, which was considered as a dose-limiting toxicity. The incidence of other adverse events is shown in table II.

Bevacizumab in combination with capecitabine was compared with capecitabine alone in 462 patients with metastatic breast cancer.[31] Approximately 85% of patients had received prior chemotherapy in the metastatic setting, with nearly 40% having received two or more lines of prior cytotoxic therapy. Despite a near doubling in objective response rate (19.1% vs 9.8%), there was no difference in PFS or OS. Safety data did not demonstrate any significant increase in capecitabine-related toxicities in the combination arm. Adverse effects associated with bevacizumab of grade 3 or 4 severity were seen in the following proportion of patients: hypertension (17.9%); bleeding (0.9%); proteinuria (0.9%); and vascular thromboembolic event (6.9%) [table II]. Quality of life was assessed in 80% of patients at baseline and at least one subsequent timepoint, with no apparent difference in the time to deterioration in either group. One possible explanation for the lack of survival benefit in this trial was that other proangiogenic mechanisms may exist during the natural history of tumour progression, such that targeting one angiogenic factor is unlikely to result in prolonged disease control. Another explanation for the short duration of response is that despite using an agent that targets one specific angiogenic factor critical in the growth of the tumour, there was an inability to select patients based on their underlying tumour molecular phenotype for those who would be most likely to respond to such therapy.

The first efficacy and safety results from a phase II study evaluating the same combination of bevacizumab and capecitabine in previously untreated patients with metastatic breast cancer has been presented.[32] In the intention-to-treat population of 106 patients, an overall response rate of 38% was seen, with a median time to

**Table II.** Percentage of grade 3 or 4 adverse events in bevacizumab (BEV) trials

| Adverse event | Cobleigh et al.[30] | Miller et al.[31] | | Sledge et al.[32] | Miller et al.[33] | | | Miles et al.[34] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BEV | CAP | CAP + BEV | CAP + BEV | PAC | PAC + BEV | p-value | DOC + PL | DOC + BEV[a] | DOC + BEV[b] |
| Asthenia | 9 | 6.6 | 7.4 | NR | 4.9 | 9.1 | 0.04 | 5.2 | 8.4 | 6.5 |
| Infection | 0 | 0.5 | 0.9 | NR | 2.9 | 9.3 | <0.001 | 0.3 | 0.8 | 0.4 |
| Headache | 7 | 0.5 | 1.7 | NR | 0.0 | 2.2 | 0.008 | NR | NR | NR |
| Hypertension | 19 | 0.5 | 17.9 | 5.0 | 0.0 | 14.8 | <0.001 | 1.3 | 0.4 | 3.2 |
| Nausea | 0 | 0.9 | 2.6 | NR | 2.0 | 3.3 | NR | NR | NR | NR |
| Diarrhoea | 1 | 10.7 | 11.8 | 7.5 | NR | NR | NR | 3.4 | 6.8 | 6.9 |
| Bleeding | 0 | 0.5 | 0.4 | NR | 0.0 | 0.5 | NR | 0.9 | 1.2 | 1.2 |
| Proteinuria | 0 | 0.0 | 0.9 | 0 | 0.0 | 3.5 | <0.001 | 0.0 | 0.0 | 0.4 |
| Sensory neuropathy | 0 | NR | NR | NR | 17.7 | 23.5 | 0.05 | 1.7 | 3.2 | 4.5 |
| Stomatitis | NR | 0.5 | 1.7 | 1.0 | 0.6 | 1.1 | NR | 0.4 | 2.8 | 3.2 |
| Thromboembolic | NR | 5.1 | 6.9 | 6.0 | 1.5 | 2.1 | NR | 3.8 | 1.2 | 1.2 |
| GIT perforation | NR | NR | NR | NR | 1.2 | 2.1 | NR | 0.9 | 0.4 | 0.4 |

a 7.5 mg/kg.

b 15 mg/kg.

**CAP** = capecitabine; **DOC** = docetaxel; **GIT** = gastrointestinal tract; **NR** = not reported; **PAC** = paclitaxel; **PL** = placebo.

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progression (TTP) of 5.7 months (95% CI 4.9, 8.4). Interestingly, the TTP for estrogen receptor (ER)-positive patients (n = 57) was significantly better than ER-negative patients (n = 49) [8.9 vs 4.0 months; $p < 0.0001$]. Biomarkers were assessed as a secondary endpoint and these results are awaited with interest to explain the marked difference in efficacy in the hormone-responsive patients.

The use of bevacizumab in the first-line metastatic breast cancer setting has been reported.[33] In the first study conducted by the Eastern Cooperative Oncology Group, patients who had not previously received chemotherapy for metastatic disease were randomized to weekly paclitaxel 90 mg/m$^2$ for 3 in every 4 weeks and bevacizumab 10 mg/kg every 2 weeks, or paclitaxel alone. Over a 26-month period, 722 patients were recruited to the study with the intention-to-treat population including 673 patients. The primary endpoint of PFS was met, with patients in the combination arm having a significantly longer median PFS of 11.8 versus 5.9 months ($p < 0.001$). Objective response rate was similarly higher in the combination arm than paclitaxel monotherapy (36.9% vs 21.2%; $p < 0.001$), while OS was not significantly different between the two groups (26.7 vs 25.2 months, respectively) at approximately 40 months median follow-up. Grade 3 or 4 adverse events that occurred more frequently in the combination arm included hypertension, proteinuria, infection, fatigue, cerebral ischaemia and sensory neuropathy. The latter finding may relate to the longer duration of paclitaxel administration when used in combination with bevacizumab (7.1 vs 5.1 months), rather than any intrinsic neuropathic toxicity from the addition of bevacizumab.

More recently, the first results of a randomized, double-blind, placebo-controlled trial of bevacizumab in combination with docetaxel was reported.[34] This trial randomized 736 patients with HER2/neu-negative metastatic breast cancer in the first-line setting to docetaxel 100 mg/m$^2$ plus placebo or bevacizumab 7.5 mg/kg or 15 mg/kg; all drugs were administered once every 3 weeks. Patients were permitted to receive up to nine cycles of docetaxel and continued placebo or bevacizumab until progression or intolerable toxicities. At a median follow-up time of approximately 11 months, the improvement in the primary endpoint PFS was significantly improved for patients receiving bevacizumab at both the 7.5 mg/kg and 15 mg/kg doses (hazard ratio 0.79 [$p = 0.032$] and 0.72 [$p = 0.009$], respectively). Similar benefits were seen irrespective of any prior adjuvant chemotherapy (non-taxane or taxane-based) or hormone receptor status. Overall, any adverse event of grade 3, 4 or 5 occurred in 67%, 74.8% and 74.1% of the docetaxel plus placebo, low and high dose bevacizumab groups, respectively. In regard to grade 3 or 4 adverse events that are most likely to be related to bevacizumab, hypertension and proteinuria were more frequent in the higher dose bevacizumab arm, bleeding was more frequent in both bevacizumab-containing groups, and gastrointestinal perforation or thromboembolic events were more frequent in the placebo arm (table II).

#### *2.1.1 Combination Therapy: Bevacizumab and Trastuzumab*

VEGF and HER2/neu levels were analyzed in breast cancer tissue from 611 women treated in one institution with a median follow-up of 50 months. A positive association was found between HER2/neu overexpression and VEGF overexpression. In univariate analysis, two isoforms of VEGF had a significant prognostic impact on OS, while in node-positive patients, VEGF expression was an independent prognostic factor.[35]

An open-label phase I study has been reported using weekly trastuzumab and bevacizumab 3–10 mg/kg every 2 weeks in HER2/neu overexpressing metastatic breast cancer patients. Pharmacokinetic analysis did not demonstrate any alteration to either agent when they were coadministered. Preliminary efficacy data showed an objective response in six of nine patients, with two further patients achieving stable disease.[36] This combination is being evaluated in an ongoing randomized adjuvant trial (CIRG 011/NSABP B-44).[37]

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### 2.2 Sunitinib

Sunitinib is a small molecule, multi-targeted kinase inhibitor, which acts as an adenosine triphosphate-competitive inhibitor of VEGFR-1, -2 and -3, PDGFR-α and -β, c-KIT, FLT-3 and RET.[38] Subsequent inhibition of the downstream signal transduction then impacts on tumour growth, progression, metastases and angiogenesis. Preclinical studies demonstrate the effectiveness of this drug as a single agent in tumour regression of several cell lines. Furthermore, combination studies with cytotoxic agents, such as doxorubicin, fluorouracil and docetaxel, show additive or synergistic effects in breast cancer.[39]

A phase II study of sunitinib was conducted in previously treated patients with metastatic breast cancer with objective response as the primary endpoint.[40] Patients were required to have received anthracyclines and taxanes, and were permitted to have up to four previous chemotherapy regimens. Of the 64 patients enrolled, 31% had triple negative tumours, 19% were HER2/neu positive and patients had received a median of 3.5 previous chemotherapy regimens. Patients received sunitinib 50 mg/day for 4 weeks followed by a 2-week rest. Partial response was seen in seven patients (11%) and three had stable disease for ≥6 months, with an overall clinical benefit of 16%. Response was seen irrespective of HER2/neu or ER status. The median duration of treatment was two cycles (i.e. 12 weeks). Median duration of response was 19 weeks (85% CI 18, 20) and the median TTP was 10 weeks (95% CI 10, 11). Uncomplicated grade 4 neutropenia occurred in one patient. Fatigue (14%), nausea (8%) and diarrhoea (6%) were the most frequent grade 3 nonhaematological adverse events. There were no treatment-related deaths. Serum biomarkers were evaluated with a cyclical rise in VEGF and decline in soluble (s)VEGFR and sKIT relative to sunitinib administration, with all plasma levels returning to near-normal levels by the end of the 2-week rest period. There was a trend for improved OS in patients who had decreases in sVEGFR and sKIT of greater than 20% and 50%, respectively, at the end of the treatment cycle. There are now several phase III trials evaluating sunitinib in first- and second-line metastatic breast cancer patients (table I).

### 2.3 Sorafenib

Preclinical studies have demonstrated that sorafenib inhibits phosphorylation of the MAP kinase pathway in several solid tumour cell lines, including breast cancer, whether or not mutant K-Ras, mutant B-Raf, or wild-type Ras or Raf are present. Furthermore, cell-based assays show that this agent can inhibit several proangiogenic tyrosine kinase receptors such as VEGFR-2, VEGFR-3, PDGFR-β, c-KIT, FLT-3 and FGFR-1. Pharmacokinetic studies confirm that the drug is metabolized via the cytochrome P450 (CYP) 3A4 system with a high degree of drug binding to plasma proteins. Sorafenib distributes evenly throughout peripheral tissues and there is some penetration across the blood-brain barrier.[41]

A review of four phase I studies evaluating sorafenib included a total of 24 patients with breast cancer (from a total of 173 patients with solid tumours, with evaluable disease present in 137 patients). The optimal dose scheduling was determined to be 400 mg twice daily without a break, with the dose-limiting toxicities including rash, hypertension, diarrhoea, fatigue, nausea, vomiting and pain. Only 2 of 137 evaluable patients achieved a partial response with 28% of patients across the trials having disease stabilization. There was evidence of decreased nuclear levels of protein extracellular signal-regulated kinase and this has been suggested to be a possible mechanism by which sorafenib exerts its antitumour activity.[42]

### 2.4 Pazopanib

Pazopanib is an oral small molecule TKI, which inhibits phosphorylation of VEGFR-1, -2 and -3, PDGFR-α and -β, and c-KIT. The subsequent downregulation of several proapoptotic molecules leads to reduced cellular proliferation, and an *in vitro* anti-tumour effect has been demonstrated in human tumour xenografts,

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including breast, colon, prostate, lung, melanoma, head and neck, and kidney.[43] A phase I study evaluating this agent in 43 patients with solid tumours demonstrated good tolerability. Doses of up to 2000 mg/day were tolerated, with mainly grade 1 and 2 toxicities occurring, which included nausea, diarrhoea, fatigue, hypertension, anorexia, vomiting and hair depigmentation. Objective tumour size reduction and stable disease for >6 months was observed in three and six patients, respectively.[44]

There are two ongoing trials evaluating pazopanib in breast cancer. VEG20007 is a randomized phase II study of pazopanib and lapatinib versus lapatinib alone in metastatic breast cancer patients with HER2/neu-positive disease who have not previously received chemotherapy or trastuzumab in the metastatic setting.[45] The primary endpoint in this trial of 141 patients with measurable disease is the rate of progressive disease at 12 weeks of therapy. Preliminary results demonstrated a lower rate of progressive disease at 12 weeks (38.9% vs 36.2%, respectively) and a higher response rate up to 12 weeks of treatment (43.5% vs 23.6%, respectively) in the combination arm versus lapatinib monotherapy. Common grade 3 and 4 adverse effects that occurred more frequently in the pazopanib and lapatinib arm included diarrhoea, increased alanine transaminase levels, hypertension and fatigue. A phase III, placebo-controlled, randomized trial of pazopanib and lapatinib versus lapatinib alone is ongoing.[46] Inclusion criteria require tumours to be HER2/neu overexpressed, with an initial diagnosis of inflammatory breast cancer, which has recurred or progressed on treatment with chemotherapy with or without trastuzumab. Primary endpoint is PFS and patients are required to have measurable disease according to RECIST (Response Evaluation Criteria in Solid Tumours) criteria or evaluable cutaneous disease. Enrolment of 320 patients is planned, with the trial expected to complete accrual by 2010.

### 2.5 Cediranib

Cediranib (AZD2171), is a potent oral inhibitor of VEGFR-2, with additional activity against VEGFR-1 and -3, c-KIT and PDGFR. Human lung tumour xenografts showed regression following treatment with this agent and immunohistochemical assessment of the tumours demonstrated a reduction in microvessel number. As tyrosine kinase signalling via VEGFR-1 can be activated by binding from two other VEGF homologues, PIGF and VEGF-B, the ability of cediranib to directly inhibit VEGFR-1 may provide additional benefits when compared with agents that only inhibit VEGF. Lymphangiogenesis results through binding of VEGF-C and -D to VEGFR-3. Thus, inhibition by cediranib at this site may potentially retard tumour cell dissemination. Pharmacokinetic studies have shown that this agent can be given daily with oral bioavailability of 60% and a very low unbound plasma portion.[47]

A phase I study involving 83 patients has been reported, which included 15 patients with breast cancer. Treatment-related grade 3 or 4 toxicities occurred in 24 patients (28%), with hypertension being the most common (16 patients); other toxicities included palmar-plantar erythrodysesthesia (3), increase in γ-glutamyl transpeptidase levels (3) and diarrhoea (2). The effect on blood pressure appeared to be dose related, but the use of standard anti-hypertensive therapy was generally successful. Overall, there were two partial responses seen and 22 patients, including two breast cancer patients, had stable disease. There was a trend for improved tumour control and reduced tumour progression as the dose range increased from 10 to 60 mg/day.[15]

An interesting aspect of anti-angiogenesis treatment that has been evaluated in cediranib studies has been an attempt to identify potential changes in perfusion as assessed by imaging techniques, to serve as a surrogate marker for measuring activity of the drug. A study has been reported that implanted MCF-$7^{neo}$ (non-VEGF expressing) and MCF-$7^{VEGF}$ cells into athymic mice. Positron emission tomography (PET) imaging was used to assess tumour blood volume with [$^{11}$C]carbon monoxide, perfusion with [$^{18}$F]fluoromethane and glucose utilization with [$^{18}$F]fluorodeoxyglucose. Subsequently, tumour histology was assessed and correlated with

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physiological imaging. Of the three radiotracers used, there was an early (24 hours) reduction in perfusion following initiation of treatment with cediranib, which further decreased at 72 hours and correlated with a decrease in tumour microvessel density and proliferation.[48]

In the phase I study of cediranib, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was performed to assess for tumour vascular density and permeability. Following treatment with cediranib, the initial area under the concentration-time curve (iAUC) over 60 seconds after contrast entry into tissues ($iAUC_{60}$) appeared to reflect the effect of the drug on acute vessel permeability, while the sustained effect on $iAUC_{60}$ at 28 and 56 days suggested a drug effect on tumour blood flow.[15]

### 2.6 Motesanib

VEGFR-1, -2 and -3, PDGFR and stem cell factor receptor are selectively inhibited by the oral, small molecule, anti-angiogenesis agent motesanib.

A phase I study to establish the maximum tolerated dose (MTD), identify dose-limiting toxicities and assess the pharmacokinetic profile of motesanib was reported in 2007.[49] An exploratory analysis was also performed to assess methods by which biomarker level changes and alteration of DCE-MRI may predict response. Seventy-one patients including four patients with breast cancer were recruited. More than two-thirds of patients had received two or more previous chemotherapy regimens. The MTD was determined at a once-daily dose of 125 mg, with the most common grade 3 events being hypertension (20%), diarrhoea (10%) and abdominal pain (7%). There were two subjects with treatment-related posterior reversible encephalopathy syndrome and three with acute cholecystitis, the aetiology of the latter event being unclear. Of the 67 assessable patients, 5 patients experienced a partial response and 35 had stable disease. A notable finding in this study was the durable stabilization of disease for >6 months seen in 16 patients (23%), which is likely to translate into a genuine clinical benefit. Biomarkers were measured at baseline and at day 50 in 62% of patients. There appeared to be correlation with serum levels of PIGF and sVEGFR-2, and the extent of change in tumour dimensions. DCE-MRI was performed in 17 patients during the course of the first cycle of treatment. There did not appear to be a significant correlation with tumour response and iAUC.[49]

Preliminary results from an open-label phase I study of motesanib in combination with either paclitaxel or docetaxel in metastatic breast cancer are available.[50] Paclitaxel was given at 90 mg/m$^2$ on a weekly schedule for 3 weeks followed by a 1-week rest. Docetaxel was given at two dose levels (100 and 75 mg/m$^2$) every 3 weeks. A starting dose of motesanib 50 mg/day was used, escalating to a maximum dosage of 125 mg/day. At the time of reporting the study, the MTD for motesanib with both taxanes had not yet been reached. Pharmacokinetic analysis does not indicate any effect of either paclitaxel or docetaxel on motesanib levels, while the AUC for both taxanes was slightly higher after exposure to motesanib. Recruitment to this study has now been completed. A phase II study is currently ongoing, which will evaluate motesanib with paclitaxel versus bevacizumab and paclitaxel in chemotherapy-naive patients in the metastatic breast cancer setting (protocol number 20050225 in table 1).

### 2.7 Aflibercept

Aflibercept, coined VEGF-Trap, is constructed from fusion of the immunoglobulin domain of VEGFR-1 and VEGFR-2, with the Fc region of human IgG1. This fusion protein has high affinity to all isoforms of VEGF and may be also bind ligands that have an affinity for VEGFR-1 and -2, such as PIGF.[17]

An early trial demonstrated marked regression of implanted human Wilm's tumour cells in response to intraperitoneal injection of aflibercept. Following 5 weeks of treatment, there was a 79% reduction in mean tumour weight. Histological examination of the tumours after treatment showed almost complete absence of blood vessels. The authors postulated that the impact of a potent and high-affinity agent to inhibiting even

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low levels of VEGF would more completely abolish the dependency that well established tumour endothelial cells have on VEGF. Furthermore, it is postulated that there may be a co-dependency between the endothelial and adjacent stromal cells, where aflibercept may destabilize this association and lead to apoptosis of both.[51,52] There are over 20 trials currently registered on the National Cancer Institute website that are evaluating aflibercept in a variety of malignancies, as well as phase I studies and an observational pilot study assessing renal and blood pressure changes in patients receiving aflibercept and other anti-angiogenic therapies.

## 3. Potential Methods of Assessing Anti-Angiogenic Efficacy

An important aspect of the use of agents that target angiogenesis is the potential to select those tumours that can be predicted to have the greatest response to treatment. Measurement of protein expression, degree of micovascular density, tumour cell apoptosis during anti-angiogenic therapy, presence of pericyte formation around tumour blood vessels and alteration in interstitial fluid pressure have all been evaluated. There is preclinical evidence that changes in these factors may reflect normalization of tumour vasculature.

Investigators have assessed VEGF expression on tumour cells and stroma, circulating VEGF levels in the plasma or urinary VEGF levels, and matrix metalloproteinase as surrogate markers of response. These have generally been in small studies with inconsistent results.[53,54] One study has demonstrated that plasma levels of VEGF can be utilized to calculate the optimal dosage needed for an anti-VEGF agent, suggesting that this may be used as a surrogate pharmacodynamic method in the clinical setting.[55]

Naturally occurring negative regulators of angiogenesis, such as thrombospondin, have been measured in patients with colorectal cancer, with expression levels having a borderline significance on survival outcome.[56]

Circulating endothelial cells and circulating endothelial progenitors have also been measured. In the preclinical setting, the levels following treatment with a VEGFR2 inhibitor were shown to correlate with tumour response.[57] In a recent study of metronomic chemotherapy, higher baseline levels of circulating endothelial cells prior to low-dose chemotherapy and bevacizumab was shown to correlate with response, clinical benefit and PFS.[58] Studies are needed to evaluate reproducible methods of measuring these circulating cells and, more importantly, they must be shown to correlate with clinical outcome in ongoing trials of anti-angiogenic agents.

Finally, imaging with DCE-MRI, PET, CT scan, ultrasound and optical technologies have been used to determine haemodynamic changes and vessel permeability in various solid tumours. These non-invasive approaches have the potential to be used to detect tumours at an earlier stage, evaluate early signs of tumour response to a given anti-angiogenic agent or radiation, or be used to select the optimal dose of a given anti-angiogenic agent. If well conducted studies confirm the accuracy of one or more of these methods, the need to standardize the imaging protocol, the availability and cost of maintaining the equipment and the reproducibility of tumour assessment amongst individual patients and across institutions, are all issues that will require further attention.[59]

## 4. Future Considerations

The understanding of the important role of angiogenesis in tumour growth, progression and dissemination has led to the exciting development of targeted agents to this pathway. The pivotal importance of the VEGF family, its receptors and other co-regulating growth factors has been the main focus of therapeutic endeavours to date. Although results with bevacizumab in heavily pre-treated breast cancer patients suggest that the use of this monoclonal antibody is more effective in earlier stages of disease, there is preclinical evidence that other anti-angiogenic agents, such as the soluble, fusion molecule, aflibercept, may be effective even in later stages of tumour growth when there is greater tumour volume.

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Research in the following areas is needed to optimize the use of these agents:

1. To identify the predominant angiogenic pathway that is responsible in a given patient's tumour to allow selection of the optimal anti-angiogenic agent. This may require biopsy of metastatic lesions for specific biomarker analysis or perhaps through measuring circulating protein products, which reflect the underlying growth pattern of the tumour. In identifying which pathway predominates in a given individual, it may then be possible to re-assess these tumour factors over time as a means of predicting tumour response or progression.
2. It has already been shown that despite the targeted action of these agents, tumour 'resistance' develops and once-effective therapy cannot maintain tumour control. In light of the multitude of prospective trials currently ongoing or planned, it is essential that these studies attempt to identify mechanisms of resistance during the course of treatment for the patients involved. This is likely to provide more a accurate understanding of the factors involved in the development of resistance than relying on interpretation from pre-clinical models. Results from these observations in current studies can then allow hypotheses to be generated and formally evaluated in the next generation of trials of targeted agents.
3. Because many of the current trials of anti-angiogenic agents are evaluating the targeted treatment in combination with cytotoxic agents, it is important to establish which partnering of agents provides the greatest synergism. Furthermore, the frequency and nature of potential interactive toxicities must be defined.
4. Prospective evaluation of biological markers and functional imaging that can predict or confirm the efficacy of these agents. Patient selection for those most likely to benefit from this class of agents will enable appropriate access and avoid unnecessary use of healthcare funding.
5. Importantly, the spectrum of adverse effects seen with anti-angiogenic agents appears to differ from those of conventional cytotoxics. Hypertension, which can occur in up to 19% of patients, may relate to a direct action of VEGF on blood vessels causing hypotension or indirectly on the reduced production of nitric oxide, an endogenous vasodilator, or a direct action of the agent on blood vessels.[60] These postulated mechanisms need to be better evaluated, methods to predict which patients will experience hypertension need to be established and optimal anti-hypertensive interventions need to be defined. Although the thromboembolic adverse effects appear less frequent in breast cancer trials than the incidence observed in colorectal and lung cancer studies, they are more frequent than with cytotoxic agents. There already exists a pro-coagulant tendency in patients with metastatic disease, and studies are needed to assess patients prior to and during anti-angiogenic therapy to minimize the occurrences of debilitating venous and arterial thromboembolic events. Close monitoring of the potential cardiac effects in the metastatic trials are necessary to define the nature and frequency of this effect, and to avoid undue toxicities of these agents when they are evaluated in the early breast cancer setting. In addition, in trials that combine anti-angiogenic agents with HER2/neu-targeted therapy, the possible additive cardiac toxicity needs to be assessed. The use of the oral tyrosine kinases introduces a new complexity to patient care, in that adequate patient education regarding concomitant medications is necessary to avoid drug interaction through the induction or inhibition of the CYP haemoprotein family.

As the understanding of the angiogenic pathways responsible for tumour development improves, the use of specific agents directed at one or more sites of growth signalling may potentially lead to significant and durable benefits for patients with metastatic breast cancer. Parallel to the trials evaluating the efficacy of anti-angiogenic agents is the need to delineate mechanisms of resistance and identify the adverse effects for this class of drugs. Finally, the greatest benefit for this approach, if proven to be successful, will be the use of these agents in the earlier stages of the disease.

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## References


1. Gasparini C, Harris A. Clinical importance of the determination of tumor angiogenesis in breast carcinoma: much more than a new prognostic tool. J Clin Oncol 1995; 13: 765-82
2. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004; 25 (4): 581-611
3. Gerber H, Dixit V, Ferrara N. Vascular endothelial growth induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. J Biol Chemother 1998; 273: 13313-6
4. Ellis L, Rosen L, Gordon M. Overview of anti-VEGF therapy and angiogenesis. Part 1: angiogenesis inhibition in solid tumor malignancies. Clin Adv Hematol Oncol 2006; 4 (1 Suppl): 1-10
5. Jain R, Duda D, Clark J, et al. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006; 3 (1): 24-40
6. Hicklin D, Lee M, Ellis L. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005; 23: 1011-27
7. Yoshiji H, Gomez D, Shibuya M, et al. Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer. Cancer Res 1996; 56 (9): 2013-6
8. Lloyd R, Scheithauer B, Kuroki T, et al. Vascular endothelial growth factor (VEGF) expression in human pituitary adenomas and carcinomas. Endocr Pathol 1999; 10: 229-35
9. Presta L, Chen H, O'Connor S, et al. Humanization of an anti-VEGF monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997; 57: 4593-9
10. Willett C, Boucher Y, Di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004; 10: 145-7
11. Wood J, Bold G, Buchdunger E, et al. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. Cancer Res 2000; 60 (8): 2178-89
12. Wedge S, Ogilvie D, Dukes M, et al. ZD4190: an orally active inhibitor of vascular endothelial growth factor signaling with broad-spectrum antitumor efficacy. Cancer Res 2000; 60 (4): 970-5
13. Prewett M, Huber J, Li Y, et al. Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. Cancer Res 1999; 59 (20): 5209-18
14. Olden S, Eckhardt S, Basser R, et al. Clinical evaluation of ZD6474, an orally active inhibitor of VEGF and EGF receptor signaling, in patients with solid, malignant tumors. Ann Oncol 2005; 16 (8): 1391-7
15. Drevs J, Siegert P, Medinger M, et al. Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. J Clin Oncol 2007; 25 (21): 3045-54
16. Podar K, Tonon G, Sattler M, et al. The small-molecule VEGF receptor inhibitor pazopanib (GW786034B) targets both tumour and endothelial cells in multiple myeloma. Proc Natl Acad Sci U S A 2006; 103 (51): 19478-83
17. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A 2002; 99 (17): 11393-8
18. Holash J, Maisonpierre P, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999; 284 (5422): 1994-8
19. Folberg R, Hendrix M, Maniotis A. Vasculogenic mimicry and tumor angiogenesis. Am J Pathol 2000; 156 (2): 361-81
20. Miller K, Sweeney C, Sledge Jr G. The snark is a boojum: the continuing problem of drug resistance in the anti-angiogenic era. Ann Oncol 2003; 14 (1): 20-8
21. Borgström P, Gold D, Hillan K, et al. Importance of VEGF for breast cancer angiogenesis in vivo: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin. Anticancer Res 1999; 19 (5B): 4203-14
22. Schirner M, Hoffmann J, Menrad A, et al. Antiangiogenic chemotherapeutic agents: characterization in comparison to their tumor growth inhibition in human renal cell carcinoma models. Clin Cancer Res 1998; 4 (5): 1331-6
23. Schirner M. Antiangiogenic chemotherapeutic agents. Cancer Metastasis Rev 2000; 19 (1-2): 67-73
24. Browder T, Butterfield C, Kräling B, et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 2000; 60 (7): 1878-86
25. Klement G, Baruchel S, Rak J, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 2000; 105 (8): R15-24
26. National Cancer Institute. Clinical trials results for active trials of anti-angiogenesis therapy in stage IV breast cancer [online]. Available from URL: http://www.cancer.gov [Accessed 2008 Dec 10]
27. 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
28. Johnson D, Fehrenbacher L, Novotny W, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 2004; 2184-91
29. Yang J, Haworth L, Sherry R, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003; 349: 427-34
30. Cobleigh M, Langmuir V, Sledge G, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Sem Oncol 2003; 30 (5 Suppl. 16): 117-24
31. Miller K, Chap L, Holmes F, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005; 23: 792-9
32. Sledge G, Miller K, Moisa C, et al. Safety and efficacy of capecitabine (C) plus bevacizumab (B) as first-line in metastatic breast cancer. Proc Am Soc Clin Oncol 2007; 25: 1013
33. 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


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34. Miles D, Chan A, Romieu G, et al. Randomised, double-blind, placebo-controlled, phase III study of bevacizumab (BV) with docetaxel (D) or D with placebo (PL) as 1st-line therapy for patients with locally recurrent or metastatic breast cancer (mBC): AVADO [abstract]. Proc Am Soc Clin Oncol 2008; 26: LBA1011
35. Konecny G, Meng Y, Untch M, et al. Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. Clin Cancer Res 2004 1; 10 (5): 1706-16
36. Pegram M, Yeon C, Ku N, et al. Phase I combined biological therapy of breast cancer using two humanized monoclonal antibodies directed against HER2 proto-oncogene and vascular endothelial growth factor (VEGF). Breast Cancer Res Treat 2004; 88: 3039
37. Breast Cancer International Research Group. TRIO-CIRG011 [online]. Available from URL: http://www.bcirg.org/Internet/Studies/011.htm [Accessed 2008 Dec 16]
38. Chow L, Eckhardt S. Sunitinib: from rational design to clinical efficacy. J Clin Oncol 2007; 25 (7): 884-96
39. Abrams T, Murray L, Pesenti E, et al. Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. Mol Cancer Ther 2003; 2 (10): 1011-21
40. Burstein H, Elias A, Rugo H, 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 (11): 1810-6
41. Beeram M, Patnaik A, Rowinsky E. Raf: a strategic target for therapeutic development against cancer. J Clin Oncol 2005; 23: 6771-90
42. Strumberg D, Clark J, Awada A, et al. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. Oncologist 2007; 12 (4): 426-37
43. Sonpavde G, Hutson T. Pazopanib: a novel multitargeted tyrosine kinase inhibitor. Curr Oncol Rep 2007; 9 (2): 115-9
44. Hurwitz H, Dowlati A, Savage S, et al. Safety, tolerability and pharmacokinetics of oral administration of GW786034 in pts with solid tumors [abstract]. J Clin Oncol 2005; 23 (16S): 3012
45. GlaxoSmithKline. Pazopanib plus lapatinib compared to lapatinib alone in subjects with advanced or metastatic breast cancer [ClinicalTrials.gov identifier NCT00347919]. US National Institutes of Health, ClinicalTrials.gov [online]. Available from URL: http://www.clinicaltrials.gov [Accessed 2009 Jan 5]
46. GlaxoSmithKline. Pazopanib plus lapatinib compared to lapatinib alone in subjects with inflammatory breast cancer [ClinicalTrials.gov identifier NCT00558103]. US National Institutes of Health, ClinicalTrials.gov [online]. Available from URL: http://www.clinicaltrials.gov [Accessed 2009 Jan 5]
47. Wedge S, Kendrew J, Hennequin L, et al. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Res 2005; 65 (10): 4389-400
48. Miller K, Miller M, Mehrotra S, et al. A physiologic imaging pilot study of breast cancer treated with AZD2171. Clin Cancer Res 2006; 12 (1): 281-8
49. Rosen L, Kurzrock R, Mulay M, et al. Safety, pharmacokinetics, and efficacy of AMG 706, an oral multikinase inhibitor, in patients with advanced solid tumors. J Clin Oncol 2007; 25 (17): 2369-76
50. DeBoer R, White S, Mainwaring et al. Safety and pharmacokinetics of motesanib diphosphate (AMG706) with paclitaxel or docetaxel for the treatment of locally recurrent, unresectable or metastatic breast cancer. Breast Can Res Treat 2007; 106 (S272): 6074
51. Hood J, Cheresh D. Building a better Trap. Proc Natl Acad Sci U S A 2003; 100 (15): 8624-5
52. Huang J, Frischer J, Serur A, et al. Regression of established tumors and metastases by potent vascular endothelial growth factor blockade. Proc Natl Acad Sci U S A 2003; 100 (13): 7785-90
53. Willett C, Boucher Y, Duda D, et al. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. J Clin Oncol 2005; 8136-9
54. Chan L, Moses M, Goley E, et al. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: a longitudinal study of protein kinetics throughout tumor progression and therapy. J Clin Oncol 2004; 22: 499-506
55. Bocci G, Man S, Green S, et al. Increased plasma vascular endothelial growth factor (VEGF) as a surrogate marker for optimal therapeutic dosing of VEGF receptor-2 monoclonal antibodies. Cancer Res 2004; 64 (18): 6616-25
56. Jubb A, Hurwitz H, 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
57. Beaudry P, Force J, Naumov G, et al. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. Clin Cancer Res 2005; 11 (9): 3514-22
58. Dellapasqua S, Bertolini F, Bagnardi V, et al. Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. J Clin Oncol 2008; 26: 4899-905
59. Miller J, Pien H, Sahani D, et al. Imaging angiogenesis: applications and potential for drug development. J Natl Cancer Inst 2005; 97: 172-87
60. Shen B, Lee D, Zioncheck T. Vascular endothelial growth factor governs endothelial nitric-oxide synthase expression via KDR/Flk-1 receptor and protein kinase. J Biol Chem 1999; 274: 33057-63

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