Angiogenesis as a therapeutic target in breast cancer

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Abstract: Current evidence indicates that angiogenesis plays an important role in the pathogenesis of several malignancies, including breast cancer. Bevacizumab is a monoclonal antibody that targets the vascular endothelial growth factor (VEGF). Recent clinical data have demonstrated that the addition of bevacizumab to first-line chemotherapy improves the progression-free survival of patients with advanced breast cancer. This review presents an update on the role of bevacizumab, as well as other anti-angiogenic agents in the management of patients with breast carcinoma.

Keywords: Angiogenesis, bevacizumab, breast cancer, treatment.

INTRODUCTION

Even though considerable improvements have been achieved in the treatment of breast cancer using chemotherapeutic agents, several limitations regarding chemotherapy still exist. Consequently, recent research has given emphasis on the investigation of specific molecular targets. Angiogenesis constitutes a crucial process in the pathogenesis of cancer, since tumors depend on the formation of their vessel network which will supply cancer cells with nutrients and oxygen. The initiation of angiogenesis requires the so-called angiogenic switch, which is activated by the prevalence of stimulatory over inhibitory angiogenic factors [1]. The vascular endothelial growth factor (VEGF) pathway represents the most dominant angiogenic pathway. The VEGF family consists of five homologous members: VEGF-A (usually referred to as VEGF), VEGF-B, VEGF-C, VEGF-D and PlGF. These factors represent ligands for VEGF tyrosine-kinase receptors (VEGFR-1, VEGFR-2 and VEGFR-3). Such receptors, which are expressed mainly by endothelial cells, are activated by VEGFs. More specifically, binding of VEGFs to the extracellular domain of VEGFRs leads to receptor dimerisation through conformational alterations. As a result, the cytoplasmatic catalytic function of the receptor is activated, leading to autophosphorylation on tyrosine residues. This autophosphorylation of the receptor stimulates a cascade of signaling pathways including phosphatidylinositol 3-kinase (PI3-K)-Akt and Ras-Raf-MEK-mitogen-activated protein kinase (MAPK)-dependent pathway. VEGFR-1 is activated by VEGF, VEGF-B and PlGF, while VEGFR-2 is activated primarily by VEGF. The production of VEGF in cancer cells is controlled by various factors. Among them, hypoxia constitutes a major mediator of VEGF expression, through hypoxia-inducible transcription factors. The principal stimulator of angiogenesis in tumors is VEGF (mainly the isoforms VEGF121 and VEGF165). The activation of VEGFR-2 by VEGF mediates vessel permeability. Moreover, signal transduction pathways activated by VEGFR-2 promote the survival, proliferation and migration of endothelial cells [2-5].

It has been demonstrated that breast cancer cells frequently overexpress VEGF [6, 7]. The significance of VEGF in adults is restricted in the female reproductive cycle and in wound healing. Therefore, the VEGF pathway represents an attractive target in the management of cancer patients [8]. At present, several agents targeting this pathway are in development, with anti-VEGF antibodies being the most extensively studied. Apart from monoclonal antibodies (mAbs) targeting VEGF, other types of agents that inhibit the VEGF pathway include: mAbs targeting the extracellular domain of VEGFR2 and VEGFR1, soluble VEGFR decoy receptors that target circulating VEGF, and tyrosine-kinase inhibitors (TKIs) that target multiple tyrosine-kinases, including VEGFRs (Fig. **1**).

MONOCLONAL ANTIBODIES TARGETING VEGF

Bevacizumab

Bevacizumab is a humanized recombinant IgG1 mAb that selectively binds to all major isoforms of human VEGF with high affinity. This binding prevents the interaction of VEGF with its receptors and neutralizes the activity of VEGF. Bevacizumab's molecular weight is approximately 149 kDa.

Mechanisms of Action

Although the exact mechanisms of action are not completely clarified thus far, recent data suggest that agents targeting VEGF are characterized by a wide spectrum of antitumor properties. Anti-VEGF therapies were initially expected to demonstrate activity by reducing the blood vessel density within tumors. It has been shown that the inhibition of VEGF by bevacizumab is associated with the regression of existing abnormal vessel network of the tumor, through apoptosis of endothelial cells [9]. Moreover, it seems that the inhibition of VEGF promotes the normalization of the structural and functional aberrations in

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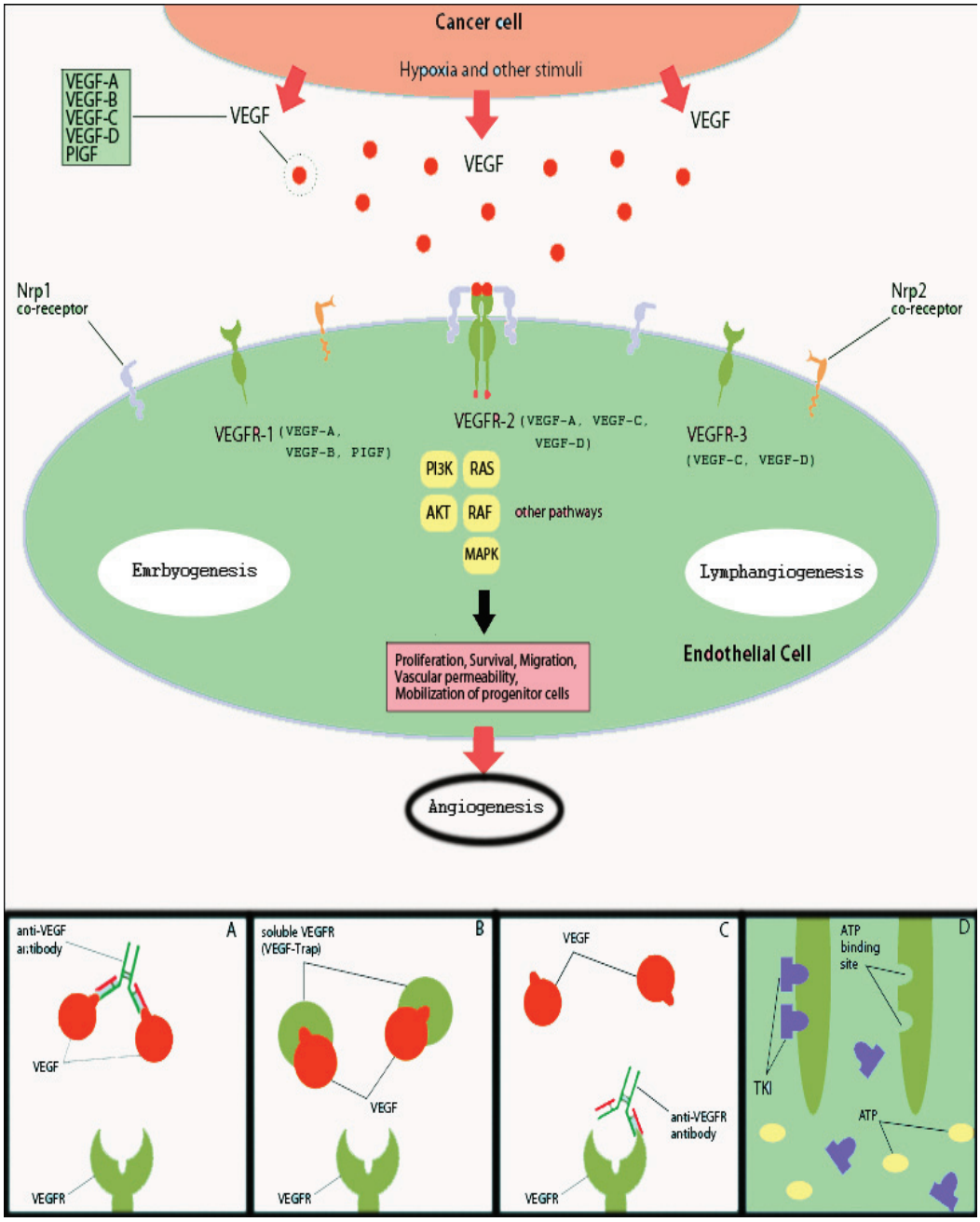

Fig. (1). Anti-angiogenic agents targeting VEGF pathway.

the residual tumor vasculature [10], which may improve the penetration of chemotherapeutic agents into the cancer cells [11]. In addition, the inhibition of the proliferation and migration of endothelial cells, suppresses the development of new vessels in the tumor. Furthermore, treatment with bevacizumab prevents the regrowth of regressed microvessels. Recently, it has been found that except for endothelial cells, VEGFR-1 and VEGFR-2 receptors are also expressed in cancer cells and thus, a direct antitumor efficacy of bevacizumab cannot be excluded [12]. Moreover, it has been demonstrated that bevacizumab may interfere with the immunological system by increasing the levels of dendritic cells, T-lymphocytes and natural-killer cells [13].

The vascular effects of VEGF inhibition have been shown clinically for bevacizumab in patients with breast cancer [14]. Preclinical data have revealed efficacy of the antibody in breast cancer cells, as well as a synergistic effect in combination with cytotoxic agents such as albumin-bound paclitaxel and capecitabine [15, 16].

Pharmacokinetics

The pharmacokinetics of bevacizumab has been evaluated in patients with various types of tumors, including breast cancer. In all clinical trials bevacizumab was administered as intravenous (IV) infusion. The rate of infusion was based on tolerability with initial infusion

duration of 90 minutes and all subsequent infusions administered over 60 minutes. Bevacizumab's pharmacokinetics has been evaluated in several studies with doses ranging from 1-20 mg/kg weekly, every 2 or 3 weeks. The pharmacokinetics of bevacizumab seems to be linear at doses ranging from 1 to 10 mg/kg. Overall, its disposition was characterized by a limited volume of the central compartment (Vc), a low clearance, and a long elimination half-life. The typical value for Vc was 2.66 L for females and 3.25L for males which is within the range reported with other mAbs. The estimated half-life of bevacizumab was approximately 20 days. The serum concentrations of bevacizumab following multiple IV doses every 2 or 3 weeks were also evaluated. The estimated time to achieve steady state concentrations appears to be approximately 100 days which is consistent with the long half-life of the agent. The clearance of bevacizumab varies by body weight and gender. The value for clearance was approximately 0.262 and 0.207 L/d for male and female subjects, respectively. Bevacizumab is supposed to be eliminated following the general route of metabolism of IgG antibodies. It seems that males have a higher clearance (+26%) than females. Moreover, patients with low albumin (<2.9g/dl) and high alkaline phosphatase (>484U/L), both indicators of advanced disease, exhibited approximately 20% higher bevacizumab clearance compared to those with median laboratory values. Significant variations were not described when the pharmacokinetics of bevacizumab was assessed in patients of different ethnicity or age. Existing data also support that bevacizumab does not have an effect on the pharmacokinetics of conventional chemotherapeutic agents [17].

Toxicity

The most frequent side effects of bevacizumab are generally mild or moderate in severity and thus, treatment with the antibody is usually well tolerated. Moreover, the toxicity profile of bevacizumab is similar across different indications [18]. Furthermore, most of the adverse events do not appear to be dose-related. Additionally, large observational studies have demonstrated that the development of serious toxicities does not increase following long-term exposure to bevacizumab [19]. The most common side effects of bevacizumab include hypertension, proteinuria and hemorrhage. It is possible that the pathogenesis of hypertension is related to a decrease in nitric oxide levels subsequently to VEGF inhibition, resulting in vasoconstriction [20]. Regarding breast cancer patients, in the randomized trials E2100 and AVF2119g the overall rate of bevacizumab-induced, grade 3 hypertension was 15.4% and 17.9%, respectively. Hypertension is usually sufficiently controlled with standard antihypertensive treatments, whereas permanent discontinuation of the antibody due to severe, uncontrolled hypertension is hardly ever necessary. Proteinuria of any grade is frequent but the incidence of grade 3 or 4 proteinuria in the abovementioned studies was below 2%. The most common hemorrhagic events include minor mucocutaneous bleeding, mainly epistaxis.

Serious adverse events such as arterial thromboembolism, wound healing complications, gastrointestinal perforation and reversible posterior leukoencephalopathy are experienced less frequently. An increased incidence of grade 3 or 4 congestive heart failure/cardiomyopathy has been observed in patients with breast cancer treated with bevacizumab (2.2% and 3.1% in the E2100 and AVF2119g studies, respectively). Chest wall irradiation and previous exposure to anthracyclines are possible risk factors. Most cases of congestive heart failure improve following medical therapy. The toxicity profile of bevacizumab in other trials in breast cancer patients (AVADO, RIBBON 1 and MO19391) was similar to that of prior phase III studies. Osteonecrosis of the jaw (ONJ) is a rare side effect which is usually associated with the administration of bisphosphonates in cancer patients. Recent data suggest that anti-angiogenic agents such as bevacizumab may induce the risk of ONJ when used along with bisphosphonates, and this observation requires additional confirmation in a prospective manner.

Clinical Efficacy in Chemotherapy-Naïve Patients

In the E2100 phase III trial, 722 patients with advanced breast cancer (mainly human epidermal growth factor receptor [HER] 2- negative) were randomized to receive paclitaxel as first-line therapy, with or without the addition of bevacizumab [21]. The dose of bevacizumab was 10 mg/kg (every 2 weeks). The results of the study showed that bevacizumab significantly improved the primary endpoint of the trial which was progression-free survival (PFS) (median, 11.8 months *vs.* 5.9 months; hazard ratio for progression, 0.6; $P<0.001$). Moreover, the response rate (RR) was also improved (36.9% *vs.* 21.2%, $P<0.001$). On the other hand, no difference was shown with the use of bevacizumab in overall survival (OS).

The AVADO trial is another randomized, placebo-controlled, phase III study, exploring the efficacy and safety of bevacizumab combined with docetaxel, as first-line treatment in patients with advanced breast cancer [22]. In this study, 736 HER2-negative patients were randomly assigned to receive docetaxel in combination with either bevacizumab (7.5 mg/kg or 15 mg/kg) or placebo. Bevacizumab 15 mg/kg, but not 7.5 mg/kg, combined with docetaxel demonstrated superior median PFS compared to placebo plus docetaxel. Response rates were also significantly increased with bevacizumab15 mg/kg.

The third randomized, placebo-controlled, phase III study of bevacizumab as first-line treatment is RIBBON 1 [23]. This study evaluated bevacizumab combined with different types of chemotherapeutic agents (single-agent capecitabine *vs.* single-agent taxane [nab-paclitaxel or docetaxel] *vs.* anthracycline-based regimen). RIBBON 1 included 1237 women with HER2-negative breast cancer. The patients were randomized (at a 2:1 ratio) to receive either bevacizumab (15 mg/kg) or placebo, every 3 weeks. Median PFS was longer for each bevacizumab combination.

A recent meta-analysis of the aforementioned phase III studies, presented at the Annual Meeting of the American Society of Clinical Oncology in 2010, demonstrated no OS benefit and a small improvement (2.5 months) in median PFS ($P<0.0001$) with the addition of bevacizumab to chemotherapy [24]. Following these data, the US Food and Drug Administration (FDA) is re-evaluating the approval of bevacizumab in metastatic breast cancer.

MO19391 is an open-label, single-arm, multicentre trial of bevacizumab in combination with first-line (mainly taxane-based) chemotherapy, in patients with HER2-negative, advanced breast carcinoma [25]. The primary objective of MO19391 was to evaluate the toxicity profile of the regimen in a broader population of breast cancer patients. An analysis of this study including 2251 patients revealed that the safety profile and the activity (PFS) of the combination in the general oncology practice context, appears to be comparable with that observed in phase III trials.

Regarding the duration of therapy, current data indicate that bevacizumab should be administered until the progression of the disease to optimize the benefit derived from the antibody. Preclinical studies have demonstrated rapid revascularization following early discontinuation of anti-VEGF treatment which might lead to re-growth of the tumor. Moreover, an exploratory analysis of the AVADO trial showed that maintenance use of bevacizumab after the discontinuation of docetaxel delayed progression of the disease, compared with placebo maintenance treatment [26].

Clinical Efficacy in Refractory Disease

The efficacy of bevacizumab in pretreated patients with metastatic breast cancer was investigated in a randomized, phase III trial (AVF2119g) [27]. In this study, 462 women previously treated with an anthracycline and a taxane, were randomized to receive capecitabine alone or in combination with bevacizumab (15 mg/kg every 3 weeks). Although the RR was significantly improved with the use of bevacizumab, neither the PFS nor the OS were affected by the addition of the antibody. These findings may imply that bevacizumab could be more efficient in earlier stages of breast cancer, when the significance of VEGF as a promoter of angiogenesis may be more pronounced.

A more recent randomized trial (RIBBON 2) investigated whether the addition of the antibody to second-line chemotherapy (single-agent taxane *vs.* gemcitabine *vs.* vinorelbine *vs.* single-agent capecitabine) could increase PFS, in 650 patients with HER2-negative advanced disease. The selection of chemotherapy regimen was at the discretion of the investigator and patients were randomized at a 2:1 ratio to receive either bevacizumab or placebo. In this study, the addition of bevacizumab significantly improved the RR and PFS [28].

A number of trials which evaluated the combination of bevacizumab with metronomic chemotherapy revealed that such regimens may be active in pretreated patients with advanced breast cancer [29, 30]. It has been shown that the administration of metronomic chemotherapy is associated with an anti-angiogenic activity through inhibiting the mobilization of circulating endothelial progenitors [31]. Consequently, metronomic chemotherapy regimens might represent attractive partners for other anti-angiogenic treatments, such as bevacizumab.

Combinations with Other Biological Agents

The majority of studies evaluating bevacizumab did not include patients with HER2-positive disease and hence, data concerning the role of this antibody in HER2-positive breast cancer are limited. Nevertheless, considerable cross-talk between HER2 and VEGF pathways seems to exist [32-35], as well as a synergistic effect between the mAbs trastuzumab and bevacizumab [36]. These observations along with the encouraging findings of phase II studies [37] represent the basis for ongoing, randomized phase III trials, which investigate the incorporation of bevacizumab into first-line, trastuzumab-based chemotherapy regimens in patients with HER2-positive, metastatic breast cancer (AVEREL, ECOG 1105). Combinations of bevacizumab with other biological agents, such as lapatinib, sunitinib or sorafenib are also currently being evaluated. Preclinical data have also demonstrated considerable cross-talk between VEGF and estrogen receptor pathways [38, 39]. Following the encouraging results of phase II trials, the role of bevacizumab plus hormonal agents is currently being evaluated in phase III studies.

Early Breast Cancer

The significance of VEGF appears to be more evident in the early steps of angiogenesis, since other inducers of this process are expressed during later stages of cancer progression. Consequently, the use of bevacizumab may be more valuable early in the process of carcinogenesis.

Following the results of phase II studies (ECOG 2104) [40] suggesting that integration of bevacizumab into anthracycline-containing adjuvant chemotherapy regimens is feasible, prospective phase III trials such as ECOG 5103 are evaluating the potential role of bevacizumab in the postoperative management of patients with early-stage breast cancer. BEATRICE is another randomized phase III study assessing the use of bevacizumab in the adjuvant treatment of patients with triple-negative early breast carcinoma, whereas BETH trial is investigating adjuvant chemotherapy plus trastuzumab, with or without the addition of bevacizumab, in patients with HER2-positive disease.

Furthermore, the potential role of bevacizumab in combination with neo-adjuvant regimens is also being evaluated in prospective, phase III studies. In two recently published clinical trials, the addition of bevacizumab to neoadjuvant chemotherapy significantly increased the rate of pathological complete response (pCR) among patients with HER2-negative early-stage breast cancer [41, 42].

OTHER ANTI-ANGIOGENIC TREATMENTS

Monolonal Antibodies Targeting VEGFRs

Apart from mAbs against VEGF, mAbs targeting VEGFRs are also under evaluation, although these agents are in less advanced stages of development, compared to bevacizumab. An ongoing phase III trial (NCT00703326) is currently investigating the addition of the antibody ramucirumab to first-line chemotherapy with docetaxel, in patients with HER2-negative, metastatic or locally-recurrent breast cancer.

VEGF-Trap

VEGF-Trap (aflibercept) is a fully recombinant, decoy fusion protein containing the extracellular domain 2 of VEGFR-1 and the extracellular domain 3 of VEGFR-2,

fused to the Fc portion of human IgG1 [43]. Aflibercept has the potential to bind to all isoforms of VEGF and to PlGF with high affinity. Preclinical models suggest that VEGF-Trap promotes the regression of existing tumor vasculature, induces the normalization of remaining vessels, and inhibits the formation of new tumor vessels. Preclinical studies demonstrated the efficacy of VEGF-Trap in a wide range of tumors. In addition, other studies have evaluated VEGF-Trap combined with several anticancer agents. Based on phase I trials, the recommended dose of VEGF-Trap for further evaluation is 4 mg/kg every 2 weeks. Phase II studies investigating the role of VEGF-Trap in a variety of solid tumors are currently under way.

Tyrosine Kinase Inhibitors

VEGFR TKIs are small molecules that block the ATP-binding pocket in the tyrosine-kinase domain of the VEGFR.

Axitinib

Axitinib is an oral inhibitor of the VEGFR, platelet-derived growth factor receptor (PDGFR) and colony stimulating factor-1 receptor tyrosinekinases. Phase II studies assessing the activity of axitinib monotherapy or combinations with chemotherapy have demonstrated efficacy in different types of tumors, including breast cancer [44].

Sorafenib

Sorafenib is also an orally administered multi-kinase inhibitor targeting VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, RAF kinase, c-Kit, and Flt-3. A randomized phase II study (SOLTI-0701) investigated sorafenib versus placebo combined with capecitabine in patients with advanced HER2-negative breast cancer [45]. A total of 229 patients who had received ≤1 prior chemotherapy regimens were included in the study. The addition of sorafenib to capecitabine significantly improved the median PFS (6.4 months *vs.* 4.1 months; P=0.0006), whereas the benefit was more pronounced in patients receiving the regimen as first-line treatment. These results indicate that sorafenib may be active in combination with chemotherapy in patients with metastatic breast cancer. These findings have led to the design of a phase III trial in order to confirm the abovementioned phase II data. Furthermore, other studies are also evaluating the efficacy and safety of sorafenib combined with chemotherapy and/or hormonal therapy in patients with HER2-negative metastatic or locally advanced breast cancer [46].

Sunitinib

Sunitinib is another TKI with anti-angiogenetic activity. Sunitinib inhibits not only VEGFRs but also PDGFR, c-Kit, RET and Flt-3. A phase II trial in heavily pretreated patients receiving sunitinib monotherapy demonstrated efficacy of the treatment, particularly in patients with triple-negative or HER2-positive disease [47]. However, a randomized phase III study which compared sunitinib with capecitabine in HER2-negative patients previously treated with anthracyclines and taxanes demonstrated that single agent sunitinib was inferior to capecitabine, in terms of PFS and RR [48]. Furthermore, two recent phase III trials evaluating the addition of sunitinib to first-line chemotherapy with docetaxel [49] or capecitabine as second or third-line therapy [50] showed no advantage for the combination, whereas toxicity was significantly increased.

Vatalanib

Vatalanib is an oral administered TKI that blocks the function of VEGFR-1, VEGFR-2 and VEGFR-3 and to some degree, PDGFR, c-Kit and Fos [51]. Phase I/II studies are currently evaluating vatalanib in combination with a variety of agents such as docetaxel, trastuzumab or letrozole in patients with advanced breast cancer.

RESISTANCE TO ANTI-VEGF AGENTS

It is well known that a considerable proportion of patients receiving anti-VEGF agents will not respond, due to inherent resistance. Furthermore, even when the disease responds to the treatment, the duration is usually short as a result of acquired resistance. The identification of pathways mediating resistance to bevacizumab and other VEGF targeted agents is expected to lead to the development of efficient strategies to overcome resistance and thus, it is great importance in the management of cancer patients.

Current data indicate the following mechanisms [52]: up-regulation of alternative pro-angiogenic pathways, including those involving the fibroblast growth factor (FGF) family of ligands [53], PlGF [54], members of the ephrin and angiopoietin families [55] and the notch ligand/receptor system [56]; recruitment of bone marrow-derived cells that secrete pro-angiogenic factors [57]; increased pericyte coverage of tumor blood vessels, supporting the survival of endothelial cells and protecting tumor vasculature [58]; and enhancement of invasion of tumor cells into adjacent normal tissue to achieve vascular sufficiency [53].

PREDICTIVE FACTORS

In patients with metastatic breast cancer, the addition of bevacizumab to chemotherapy improves the efficacy over chemotherapy alone, in terms of PFS. However, although treatment with bevacizumab is generally well tolerated, a small percentage of patients will develop serious, potentially life-threatening toxicities. Moreover, the cost of bevacizumab therapy is considerably high. Consequently, the detection of those patients who may benefit from the antibody is considered crucial in the treatment of breast cancer.

In December 2010 the FDA revoked the approval of bevacizumab in patients with metastatic breast cancer, concluding that the benefits of the treatment do not outweigh the risks. On the other hand, the European Medicines Agency (EMA) supported the use of bevacizumab in metastatic breast cancer combined with paclitaxel. Recently, the EMA also recommended the use of bevacizumab plus capecitabine as first-line therapy for patients who are not suitable for other options, such as anthracyclines or taxanes.

Thus far, no definitive biomarkers have been identified that can reliably predict benefit from bevacizumab or other anti-VEGF agents. Retrospective studies have evaluated genetic, molecular or biological markers for bevacizumab

efficacy. Recent data indicate a potential predictive significance of *VEGF* genotypes. A retrospective analysis investigated the correlation of *VEGF* genotypes with activity of the treatment in breast cancer patients involved in the E2100 study [59]. In this analysis, patients with *VEGF*-1154 AA and *VEGF*-2578 AA genotypes treated with paclitaxel and bevacizumab had significantly improved median OS versus those with alternative genotypes. Furthermore, these findings were not demonstrated in the control arm of patients receiving paclitaxel only, suggesting the predictive value of these genotypes. Another study in patients with ovarian cancer treated with cyclophosphamide and bevacizumab showed that the *VEGF*-936 CT genotype was associated with a longer median PFS, compared with the CC and TT genotype [60].

Recently, we showed that the *VEGF*-1154 GG genotype was associated with significantly shorter OS in patients with colorectal cancer receiving irinotecan-containing chemotherapy plus bevacizumab [61]. Moreover, the *VEGF*-1154 GG genotype was more frequent in patients not responding to treatment compared with responders. It seems that the genetic variability of *VEGF,* which is a highly polymorphic gene, potentially affects the expression and function of VEGF. The *VEGF*-1154 GG genotype has been correlated with higher production of VEGF [62]. In another study in metastatic colorectal cancer evaluating the administration of irinotecan, gemcitabine and bevacizumab in pretreated patients, low VEGF level-associated single nucleotide polymorphisms (SNPs) were associated with an improved time to disease-progression [63]. Additionally, in the above mentioned study considering breast cancer patients [59], the *VEGF*-1154 AA and *VEGF*-2578 AA genotypes were associated with a trend for lower VEGF expression. These findings suggest a potential predictive role of *VEGF* genotypes affecting the expression levels of VEGF, for patients treated with bevacizumab-containing regimens. However, the predictive significance of the afore mentioned genotypes needs to be prospectively validated in larger cohorts.

In addition, recent evidence supports the predictive value of circulating endothelial cells (CECs) in cancer patients treated with bevacizumab-based therapy. It has been suggested that the level of CECs may reflect the angiogenic turnover and therefore may serve as a biomarker. A neoadjuvant trial which evaluated the combination of bevacizumab with letrozole and chemotherapy in patients with locally advanced breast carcinoma showed that basal circulating progenitors were positively correlated with clinical response [64]. Another study investigated the combination of bevacizumab with metronomic chemotherapy in pretreated patients with advanced breast cancer. In this study, baseline CECs and viable CECs were significantly increased in patients experiencing clinical response and clinical benefit. Moreover, baseline apoptotic CECs were associated with a significantly improved PFS [29]. Nevertheless, prognostic effects of CECs have also been demonstrated. In addition, the markers used to identify these cells vary considerably among different studies.

The occurrence of hypertension during therapy with bevacizumab may represent a surrogate biological marker of treatment efficacy, reflecting sufficient VEGF inhibition. Small studies in different tumor types support the predictive role of hypertension for treatment with bevacizumab [65-67]. Moreover, in E2100 phase III study, patients developing grade 3 or 4 hypertension, had a significantly improved median OS compared with those not developing hypertension [59]. Other retrospective analyses of phase III trials in patients with advanced non-small cell lung cancer and renal-cell carcinoma have demonstrated similar results, regarding the predictive value of hypertension for bevacizumab efficacy [68, 69]. On the other hand, another recent retrospective analysis of six studies showed that hypertension was predictive in only one trial [70]. It is obvious that the retrospective nature of the above mentioned analyses underlines the need for prospective studies in order to elucidate the potential predictive significance of hypertension. However, a major limitation is that the predictive value of hypertension can only be evaluated after the initiation of treatment. It is clear that useful biomarkers in the clinical practice could be only those that enable the prospective identification of candidate patients for bevacizumab therapy.

Finally, recent evidence suggests that imaging tools such as dynamic contrast enhanced (DCE)-MRI or PET may be useful to assess the activity of bevacizumab. However, the predictive value of these imaging criteria, as well as other circulating or in-situ biomarkers for the efficacy of bevacizumab is not validated thus far [71].

CONCLUSIONS AND FUTURE PERSPECTIVES

Current evidence indicates that the incorporation of bevacizumab in the treatment of unselected patients with advanced breast cancer offers a modest improvement in PFS, with no effect on OS. Since bevacizumab may be active in a specific subset of breast cancer patients, it is of great importance to identify reliable predictive markers for the individualized selection of patients who might benefit from the treatment. Nevertheless, even though accumulating data suggest predictive value for several biomarkers, no definitive factors have been identified so far. Moreover, the underlying pathways mediating resistance to treatment need to be further elucidated. Apart from chemotherapy, ongoing studies are evaluating the safety and activity of bevacizumab combined with various agents, such as hormonal treatments and other targeted therapies. Moreover, other trials are investigating the potential role of bevacizumab in earlier stages of the disease. Finally, alternative strategies to inhibit the VEGF pathway such as anti-VEGFR mAbs, VEGF-Traps and anti-VEGFR TKIs are currently under clinical evaluation. The failure of bevacizumab studies to show survival benefit, along with the negative results of other trials investigating agents targeting the VEGF pathway (such as sunitinib), lead to the conclusion that targeted agents should not be studied in unselected patients.

Concerning other types of cancer, results of prospective clinical trials have been variable, ranging from negative studies to trials demonstrating PFS or OS benefits. The addition of bevacizumab to chemotherapy has been associated with a significant improvement in OS in patients

with metastatic colorectal cancer in first-line and second-line treatment, as well as in patients with advanced non-small cell lung cancer receiving first-line chemotherapy. In patients with advanced ovarian, renal and gastric carcinoma, an advantage in PFS has been demonstrated, whereas in recurrent glioblastoma the use of bevacizumab has been associated with a considerably high RR and 6-month PFS. Regarding anti-VEGFR TKIs, sunitinib has been associated with a PFS benefit in first-line treatment of advanced renal cancer, whereas sorafenib has shown an OS advantage in patients with hepatocellular carcinoma. Moreover, a phase III trial demonstrated an OS benefit from the addition of the VEGF-Trap aflibercept to second-line chemotherapy, in patients with metastatic colorectal cancer. It is well documented that the dependence of cancer pathogenesis on VEGF pathway differs considerably among several types of cancer, and this might explain the difference in activity of anti-angiogenic treatments in solid tumors. Moreover, there has been a debate whether PFS represents a valid endpoint in clinical trials. However, it is possible that the effect on OS may be influenced by several factors including the statistical power of the study to detect an OS benefit, cross-over to the investigational agent, treatments administered following disease progression, as well as duration of survival post progression.

In conclusion, it is clear that inhibition of angiogenesis works. It may not be a curative strategy, but it represents an important step forward in the treatment of a disease that is difficult to treat.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

MEK = MAPK/extracellular signal-regulated kinase (Erk) kinase

MAPK = Mitogen-activated protein kinase

PI3-K = Phosphatidylinositol 3-kinase

VEGF = Vascular endothelial growth factor

PlGF = Placental growth factor

Nrp1 = Neuropilin 1

Nrp2 = Neuropilin 2

TKI = Tyrosine-kinase inhibitor

VEGFR = Vascular endothelial growth factor receptor

REFERENCES

[1] Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.*, **1996**, *1*, 27-31.

[2] Ferrara, N. VEGF and the quest for tumour angiogenesis factors. *Nat. Rev. Cancer*, **2002**, *2*, 795-803.

[3] Hicklin, D.J.; Ellis, L.M. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J. Clin. Oncol.*, **2005**, *23*, 1011-1027.

[4] Shibuya, M.; Claesson-Welsh, L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp. Cell Res.*, **2006**, *312*, 549-560.

[5] Kerbel, R.S. Tumor Angiogenesis. *N. Engl. J. Med.*, **2008**, *358*, 2039-2049.

[6] Relf, M.; LeJeune, S.; Scott, P.A.; Fox, S.; Smith, K.; Leek, R.; Moghaddam, A.; Whitehouse, R.; Bicknell, R.; Harris, A.L. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res.*, **1997**, *57*, 963-969.

[7] Yoshiji, H.; Gomez, D.E.; Shibuya, M.; Thorgeirsson, U.P. Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer. *Cancer Res.*, **1996**, *56*, 2013-2016.

[8] Ferrara, N.; Gerber, H.P.; LeCouter, J. The biology of VEGF and its receptors. *Nat. Med.*, **2003**, *9*, 669-676.

[9] Inai, T.; Mancuso, M.; Hashizume, H.; Baffert, F.; Haskell, A.; Baluk, P.; Hu-Lowe, D.D.; Shalinsky, D.R.; Thurston, G.; Yancopoulos, G.D.; McDonald, D.M. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. *Am. J. Pathol.*, **2004**, *165*, 35-52.

[10] Jain, R.K. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science*, **2005**, *307*, 58-62.

[11] Wildiers, H.; Guetens, G.; De Boeck, G.; Verbeken, E.; Landuyt, B.; Landuyt, W.; de Bruijn, E.A.; van Oosterom, A.T. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. *Br. J. Cancer*, **2003**, *88*, 1979-1986.

[12] Dallas, N.A.; Fan, F.; Gray, M.J.; Van Buren, G. 2nd.; Lim, S.J.; Xia, L.; Ellis, L.M. Functional significance of vascular endothelial growth factor receptors on gastrointestinal cancer cells. *Cancer Metast. Rev.*, **2007**, *26*, 433-441.

[13] Rovati, B.; Manzoni, M.; Bencardino, K.; Ronzoni, M.; Mariucci, S.; Loupakis, F.; Gattoni, E.; Villa, E.; Falcone, A.; Danova, M. In: *The effect of bevacizumab plus chemotherapy on the immunological profile of advanced colorectal cancer patients* In: Proceedings of the 2008 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, May 30-June 3, 2008; *Journal of Clinical Oncology*: **2008**; Abstr 14579.

[14] Wedam, S.B.; Low, J.A.; Yang, S.X.; Chow, C.K.; Choyke, P.; Danforth, D.; Hewitt, S.M.; Berman, A.; Steinberg, S.M.; Liewehr, D.J.; Plehn, J.; Doshi, A.; Thomasson, D.; McCarthy, N.; Koeppen, H.; Sherman, M.; Zujewski, J.; Camphausen, K.; Chen, H.; Swain, S.M. Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. *J. Clin. Oncol.*, **2006**, *24*, 769-777.

[15] Ran, S.; Bivens, C.; Trieu, V.; Desai, N. Synergistic effect of albumin-bound paclitaxel (abraxane) and anti-VEGF-A antibody (avastin) on growth of orthotopic MDA-MB-231 breast tumors as well as lymphatic and pulmonary disease. Program and abstracts of the 29th Annual San Antonio Breast Cancer Symposium; December 14-17, **2006**; San Antonio, Texas. [Abstract 1094].

[16] Higgins, B.; Kolinsky, K.; Zhang, Y-E.; Moisa, C.; Dugan, U.; Packman, K. Antitumor activity of a combined regimen of capecitabine and bevacizumab in a xenograft model of human breast cancer. Program and abstracts of the 29th Annual San Antonio Breast Cancer Symposium; December 14-17, **2006**; San Antonio, Texas. [Abstract 6116].

[17] European Medicines Agency, **2005**.

[18] Miles, D. Management of toxicity in patients receiving therapy with bevacizumab. *Eur. J. Cancer Suppl.*, **2008**, *6*, 29-39.

[19] Purdie, D.M.; Berlin, J.D.; Flynn, P.J.; Grothey, A.; Kabbinavar, F.F.; Kozloff, M.F.; Dong, W.; Sugrue, M.M. In: *The safety of long-term bevacizumab use: Results from the BRiTE observational cohort study (OCS)*, Proceedings of the 2008 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, May 30-June 3, 2008; *Journal of Clinical Oncology*: **2008**; Abstr 4103.

[20] Hood, J.D.; Meininger, C.J.; Ziche, M.; Granger, H.J. VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells. *Am. J. Physiol.*, **1998**, *274(3 Pt 2),* H1054-1058.

[21] Miller, K.; Wang, M.; Gralow, J.; Dickler, M.; Cobleigh, M.; Perez, E.A.; Shenkier, T.; Cella, D.; Davidson, N.E. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N. Engl. J. Med.*, **2007**, *357*, 2666-2676.

[22] Miles, D.W.; Chan, A.; Dirix, L.Y.; Cortés, J.; Pivot, X.; Tomczak, P.; Delozier, T.; Sohn, J.H.; Provencher, L.; Puglisi, F.; Harbeck, N.; Steger, G.G.; Schneeweiss, A.; Wardley, A.M.; Chlistalla, A.; Romieu, G. 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*, 3239-3247.

[23] Robert, N.J.; Diéras, V.; Glaspy, J.; Brufsky, A.M.; Bondarenko, I.; Lipatov, O.N.; Perez, E.A.; Yardley, D.A.; Chan, S.Y.; Zhou, X.; Phan, S.C.; O'Shaughnessy, J. 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*, 1252-1260.

[24] O'Shaughnessy, J.; Miles, D.; Gray, R.J.; Dieras, V.; Perez, E.A.; Zon, R.; Cortes, J.; Zhou, X.; Phan S.; Miller K. In: *A meta-analysis of overall survival data from three randomized trials of bevacizumab (BV) and first-line chemotherapy as treatment for patients with metastatic breast cancer (MBC)*. In: Proceedings of the 2010 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, June 4-8, 2010; *J. Clin. Oncol.*, **2010**; Abstr 1005.

[25] Smith, I.E.; Pierga, J.Y.; Biganzoli, L.; Cortés-Funes, H.; Thomssen, C.; Pivot, X.; Fabi, A.; Xu, B.; Stroyakovskiy, D.; Franke, F.A.; Kaufman, B.; Mainwaring, P.; Pienkowski, T.; De Valk, B.; Kwong, A.; González-Trujillo, J.L.; Koza, I.; Petrakova, K.; Pereira, D.; Pritchard, K.I.; ATHENA Study Group. First-line bevacizumab plus taxane-based chemotherapy for locally recurrent or metastatic breast cancer: safety and efficacy in an open-label study in 2,251 patients. *Ann. Oncol.*, **2011**, *22*, 595-602.

[26] Fumoleau, P.; Greil, R.; Rayson, D.; Müller, V.; Barni, S.; Aleknavicius, E.; Tellez, E.; Wilson, C.; Miles, D.W. In: Bevacizumab (BV) maintenance therapy significantly delays disease progression (PD) or death compared with placebo (PL) in the AVADO trial (BV + docetaxel [D]vs D + PL in 1st-line HER2-negative locally recurrent [LR]or metastatic breast cancer [mBC]). In: Program and abstracts of the 31st Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, December 10-14, **2008**; Abstr 903.

[27] Miller, K.D.; Chap, L.I.; Holmes, F.A.; Cobleigh, M.A.; Marcom, P.K.; Fehrenbacher, L.; Dickler, M.; Overmoyer, B.A.; Reimann, J.D.; Sing, A.P.; Langmuir, V.; Rugo, H.S. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J. Clin. Oncol.*, **2005**, *23*, 792-799.

[28] Brufsky, A.; Bondarenko, I.; Smirnov, V.; Hurvitz, S.; Perez, E.; Ponomarova, O.; Vynnychenko, I.; Swamy, R.; Mu, H.; Rivera, R. In: *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 HER2-negative metastatic breast cancer*. In: Program and abstracts of the 32nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, December 10-13, **2009**; Abstr 42.

[29] Dellapasqua, S.; Bertolini, F.; Bagnardi, V.; Campagnoli, E.; Scarano, E.; Torrisi, R.; Shaked, Y.; Mancuso, P.; Goldhirsch, A.; Rocca, A.; Pietri, E.; Colleoni, M. Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. *J. Clin. Oncol.*, **2008**, *26*, 4899-4905.

[30] García-Sáenz, J.A.; Martín, M.; Calles, A.; Bueno, C.; Rodríguez, L.; Bobokova, J.; Custodio, A.; Casado, A.; Díaz-Rubio, E. Bevacizumab in combination with metronomic chemotherapy in patients with anthracycline- and taxane-refractory breast cancer. *J. Chemother.*, **2008**, *20*, 632-639.

[31] Shaked, Y.; Emmenegger, U.; Man, S.; Cervi, D.; Bertolini, F.; Ben-David, Y.; Kerbel, R.S. Optimal biologic dose of metronomic chemotherapy regimens is associated with maximum antiangiogenic activity. *Blood*, **2005**, *106*, 3058-3061.

[32] Russell, K.S.; Stern, D.F.; Polverini, P.J.; Bender, J.R. Neuregulin activation of ErbB receptors in vascular endothelium leads to angiogenesis. *Am. J. Physiol.*, **1999**, *277*(Pt 2), H2205-11.

[33] Yen, L.; You, X.L.; Al Moustafa, A.E.; Batist, G.; Hynes, N.E.; Mader, S.; Meloche, S.; Alaoui-Jamali, M.A. Heregulin selectively upregulates vascular endothelial growth factor secretion in cancer cells and stimulates angiogenesis. *Oncogene*, **2000**, *19*, 3460-3469.

[34] Finkenzeller, G.; Weindel, K.; Zimmermann, W.; Westin, G.; Marmé, D. Activated Neu/ErbB-2 induces expression of the vascular endothelial growth factor gene by functional activation of the transcription factor Sp 1. *Angiogenesis*, **2004**, *7*, 59-68.

[35] Klos, K.S.; Wyszomierski, S.L.; Sun, M.; Tan, M.; Zhou, X.; Li, P.; Yang, W,; Yin, G.; Hittelman, W.N.; Yu, D. ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. *Cancer Res.*, **2006**, *66*, 2028-2037.

[36] Scheuer, W.; Friess, T.; Hasmann, M. Enhanced antitumour effect by combination of HER2-targeting antibodies with bevacizumab in a human breast cancer xenograft model. *Eur. J. Cancer*, **2006**, *4*(Suppl), 66 [Abstr 213].

[37] Pegram, M.; Chan, D.; Dichmann, R.A.; Tan-Chiu, E.; Yeon, C.; Durna, L.; Lin, L.S.; Slamon, D. In: Phase II combined biological therapy targeting the HER2 protooncogene and the vascular endothelial growth factor using trastuzumab (T) and bevacizumab (B) as first line treatment of HER2-amplified breast cancer. In: Program and abstracts of the 29th Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, December 14-17, **2006**; Abstr 301.

[38] Hyder, S.M.; Nawaz, Z.; Chiappetta, C.; Stancel, G.M. Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. *Cancer Res.*, **2000**, *60*, 3183-3190.

[39] Takei, H.; Lee, E.S.; Jordan, V.C. In vitro regulation of vascular endothelial growth factor by estrogens and antiestrogens in estrogen-receptor positive breast cancer. *Breast Cancer*, **2002**, *9*, 39-42.

[40] Miller, K.D.; O'Neill, A.; Perez, E.A.; Seidman, A.D.; Sledge, G.W. In: Phase II feasibility trial incorporating bevacizumab into dose dense doxorubicin and cyclophosphamide followed by paclitaxel in patients with lymph node positive breast cancer: a trial of the Eastern Cooperative Oncology Group (E2104). In: Proceedings of the 2008 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, May 30 - June 3, 2008; Journal of Clinical Oncology: **2008**; Abstr 520.

[41] von Minckwitz, G.; Eidtmann, H.; Rezai, M.; Fasching, P.A.; Tesch, H.; Eggemann, H.; Schrader, I.; Kittel, K.; Hanusch, C.; Kreienberg, R.; Solbach, C.; Gerber, B.; Jackisch, C.; Kunz, G.; Blohmer, J-U.; Huober, J.; Hauschild, M.; Fehm, T.; Müller, B.M.; Denkert, C.; Loibl, S.; Nekljudova, V.; Untch, M.; German Breast Group; Arbeitsgemeinschaft Gynäkologische Onkologie–Breast Study Groups. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. *N. Engl. J. Med.,* **2012**, *366*, 299-309.

[42] Bear, H.D.; Tang, G.; Rastogi, P.; Geyer, C.E. Jr.; Robidoux, A.; Atkins, J.N.; Baez-Diaz, L.; Brufsky, A.M.; Mehta, R.S.; Fehrenbacher, L.; Young, J.A.; Senecal, F.M.; Gaur, R.; Margolese, R.G.; Adams, P.T.; Gross, H.M.; Costantino, J.P.; Swain, S.M.; Mamounas, E.P.; Wolmark, N. Bevacizumab added to neoadjuvant chemotherapy for breast cancer. *N. Engl. J. Med.,* **2012**, *366*, 310-320.

[43] Teng, L.S.; Jin, K.T.; He, K.F.; Zhang, J.; Wang, H.H.; Cao, J. Clinical applications of VEGF-trap (aflibercept) in cancer treatment. *J. Chin. Med. Assoc.*, **2010**, *73*, 449-456.

[44] Choueiri, T.K. Axitinib, a novel anti-angiogenic drug with promising activity in various solid tumors. *Curr. Opin. Investig. Drugs*, **2008**, *9*, 658-671.

[45] Baselga, J.; Grupo Español de Estudio Tratamiento y Otras Estrategias Experimentales en Tumores Sólidos, Roché H. In: *SOLTI-0701: a multinational double-blind, randomized phase 2b study evaluating the efficacy and safety of sorafenib compared to placebo when administered in combination with capecitabine in patients with locally advanced or metastatic breast cancer (BC)*. In: Program and abstracts of the 32nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, December 10-13, **2009**; Abstr 45.

[46] Baselga, J.; Gianni, L.; Gradishar, W.J.; Hudis, C.; Perez, E.A.; Piccart-Gebhart, M.; Schwartzberg, L.S.; Sledge, G.; Fleming, T.R. In: *Phase IIb double-blind, randomized, placebo-controlled trials for the efficacy and safety of sorafenib in patients (pts) with metastatic or locally advanced breast cancer (BC): Review of the Trials to Investigate the Effects of Sorafenib in BC (TIES) program***.** In: Proceedings of the 2009 American Society of Clinical Oncology Annual Meeting, Orlando, Florida, May 29 - June 2, 2009; *Journal of Clinical Oncology*: **2009**; Abstr e12000.

[47] Burstein, H.J.; Elias, A.D.; Rugo, H.S.; Cobleigh, M.A.; Wolff, A.C.; Eisenberg, P.D.; Lehman, M.; Adams, B.J.; Bello, C.L.; DePrimo, S.E.; Baum, C.M.; Miller, K.D. 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*, 1810–1816.

[48] Barrios, C.; Liu, M.; Lee, S.; Vanlemmens, L.; Ferrero, J.; Tabei, T.; Pivot, X.; Iwata, H.; Aogi, K.; Brickman, M.; Zhang, K.; Kern K.; Martin, M. In: *Phase III randomized trial of sunitinib (SU) vs. capecitabine (C) in patients (Pts) with previously treated HER2-negative advanced breast cancer (ABC).* In: Program and abstracts of the 32nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, December 10-13, **2009**; Abstr 46.

[49] Bergh, J.; Greil, R.; Voytko, N.; Makhson, A.; Cortes, J.; Lortholary, A.; Huang, X.; Giorgetti, C.; Kern, K.A.; Lichinitser, M. In: *Sunitinib (SU) in combination with docetaxel (D) versus D alone for the first-line treatment of advanced breast cancer (ABC)*. In: Proceedings of the 2010 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, June 4-8, 2010; *Journal of Clinical Oncology*: **2010**; Abstr LBA 1010.

[50] Crown, J.; Dieras, V.; Staroslawska, E.; Yardley, D.A.; Davidson, N.; Bachelot, T.D.; Tassell, V.R.; Huang, X.; Kern, K.A.; Romieu, G. In: *Phase III trial of sunitinib (SU) in combination with capecitabine (C) versus C in previously treated advanced breast cancer (ABC)*. In: Proceedings of the 2010 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, June 4-8, 2010; *Journal of Clinical Oncology*: **2010**; Abstr LBA 1011.

[51] Giovannini, M.; Aldrighetti, D.; Zucchinelli, P.; Belli, C.; Villa, E. Antiangiogenic strategies in breast cancer management. *Crit. Rev. Oncol. Hematol.*, **2010**, *76*, 13-35. Epub 2010 Aug 11.

[52] Ellis, L.M.; Hicklin, D.J. Pathways mediating resistance to vascular endothelial growth factor – targeted therapy. *Clin. Cancer Res.*, **2008**, *14*, 6371-6375.

[53] Casanovas, O.; Hicklin, D.J.; Bergers, G.; Hanahan, D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell.*, **2005**, *8*, 299-309.

[54] Fischer, C.; Jonckx, B.; Mazzone, M.; Zacchigna, S.; Loges, S.; Pattarini, L.; Chorianopoulos, E.; Liesenborghs, L.; Koch, M.; De Mol, M.; Autiero, M.; Wyns, S.; Plaisance, S.; Moons, L.; van Rooijen, N.; Giacca, M.; Stassen, J.M.; Dewerchin, M.; Collen, D.; Carmeliet, P. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell*, **2007**, *131*, 463-475.

[55] Bergers, G.; Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer*, **2008**, *8*, 592-603.

[56] Ridgway, J.; Zhang, G.; Wu, Y.; Stawicki, S.; Liang, W.C.; Chanthery, Y.; Kowalski, J.; Watts, R.J.; Callahan, C.; Kasman, I.; Singh, M.; Chien, M.; Tan. C.; Hongo, J.A.; de Sauvage, F.; Plowman, G.; Yan, M. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature*, **2006**, *444*, 1083-1087.

[57] Grunewald, M.; Avraham, I.; Dor, Y.; Bachar-Lustig, E.; Itin, A.; Jung, S.; Chimenti, S.; Landsman, L.; Abramovitch, R.; Keshet E. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell*, **2006**, *124*, 175-189.

[58] Reinmuth, N.; Liu, W.; Jung, Y.D.; Ahmad, S.A.; Shaheen, R.M.; Fan, F.; Bucana, C.D.; McMahon, G.; Gallick, G.E.; Ellis, L.M. Induction of VEGF in perivascular cells defines a potential paracrine mechanism for endothelial cell survival. *FASEB J.*, **2001**, *15*, 1239-1241.

[59] Schneider, B.P.; Wang, M.; Radovich, M.; Sledge, G.W.; Badve, S.; Thor, A.; Flockhart, D.A.; Hancock, B.; Davidson, N.; Gralow, J.; Dickler, M.; Perez, E.A.; Cobleigh, M.; Shenkier, T.; Edgerton, S.; Miller, K.D. 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*, 4672-4678.

[60] Schultheis, A.M.; Lurje, G.; Rhodes, K.E.; Zhang, W.; Yang, D.; Garcia, A.A.; Morgan, R.; Gandara, D.; Scudder, S.; Oza, A.; Hirte, H.; Fleming, G.; Roman, L.; Lenz, H.J. Polymorphisms and clinical outcome in recurrent ovarian cancer treated with cyclophosphamide and bevacizumab. *Clin. Cancer Res.*, **2008**, *14*, 7554-7563.

[61] Koutras, A.K.; Antonacopoulou, A.G.; Eleftheraki, A.G.; Dimitrakopoulos, F.I.; Koumarianou, A.; Varthalitis, I.; Fostira, F.; Sgouros, J.; Briasoulis, E.; Bournakis, E.; Bafaloukos, D.; Bompolaki, I.; Galani, E.; Kalogeras, K.T.; Pectasides, D.; Fountzilas, G.; Kalofonos, H.P. Vascular endothelial growth factor polymorphisms and clinical outcome in colorectal cancer patients treated with irinotecan-based chemotherapy and bevacizumab. *Pharmacogenomics J.,* **2011**, Aug 16. doi: 10.1038/tpj.2011.37. [Epub ahead of print]

[62] Mohammadi, M.; Ollier, W.; Hutchinson, I.V. A functional association study of VEGF gene promoter polymorphisms with VEGF expression by stimulated pbm cells. *Hum. Immunol.*, **2003**, *64*, S125.

[63] Abajo, A.; Rodriguez, J.; Bitarte, N.; Zarate, R.; Boni, V.; Ponz, M.; Chopitea, A.; Bandres, E.; Garcia-Foncillas, J. Dose-finding study and pharmacogenomic analysis of fixed-rate infusion of gemcitabine, irinotecan and bevacizumab in pretreated metastatic colorectal cancer patients. *Br. J. Cancer*, **2010**, *103*, 1529-1535.

[64] Torrisi, R.; Bagnardi, V.; Cardillo, A.; Bertolini, F.; Scarano, E.; Orlando, L.; Mancuso, P.; Luini, A.; Calleri, A.; Viale, G.; Goldhirsch, A.; Colleoni, M. Preoperative bevacizumab combined with letrozole and chemotherapy in locally advanced ER- and/or PgR-positive breast cancer: clinical and biological activity. *Br. J. Cancer*, **2008**, *99*, 1564-1571.

[65] Friberg, G.; Kasza, K.; Vokes, E.E.; Kindler, H.L. In: Early hypertension (HTN) as a potential pharmacodynamic (PD) marker for survival in pancreatic cancer (PC) patients (pts) treated with bevacizumab (B) and gemcitabine (G). In: Proceedings of the 2005 American Society of Clinical Oncology Annual Meeting, Orlando, Florida, May 13-17, 2005; Journal of Clinical Oncology: **2005**; Abstr 3020.

[66] Scartozzi, M.; Galizia, E.; Chiorrini, S.; Giampieri, R.; Berardi, R.; Pierantoni, C.; Cascinu, S. Arterial hypertension correlates with clinical outcome in colorectal cancer patients treated with first-line bevacizumab. *Ann. Oncol.*, **2009**, *20*, 227-230.

[67] Bono, P.; Elfving, H.; Utriainen, T.; Osterlund, P.; Saarto, T.; Alanko, T.; Joensuu, H. Hypertension and clinical benefit of bevacizumab in the treatment of advanced renal cell carcinoma. *Ann. Oncol.*, **2009**, *20*, 393-394.

[68] Dahlberg, S.E.; Sandler, A.B.; Brahmer, J.R.; Schiller, J.H.; Johnson, D.H. Clinical course of advanced non-small-cell lung cancer patients experiencing hypertension during treatment with bevacizumab in combination with carboplatin and paclitaxel on ECOG 4599. *J. Clin. Oncol.*, **2010**, *28*, 949-954.

[69] Rini, B.I.; Halabi, S.; Rosenberg, J.E.; Stadler, W.M.; Vaena, D.A.; Archer, L.; Atkins, J.N.; Picus, J.; Czaykowski, P.; Dutcher, J.; Small, E.J. 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*, 2137-2143.

[70] Hurwitz, H.; Douglas, P.S.; Middleton, J.P.; Sledge, G.W.; Johnson, D.H.; Reardon, D.A.; Chen, D.; Rosen, O. In: *Analysis of early hypertension (HTN) and clinical outcome with bevacizumab (BV)*. In: Proceedings of the 2010 American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, June 4-8, 2010; *Journal of Clinical Oncology*: **2010**; Abstr 3039.

[71] Jubb, A.M.; Harris, A.L. Biomarkers to predict the clinical efficacy of bevacizumab in cancer. *Lancet Oncol.*, **2010**, *11*, 1172-1183.

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