Bevacizumab in the treatment of patients with advanced breast cancer: where have we landed?

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Abstract: Vast preclinical and clinical evidence has made angiogenesis one of the hallmarks of cancer. In many human tumours, vascular endothelial growth factor (VEGF) has been identified as the crucial mediator of this process. Initial studies suggested that angiogenesis, and VEGF in particular, could be inhibited without the risk of major side effects. After the pivotal data in first-line studies in patients with colorectal cancer, numerous clinical trials have been undertaken in patients with breast cancer. This review attempts to update these investigations and define the role of anti-VEGF antibody treatment in advanced breast cancer.

Keywords: advanced breast cancer, bevacizumab, chemotherapy, vascular endothelial growth factor

Introduction

Angiogenesis is defined as the sprouting of new blood vessels from pre-existing vessels. It is considered to be a prerequisite for tumour growth both for solid tumours and for haematological malignancies [Vermeulen *et al.* 2002; Perez-Atayde *et al.* 1997]. Quantification of blood vessels in breast cancer specimens and other human tumours is predictive for both relapse-free and overall survival [Borre *et al.* 1998; Dirix *et al.* 1997; Weidner *et al.* 1992]. Similarly, breast cancer vascularization has been found to predict for tumour cell dissemination in patients with early stage disease [Dhakal *et al.* 2008; Benoy *et al.* 2005]. The process of developing a high-density blood vessel network that connects the tumour with the host circulation has been termed the ‘angiogenic switch’. Both preclinical breast cancer models such as MMTV-PyMT mice, as well as pathological studies in noninvasive carcinoma, have revealed that the activation of the angiogenic phenotype is both an early event in tumour development and a rate-limiting step for tumour progression and dissemination [Bluff *et al.* 2009; Lin and Pollard, 2007; Jensen *et al.* 1982]. A crucial factor involved in angiogenesis in nearly all human tumours is a protein identified as vascular endothelial growth factor (VEGF) [Ferrara, 2009; Ferrara and Davis-Smith, 1997]. Nevertheless, an increasing

number of pathways are being elucidated that mediate VEGF-independent angiogenesis in cancer [Ferrara, 2010; Trinh *et al.* 2009]. Even alternative nonangiogenesis-dependent mechanisms of tumour growth are being reported [Dome *et al.* 2007; Vermeulen *et al.* 2001].

VEGF biology

The VEGF family of growth factors consists of five members in mammals: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) [Lohela *et al.* 2009]. The VEGF homologues produced by Orf viruses are collectively termed VEGF-E, and a homologue isolated from snake venom is called VEGF-F. VEGF-A alternative splicing has been shown to result in the generation of different isoforms, with differences in matrix and coreceptor binding. The VEGF ligands bind to and activate three structurally similar type III receptor tyrosine kinases. VEGF-A functions by binding to VEGFR-1 (Flt-1) and VEGFR-2 (KDR). VEGFR-2 is thought to mediate the majority of the pro-angiogenic properties of VEGF-A. VEGFR-1 has a higher affinity for VEGF-A, but its role remains somewhat unclear, being mainly thought of as a decoy receptor, limiting the availability of VEGF-A for VEGFR-2. The neuropilins 1 and 2, originally identified as semaphorin receptors involved in neuronal guidance, function as coreceptors for

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specific VEGFs. Both VEGF-A and VEGFR-2 are essential for embryonic development and the deletion of either one is embryonically lethal [Ferrara *et al.* 2006; Carmeliet *et al.* 1996].

VEGF and VEGF signalling in human breast cancer

In ductal carcinoma *in situ*, microvessel density (MVD) has been shown to be increased in pathologically aggressive lesions and this was associated with increased VEGF protein levels [Guidi *et al.* 1997]. VEGF protein content is also increased in invasive breast cancer and this overexpression has prognostic significance in patients with either node-positive or node-negative disease for both relapse-free and overall survival [Toi *et al.* 2001; Linderholm *et al.* 1998]. High VEGF protein levels are related to vascular density and inversely related to steroid receptor expression. Increased levels of VEGF and/or VEGFR-2 are predictive of a poor response to both tamoxifen and chemotherapy in patients with metastatic breast cancer (MBC) [Ryden *et al.* 2005; Foekens *et al.* 2001]. Primary tumour VEGF content seems of less importance for the beneficial effect of adjuvant chemotherapy but, for adjuvant tamoxifen, high levels of VEGFR-2 seem to predict for an impaired effect [Linderholm *et al.* 2004]. In patients with human epidermal growth factor receptor (HER)-2 overexpressing tumours, a higher VEGF content was demonstrated, confirming that VEGF is a downstream target of HER-2 activation [Masood *et al.* 2001]. The crucial VEGF-A/VEGFR-2 axis is not strictly confined to the endothelial compartment of solid tumours. In a number of different breast cancer cell lines, the presence of both VEGFR-1 and -2, the neuropilins and the autocrine production of VEGF-A, -B and -C have been demonstrated [Weigand *et al.* 2005; Presta *et al.* 1997]. These investigators have further shown that this axis is functional and that increased VEGF-A exposure, increases VEGFR-2 phosphorylation and activation of ERK1/2 and Akt [Presta *et al.* 1997].

Clinical studies

The first anti-angiogenic agent that gained approval was bevacizumab (Avastin, Genentech, Inc., South San Francisco, CA). It is the humanized version of a murine antihuman VEGF monoclonal antibody, A.4.6.1., used in early proof-of-concept studies. It is directed at all VEGF-A isoforms. Bevacizumab is humanized (93% human) by incorporating murine

VEGF-binding residues into a human IgG1 framework [Kowanzetz and Ferrara, 2006; Wang *et al.* 2004]. Bevacizumab inhibits the growth of cultured endothelial cells by 90% at 3 nmol/L. It does not exhibit antibody-dependent cell-mediated cytotoxic effects on VEGF-producing or malignant cells. In the range of 0.1–10 mg/kg, bevacizumab demonstrates linear pharmacokinetics. Consistent with a long terminal half-life (>20 days), a tendency for accumulation exists with steady-state levels estimated to occur at 100 days [Gordon *et al.* 2001; Margolin *et al.* 2001]. Bevacizumab either alone or in combination with other anticancer agents is generally well tolerated. Specific and sometimes life-threatening side effects do occur with hypertension, proteinuria, thrombosis and bleeding being most reported [Kamba and McDonald, 2007].

Single-agent activity

In a phase I/II dose escalation study of single-agent bevacizumab at 3, 10 and 20 mg/kg every 2 weeks in 75 patients with variable but in general extensively pretreated MBC, objective responses were obtained in 5.6%, 7.3% and 6.3%, according to the respective dosing [Cobleigh *et al.* 2003]. Responses were observed at all sites (lymph nodes, liver, lung, skin and bone). Interestingly 16% (12/75) of patients continued bevacizumab without progression for at least 5 months of therapy. The choice of the optimal dose was dictated by excessive adverse events at 20 mg/kg; mainly severe headaches with nausea and vomiting in 4/16 patients treated at that dose.

Anthracycline and taxane pretreated patients

In the first phase III study with bevacizumab and chemotherapy, 462 patients with MBC were randomly assigned to capecitabine alone (1250 mg/m² twice daily every 2 or 3 weeks) or capecitabine at the same dose combined with bevacizumab 15 mg/kg every 3 weeks [Miller *et al.* 2005]. Patients were eligible if pretreated with an anthracycline and a taxane and no more than two regimens for MBC. If progression of disease occurred within 12 months of completing adjuvant anthracycline and taxane therapy, patients were also eligible (71/462). Patients with HER-2 positive disease must have suffered progressive disease after prior trastuzumab treatment. Patients also needed to have measurable disease. No crossover was permitted, and patients in the bevacizumab arm could continue after interruption of capecitabine for either

Table 1. First-line phase III studies in metastatic breast cancer with bevacizumab and chemotherapy. The RIBBON1 study consists of three substudies based on the type of chemotherapy that was used.

Trial acronym	E2100	AVADO	RIBBON1	RIBBON1	RIBBON1
Number of patients	673	736	615	307	315
Randomized	Yes	Yes	Yes	Yes	Yes
Placebo	No	Yes	Yes	Yes	Yes
Bevacizumab dose	10 mg/kg every 2 weeks	7.5/15 mg/kg every 3 weeks	15 mg/kg every 3 weeks	15 mg/kg every 3 weeks	15 mg/kg every 3 weeks
Randomization ratio	1:1	1:1:1	1:2	1:2	1:2
Bevacizumab crossover	No	Yes	Yes	Yes	Yes
Chemotherapy	Paclitaxel 80 mg/m ² weekly	Docetaxel 100 mg/m ² every 3 weeks	Capecitabine	Taxane	Anthracyclin
Primary endpoint	PFS	PFS	PFS	PFS	PFS
IRF	Retrospective	Yes	Yes	Yes	Yes
RR	21.2 vs 36.9	45.9 vs 64.1	23.6 vs 35.4	35.3 vs 50.3	40.2 vs 52.2
PFS (months)	5.9 vs 11.8	8.1 vs 9.0 vs 10.0	5.7 vs 8.6	8.0 vs 9.2	8.0 vs 9.2
OS (weeks)		31.9 vs 30.8 vs 30.2	21.2 vs 29	23.8 vs 25.2	

IRF, independent review facility; OS, overall survival; PFS, progression-free survival; RR, response rate; vs, *versus*.

toxicities or progression. Only 11 patients (4.8%) did continue bevacizumab after discontinuing capecitabine. Seventy patients continued bevacizumab alone or with other therapies after initial progression. The primary endpoint of this open study was an improvement in progression-free survival (PFS) for the combination of 33%, increasing from 4 to 6 months. The objective response rate (RR) was significantly improved from 9.1% to 19.8% for the independent reviewers and from 19.1% to 30.2% for the investigators. The median duration of responses and the proportion of patients with responses lasting more than 4 months were similar in both groups. The primary endpoint was unaffected by bevacizumab with median PFS of 4.2 months for capecitabine and 4.9 months for the combination. Most toxicities were as expected, but interestingly an apparent association between proteinuria and hypertension was observed. A numerical difference in the number of patients suffering from congestive heart failure (two versus seven) was also observed, but considered of unclear relationship with bevacizumab exposure. There were no pharmacokinetic interactions, neither was there any suggestion of increased capecitabine toxicity in the combination arm.

First-line HER2-negative advanced disease

Different phase II studies have evaluated the feasibility of combining cytotoxic agents with bevacizumab. The most elaborate program has been executed with the different taxanes (docetaxel, paclitaxel and nab-paclitaxel) in patients to be treated in the first-line metastatic setting

[Conlin *et al.* 2009; Ramaswamy *et al.* 2006]. All of these studies led to the conclusion that taxanes could be combined safely with bevacizumab and that RRs suggested some additional benefit from their combined use. Similarly, combination therapy with either single-agent capecitabine, vinorelbine, or pegylated liposomal doxorubicin proved feasible, without any unexpected safety signal [Rochlitz *et al.* 2009; Burstein *et al.* 2008]. A number of more recent studies have investigated the feasibility of two cytotoxic compounds combined with bevacizumab. In one of these, gemcitabine, paclitaxel and bevacizumab were administered as first-line treatment for HER-2 negative MBC [Guardino *et al.* 2009]. Among 17 evaluable patients, the clinical benefit rate was 88%. In addition, the combination treatment with bevacizumab has been well tolerated. Another single-arm study in 45 patients combined 3-week cycles of bevacizumab 15 mg/kg, with docetaxel 75 mg/m² and capecitabine 825 mg/m² twice daily (days 1–14) [Perez *et al.* 2010]. This resulted in a RR of 49% (44% partial response [PR], 4% complete response [CR]) with an impressive median duration of response of nearly 1 year, 11.8 months, and a median overall survival (OS) of 28.4 months.

Three multicentre, randomized phase III trials have been reported in patients with MBC receiving first-line chemotherapy with or without bevacizumab (Table 1). With the exception of 8/673 patients with known HER-2 overexpression and 51/673 with unknown HER-2 status in the

E2100 study, all remaining patients were suffering from HER-2 negative MBC.

In the E2100 study, paclitaxel was given at 90 mg/m² on days 1, 8 and 15 every 4 weeks [Miller *et al.* 2007b]. Treatment was continued until progression or unacceptable toxicity. In the combination arm, patients could continue bevacizumab after discontinuing paclitaxel without disease progression. In total, 722 patients were randomized, but 49 patients were excluded from the Eastern Cooperative Oncology Group (ECOG) analysis as they did not meet the pre-specified eligibility criteria. Although differences in analysis have somewhat obscured this study (differences based on either the intent-to-treat population or the eligible population, the different use of stratification factors by the ECOG and the regulatory analysis and different dates of cutoff), the main conclusions prevail in all of these analysis. The addition of bevacizumab improved the primary endpoint with a median to 11.8 months compared with 5.9 months. Paclitaxel was discontinued in 295 patients (43.5%) before disease progression (117 in the paclitaxel alone and 178 in the combination arms). In total, 74 (21.3%) of patients continued bevacizumab as sole therapy for a median of 3.7 months. If one theme is constant in all bevacizumab studies in MBC, RRs have been improved substantially in patients treated within the combination arms. In the E2100 study, the RR improved from 21.2% to 36.9%. The benefit of bevacizumab was similar in all age groups, was independent of the disease-free interval, oestrogen receptor (ER) status, number of metastatic sites, measurability of disease, prior adjuvant chemotherapy, prior anthracycline or taxane therapy. Of the toxic effects observed in the combination arm, a number were chemotherapy related and most likely the direct consequence of the longer paclitaxel exposure in the combination arm. Grade 3–4 hypertension, proteinuria, arterial thromboembolic events, bleeding, congestive heart failure and gastrointestinal perforations were increased by the addition of bevacizumab. No difference in OS was observed, even though combined therapy increased 1-year survival rate (81.2% versus 73.4%, $p=0.01$).

Because docetaxel is considered the more potent taxane and also has demonstrated anti-angiogenic activity while potentiating the activity of anti-angiogenic drugs, the combination of docetaxel with bevacizumab made a logical choice.

A phase II study of weekly docetaxel 30 mg/m² on days 1, 8 and 15 of a 28-day cycle was combined with bevacizumab at 10 mg/kg on days 1 and 15 in 27 mainly untreated patients [Ramaswamy *et al.* 2006]. This combination proved safe and resulted in a 52% RR with a median PFS of 6.0 months.

The AVADO study is a controlled, three-arm, international phase III trial, randomizing patients with HER-2 negative advanced breast cancer to docetaxel 100 mg/m² every 3 weeks with either placebo, or combined with bevacizumab at 7.5 or 15 mg/kg, as their first-line chemotherapy regimen [Miles *et al.* 2010]. The study was designed to detect a 30% reduction in the risk of disease progression or death as the primary endpoint. The protocol-specified analysis was an unstratified comparison using the log-rank test of the PFS in the three arms. At progression, the patients in the single-agent docetaxel arm could be crossed to bevacizumab in combination with a number of other agents. At a median follow up of 25 months there were no differences in OS between the different treatment groups. The 1-year survival rate increased numerically from 76% for single-agent docetaxel to 81% and 84% in the combination arms. The primary endpoint was improved from 8.1 months to 9.0 and 10.1 months for the high-dose bevacizumab arm. For the 15 mg/kg bevacizumab arm the hazard ratio (HR) for PFS was 0.67. Similar to the E2100 study, in the AVADO trial RRs improved from 46.4% to 55.2% ($p=0.074$) and 64.1% ($p=0.0003$). In conclusion, the addition of bevacizumab to maximally dosed docetaxel, did improve RRs and PFS, but failed to influence OS. In this study, crossing over and postdocetaxel chemotherapy and endocrine agents might have influenced this result. Importantly, this blinded study does suggest an improved benefit both in RR and PFS for the higher-dosed bevacizumab arm.

The RIBBON1 study is also a randomized, placebo-controlled, double-blind phase III trial exploring the efficacy and safety of bevacizumab in combination with capecitabine ($n=615$), taxane ($n=307$) or anthracycline ($n=315$) based chemotherapy as first-line treatment for advanced HER-2 negative breast cancer [Robert *et al.* 2009]. This study enrolled 1237 patients, with a two-to-one randomization to receive either placebo or bevacizumab at 15 mg/kg every 3 weeks. This is the higher dose used in the

AVADO study. As in AVADO, patients with progression of disease were eligible for bevacizumab in combination with second-line chemotherapy. In this study, the median PFS improved by adding bevacizumab with a HR of 0.69 for capecitabine, and in the taxane/anthracycline cohort the HR was 0.64. Similarly, as in the E2100 and AVADO studies, the RR improved by 11.8% (capecitabine) to 12% (anthracyclins) to 15% (taxanes) (Table 1).

A comparison of the benefit of adding bevacizumab to first-line chemotherapy, compiling all three randomized studies, shows that the added benefit is independent of patient age, prior neoadjuvant or adjuvant use of taxanes, hormone-receptor status, presence of visceral disease or triple-negative phenotype.

The M019391 study is an open-label first-line study adding bevacizumab to taxane-based chemotherapy, in advanced, mainly HER-2 negative, advanced breast cancer [Smith *et al.* 2009]. In total 2027 patients were treated. The median time-to-progression (TTP) was 9.5 months, with a reassuring safety profile. This type of analysis suggests that the results obtained in well-controlled phase III studies are being duplicated in general oncology practice, without major safety issues.

Another approach to enhance the effect of VEGF targeting relates to the apparent utility of double inhibition of the VEGF pathway with both a receptor tyrosine kinase inhibitor (RTKI) and a monoclonal antibody (MoAb). One of these studies combined paclitaxel with bevacizumab as in E2100, but added sunitinib, a potent VEGFR-2 RTKI. Unfortunately, this double targeting of the VEGFR-2 pathway proved too toxic and the study was stopped [Mayer *et al.* 2009].

First-line HER-2 positive disease

The interaction between HER-2 and VEGF is based on extensive preclinical studies [Finkenzeller *et al.* 2004; Laugner *et al.* 2001]. This cross-talk analysis suggests that VEGF is a downstream target of HER-2 activation. The addition of bevacizumab to trastuzumab confirms the predicted synergism of preclinical models. A phase II study enrolled 50 patients, with HER-2 positive (fluorescent *in situ* hybridization positive), measurable, untreated advanced breast cancer [Hurvitz *et al.* 2009]. Objective clinical responses were documented in

24 patients (48%) with 15 patients (30%) having stable disease, six of which lasted ≥ 6 months (12%) for a clinical benefit rate of 60%. The median TTP was 9.2 months. No difference in TTP was detected between hormone-receptor negative and positive tumour types. Median OS was 43.8 months. These extraordinary results show that this combination is clinically feasible and very active despite the absence of chemotherapy in HER-2 positive advanced breast cancer. They formed the clinical basis to embark on the AVEREL trial which has randomized 410 patients to docetaxel and trastuzumab with or without bevacizumab. Other studies have randomized patients to investigate the added value of bevacizumab to either docetaxel and trastuzumab with carboplatin. In an original design, a Dutch study has randomized patients to either trastuzumab and bevacizumab until progression, compared with the same drugs combined with a weekly paclitaxel regimen.

Oestrogen receptor and/or progesterone receptor positive MBC

Detailed insight into the quantitative crosstalk among different pathways responsible for tumour progression and treatment resistance is of critical importance in the design of rational combinations of targeted agents in the clinic. The human VEGF gene contains functional oestrogen and progesterone responsive elements. Progesterone and oestrogen affect VEGF transcription and posttranscriptional modification of VEGF mRNA and VEGF receptor expression in breast cancer cell lines. Multiple points of interaction between the ER and the VEGF pathways, all suggest that overexpression or overactivation of the VEGFR pathway results in resistance to endocrine treatment. The clinical correlative studies support these findings, suggesting that VEGF content and/or VEGFR overexpression are related to tamoxifen resistance. Preclinical studies have confirmed that exogenous VEGF both increases VEGFR-2 positive breast cancer cell proliferation and overrides the growth-suppressive effects of antihormones (anti-oestrogen and antiprogesterone) [Hyder, 2006; Liang *et al.* 2006; Hyder *et al.* 2000].

Forty three patients with extensively pretreated MBC were enrolled in a feasibility study of letrozole, a nonsteroidal aromatase inhibitor (NSAI) 2.5 mg daily, in combination with bevacizumab 15 mg/kg every 3 weeks [Traina *et al.* 2010]. Patients needed not to have progressed on a

NSAI upon study entry, making efficacy results difficult to interpret. Nonetheless, a median PFS of 17 months remains impressive. Hypertension (58% all grades) and proteinuria (67% all grades) were the expected side effects. Interestingly, three patients receiving angiotensin-receptor blockers and angiotensin-converting enzyme inhibitors remained on study with improvement of their proteinuria. Two patients developed osteonecrosis of the jaw. These data suggests an increased incidence of hypertension and proteinuria with this combination, compared to other studies with bevacizumab. As already alluded to in the phase III capecitabine study, the relationship between proteinuria and hypertension needs careful quantitative examination. In two other studies with letrozole and bevacizumab, one neoadjuvant and the other adding bevacizumab to an endocrine agent failing to control disease progression (21 patients with 38% on letrozole), the increased incidence of hypertension and proteinuria was not observed [Forero *et al.* 2009]. In the placebo-controlled, phase III study CALGB 40503, with either tamoxifen or letrozole as the first-line endocrine agent with or without bevacizumab, these side effects and their possible association with variants of the VEGF and/or VEGFR gene will be explored. A very similar study is being performed by the GEICAM group in Spain (GEICAM 2006-11).

Another recurring theme is whether bevacizumab in combination with another endocrine agent is able to improve disease control in patients having progression of their disease after treatment with an aromatase inhibitor. A single-stage phase II study of fulvestrant and bevacizumab was conducted with these objectives, but failed to meet the specified endpoint. Among 18 patients with measurable disease, two (11%) patients had a confirmed tumour response (both partial responses). In addition, two patients had stable disease for greater than 6 months, for a clinical benefit rate of 22% [Tan *et al.* 2009].

Neoadjuvant studies in patients with locally advanced breast cancer

In one study, 21 previously untreated patients with inflammatory breast cancer or locally advanced breast cancer were treated with neoadjuvant bevacizumab (15 mg/kg) on day 1 followed by bevacizumab plus docetaxel 75 mg/m² and doxorubicin 50 mg/m² resulting in an objective RR of 67% [Wedam *et al.* 2006]. The first cycle of single-agent bevacizumab resulted in

substantial changes in MRI-defined parameters related to tumour vasculature and blood flow. Single-agent bevacizumab administration resulted in a median decrease of 66.7% in phosphorylation of VEGFR-2 on tumour cells and a median increase of 128.9% in tumour cell apoptosis without affecting tumour cell proliferation. In-depth pathology analysis and gene expression profiling of tumour biopsies taken at different stages of the study showed that baseline CD31 and platelet-derived growth factor receptor-beta expression, and gene ontology classes related with proliferation and mitosis, with VEGFR activity and cell motility associated with response to bevacizumab [Yang *et al.* 2008].

Similarly, in a randomized phase II study of 49 patients who were given docetaxel 35 mg/m² weekly with or without bevacizumab, the addition of bevacizumab resulted in a greater reduction of tumour perfusion on dynamic contrast-enhanced MRI [Baar *et al.* 2009]. Clinical outcomes seemed unaffected by the addition of bevacizumab.

In a single-arm study in 51 patients with triple-negative breast cancer, three 3-week cycles of bevacizumab 15 mg/kg (day 1) plus cisplatin 75 mg/m² (day 1), resulted in a pCR of 16% in 45 evaluable patients [Ryan *et al.* 2009]. In a similar single-arm study with four 3-week cycles of bevacizumab 15 mg/kg (day 1) plus docetaxel 75 mg/m² (day 1) plus cyclophosphamide 500 mg/m² (day 1), followed by four 3-week cycles of doxorubicin 60 mg/m² (day 1), a pCR rate of 41% was obtained [Makhoul *et al.* 2009].

Metronomic regimens

Metronomic treatment refers to the frequent daily continuous treatment with low-dose toxic compounds, based on the assumption that the genetically normal endothelial cells in tumours are both more sensitive and better exposed to cytotoxic compounds than the actual cancer cell compartment. This type of approach has been applied in the clinic with low-dose combinations of methotrexate and cyclophosphamide. In pre-clinical models, the addition of VEGFR-2 antibodies resulted in enhanced tumour regression. At least three clinical studies have been reported on metronomic regimens in breast cancer with the addition of bevacizumab. In a phase II study, Spanish investigators applied a 2-week schedule consisting of bevacizumab 10 mg/kg on day 1 combined with cyclophosphamide 50 mg

daily and methotrexate 1 mg/kg only on day 1 [García-Sáenz *et al.* 2008]. Trastuzumab was added for HER-2 positive tumours. In 22 evaluable, pretreated patients, this regimen resulted in an overall RR of 31.8%, with stable disease (≥ 24 weeks) amounting to 31.8%, and a combined clinical benefit rate of 63.6%. The anticipated low toxicity of this regimen was confirmed. Only the expected side effects of full-dose bevacizumab were observed. The Milan group, who pioneered the methotrexate/cyclophosphamide combination added bevacizumab at the two weekly 10 mg/kg level to fixed-dose capecitabine (500 mg three times daily) plus cyclophosphamide 50 mg once daily [Calleri *et al.* 2009; Dellapasqua *et al.* 2008]. Of the 46 evaluable patients, 11 (24%) had never received any treatment and 16 (35%) had only endocrine treatment for metastatic disease. Nevertheless, a RR of 48% (46% PR, 2% CR) and additional disease stability of at least 24 weeks was obtained in eight patients (17%) resulting in a clinical benefit rate of 68%. Toxicity was mild. Interestingly, patients with higher CEC (circulating endothelial cells) had an increased TTP. A third study randomized patients between bevacizumab 10 mg/kg every 2 weeks, cyclophosphamide 50 mg daily plus methotrexate 2.5 mg twice daily (days 1 and 2 each week) *versus* cyclophosphamide plus methotrexate alone [Burstein *et al.* 2005]. Similarly, recent data suggest the feasibility of adding bevacizumab to dose-dense regimens, obviously intended for adjuvant use: these regimens might have a limited role in instances where rapid tumour shrinkage is of paramount importance in the metastatic setting [Miller *et al.* 2007a].

Specific sites of disease

The incidence of central nervous system (CNS) relapse seems to be increasing and its time course is apparently related to specific breast cancer phenotypes. The recent safety update from the double-blind, first-line study is reassuring in this respect [Dirix *et al.* 2009]. Of particular interest are leptomeningeal metastases [Chamberlain, 2005]. Increasing VEGF levels in the CSF seem to have prognostic significance [Herrlinger *et al.* 2004]. Phase II studies both at our own institution and elsewhere are exploring the role of bevacizumab in this setting [Dirix, Personal communication].

Discussion

VEGF-interfering agents in general, and bevacizumab in particular, have acquired a definite and

evolving role in the treatment of human cancers. These agents have also confronted oncologists with an unfamiliar group of specific side effects: proteinuria, hypertension, impaired wound healing, gastrointestinal perforation, haemorrhage and thrombosis, reversible posterior leukoencephalopathy, and for the RTKIs some new types of cardiac impairment and endocrine dysfunction [Bhargava, 2009; Kamba and McDonald, 2007; Zhu *et al.* 2007]. The study of the monotarget agent bevacizumab demonstrated that a substantial number of these toxicities are related to VEGF-signalling inhibition. These observations inevitably led to the conclusion that anti-angiogenesis, VEGF-signalling inhibition is not devoid of serious side effects and that the physiological role of this target is not restricted, as was anticipated, to embryonic development, growth, the menstrual cycle and wound healing. Even if active angiogenesis might be restricted in a non-pregnant adult, clearly VEGF is needed for vascular homeostasis. The clinical development of anti-VEGF agents also demonstrated new and critical roles for VEGF in human physiology, in addition to those related with vascular homeostasis. Most critical is the renal function of VEGF [Eremina *et al.* 2008, 2003]. In the kidney, VEGF is predominantly expressed in the glomerular podocytes. Targeted heterozygous deletion of VEGF in these podocytes results primarily in phenotypic changes in the endothelial cells across the glomerular membrane. This is followed by mesangiolysis, endothelial swelling and even thrombotic microangiopathy (TMA). TMA has been reported as an anecdotal observation in patients treated with bevacizumab, VEGF trap and sunitinib [Frangié *et al.* 2007; Izzidine *et al.* 2007]. Clearly inhibition of VEGF-dependent interactions between podocytes and glomerular endothelial cells disrupts the filtration barrier and leads to a dose-dependent increase in proteinuria. The relationship between hypertension and proteinuria is unclear at this stage, although an association was reported in the capecitabine-bevacizumab phase III study [Zhu *et al.* 2007; Miller *et al.* 2005].

Clinical studies and most notably the neoadjuvant studies have demonstrated that the VEGF/VEGFR-2 axis is clearly not confined to the stromal compartment of human cancer in general, and most clearly in locally advanced breast cancer [Wedam *et al.* 2006]. Preclinical studies have corroborated the autocrine role of VEGF in human breast cancer cell lines. In conclusion,

both in the healthy human body and in numerous human tumours, the VEGF-driven signalling cascade is ubiquitous and active. The clinical development of even a monotargeted agent against a ubiquitous target obviously poses limits on dosing and schedule. It also provides a unique and simple means of assessing pharmacodynamics (PD) of this family of agents. These PD endpoints might be applied in creative randomized phase II designs investigating different schedules and dosages of eventually even combinations of different VEGF-interfering agents.

In patients with extensively pretreated human breast cancer, bevacizumab has a definite, albeit limited activity, as a single agent. Furthermore, nearly one in six of these patients had their disease stabilized for over 5 months. This antitumour activity became even more obvious in the neoadjuvant study. Single-agent bevacizumab had an influence on vascularization as assessed by MRI, but more clearly affected VEGFR-2 phosphorylation on tumour cells, resulting in minor changes of tumour cell proliferation but a steep increase in tumour cell apoptosis.

The numerous studies of bevacizumab in patients with breast cancer have shown that this agent can safely be combined with all currently used chemotherapy agents. It furthermore seems to be safe in patients with brain metastasis. There has been some concern of an increased risk for osteonecrosis of the jaw (ONJ), by adding bevacizumab to letrozole in patients with extensive prior exposure to bisphosphonates. In an analysis of first-line chemotherapy studies, incidence of ONJ was not increased by the addition of bevacizumab [Guarneri *et al.* 2009]. In general, the safety profile of bevacizumab combinations seems to be superior in patients with breast cancer compared with patients suffering from other types of cancer. This is probably a reflection of both disease specific characteristics, for example, gastrointestinal perforations in ovarian and colorectal cancer, and the cardiovascular risk profile of breast cancer patients in comparison to non-small cell lung cancer patients.

In all phase III clinical studies, the addition of bevacizumab resulted in a substantial increase in the objective RR, irrespective of disease sites and irrespective of the clinical observer. The increase by 20% in tumour regression by adding a noncytotoxic agent in any one regimen is impressive, and of a similar magnitude to what

was observed in the pivotal trastuzumab trials in patients selected for HER-2 overexpressing disease. This clearly should lead to the conclusion that irrespective of site of disease, in patients with high disease burden of the HER-2 negative phenotype, bevacizumab should be included in first-line treatment. Patients with a disease of the triple-negative phenotype and/or those pretreated with an adjuvant regimen consisting of both an anthracycline/taxane combination should also be considered for this approach. Although the benefit of adding bevacizumab to a first-line chemotherapy regimen to patients with ER and/or PR positive disease is of a similar magnitude, the need to use this combination seems to us to depend on residual endocrine treatment options, the anticipated degree of endocrine sensitivity, the bulk disease to be treated and patients' risk factors to specific side effects of bevacizumab.

It also seems clear that at this stage that the use of bevacizumab in patients to be treated with second or other lines of chemotherapy, or in combination with HER-2 or ER-targeted agents, cannot be justified outside of clinical trials. The preliminary results of other VEGF-A inhibitors, although most of these are multitargeted, are inline with the results obtained with bevacizumab.

At present we know with certainty that in the first-line setting, the addition of bevacizumab acts synergistically with chemotherapy. We are still unclear on its role as a useful agent in consolidating the antitumour effect, but then this has never been assessed prospectively in any breast trial design.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

References

- Baar, J., Silverman, P., Lyons, J., Fu, P., Abdul-Karim, F., Ziats, N. *et al.* (2009) A vasculature-targeting regimen of preoperative docetaxel with or without bevacizumab for locally advanced breast cancer: impact on angiogenic biomarkers. *Clin Cancer Res* 15: 3583–3590.

- Benoy, I.H., Salgado, R., Elst, H., van Dam, P., Weyler, J., van Marck, E. *et al.* (2005) Relative microvessel area of the primary tumor, and not lymph node status predicts the presence of bone marrow micrometastases detected by reverse transcriptase polymerase chain reaction in patients with clinically non-metastatic breast cancer. *Breast Cancer Res* 7: R210–R219.
- Bhargava, P. (2009) VEGF kinase inhibitors: how do they cause hypertension? *Am J Physiol Reg Integr Comp Physiol* 297: R1–R5.
- Bluff, J.E., Menakuru, S.R., Cross, S.S., Higham, S.E., Balasubramanian, S.P., Brozwn, N.J. *et al.* (2009) Angiogenesis is associated with the onset of hyperplasia in human ductal breast disease. *Br J Cancer* 101: 666–672.
- Borre, M., Offersen, B.V., Nerstrom, B. and Overgaard, J. (1998) Microvessel density predicts survival in prostate cancer patients subjected to watchful waiting. *Br J Cancer* 78: 940–944.
- Burstein, H.J., Chen, Y.H., Parker, L.M., Savoie, J., Younger, J., Kuter, I. *et al.* (2008) VEGF as a marker for outcome among advanced breast cancer patients receiving anti-VEGF therapy with bevacizumab and vinorelbine chemotherapy. *Clin Cancer Res* 14: 7871–7877.
- Burstein, H.J., Spigel, D., Kindsvogel, K., Parker, L.M., Bunnell, C.A., Partridge, A.H. *et al.* (2005) Metronomic chemotherapy with and without bevacizumab for advanced breast cancer: a randomized phase II trial. *Breast Cancer Res Treat* 94(Suppl. 1): S6 (Abstract 4).
- Calleri, A., Bono, A., Bagnardi, V., Quarna, P., Mancuso, P., Rabascio, C. *et al.* (2009) Predictive potential of angiogenic growth factors and circulating endothelial cells in breast cancer patients receiving metronomic chemotherapy plus bevacizumab. *Clin Cancer Res* 15: 7652–7657.
- Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M. *et al.* (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 380: 435–439.
- Chamberlain, M.C. (2005) Neoplastic meningitis. *J Clin Oncol* 15: 3605–3613.
- Cobleigh, M.A., Langmuir, V.K., Sledge, G.W., Miller, K.D., Haney, L., Novotny, W.F. *et al.* (2003) A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. *Sem Oncol* 30: 117–124.
- Conlin, A.K., Hudis, C.A., Bach, A., Moynahan, M., Lake, D., Forero-Torres, A. *et al.* (2009) Randomized phase II trial of nanoparticle albumin-bound paclitaxel in three dosing schedules with bevacizumab as first-line therapy for HER2-negative metastatic breast cancer. *J Clin Oncol* 27: 42s (Abstract 1006).
- Dellapasqua, S., Bertolini, F., Bagnardi, V., Campagnoli, E., Scarano, E., Torrisi, R. *et al.* (2008) Metronomic cyclophosphamide and capecitabine combined with bevacizumab in advanced breast cancer. *J Clin Oncol* 26: 4899–4905.
- Dhokal, H.P., Naume, B., Synnestvedt, M., Borgen, E., Kaaresen, R. and Schlichting, E. (2008) Vascularization in primary breast carcinomas: its prognostic significance and relationship with tumor cell dissemination. *Clin Cancer Res* 14: 2341–2350.
- Dirix, L.Y., Romieu, G., Provencher, L., Grimes, D., de Souza Viana, L., Paterson, A. *et al.* (2009) Safety of bevacizumab (BV) plus docetaxel (D) in patients (pts) with locally recurrent (LR) or metastatic breast cancer (mBC) who developed brain metastases during the AVADO phase III study. *Cancer Res* 69(Suppl.): 292s (Abstract #4116).
- Dirix, L.Y., Vermeulen, P.B., De Wever, I. and Van Oosterom, A.T. (1997) Soft tissue sarcoma in adults. *Curr Opin Oncol* 9: 348–359.
- Dome, B., Hendrix, M.J.C., Paku, S., Tovari, J. and Timar, J. (2007) Alternative vascularization mechanisms in cancer. *Am J Pathol* 170: 1–15.
- Eremina, V., Jefferson, J.A., Kowalewska, J., Hochster, H., Haas, M., Weisstuch, J. *et al.* (2008) VEGF inhibition and renal thrombotic microangiopathy. *N Eng J Med* 358: 1129–1136.
- Eremina, V., Sood, M., Haigh, J., Nagy, A., Lajoie, G., Ferrara, N. *et al.* (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal disease. *J Clin Invest* 111: 707–716.
- Ferrara, N. (2009) Vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol* 29: 789–791.
- Ferrara, N. (2010) Pathways mediating VEGF-independent tumor angiogenesis. *Cyto Growth Fact Rev* 21: 21–26.
- Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K.S. *et al.* (2006) Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 380: 439–442.
- Ferrara, N. and Davis-Smith, T. (1997) The biology of vascular endothelial growth factor. *Endocrine Rev* 18: 4–25.
- Finkenzeller, G., Wendel, K., Zimmermann, W., Westin, G. and Marmé, D. (2004) Activated Neu/ErbB-2 induces expression of the vascular endothelial growth factor gene by functional activation of the transcription factor Sp1. *Angiogenesis* 7: 59–68.
- Foekens, J.A., Peters, H.A., Grebenchtchiklov, N., Look, M.F., Meijer-Van Gelder, M.E., Geerts-Moespot, A. *et al.* (2001) High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. *Cancer Res* 61: 5407–5414.
- Forero, A., Saleh, M., Galleshaw, J., Jones, C., Shah, J., Percent, I. *et al.* (2009) A pilot trial of pre-operative (neoadjuvant) letrozole in combination with bevacizumab in post-menopausal women with newly diagnosed estrogen and/or progesterone receptor positive

- breast cancer. *Cancer Res* 69(Suppl.): 24s (Abstract #1088).
- Frangié, C., Lefaucheur, C., Medioni, J., Jacquot, C., Hill, G.S. and Nochy, D. (2007) Renal thrombotic microangiopathy caused by anti-VEGF-antibody treatment for metastatic renal-cell carcinoma. *Lancet Oncol* 8: 177–178.
- García-Sáenz, J.A., Martín, M., Calles, A., Bueno, C., Rodriguez, L., Bobokova, J. *et al.* (2008) Bevacizumab in combination with metronomic chemotherapy in patients with anthracycline- and taxane-refractory breast cancer. *J Chemother* 20: 632–639.
- Gordon, M.S., Margolin, K., Talpaz, M., Sledge, G.W., Holmgren, E., Benjamin, R. *et al.* (2001) Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. *J Clin Oncol* 19: 843–850.
- Guardino, A.E., Chen, M., Levy, M. and Chen, M. (2009) Phase II trial with gemcitabine, paclitaxel and bevacizumab for the first-line treatment of metastatic breast cancer. *Cancer Res* 69: 853s (Abstract 6089).
- Guarneri, V., Miles, D., Robert, N.J., Dieras, V.C., Glaspy, J., Smith, I.E. *et al.* (2009) Analysis of bevacizumab (Bev) therapy, bisphosphonate use and osteonecrosis of the jaw (ONJ) in >1900 patients treated in two randomized, controlled trials. *Cancer Res* 69(Suppl.): 512s (Abstract #208).
- Guidi, A.J., Schnitt, S.J., Fisher, L., Tognazzi, K., Harris, J.R., Dvorak, H.F. *et al.* (1997) Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. *Cancer* 80: 1945–1954.
- Herrlinger, U., Wiendl, H., Renninger, M., Förschler, H., Dichgans, J. and Weller, M. (2004) Vascular endothelial growth factor in leptomeningeal metastasis: diagnostic and prognostic value. *Br J Cancer* 91: 219–224.
- Hurvitz, S.A., Pegram, M.D., Lin, L.-S., Chan, D.S., Allen, H.J., Dichmann, R.A. *et al.* (2009) Final results of a Phase II trial evaluating trastuzumab and bevacizumab as first line treatment of HER2-amplified advanced breast cancer. *Cancer Res* 24(Suppl.): 854s (Abstract #6094).
- Hyder, S.M. (2006) Sex-steroid regulation of vascular endothelial growth factor in breast cancer. *Endocrine-related Cancer* 13: 667–687.
- Hyder, S.M., Nawaz, Z., Chiapetta, C. and Stancel, G.M. (2000) Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. *Cancer Res* 60: 3183–3190.
- Izzidine, H., Brocheriou, I., Deray, G. and Rixe, O. (2007) Thrombotic microangiopathy and anti-VEGF agents. *Nephrol Dial Transplant* 24: 682–685.
- Jensen, H.M., Chen, I., Devault, M.R. and Lewis, A.E. (1982) Angiogenesis induced by normal human breast tissue: a probable marker for precancer. *Science* 218: 293–295.
- Kamba, T. and McDonald, D.M. (2007) Mechanisms of adverse effects of anti-VEGF therapy in cancer. *Br J Cancer* 96: 1788–1795.
- Kowanetz, M. and Ferrara, N. (2006) Vascular endothelial growth factor signaling pathways: therapeutic perspective. *Clin Cancer Res* 12: 5018–5022.
- Laugner, E., Taghavi, P., Chiles, K., Mahon, P.C. and Semenza, G.L. (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 21: 3995–4004.
- Liang, Y., Brekken, R.A. and Hyder, S.M. (2006) Vascular endothelial growth factor induces proliferation of breast cancer cells and inhibits the anti-proliferative activity of anti-hormones. *Endocrine-Related Cancer* 13: 905–919.
- Lin, E.Y. and Pollard, J.W. (2007) Tumor associated macrophages press the angiogenic switch in breast cancer. *Cancer Res* 67: 5064–5066.
- Linderholm, B., Andersson, J., Lindh, B., Beckman, L., Erlanson, M., Edin, K. *et al.* (2004) Overexpression of c-erbB-2 is related to a higher expression of vascular endothelial growth factor (VEGF) and constitutes an independent prognostic factor in primary node-positive breast cancer after adjuvant systemic therapy. *Eur J Cancer* 40: 33–42.
- Linderholm, B., Tavelin, B., Grankvist, K. and Henriksson, R. (1998) Vascular endothelial growth factor is of high prognostic significance in node negative breast carcinoma. *J Clin Oncol* 16: 3121–3128.
- Lohela, M., Bry, M., Tammela, T. and Alitalo, K. (2009) VEGFs and receptors in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol* 21: 154–165.
- Makhoul, I., Vicki, K.S., Korourian, S., Henry-Tillman, R.S., Siegel, E.R., Westbrook, K.C. *et al.* (2009) Bevacizumab combined with chemotherapy significantly improves pathologic complete response in patients with operable or locally advanced breast cancer. *Cancer Res* 69(Suppl.): 343s (Abstract #5114).
- Margolin, K., Gordon, M.S., Holmgren, E., Gaudreault, J., Novotny, W., Fyfe, G. *et al.* (2001) Phase Ib trial of intravenous recombinant growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. *J Clin Oncol* 19: 851–856.
- Masood, R., Cal, J., Zheng, T., Smith, D.L., Hinton, D.R. and Gill, P.S. (2001) Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors. *Blood* 98: 1904–1913.
- Mayer, E.L., Kozloff, M., Qamar, R., Klencke, B., Balkissoon, J., Parmar, H. *et al.* (2009) SABRE-B: a randomized phase II trial evaluating the safety and efficacy of combining sunitinib with

- paclitaxel + bevacizumab as first-line treatment for HER2-negative metastatic breast cancer (MBC): final results. *Cancer Res* 69(Suppl.): 245s (Abstract #3126).
- Miles, D., Chan, A., Dirix, L.Y., Cortés, J., Pivot, X., Piotr Tomczak, P. *et al.* (2010) Randomized, double-blind, placebo-controlled, phase III study of bevacizumab with docetaxel or docetaxel with placebo as first-line therapy for patients with locally recurrent or metastatic breast cancer (mBC): AVADO. *J Clin Oncol*, DOI: 10.1200/JCO.2008.21.6457.
- Miller, K.D., Chap, L.I., Holmes, F.A., Cobleigh, M.A., Marcon, P.K., Fehrenbach, L. *et al.* (2005) Randomized phase III trial of capecitabine compared with bevacizumab and capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* 23: 792–799.
- Miller, K.D., O'Neill, A., Perez, E.A., Seidman, A.D. and Sledge, G.W. (2007a) 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). *Breast Cancer Res Treat* 106(Suppl. 1): S147 (Abstract 3063).
- Miller, K., Wang, M., Gralow, J., Dickler, M., Cobleigh, M., Perez, E.A. *et al.* (2007b) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 357: 2666–2676.
- Perez, E.A., Hillman, D.W., Dentchev, T., Le-Lindqwister, N.A., Geeraerts, L.H., Fitch, T.R. *et al.* (2010) North Central Cancer Treatment Group (NCCTG) N0432: phase II trial of docetaxel with capecitabine and bevacizumab as first-line chemotherapy for patients with metastatic breast cancer. *Ann Oncol* 21: 269–274.
- Perez-Atayde, A.R., Sallan, S.E., Tedrow, U., Connors, S., Allred, E. and Folkman, J. (1997) Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. *Am J Pathol* 150: 815–821.
- Presta, L.G., Chen, H., O'Connor, S.J., Chisholm, V., Meng, Y.G., Krummen, L. *et al.* (1997) Humanization of anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Can Res* 57: 4593–4599.
- Ramaswamy, B., Elias, A.D., Kelbick, N.T., Dodley, A., Morrow, M., Hauger, M. *et al.* (2006) Phase II trial of bevacizumab in combination with weekly docetaxel in metastatic breast cancer patients. *Clin Cancer Res* 12: 3124–3129.
- Robert, N., Dieras, V., Glaspy, J., Brufsky, A., Bondarenko, I.N., Lipatov, O. *et al.* (2009) Clinical benefit rate and time to response in RIBBON-1, a randomized, double-blind, phase III trial of chemotherapy with or without bevacizumab(b) for the first-line treatment of HER2-negative locally recurrent or metastatic breast cancer (MBC). *Cancer Res* 69(Suppl.): 851s(Abstract #6085).
- Rochlitz, C., von Moos, R., Mannhart-Harms, M., Winterhalter, R., Rauch, D., Müller, A. *et al.* (2009) Pegylated liposomal doxorubicin and bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer. A multicenter, single-arm phase II trial (SAKK 24/06). *Eur J Cancer Suppl* 7: 266 (Abstract 5019).
- Ryan, P.D., Tung, N.M., Isakoff, S.J., Golshan, M., Richardson, A., Corben, A.D. *et al.* (2009) Neoadjuvant cisplatin and bevacizumab in triple negative breast cancer (TNBC): safety and efficacy. *J Clin Oncol* 27(Suppl.): 18s (Abstract 551).
- Ryden, L., Jirstrom, K., Bendahl, P.-A., Ferno, M., Nordenskjöld, B. *et al.* (2005) Tumor-specific expression of vascular endothelial growth factor 2 but not vascular endothelial growth factor or human vascular endothelial growth factor receptor 2 is associated with impaired response to adjuvant tamoxifen in premenopausal breast cancer. *J Clin Oncol* 23: 4695–4704.
- Smith, I.E., Biganzoli, L., Cortes-Funes, H., Stroyakovskiy, D., Franke, F.A., Chlistalla, A. *et al.* (2009) Primary analysis of study MO19391, a 2000-patient open-label safety study of bevacizumab plus taxane-based therapy as first-line treatment of patients with locally recurrent or metastatic breast cancer. *Cancer Res* 69(Suppl.): 292s (Abstract #4118).
- Tan, W.W., Dueck, A.C., Flynn, P., Steen, P., Anderson, D., Rowland, K. *et al.* (2009) Phase II Trial of fulvestrant and bevacizumab in patients with metastatic breast cancer previously treated with an aromatase inhibitor: a North Central Cancer Treatment Group Trial. *Cancer Res* 69(Suppl.): 738s (Abstract #4096).
- Toi, M., Matumoto, T. and Bando, H. (2001) Vascular endothelial growth factor: its prognostic, predictive and therapeutic implications. *Lancet Oncol* 2: 667–673.
- Traina, T.A., Rugo, H.S., Caravelli, J.F., Patil, S., Yeh, B., Melisko, M.E. *et al.* (2010) Feasibility of Letrozole with bevacizumab in patients with metastatic breast cancer. *J Clin Oncol* 28: 628–633.
- Trinh, B., Van Dam, P., Prové, A., Maes, H., Van Laere, S., Tjalma, W. *et al.* (2009) Angiogenic escape and tumour progression in two patients with metastatic breast cancer receiving bevacizumab treatment. *Cancer Res* 69(Suppl.): 112s (Abstract #1029).
- Vermeulen, P.B., Colpaert, C., Salgado, R., Royers, R., Hellems, H., Van Den Heuvel, E. *et al.* (2001) Liver metastases from colorectal adenocarcinoma grow in three patterns with different angiogenesis and desmoplasia. *J Pathol* 195: 336–342.
- Vermeulen, P.B., Gasparini, G., Fox, S.B., Colpaert, C., Marson, L.P., Gion, M. *et al.* (2002) Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur J Cancer* 38: 1564–1579.
- Wang, Y., Fei, D., Vanderlaan, M. and Song, A. (2004) Biological activity of bevacizumab, a humanized anti-VEGF antibody in-vitro. *Angiogenesis* 7: 335–345.

Wedam, S.B., Low, J.A., Yang, S.X., Chow, C.K., Choyke, P., Danforth, D. *et al.* (2006) Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. *J Clin Oncol* 24: 769–777.

Weidner, N., Folkman, J., Pozza, F., Bevilacqua, P., Allred, E.N., Moore, D.H. *et al.* (1992) Tumor angiogenesis: a new significant and independent prognostic indicator in early stage breast carcinoma. *J Natl Cancer Inst* 84: 1875–1887.

Weigand, M., Hantel, P., Kreienberg, R. and Waltenberger, J. (2005) Autocrine vascular endothelial growth factor signalling in breast cancer. Evidence

from cell lines and primary breast cancer cultures in vitro. *Angiogenesis* 8: 197–204.

Yang, S.X., Steinberg, S.M., Nguyen, D., Du, T.D., Modrusan, Z. and Swain, S.M. (2008) Gene expression profile and angiogenic markers correlate with response to neoadjuvant bevacizumab followed by bevacizumab plus chemotherapy in breast cancer. *Clin Cancer Res* 14: 5893–5899.

Zhu, X., Wu, S., Dahut, W.L. and Parikh, C.R. (2007) Risks for proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. *Am J Kidney Dis* 49: 186–193.

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