EJC SUPPLEMENTS 6 (2008) 26–29

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

The role of bevacizumab in breast cancer

*Gennaro Daniele, Roberta Marciano, Giampaolo Tortora**

Cattedra di Oncologia Medica, Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università di Napoli Federico II, Via S. Pansini 5, 80131 Naples, Italy

ARTICLE INFO

Article history:
Received 6 June 2008

Keywords:
Breast cancer
Angiogenesis
Bevacizumab

ABSTRACT

Neoangiogenesis and expression of the main angiogenic factor Vascular endothelial growth factor (VEGF) are critical steps already present at an early stage of breast cancer development. As tumor growth progresses other pro-angiogenic factors, including bFGF, TGFa and IL-8, are overexpressed and act in addition to VEGF. VEGF expression has been associated to poor prognosis and therapy outcome in breast cancer. Moreover, several studies have documented that HER-2 overexpression induces VEGF expression/secretion and neovascularization and that VEGF expression increases when tumors become resistant to treatment. On these bases the anti-VEGF drug Bevacizumab has been evaluated in the clinical setting in breast cancer patients. Recently, phase III studies have demonstrated that Bevacizumab in combination with taxanes produces a high rate of responses and increases the Progression Free survival of breast cancer patients. A large array of studies is currently ongoing with Bevacizumab in combination with chemotherapy and hormone-therapy in the metastatic, neoadjuvant and adjuvant setting.

1. Angiogenesis and breast cancer

Breast cancer is the most common type, accounting for 26% of all female cancers in the United States. In 2007, approximately 178,480 women and 2030 men will be diagnosed with invasive breast cancer and 40,460 women and 480 men will die from the disease.

Recently, our understanding of breast cancer biology is increasing by the findings that the breast cancer represents a family of tumours, sharing the same origin organ, with specific molecular features and prognostic implications. In this regard, molecular markers as hormonal receptors (HRs) and HER2/neu are the most important tools for therapeutic decision, albeit genomic profiling, performed with tissue chip microarrays, are a very interesting and promising approach.

During the last three decades, since Judah Folkman hypothesis in early 1970s,[1] accumulating data concerning tumour biology suggest that angiogenesis is a crucial step in the growth, invasion and metastatic dissemination in cancer.

Now it is increasingly clear that the cancer cell is only partially responsible for tumour progression and that the tumour microenvironment, formed by endothelial and bone marrow derived cells, has a crucial role in disease development. Therefore, acting on these cells can augment the efficacy of cancer cell targeted therapy as chemotherapeutic agents and molecular targeted drugs.

The angiogenesis is an important developmental mechanism implicated in many physiological and pathological processes. Amongst the many factors implicated in angiogenesis most attention is catalysed, during these years, by VEGF (vascular endothelial growth factor) system.

VEGF-A (commonly referred as VEGF), the major proangiogenic factor, circulating as various isoforms (VEGF121 and VEGF165), belong to the VEGF family of growth factors, comprising VEGF-A (referred as VEGF), VEGF-B, VEGF-C, VEGF-D VEGF-E and PlGF.[2,3]

VEGF production is finely regulated by various stimuli as hypoxia (via hypoxia-inducible factor 1α, HIF-1α),[4] oncogenes

* *Corresponding author:* Tel.: +39 081 7462061; fax: +39 081 2203147.
E-mail address: gtortora@unina.it (G. Tortora).
1359-6349/$ - see front matter
doi:10.1016/j.ejcsup.2008.06.006

and tumour suppressor genes, nitric oxide, and EGFR family receptors (EGFR and HER2).[5]

VEGF activity is mediated by binding on cell surface expressed tyrosine-kinase receptors, called VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR) and VEGFR-3 (flt-4) and Neuropilin1–2.[6]

Although VEGFR-2 is classically defined as the principal mediator of VEGF transduction activity, increasing evidence suggests a central role in many processes also for VEGFR-1.

VEGF promotes the growth of structurally chaotic and functional aberrant vessels into tumour burden,[7] increases permeability, raising intratumoural pressure that, in turn, prevents the penetration of chemotherapeutic agents into the tumour,[8] and prevents the apoptosis of immature pericyte on vessels critical for the tumour.[9]

All these features, added to the limited role that VEGF plays in healthy adults, explain why its targeting is strongly pursued for therapeutic purposes.

The importance of VEGF in breast cancer was first proved in preclinical and in clinical setting.

In the preclinical models VEGF expressing breast cancer cells demonstrated increased growth and metastatic potential in human tumour xenografts.[10] In the clinical setting VEGF overexpression in the early stage breast cancer was related to worse distant Disease-free survival, accounting for a major metastatic potential, and with the resistance to systemic chemotherapy and hormonal therapy.[11–14] Moreover, HER2 overexpression in breast cancer is associated with VEGF overexpression.[15–17]

Recent preclinical studies show that VEGFR-2 is overexpressed on some breast cancer cells and that VEGF can act as an autocrine loop on this tumour cells, associated with a paracrine loop involving cancer and endothelial cells. This observation, albeit very interesting, has not been yet confirmed in clinical setting, though many reports show the presence of either VEGFR1 and/or -2 on breast cancer cell surface.[18,19]

The first VEGF antagonist was a monoclonal antibody approved by FDA for the treatment of metastatic colorectal patients in February 2004 and, after few months, worldwide with the same indication: bevacizumab (Avastin®).

Parallel to colorectal cancer, bevacizumab demonstrated activity in many type of cancer such as renal cell cancer (RCC), non-small cell lung cancer (NSCLC) and breast cancer.

In this regard, bevacizumab was approved by EMEA on the 27th March 2007 for the treatment of previously untreated metastatic breast cancer patients.

Bevacizumab is a humanised (93% human and 7% murine) monoclonal antibody designed to efficiently recognise and bind all the major isoforms of circulating VEGF. With a terminal half-life of 17–21 d it can be associated with the most common chemotherapeutic administration schedules.[20]

2. Preclinical data with bevacizumab

In human breast cancer xenograft models either the A4.6.1, the murine precursor of bevacizumab or bevacizumab has demonstrated a certain activity in inhibiting angiogenesis and tumour growth and spreading, given alone or in combination with chemotherapeutics and other targeted agents (trastuzumab).[21–23]

3. Clinical data with bevacizumab: focus on mBC

On the basis of preclinical data some early clinical studies were conducted in humans to assess the feasibility of bevacizumab administration. Following phase I studies some phase I/II studies were performed in breast cancer patients to define the optimal dose and obtain an early assessment of the drug activity in this setting.

Cobleigh and colleague conducted a phase I/II trial in 75 pretreated mBC patients to primarily assess the overall response rate and the safety of three different doses of bevacizumab (3, 10, 20 mg/kg every 2 weeks) administered alone. At the dose of 10 mg/kg, there was one CR and four PR versus zero and one, respectively, for the 20 mg/kg dose. Although the overall response rate (ORR) was very low, this study showed a median duration of response of 5.6 months and provided better data for the antiangiogenic drug alone than was observed in the early phase II studies with capecitabine.[24] The safety profile was favourable with hypertension G3 in 17% of patients treated with the intermediate dose comparing with 22% and 19% of patients treated with 3 mg/kg and 20 mg/kg, respectively. Based on these data 10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks was selected as the dose for the further phases II and III studies.

These data support the investigation of bevacizumab combined with chemotherapy. The first phase III study was designed and performed. In this study, 462 pretreated mBC patients were randomised to receive capecitabine plus bevacizumab or capecitabine alone. The progression free survival (PFS) was the primary end-point and there was no statistically significant difference between the two groups of patients (4.2 versus 4.9 for those treated with capecitabine + bevacizumab). Similarly, also median overall survival (OS), 14.5 versus 15.1 for capecitabine versus capecitabine + bevacizumab, respectively, was not reached a statistical difference. The ORR (assessed by an independent review facility) was higher for the combination treatment arm: 19.8 months versus 9.1 months (p 0.001).[25] The key explanation of the failure to achieve PFS and OS advantages for this study was that VEGF is expressed throughout the tumour lifecycle, but other factors (i.e. bFGF, TGF-β, PlGF and pleiotrophin) come into play at later stages. Thus tumours may escape anti-VEGF treatment by using alternative pro-angiogenic pathways. A direct consequence of this theory is that VEGF-driven angiogenesis plays a more important role in early stage of tumour formation, thus the anti-VEGF treatments should be administrated early in cancer therapy.

A more recent phase III trial evaluated weekly paclitaxel alone or in combination with bevacizumab, given at the dose of 10 mg/kg every 2 weeks, as first-line treatment in locally advanced mBC patients. The trial was early stopped due a scheduled interim analysis by an independent data monitoring committee which concluded that was met the PFS, primary end-point of the study. Indeed at the date of the interim analysis PFS was 5.8 months for patients who received paclitaxel alone and 11.4 months for those who received combination therapy ($p < 0.0001$). In addition, the ORR was increased from 23.4% for paclitaxel arm to 48% for combination arm ($p < 0.0001$). This meaningful increase in PFS and

ORR was observed in all pre-specified subgroups of patients. Although the increased trend for OS observed for patients who received combination therapy was not significantly different compared to those receiving paclitaxel alone (26.6 versus 24.8, respectively; p 0.14), an exploratory analysis reported that the addition of bevacizumab to paclitaxel led to higher 1 year OS (82.3 versus 72.8%, p 0.007).[26]

To explain the lack in survival advantage it may be argued that in the study analysis were not considered the different subsequent therapies that may affect the results of first-line therapy.

However, the magnitude of the PFS and ORR data, higher than other ever observed in first-line mBC treatment, led to EMEA and FDA approval of the combination of bevacizumab with paclitaxel in first-line mBC patients.

Other studies assessing efficacy and feasibility of the combination of bevacizumab with other targeted and conventional agents were performed in these years or are ongoing.[27–31]

In particular the combination of bevacizumab and docetaxel, another drug widely used in mBC treatment, was investigated by some phase II and III studies with some successes.[27,28] A phase III study, known as AVADO, randomised 736 patients until March 2007 to receive docetaxel plus placebo or bevacizumab every 3 weeks at the dose of 7.5 mg/kg or 15 mg/kg in combination with docetaxel. The AVADO trial demonstrated a higher ORR, Time to Treatment Failure and PFS in the bevacizumab 7.5 mg/kg and 15 mg/kg as compared to placebo arm.

Other ongoing two phase III studies, RIBBON1 and 2 are evaluating the role of bevacizumab combination with some chemotherapy regimens. In particular, RIBBON1 study will evaluate the safety and the efficacy of the bevacizumab combined with anthracycline-based, capecitabine and taxane as first-line therapy for mBC patients. The RIBBON2 study is designed to assess the efficacy of bevacizumab when associated with standard non-anthracycline-based chemotherapy in previously treated mBC patients.

After the approval by major regulatory agencies (i.e. FDA and EMEA) of the first-line combination of bevacizumab and paclitaxel, a large phase IV started assessing the safety profile of this treatment in locally recurrent mBC patients (MO19391).

Based on the abovementioned evidence of HER2 and VEGF co-expression on breast cancer cell surface and the crosstalk between HER2 pathway and VEGF,[23] a phase I/II study was performed with bevacizumab associated to trastuzumab in HER2 positive mBC patients. Early results on 37 patients suggest that the combination is well tolerated with only few G3/G4 events reported. Preliminary efficacy data showed 19 PR and one CR with 11 patients with stable disease.[32,33] These interesting data represent the basis on which was designed and has been started a phase III study (AVEREL) investigating the efficacy of bevacizumab addition to first-line trastuzumab and docetaxel therapy for HER2 mBC patients (n = 412).

4. Neoadjuvant and adjuvant

Ongoing trials are now evaluating the role of bevacizumab in neoadjuvant and adjuvant setting.

In neoadjuvant setting, the phase III ongoing trial is investigating the role of adding bevacizumab to sequential combinatorial therapy with 3×2 arms of treatment: docetaxel +/– bevacizumab or docetaxel + capecitabine +/– bevacizumab or docetaxel + gemcitabine +/– bevacizumab followed by two arms epirubicin + cyclophosphamide (AC) +/– bevacizumab. The primary end-point is the pathological complete response rate (CRR), the secondary end-points are ORR, DFS and safety.

In adjuvant setting three sets of trials are ongoing for triple negative breast cancer patients, HER2 negative and HER2 positive breast cancer patients.

In HER2 negative group two studies are investigating different integration schedules of bevacizumab in adjuvant therapy. The E2104 study is designed to provide the 'proof of concept' that bevacizumab is feasible with an anthracycline-based therapy in adjuvant setting. E2104 is a two step phase II trial in which node-positive patients receive chemotherapy with doxorubicin plus cyclophosphamide followed by paclitaxel and receive bevacizumab beginning into the doxorubicin/cyclophosphamide phase of treatment or in the paclitaxel phase, in this case the subsequential maintenance with bevacizumab alone is for 22 cycles instead of 18.

The second study (E5103) is designed to primarily evaluate the efficacy in DFS prolongation of adding bevacizumab to sequential schedule with AC followed by paclitaxel in node-positive or high risk node-negative, HER2-negative, resected BC patients. Secondary end-points of this study are to comparing the OS of the patients treated with this regimen, to evaluate the toxicity profile of the combination therapy and to compare the impact of short-term (20–24 weeks) versus long-term (50–54 weeks) administration of bevacizumab on the DFS for this patient.

Based on the cooperative effect showed in the phase II study performed by Pegram et al. between trastuzumab and bevacizumab in mBC patients BETH study is evaluating the efficacy of this combination the adjuvant setting for HER2-positive fully resected BC patients.

BEATRICE study will evaluate bevacizumab therapy in association with standard chemotherapy regimens as adjuvant treatment in triple negative BC patients comparing standard chemotherapy alone. The primary end-point is invasive-DFS, and secondary end-points are OS, DFS, distant-DFS, tolerability and safety of the combination.

Breast cancer is often an hormone-dependent disease in early phases. Hormones can play a role in angiogenesis, too. In particular, regulatory regions of the VEGF gene contain elements responsive to oestrogen,[34] estrogens increase VEGF levels in breast cancer cell lines.[35] For all these reasons many ongoing studies are investigating the role of bevacizumab in association with hormonal therapy. The first, GEICAM 2006-11, is evaluating the efficacy and safety of combination of letrozole plus bevacizumab versus letrozole alone in previously untreated HR positive mBC patients suitable for a first-line hormonal treatment. In the second, the double-blinded study CALGB40503 was evaluated the efficacy of the bevacizumab combined with AI or tamoxifen versus AI or tamoxifen alone.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;**285**(21):1182–6.
2. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997;**18**(1):4–25.
3. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 2005;**23**(5):1011–27.
4. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 2003;**3**(10):721–32.
5. Kerbel L, Folkman J. Clinical translation of angiogenesis inhibitors. *Nat Rev Cancer* 2002;**2**(10):727–39.
6. Shibuya M, Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006;**312**(5):549–60.
7. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995;**1**(10):1024–8.
8. Netti PA, Hamberg LM, Babich JW, et al. Enhancement of fluid filtration across tumor vessels: implication for delivery of macromolecules. *Proc Natl Acad Sci USA* 1999;**96**(6):3137–42.
9. Benjamin LE, Golijanin D, Itin A, et al. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J Clin Invest* 1999;**103**(2):159–65.
10. McLeskey SW, Tobias CA, Vezza PR, Filie AC, Kern FG, Hanfelt J. Tumor growth of FGF or VEGF transfected MCF-7 breast carcinoma cells correlates with density of specific microvessels independent of the transfected angiogenic factor. *Am J Pathol* 1998;**153**(6):1993–2006.
11. Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Henriksson R. Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. *J Clin Oncol* 2000;**18**(7):1423–31.
12. Gown A, Rivskin SE, Hunt HN, et al. Prognostic factor of vascular endothelial growth factor (VEGF) expression in node-positive breast cancer. *Proc Am Soc Clin Oncol* 2001;**20**. abstract 1703.
13. Foekens JA, Peters HA, Grebenchtchikov N, et al. High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. *Cancer Res* 2001;**61**(14):5407–14.
14. Gasparini G, Toi M, Gion M, et al. Prognostic value of vascular endothelial growth factor in breast cancer. *Oncologist*(5 Suppl. 1): S37–44.
15. 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**(1):59–68.
16. Konecny GE, Meng YG, Untch M, et al. Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. *Clin Cancer Res* 2004;**10**(5):1706–16.
17. Linderholm B, Andersson J, Lindh B, et al. 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 treatment. *Eur J Cancer* 2004;**40**(1):33–42.
18. Huh JI, Calvo A, Stafford J, et al. Inhibition of VEGF receptors significantly impairs mammary cancer growth in C3(1)/Tag transgenic mice through antiangiogenic and non-antiangiogenic mechanisms. *Oncogene* 2005;**24**:790–800.
19. Dales J, Garcia S, Bonnier P, et al. Prognostic significance of VEGF receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) in breast carcinoma. *Ann Pathol* 2003;**23**:297–305.
20. Scott LJ. Bevacizumab: in first-line treatment of metastatic breast cancer. *Drugs* 2007;**67**(12):1793–9.
21. Borgström P, Gold DP, Hillan KJ, Ferrara N. Importance of VEGF for breast cancer angiogenesis *in vivo*: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin. *Anticancer Res* 1999;**19**(5B):4203–14.
22. Borgström P, Hillan KJ, Sriramarao P, Ferrara N. Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. *Cancer Res* 1996;**56**(17):4032–9.
23. Epstein M, Ayala RE, Tchekmedyian N, Borgstrom P, Pegram DJ, Slamon DJ. HER2-overexpressing human breast cancer xenografts exhibit increased angiogenic potential mediated by vascular endothelial growth factor (VEGF). *Breast Cancer Res Treat* 2002;**76**(Suppl. 1):S143. abstract 570.
24. Cobleigh MA, Langmuir VK, Sledge GW, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. *Semin Oncol* 2003;**30**(5 Suppl. 16): 117–24. October.
25. Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* 2005;**23**:792–9.
26. Miller KD, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007;**357**(26):2666–76.
27. Ramaswamy B, Elias AD, Kelbick NT, et al. Phase II trial of bevacizumab in combination with weekly docetaxel in metastatic breast cancer patients. *Clin Cancer Res* 2006;**12**(10):3124–9.
28. Burstein HJ, Parker LM, Savoie J, et al. Phase II trial of the anti-VEGF antibody bevacizumab in combination with vinorelbinefor refractory advanced breast cancer. *Breast Cancer Res Treat* 2002;**76**(Suppl. 1):S115. abstract 446.
29. Burstein HJ, Spigel DR, Kindsvogel K, et al. Metronomic chemotherapy with and without bevacizumab for advanced breast cancer: a randomized phase II study. *Breast Cancer Res Treat* 2005;**94**(Suppl. 1):S6. abstract 4.
30. Traina TA, Rugo H, Caravelli J, et al. Letrozole (L) with bevacizumab (B) is feasible in patients (pts) with hormone receptor-positive metastatic breast cancer (MBC). *J Clin Oncol* 2006;**24**(Suppl. 18):133S. abstract 3050.
31. Pegram M, Yeon C, Durna LN, et al. Phase 1 combined biologic therapy of breast cancer using 2 humanized monoclonal antibodies directed against HER2 protooncogene and vascular endothelial growth factor. *Breast Cancer Res Treat* 2004;**88**(Suppl. 1):S124. abstract 3039.
32. Pegram M, Chan D, Dichmann RA, et al. Phase II combined biological therapy targeting the HER2 proto-oncogene and the vascular endothelial growth factor using trastuzumab (T) and bevacizumab (B) as first line treatment of HER2-amplified breast cancer. *Breast Cancer Res Treat* 2006;**100**(Suppl. 1):S28. abstract 301.
33. Hyder SM, Nawaz Z, Chiappetta C, Stancel GM. Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. *Cancer Res* 2000;**60**(12):3183–90.. June 15.
34. Takei H, Lee ES, Jordan VC. *In vitro* regulation of vascular endothelial growth factor by estrogens and antiestrogens in estrogen-receptor positive breast cancer. *Breast Cancer* 2002;**9**(1):39–42.
35. Nakamura J, Savinov A, Lu Q, Brodie A. Estrogen regulates vascular endothelial growth/permeability factor expression in 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors. *Endocrinology* 1996;**137**(12):5589–96.