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VEGF and angiogenesis: implications for breast cancer therapy

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ABSTRACT

Angiogenesis is essential for tumour growth, progression and metastasis. Vascular endothelial growth factor (VEGF) is a key angiogenic mediator, and has been shown preclinically to play multiple roles in the development and maintenance of abnormal tumour vasculature, while playing a limited role in adults. VEGF is thus an attractive therapeutic target. Bevacizumab is a humanised anti-VEGF monoclonal antibody and has extensive effects on tumour vasculature as demonstrated by a number of preclinical studies. Its mechanism of action and preclinical evidence indicate that it regresses new tumour vasculature, normalises the dysfunctional and chaotic existing tumour vasculature, and prevents the formation of additional tumour vasculature. Although the drug target of bevacizumab is well known, no biomarkers have been identified that predict the clinical benefit from targeting circulating VEGF with bevacizumab. The mechanism of action of bevacizumab with regards to direct antitumour effects has not been elucidated. Future clinical studies will provide additional evidence for the effectiveness of bevacizumab in combination with a variety of different chemotherapy and biological agents.

1. Introduction

Angiogenesis is the process by which new blood vessels are formed from pre-existing vasculature. Angiogenesis occurs physiologically during embryogenesis and early post-natal development, and also during the process of wound healing and the reproductive cycle in adults. However, pathological angiogenesis is a key factor in cancer development, where the development of new vessels provides essential nutrients and oxygen to tumours >2 mm in size (Figure 1), i.e. those that have grown too large to be supported by passive diffusion alone, [1,2] allowing them to grow and metastasise. Angiogenesis is a complex process that is tightly regulated by stimulatory and inhibitory factors. [4] It is initiated when there is a predominance of angiogenic factors that favour new vessel growth (e.g. vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF], and transforming growth factors alpha and beta), commonly known as the 'angiogenic switch'. [5,6]

VEGF is one of the most potent regulators of angiogenesis, both physiologically and pathophysiologically. [7] It is a potent mitogen, a survival factor for endothelial cells, and also mediates vessel permeability and migration of endothelial progenitor cells from the bone marrow. As VEGF is secreted by tumours, but has a limited role in adults, it is a rational therapeutic target. [5]

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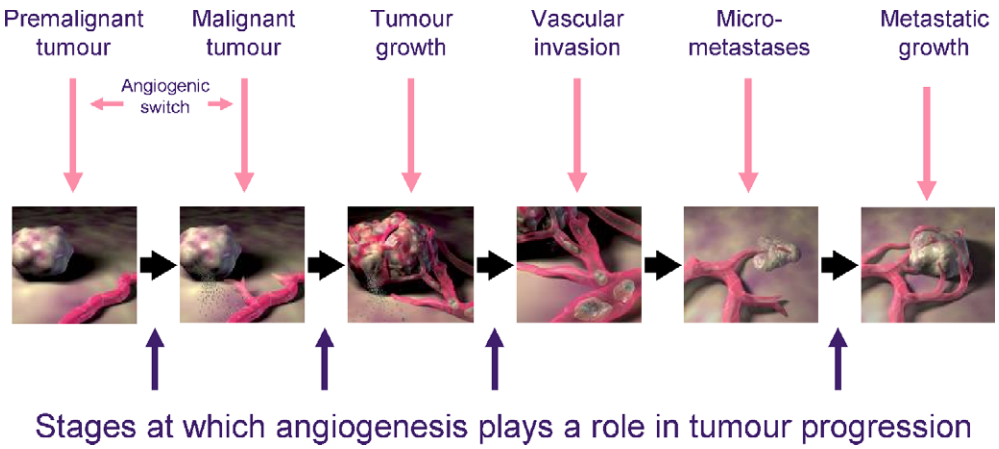

Fig. 1 – Role of angiogenesis at various stages of tumour development. Adapted from Poon et al.,[3] *J Clin Oncol* 2001, with permission from the American Society of Clinical Oncology.

2. VEGF and tumour growth

VEGF promotes the growth of tumour vasculature to allow oxygen and nutrients to reach the rapidly dividing cancer cells. However, this tumour vasculature is abnormal both in structure and function, with the vessels being immature, leaky and tortuous, with a reduction or absence of supporting cells. The effect of VEGF on endothelial cells is important in the development of these abnormal vessels. In a mouse xenograft model, endothelial cells undergo apoptosis and newly formed vessels disintegrate in the absence of VEGF.[8] However, the presence of VEGF promotes the survival of the new vasculature by increasing the expression of the anti-apoptotic proteins Bcl-2, XIAP and survivin in vitro.[9,10] High VEGF expression also promotes vascular permeability in vitro, leading to high interstitial and intratumoral pressure, which may allow tumour cells to enter the bloodstream and metastasise[11] as well as impairing the delivery of chemotherapy to the tumour.[12,13] The chaotic layout of tumour vasculature leads to inconsistent oxygen delivery within the tumour; this creates regions of hypoxia, which are resistant to radiotherapy.[14] Tumour blood vessels also have a reduction or absence of supporting pericyte and smooth muscle cells, which are essential to the functioning of the vasculature by stabilising vessel walls and helping to regulate microcirculatory blood flow, as well as influencing endothelial permeability, proliferation, survival, migration and maturation. An absence of pericytes sensitises tumour vessels to VEGF inhibitors, as shown in a number of mouse xenograft models.[15–17]

Notch 1–4 are transmembrane protein receptors that interact with five transmembrane ligand proteins (Jagged 1/2 and Delta-like ligand [DII] 1/3/4).[18,19] Notch signalling is thought to regulate tumour vascular formation and remodelling, as well as endothelial cell migration and differentiation, as evidenced by preclinical studies.[4,20] It appears that VEGF is involved in this process by regulating these proteins in vitro and this pathway may be responsible for some of the characteristic tumour vasculature abnormalities.[20–22]

In addition to effects on existing vessels, VEGF also stimulates neovascularisation and/or revascularisation by inducing vascular sprouting from existing vessels in vitro; transgenic mouse models have confirmed that it is also a survival factor for these newly formed vessels.[23,24] Thus, throughout tumour development, the formation of new vessels remains dependent on VEGF expression.[10] The multiple roles of VEGF in the development and maintenance of tumour vasculature therefore make it an attractive therapeutic target.

3. Approaches to targeting the VEGF ligand and VEGF receptors

A wide range of drugs that target the VEGF system are currently in development. Those that show most promise are monoclonal antibodies directed at the VEGF ligand or receptor and VEGF receptor tyrosine kinase inhibitors (TKIs). VEGF ligand monoclonal antibodies are highly specific and bind to free-circulating VEGF to eliminate interaction with all its receptors. Furthermore, despite the high levels of VEGF released by the tumour and stromal cells, anti-VEGF antibodies are able to neutralise all free VEGF.[25] In contrast, VEGF receptor TKIs are non-specific and have activity against multiple receptors in addition to those for VEGF. Principal differences between anti-VEGF monoclonal antibodies and VEGF receptor TKIs are highlighted in Table 1.

The agents in these classes that are most advanced in development for the treatment of breast cancer are:

- Bevacizumab (Avastin®): a humanised monoclonal antibody developed from a mouse monoclonal antibody (A4.6.1) targeting all major isoforms of VEGF. It is the first anti-VEGF agent to be approved for cancer treatment.[26]

Table 1 – Principal differences between anti-VEGF monoclonal antibodies and VEGF receptor TKIs

Anti-VEGF monoclonal antibodies	VEGF receptor TKIs
Directly block interactions between VEGF and its receptors	Block signalling by activated VEGF receptors
Prevent activation of downstream signals	Down-regulate signalling pathways which are already activated
Bind specifically to VEGF – minimal effects on normal physiology	Interact with other receptor tyrosine kinases; effects are not all specific to angiogenesis inhibition – non-specific action could produce unexpected side effects
Inhibit all functions of VEGF on endothelial and non-endothelial cells	May not inhibit all functions of VEGF
May not affect the function of other VEGF family ligands (e.g. VEGF-B)	Inhibit the activity of other VEGF family members signalling through the same receptor
Additive effects when combined with chemotherapy	Additive effects when combined with chemotherapy
Size may limit penetration	Smaller size, so potentially better penetration

VEGF = vascular endothelial growth factor; TKIs = tyrosine kinase inhibitors.

- Sunitinib malate (Sutent®): a small molecule TKI of platelet-derived growth factor receptor (PDGFR)-α and -β, VEGF receptors -1, -2 and -3, Flt-3 and c-Kit. The current relevance of the VEGF activity of this drug remains unclear.
- Sorafenib (Nexavar®): a small molecule TKI of Raf-1, VEGF receptors -2 and -3, PDGFR-β, Flt-3 and c-Kit. The current relevance of the VEGF activity of this drug remains unclear.
- Motesanib (AMG706): a TKI with activity against VEGF receptors -1, -2 and -3, PDGFR and c-Kit.
- Pazopanib (GW786 034): a TKI of VEGF receptors -1 and -2, PDGFR-β and c-Kit.

Professor Nadia Harbeck will review these agents in detail elsewhere in the supplement (see 'Clinical data for anti-angiogenic agents in previously treated advanced breast cancer').

4. Effects of anti-VEGF therapy: preclinical data

During the 1970s, the development of anti-angiogenic agents was proposed as an effective anticancer strategy. [1] Evidence shows that anti-VEGF agents possess a variety of *in-vivo* mechanisms of action comprising early effects of the regression of existing tumour microvasculature and normalisation of remaining tumour vasculature, as well as continued effects such as inhibition of new tumour vasculature. The mechanism of action of the anti-VEGF agents with regards to direct antitumour effects has not been fully elucidated, although it is suggested that inhibition of the effects of VEGF on dendritic cell maturation may improve the immune response to tumours. [27]

4.1. *Early effects*

Anti-VEGF therapy removes endothelial cell survival signals in vitro, thereby causing the endothelial cells to shrivel, die and fragment (vessel regression). [28] Inai et al. examined the effects of two VEGF inhibitors, VEGF-Trap and AG-013736, on blood vessel structure in spontaneous islet-cell tumours of RIP-Tag2 transgenic mice and in subcutaneously implanted Lewis lung carcinomas. [29] The effect of VEGF inhibition was rapid (<24 hours), as evidenced by reduced tumour blood vessel flow and loss of luminal patency leading to a significant reduction in microvascular density. [16,29]

In addition to regression, a key role of anti-VEGF therapy is the reversal of the structural and functional abnormalities of the remaining tumour vasculature to produce a more organised and efficient system (normalisation) (Figure 2). [12,13] Such changes comprise a more uniform vessel size, improved shape and permeability, reduced intratumoral pressure, improved oxygenation and increased pericyte coverage. [16,30–32] The relationship between anti-VEGF therapy and the uptake of anticancer drugs was examined by Wildiers et al. [33] Nude NMRI mice bearing colon adenocarcinoma were treated with the anti-VEGF monoclonal antibody A4.6.1 or placebo. CPT-11 (irinotecan) was administered 1 hour before the animals were sacrificed a week later. A4.6.1-treated tumours displayed a significant decrease in vessel density compared with placebo, and a trend towards increased intratumoral CPT-11 concentration ($p = 0.09$). These data suggest that anti-VEGF-mediated vascular changes may facilitate the uptake and penetration of anticancer agents into the tumour. [33]

4.2. *Continued effects*

Anti-VEGF therapy inhibits endothelial cell proliferation and migration in vitro. [16,29] A number of preclinical studies have also shown that it inhibits neovascularisation (new vessel growth/vascular sprouts) that is required for further tumour growth and micrometastases. [34–36] Continued suppression of new tumour vasculature may be a key factor in preventing or delaying disease progression.

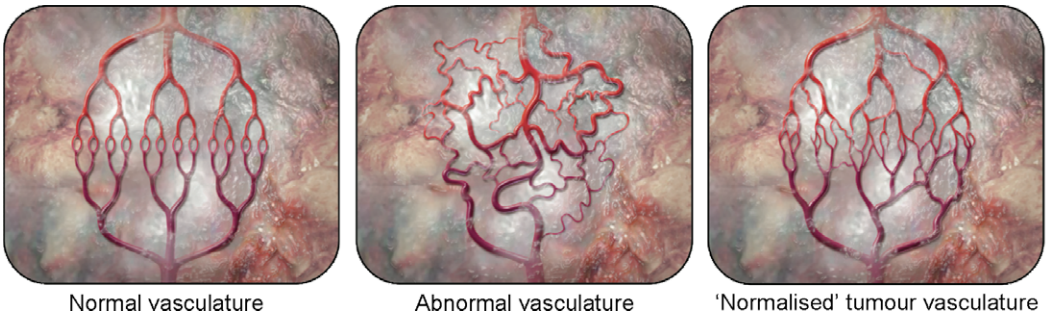

Fig. 2 – Effect of anti-VEGF therapy on abnormal tumour vasculature. Reprinted from Jain,[12] *Nat Med* 2001, by permission from Macmillan Publishers Ltd: © 2001.

Anti-VEGF therapy may also inhibit revascularisation (regrowth of regressed microvessels), in which basement membrane tracks or 'ghosts' serve as pathways for rapid vessel regrowth in vitro.[16,29] Recent evidence from mouse tumour models suggests that early withdrawal of anti-VEGF therapy results in rapid vessel regrowth.[26,37,38] Kamba et al. treated wild-type C57BL/6 mice with a small molecule VEGF receptor TKI (AG-013736) for 7 days and examined the thyroid vasculature at the end of treatment and 14 days later. A 46% decrease in vascularity was noted during the treatment period, with complete regrowth of perifollicular capillaries 14 days after withdrawal of anti-VEGF therapy.[37] These data demonstrate the importance of continuing anti-VEGF therapy until disease progression to prevent rapid revascularisation and regrowth of tumours.

4.3. *Additional effects*

Angiogenic inhibition with anti-VEGF agents does not totally prevent vessel regrowth since patients receiving this therapy do eventually experience disease progression.[39,40] This may be due to several 'escape mechanisms', including the induction of alternative pro-angiogenic molecules (e.g. FGF) and protection of more mature tumour blood vessels through increased pericyte coverage. However, since VEGF has a key role in angiogenesis, it is possible that removal of VEGF inhibition would result in accelerated angiogenesis and tumour regrowth due to an induction of both VEGF and alternative pro-angiogenic pathways.

Evidence suggests that functional VEGF receptors are not restricted to endothelial cells but are also found on tumour cells.[41] This is supported by several studies that have observed the presence of functional VEGF receptors on various cancer lines and suggests that VEGF may stimulate tumour cell growth by binding to VEGF receptors on their surface. Anti-VEGF therapy may provide direct antitumour effects through inhibition of tumour cell growth or promotion of apoptosis via a VEGF/VEGF receptor autocrine pathway. Therefore, even when the tumour is independent of VEGF for growth and progression, anti-VEGF therapy may still enhance anticancer therapy as well as elicit direct antitumour effects.

5. Preclinical data on anti-VEGF therapy in breast cancer

In a study of athymic rats xenografted with a human breast carcinoma cell line (MDA-MB-435), enhanced magnetic resonance imaging (MRI) scanning revealed that 24 hours following treatment with a single dose of anti-VEGF antibody (A4.6.1) a significant decrease ($p < 0.05$) of tumour permeability surface area product was seen, although no significant change was observed in tumour fractional blood volume.[42] Similarly, Pham et al. found significant reductions in tumour growth rates ($p < 0.05$) and in microvascular permeabilities ($p < 0.05$) in nude rats implanted with human breast cancer cells (MDA-MB-435) following administration of anti-VEGF antibody (A4.6.1) (three 1 mg doses at 3-day intervals), as evidenced by macromolecular contrast medium-enhanced MRI and tumour volume measurements.[43] Borgstrom et al. demonstrated significant suppression of tumour angiogenesis following anti-VEGF monoclonal antibody treatment (A4.6.1) in nude mice implanted with tumour spheroids of human breast cancer cell lines (MCF-7, ZR-75 and SK-BR-3), but in addition, showed that when A4.6.1 was combined with a conventional chemotherapeutic drug (doxorubicin 5 mg/kg, weekly), significant tumour regression was observed for all cell lines.[44] The tumour cells were pre-labelled with a fluorescent vital dye, which allowed the estimation of growth of the implanted tumour spheroids and tumour site neovasculature was assessed using fluorescein isothiocyanate (FITC)-Dextran. Treatment with anti-VEGF monoclonal antibody alone caused significant suppression of tumour angiogenesis and treatment with doxorubicin alone reduced the growth rate of MCF-7 cells, but did not significantly affect angiogenesis.[44] However, results showed that combination treatment resulted in a synergistic inhibition of tumour growth compared with that seen with either agent administered alone ($p < 0.001$).[44,45]

The humanised form of A4.6.1, bevacizumab, has also been investigated in the preclinical setting in combination with a variety of chemotherapeutic agents. Ran et al. performed a study in nude mice implanted with MDA-MB-231 human breast carcinoma cells to investigate

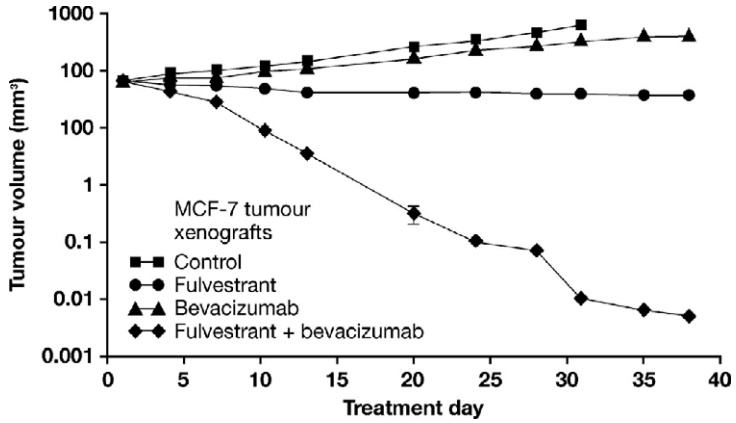

Fig. 3 – Synergistic effect of bevacizumab and fulvestrant in a breast cancer xenograft model. Reprinted with permission from Pietras et al.,[47] poster presented at SABCS 2006.

the effects of albumin-bound paclitaxel (Abraxane®) and bevacizumab as single or combination therapy on tumour growth and metastatic spread. Bevacizumab significantly improved the antitumour effect of albumin-bound paclitaxel treatment, with the combination treatment having a synergistic effect on tumour metastasis, in particular in association with increased bevacizumab dose.[46] Pietras et al. investigated the effect of combining VEGF inhibitors with anti-oestrogen therapy in the hope of creating a more effective antitumour treatment strategy. Oestrogens not only stimulate the proliferation of human breast cancer cells through binding and activation of tumour cell oestrogen receptors (ER), but may also contribute to tumour angiogenesis by causing increased VEGF secretion, as well as inducing the proliferation of nearby vascular endothelial cells through ER-induced signalling in both the tumour and on the neighbouring vasculature. Results using the VEGF inhibitor in combination with anti-oestrogen therapy showed that tumour and vascular cells exhibit proliferative responses in response to oestradiol and this effect was blocked by treatment with the anti-oestrogen, fulvestrant (Faslodex®). Furthermore, reduced VEGF secretion was observed, and, when bevacizumab and fulvestrant were combined, a greater antitumour effect was obtained than demonstrated with either agent alone (Figure 3).[47] Similarly, the combination of capecitabine (Xeloda®) with bevacizumab showed high antitumour activity in a preclinical xenograft breast cancer model in nude mice compared with single-agent administration of either therapy in terms of both tumour growth inhibition ($p < 0.05$) and survival ($p < 0.0001$). The two agents acted synergistically with respect to tumour growth inhibition and increased life span, this being most evident at the higher dose of capecitabine (400 mg/kg).[48]

6. Anti-angiogenic effects of anti-VEGF therapy

Though there is a question as to whether preclinical data accurately reflect the clinical situation, there is a growing accumulation of clinical data that supports the hypothesised mechanism of action of bevacizumab. Consistent and significant improvements in tumour response rate have been demonstrated with bevacizumab, whether used alone or in combination with different chemotherapeutic agents in patients with breast cancer.[49–51]

Wedam et al. conducted a pilot study investigating the anti-angiogenic and antitumour effects of bevacizumab (15 mg/kg every 3 weeks), either alone or in combination with doxorubicin and docetaxel, in 21 patients with previously untreated stage III or IV inflammatory and locally advanced breast cancer.[52] Assessments comprised immunohistochemistry, activated VEGF receptor-2 status, total VEGF receptor-2, tumour microvascular density, tumour cell apoptosis and proliferation. Also, vascular permeability (K^{trans}) was assessed using serial investigations with dynamic contrast-enhanced MRI (DCE-MRI). Results showed measurable effects of bevacizumab alone on tumour angiogenesis, with a median decrease of 66.7% in phosphorylated VEGF receptor-2 in tumour cells ($p = 0.004$) and a median increase of 128.8% in tumour apoptosis ($p = 0.0008$). These changes persisted with the addition of chemotherapy. DCE-MRI results showed a median decrease of 34.4% in the inflow transfer rate constant ($p = 0.003$), 15.0% in the backflow extravascular-extracellular rate constant ($p = 0.0007$) and 14.4% in extravascular-extracellular volume fraction ($p = 0.002$) following bevacizumab monotherapy. This study concluded that bevacizumab has inhibitory effects on VEGF receptor activation and vascular permeability and induces tumour cell apoptosis. Significantly, this was the first study to demonstrate that bevacizumab has a direct inhibitory effect on tumour cell angiogenic parameters.[52] Wedam et al. discussed the issue of establishing predictive markers to bevacizumab treatment, although they acknowledged that patient numbers in this trial were very small. Indeed, no correlations have been identified between response and VEGF levels or VEGF receptor levels in clinical studies across several

tumour types. In addition, a dose-response relationship between bevacizumab dose and number of targets has not been studied in the preclinical or clinical setting.

Efficacy and safety data from a large pivotal phase III study (E2100) of bevacizumab combined with paclitaxel for first-line treatment of patients with metastatic breast cancer will be reviewed by Professor David Cameron and Dr David Miles in additional articles in this supplement.

7. Summary

Preclinical data illustrate that VEGF is a potent stimulator of angiogenesis and is a key factor in tumour growth, progression and metastasis. VEGF-induced tumour vasculature has a number of structural and functional abnormalities that support tumour growth including increased permeability and chaotic structure, as demonstrated preclinically. Bevacizumab is a humanised monoclonal antibody directed against VEGF; in preclinical models it leads to the rapid regression, normalisation and inhibition of tumour vasculature, which in turn prevents or reverses tumour growth and reduces risk of metastasis. The actions of bevacizumab also enhance the effects of any concomitant chemotherapy regimen in both the preclinical and clinical setting. The mechanism of action of bevacizumab with regards to direct antitumour effects has not been elucidated.

No correlations have been identified between response and VEGF levels or VEGF receptor levels in clinical studies. However, future clinical studies will provide additional evidence for the effectiveness of bevacizumab in combination with a variety of different chemotherapy and biological agents.

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Conflict of interest statement

Dr Drevs has provided part-time consultancy to Roche.

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