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The role of antiangiogenesis therapy: Bevacizumab and beyond*

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Abstract The importance of angiogenesis in tumour growth and development is well known. Overexpression of vascular endothelial growth factor (VEGF), the key mediator of angiogenesis, is associated with poor prognosis in cancer. As a result, several therapeutic agents that inhibit the actions of VEGF or its receptors are currently in development for use in advanced solid tumours, such breast, colorectal, lung and renal cancer. Clinical data from trials of anti-VEGF agents in this group of tumours are discussed, with a particular focus on the efficacy and safety of bevacizumab, the anti-VEGF agent at the most advanced stage of development in those tumour types. Future potential uses of bevacizumab in cancer therapy will be discussed.

Keywords Angiogenesis · VEGF · Angiogenesis inhibitor · Metastasis

Angiogenesis, the growth of new blood vessels from existing vasculature, is known to be essential for the development and progression of cancer [1, 2]. This has led to the development of many anti-angiogenic agents, which were reviewed recently for breast cancer [3] and is updated here due to their potential and the widespread interest in their use to treat solid tumours.

Key role of vascular endothelial growth factor (VEGF) in tumour angiogenesis

Vascular endothelial growth factor (VEGF) is the key proangiogenic factor that promotes tumour angiogenesis and the survival of tumour endothelial cells. VEGF-A, generally referred to as VEGF, is a member of a family of related molecules, six of which have been identified to date [4, 5]. Due to alternative splicing of VEGF messenger RNA (mRNA), the VEGF protein occurs as four main isoforms [5, 6]. The physiological significance of the different isoforms remains uncertain, but the 165-amino acid molecule, VEGF165, is the most abundant [7]. VEGF gene expression is regulated by a number of stimuli, including hypoxia, growth factors, tumour suppressor genes, oncogenes, nitric oxide and human epidermal growth factor receptor-2 (HER-2) [8–13]. Physiological effects of VEGF are mainly mediated through binding to an endothelial cell surface receptor, VEGF receptor-2, which also serves as a receptor for other VEGF family members [14]. VEGF binds to VEGF receptor-1 (flt1) with higher affinity than VEGF-receptor 2 (KDR) (1, 15, 16), but the role of this receptor in angiogenesis remains unclear.

In addition to its role in normal physiological processes (such as embryogenesis and early postnatal growth), VEGF is the key mediator of tumour angiogenesis and thus of tumour development and metastasis. Tumours are unable to grow beyond a diameter of 2 mm in the absence of angiogenesis, due to the inability of oxygen and nutrients to diffuse beyond this distance [17]. Preclinical studies using antibodies against VEGF have demonstrated its importance

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Table 1 Clinical benefit with bevacizumab in phase III studies

Tumour type	Traditional therapy	PFS (months)	OS (months)	T. therapy+bevacizumab[a]
Metastatic CRC	CT doublets	~9[1,2]	~16[1,2]	PFS>5 mos [34, 35] OS>4 mos [35, 36]
Advanced NSCLC	CT doublets	~4[3]	~10[3]	PFS>2 mos [43] OS>2 mos [45]
Metastatic	CT doublets	~9[4,5]	~22[4,5]	PFS>5 mos [41]
Metastatic RCC	IFN/IL-2	–	~13[6]	OS>5 mos [47]

[1]Colucci, et al JCO 2005; [2]de Gramont, et al JCO 2000; [3]Schiller, et al NEJM 2002; [4]Sledge, et al JCO 2003; [5]Alba, et al JCO 2004; [6]Coppin, et al Cochrane Database Syst Rev 2005
[a]References in this article

in tumour angiogenesis. When VEGF is inhibited, both neovascularisation and tumour growth are suppressed in animal models [18, 19]. VEGF has other effects on tumours, including inhibition of tumour cell apoptosis [20], stimulation of metastasis [21], suppression of the antitumour immune response and increasing the permeability of tumour blood vessels, causing a rise in interstitial pressure, which reduces the delivery of chemotherapeutic agents to the tumour [22, 23].

VEGF as a therapeutic target

VEGF has a limited role in normal adult physiology, with functions restricted to wound healing and the female reproductive cycle [24]. Tumour-derived VEGF can therefore be inhibited with minimal effects in adults, and represents a novel approach to anticancer therapy. Vascular regression has been demonstrated to occur rapidly after initiation of anti-VEGF therapy in preclinical models and cancer patients [25, 26]. The morphology and function of surviving tumour blood vessels are transiently 'normalised' so that they more closely resemble the normal vasculature [27, 28].

These changes reduce intratumoral pressure, facilitating the delivery of other anticancer therapies to the tumour [29].

Furthermore, neovascularisation and vascular regrowth are both inhibited. Revascularisation occurs rapidly following the withdrawal of anti-VEGF therapy [30–32]. Interestingly, increased VEGF expression, at both the mRNA and protein levels, has been observed in patients with breast cancer and other tumours [33]. High VEGF expression is associated with poor clinical outcomes, including reduced survival [34].

Bevacizumab: clinical data

Bevacizumab is a humanised recombinant antibody that prevents VEGF receptor binding and inhibits angiogenesis and tumour growth and has been tested in many tumours. Efficacy of bevacizumab as a single agent was seen in early phase I/II in relapsing metastatic breast cancer (mBC) with a 6.7% overall response rate, median duration of response of 5.6 months and overall survival (OS) of 10.2 months. When was added to almost different chemotherapy regimen, bevacizumab produce a significantly increase in response rate. Bevacizumab was well tolerated, producing very few incidences of hypertension and proteinuria and occasional cases of bleeding and thromboembolism.

An important clinical research programme was developed in the main solid tumours with significant positive results that, in some diseases, have changed the standard clinical practice. The most relevant data came from nine phase III studies done in colorectal, breast, lung and renal cancer (Table 1).

In *metastatic colorectal cancer* (mCRC) patients receiving the IFL regimen (irinotecan plus fluorouracil/leucovorin at first-line treatment), the addition of bevacizumab significantly increased the progression-free survival (PFS) and OS in more than 4 months [35]. In the second-line treatment, patients who received bevacizumab in combination with a fluorouracil/leucovorin plus oxaliplatin (FOLFOX4) regimen had an OS time 2 months longer than that in patients receiving FOLFOX4 alone [36]. Bevacizumab was tested in neoadjuvant setting in patients with locally advanced rectal cancer. In a phase II study, 24 patients with late-stage non-metastatic rectal cancer completed four cycles of neoadjuvant therapy including bevacizumab infusion (5 or 10 mg/kg) on day 1 of each cycle, additional standard chemotherapy with 5-fluorouracil, external beam irradiation, and surgery 7 to 9 weeks after completion of all neoadjuvant therapy. At 4 years, local tumour control was achieved in all patients and disease-free survival in 88% [37]. The addition of bevacizumab to either FOLFOX4 or XELOX (oxaliplatin, Xeloda®) demonstrated a significant benefit to either of these chemotherapy regimens in a placebo control phase III study. Median PFS was 9.3 months in the two treatment arms of bevacizumab plus XELOX or FOLFOX, compared with 8.0 months for the placebo arms (p=0.0023, HR 0.83) [38].

Continuation of bevacizumab after mCRC progression to improve survival?

A large observational trial has shown substantially increased survival among patients who continued to take bevacizumab after their mCRC progressed [39]. Besides offering a hypothesis, it was revealed that about 40% of patients did not receive all three chemotherapeutic drugs (fluorouracil, irinotecan and oxaliplatin) previously shown to be active against this malignancy. Only half took EGFR inhibitors after progression.

In *metastatic breast cancer* (mBC) the most relevant evidence of bevacizumab efficacy is coming from two large phase III trials. The first one compared a combination of bevacizumab plus capecitabine vs. capecitabine alone in 462 women previously treated with an anthracycline and taxanes. Although the addition of bevacizumab to capecitabine did not show a statistically significant improvement in PFS nor OS, there was a significant increase in overall response rate in the capecitabine plus bevacizumab arm when the data were analysed both by the investigators (30.2% vs. 19.1%; p=0.006) and by an independent review facility (19.8% vs. 9.1%; p=0.001). The combination was found to be well tolerated [40].

In the other phase III trial (E2100) bevacizumab is being evaluated in combination with weekly paclitaxel as first-line therapy for mBC. Results from the final analysis showed a significant increase in median PFS in patients receiving bevacizumab plus paclitaxel compared with paclitaxel alone (11.4 vs. 6.11 months; p<0.0001). The overall response rate for all patients was 36.9% (bevacizumab/paclitaxel) vs. 21.2% (paclitaxel) (p<0.0001) and 49.2% (bevacizumab/paclitaxel) vs. 25.2% (paclitaxel) (p<0.0001) for patients with measurable disease only. No benefit was observed in survival [41]. Another phase III trial recently reported (AVADO) randomised 705 patients to receive docetaxel either with placebo or bevacizumab at 7.5 mg/kg or 15 mg/kg doses every three weeks. Both bevacizumab arms showed a significant increase in PFS (primary end-point) in comparison with docetaxel and placebo. Overall response rate was also significantly better for the bevacizumab-treated patients (55% and 63% vs. 44%, p=0.0295 and 0.0001 respectively) [42].

In *lung cancer* two large randomised phase III trials have now demonstrated improved outcomes when bevacizumab is combined with chemotherapy vs. chemotherapy alone. In the E4599 trial 878 Stage IIIB/IV recurrent or advanced patients were randomised to receive the combination of paclitaxel and carboplatin, with or without bevacizumab. Combining bevacizumab with platinum-based chemotherapy significantly increased OS (12.3 months compared to 10.3 months), as well as PFS (6.2 months compared to 4.5 months) and response rate representing the most significant achievement in improving clinical outcomes in first-line advanced NSCLC [43]. Bevacizumab-based therapy until progression is the first treatment to achieve OS beyond 1 year. The AVAiL trial is a double-blinded, randomised, phase III study which enrolled 1043 first-line advanced non-squamous NSCLC patients treated with cisplatin plus gemcitabine, in combination with either bevacizumab at two doses (15 mg/kg and 7.5 mg/kg every 3 weeks or placebo) until disease progression. The results reported a statistically significant improvement in PFS, the primary endpoint of the study, in the two arms receiving bevacizumab. However, there was not a statistically significant improvement in OS, the secondary endpoint of the trial [44].

In metastatic renal cell cancer (mRCC), bevacizumab has shown a clinical benefit in phase II studies as a single agent, having a median PFS of 8.5 months in previously untreated patients and 4.8 months vs. 2.5 months of placebo (p<0.001) in previously treated patients. A number of patients have had durable responses lasting 3–5 years with continued bevacizumab therapy [45, 46]. In a multicentric, randomised, double-blind, phase III trial (AVOREN), patients with previously untreated mRCC were randomised to receive interferon alpha-2a and bevacizumab or placebo and interferon alpha-2a. The primary endpoint was OS. Median duration of PFS was significantly longer in the bevacizumab plus interferon alpha group than it was in the control group (10.2 months vs. 5.4 months; HR 0.63, p=0.0001). Increases in PFS were seen with bevacizumab plus interferon alpha irrespective of risk group or whether reduced-dose [47].

In advanced epithelial *ovarian cancer*, bevacizumab as a single agent possesses more activity than in any other epithelial cancer, apart from renal cancer, where the vascular biology is specifically relevant to this therapeutic approach. A single case report study described how single-agent bevacizumab induced an objective durable response in a patient with recurrent and refractory serous carcinoma of the ovary after failing the 11th-line cytotoxic therapy [48]. In a phase II trial, bevacuzimab was evaluated as a single agent in the second- or third-line treatment of patients with persistent or recurrent ovarian or primary peritoneal cancer [49]. The treatment was well tolerated and efficacious with 13/62 (21.0%) clinical responses and 25/62 (40.3%) patients who survived progression-free for at least 6 months. Another study recruited a total of 44 patients, all of whom were platinum-resistant, as well as resistant to either topotecan or liposomal doxorubicin; 27 (64%) of these cases achieved stable disease with median PFS of 4.4 and 4.5 months [50]. Another study, from the California Consortium, evaluated 29 patients with recurrent ovarian cancer resistant up to three lines of chemotherapy who were treated with daily low dose of cyclophosphamide and bevacizumab. Nearly half of the patients had no tumour progression for six months. Over 20% of the patients have a partial response of long duration [51]. A recent review of the role of bevacizumab in resistant ovarian cancer, based on these studies, was published as an editorial by Kaye, an expert in this disease [52].

In glioblastoma, a tumour characterised by markers of hypoxia and necrosis, some positive results have been

Table 2 Anti-VEGF therapeutic agents in clinical development

Drug (trade mark)	Molecular target(s)	Stage clinical development
Bevacizumab (Avastin)	VEGF ligand	Approved
Sunitinib (Sutent)	VEGFR 1-2-3, PDGFR-αß, Fit3, c-kit	Approved
Sorafenib (Nexavar)	Raf-1, VEGFR 2-3, PDGFR-ß, Fit3, c-kit	Approved
Vatalanib (PTK/ZK)	VEGFR 1-2-3, PDGFR-ß, c-kit, c-Fms	Phase II-III
Vandetanib (Zactima)	VEGFR 1-3, EGFR	Phase II
Cediranib (Recentin)	VEGFR 1-2-3	Phase II–III
Motesanib (AMG 706)	VEGFR 1-2-3, PDGFR, c-kit	Phase II
Pazopanib (Armala)	VEGFR 1-2, PDGFR-ß, c-kit	Phase II-III
Axitinib (AG-013736)	VEGFR 1-2-3, PDGFR, Fit3, c-kit	Phase II-III
Aflibercept (VEFGR/Trap)	VEGF-trap	Phase II

obtained, but bevacizumab has yet to become an integral part of the initial therapeutic strategy for drug approval. Bevacizumab in combination with irinotecan is an effective treatment for recurrent glioblastoma multiforme, with acceptable toxicity.

In a phase II trial, 23 patients received bevacizumab at 10 mg/kg plus irinotecan every 2 weeks and irinotecan at variable doses ranging from 125 to 340 mg/m^2, depending on concomitant treatment with enzyme-inducing anti-epileptic drugs. Each cycle was 6 weeks long and concluded with patient evaluations, including magnetic resonance imaging. The 6-month PFS among all 35 patients was 46% (95% CI, 32–66%). The 6-month OS was 77% (95% CI, 64–92%). Twenty of the 35 patients (57%; 95% CI, 39–74%) had at least a partial response [53, 54].

A recent trial presented at the last ASCO meeting combined daily temozolomide at 50 mg/m^2/day with bevacizumab at 10 mg/kg every 14 days for patients with recurrent GBM. Thirty-two patients were enrolled and all had received radiation therapy and 63% progressed to 5-day temozolomide. The treatment had acceptable toxicity; there was no >grade 3 haematologic toxicity and no CNS haemorrhages. Twelve patients (37.5%) had a partial response and another 12 (37.5%) had stable disease [55]. Based on these data, the US Food and Drug Administration (FDA) has recently granted accelerated approval for bevacizumab for use in patients with glioblastoma that has progressed despite previous therapy.

The future of bevacizumab and other angiogenesis therapies

In spite of all the clinical data mentioned above, which confirm the important activity of bevacizumab in solid tumours, there are still many research questions that need to be answered at the present time. Some of them will be solved by a large number of ongoing trials, with special regards to the role of bevacizumab in neoadjuvant and adjuvant settings, in breast and colorectal cancer, but also in other tumours such as lung, renal, ovarian, etc. Several studies confirm the activity of bevacizumab in other solid tumours as hepatocellular carcinoma [56], sarcomas [57], head and neck [58] and neuroendocrine tumours [59].

The second question to be answered is the role of the other antiangiogenic agents, tyrosine kinase inhibitors (TKI), in comparison with the already known data from bevacizumab, mentioned above. These small molecules are active in solid tumours and have some differences from the monoclonal antibodies (Table 2). In order to complete this review we will give a resume of the most relevant data on these new antiangiogenic agents, which are already registered for clinical practice.

Sorafenib

The small-molecule kinase inhibitor sorafenib targets wild-type and mutated B-Raf, VEGFR2, VEGFR3, PDGFR-ß, c-KIT, FLT-3 and p38. It induces growth arrest and apoptosis of endothelial cells and some tumour cell types [60]. Activity was first reported in a phase II randomised discontinuation trial in patients with RCC, whereby PFS was prolonged compared with placebo (24 vs. 6 weeks). Patients were treated for 12 weeks (run-in treatment) and at the end of that period those with stable disease were subsequently randomised to sorafenib or placebo [61]. In a phase III first-line placebo-controlled trial, sorafenib prolonged PFS of patient with metastatic RCC and was approved by the FDA and the EMEA for the treatment of advanced and metastatic RCC [62]. Retrospective analysis showed that high basal VEGF levels (>131 pg/ml) correlated with a poor prognosis and a trend towards greater PFS benefit in sorafenib vs. placebo-treated patients [63].

In *hepatocellular cancer*, sorafenib is effective in previously untreated patients. In one large placebo-controlled trial, 602 patients with advanced hepatocellular carcinoma received sorafenib at 400 mg twice daily, achieving a median OS of 10.7 months in comparison with 7.9 months in the placebo group $p<0.001$. The median time to radiologic progression was 5.5 months in the sorafenib group and 2.8 months in the placebo group ($p<0.001$). Seven patients in the sorafenib group (2%) and two patients in the placebo

group (1%) had a partial response. This unprecedented impact on survival was presumably mainly due to VEGFR2 inhibition [64].

A phase I/II trial of a combination of sorafenib with bevacizumab, in advanced platinum-resistant/refractory epithelial ovarian carcinoma reported a 47 % partial response with a manageable toxicity [65].

Sunitinib

Sunitinib is a TKI that targets VEGFR1, VEGFR2, VEGFR3, PDGFR-α/ß, FLT-3, c-KIT and c-RET [66]. Sunitinib showed evidence of activity in a phase I trial in many tumours, including thyroid and neuroendocrine cancers, soft tissue sarcoma, gastrointestinal stromal tumour and RCC [67]. In a phase II trial in patients with cytokine-refractory RCC, the overall response rate was 34% and median PFS was 8.3 months [68]. A phase III trial in patients with metastatic RCC showed that sunitinib improved PFS and overall response rate compared with IFNα as first-line therapy, and sunitinib was subsequently approved for the treatment of advanced and metastatic RCC [69]. Retrospective analysis from a phase II trial in bevacizumab-refractory RCC suggests that circulating levels of VEGF and sVEGFR3 might be predictive of response to sunitinib: responding patients had lower basal levels of sVEGFR3 ($p<0.0318$) and a trend towards a greater decrease upon treatment levels ($p<0.10$) than non-responding patients [70].

Temsirolimus

Temsirolimus inhibits mTOR, an Akt-target kinase downstream of VEGFR2, which controls cell proliferation, cellular metabolism and survival [71]. Activity was demonstrated in a phase I trial in patients with RCC and was confirmed in phase II studies, improving outcomes when administered either as a single agent or in combination with IFNα [72]. A three-arm, randomised, phase III trial compared temsirolimus, IFNα and a combination of the two drugs as first-line treatment in aggressive RCC. Patients who received temsirolimus alone had longer OS and PFS than did patients in either of the other two treatment arms [73].

Other studies of antiangiogenic agents

Some of the kinases targeted by sunitinib and sorafenib such as PDGFR, p38, FLT-3, c-KIT and c-RET are active in cancer cells and promote tumour cell proliferation, survival and motility. mTOR, the temsirolimus target, is often activated in cancer and has a central role in regulating metabolic events, in particular protein synthesis, which is essential for tumour growth [71]. Thus, in addition to antiangiogenic effects, these drugs may have direct effects on cancer cells that contribute to their global antitumour activities. The use of anti-VEGF agents in CRC has provided varied results. While bevacizumab, as was mentioned before, in combination with irinotecan, fluorouracil and leucovorin induced a significant increase in OS in patients with advanced, previously untreated CRC [35], the pan-VEGFR TKI vatalanib (PTK787), combined with the FOLFOX4 regimen, did not produce a survival benefit. In two phase I trials, vatalanib decreased vascular permeability and vascularity, as measured by dynamic contrast-enhanced (DCE) MRI in patients receiving ≥750 mg/day [72]. Although this effect occurred only in patients with liver metastases, it was interpreted as validation of vatalanib activity and was used to support the case for further clinical development. All four above-mentioned approved drugs demonstrated activity in RCC and target the VEGF/VEGFR pathway, albeit to different extents. RCC is the prototype cancer with VEGF-driven angiogenesis owing to frequent mutations in the von *Hippel Lindau* (VHL) tumour suppressor gene, a negative regulator of hypoxia-inducible factor 1 (HIF-1) activity and VEGF expression [73]. These findings suggest that the match between the targeted pathway and the cancer chosen may be relevant or possibly critical to the success of these drugs. Second, biomarkers are dispensable when clinical results obtained in phase I/II trials are already convincing. By contrast, when the tested agent does not achieve tumour control during an early stage of clinical development, a pharmacodynamic biomarker reporting biological activity would constitute an essential requirement to pursue clinical development. The use of a pharmacodynamic biomarker that is irrelevant to the main biological activity of the drug or representative of activity in only a specific patient subpopulation, however, might compromise further development.

Conclusions

Based on these data and without going into detail, we would like to raise the following issues for future studies based on presentations in a recent ESOC new drugs meeting [74]. In relation to tumour-specific determinants of winning strategies in renal cell carcinoma, clear cell type and its link to VHL mutations constitutively driving angiogenesis has been the prototypic target of antiangiogenic therapy. A number of single biologic agents such as α-interferon, bevacizumab, sorafenib, sunitinib and temsirolimus have shown antitumour activity against renal cell carcinoma that has very limited chemosensitivity [62–69]. In relation to select drugs leading to these winning strategies, the experience is currently very limited, with the application of a vertical strategy to suppress angiogenesis (e.g., combining binders of VEGF with small molecule inhibitors of VEGFR2) or horizontal strategies targeting inhibition of endothelium and the stroma, as well as the tumour. Some types of chemotherapy, such as taxanes, the

pegylated liposomal doxorubicin or low-dose (metronomic) cyclophosphamide, potentially enhance antiangiogenic activity via such inhibition [51].

It is important in studies with new biologic therapies to avoid toxicities and complications. Bevacizumab-induced hypertension and proteinuria are shared by other drugs involved in antiangiogenic therapies. Conditions contributing to more serious complications such as visceral perforations, haemoptysis and other forms of bleeding, and thromboembolism are being scrutinised to improve safety and patient selection. More recently, a "tumour flare" phenomenon has been identified following discontinuation of an antiangiogenic drug. Therefore, an understanding of the dynamic consequences of antiangiogenics is highly relevant to the safety as well as efficacy of this intervention.

Finally, in order to understand the translational endpoints that can guide early development in translational studies, we must seek to define how antiangiogenics yields the antitumour effects. The application of functional and anatomic imaging, as well as the study of interstitial pressure, has shed some light on drug effects and drug sequencing, an important issue in the implementation of future strategies.

Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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