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Review

# Mechanisms of resistance to antiangiogenesis therapy

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ABSTRACT

Angiogenesis, the formation of new blood vessels from existing vasculature, plays an essential role in tumour growth, invasion and metastasis. Vascular endothelial growth factor (VEGF) is one of the key factors responsible for its regulation. High expression of VEGF has been observed in many cancers, and is associated with worse survival. When antiangiogenic agents are used alone they typically initially cause reduction in blood flow or vascular permeability, in many types of cancer. In some cases tumour regression occurs, mainly in renal cancer. In combination with chemotherapy, progression-free survival is often prolonged, but overall survival is not. Many tumours fail to respond initially – de novo resistance. Others develop resistance over time, with progression after a few months of treatment. The mechanisms of resistance are not well understood. The theoretical benefits of VEGF inhibitors are more likely to be realised by understanding these mechanisms and modifying therapy accordingly. This article reviews current knowledge on resistance mechanisms and the therapeutic implications.



## 1. Introduction

Angiogenesis plays an essential role in tumour progression and metastasis. Tumours require persistent new blood vessels for growth and spread, and acquire these by co-option of neighbouring blood vessels, capillary sprouting from existing vessels and new vessel formation from endothelial precursor cells.[1] A balance between pro-angiogenic and anti-angiogenic factors[2,3] regulates angiogenesis. The disruption of the normal balance has been called the 'angiogenic switch' and is necessary for tumours to grow beyond a volume of about 1 $mm^3$. VEGF is one of the key factors responsible for regulation of angiogenesis.[4] Antiangiogenic agents have been recognised to alter different stages of angiogenesis,[5] and have become an attractive target for anticancer therapy. These agents are mostly effective in combination with chemotherapy for the treatment of cancer, with an exception of renal cell carcinoma, where between 30% and 40% of patients have improved progression-free survival with single agents.[6,7]

Although antiangiogenic therapy is considered as an important option for the treatment of cancer, its use is still limited because of the lack of understanding of which patients will show the benefit of these expensive agents and occurrence of resistance.[8–12] To date the only licensed angiogenesis inhibitors are bevacizumab, sorafenib, sunitinib and thalidomide.[13–16] Clinical studies have shown benefits in relapse-free survival for metastatic colorectal cancer, advanced non-small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma, metastatic breast cancer, gastrointestinal stromal tumours (GIST), and perhaps in glioblastoma,[17,18] but overall survival benefit has not yet been seen.[19]

The mechanisms of tumour escape from antiangiogenic therapy are not known, but if the clinical promise of antiangiogenic strategies is to be fully realised these compensatory

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mechanisms must be understood. This review focuses on the possible modes of resistance to antiangiogenesis therapies.

## 2. Vascular endothelial growth factor (VEGF) angiogenic signalling (Fig. 1)

VEGF stimulates quiescent endothelial cells to divide and form new blood vessels; tumour endothelial cells may divide as rapidly as every 7–10 days, as opposed to the normal time of approximately every 10 years. Binding of VEGF to its receptors begins the signalling cascade that regulates cellular events, including endothelial cell mitogenesis and migration, induction of proteinases leading to remodelling of the extracellular matrix, increased vascular permeability, maintenance and survival of newly formed blood vessels, enhanced chemotaxis and homing of bone-marrow progenitors to 'prepare' an organ for subsequent metastasis.[20,21]

The VEGF family is encoded by seven genes (VEGF-A, B, C, D, E [a viral gene], PlGF-1 and -2 (Placenta Growth Factor). These VEGF ligands specifically bind to their cognate receptors on endothelial cells. The receptors have an extracellular immunoglobulin (Ig)-like domain, a transmembrane region and an intracellular tyrosine-kinase domain. All VEGF-A isoforms bind both VEGFR-1 (FLT1) and VEGFR-2 (FLK1). Binding affinity of VEGF-A to FLT1 is higher compared to FLK1 but it induces weaker tyrosine-kinase activity in FLT1.[22] PlGF-1 and -2, and VEGF-B isoforms bind only VEGFR-1. VEGF-E binds VEGFR-2. VEGF-C and -D bind both VEGFR-2 and 3. Two co-receptor proteins in the cell membrane, neuropilin (NRP)-1 and NRP-2, interact with VEGFR proteins to increase the affinity of the latter for their ligands.[23] They differ from VEGFR proteins in not having well-defined intracellular signalling domains.[24,25]

VEGFR-1 binds VEGF-A, VEGF-B, and PlGF homodimers and is required for normal angiogenesis and haematopoiesis. VEGFR-2 binds VEGF-A, C and D homodimers and is the primary mediator of the physiological effects of VEGF-A in angiogenesis, including microvascular permeability, endothelial cell proliferation, invasion, migration, and survival. VEGFR-3 preferentially binds VEGF-C and D. Mutations of the VEGFR-3 tyrosine kinase domain are seen in human hereditary lymphoedema. VEGFR-3 expression has been correlated with transient lymphangiogenesis in wound healing and may modulate VEGFR-2 signalling in maintaining vascular integrity.[24] Higher affinity and weak tyrosine-kinase activity of VEGF-A compared to VEGFR-1 have led to the development of a model in which VEGFR-1 acts as a decoy receptor and modulates angiogenesis through its ability to sequester VEGF-A, and thereby reduces signalling through FLK1. VEGFR-1 in this way functions as a negative regulator of angiogenesis, by binding VEGF and preventing its binding to VEGFR-2. Studies suggest that dual targeting of the vasculature with antibodies to VEGF and NP1 is more effective than single-agent therapy[26] (Fig. 1).

Apart from VEGF pathway, there are many other signalling pathways, which play an important role in angiogenesis, such as angiopoietins (Ang-1 and Ang-2), Notch pathways and integrin pathways. It is beyond the scope of this review to describe all these pathways, but clearly they may provide a source for alternative growth stimuli (reviewed by Ferrara 2009),[27] when one pathway is blocked. Examples include Ang-1 binding to Tie2 tyrosine kinase receptor, which has a stabilising effect on the vasculature, whilst Ang-2 results in destabilisation.[28] Binding jagged and Delta-like ligands, such as Dll4, to Notch receptors on adjacent endothelial cells triggers angiogenic signalling. Blocking Dll4 activity results in the

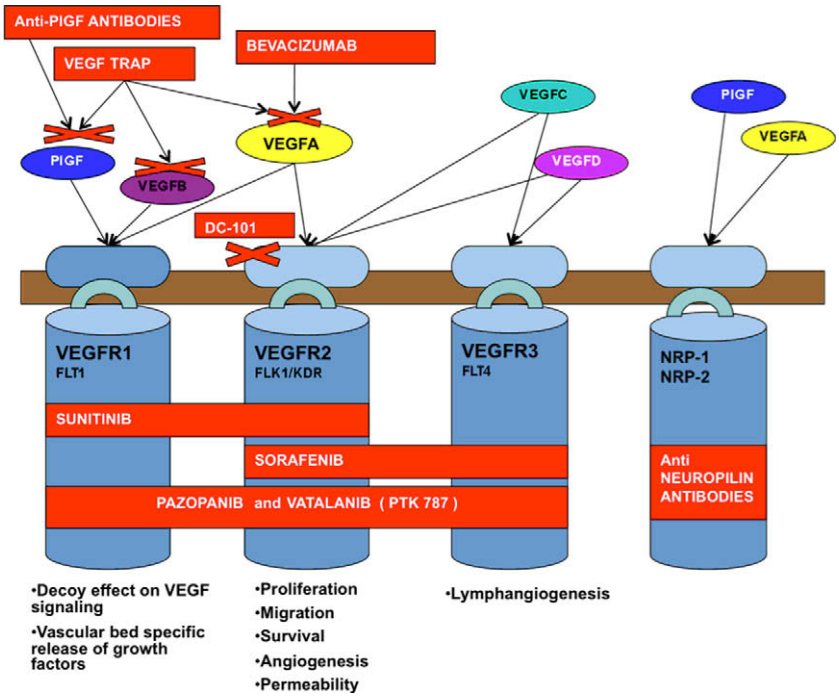


**Fig. 1 – Antiangiogenic drugs and their targets. Sunitinib blocks VEGFR-1, 2. Sorafenib blocks VEGFR-2, 3. Pazopanib blocks VEGFR-1, 2, 3. DC101 blocks extracellular part of VEGFR-2. Bevacizumab blocks VEGF-A. VEGF Trap blocks VEGF-A, VEGF-B and PlGF.**

formation of functionally abnormal neovasculature.[29] Notch signalling creates a negative feedback loop to block VEGF-A-dependent proliferation. Increased DLL4/Notch signalling results in the transcriptional inhibition of both VEGFR-2 and its co-receptor NRP-1.[30] Notch signalling favours EC survival and activates the expression of VEGFR-3, which responds to VEGF-C, to protect EC from apoptosis.[31] In addition, programmed cell death is inhibited in EC by Notch signalling through the induction of the anti-apoptotic protein BCL2.[32]

Integrins transmit signals via the extracellular matrix and affect cell survival and proliferation.[33] They are targets of both angiogenic activators and inhibitors. VEGF and bFGF are known to enhance the expression and activation of several integrins involved in angiogenesis.[34] Several endogenous angiogenic inhibitors seem to exert their function by blocking integrins. Integrin role in angiogenesis is probably through both their adhesive and signalling functions.

## 3. Mechanisms of resistance to antiangiogenic agents

### 3.1. VEGF pathways (Fig. 1)

As described above, VEGF and its receptors represent the most commonly targeted signalling pathway in angiogenesis.[35] All these signalling pathways contribute to the process of angiogenesis synergistically. Antiangiogenic drugs exert therapeutic effects by blocking certain specific receptors (Table 1), but none will fully block all the components of VEGF signalling, and so the angiogenesis signalling may continue through the others. For example sunitinib blocks signalling through VEGFR-1, VEGFR-2, FLT3, PDGFRa, b, c-Kit and c-RET, but not through VEGFR-3, NRP1, NRP2. Sorafenib blocks VEGFR-2 and VEGFR-3 but not VEGFR-1. Fig. 1 demonstrates targets for various antiangiogenic drugs.

### 3.2. Placental growth factor (PlGF)

PlGF activates pathological angiogenesis by directly stimulating endothelial cells, pericytes and smooth-muscle cells (SMCs) and indirectly by attracting macrophages and bone-marrow progenitor cells.[36] It displaces VEGF-A from FLT1 and liberates it to activate FLK1. Its binding to FLT1 also leads to intermolecular crosstalk between FLT1 and FLK1, which amplifies FLK1 signalling and consequently enhances VEGF-driven angiogenesis.[37] PlGF can also upregulate the expression of VEGF-A, fibroblast growth factor 2 (FGF2), platelet-derived growth factor- B (PDGFB), matrix metalloproteinases (MMPs) and other angiogenic factors.[38] Preclinical animal studies have shown effectiveness of anti-PlGF antibodies and combined use will be of interest with anti-VEGF antibodies.[39]

**Table 1 – Targets for antiangiogenic drugs.**

| DRUGS | TARGETS |
|---|---|
| Bevacizumab | VEGF |
| Sunitinib | VEGFR-1, VEGFR-2, FLT3, PDGFRa,b, c-kit, c-RET |
| Sorafenib | VEGFR-2, VEGFR-3, PDGFR, c-kit, FGFR1, B-raf |
| Pazopanib | VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, c-kit |
| Antibodies | VEGFR-1, VEGFR-2, Neuropilin, PDGFR |
| Vatalanib | VEGFR-1, VEGFR-2, VEGFR-3 |
| PTK 787 | |
| DC101 | VEGFR-2 |
| VEGF Trap | VEGF-A, VEGF-B, PlGF |

### 3.3. VEGF Trap

Combined blockade of several receptor agents might be more effective against VEGF signalling. An example is combination of anti-PlGF with anti-FLK1 antibodies or with sFLK1 producing a more sustained antitumour effect than either agent alone.[40] VEGF Trap is another example of inhibiting multiple targets. It binds VEGF-A, PlGF and VEGF-B.[41] VEGF Trap was shown to be effective in the management of age-related macular degeneration (AMD), a condition in which an angiogenesis play a major role.[42] But it did not inhibit the growth of tumours that are resistant to an anti-VEGF-A antibody.[43]

### 3.4. VEGF splice variants

Alternative splicing generates many VEGF isoforms. VEGF189, VEGF165, and VEGF121 are commonly overexpressed, but VEGF-A165 predominates. VEGF-A165 and VEGF-A165b are structurally identical except for the unique COOH-terminal sequence of six amino acid residues, CDKPRR in VEGF-A165 compared with SLTRKD in VEGF-A165b, encoded by alternative exons 8a and 8b.[44] VEGF-A165b binds VEGFR-2 with the same affinity as VEGF-A165, but it inhibits angiogenesis. It is possible that VEGF-A165b produced in excess would block binding of VEGF-A165 to VEGFR-2.[45] Several studies reported blockade of VEGF-A165 *in vivo* responses by VEGF-A165b, such as vascularisation in the rabbit cornea, the rat mesentery, the chicken embryo chorioallantoic membrane, and xenografts in mice.[46–49] There is a possibility that anti-VEGF antibodies, blocking VEGF-A 165b (inhibitor of angiogenesis) could produce more pro-angiogenic affects if it was a predominate isoform.

### 3.5. VEGF polymorphisms

VEGF single nucleotide polymorphisms (SNPs) are a possible factor in cancer progression, anti-VEGF therapeutic responses and susceptibly to side-effects.[50–54] Several polymorphisms have been reported within the promoter region (−2578C>A, −2489C>T, −1498C>T, and −1154G>A), 5′-UTR (−634G>C and −7C>T), and 3′-UTR (936C>T and 1612G>A). However, it is likely that only a small number of these polymorphisms and haplotypes (linearly linked SNPs) actually have a functional effect on VEGF translation.[55] The variant allele for −1154G>A and 936C>T results in lower VEGF expression, whereas the variant allele for −1498C>T and −7C>T results in increased concentrations of VEGF mRNA. Haplotype −2578A/−1154A/−634G and −1154A/−634G predicted a reduced risk of breast cancer.[56] Whilst carriers of high-risk genotypes, i.e. VEGF −1154AA/VEGF −634GG/MMP9CC, had a higher risk of prostate cancer than VEGF −1154GG/VEGF −634CC/MMP9TT.[57] Recently, the ECOG 2100 study has shown an association between the

VEGF-2578 AA genotype with superior median overall survival in paclitaxel plus bevacizumab arm in advanced breast cancer.[58] Another study has reported longer median PFS in ovarian cancer patients with VEGF C+ 936 T polymorphism when treated with cyclophosphamide and bevacizumab.[59] As angiogenesis is mainly a host-mediated event, the heterogeneity in responses with antiangiogenic therapy could be explained by host-imprinted variability. Germline SNPs in VEGF and other angiogenic genes might be inherited predictive factors of response to anti-VEGF agents.

## 4. Hypoxia

Hypoxia is one of the main factors involved in the resistance to anticancer treatment (radiation therapy and chemotherapy). It is associated with poor prognosis and more invasiveness.[60] Hypoxia inducible factor-1 (HIF-1) acts as a survival factor of cancer cells by activating transcription of genes involved in angiogenesis, glycolytic metabolism, oxygen consumption, migration and invasion.[61] In normoxia, HIF-1α is hydroxylated and targeted for ubiquitination and proteasomal degradation.[62] In hypoxic conditions it translocates to the nucleus where it dimerises with HIF-1β and binds to hypoxia responsive elements and activates transcription. Expression of HIF-1α is upregulated in many human cancers and is associated with treatment failure.[63,64]

Hypoxia and HIF-dependent responses may have an important role in resistance to antiangiogenic agents. For example, elevated CA9 (carbonic anhydrase 9, a HIF-1 target gene) and HIF-2α levels are associated with poor prognosis in malignant astrocytoma and there elevation is inversely correlated with response to bevacizumab and irinotecan.[65] Recently it has been suggested that hypoxia generated by angiogenesis inhibition triggers pathways that make tumours more aggressive and metastatic.[66,67] These results generated in pre-clinical studies have important implications for the clinic. Single agent antiangiogenic therapy-induced tumour hypoxia may result in switching to other pro-angiogenic factors, and resistance to the initial drug. VEGF blockade induced tumour hypoxia will lead to further elevation of VEGF expression in tumours.[26,68] Thus, withdrawal of a VEGF inhibitor could also lead to a rebound effect of tumour angiogenesis, yet intermittent therapy is the usual way to use low molecular weight inhibitors.

## 5. Upregulation of pro-angiogenic stromal cells

The tumour microenvironment has a complex mixture of stromal cells including fibroblasts, pericytes, mesenchymal and hematopoietic cells. They support tumour growth by several mechanisms, including direct contribution to the tumour vasculature and release of VEGF and MMP9.[12] Other pro-angiogenic growth factors secreted are: b-FGF, angiopoietin-1, hepatocyte growth factor and pro-tumourigenic cytokines.[10] Tumour-associated fibroblasts (TAFs) may play a role in resistance to antiangiogenic therapy. Xenograft studies have demonstrated that blocking only human VEGF is not sufficient to inhibit tumour growth as stromal cells, including TAFs, produce murine VEGF.[69] In a recent study, an up-regulation of PDGF-C production by TAF has been noted in tumours resistant to anti-VEGF therapy. PDGF-C was thought to be responsible for continuing tumour growth and angiogenesis and hence causing resistance to anti-VEGF therapy. Vascular disrupting agents, which cause acute hypoxia, result in accumulation of endothelial progenitor cells at the tumour margins.[70] Macrophages accumulates in hypoxic tumour areas and produce VEGF, so contributing to the tumour angiogenesis.[71] Infiltrating neutrophils are also important contributors to an angiogenic and invasive cancer phenotype.[72]

### 5.1. *Bone marrow-derived cells*

Hypoxia due to vessel regression after antiangiogenic therapy causes an increase in bone marrow-derived cells (BMDCs),[11] which can form tumour endothelium. VEGF attracts BMDCs consisting of vascular progenitors and pro-angiogenic monocytic cells[73] and immature monocytic cells including TIE2$^+$, monocytes,[74] VEGFR-1$^+$ hemangiocytes,[75,76] CD11b$^+$myeloid cells. These express cytokines, growth factors and proteases and function as vascular modulators.[20,77] In an angiogenesis study of glioblastoma multiforme (GBM), HIF-1 was found to promote angiogenesis and tumour growth by recruiting various pro-angiogenic bone marrow-derived CD45$^+$myeloid cells, including TIE2, VEGFR-1, CD11b and mature F4/80$^+$tumour-associated macrophages. Endothelial and pericyte progenitor cells were also in abundance.[74] GBM tumours lacking HIF-1 had fewer BMDCs, and were severely impaired in their angiogenic and tumour growth phenotype.[77] Up regulation of pro-angiogenic factors like FGF2 and recruitment of BMDCs by SDF1α[78] also contributed.

VEGF inhibitors induce SDF1a, PlGF, SCF, IL-6, erythropoietin, osteopontin, and other cytokines in non-tumour tissues, and can stimulate metastasis and angiogenesis in a VEGF-independent manner.[26] These cytokines recruit angiogenic bone marrow-derived endothelial cells and myeloid progenitors that promote the formation of a premetastatic niche.[76] Some of these recruited cells only express VEGFR-1 and so are resistant to those VEGF inhibitors that specifically act on other receptors (such as anti-VEGFR-2). Changes in the endothelium due to these cytokines facilitate adhesion, permeability, and egression of tumour cells from the vasculature.[79]

### 5.2. *CD11b$^+$Gr1$^+$ myeloid cells*

Tumour-associated BMD cell type CD11b$^+$Gr1$^+$ myeloid cells are considered to confer refractoriness to anti-VEGF in mouse models.[43] These cells are increased in the tumours and the peripheral blood of tumour-bearing animals. CD11b$^+$Gr1$^+$ cells produce several angiogenic factors, including Bv8, which promote tumour angiogenesis.[80] Other factors implicated in the recruitment and activation of CD11b$^+$Gr1$^+$ cells are GM-CSF, M-CSF and IL-6.[81] In a recent xenograft study Shojaei et al. reported that Bv8 and G-CSF are preferentially expressed in the refractory tumours. Both anti-Bv8 and anti-G-CSF inhibited growth of the tumours in single or combination treatments. However, only anti-G-CSF treatment resulted in a dramatic suppression in the number of circulating and tumour-associated CD11b$^+$Gr1$^+$ cells.[82]

## 6. Upregulation of pro-angiogenic pathways

### 6.1. Signalling by angiogenic factors besides VEGF

Multiple signalling pathways can be simultaneously dysregulated in the cancer cells.[83,84] Use of different angiogenesis inhibitors in combination may be more effective, e.g. in human renal cell carcinoma xenografts, the use of anti-VEGF therapy in combination with endogenous tumstatin peptide caused tumour growth delay, whilst individually these compounds yielded very little tumour response.[78] Antiangiogenic treatment targeting only those angiogenic factors that are expressed at high levels may still not be effective, as others with a low level of expression may be synergistic, e.g. PDGF-BB and FGF-2 in combination has been shown to elicit a robust angiogenic response.[85] Casanovas et al reported from a study on Rip1-Tag2 mouse model of pancreatic neuroendocrine cancer that a DC101 antibody that blocks signalling through VEGFR-2, induced tumour stasis and a decrease in vascular density initially but the tumour regrew, with an increase in tumour vasculature and active tumour angiogenesis after a month of continuous therapy. Compensatory up-regulation of several pro-angiogenic growth factors was noted. Basic FGF, angiopoietin 1 and ephrin-A1 was found responsible for resuming tumour angiogenesis growth.[86] High levels of FGF2, SDF-1 and PlGF are reported in progressive tumours treated with anti-VEGF therapy.[87]

### 6.2. Delta like ligand4 (DLL4)-Notch signalling

The Notch ligand/receptor system also plays an important role in the development of resistance to antiangiogenic therapy. Its activation leads to a more mature vasculature, whilst inhibition induces an increase in vessel density and decrease in tumour perfusion. This leads to decrease in vessel function and so increase in intra-tumour hypoxia.[88,89] Dll4 is downstream of VEGF; there is a negative feedback loop by which Dll4-mediated Notch signalling restrains the response to VEGF. Up-regulation of VEGF receptor 2 were noted following blockade of Dll4/Notch signalling in cultured human umbilical vascular endothelial cells, whereas down-regulation of VEGF receptor 2 after activation of Notch by Dll4.[30,90] Tumours with an intrinsic resistance to anti-VEGF agents appear to be sensitive to inhibition of Dll4.[91] Dll4 has been shown to be under control of HIF-1[92], emphasising the role of hypoxic pathway in mediating resistance to antiangiogenic therapy.

## 7. Pericytes and endothelial cells (EC)

Pericytes are peri-endothelial support cells of microvasculature. They are responsible for survival of the tumour blood vessels.[68,93] Pericytes play a major role in retaining the vascular function by regulating EC proliferation and providing a scaffold (along with remaining basement membrane-associated cells) for rapid revascularisation after cessation of therapy. VEGF and PDGF coordinate this process through their receptors on ECs. Pericytes release pro-angiogenic mediators, which induces neovascularisation after priming by PDGF.[94,95]

### 7.1. VEGF as a negative regulator of pericytes

PDGF-Rβ signalling in pericytes is suppressed by VEGF-mediated activation of VEGF-R2 through the assembly of a receptor complex consisting of PDGF-Rβ and VEGF-R2. VEGF-R2 inhibition prevents assembly of this receptor complex and restores angiogenesis in tissues exposed to both VEGF and PDGF. Genetic deletion of tumour cell VEGF also disrupts this complex formation and increases tumour vessel maturation. Thus VEGF and VEGF-R2 signalling act as a negative regulator of pericytes and vessel maturation, whilst promoting EC functions.[96] Greenberg et al. reported restoration of pericyte coverage and neovascularisation following VEGF-R2 inhibition, suggesting that VEGF-R2 negatively regulates this response.

In a study on the Rip1-Tag2 pancreatic islet tumour model, genetic disruptions of pericyte coverage elicited increased metastasis.[97] Sunitinib not only inhibits VEGFR and PDGFR signalling but also targets endothelial cells and their supporting pericytes.[98,99] This action can destabilise vessels, makes them more leaky and immature and facilitates intravasation of tumour cells and metastasis and so can lead to a failure of antiangiogenic therapy.[10] This behaviour of tumour cells after treatment with VEGF-targeted therapy could contribute to cancer progression after a transitory period of primary tumour growth inhibition and prolongation of progression-free survival but limiting the benefit for overall survival.[11]

## 8. Hormesis

Inhibitory drug concentrations may not be maintained during therapy. This has been evidenced in clinical trials using αvβ3/αvβ5 inhibitors. They were administered as twice-weekly infusions and the plasma drug concentrations were noted to fall to nanomolar levels between administration sessions.[100] Interestingly, some of these compounds inhibited tumour growth at high concentration but stimulated tumour growth at lower concentrations, that is a hormetic/'bell-shaped' dose-response curve was observed.[101] Reynolds et al. demonstrated that nanomolar concentrations of RGD mimetic αvβ3/αvβ5 inhibitors enhanced tumour growth and tumour angiogenesis *in vivo* by directly stimulating VEGF-mediated tumour angiogenesis and promoting VEGF-stimulated endothelial cell migration.[102] This has strong implications for clinical schedules and pharmacokinetic mechanisms of resistance.

## 9. Vascular changes and resistance

### 9.1. Co-option

It is generally accepted that tumour growth is heavily dependent on angiogenesis, but is not always a prerequisite for tumour growth because tumour cells may exploit pre-existent vasculature, a process known as vascular co-option.[103] A

xenograft study of intracranial glioblastoma model in nude rats reported tumour adaption to angiogenesis inhibition by increased infiltration and co-option of the host vasculature after treatment with an anti-VEGF antibody.[104] Leenders et al. also reported in a mouse model of brain colonisation by human melanoma cell lines that the angiogenesis could be effectively blocked by anti VEGF therapy, but in vessel-dense organs this may result in sustained tumour progression via co-option, rather than in tumour dormancy.[105] They have found that those co-opting tumours still expressed high levels of VEGF-A, excluding the possibility that the development of this phenotype was due to loss of VEGF-A expression.

### 9.2. *Sprouting and intussusception*

Sprouting angiogenesis happens with endothelial cells proliferation, migration and maturation into new vessels. In the absence of VEGF, the blood vessels split into new vessels without endothelial cells proliferation. This process is called intussusception and has been demonstrated in various tumours.[106] Anti-VEGF antibodies can stop sprouting angiogenesis but not intussusception. Clinically, accumulation of tumour blood vessels by intussusceptive vessel growth is associated with a poor outcome for various types of cancers.

## 10. p53 and resistance

The p53 tumour suppressor protein has been recognised as one of the central factors in protecting humans from cancer by different processes, including inhibition of angiogenesis.[107] Tumours with mutated p53 exhibit a significant higher vascular density compared to p53 wild-type tumours. Recently a preclinical study on glioblastoma cell lines, LN229, reported significant increase in MVD and absolute vessel number in p53 mutated low-grade astrocytomas as compared to p53 wild-type low-grade astrocytomas. Furthermore an angiogenesis protein array detected a significant increase in thrombospondin-1 (TSP-1), coagulation factor (CF) III, serpin E1 and a significant decrease of MMP-9 in wild-type p53 transfected LN229 cells.[108] The loss of p53 leads to a deficiency in TSP-1 expression, and subsequently an inability to shut off angiogenesis.[109] Isogenic p53 wild-type and mutant tumours were compared and the latter showed *in vivo* resistance to antiangiogenic therapy because of resistance to apoptosis.[110]

## 11. Role of drug transport in resistance

ABC (ATP-binding cassette) transporters are known to play a crucial role in the development of multidrug resistance (MDR). P-glycoprotein is the best-studied efflux pump. ABC transporters are expressed on hematopoietic and leukaemic stem cells and can mediate antiangiogenic drug resistance. For example, chronic myeloid leukaemia cells transduced with ABCG2 exhibited lower intracellular accumulation of imatinib and nilotinib and hence suggest a role of ABC transporters in stem cell resistance to tyrosine kinase inhibitors.[111] Thus resistance to small molecules may be mediated by other well known drug resistance mechanisms. Sunitinib can partially reverse drug resistance mediated by P-gp and completely reverse resistance mediated by ABCG2.[112] Sunitinib may affect the bioavailability of drugs coadministered with it by inhibiting the transport mediated by ABC drug transporters.

## 12. Tumour endothelial properties as a cause of drug resistance

Although controversial, endothelial cells from human tumours have been reported to have abnormal karyotypes with excessive number of centrosomes. When grown in nude mice[113], the extent of aneuploidy increased compared to normal tissue endothelial cells, indicating an inherently unstable genome and potential for resistance to antiangiogenesis therapy. Other studies have shown endothelial cells derived from tumours differ from normal tissue endothelial cells and are more resistant to therapy *in vitro*.[114]

## 13. Increased malignant progression of tumours after antiangiogenic therapy

Although VEGF-targeted drugs prolong progression-free survival of cancer patients by months, it can also cause increase in local tumour invasion and metastasis experimentally.[66,67,115] However, in spite of this, when used according to clinical schedules, they still extended survival of mice. This increased invasiveness was first described in mouse models of orthotopic glioblastoma multiforme (GBM) in which neovascularisation was blocked by genetically deleting angiogenic factors such as VEGF, HIF1 and matrix metalloproteinase 9, or inhibited pharmacologically with the VEGF inhibitor SU5416 (semaxanib) but tumours eventually became more invasive and continued to grow.[77,104,116] Clinical studies of GBM treatment with Bevacizumab reported the same results of multifocal recurrence during the course of anti-VEGF therapy.[117,118]

These findings help to explain resistance to these drugs but also raise a number of questions of how to best combat cancer with antiangiogenic drugs in future. Clinical studies using Bevacizumab as a first line treatment for metastatic breast cancer in combination with paclitaxel reported an improved progression-free survival as compared with paclitaxel alone (median, 11.8 vs. 5.9 months; hazard ratio for progression, 0.60; $P < 0.001$) and increased the objective response rate (36.9% vs. 21.2%, $P < 0.001$) but no benefit in overall survival rate (median, 26.7 vs. 25.2 months; hazard ratio, 0.88; $P = 0.16$).[119] Results showed an increase in response, but not progression time, when used as a second line in a breast cancer.[120] These results suggest that the pretreated tumours might have already activated mechanisms that could convey intrinsic resistance to subsequent antiangiogenic therapy.

## 14. Conclusions

Research into the inhibition of angiogenesis has produced several effective anticancer treatments but failed to provide

improvements in long-term survival for cancer patients. VEGF inhibitors are considered to be an effective antiangiogenic treatment but upregulation of other pro-angiogenic factors, vascular changes, genetic mutations and dysregulation in multiple signalling pathways reduce their effectiveness.

Key issues are the initial selection of patients who may show de novo resistance, then the mechanism(s) of progression in an individual patient on therapy. Combined profiling of tumours and imaging of early vascular changes, with repeat analyses on progression will yield more insight, currently peripheral markers have been of little value. It is clear from preclinical studies a much more detailed analysis is needed in the clinic, on the tumour, defining heterogeneity and mechanism of response. Combined or sequential blockade of validated resistance pathways will be an important direction. Further trials involving prospective investigation of the pathways described above are needed to optimise the available angiogenic treatments, find new ways to overcome the resistance and to find reliable markers that can predict the relapse and response to these targeted therapies.

## Conflict of interest statement

None declared.

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## REFERENCES

[1]. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;**407**:249–57.
[2]. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. *Semin Cancer Biol* 2009.
[3]. Italiano Jr JE, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and anti-angiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood* 2008;**111**:1227–33.
[4]. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol* 2002;**20**:4368–80.
[5]. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;**86**:353–64.
[6]. Schmidt C. Why do tumors become resistant to antiangiogenesis drugs? *J Natl Cancer Inst* 2009.
[7]. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 2008;**8**:579–91.
[8]. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* 2006;**3**:24–40.
[9]. Burris 3rd H, Rocha-Lima C. New therapeutic directions for advanced pancreatic cancer: targeting the epidermal growth factor and vascular endothelial growth factor pathways. *Oncologist* 2008;**13**:289–98.
[10]. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008;**8**:592–603.
[11]. Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008;**358**:2039–49.
[12]. Shojaei F, Ferrara N. Role of the microenvironment in tumor growth and in refractoriness/resistance to anti-angiogenic therapies. *Drug Resist Updat* 2008;**11**:219–30.
[13]. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006;**24**:16–24.
[14]. Escudier BSC, Eisen T, et al. Randomized phase III trial of Raf kinase and VEGFR inhibitor in patients with advanced renal cell cancer. *J Clin Oncol* 2005;**23**:15–6.
[15]. Smith JK, Mamoon NM, Duhe RJ. Emerging roles of targeted small molecule protein–tyrosine kinase inhibitors in cancer therapy. *Oncol Res* 2004;**14**:175–225.
[16]. Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. *Biochem Biophys Res Commun* 2005;**333**:328–35.
[17]. Ellis LM, Hicklin DJ. Pathways mediating resistance to vascular endothelial growth factor-targeted therapy. *Clin Cancer Res* 2008;**14**:6371–5.
[18]. Shojaei F, Ferrara N. Antiangiogenic therapy for cancer: an update. *Cancer J* 2007;**13**:345–8.
[19]. Miller KD. E2100: a phase III trial of paclitaxel versus paclitaxel/bevacizumab for metastatic breast cancer. *Clin Breast Cancer* 2003;**3**:421–2.
[20]. Grunewald M, Avraham I, Dor Y, et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006;**124**:175–89.
[21]. Zwaans BM, Bielenberg DR. Potential therapeutic strategies for lymphatic metastasis. *Microvasc Res* 2007;**74**:145–58.
[22]. Fischer C, Mazzone M, Jonckx B, Carmeliet P. FLT1 and its ligands VEGFB and PlGF: drug targets for anti-angiogenic therapy? *Nat Rev Cancer* 2008;**8**:942–56.
[23]. Neufeld G, Kessler O, Herzog Y. The interaction of Neuropilin-1 and Neuropilin-2 with tyrosine-kinase receptors for VEGF. *Adv Exp Med Biol* 2002;**515**:81–90.
[24]. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 2005;**23**:1011–27.
[25]. Shibuya M. Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. Angiogenesis 2006;9:225–30 [discussion 31].
[26]. Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. *Proc Natl Acad Sci USA* 2007;**104**:17069–74.
[27]. Ferrara N. Pathways mediating VEGF-independent tumor angiogenesis. Cytokine Growth Factor Rev 2009.
[28]. Oliner J, Min H, Leal J, et al. Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. *Cancer Cell* 2004;**6**:507–16.
[29]. Noguera-Troise I, Daly C, Papadopoulos NJ, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature* 2006;**444**:1032–7.
[30]. Williams CK, Li JL, Murga M, Harris AL, Tosato G. Up-regulation of the Notch ligand Delta-like 4 inhibits VEGF-induced endothelial cell function. *Blood* 2006;**107**:931–9.

[31]. Shawber CJ, Funahashi Y, Francisco E, et al. Notch alters VEGF responsiveness in human and murine endothelial cells by direct regulation of VEGFR-3 expression. *J Clin Invest* 2007;**117**:3369–82.
[32]. MacKenzie F, Duriez P, Wong F, Noseda M, Karsan A. Notch4 inhibits endothelial apoptosis via RBP-Jkappa-dependent and -independent pathways. *J Biol Chem* 2004;**279**:11657–63.
[33]. Guo W, Giancotti FG. Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol* 2004;**5**:816–26.
[34]. Byzova TV, Goldman CK, Pampori N, et al. A mechanism for modulation of cellular responses to VEGF: activation of the integrins. *Mol Cell* 2000;**6**:851–60.
[35]. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;**9**:669–76.
[36]. Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. *Cancer Res* 2002;**62**:2749–52.
[37]. Autiero M, Waltenberger J, Communi D, et al. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med* 2003;**9**:936–43.
[38]. Marcellini M, De Luca N, Riccioni T, et al. Increased melanoma growth and metastasis spreading in mice overexpressing placenta growth factor. *Am J Pathol* 2006;**169**:643–54.
[39]. Taylor AP, Goldenberg DM. Role of placenta growth factor in malignancy and evidence that an antagonistic PlGF/Flt-1 peptide inhibits the growth and metastasis of human breast cancer xenografts. *Mol Cancer Ther* 2007;**6**:524–31.
[40]. Fischer C, Jonckx B, Mazzone M, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* 2007;**131**:463–75.
[41]. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci USA* 2002;**99**:11393–8.
[42]. Nguyen QD, Shah SM, Browning DJ, et al. A phase I study of intravitreal vascular endothelial growth factor trap-eye in patients with neovascular age-related macular degeneration. *Ophthalmology* 2009;**116**:2141–8. e1.
[43]. Shojaei F, Wu X, Malik AK, et al. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol* 2007;**25**:911–20.
[44]. Kawamura H, Li X, Harper SJ, Bates DO, Claesson-Welsh L. Vascular endothelial growth factor (VEGF)-A165b is a weak in vitro agonist for VEGF receptor-2 due to lack of coreceptor binding and deficient regulation of kinase activity. *Cancer Res* 2008;**68**:4683–92.
[45]. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling – in control of vascular function. *Nat Rev Mol Cell Biol* 2006;**7**:359–71.
[46]. Varey AH, Rennel ES, Qiu Y, et al. VEGF 165b, an antiangiogenic VEGF-A isoform, binds and inhibits bevacizumab treatment in experimental colorectal carcinoma: balance of pro- and anti-angiogenic VEGF-A isoforms has implications for therapy. *Br J Cancer* 2008;**98**:1366–79.
[47]. Rennel E, Waine E, Guan H, et al. The endogenous anti-angiogenic VEGF isoform, VEGF165b inhibits human tumour growth in mice. *Br J Cancer* 2008;**98**:1250–7.
[48]. Bates DO, Cui TG, Doughty JM, et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res* 2002;**62**:4123–31.
[49]. Cebe Suarez S, Pieren M, Cariolato L, et al. A VEGF-A splice variant defective for heparan sulfate and neuropilin-1 binding shows attenuated signaling through VEGFR-2. *Cell Mol Life Sci* 2006;**63**:2067–77.
[50]. Jain RK, Duda DG, Willett CG, et al. Biomarkers of response and resistance to antiangiogenic therapy. *Nat Rev Clin Oncol* 2009;**6**:327–38.
[51]. Masago K, Fujita S, Kim YH, et al. Effect of vascular endothelial growth factor polymorphisms on survival in advanced-stage non-small-cell lung cancer. *Cancer Sci* 2009;**100**:1917–22.
[52]. Guan X, Zhao H, Niu J, Tang D, Ajani JA, Wei Q. The VEGF –634G>C promoter polymorphism is associated with risk of gastric cancer. *BMC Gastroenterol* 2009;**9**:77.
[53]. Smerdel MP, Waldstrom M, Brandslund I, Steffensen KD, Andersen RF, Jakobsen A. Prognostic importance of vascular endothelial growth factor-A expression and vascular endothelial growth factor polymorphisms in epithelial ovarian cancer. *Int J Gynecol Cancer* 2009;**19**:578–84.
[54]. Schneider BP, Radovich M, Sledge GW, et al. Association of polymorphisms of angiogenesis genes with breast cancer. *Breast Cancer Res Treat* 2008;**111**:157–63.
[55]. Pander J, Gelderblom H, Guchelaar HJ. Pharmacogenetics of EGFR and VEGF inhibition. *Drug Discov Today* 2007;**12**:1054–60.
[56]. Kataoka N, Cai Q, Wen W, et al. Population-based case-control study of VEGF gene polymorphisms and breast cancer risk among Chinese women. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:1148–52.
[57]. Sfar S, Saad H, Mosbah F, Chouchane L. Combined effects of the angiogenic genes polymorphisms on prostate cancer susceptibility and aggressiveness. *Mol Biol Rep* 2009;**36**:37–45.
[58]. Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. *J Clin Oncol* 2008;**26**:4672–8.
[59]. Schultheis AM, Lurje G, Rhodes KE, et al. Polymorphisms and clinical outcome in recurrent ovarian cancer treated with cyclophosphamide and bevacizumab. *Clin Cancer Res* 2008;**14**:7554–63.
[60]. Harris AL. Hypoxia – a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002;**2**:38–47.
[61]. Semenza GL. Hypoxia and cancer. *Cancer Metastasis Rev* 2007;**26**:223–4.
[62]. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 2006;**441**:437–43.
[63]. Koukourakis MI, Bentzen SM, Giatromanolaki A, et al. Endogenous markers of two separate hypoxia response pathways (hypoxia inducible factor 2 alpha and carbonic anhydrase 9) are associated with radiotherapy failure in head and neck cancer patients recruited in the CHART randomized trial. *J Clin Oncol* 2006;**24**:727–35.
[64]. Bos R, van der Groep P, Greijer AE, et al. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer* 2003;**97**:1573–81.
[65]. Sathornsumetee S, Cao Y, Marcello JE, et al. Tumor angiogenic and hypoxic profiles predict radiographic response and survival in malignant astrocytoma patients treated with bevacizumab and irinotecan. *J Clin Oncol* 2008;**26**:271–8.
[66]. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell* 2009;**15**:232–9.
[67]. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to

increased local invasion and distant metastasis. *Cancer Cell* 2009;**15**:220–31.
[68]. Mancuso MR, Davis R, Norberg SM, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest* 2006;**116**:2610–21.
[69]. Liang WC, Wu X, Peale FV, et al. Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF. *J Biol Chem* 2006;**281**:951–61.
[70]. Shaked Y, Ciarrocchi A, Franco M, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. *Science* 2006;**313**:1785–7.
[71]. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006;**124**:263–6.
[72]. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci USA* 2006;**103**:12493–8.
[73]. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009;**9**:239–52.
[74]. De Palma M, Venneri MA, Galli R, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005;**8**:211–26.
[75]. Hattori K, Heissig B, Wu Y, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med* 2002;**8**:841–9.
[76]. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 2005;**438**:820–7.
[77]. Du R, Lu KV, Petritsch C, et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008;**13**:206–20.
[78]. Eikesdal HP, Sugimoto H, Birrane G, et al. Identification of amino acids essential for the antiangiogenic activity of tumstatin and its use in combination antitumor activity. *Proc Natl Acad Sci USA* 2008;**105**:15040–5.
[79]. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell* 2006;**127**:679–95.
[80]. Shojaei F, Singh M, Thompson JD, Ferrara N. Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proc Natl Acad Sci USA* 2008;**105**:2640–5.
[81]. Marigo I, Dolcetti L, Serafini P, Zanovello P, Bronte V. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol Rev* 2008;**222**:162–79.
[82]. Shojaei F, Wu X, Qu X, et al. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci USA* 2009;**106**:6742–7.
[83]. Pham NA, Schwock J, Iakovlev V, Pond G, Hedley DW, Tsao MS. Immunohistochemical analysis of changes in signaling pathway activation downstream of growth factor receptors in pancreatic duct cell carcinogenesis. *BMC Cancer* 2008;**8**:43.
[84]. Faivre S, Djelloul S, Raymond E. New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. *Semin Oncol* 2006;**33**:407–20.
[85]. Cao R, Brakenhielm E, Pawliuk R, et al. Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nat Med* 2003;**9**:604–13.
[86]. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* 2005;**8**:299–309.
[87]. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 2007;**11**:83–95.
[88]. Sainson RC, Harris AL. Regulation of angiogenesis by homotypic and heterotypic notch signalling in endothelial cells and pericytes: from basic research to potential therapies. *Angiogenesis* 2008;**11**:41–51.
[89]. Thurston G, Noguera-Troise I, Yancopoulos GD. The delta paradox: DLL4 blockade leads to more tumour vessels but less tumour growth. *Nat Rev Cancer* 2007;**7**:327–31.
[90]. Ridgway J, Zhang G, Wu Y, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature* 2006;**444**:1083–7.
[91]. Yan M, Plowman GD. Delta-like 4/Notch signaling and its therapeutic implications. *Clin Cancer Res* 2007;**13**:7243–6.
[92]. Diez H, Fischer A, Winkler A, et al. Hypoxia-mediated activation of Dll4-Notch-Hey2 signaling in endothelial progenitor cells and adoption of arterial cell fate. *Exp Cell Res* 2007;**313**:1–9.
[93]. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005;**307**:58–62.
[94]. Pietras K, Pahler J, Bergers G, Hanahan D. Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. *PLoS Med* 2008;**5**:e19.
[95]. Jechlinger M, Sommer A, Moriggl R, et al. Autocrine PDGFR signaling promotes mammary cancer metastasis. *J Clin Invest* 2006;**116**:1561–70.
[96]. Greenberg JI, Shields DJ, Barillas SG, et al. A role for VEGF as a negative regulator of pericyte function and vessel maturation. *Nature* 2008;**456**:809–13.
[97]. Xian X, Hakansson J, Stahlberg A, et al. Pericytes limit tumor cell metastasis. *J Clin Invest* 2006;**116**:642–51.
[98]. Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J Clin Oncol* 2005;**23**:939–52.
[99]. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 2003;**111**:1287–95.
[100]. Hariharan S, Gustafson D, Holden S, et al. Assessment of the biological and pharmacological effects of the alpha nu beta3 and alpha nu beta5 integrin receptor antagonist, cilengitide (EMD 121974), in patients with advanced solid tumors. *Ann Oncol* 2007;**18**:1400–7.
[101]. Calabrese EJ. Cancer biology and hormesis: human tumor cell lines commonly display hormetic (biphasic) dose responses. *Crit Rev Toxicol* 2005;**35**:463–582.
[102]. Reynolds AR, Hart IR, Watson AR, et al. Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. *Nat Med* 2009;**15**:392–400.
[103]. Dome B, Paku S, Somlai B, Timar J. Vascularization of cutaneous melanoma involves vessel co-option and has clinical significance. *J Pathol* 2002;**197**:355–62.
[104]. Rubenstein JL, Kim J, Ozawa T, et al. Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia* 2000;**2**:306–14.

[105]. Leenders WP, Kusters B, Verrijp K, et al. Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option. *Clin Cancer Res* 2004;**10**:6222–30.
[106]. Hillen F, Griffioen AW. Tumour vascularization: sprouting angiogenesis and beyond. *Cancer Metastasis Rev* 2007;**26**:489–502.
[107]. Teodoro JG, Evans SK, Green MR. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J Mol Med* 2007;**85**:1175–86.
[108]. Gaiser T, Becker MR, Meyer J, Habel A, Siegelin MD. P53-mediated inhibition of angiogenesis in diffuse low-grade astrocytomas. *Neurochem Int* 2009;**54**:458–63.
[109]. Giuriato S, Ryeom S, Fan AC, et al. Sustained regression of tumors upon MYC inactivation requires p53 or thrombospondin-1 to reverse the angiogenic switch. *Proc Natl Acad Sci USA* 2006;**103**:16266–71.
[110]. Yu JL, Rak JW, Coomber BL, Hicklin DJ, Kerbel RS. Effect of p53 status on tumor response to antiangiogenic therapy. *Science* 2002;**295**:1526–8.
[111]. Brendel C, Scharenberg C, Dohse M, et al. Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. *Leukemia* 2007;**21**:1267–75.
[112]. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. *Drug Metab Dispos* 2009;**37**:359–65.
[113]. Hida K, Hida Y, Amin DN, et al. Tumor-associated endothelial cells with cytogenetic abnormalities. *Cancer Res* 2004;**64**:8249–55.
[114]. Xiong YQ, Sun HC, Zhang W, et al. Human hepatocellular carcinoma tumor-derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells. *Clin Cancer Res* 2009;**15**:4838–46.
[115]. Ebos JM, Lee CR, Kerbel RS. Tumor and host-mediated pathways of resistance and disease progression in response to antiangiogenic therapy. *Clin Cancer Res* 2009;**15**:5020–5.
[116]. Blouw B, Song H, Tihan T, et al. The hypoxic response of tumors is dependent on their microenvironment. *Cancer Cell* 2003;**4**:133–46.
[117]. Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology* 2008;**70**:779–87.
[118]. Narayana A, Kelly P, Golfinos J, et al. Antiangiogenic therapy using bevacizumab in recurrent high-grade glioma: impact on local control and patient survival. *J Neurosurg* 2009;**110**:173–80.
[119]. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007;**357**:2666–76.
[120]. 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.