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Controlling escape from angiogenesis inhibitors

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Abstract

Selective inhibition of vascular endothelial growth factor (VEGF) increases the efficacy of chemotherapy and has beneficial effects on multiple advanced cancers, but response is often limited and the disease eventually progresses. Changes in the tumour microenvironment — hypoxia among them — that result from vascular pruning, suppressed angiogenesis and other consequences of VEGF inhibition can promote escape and tumour progression. New therapeutic approaches that target pathways that are involved in the escape mechanisms add the benefits of blocking tumour progression to those of slowing tumour growth by inhibiting angiogenesis.

Most of the angiogenesis inhibitors that are currently used for the treatment of cancer achieve their effects by blocking vascular endothelial growth factor (VEGF), a cytokine that promotes blood vessel growth and survival[1-3]. Inhibitors of VEGF not only stop angiogenesis and destroy part of the tumour vasculature, but they also normalize some tumour vessels[3-5].

Through rapid and robust effects on the tumour vasculature, angiogenesis inhibitors slow the growth of many primary tumours and metastases, and selective VEGF blockade increases the efficacy of certain types increases the efficacy of certain types of chemotherapy[6,7]. Clinical benefit is reflected by lengthening of progression-free survival in advanced colorectal, lung, renal, pancreatic neuroendocrine and ovarian cancer, and by longer overall survival in metastatic colorectal and renal cancer. Although the clinical benefit is not usually sustained, and is small or absent in some types of cancer, these limitations are not unique to angiogenesis inhibitors. Many cancer therapies have modest effects on overall survival. Improved overall survival was found in only 12% of 73 randomized Phase III trials of bevacizumab, trastuzumab and other targeted therapies, as well as a range of chemotherapeutic agents for metastatic breast cancer over the past 30 years[8].

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Competing interests statement
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Experience shows that most advanced cancers can escape from therapy. When a VEGF inhibitor is combined with chemotherapy or radiation, escape can be from one or both. Preclinical studies raise the additional possibility that VEGF signalling inhibitors that slow tumour growth can also promote tumour escape and progression[9].

Multiple strategies for preventing escape are being developed and evaluated in the laboratory and in clinical trials. This Opinion article explores the rationale, evidence and potential strategies for treating advanced cancers by targeting angiogenesis concurrently with mechanisms of tumour progression.

Benefits and limitations

Hundreds of thousands of patients worldwide are being treated with angiogenesis inhibitors for cancer. Angiogenesis inhibitors have been approved for a wide range of cancer types, including hepatocellular carcinoma and renal cell carcinoma that respond poorly to other agents. Bevacizumab, a function-blocking antibody to VEGF, is approved for use with chemotherapy to treat metastatic colorectal cancer and non-small-cell lung cancer; with interferon-α to treat metastatic renal cell cancer; and as a single agent for recurrent glioblastoma (see the Genentech website; see Further information) (TABLE 1). Bevacizumab with chemotherapy significantly prolongs overall survival as first-line treatment for metastatic colorectal cancer[10]. Ziv-aflibercept, a recombinant fusion protein that as a decoy VEGF receptor (VEGFR) binds VEGFA, VEGFB and placental growth factor (PLGF; also known as PGF), is approved for use with chemotherapy to treat metastatic colorectal cancer (see the Regeneron website; see Further information) (TABLE 1).

Multiple other angiogenesis inhibitors that are approved for cancer therapy (such as sorafenib, sunitinib, axitinib, pazopanib and vandetanib) inhibit VEGF signalling by targeting receptor tyrosine kinases[11,12] or reduce VEGF production by blocking the mTOR pathway (for example, everolimus and temsirolimus)[13-15] (TABLE 1). Rapamycin analogues that block both mTOR complex 1 (mTORC1) and mTORC2 — OSI-027 being an example[16] — have a more potent antiangiogenic action in preclinical models than agents that block only mTORC1 (REFS 15,17).

The responses to VEGF inhibition that are found in metastatic colorectal or lung cancer are not matched in metastatic breast cancer, for which the addition of bevacizumab to chemotherapy extends progression-free survival by a few months but does not increase overall survival[18-22]. However, although the patient population as a whole has a limited benefit from this treatment, some patients have striking responses and live significantly longer. These exceptions highlight the importance of finding biomarkers that predict response[23-29].

The search for biomarkers (BOX 1) is providing insight not only into approaches for identifying patients who will respond favourably but also into mechanisms of escape in patients who respond initially but then progress during treatment[27,30]. As an example, a single nucleotide polymorphism (SNP) in the tyrosine kinase domain of the *VEGFR1* gene in genomic DNA was found to be significantly correlated with progression-free survival and overall survival in bevacizumab-treated patients with metastatic pancreatic cancer in the Avastin and Tarceva (AViTA) trial[29]. Bevacizumab-treated patients with the AA genotype — but not placebo-treated patients with this genotype — lived longer than those with AC or CC genotypes, and also longer than the entire cohort of bevacizumab-treated patients undivided by genotype.

Angiogenesis inhibitors are approved for cancer therapy in many countries. The approved indications are generally the same but can differ in some cases (TABLE 1). Bevacizumab has received approval for use in the treatment of metastatic breast cancer and metastatic ovarian cancer by the European Medicines Agency (EMA) but not by the US Food and Drug Administration (FDA), and for recurrent glioblastoma by the FDA but not by the EMA. Regulatory agencies can have different views of whether the beneficial effects of angiogenesis inhibitors are sufficient in some types of cancer to balance the risk and expense. For example, the EMA did not follow the recent FDA decision to revoke the approval of bevacizumab plus paclitaxel for metastatic breast cancer.

Overcoming resistance

Disease progression, as reflected by tumour growth and metastasis during treatment with inhibitors of VEGF signalling, is attributed to multiple interacting mechanisms (BOX 2). Among them are compensatory actions of angiogenic growth factors that are not blocked by inhibitors of VEGF signalling, blood flow alterations owing to tumour-vessel pruning and normalization, co-option of normal peritumoural blood vessels, exaggeration of intratumoural hypoxia, activation of pathways that favour epithelial–mesenchymal transition (EMT), promotion of tumour invasiveness, suppression of immune surveillance, induction of tolerance and activation of cancer stem cells[9,25,30,31].

Other proposed mechanisms that contribute to resistance include changes in the dominant VEGF isoform ($VEGF_{121}$, $VEGF_{165}$ or $VEGF_{189}$) or in neuropilin 1 as a VEGFR co-receptor[28], and loss of endothelial cell VEGF dependence from downregulation of VEGFR2 (REFS 32,33). For small-molecule receptor tyrosine kinase inhibitors, additional mechanisms include under-dosing owing to increasingly rapid clearance of the inhibitor[34], and inhibitor inactivation by uptake and lysosomal sequestration in tumour cells[35].

Disease progression during treatment with bevacizumab or ziv-aflibercept paired with chemotherapy does not necessarily mean that the inhibitor has lost efficacy. The resistance could be to the chemotherapy[36]. Evidence of better overall survival in metastatic colorectal cancer, when bevacizumab is continued beyond progression in the presence of diverse types of chemotherapy, reflects the continued involvement of VEGF[37,38].

Preventing, decreasing or reversing resistance are major unmet needs in cancer therapy and are necessary steps towards reducing morbidity and mortality[39]. This is a serious challenge because of the complex underlying biology and patient diversity. Fortunately, advances in understanding the effects of angiogenesis inhibitors on blood vessels and tumour cells and the mechanisms of tumour invasion and metastasis have led to new treatment strategies. One approach is to target tumour angiogenesis and progression together (FIG. 1).

Contribution of chemotherapy

Chemotherapy used together with angiogenesis inhibitors can contribute to resistance and can potentially be used to overcome resistance[36]. Chemotherapy and anti-VEGF therapy have complex — and not simply additive — actions when combined. The value of using bevacizumab with chemotherapy is well documented[40], but its benefit depends on the type, dose and the treatment schedule of the chemotherapeutic agent or drug combination, as well as on the tumour type[40,41]. Ziv-aflibercept is also more effective when combined with chemotherapy[42].

Box 1

Biomarkers to predict response to angiogenesis inhibitors

Functional imaging

Although no biomarker currently available is uniformly predictive of clinical response to inhibition of vascular endothelial growth factor (VEGF) signalling, changes in tumour vascular perfusion and leakage, as surrogate indices of bevacizumab efficacy, can be monitored by dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI)[174], and changes in proliferation, metabolism and hypoxia can be assessed by positron emission tomography (PET)[175-177].

Hypertension

Treatment-associated hypertension, which results from the suppression of VEGF-mediated vasodilatation, is a surrogate marker of VEGF signalling inhibition that is predictive of survival benefit in some trials but not in others[27,178-180].

Circulating proteins

Baseline plasma VEGF concentration is higher in many patients who respond to bevacizumab[28]. Plasma levels of short VEGF isoforms, $VEGF_{110}$ and $VEGF_{121}$, can be particularly informative[28]. Baseline plasma levels or treatment-induced changes in placental growth factor (PLGF), soluble VEGF receptor 2 (VEGFR2) and multiple other factors can predict response or signal progression of some tumours[73,74,181-185].

Circulating cells

The relationship of circulating endothelial cells or tumour cells to therapeutic response has not been consistent in clinical trials[74,186-188]. One challenge is identifying small numbers of cells in the blood, but more sensitive methods are being developed, and mechanistic insights are coming from preclinical studies[171].

Polymorphisms

Single nucleotide polymorphisms (SNPs) in genes that are relevant to VEGF signalling can be predictive of response to bevacizumab in some cancers[29].

Tumour biomarkers

Tumour vascularity, VEGF pathway components, and markers of tumour cells, endothelial cells and inflammatory cells can help in assessing the response to inhibitors of VEGF signalling. One dose of bevacizumab reduces CD31-positive tumour vessels and DCE-MRI indices of tumour vascularity and leakiness, and increases tumour cell apoptosis in inflammatory breast cancer[189,190]. Partial responses to bevacizumab plus chemotherapy correlate with the abundance of CD31-positive tumour vessels at baseline[190], and overall survival correlates with the baseline presence of tumour cells that are p53-negative and that have low apoptosis rates[191]. Tumour neuropilin 1 immunoreactivity has prognostic value in gastric cancer[28], and plasma levels of intercellular adhesion molecule 1 (ICAM1) correlate with clinical outcome in non-small-cell lung cancer[23].

Outcome is better when bevacizumab is paired with uninterrupted, low-dose (metronomic) chemotherapy, both in preclinical models and in some clinical settings[40,43-45]. The underlying mechanism of the added benefit is currently under debate, but metronomic administration of a topoisomerase I (TOP1) inhibitor or anthracycline chemotherapy as a

single agent inhibits hypoxia-inducible factor 1α (HIF1α)[46,47]. HIF1α blockade could offset the hypoxic effects of vascular pruning when paired with an angiogenesis inhibitor[48].

Unlike bevacizumab and ziv-aflibercept, small-molecule tyrosine kinase inhibitors of angiogenesis are not so effectively paired with chemotherapy. Most clinical trials of sunitinib, axitinib or sorafenib have found dose-limiting toxicities or no further benefit when the agent was combined with chemotherapy[49-54].

Inhibition of multiple angiogenic factors

Compensatory actions of multiple growth factors can contribute to escape by promoting angiogenesis in the presence of inhibitors that block VEGF signalling. Angiopoietins, angiopoietin 1 (ANG1) and ANG2, PLGF, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) family members are among those linked to escape from the effects of inhibition of VEGF signalling[55-60]. VEGFC and VEGFD, which activate signalling of VEGFR3 and VEGFR2, have been implicated in resistance, and the inhibition of both pathways together has been proposed as a solution[61-63]. In addition to factors that drive angiogenesis directly[64-66], some released substances function by recruiting myeloid cells and macrophages that release angiogenic and other factors[67]. Inhibition of FGF receptor signalling or ANG2 can increase the efficacy of VEGF signalling inhibitors in preclinical tumour models[55,68].

Function-blocking antibodies to PLGF reduce tumour angiogenesis, growth and metastasis, and can render some preclinical tumour models more responsive to inhibitors of VEGF signalling[69,70]. Other evidence raises questions about the anti-angiogenic potency of PLGF-specific antibodies in tumours but supports the anti-metastatic effects of PLGF blockade[71]. Inhibition of PLGF does not seem to exaggerate intratumoural hypoxia.

Plasma PLGF levels increase in patients with colorectal cancer, renal cell cancer or glioblastoma after treatment with an inhibitor of VEGF signalling[72-74]. The fusion protein sFLT01, which sequesters both VEGF and PLGF, slows tumour growth and prolongs survival in mice, but circulating PLGF levels remain elevated, raising the question of whether tumour progression could eventually be promoted[75]. The antibody RO5323441 against human PLGF in combination with bevacizumab for recurrent glioblastoma or hepatocellular carcinoma had an acceptable tolerability in an early clinical trial, but it is unclear whether PLGF blockade adds to the benefit of blocking VEGF signalling (TABLE 2). Whether an anti-PLGF strategy is an effective treatment option for cancers that are refractory to VEGF inhibition is still to be determined.

Inhibition of PLGF, ANG2 or FGF along with VEGF could overcome some aspects of resistance to VEGF blockade, but the diversity of other growth factors that can drive angiogenesis and promote escape raises questions about the durability of this approach.

Destabilization of resistant tumour blood vessels

Tumour vessels that do not regress after inhibition of VEGF signalling tend to have more normal endothelial cells, more complete and tighter pericyte coverage, and less leakiness[4,76]. This ‘normalization’ is considered by some to be therapeutically beneficial because more efficient vessels and lower tumour interstitial fluid pressure owing to less leakage could improve drug delivery[77-82]. Vessels with normalized endothelial cells could also be less vulnerable to tumour cell intravasation[30].

Some clinical studies have been interpreted as indicating that vessel normalization after treatment with bevacizumab can improve the efficacy of radiation therapy[83], perhaps by improving tumour oxygenation and radiosensitivity. An alternative mechanism, whereby

radiotherapy sensitizes endothelial cells to bevacizumab, has been suggested by preclinical studies showing that bevacizumab causes greater slowing of tumour growth when preceded by radiation than when followed by radiation[84]. Neoadjuvant (pre-surgery) bevacizumab sequenced with chemotherapy plus radiation followed by adjuvant (post-surgery) chemotherapy has shown favourable results in a trial of locally invasive rectal cancer[85]. These promising findings await confirmation by randomized prospective trials that determine long-term outcomes.

Vessel normalization also has potential downsides. Stabilized tumour vessels with tight pericyte coverage can resist regression and support tumour growth with little or no angiogenesis[86,87]. Reduced tumour vascularity and tighter endothelial barrier function after inhibition of VEGF signalling impairs the delivery of some agents[88-91]. The measurement of radiolabelled water and docetaxel in ten patients with non-small-cell lung cancer by positron emission tomography (PET) revealed significant reductions in both tumour perfusion and tumour drug delivery at 5 hours after bevacizumab infusion[90]. Systemic exposure to docetaxel increased owing to slower plasma clearance despite the decrease in tumour accumulation that persisted for at least 4 days.

Box 2

Putative mechanisms of resistance to angiogenesis inhibitors

- Actions of angiogenic growth factors other than vascular endothelial growth factor (VEGF)
- Blood flow alterations owing to vessel pruning and normalization
- Co-option of normal peritumoural blood vessels
- Exaggeration of intratumoural hypoxia
- Activation of pathways that favour epithelial-mesenchymal transition (EMT)
- Promotion of tumour invasiveness
- Suppression of immune surveillance and induction of tolerance
- Activation of cancer stem cells
- Changes in dominant VEGF isoform $VEGF_{121}$, $VEGF_{165}$ or $VEGF_{189}$
- Changes in neuropilin 1 as a co-receptor of VEGF receptor (VEGFR)
- Loss of endothelial cell VEGF dependence resulting from VEGFR2 downregulation
- Under-dosing owing to more rapid clearance of small-molecule tyrosine kinase inhibitors
- Tyrosine kinase inhibitor inactivation by uptake and lysosomal sequestration in tumour cells

Co-option of peritumoural blood vessels by invading tumour cells, which is a common feature of glioblastoma[92,93] and lung cancer[94,95], enables tumour growth without angiogenesis by exploiting existing normal vasculature. Although this process has not been studied in detail, co-opted blood vessels seem to be less sensitive than tumour vessels to inhibition of VEGF signalling[96].

Therapeutic destabilization ('abnormalization') of tumour vessels is a potential approach for overcoming resistance to VEGF inhibition. The inhibition of Notch signalling by blocking delta-like protein 4 (DLL4) stimulates the growth of abundant vascular structures but slows tumour growth in preclinical models because the vessels that are derived from endothelial hypersprouting are poorly functional and intratumoural hypoxia is increased[97-99]. The abnormal vasculature is more responsive to inhibition of VEGF signalling, and tumour growth is slowed even in some models that are usually unresponsive to VEGF inhibition[98]. Broad inhibition of Notch signalling by the γ-secretase inhibitor dibenzazepine (DBZ) also increases the efficacy of bevacizumab in preclinical models[100].

Clinical studies of DLL4 inhibitors such as REGN421 should determine whether this strategy can overcome resistance to inhibitors of VEGF signalling. However, a report of vascular neoplasms and other pathologies in mice, rats and monkeys after administration of a function-blocking DLL4 antibody, soluble DLL4 fusion protein or DBZ[101] has slowed the development of DLL4 inhibitors.

High levels of DLL4 are correlated with resistance to inhibitors of VEGF signalling in some tumour types. Patients with breast cancer who are treated with capecitabine plus bevacizumab and who have tumours with little or no expression of DLL4 have significantly longer progression-free survival than those treated with capecitabine alone[102]. This benefit of bevacizumab is not found in similarly treated patients when the tumours have high DLL4 expression[102].

Inhibition of immune cell recruitment.

The recruitment of bone marrow-derived myeloid cells and other immune cells that produce angiogenic factors and that contribute to the suppression of immune surveillance can accompany escape from inhibition of VEGF signalling in preclinical tumour models[31,103-107]. Tumour-infiltrating immune cells are an important source of matrix metalloproteinases that degrade the extracellular matrix, disrupt cell–matrix contacts and promote tumour cell invasion[108,109]. These proteases also release growth factors from the matrix and activate growth factors secreted as propeptides.

Some tumours resistant to inhibitors of VEGF signalling secrete cytokines that recruit myeloid cells and other cells that promote angiogenesis and immune tolerance[110]. Among these are stromal cell-derived factor 1 (SDF1; also known as CXCL12), colony-stimulating factors (CSFs) M-CSF, G-CSF and GM-CSF, BV8 (also known as prokineticin 2), interleukin-8 (IL-8) and C-C motif chemokine 28 (CCL28) (REFS 111-114). Cancer-associated fibroblasts (CAFs) are another source of SDF1 and angiogenic factors[109,115-117].

Plasma levels of SDF1 correlate with metastasis in bevacizumab-treated patients with advanced rectal cancer[118]. Plasma SDF1 levels are also increased in patients with glioblastoma who have radiographic evidence of progression on treatment with cediranib[74]. SDF1, a ligand for CXC motif receptor 4 (CXCR4), promotes myeloid cell recruitment to the hypoxic regions of tumours. After radiation therapy, glioblastoma xenografts become revascularized and regrow through a HIF1α-mediated process that leads to CXCR4-driven recruitment of myeloid cells[119,120]. Tumour regrowth does not occur when myeloid cell influx is blocked by the inhibition of CXCR4 using plerixafor (AMD3100)[119,120]. Plerixafor, which is approved for use in non-Hodgkin's lymphoma and multiple myeloma, is currently being used in combination with bevacizumab in early clinical trials of glioblastoma (TABLE 2). These trials will test whether escape from inhibition of VEGF signalling can be prevented by blocking SDF1-driven recruitment of myeloid cells.

Myeloid cell recruitment can also be suppressed by inhibition of M-CSF-mediated signalling through its receptor CSF1R (also known as c-FMS). Lung cancer growth in preclinical studies is reduced to a greater extent by blocking VEGF signalling (using the VEGFR2-specific antibody DC101) together with CSF1R signalling (using GW2580) than by blocking VEGF signalling alone[121].

Inhibition of processes that favour tumour progression

Some effects of VEGF signalling inhibitors that slow tumour growth can also promote invasion and metastasis in some preclinical models[122-126]. The propensity for these effects seems to be dependent on multiple factors, including the tumour type and microenvironment, as well as drug-specific properties, such as dose and schedule, as these effects have been found in some studies[122-132] but not in others[7,133,134].

Among the mechanisms that could causally link angiogenesis inhibitors to tumour progression are vascular pruning, intratumoural hypoxia and other changes in the tumour microenvironment. Additional factors include conditions that favour crosstalk between VEGF receptors and other receptor tyrosine kinases (for example, MET, the receptor for hepatocyte growth factor) through heterodimerization, receptor complex formation, transphosphorylation, shared endocytosis and recycling or other interactions[126,135-138]. Changes in VEGF signalling can influence other receptors and their downstream pathways through these processes.

Inhibitors of VEGF signalling slow tumour growth by stopping angiogenesis and causing the rapid pruning of tumour vessels. Vascular pruning can exaggerate intratumoural hypoxia and can stabilize HIF1α[47,139]. Intratumoural hypoxia selects for tumour cells that survive in a low oxygen environment, undergo EMT, are more motile and invasive, and have gene expression changes that are driven by HIF1α activation[140]. Downstream effects of HIF1α are influenced by the tumour microenvironment. Deletion of HIF1α in astrocytoma cells leads to rapidly growing, invasive tumours in the brain but results in poorly vascularized, slowly growing, necrotic tumours when grown subcutaneously[141].

The association of intratumoural hypoxia with evasive resistance to inhibitors of VEGF signalling makes the hypoxic microenvironment an attractive therapeutic target. Targeting HIF1α and angiogenesis together is a potential strategy for preventing escape from inhibitors of VEGF signalling[142,143]. Preclinical and clinical trials are currently underway to test whether HIF1α block-ade increases the therapeutic benefit of inhibitors of VEGF signalling. The TOP1 inhibitor topotecan blocks the accumulation of α-subunits of HIF1 through a TOP1-dependent effect on RNA transcription that is independent of DNA replication and proteasome degradation of HIF1α[144]. Daily low-dose topotecan in combination with bevacizumab reduces HIF1α accumulation, decreases tumour cell proliferation, increases apoptosis and promotes tumour regression in preclinical models[47]. EZN-2208, a pegylated TOP1 inhibitor that results in sustained downregulation of HIF1α[145], is being tested in combination with bevacizumab in patients with refractory solid tumours (TABLE 2).

Like hypoxia, insulin and insulin-like growth factor I (IGF1) increase HIF1α activity and expression of VEGF[146,147]. IGF1–IGF1 receptor (IGF1R) signalling also increases tumour cell proliferation, decreases apoptosis and promotes progression to a more invasive and metastatic phenotype[148]. Downregulation of IGF1R suppresses the growth of some tumours through a mechanism that is complementary to VEGF blockade[147]. The suppression of tumour growth can also require inhibition of IGF2 or insulin receptors[149]. A function-blocking antibody to IGF1R in combination with bevacizumab is being tested on advanced solid tumours in early clinical trials (TABLE 2).

Through HIF1α, hypoxia increases the expression of various proteins that are involved in glycolytic metabolism, oxygen consumption, resistance to apoptosis, immune evasion, angiogenesis, invasion and metastasis[140]; these proteins include SDF1, CXCR4 and MET[150-152].

Tumours with high MET expression or activating mutations of MET are generally more aggressive and have a less favourable prognosis[153]. The activation of MET can promote EMT and tumour invasiveness[151,154], partly by increasing the activity of transcriptional repressors, such as snail homolog 1 (*SNAI1*; also known as SNAIL), *ZEB1* and *TWIST1*, that reduce E-cadherin expression, increase N-cadherin expression and turn on the expression of other mesenchymal markers[155]. Inhibition of VEGF signalling can result in decreased expression of epithelial markers and increased expression of *SNAI1*, *TWIST1* and other mesenchymal markers in some preclinical models[124-126,129]. The expression of the mesenchymal marker fascin increases in some glioblastomas after treatment with bevacizumab[156].

Targeting tumour progression and angiogenesis together has recently shown promise as a strategy for preventing escape from inhibitors of VEGF signalling in preclinical models[124-126,157,158]. One approach is the inhibition of MET and VEGF signalling together, either by concurrent administration of selective inhibitors (PF-04217903 plus a VEGF-specific antibody) or by single agents that block both receptors (cabozantinib (also known as XL184) or foretinib (also known as XL880))[125,158]. Concurrent inhibition of MET and VEGF signalling can slow tumour growth, decrease invasion and metastasis, and change invasive tumours into a shape with a more ball-like appearance (FIG. 2a,b) in preclinical models[125,158]. The reduction in tumour progression is attributed to synergistic effects of blocking MET and VEGF receptor signalling and crosstalk[125,126,135,158,159]. The therapeutic benefit of blocking MET and VEGF together is currently being evaluated in clinical trials of multiple tumour types (TABLE 2).

Administration of semaphorin 3A (SEMA3A), a secreted ligand for neuropilin 1, plexins and integrins via RHO-family GTPases, is another approach that has been shown to prevent escape from the inhibition of VEGF signalling[124]. Neuroendocrine tumours of the pancreas and carcinomas of the uterine cervix are more invasive and metastatic in mice treated with sunitinib or with the VEGFR2-specific antibody DC101. However, administration of SEMA3A by adeno-associated virus along with a VEGF signalling inhibitor improves tumour vascular function, reduces intratumoural hypoxia, MET expression and EMT, decreases invasion (FIG. 2c,d) and metastasis, and prolongs survival[124].

AXL, a member of the TYRO-AXL-MER (TAM) family of receptor tyrosine kinases, is a further target currently under examination for the prevention of escape from VEGF signalling inhibitors[160]. AXL and its ligand, growth arrest-specific gene 6 (GAS6), promote tumour cell survival, proliferation and migration[161]. Expression of AXL increases during EMT, contributes to invasion and metastasis, and is a negative predictor of overall survival in breast cancer[160]. Knockdown of AXL by short hairpin RNA eliminates the metastatic potential of MDA-MB-231 breast cancer xenograft tumours[160]. Similarly, a small-molecule inhibitor of AXL (R428) reduces invasion and metastasis in multiple tumour models[162], and the AXL-specific antibody YW327.6S2 reduces metastasis and increases the efficacy of inhibitors of VEGF signalling in lung and breast cancer xenograft tumours[157].

Cabozantinib, which inhibits MET, AXL and VEGF receptors, as well as multiple other receptor tyrosine kinases, is a potent inhibitor of invasion and metastasis in spontaneous and xenograft tumours in mice[125,158,159]. Cabozantinib has greater effects on tumour angiogenesis and overall survival than those found with combinations of selective inhibitors

of MET and VEGF signalling in the same preclinical model, suggesting that AXL or other targets (such as RET, KIT and TIE2) contribute to the efficacy of cabozantinib[125,159]. Cabozantinib is showing promising results in clinical trials of castration-resistant metastatic prostate cancer (FIG. 2e; TABLE 2), medullary thyroid cancer, breast cancer, non-small-cell lung cancer, melanoma, liver cancer and ovarian cancer[163-166].

Inhibition of cancer stem cell activation.

Treatment with angiogenesis inhibitors can increase the population of cancer cells with stem cell-like properties in hypoxic regions of a tumour[128,154,167,168]. A recent study has shown that breast cancer xenograft tumours in mice treated with sunitinib or bevacizumab have less vascularity, more hypoxia, slower growth and more abundant stem cells — identified by aldehyde dehydrogenase immunofluorescence — in hypoxic regions[128]. This process is dependent on HIF1α activation of AKT–β-catenin signalling. These findings are consistent with a contribution of breast cancer stem cells to hypoxia-related mechanisms of escape from inhibitors of VEGF signalling. The presence of MET overexpression, EMT, invasion and metastasis and potential strategies for inhibiting this escape mechanism are yet to be examined.

Future directions

The expectations for the use of angiogenesis inhibitors as cancer therapeutics have evolved during the years following their approval for clinical use. Many preclinical experiments and clinical trials have documented their potential, as well as their limitations, as with other anticancer drugs, and have shown that VEGF blockade by bevacizumab or zivaflibercept is usually more effective when used in combination with chemotherapy.

As the actions of angiogenesis inhibitors are becoming better understood, targeted therapies are being developed to block key steps in tumour growth, invasion and metastasis, and the range of drugs is expanding as mechanisms of tumour progression are elucidated. Preclinical studies have already provided proof of concept for inhibiting mechanisms of escape together with VEGF-driven angiogenesis, with the goal of slowing both tumour growth and progression. Although initial results are promising, important questions remain about how well the preclinical findings will translate to human cancer and whether the benefits will be durable, apply to multiple tumour types and not be limited by other escape mechanisms. More work is needed to identify inhibitors of escape mechanisms that can be used safely and effectively in combination with inhibitors of VEGF signalling over extended periods.

Among other issues to be resolved is why chemotherapy is required for meaningful clinical benefit of bevacizumab and zivaflibercept in multiple tumour types. Do selective inhibitors of VEGF improve the delivery of other agents and sensitivity to radiotherapy by normalizing tumour blood vessels? Or does chemotherapy or radiation augment the effects of VEGF inhibitors by sensitizing endothelial cells to their actions? Does chemotherapy suppress hypoxia-driven tumour progression that would otherwise develop with a VEGF inhibitor used as monotherapy? How does VEGF blockade influence myeloid cell recruitment and immune surveillance? Why do most small-molecule tyrosine kinase inhibitors not pair well with chemotherapy? Is this due to drug–drug interactions, their multi-targeted nature or due to additive toxicities? As the number of druggable targets increases, the relative cost, benefit and safety of combinations of expensive targeted therapies will need to be balanced against the efficacy and toxicity profile of single agents that block multiple targets.

The 90% of cancer patients who die as a consequence of tumour invasion and metastasis[39,169] are telling reminders of the importance of these processes in outcome. In colorectal cancer, in which the primary tumour is generally removed by surgery, metastases

can occur despite adjuvant bevacizumab treatment plus chemotherapy[170], presumably because tumour cell dissemination occurs before surgery[171] or because bevacizumab promotes evasive resistance via heightened invasion and metastasis[9].

If the growth of metastases in humans is angiogenesis dependent, and selective VEGF blockade can stop angiogenesis in humans as in preclinical models[6,7,133,134], why does bevacizumab not prolong disease-free survival in the adjuvant setting? In the 3-year Phase III C-08 trial of adjuvant therapy after surgery for colorectal cancer, the addition of bevacizumab significantly improved disease-free survival during the initial year of treatment but not during the remaining period after treatment ended[170]. This finding illustrates an effect of treatment duration on outcome and supports the continued administration of bevacizumab beyond progression. Withdrawal of VEGF signalling inhibition is followed by tumour revascularization and regrowth as the actions of VEGF resume[7,172,173]. In the Bevacizumab Regimens: Investigation of Treatment Effects and Safety (BRiTE) trial in metastatic colorectal cancer, 74% of 1,953 patients who experienced disease progression while on bevacizumab and chemotherapy first-line had significantly better survival when bevacizumab was continued beyond progression[37]. Bevacizumab treatment beyond progression also resulted in prolonged overall survival in a retrospective study of metastatic colorectal cancer[38].

More needs to be learned about the effects of sustained inhibition of VEGF signalling on the growth and further spreading of metastases. Knowing whether sustained anti-VEGF therapy in the adjuvant setting can slow the appearance of metastases and whether treatment beyond progression can slow further growth and dissemination of metastases would help to resolve these issues.

Blocking both angiogenesis and escape pathways that drive tumour progression is now an attainable step in the evolution of the use of agents that inhibit VEGF signalling together with other targets. This approach takes advantage of the current knowledge of tumour vascular biology and mechanisms of tumour growth, invasion and metastasis. Key steps that still need to be taken include learning more about escape mechanisms and how to control them, identifying additional targeted drugs that act synergistically with angiogenesis inhibitors and finding predictive biomarkers to identify patients who will have sustained benefit.

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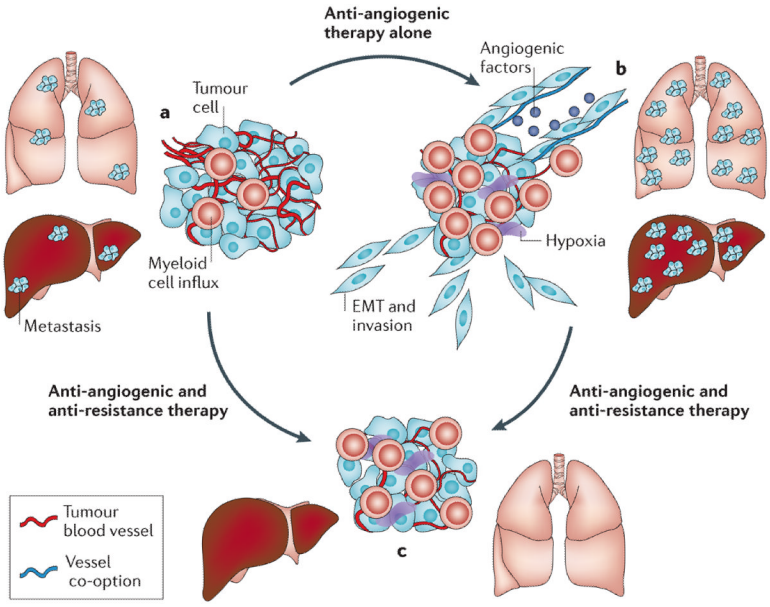

Figure 1. Overcoming resistance to inhibitors of VEGF signalling by blocking angiogenesis and tumour progression.

A schematic representation of the development and resolution of resistance to angiogenesis inhibitors, based on evidence from preclinical studies, is shown. **a** | Before treatment with an angiogenesis inhibitor the tumour is highly vascular, rapidly growing and accompanied by metastases. **b** | After treatment with an inhibitor of vascular endothelial growth factor (VEGF) signalling, the main tumour is smaller and less vascular but is more hypoxic, has more myeloid cells and is more invasive. Tumour growth continues without angiogenesis by co-option of normal blood vessels. Tumour cells undergo epithelial–mesenchymal transition (EMT), become more mesenchymal, invade surrounding tissues and metastasize. **c** | After inhibition of the pathways involved in tumour progression together with VEGF signalling, the tumour is smaller, has a ball-like shape, is less invasive and has no metastases.

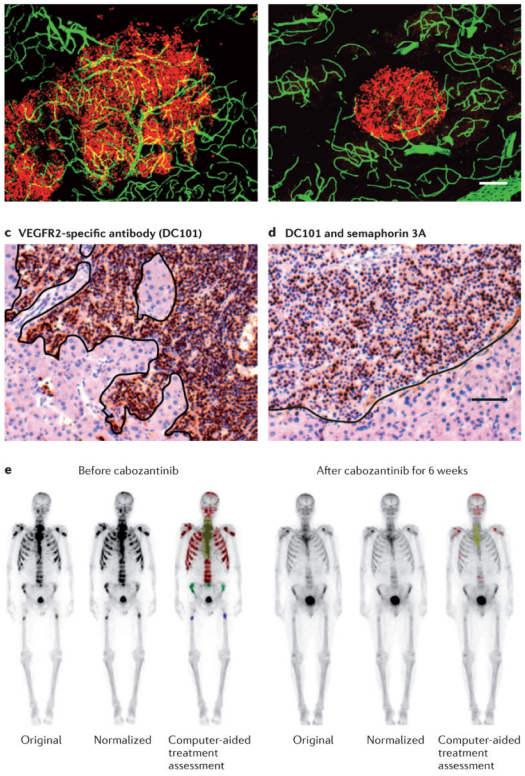

Figure 2. Reversal of tumour progression.

Pancreatic neuroendocrine tumours from RIP1-Tag2 transgenic mice are shown (parts **a**–**d**). Tumour cells stained for SV40 T- antigen are indicated (shown in red in parts **a,b**; shown in brown in parts **c,d**). Blood vessels stained for CD31 are shown (green in parts **a,b**). The scale bar shown in part **b** (80 μm) also applies to part **a**. The scale bar in part **d** (50 μm) also applies to part **c**. The irregular borders of invasive tumours treated with a vascular endothelial growth factor (VEGF)-specific antibody (part **a**) or a VEGF receptor 2 (VEGFR2)-specific antibody DC101 (part **c**) contrast with the smooth borders of tumours treated with cabozantinib, which is a small-molecule tyrosine kinase inhibitor targeting VEGF receptors and MET (part **b**), or with VEGFR2-specific antibody plus semaphorin 3A[124] (part **d**) (parts **a** and **b** are similar to data reported in REF. 125). Bone scans of a patient with castration-resistant metastatic prostate cancer before and after treatment with cabozantinib are shown (part **e**)[166]. Metastases are highlighted and are coloured (red, green and blue) by computer-aided normalization and enhancement algorithms. Parts **c** and **d** reproduced, with permission, from REF. 124 © Am. Soc. Clin. Investigation (2012). Part **e** reproduced, with permission, from REF. 166 © Lippincott Williams & Wilkins (2012).

Table 1

Angiogenesis inhibitors currently approved for use in cancer patients

Angiogenesis inhibitor	Developer	Type of inhibitor	Targets	Use	Indication (approval region*)
Bevacizumab (Avastin)	Genentech/ Roche	Monoclonal antibody	VEGFA	First or second line	Metastatic colorectal cancer (United States and Europe)
				First line	Metastatic non-small-cell lung cancer (United States and Europe)
				First line	Metastatic renal cell cancer (United States and Europe)
				First line	Recurrent glioblastoma (United States)
				First line	Metastatic breast cancer (Europe)
				First line	Advanced ovarian cancer (Europe)
Ziv-aflibercept (Zaltrap, VEGF Trap)	Regeneron/ Sanofi	Recombinant fusion protein acting as a soluble decoy receptor	VEGFA, VEGFB and PLGF	Second line	Metastatic colorectal cancer (United States)
Sorafenib (Nexavar, Bay 43–9006)	Bayer	Tyrosine kinase inhibitor	VEGFR2, VEGFR3, PDGFRβ, FGFR1, KIT and RAF	First line	Advanced renal cell carcinoma (United States and Europe)
				First line	Unresectable hepatocellular carcinoma (United States and Europe)
Sunitinib (Sutent, SU11248)	Pfizer	Tyrosine kinase inhibitor	VEGFRs, PDGFRs, FLT3, KIT and RET	First line	Advanced renal cell carcinoma (United States and Europe)
				Second line	Gastrointestinal stromal tumour (United States and Europe)
				First line	Unresectable pancreatic neuroendocrine tumours with locally advanced or metastatic disease (United States and Europe)
Axitinib (Inlyta, AG-013736)	Pfizer	Tyrosine kinase inhibitor	VEGFRs	Second line	Advanced renal cell carcinoma (United States and Europe‡)
Pazopanib (Votrient, GW786034B)	GlaxoSmithKline	Tyrosine kinase inhibitor	VEGFRs, PDGFRs and KIT	First line	Renal cell carcinoma (United States and Europe)
				Second line	Advanced soft tissue sarcoma (United States and Europe‡)
Vandetanib (Caprelsa, ZD6474)	AstraZeneca	Tyrosine kinase inhibitor	VEGFRs, EGFR and RET	First line	Late-stage medullary thyroid cancer (United States and Europe)
Everolimus (Afinitor, RAD001)	Novartis	Rapamycin derivative	mTOR	First line	Advanced pancreatic neuroendocrine tumours (United States and Europe)
				First line	Subependymal giant cell astrocytoma with tuberous sclerosis (United States and Europe)
				First line	Renal angiomyolipoma associated with tuberous sclerosis complex (United States)
				Second line	Advanced HER2-negative breast cancer (United States and Europe)
				Second line	Advanced renal cell carcinoma (United

Angiogenesis inhibitor	Developer	Type of inhibitor	Targets	Use	Indication (approval region*)
		inhibitor of mTORC1			States and Europe)

EGFR, epidermal growth factor receptor; FGFR1, fibroblast growth factor receptor 1; FLT3, fms-related tyrosine kinase 3; mTORCl, mTOR complex 1; PDGFR, platelet-derived growth factor receptor; PLGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

* Europe refers to approval by the European Medicines Agency (EMA); United States refers to approval by the US Food and Drug Administration (FDA).

‡ A positive opinion was received in May 2012 for the use in this indication from the Committee for Medicinal Products for Human Use (CHMP) of the EMA but final approval has not yet been granted.

Table 2

Clinical trials of agents targeting VEGF signalling in combination with other targeted agents

ClinicalTrials.gov identifier	Combination strategy	Stage	Cancer type
NCT01496742	Bevacizumab plus MET-specific antibody onartuzumab (MetMAb) and standard therapies	Phase II	Non-small-cell lung cancer
NCT01186991	Bevacizumab plus MET-specific antibody onartuzumab (MetMAb) and paclitaxel	Phase II	Metastatic triple-negative breast cancer
NCT01418222	Bevacizumab plus MET-specific antibody onartuzumab (MetMAb) and standard therapies	Phase II	Metastatic colorectal cancer
NCT01339039	Bevacizumab plus CXCR4 inhibitor plerixafor (mozobil and AMD3100)	Phase I	Recurrent high-grade glioma
NCT01251926	Bevacizumab plus topoisomerase I inhibitor EZN-2208 (pegylated SN-38)	Phase I	Refractory solid malignancies
NCT00811993	Bevacizumab plus IGF1R inhibitor R1507 and standard therapies	Phase I	Advanced solid malignancies
NCT01308684	Bevacizumab plus PLGF-specific antibody RO5323441 (TB-403)	Phase I	Recurrent high-grade glioma
NCT01605227	Cabozantinib (XL184, inhibitor of VEGFR, MET, AXL and other kinases) versus prednisone (COMET-1 trial)	Phase III	Castration-resistant prostate cancer metastatic to bone
NCT01522443	Cabozantinib (XL184, inhibitor of VEGFR, MET, AXL and other kinases) versus mitoxantrone and prednisone (COMET-2 trial)	Phase III	Castration-resistant prostate cancer metastatic to bone
NCT00726323	Foretinib (GSK1363089, XL880, inhibitor of VEGFR, MET and other kinases)	Phase II	Papillary renal cell carcinoma
NCT01468922	Pazopanib (inhibitor of VEGFRs, PDGFRs and other kinases) and tivantinib (ARQ 197, inhibitor of MET)	Phase IB	Refractory advanced solid tumours

CXCR4, chemokine (C-X-C motif) receptor 4; IGF1R, insulin-like growth factor 1 receptor; PDGFR, platelet-derived growth factor receptor; PLGF, placental growth factor; VEGFR, vascular endothelial growth factor receptor.