Antiangiogenic therapy: impact on invasion, disease progression, and metastasis

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Abstract | Antiangiogenic drugs targeting the VEGF pathway have slowed metastatic disease progression in some patients, leading to progression-free survival (PFS) and overall survival benefits compared with controls. However, the results are more modest than predicted by most preclinical testing and benefits in PFS are frequently not accompanied by overall survival improvements. Questions have emerged about the basis of drug resistance and the limitations of predictive preclinical models, and also about whether the nature of disease progression following antiangiogenic therapy is different to classic cytotoxic therapies—in particular whether therapy may lead to more invasive or metastatic behavior. In addition, because of recent clinical trial failures of antiangiogenic therapy in patients with early-stage disease, and the fact that there are hundreds of trials underway in perioperative neoadjuvant and adjuvant settings, there is now greater awareness about the lack of appropriate preclinical testing that preceded these studies. Improved preclinical assessment of all stages of metastatic disease should be a priority for future antiangiogenic drug discovery and development.

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Introduction

Antiangiogenic therapy is based on the theory that blocking new blood vessel formation in tumors will stop or slow their growth. Currently, four molecular-targeted drugs are approved by the FDA for six tumor indications; all act to disrupt the VEGF pathway.[1] Thus, nearly four decades after the antiangiogenesis concept was introduced by Judah Folkman,[2] antiangiogenic therapy is considered a major anticancer treatment modality.[3] However, with hundreds of clinical trials currently underway in multiple cancer indications and pathological stages, and dozens of other VEGF and other angiogenic-pathway-targeted agents now in experimental or clinical testing, an urgent issue is understanding why the majority of patients stop responding—or do not respond at all—to such drugs and how such limitations can be overcome. Numerous mechanisms of resistance to antiangiogenic therapy have been proposed[4] highlighting that over two decades of positive preclinical studies have yielded only modest incremental changes in the clinic. While this is an unfortunate and common occurrence among cancer treatments, the question remains: are the challenges facing antiangiogenic drugs unique?

In theory, targeting the host 'tumor-supporting' angiogenic processes has many benefits but it might also have limitations. Antiangiogenic therapies might initiate an array of stromal and microenvironmental defense mechanisms[4] that contribute to eventual drug inefficacy and, more provocatively, may lead to a more aggressive and invasive tumor phenotype—one with an increased ability to metastasize. Though perhaps surprising, this latter property is not distinct from other anticancer treatment modalities—surgery, radiation and chemotherapy can also produce similar unwanted 'prometastatic' effects in certain isolated experimental settings (Box 1). However, the possibility that VEGF-pathway inhibitors, and perhaps other 'host-targeted' drugs as well, could augment invasive or metastatic potential (despite controlling primary tumor growth or initially slowing the growth of metastasis) could be significant and has become a topic of considerable controversy. The debate has been fuelled by modest clinical benefits, high drug cost, and adverse side effects, in addition to converging findings published in the past 2 years, which relate to limited drug efficacy in early-stage disease. The first finding comes from two preclinical studies showing that the benefits from VEGF-pathway-inhibitor monotherapy can depend on disease stage and treatment circumstances and can, in certain settings, be offset by increased aggressive invasiveness and augmented metastatic potential.[5,6] The second finding comes from two large phase III clinical trials involving bevacizumab, a monoclonal antibody to VEGF, used in combination with chemotherapy and administered as adjuvant therapy to patients with early-stage colorectal carcinoma; the treatment combination showed no benefit in the primary end point of disease-free survival (DFS) compared with the chemotherapy-alone arm.[7,8] These studies have raised questions about the expectations for antiangiogenic agents in blocking different stages of tumor progression and, in particular, the benefits of these drugs in micrometastatic disease settings.

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We summarize evidence that suggests antiangiogenic drugs might alter the natural history of disease progression, depending on the disease stage and tumor type, and focus on limitations that antiangiogenic drugs might have to overcome to bring about greatly improved clinical benefits. It is possible that antiangiogenic therapy may induce a different disease progression pattern than cytotoxics and lead to worse outcomes in terms of progression, invasion, and metastasis. However, this result might never materialize outside of certain limited preclinical scenarios. It remains theoretically possible that such 'evasive resistance' mechanisms have a role in the clinical limitations of successful antiangiogenic drugs and, perhaps most importantly, might provide a clue as to how they can be made more effective. There is no compelling clinical evidence that antiangiogenesis treatment will make disease worse or decrease survival;[9] however, neither is there a large pool of supporting preclinical evidence that such therapy will be beneficial in blocking early-stage disease, particularly in potentially curative and preventive settings where detailed analysis is rarely performed. With thousands of patients projected to be enrolled to clinical trials over the next 5 years to assess neoadjuvant or long-term adjuvant use of VEGF-pathway-targeted drugs,[10] a rigorous assessment of actual and predicted outcomes for antiangiogenic therapy should be conducted using improved preclinical models to better understand when and to what extent these new drugs are likely to work.

Key points

- Successful clinical trials with various VEGF-pathway inhibitors have been accompanied by numerous phase III failures
- Trial failures in adjuvant disease, and ongoing trials in early-stage settings, could highlight differences in antiangiogenic drug efficacy depending on disease stage
- There is a gap between how antiangiogenics are usually tested in the clinic (late-stage metastatic) and in preclinical mouse models (localized primary tumors)
- There is debate whether anti-VEGF therapy may lead to 'rebound growth' when halted or if it may fuel more invasive and metastatic disease phenotypes
- Future testing of antiangiogenic therapies should be conducted in clinically relevant animal models of all disease stages

Box 1 | Therapy-accelerated tumor growth and metastasis—not a new phenomenon

Nearly all anticancer treatments have been shown in some preclinical settings to enhance or facilitate metastatic disease growth and distribution (Supplementary Table 1 online). For example, antitumor effects of radiation can be offset by effects on adjacent 'bystander' tissues (the radiation-induced 'tumor bed effect') that, in turn, allow for a more hospitable site for tumor extravasation and metastatic growth.[130,131] However, preclinical studies involving therapy-induced metastasis must be put into context. This phenomenon only occurs under certain conditions, and can be directly contrasted with positive preclinical examples of beneficial effects in cancer where treatment is sustained. Moreover, decades of clinical use of chemotherapy and radiation clearly demonstrate that antitumor effects outweigh any potential prometastatic effects. Nevertheless, no anticancer therapy has been consistently curative for patients, and prometastatic effects could counteract, or limit, the beneficial antitumor effects of any treatment strategy. Molecular host-targeted drugs such as antiangiogenics could warrant more careful consideration—particularly in micrometastatic disease settings. Chemotherapy and radiation mainly act by direct tumor cytotoxicity and are administered for defined periods (usually brief), whereas antiangiogenic agents are typically cytostatic inhibitors and meant to be administered for longer periods because of their reduced toxic effects.

Successful therapy—but challenges remain

Bevacizumab was the first molecular-targeted antiangiogenic therapy approved by the FDA and is used as first-line therapy in colorectal cancer (CRC), metastatic breast cancer (MBC), non-small-cell lung cancer (NSCLC), and metastatic renal cell carcinoma (mRCC), and as second-line therapy in CRC and glioblastoma multiforme (GBM).[11] With the exception of GBM, bevacizumab is only approved when combined with chemotherapy or cytokine therapy, as monotherapy failed to show robust activity in most instances of advanced-stage disease.[12] A second class of approved inhibitors (sunitinib, sorafenib and pazopanib) include oral small-molecule tyrosine kinase inhibitors (TKIs) that target VEGFRs, platelet-derived growth factor (PDGF) receptors, and other kinases including KIT, Ret, BRAF and Flt-3.[13] All three of these VEGFR TKIs have been approved as monotherapies for the treatment of mRCC; sunitinib is approved to treat imatinib-refractory gastrointestional stromal tumors (GIST), and sorafenib is approved for hepatocellular carcinoma (HCC). But, these clinical successes have been accompanied by questions that have emerged in the phase III trial setting, which represent potential challenges that must be addressed in order to overcome the limited efficacy of VEGF-pathway inhibitors (Tables 1 and 2).

PFS gains and overall survival

In terms of objective benefits, such as disease stabilization and progression-free survival (PFS) or overall survival, VEGF-pathway-targeted therapy has largely yielded only modest gains. Despite the presence of VEGF and VEGFR2, tumors either do not respond or eventually become unresponsive with PFS or overall survival benefits in patients receiving antiangiogenic therapy being, in most cases, measured in months.[14] In some instances, trials in indications that initially yielded significant improvements in overall survival when bevacizumab was combined with chemotherapy have sometimes not shown similar benefits when compared with more-effective chemotherapies in follow-up studies.[15]

Perhaps more concerning, however, is the emerging trend where patient response rate and PFS does not translate into significantly increased overall survival in phase III trials (Tables 1 and 2 and Supplementary Box 1 online). Currently, overall survival remains the gold standard for determining therapeutic benefit but the potential use of PFS as an 'overall survival surrogate' has been introduced because of a typically strong correlation between the hazard ratios for overall survival and PFS.[16] However, there remains a lack of consensus on the use of PFS in this manner and results with antiangiogenic drugs suggest an example where PFS benefits are often not translated into overall survival benefits. It remains a major question as to why such robust gains in PFS seen in the majority of completed phase III trials with bevacizumab and chemotherapy, or VEGFR TKIs as monotherapy, have not frequently corresponded to robust gains in overall survival.

The paradox of chemotherapy combination

Failed trials with VEGF-pathway inhibitors have uncovered a disparity between the efficacy of different treatment

Table 1 | Successful completed phase III trials with anti-VEGF pathway agents

Combined with	Tumor (setting)	↑ PFS?	↑ OS?	Trial identifier
Bevacizumab				
IFL	CRC (1st)	Yes	Yes*	AVF2107[96]
FOLFOX or XELOX	CRC (1st)	Yes*	Yes	NO16966[15]
FOLFOX	CRC (2nd)	Yes	Yes*	E3200[97]
Paclitaxel	MBC (1st)	Yes*	No	E2100[98]
Docetaxel	MBC (1st)	Yes*	NA	AVADO[99]
Capecitabine, taxane or anthracycline	MBC (1st)	Yes*	No	Ribbon1[100]
Chemotherapy‡	MBC (2nd)	Yes*	NA	Ribbon2[101]
Carboplatin and paclitaxel	NSCLC (1st)	Yes	Yes*	E4599[102]
Cisplatin and gemcitabine	NSCLC (1st)	Yes*	No	AVAiL[103]
Erlotinib	NSCLC (2nd)	Yes*	NA	ATLAS[104]
Interferon-2α	RCC (1st)	Yes	No*	AVOREN[105]
Interferon-2α	RCC (1st)	Yes	No*	CALGB90206[106]
Carboplatin and paclitaxel	OC (1st)	Yes*	NA	GOG 0218[37]
Monotherapy	GBM (2nd)§	Yes‖	Yes‖	AVF3708[107]
Sunitinib				
Monotherapy	RCC (1st)	Yes*	Yes	NCT00083889[108]
Monotherapy	GIST (2nd)	Yes¶	NA	SUN 1112[109]
Monotherapy	PIC (2nd)	Yes*	Yes	NCT00428597[110]
Sorafenib				
Monotherapy	RCC (1st)	Yes	No*#	TARGET[111]
Monotherapy	HCC (1st)	No	Yes*	SHARP[112]
Pazopanib				
Monotherapy	RCC (1st and 2nd)	Yes*	NA	VEG105192[113]
Vandetanib				
Docetaxel	NSCLC (2nd)	Yes*	No	ZODIAC[114]

*Primary end point. ‡Various chemotherapies including paclitaxel, protein-bound paclitaxel, docetaxel, gemcitabine, capecitabine, and vinorelbine. §A phase II trial. ‖Study evaluated the outcome of each arm relative to historical control. ¶Objective response rate improved. #Benefit seen with crossover. Abbreviations: ↑, increased; 5-fluorouracil, 5-FU; CRC, colorectal cancer; FOLFOX, 5-FU, leucovorin and oxaliplatin; GBM, glioblastoma muliforme; GIST, gastrointestinal stromal tumors; HCC, hepatocellular carcinoma; IFL, 5-FU (bolus), leucovorin and irinotecan; MBC, metastatic breast cancer; NA, not available (pending, unknown or not reported); NSCLC, non-small-cell lung cancer; OC, ovarian cancer; OS, overall survival; PFS, progression-free survival; PIC, pancreatic islet cell; RCC, renal cell carcinoma; XELOX, capecitabine and oxaliplatin.

modalities with and without chemotherapy. With only two exceptions to date (Supplementary Box 2 online), bevacizumab monotherapy has proven ineffective and VEGFR TKIs have failed to improve results obtained with chemotherapy when given in combination in randomized phase III trials. Nevertheless, inhibition of the VEGF pathway can have striking effects (Table 1) but the molecular basis of why this effect is dependent on the drug strategy employed is unknown (Supplementary Box 3 online). It is also not clear why clinical limitations of bevacizumab monotherapy contrast with preclinical data that indicated efficacy, or why the effects of VEGFR TKIs are not at least additive with chemotherapy despite efficacy in some indications, such as mRCC or HCC, when used as monotherapies (Table 1).

Is disease bound to 'rebound'?

The potential that sustained suppression of the VEGF pathway, once discontinued, may lead to a 'rebound' in tumor growth is important—raising the possibility that initial positive effects of treatment, such as rapid reduction in tumor vascularity and inhibition of tumor growth (which could lead to improved PFS), may be negated or reversed (which could influence overall survival). In the case of VEGFR TKIs, such rebounds have been reported during 'drug holiday' periods in the 6-week sunitinib cycle and when treatment is stopped in patients with RCC.[17,18] Enhanced tumor regrowth rates after therapy cessation was noted with liver metastases in patients with CRC when bevacizumab was combined with chemotherapy,[19] and in patients with RCC treated with bevacizumab alone.[20] There is also preclinical evidence for rapid revascularization[21] and rebound tumor growth[22,23] in studies using imaging and immunohistochemical techniques in mice. Though similar rebounds have not been observed in all instances,[24] further study of this concept is critical because drug discontinuation or dose reduction can occur with high frequency, as shown in RCC where 30–50% of patients halted therapy either because of inefficacy or toxic effects.[25–27] Moreover, drug discontinuation rates are reported to be higher outside of clinical trials[28] and in patients with a genetic background that makes them susceptible to toxic effects.[29] Disease stage and treatment combinations could be important in the observation of rebound; Miles *et al.*[9] demonstrated using data from phase III trials in metastatic diseases that halting bevacizumab (and chemotherapy) did not alter mortality rates (Box 2).[9]

A case for treatment beyond progression?

Also of potential importance is that rebounds (if real) may be reversed; in the case of VEGFR TKI-treated patients that have been taken off therapy or fail to respond, benefits have been observed from treatment resumption after a break period,[30] or from switching drugs (for example sunitinib to sorafenib, or vice versa),[31–33] suggesting resistance may be transient in some cases.[34] An observational study of nearly 2,000 patients with CRC (BRiTE Registry) suggests that continuation of bevacizumab treatment while discontinuing and/or switching to additional chemotherapy may substantially increase survival times, indicating that treatment beyond progression may have value.[35] This finding was recently confirmed in another study (ARIES observational cohort study).[36] Indeed, even without progression, bevacizumab as a maintenance therapy significantly improved PFS in two phase III trials (GOG0218 and ICON7) in the primary treatment of advanced ovarian cancers when chemotherapy (carboplatin or paclitaxel) was halted but bevacizumab treatment continued—raising the question of whether administration of an anti-VEGF therapy should continue for longer periods.[37,38] Thus, continued dosing and/or alternative antiangiogenic drug 'switching' might reduce any rebound effects, giving insight into resistance mechanisms and providing a clue as to how such rebounds (if any) may be minimized.

VEGF-pathway inhibition—disease progression

Effect on local tumor invasion

There is a small but growing supportive body of literature that indicates initial tumor response, and even tumor shrinkage, during or after antiangiogenic therapy can sometimes be followed not only by eventual relapse and regrowth,

but also an enhanced invasive or infiltrative phenotype.[39] Supportive evidence is largely anecdotal and limited to small studies or case reports; therefore the concept remains speculative (Box 2). GBM is the most notable example, as 30–50% of patients treated with bevacizumab develop progressive disease accompanied by a high rate of diffuse infiltrative lesions.[40,41] Although GBM is already a highly infiltrative, invasive tumor, this finding has been noted in several studies[42–50] and suggests an adaptive response to anti-angiogenic therapy that leads to more invasive behavior. In preclinical mouse models of GBM where VEGF or hypoxia inducible factor 1α is genetically or therapeutically blocked, initial tumor stabilization and/or shrinkage can be followed by recurrent or existing tumor regrowth, as well as increases in new microsatellite lesions in adjacent sites with infiltrative behavior and wide fronts of invasion (Supplementary Box 4 online).[6,51–59] The caveat is that such findings are not uniformly observed[60] and could manifest primarily from the initial success of therapy rather than from a direct negative effect. If patients with GBM survive longer because of bevacizumab treatment, then this could create more time for tumors to become invasive. Thus, a PFS benefit might have uncovered progression patterns of a rapidly progressing tumor type that had not been observed as frequently and that may shorten the period between relapse and death and compromise overall survival benefits (Figure 1).

Effect on tumor dissemination and metastasis

Paez-Ribes *et al.*[6] observed increased numbers of metastases in distant organs after VEGF-pathway inhibition. It is critical to note that—as for previous preclinical studies—these results were observed only after objective tumor growth inhibition in localized disease that led to prolonged overall survival.[6] Therefore, despite an initial and overall benefit in survival after treatment, tumor-response mechanisms to therapy may eventually facilitate induction of invasive and metastatic tumor outgrowths. This, in turn, might limit the overall benefits in survival times. If these findings suggest a tumor-dependent response to therapy, then it is also possible that host-dependent responses to VEGF-pathway inhibition could facilitate metastasis. Similar potent antitumor properties were observed using short-term and sustained VEGFR TKI monotherapy treatment in orthotopically implanted tumors, but when mice were treated before intravenous inoculation (experimental metastasis) or immediately following primary tumor removal (spontaneous metastasis) an increase in metastatic disease was observed that translated into shortened survival times for mice receiving therapy.[5] Thus, short-term treatment could influence early-stage micrometastatic disease initiation, independent of direct effects of drug on tumor cells, suggesting that systemic reactions to VEGF-pathway disruption could facilitate tumor dissemination. These preclinical studies demonstrate that early-stage micrometastatic growth, under certain conditions, can be elicited rather than inhibited by VEGF-pathway inhibition, and might involve both adaptive tumor-dependent and tumor-independent (host-mediated) mechanisms.[5,6]

The clinical relevance of these findings is unclear; however, clinical results that seem consistent with these preclinical findings have emerged. For VEGFR TKIs, similar instances where treatment cessation and rebound regrowth has been accompanied by increases in local foci and/or distant metastasis in retrospective analyses of patients with RCC who discontinued either sunitinib

Table 2 | Unsuccessful or terminated phase III trials with anti-VEGF pathway agents

Combined with	Tumor (setting)	↑ PFS?	↑ OS?	Identifier
Bevacizumab				
XELOX and cetuximab	CRC (1st)	No*‡	NA	CAIRO2[115]
Oxaliplatin- or irinotecan-based chemotherapy and panitumumab	CRC (1st)	No*‡	NA	PACCE[116]
FOLFOX	CRC (adjuvant)	No§	NA	NSABP-C-08[89]
Capecitabine	MBC (2nd)	No*	No	AVF2119[117]
Erlotinib	NSCLC (2nd)	Yes	No*	BeTa[118]
Capecitabine or 5-FU and cisplatin	AGC (1st)	Yes	No*	AVAGAST[119]
Gemcitabine	PC (1st)	No	No*	CALGB80303[120]
Gemcitabine and erlotinib	PC (1st)	Yes	No*	AviTA[121]
Docetaxel and prednisone	PR (1st)	Yes	No*	CALGB90401[122]
FOLFOX or XELOX	CRC (adjuvant)	No§	NA	AVANT[24]
Aflibercept				
Gemcitabine	PC (1st)	NA	No*	VANILLA‖
Sunitinib				
Paclitaxel	MBC (1st)	No*	NA	SUN 1094‖
Capecitabine	MBC (2nd)	No*	No	SUN 1099[123]
Docetaxel	MBC (1st)	No*	NA	SUN 1064‖
FOLFIRI	CRC (1st)	No*	NA	SUN 1122‖
Erlotinib	NSCLC (2nd)	Yes	No*	SUN 1087‖
Monotherapy	MBC (2nd)	No*	No	SUN 1107[124]
Monotherapy	HCC (2nd)	NA	No	SUN 1170‖
Prednisone	PR (2nd)	NA	No*	SUN 1120‖
Sorafenib				
Carboplatin and paclitaxel	MM (2nd)	No*	NA	PRISM‖
Carboplatin and paclitaxel	NSCLC (1st)	No	No*	ESCAPE[125]
PTK787				
FOLFOX	CRC (2nd)	Yes	No*	CONFIRM 2[126]
FOLFOX	CRC (1st)	No*	No	CONFIRM 1‖
Semaxanib				
FOLFIRI	CRC (1st)	NA	No*	NCT00021281‖
Leucovorin and 5-FU	CRC (1st)	NA	No*	NCT00004252‖
Axitinib				
Gemcitabine	PC (1st)	NA	No*	A4061028‖
Vandetanib				
Monotherapy	NSCLC (2nd)	No*	No	ZEST[127]
Pemetrexed	NSCLC (2nd)	No*	No	ZEAL[128]
Cediranib				
FOLFOX	CRC (1st)	No*	NA	HORIZON III‖
Monotherapy or lomustine	GBM (2nd)	No*	No	REGAL[129]

*Primary end point. ‡PFS worse in experimental arm; all patients received bevacizumab. §Disease-free survival. ‖No citation available, study terminated. Abbreviations: ↑, increased; 5-FU, 5-fluorouracil; AGC, advanced gastric cancer; CRC, colorectal cancer; FOLFIRI, 5-FU, leucovorin and irinotecan; FOLFOX, 5-FU, leucovorin and oxaliplatin; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; MBC, metastatic breast cancer; MM, metastatic melanoma; NA, not available (pending, unknown or not reported); NSCLC, non-small-cell lung cancer; OS, overall survival; PC, pancreatic cancer; PFS, progression-free survival; PR, prostate cancer; XELOX, capecitabine and oxaliplatin.

Box 2 | Therapy-induced metastasis—preclinical anomaly or clinical reality?

It remains a controversial issue whether mechanisms of resistance to antiangiogenic therapy might involve increased invasive behavior with enhanced metastatic potential and there is debate about how to make the proper assessments. In terms of tumor rebound when VEGF-targeted therapy is stopped, there is no consensus in preclinical studies. Revasularization and regrowth has been observed when treatment with VEGFR tyrosine kinase inhibitors (TKIs) is stopped,[21–23] but similar rebounds were not observed in localized tumors when treated with different TKIs[24] or with anti-VEGF antibodies.[132] Perhaps the critical distinction is that the latter studies did not monitor micrometastatic disease progression. Increases in invasive characteristics have been confirmed after treatment with VEGFR TKIs;[79] however, acceleration of metastasis has not been observed in similar circumstances,[133,134] including with antibody treatment.[82] Crucially, overall survival improvement in mouse models of clinically relevant metastasis is not regularly tested or observed (Supplementary Table 2 online).

In a meta-analysis of phase III trial data from over 4,000 patients with colorectal (NO16966 and AVF2107g), breast (AVADO), renal (AVOREN), and pancreas (AViTA) cancer treated with bevacizumab, disease progression was not accelerated when therapy was stopped.[9] Unfortunately, there are caveats. First, the trials incorporate chemotherapy or immunotherapy whereas preclinical studies tested antiangiogenic drugs as monotherapy, using anti-VEGFR2 antibodies or VEGFR TKIs. Second, the patients included have established metastatic (often refractory) disease, and there are no preclinical equivalents that mirror such clinical trials. Thus the question of whether VEGF-pathway inhibition could negatively influence micrometastatic disease remains outstanding[135,136] and further testing is required.

or sorafenib,[30] and in isolated case reports.[61] In one study, the anatomical sites of disease progression were similar in patients who eventually failed to respond to either interferon or VEGF-pathway inhibitors, however, in the latter group there was an increase in metastases in previously uninvolved anatomical sites, suggesting that efficacy of therapy in sites of established metastases is superior to the prevention or inhibition of microscopic tumor growth in new ones.[62]

Mechanisms of evasive resistance

Modes of resistance to VEGF-pathway inhibition have been discussed,[4] and themes have emerged that could be related to disease progression changes in response to therapy, including tumor and host responses (Box 3 and Supplementary Box 5 online).[39,63] For cytotoxic therapies, drug-resistance mechanisms involve a multitude of tumor-dependent changes, including multidrug-resistance gene or protein upregulation; clonal selection or repopulation; and resistance of cancer stem cells.[4] The microenvironment can also be affected by cytotoxic therapies; however, for anti-angiogenic agents—where the microenvironment is the primary target—it is clearly possible that microenvironment effects are of greater influence (Box 3). Disruption of the VEGF pathway could affect these functions with eventual tumor progression and disease relapse.

A change in the seed, the soil, or both?

Although acquired drug resistance is an accepted reality for antiangiogenic therapy, how would resistance lead to a tumor phenotype of increased invasion or metastasis? When a locally growing primary tumor progresses to form distant metastases, several steps are involved including loss of cellular adhesion; enhanced motility and invasion capabilities; intravasation into the bloodstream; homing and survival; extravasation and seeding of micrometastases; and colonization and growth in a distant site.[64] Critically, as Stephen Paget theorized as the 'seed and soil hypothesis,'[65] both the tumor (seed) and host organ environment (soil) must allow for dissemination of disease. There are many preclinical studies showing that anticancer treatments can facilitate the dissemination of tumor cells and metastases (Box 1), and there are mechanisms that could account for antiangiogenic-therapy-induced invasion or metastasis (Box 2), some driven by the host and others by the tumor, though it is likely that both have a role.

Perhaps the most important compensatory mechanism a tumor can acquire in response to VEGF-pathway inhibition is an elevation in tumor hypoxia, which could select for tumor populations able to grow in low oxygen environments[66,67] and/or provide alternate compensatory proangiogenic pathways to allow persistent neovascularization.[68] Though the connection between antiangiogenic therapy and an increase in invasive and metastatic phenotypes needs further validation, the evidence linking hypoxia to a more aggressive metastatic phenotype is established. Both acute and systemic oxygen deprivation facilitate tumor metastasis and studies have demonstrated that hypoxia-induced mechanisms, such as c-met upregulation (among others), can force tumors to branch and disseminate despite therapy-induced hypoxia being a key initial controller of tumor growth.[69–71]

A second important potential mediator of increased metastatic potential after therapy could include inflammatory mechanisms of the host, perhaps as a result of alteration (or injury) to the endothelial microenvironment, which assist in both the intravasive and extravasive potential of tumors.[72,73] It is possible (though unproven), that such favorable conditions (or 'premetastatic niches') could differ significantly depending on the therapy. Chemotherapy and radiation, for example, could primarily act in this manner to promote metastasis (Box 1 and Supplementary Table 1 online), and it is possible that this effect could differ between VEGF-pathway inhibitors. For example, the less-specific multitargeted small-molecule TKIs could cause an increased metastatic potential, whereas antibodies or other large-molecule inhibitors, which may not evoke a systemic inflammatory response, could lack or have attenuated 'prometastatic' capacity. In addition, perivascular pericytes might act as a barrier to limit tumor cell intravasation and extravasation and targeting these cells using VEGFR TKIs (which block PDGFRs) could promote aspects of the metastatic process.[74] Future investigations could illuminate the differences between how the tumor and microenvironment react to therapy, whether positively or negatively, with respect to tumor growth and metastatic dissemination.

Early-stage disease

There are several important ramifications for the field of antiangiogenesis therapy if one or more of the theoretical mechanisms of resistance and/or preclinical findings manifest into altered disease progression in the clinical setting. The most obvious question is how can such data be reconciled with the numerous preclinical and clinical

data indicating that antiangiogenic therapy inhibits, not promotes, disease progression in localized and metastatic settings? Indeed, experimental conditions (such as animal model, tumors, drugs, doses, treatment duration, or combinations with chemotherapy) may explain some differences in outcomes; however, antiangiogenic therapies may have different efficacies in established localized primary tumors and micrometastatic and macrometastatic disease.

The gap between bedside and bench

Perhaps foremost among the challenges in predicting disease-progression patterns and mechanisms of drug resistance to antiangiogenic therapy is a general disconnect between how VEGF-pathway inhibitors (and all anticancer therapies for that matter) are tested in experimental versus clinical settings. In preclinical evaluations, the majority of analyses have been conducted either in genetically engineered mouse models (GEMMS) or, more frequently, in locally grown primary ectopic (or orthotopic) tumors using human (xenograft) or mouse (syngenic) models.[75] Conversely, most cancer patients receiving VEGF-pathway inhibitors have late-stage (sometimes refractory) disease involving established metastases in more than one site.[71] In the preclinical setting, only a negligible fraction of studies have tested VEGF-pathway inhibitors in similar late-stage models and even fewer have compared directly antitumor efficacy in such circumstances to locally grown primary tumors (Figure 2). Also, preclinical metastasis models are often quantitatively assessed (for example visual nodule counts, immunohistochemistry, and imaging[76]) and disease is measured at a defined end point—usually when a primary or localized tumor has reached an institutional ethical limit. This means studies are stopped short of overt systemic metastatic disease, and therefore the majority of preclinical studies involving VEGF-pathway inhibitors and metastasis have included non-survival-based analyses. These limitations have resulted in relatively few studies that are designed to investigate the impact of VEGF-pathway inhibitors on established metastasis when compared with the hundreds of publications dedicated to localized or primary disease. In addition, there are even fewer studies that include clinically relevant, survival-based evaluations of therapy in models of metastasis, and there is disparity in the tumor models employed and modes of metastasis quantification used (Figure 2 and Supplementary Table 2 online). These preclinical studies have shown that VEGF-pathway-targeted therapy leads to the inhibition of metastasis when quantified empirically, either after short treatment periods or when studies are terminated because of primary tumor growth. However, considering that the vast majority of patients receiving similar drugs in the clinic have established metastases, more relevant preclinical analyses should be conducted to determine the consequences of this on overall survival. In such rare preclinical studies, the results have been mixed, with some reporting treatment benefit[77] and others noting more moderate or negligible effects[78,79] (Supplementary Table 2 online). The limitations of these studies are of particular relevance because metastasis is generally the cause of patient mortality,[80] and antiangiogenic agents are now being evaluated in earlier stages of disease, such as the adjuvant setting, which may involve treating early-stage occult micrometastatic disease. Moreover, as the studies by Paez-Ribes *et al.*[6] and Ebos *et al.*[5] show, positive effects in primary tumor models do not always translate into beneficial effects in blocking hematogenous micrometastatic-disease progression (the outcomes may even be worse), and comparisons of drug effects in the primary tumor and micrometastasis treatment settings can be very different. Results from similar studies have varied,[79,81,82] and thus it is critical when interpreting potential conflicting data sets from preclinical studies using inhibitors of the VEGF pathway to consider variables such as disease stage, the types of drug employed (antibodies versus TKIs), and the models of metastasis that are used.

The need for optimal mouse models to study metastasis has taken on a greater urgency, particularly in the setting of micrometastatic disease. A recent Review covered this topic in detail and listed models that could be employed[10]—an example is a model of NSCLC where sunitinib prolonged survival but longer treatments (initiated earlier) did not translate into greater benefit.[83,84] This situation emphasizes the importance of developing models that can clearly distinguish between macroscopic and microscopic disease. In addition, use of models that employ clinically relevant end points such as PFS are promising for improving their predictions of clinical potential.[85]

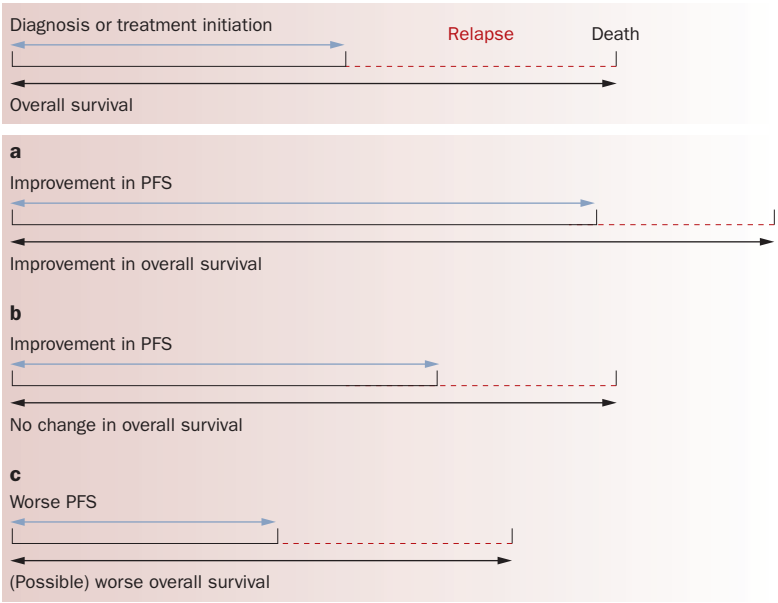

Figure 1 | Clinical results of combinations of PFS and overall survival. There are several different combinations of PFS and overall survival, including no change in either (not shown here). **a** | Improvement in PFS translates into improved overall survival. In completed phase III trials with anti-VEGF-pathway therapy (Tables 1 and 2), two additional scenarios have occurred: **b** | PFS benefit does not translate into improved overall survival, and **c** | reduced PFS (Table 2). Worse overall survival has not been shown in a phase III trial though a recent interim analysis of the AVANT trial indicated that this trend is possible.[24] It is possible that response to anti-VEGF therapy (even if leading to improved PFS) can change the natural history of disease progression to include a more aggressive phenotype—possibly explaining lack of changes in overall survival. This figure is based on conceptual ideas outlined by David Reardon. Abbreviation: PFS, progression-free survival.

Box 3 | Possible mechanisms influencing invasion and metastasis after therapy

Tumor-dependent mechanisms

- Increased expression of prometastatic proteins: c-met,[69,70] interleukin (IL)-6,[137] IL-8,[138] and urokinase-type plasminogen activator receptor[139]
- Suppression of antimetastatic mediators: myoglobulin[140]
- Altered adhesion: upregulation or activation and secretion of exomal proteolytic enzymes, such as matrix metalloproteinases[141,142]
- Bone-marrow-derived dendritic cell (BMDC) mobilization creates 'premetastatic niches'[143]
- Acute hypoxic stress[144–146]
- Instigation of tumor epithelial–mesenchymal transition[147]
- Increased vascular co-optive behavior[133]
- Activation of alternative angiogenic pathways: FGF and ephrin[148]
- Induction of stromal autophagy[149]
- Vascular mimicry or cancer stem cells[150,151]

Tumor-independent—host-mediated—mechanisms

- Compensatory upregulation of proangiogenic or prometastatic factors contribute to 'rebound'[21] and/or increased extravasive potential: VEGF, PlGF, G-CSF, osteopontin, Bv8 (prokineticin), G-CSF, angiopoieten2, PDGFA and SDF1α[14,152–158]
- BMDC mobilization recruits VEGFR1-positive bone marrow cells to distant sites to facilitate 'premetastatic niches';[159,160] this has not been confirmed in all cases[92]
- BMDC mobilization of $Gr1^{+}CD11b^{+}$ myeloid suppressor-type cells, TIE2 expressing monocytes, and tumor-associated macrophages to home to the tumor microenvironment and produce compensatory proangiogenic factors[14,155–158]
- Pericyte dysfunction increases vessel leakiness and allows for increased extravasive and metastatic tumor potential[4,74,161,162]
- Increased prothrombotic events caused by vessel damage as a result of therapy allows for increased tumor cell 'seeding' and growth in distant organs[163]
- Altered endothelial cell adhesion molecule function may enhance VEGF-driven angiogenesis and tumor growth[164]
- Inflammatory pathway activation alters the endothelial microenvironment increasing intravasive and extravasive potential of tumor cells[72]

The perioperative setting

Perhaps the area where disease progression after therapy presents the biggest challenge (and the potential to show benefit) is in the perioperative setting, when treatments are administered either before (neoadjuvant) or after (adjuvant) surgery to remove the tumor. With studies underway in patients with CRC, RCC, NSCLC, breast and central nervous system cancers, it will be critical to determine safety parameters for wound healing and therapy toxicity to optimize guidelines,[86] and to determine the efficacy of VEGF-pathway inhibition in these settings.

Adjuvant therapy

Currently, there are over 200 adjuvant clinical trials planned or underway assessing antiangiogenesis drugs either alone or in combination with chemotherapy in cancer types including breast, RCC, prostate, head and neck, NSCLC, and ovarian.[87] The rationale for therapeutic intervention with VEGF-pathway inhibitors in the postoperative setting was summarized by Bagri *et al.*[10] who highlighted the advantages of antiangiogenic blockade in preventing occult micrometastatic growth in distant sites. Most obvious is that because of the integral role of the vasculature in the stepwise process of metastasis, antiangiogenic therapy could compromise some of these steps in primary tumors such as preventing or delaying intravasation (for example via the destruction of the immature vasculature) and the 'angiogenic switch' in avascular metastases at distant sites.[10] Recently, two phase III postoperative adjuvant trials (C-08 and AVANT) that assessed bevacizumab in patients with stage II–III CRC were completed. Patients in both trials received either bevacizumab for 1 year (in combination with chemotherapy for the first 6 months) or 6 months of chemotherapy alone. The chemotherapy regimen FOLFOX (5-fluorouracil, leucovorin and oxaliplatin) was compared with bevacizumab in C-08, and FOLFOX or XELOX (oxaliplatin and capecitabine) was compared with bevacizumab in AVANT. The primary end point of a benefit in DFS after 3 years was not met in either trial, although in both the C-08 and AVANT trials indications of DFS improvement was observed following the 6-month bevacizumab maintenance period at the 1-year interim analysis, and at subsequent interim analyses (in C-08 only)—but the extent of the benefit diminished over time in both trials.[88,89] The basis of this 'fading' effect is unknown, and questions remain as to whether long-term bevacizumab maintenance should be tested in follow-up studies to potentially prolong the observed DFS benefits (as was seen in the GOG0218 and ICON7 ovarian cancer trials[37]). However, it is important to question if DFS benefits translate into overall survival benefits and, if not, do they justify the associated costs and toxicity of using a drug such as bevacizumab? As well, and perhaps overshadowing such questions, the AVANT trial results demonstrated that patients receiving bevacizumab with chemotherapy had numerical increases in disease relapse and death compared with chemotherapy alone.[24] Though firm conclusions cannot be made based on early reporting of trial results, and it is possible that patient crossover in the control group may have had a role in these observations (these patients later received bevacizumab), it remains an open question whether bevacizumab was a detriment in this trial—a point raised by the trial organizers.[8] Regardless, in both trials, the fact that DFS changed rapidly after bevacizumab was halted requires further study and highlights the importance of undertaking appropriate preclinical studies to examine the mechanisms by which antiangiogenic treatments lose their activity and/or alter tumor progression and metastasis over time, especially in the adjuvant setting (Figure 2).

Neoadjuvant therapy

In neoadjuvant therapy, the theoretical advantages of antiangiogenesis treatment are twofold. First, to elicit an objective reduction in tumor size, usually to downstage an unresectable tumor or improve the impact of surgery of resectable tumors and second, to prevent micrometastatic outgrowth, increasing the potential for PFS and overall survival benefits.[90,91] There are over 100 ongoing neoadjuvant trials using VEGF-pathway inhibitors, either alone or in combination with chemotherapy, radiation, or other therapies (Table 3 and Supplementary Box 6 online).[87]

To date, there are few, if any, preclinical studies that have been conducted with antiangiogenic drugs (or any other drug types) that analyze neoadjuvant or presurgical treatments,[81,92] and virtually none that compare treatment effects on primary tumors to metastatic-disease progression (or

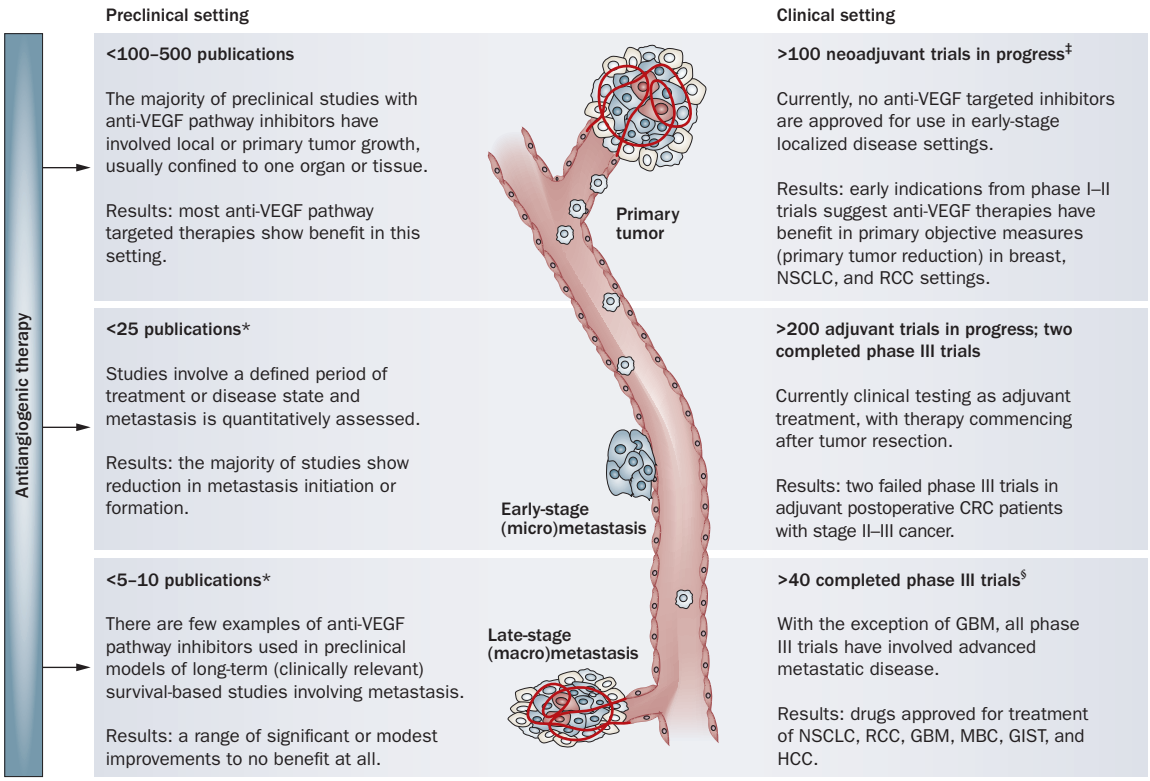

Figure 2 | Variable efficacy of VEGF pathway-targeted therapies: exposing the gap between preclinical and clinical testing. The number of studies that have been completed in the clinic in each setting are inversely correlated with the number of preclinical publications that model each setting. *See Supplementary Table 2 online. ‡See Table 3. §See Table 1. Abbreviations: CRC, colorectal cancer; GBM, glioblastoma muliforme; GIST, gastrointestional stromal tumors; HCC, hepatocellular carcinoma; MBC, metastatic breast cancer; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma.

prevention) after resection. Similar to post-operative adjuvant studies, preclinical neoadjuvant studies could be useful to distinguish between relative efficacy effects of a drug in both the primary and metastatic settings and to evaluate the usefulness of antiangiogenic therapies in this treatment setting (Supplementary Box 7 online).

Opportunities and challenges

With the success of VEGF-pathway inhibitors in the clinic, and the discovery of limitations to their use, what should be the priority for researchers and clinicians? First, clearly there is a need to evaluate new therapies in the most appropriate cancer models possible, at various stages of disease and metastatic spread, even if this means observed benefits may not be as significant as has traditionally been the case in preclinical testing. Less impressive gains in more challenging disease models may equate to more clinical relevance.[93] Second, in using such models it might be possible to uncover therapeutic agents that have differential activity in different settings, for example, efficacy in localized tumor growth but not in slowing (or preventing) micrometastatic or macrometastatic disease. Of course, uncovering drugs or therapies that have the opposite properties could be extremely important as well, that is, no effect in established tumors but with antimetastatic activity.[94] Several examples of inhibitors or treatment strategies with such effects have been noted, including those targeting the TGF-β, integrin, and c-met/HGF pathways; nuclear and cellular protein inhibitors (for example, agents targeting NF-κB, Grb-2, and RhoC); and other compounds, such as propranolol and cyclopamine.[80] Such antimetastasic (but not 'antiprimary') properties have also been noted with chemotherapy regimens administered continuously at low doses (termed 'metronomic chemotherapy') in preclinical models of MBC (cyclophosphomide and UFT, a 5-fluorouracil prodrug), and melanoma (cyclophosphomide and vinblastine).[75] These findings raise the possibility of more rational combination studies; for example, could the limitations of antiangiogenic agents be overcome (or delayed) by combination with an antimetastatic agent, which itself may not have potent antitumor properties? Third, it is critical to perform studies in preclinical models that assess drug treatments or combinations that closely mimic phase III clinical trials, ideally using similar (or equivalent) quantitative assessments to PFS and overall survival. An example was performed by Singh *et al.*[85] who used two different GEMMs involving *KRAS* mutations (modeling NSCLC and pancreatic cancer) to compare 'standard of care' chemotherapy regimens with inhibitors of EGFR (erlotinib) and VEGF, using a bevacizumab-equivalent mouse-specific antibody. Such large-scale preclinical studies, despite a high expense, could be used as surrogates for clinical trials as retrospective study tools to understand failure (as in the Singh *et al.*[85] study) or prospectively to assess and predict results for ongoing or planned phase III trials.[95] Finally, with respect to determining whether VEGF-pathway inhibitors can lead

Table 3 | VEGF pathway-targeted drugs currently in neoadjuvant clinical trials

Search criteria*	Cancer	Number of trials (drugs used)					
		Total	Mono-therapy‡	With MTT	With hormone therapy	With chemotherapy§	With radiation
Neoadjuvant and sunitinib	Breast, renal, bladder, soft-tissue sarcoma, GIST, prostate	17	10	–	2 (exemestane, LHRH agonist)	4 (TCARB, gemcitabine, cisplatin, docetaxel)	2
Neoadjuvant and sorafenib	Breast, renal, soft-tissue sarcoma, prostate, rectal, SCCHN	12	5	–	1 (letrozole)	6 (cisplatin, capecitabine, IE, ifosfamide, ixabepilone, LDM CTX)	5
Neoadjuvant and pazopanib	Breast, NSCLC	3	3	–	–	1 (docetaxel)	–
Neoadjuvant and zactima	Breast, NSCLC, esophageal	3	2	–	1 (anastrozole)	1 (docetaxel and carboplatin combination)	1
Neoadjuvant and cediranib	Breast	1	–	–	–	1 (docetaxel, doxorubicin and cyclophosphamide combination)	–
Neoadjuvant and semaxinib	Soft-tissue sarcoma	1	–	–	–	1 (doxorubicin, ifosfamide and dacarbazine combination)	1
Neoadjuvant and bevacizumab	Breast, renal, bladder, soft-tissue sarcoma, prostate, esophageal, cervical, colorectal, urothelial, rectal, NSCLC, glioblastoma, pancreatic, ovarian, uveal melanoma, gastric or adrenal	63	2	3 (trastuzumab, cetuximab)	4 (letrozole, AI)	65 (docetaxel, carboplatin, capecitabine, gemcitabine, irinotecan, cisplatin, 5-FU, XELOX, FOLFOX, FOLFOXIRI, TC, M-VAC, TAC, TCARB, DC, AC, FEC, IC, DG, OCFL-BC, DECNC, ECX)	10

*Searched in www.clinicaltrials.gov, only active or completed studies (recruiting or non-recruiting) included. Studies include local (primary) disease and metastasis deemed surgically operable. If perioperative therapy included separate regimens, only the VEGF-pathway inhibitor was included in the table. In all studies overall survival is a secondary end point. All searches filtered for 'intervention' trials only. ‡At least one study arm testing drug alone. §Docetaxol and paclitaxol used interchangeably unless otherwise indicated. Abbreviations: 5-FU, 5-fluorouracil; AC, doxorubicin and cyclophosphamide; AI, undefined aromatase inhibitor; DC, docetaxel and capecitabine; DECNC, docetaxel, epirubicin, cyclophosphamide, navelbine and capecitabine; DG, docetaxol and gemcitabine; ECX, epirubicin, cisplatin and capecitabine; FEC, 5-FU, epirubicin and cyclophosphamide; FOLFOX, 5-FU, leucovorin and oxaliplatin; FOLFOXIRI, 5-FU, leucovorin, oxaliplatin and irinotecan; GIST, gastrointestinal stromal tumors; IC, irinotecan and cisplatin; IE, epirubicin and ifosfamide; LDM CTX, low-dose metronomic cyclophosphamide; LHRH, luteinizing hormone-releasing hormone; MTT, molecular-targeted therapy; M-VAC, methotrexate, vinblastine, adriamycin and cisplatin; NSCLC, non-small-cell lung cancer; OCFL-BC, oxaliplatin-CPT-11, 5-FU, leucovorin, bevacizumab and cetuximab; SCCHN, squamous cell carcinomas of the head and neck; TC, docetaxel and cyclophosphamide; XELOX, capecitabine and oxaliplatin.

to more invasive and metastatic phenotypes (despite initial positive effects in terms of tumor shrinkage, PFS or overall survival benefits), it will be critical to properly assess (and compare) different modes of inhibition (antibodies versus TKIs) in different disease stages (localized verses micrometastasis and macrometastasis) to understand disease progression on (or off) therapy. This will be essential for understanding the basis of any future rationale for extended treatments in patients, such as the initial benefits seen in certain settings where treatment extends beyond disease progression. In addition, the differences between PFS and overall survival benefits in clinical trials investigating anti-angiogenic drugs could have important implications for the future use of VEGF-pathway-targeted agents. For example, in 2010, the FDA rejected full approval of bevacizumab with chemotherapy in patients with MBC based on toxicities and the lack of overall survival benefits and diminishing PFS benefits in the AVADO and RIBBON-1 trials compared to the earlier E2100 trial (Tables 1 and 2 and Box 3).

Conclusions

While efficacies of VEGF-pathway-targeted therapies in certain cancer settings represent a conceptual and practical medical success, the lack of substantial benefits for the vast majority of patients in terms of increased long-term overall survival times remains an ongoing challenge. Understanding the basis of these treatment limitations will likely be key to devising improved strategies and to overcome the possible difficulties facing further development of antiangiogenic therapies used at all stages of tumor progression.

Review criteria

Data in this Review were compiled from databases searched before 1 February 2011, including PubMed and MEDLINE; conference proceedings (AACR, ASCO, and others); company website and trial information releases; and online search engines for press releases of failed or terminated trials. Information on phase III trials and drug approvals was from NIH databases (www.clinicaltrials.gov, www.cancer.gov/clinicaltrials) and the FDA website (www.fda.gov). Searches used every drug name in this article, as well as combinations of biological and disease-stage terms such as 'VEGF- or VEGFR-inhibitors', 'antiangiogenic therapy', 'metastasis', 'adjuvant', 'neoadjuvant', 'perioperative'.

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Author contributions

J. M. L. Ebos researched the data for the article and wrote the manuscript. Both authors had a substantial contribution to discussion and editing of content.

Supplementary information is linked to the online version of the paper at www.nature.com/nrclinonc

CORRECTION

Antiangiogenic therapy: impact on invasion, disease progression, and metastasis

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In the version of this article initially published online, the title of Table 2 should have read 'Unsuccessful or terminated phase III trials with anti-VEGF pathway agents'; in the last row of Table 2 an asterisk has been added to the PFS column; the title of Box 3 should have read 'Possible mechanisms influencing invasion and metastasis after therapy'; the sentence on page 7, column 2 beginning 'In both trials, the rapid change in PFS after bevacizumab was halted, which requires...' should have read 'Regardless, in both trials, the fact that PFS changed rapidly after bevacizumab was halted requires...'; and in Table 3 'CAPOX' has been deleted. The errors have been corrected for the print, HTML and PDF versions of the article.

CORRECTION

Antiangiogenic therapy: impact on invasion, disease progression, and metastasis

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In the Review article published in the April issue of *Nature Reviews Clinical Oncology* there were errors in the clinical data presented in Tables 1 and 2. In addition, the primary end point of the adjuvant AVANT and NSABP-C-08 trials should have been disease-free survival (DFS) instead of progression-free survival (PFS). The errors have been corrected for the HTML and PDF versions of the article.