CLINICAL INVESTIGATION

Therapeutic targeting of tumor angiogenesis: how far have we come?

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Angiogenesis, the formation of new blood vessels from existing ones, is a potentially important therapeutic target. The discovery of the role of VEGF in angiogenesis prompted research on two major classes of antiangiogenic drugs: those acting through VEGF itself and those acting on VEGF receptors. Combination trials, particularly with cytotoxic drugs, followed single-agent clinical trials in several cancer types including colorectal, breast and non-small-cell lung cancer. Positive findings of tumor responses were balanced by later analyses showing in many trials that overall survival was not significantly increased. The challenge for the future is to identify appropriate biomarkers that will allow patient selection for optimal therapy.

Keywords: angiogenesis • antiangiogenic • bevacizumab • combination chemotherapy • sunitinib • VEGF • VEGFR

Tumor tissue, like normal tissue, is dependent on a functioning vascular network for the delivery of oxygen and nutrients. However, tumor tissue differs from most normal tissues by being in a state of net expansion. Angiogenesis, the formation of new blood vessels from existing ones [1], was hypothesized to be necessary for tumors to grow to a diameter of more than 1 mm, prompting the concept that inhibition of tumor angiogenesis would provide effective therapy for a broad range of malignancies [2]. Most research at that time involved studies with murine tumor models and the antiangiogenic compounds identified included angiostatin, a 38 kDa internal fragment of plasminogen released by enzymatic cleavage [3], endostatin, a 20 kDa C-terminal fragment of collagen XVIII that is stored in platelets and released by inhibition of cyclooxygenases [4] and TNP-470, a synthetic fumagillin analog that inhibits methionine aminopeptidase [5]. Further work identified a number of further compounds including neovastat [6], SU6668 [7], ABT-510 [8] and squalamine [9], which showed evidence of antiangiogenic effects in mouse tumor models. In this review, we would like to provide an overview of the work and thinking that has occurred since that time. The clinical literature on antiangiogenic agents is vast and we can only cover some of it, pointing out both positive and negative aspects. We have provided some commentary on possible reasons for negative results and finish by briefly describing the differences between antiangiogenic drugs and vascular disrupting agents, the other class of anticancer drug that is directed to the tumor vasculature.

VEGF as a target

Against this background of results with antiangiogenic compounds that had poorly defined targets of action, the discovery [10] that the cytokine VEGF was preferentially expressed in tumor tissue provided a large impetus to the development of antiangiogenic therapy. VEGF, also called vascular permeability factor because of its action on tumor vasculature, was found to be capable of inducing angiogenesis in experimental systems [11] and also of protecting the vascular endothelium from apoptosis [12]. There are five structurally related members of the VEGF family forms (VEGFA, VEGFB,

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VEGFC, VEGFD and placental growth factor/PIGF) but this diversity is increased by alternative splicing and processing. These ligands act on a series of tyrosine kinase receptors including VEGFR-1, VEGFR-2 and VEGFR-3 [13] as well as on neuropilins and integrins that act as co-receptors [14]. Dvorak and colleagues introduced the principle of blocking either VEGF or its receptors as an approach to antitumor therapy.

The development of mouse antibodies to VEGF [15] was followed by the clinical introduction in 1997 of bevacizumab (Avastin®), a humanized antibody that targeted VEGF-A. Bevacizumab had a long plasma half-life and could therefore effectively reduce circulating VEGF-A, and thus corresponding VEGFR responses, for a long period of time. Bevacizumab became widely used in combination with conventional anticancer drugs such as 5-fluorouracil, carboplatin and paclitaxel in a variety of malignancies [16], more recently including glioblastoma [17,18], cervical cancer [19] and ovarian cancer [20]. Ziv-aflibercept, an engineered soluble VEGF 'decoy' receptor comprising the extracellular domains of VEGFR1 and VEGFR2 fused to an Fc segment of immunoglobulin G1, provided a further addition to the group of antiangiogenic drugs with an action that was potentially similar to that of bevacizumab [21].

Therapies targeting VEGF or the extracellular portion of the VEGF receptor were complemented by the development of drugs that targeted the VEGF receptor tyrosine kinase; this included ramucirumab, an antibody that targets the extracellular domain of VEGFR-2 [22].

Approved anticancer drug therapies targeting vascular growth factors or their receptors are shown in Table 1. The rationales developed with earlier work on drugs targeting EGF receptor tyrosine kinase in cancer cells were applied to VEGFR [23] leading to a number of drugs including sunitinib [24], sorafenib [25,26], pazopanib [27], vandetinib [28], axitinib [29], cabozantinib [30] and TAS-115 [31]. A further strategy was based on the principle of inhibiting cellular receptors other than VEGFR which contribute to angiogenesis, such as PDGFR. This strategy included agents such as dasatinib [32], nilotinib [33], regorafenib [34] and ponatinib [35].

Rationales for combination clinical trials with antiangiogenic agents

The efficacy of a two-pronged approach, combining inhibition of tumor cell proliferation and inhibition of tumor angiogenesis, was suggested by combination studies in mice [44]. This concept was further bolstered by the hypothesis that 'vascular normalization,' would provide a possible rationale for the administration of combination therapy. It was known from clinical as well as experimental studies that the many tumors were characterized by a low vascular density, which in turn led to a situation where the consumption of oxygen by some regions occurred at rates that were greater than those of oxygen diffusion across the tumor, resulting in increased hypoxia among tumor cells most distant to the vasculature. This in turn activated HIF-1α, leading to the transcription of a number of genes including that for VEGF. Increased tumor tissue concentrations of VEGF lead to both sprouting of the vasculature and to increased vascular permeability; the vascular normalization hypothesis postulates that inhibition of VEGF action, as a consequence of either blocking the external VEGF signal or blocking the corresponding tyrosine kinase, reduces both angiogenic sprouting and vascular permeability, correspondingly increasing blood flow through individual tumor vessels and thus improving delivery of cytotoxic drugs, targeted antitumor drugs and immunotherapy to tumor cells [45,46]. Thus, an antiangiogenic drug might exert multiple effects with different time courses in a combination framework, improving drug delivery at earlier times and inhibiting vascular expansion at later times.

The normalization hypothesis was supported by experimental studies in mice, as well as by limited studies in human cancer. For instance, glioblastoma patients have been reported to experience increased tumor vascular perfusion and improved tumor oxygenation following administration of the antiangiogenic agent cediranib in conjunction with temozolomide as a standard cytotoxic agent [47]. However, some experimental studies have also indicated that administration of antiangiogenic drugs reduces tumor oxygenation [48]. The observation that some antiangiogenic therapies can render tumors hypoxic while others show improved tumor oxygenation has been discussed in terms of the heterogeneity of individual capillary transit times [48]. Thus the two effects of antiangiogenic agents, increased tumor blood flow and decreased vascular area, can act in opposite pharmacological directions. The overall effect of administration of an antiangiogenic drug on uptake of an antitumor drug may therefore depend on both the vascular organization of the tumor and the pharmacological properties of the antitumor drug.

Antiangiogenic agents in clinical combination chemotherapy

An early pivotal study in metastatic colorectal cancer demonstrated that addition of bevacizumab to a combination regimen involving irinotecan, 5-fluorouracil and leucovorin produced statistically significant ($p < 0.001$) increases in the duration of progression-free survival (6.2–10.6 months), and median survival

Table 1. Antiangiogenic protein-based therapies targeting VEGFs or receptors and their pivotal clinical trials and approvals in treating cancer.

Drug	Mode of action	Approved disease indication	Clinical setting	Pivotal clinical trial		
				Treatment and sample size	*Primary endpoint: result*	Ref.
Bevacizumab (Avastin®)	Human VEGF-binding monoclonal antibody	Metastatic colorectal cancer	With 5-fluorouracil based chemotherapy for first- or second-line treatment	IFL chemotherapy plus bevacizumab (n = 402) or placebo (n = 411)	OS: median, 20.3 vs 15.6 months; HR, 0.66; $p < 0.001$	[36]
			With fluoropyrimidine-irinotecan- or fluoropyrimidine-oxaliplatin-based chemotherapy for second-line treatment after progression on first-line bevacizumab-containing therapy	FOLFOX4 chemotherapy with (n = 286) or without bevacizumab (n = 291)	OS: median, 13 vs 10.9 months; HR, 0.75; $p = 0.001$	[37]
				Fluoropyrimidine-based chemotherapy with (n = 409) or without bevacizumab (n = 411)	OS: median, 11.2 vs 19.8 months; HR, 0.81; $p = 0.0057$	[38]
		Glioblastoma	As single agent after progressive disease following prior therapy	Bevacizumab alone (n = 85)	Tumor response: rate, 25.9%; median duration, 4.2 months	[39]
		Nonsquamous non-small-cell lung cancer	With carboplatin and paclitaxel as first line treatment of unresectable, locally advanced or metastatic disease	Carboplatin and paclitaxel with (n = 434) or without bevacizumab (n = 444)	OS: median, 12.3 vs 10.3 months; HR, 0.80; $p < 0.013$	[40]
		Metastatic renal cell carcinoma	With IFN-α	IFN2a plus bevacizumab (n = 327) or placebo (n = 222)	PFS: median, 10.2 vs 5.4 months; HR, 0.6; $p < 0.0001$	[41]
Aflibercept (Zaltrap®)	Human VEFG-A, -B, -C and PIGF-binding soluble receptor	Metastatic colorectal cancer	Disease resistance or progressive following an oxaliplatin-containing regimen	FOLFIRI chemotherapy plus ziv-aflibercept (n = 612) or placebo (n = 614)	OS: median, 13.15 vs 12.06 months; HR, 0.817; $p = 0.0032$	[42]
Ramucirumab (Cyramza®)	VEGFR2-binding monoclonal antibody	Advanced gastric or gastro-esophageal junction adenocarcinoma	As single agents after prior fluoropyrimidine- or platinum-containing chemotherapy	Ramucirumab (n = 238) or placebo (n = 117)	OS: median, 5.2 vs 3.8 months; HR, 0.776; $p = 0.047$	[43]

HR: Hazard ratio; IFL: Irinotecan, leucovorin (folinic acid), and fluorouracil; OS: Overall survival; PFS: Progression-free survival.

(15.6–20.3 months) [36]. Many clinical combination studies have subsequently reported positive results and a number of agents are now used as part of standard clinical practice. Table 1 lists approved protein-based antiangiogenic agents, together with key trials that led to their approval. In addition, a recent study of bevacizumab in combination with standard pelvic chemoradiation therapy for locally advanced cervical cancer has shown promising results [49].

The analysis of clinical trials of the small molecular-weight tyrosine kinase inhibitor class of antiangiogenic drugs is complex because any observed antitumor responses may reflect, as they do for antibodies such as bevacizumab, effects of the drug on tumor cells rather than on tumor endothelial cells. While VEGFR is expressed by tumor vascular endothelial cells, it is also expressed by tumor cells [50,51], and the growth of such cells in culture is potentially inhibited by cellular actions of tyrosine kinase inhibitors that act on VEGFR (it is worthwhile mentioning that depletion of circulating VEGF by bevacizumab may also affect the behavior of tumor cells expressing VEGFR). Table 2 lists approved kinase inhibitors together with key trials that led to their approval; only agents acting on renal cell carcinoma are included as this disease is considered to be clearly driven by aberrant angiogenic signaling. In cancers, such as gastrointestinal stromal tumors, leukemias and thyroid tumors where kinase inhibitors are approved, the exact contribution of inhibition of angiogenesis is less clear and these agents have therefore been omitted from the table.

Negative results in clinical trials of angiogenic agents

Against the above background of positive results, a key retrospective survey in 2011 highlighted a number of clinical combination chemotherapy studies, including those for breast, prostate, ovary, lung, gastric, pancreatic and colorectal cancer, where addition of bevacizumab to drug combinations failed to provide a consistent increase in overall survival [53]. Since that time, a number of Phase III combination clinical trials have been reported that also failed to demonstrate a survival advantage. In metastatic colorectal cancer, a Phase III randomized trial (AVANT) concluded that addition of bevacizumab did not improve the efficacy of oxaliplatin-based chemotherapy in the adjuvant treatment of patients with resected stage 3 or high-risk stage 2 colon carcinoma [54]. A randomized Phase II study testing the capacity of bevacizumab or axitinib in combination with FOLFOX (5-fluorouracil/leucovorin/oxaliplatin) or FOLFIRI (5-fluorouracil/leucovorin/irinotecan) to extend survival in second-line treatment of metastatic colorectal cancer also produced negative results [55]. A double-blind, Phase III study concluded that sunitinib plus FOLFIRI (fluorouracil, leucovorin and irinotecan) was not superior to FOLFIRI alone in previously untreated patients. In non-small-cell lung cancer, a multicenter, randomized, placebo-controlled trial assessed the efficacy of sorafenib in combination with carboplatin and paclitaxel in first-line therapy of patients with unresectable stage IIIB or stage IV non-small-cell lung cancer, and found no survival advantage [56].

Table 2. Antiangiogenic small molecular-weight inhibitors of vascular receptor kinases & their pivotal clinical trials and approvals in treating renal cell carcinoma.

Drug	Mode of action	Pivotal clinical trial		Ref.
		Treatment and sample size	Primary endpoint: result	
Axitinib (Inlyta®)	Inhibits multiple tyrosine kinases including VEGFR-1, VEGFR-2 and VEGFR-3	Axitinib (n = 361) or sorafenib (n = 362)	PFS: median, 6.7 vs 4.7 months; HR, 0.67; p < 0.0001	[29]
Pazopanib (Votrient®)	Inhibits multiple tyrosine kinases including VEGFR-1, VEGFR-2, VEGFR-3, PDGFRA, PDGFRB, FGFR1, FGFR3, ITK, KIT, LCK and CSF1R	Pazopanib (n = 290) or placebo (n = 145)	PFS: median, 9.2 vs 4.2 months; HR, 0.46; p < 0.001	[27]
Sorafenib (Nexavar®)	Inhibits multiple kinases including CRAF, BRAF, mutant BRAF, KIT, FLT-3, RET, VEGFR-1, VEGFR-2, VEGFR-3, and PDGFRB	Sorafenib (n = 384) or placebo (n = 385)	PFS: median, 167 vs 84 days; HR, 0.44; p < 0.000001	[52]
Sunitinib (Sutent®)	Inhibits multiple kinases including PDGFRA, PDGFRB VEGFR-1, VEGFR-2, VEGFR-3, KIT, FLT-3, RET and CSF1R	Sunitinib (n = 375) or IFNA (n = 375)	PFS: median, 47.3 vs 22.0 weeks; HR, 0.415; p < 0.000001	[24]

HR: Hazard ratio; PFS: Progression-free survival.

In breast cancer, an analysis of early results concluded that no Phase III trial had yet demonstrated overall and progression-free survival benefits [57]. A randomized, placebo-controlled, double-blind, Phase II study also showed that combination of axitinib did not improve response to docetaxel in first-line treatment of metastatic breast cancer [58] and a Phase III study showed that the addition of sunitinib did not improve responses to capecitabine [59]. A Phase III trial examining capecitabine in combination with sorafenib for the treatment of locally advanced or metastatic HER2-negative breast cancer (RESILIENCE trial) is underway [60]. In ovarian cancer, analysis of a large series of combination trials concluded that antiangiogenic therapy was beneficial to tumor response but its effects on overall survival were not clear [61]. A meta-analysis of four Phase III randomized controlled trials suggested that the addition of bevacizumab to chemotherapy offered meaningful improvement in objective response rate but not in overall survival [62]. A recent randomized placebo-controlled Phase III trial (OCEANS) concluded that addition of bevacizumab to gemcitabine and carboplatin significantly improved investigator-determined progression-free survival and objective response rate in platinum-sensitive, recurrent ovarian cancer [63]. In glioblastoma, a randomized, double-blind, placebo-controlled trial demonstrated prolonged progression-free survival but not overall survival in bevacizumab-treated patients undergoing radiotherapy [18]. A Phase III double-blind, placebo-controlled trial in patients with newly diagnosed glioblastoma showed that bevacizumab did not significantly increase progression-free survival [64]. A further trial in patients treated with radiotherapy and temozolomide treatment concluded that addition of bevacizumab improved quality of life and performance status but not overall survival [17].

The current clinical picture of combination antiangiogenic therapy thus provides a balance of positive and negative results. It is also worth emphasizing that patient cross-over design in randomized clinical trials, as well as the availability or other therapies administered after patients progress on the experimental drug assessment, makes assessment of overall survival difficult. Nevertheless, the earlier optimism that such therapy would greatly benefit a wide variety of cancer types has given way to a view that we need to understand more of the factors involved in clinical response, and to develop some form of patient selection strategy to maximize the benefits of therapy.

Reasons for negative clinical results for antiangiogenic therapy

One possible reason for a lack of response to antiangiogenic agents is that the antiangiogenic agent utilized may not have the correct specificity for the receptors expressed in tumor vasculature [65]. Angiogenesis may be dependent on only one VEGFR type, or on receptors for other angiogenic factors such as ephrinB2, PDGF-β and FGF [66,67]. Randomized Phase III trials in which patients are selected or stratified on the basis of biomarkers such as elevated VEGF expression have not yet been reported and further clinical work to evaluate the expression of vascular receptors to angiogenic factors by individual tumors is required.

A second possible reason for the failure of antiangiogenic therapy is that nonresponding human cancers are not utilizing angiogenesis for growth. Angiogenesis is only one of several possible mechanisms for tumor expansion; other mechanisms include invasion by tumor cells of existing normal vasculature, recruitment of circulating endothelial precursor cells to the vasculature and phenotypic conversion (vasculogenic mimicry) of tumor cells to those expressing endothelial function [68]. Angiogenesis may therefore be a feature of more rapidly growing tumors, meaning that antiangiogenic therapy could be improved by selecting for rapidly growing tumors. A number of tumor imaging studies have been carried out to estimate tumor growth [69,70] and the diagram in Figure 1 indicates the approximate distribution of human tumor doubling times obtained in these studies. Some human malignancies, particularly germ cell tumors, pediatric tumors and leukemias, have relatively short tumor volume doubling times [70]. Furthermore, there is evidence that markers of angiogenesis in human tumors decline with increased age [71], suggesting that the efficacy of antiangiogenic therapy might also decline with age, suggesting that tumors from older patients have reduced reliance on angiogenesis. It should be noted that the cycle times of human tumor cells, measured either *in vivo* [69] or after initial transfer to culture [72], are shorter (typically in the range 3 days to 3 weeks) than the volume doubling times of tumors, indicating that measurement of tumor cytokinetics is unlikely to predict the rate of tumor angiogenesis.

It is pertinent to compare the volume doubling times of human tumors with those for murine tumors, which have been used extensively in the development of antiangiogenic drugs. As indicated in Figure 1, tumor volume doubling times of murine tumors, as well as those of human tumor xenografts in murine hosts, are generally in the range 2–5 days, about 30-fold shorter than that for human tumors. Angiogenesis may therefore be more active in murine tumors than in human tumors, helping to explain why antiangiogenic drugs have generally been found to be highly effective against murine tumors. Measurement of the rates of proliferation of tumor endothelial cells would be of great help in

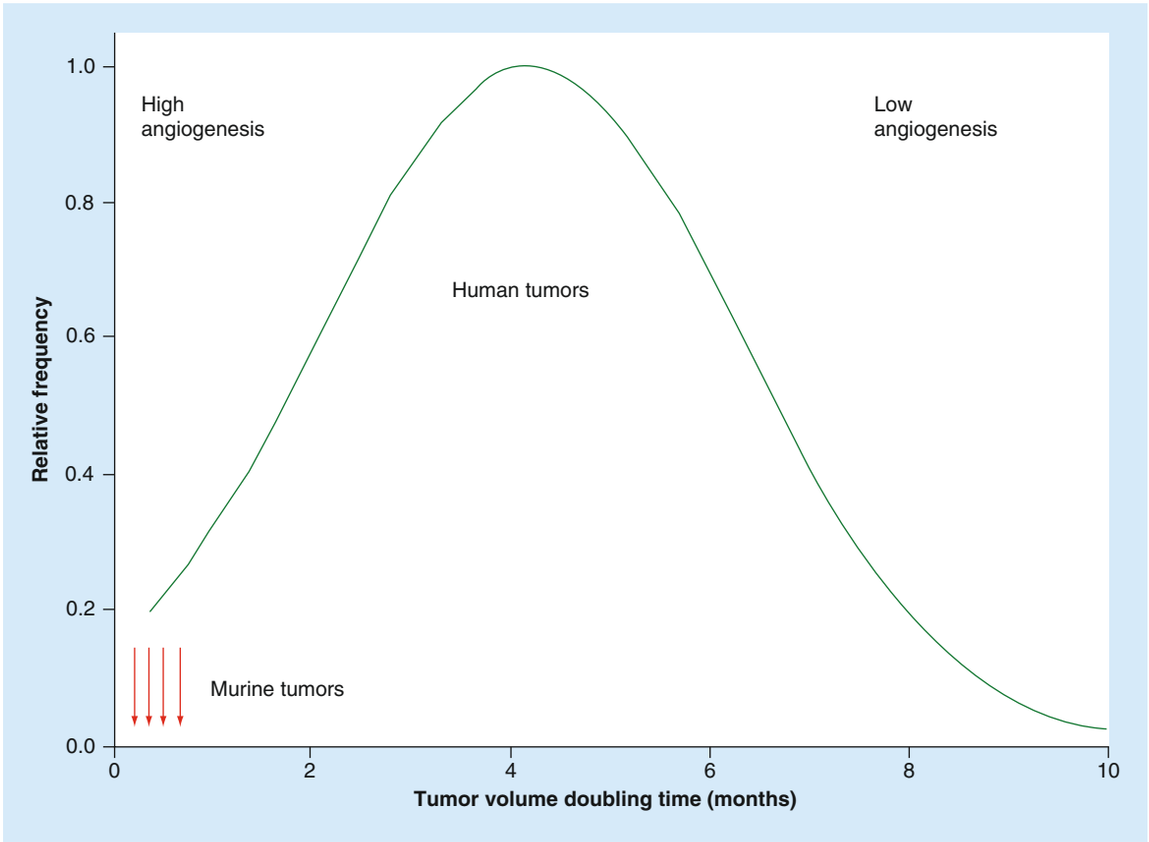

Figure 1. Approximate distribution of human tumor volume doubling times (green line), based on published data [70] as compared with those of murine tumors and of human tumor xenografts growing in immunodeficient mice (red arrows). More rapid tumor growth rates (shorter doubling times, on left) may reflect higher rates of tumor angiogenesis and correspondingly higher susceptibility to antiangiogenic agents.

comparing the contribution of angiogenesis to tumor growth in different species. A study in murine tumors estimated endothelial cell doubling times between 2.4 and 13 days [73]. A study of human tumors could not measure endothelial cell doubling times directly but compared proliferating capillary indices in tissue sections. Glioblastomas showed the highest index, followed by renal cell carcinomas, colon carcinomas, mammary carcinomas, lung carcinomas and prostate carcinomas [74].

Antiangiogenic therapy in comparison to vascular disrupting therapy

Tumor vasculature is generally thought to show increased vascular permeability in comparison with normal tissue [75], which is in turn related to increased VEGF production in response to hypoxia and other factors [76]. Such increased endothelial permeability leads to higher interstitial pressures and reduced blood vessel diameters, which combine to decrease tumor blood flow [77]. This gives rise to two main treatment strategies, as shown in Figure 2; antiangiogenic agents, reviewed here, decrease tumor vascular permeability by 'normalizing' the tumor vasculature, while tumor vascular disrupting agents (VDAs) further increase tumor vascular permeability to disrupt vascular function [78]. It should be noted however that antiangiogenic drugs, by decreasing microvascular density and increasing hypoxia, can also induce regional VEGF production with consequent increased tumor vascular permeability. It is pertinent to mention here the clinical results of trials with the two main tumor VDAs that have been advanced to clinical trial, vadimezan (DMXAA) and fosbretabulin (combretastatin-A4 phosphate) [79]. Vadimezan was tested in two Phase III combination therapy trials in patients with non-small-cell cancer but failed to induce a significant increase in survival [79,80]. Fosbretabulin was tested in a Phase II/III combination therapy trial in patients with anaplastic thyroid cancer but also failed to induce a significant increase in survival [81]. Increased overall survival has not yet been demonstrated in a Phase III trial.

Conclusion

It is evident that incorporation of antiangiogenic agents into combination therapy has resulted in clini-

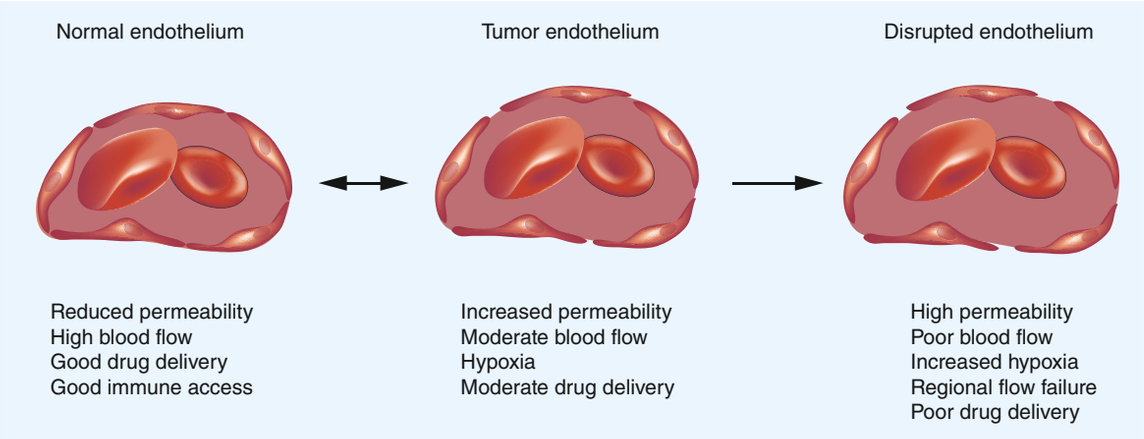

Figure 2. Comparison of the actions of two main classes of drugs that act on tumor vasculature. Antiangiogenic agents and tumor vascular disrupting agents tend to have opposite effects on vascular function, one improving function and the other further compromising it.

cal responses, with the result that several of these agents are now included in standard chemotherapy regimens. On the other hand, it is also clear that a number of Phase III trials have failed to demonstrate a significant increase in overall survival. There is now a need to study underlying mechanisms of tumor angiogenesis in individual cancer patients, so that antiangiogenic treatment can be tailored for maximum effect.

Future perspective

The main challenge for the future is to identify definitive biomarkers that will select patients who can potentially benefit from antiangiogenic therapy. In particular, it is important to utilize biomarkers to determine, in cases where a clinical benefit has been obtained, whether it has resulted from an effect on the tumor vasculature or from an effect on tumor cells. Circulating VEGF and tumor neuropilin-1 (a co-receptor for VEGF) have both been suggested as potential predictive biomarkers for sensitivity to bevacizumab [82,83]. Clear cell renal carcinomas lacking a VHL gene are known to activate the HIF-1α pathway and to overexpress VEGF [84], and these are clearly worthy of further studies.

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Executive summary

- Tissues depend on a vascular network for the delivery of oxygen and nutrients.
- Vascular function in tumor tissue is compromised, mainly because of increased vascular permeability and consequent reduced blood flow.
- Reduced blood flow leads to reduced delivery of oxygen and nutrients, and to hypoxia
- Hypoxia in turn leads to increased production of VEGF, which acts on receptors on vascular endothelial cells to increase angiogenesis, formation of new blood vessels from existing ones.
- Antiangiogenic agents such as bevacizumab and aflibercept act to prevent VEGF from activating its receptors.
- Antiangiogenic agents such as sunitinib and axitinib act on VEGF receptors to prevent their downstream signaling.
- Both types of antiangiogenic agents have generally been employed in combination with other types of anticancer agent.
- While some clinical trials have shown that addition of antiangiogenic drugs to standard therapy has a clinical benefit, others have not.
- Possible reasons for this result are that nonresponding tumors do not require VEGF for angiogenesis, or that nonresponding tumors do not require angiogenesis for growth.
- Future work in this area is needed to assess the mechanisms utilized by individual tumors for growth and to tailor therapy to these mechanisms.

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