The Snark is a Boojum: the continuing problem of drug resistance in the antiangiogenic era

K. D. Miller*, C. J. Sweeney & G. W. Sledge Jr

Division of Hematology and Oncology, Indiana University, Indianapolis, USA

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If your Snark be a Snark, that is right:
Fetch it home by all means—you may serve it with greens,
And it’s handy for striking a light . .
“But oh, beamish nephew, beware of the day,
If your Snark be a Boojum! For then
You will softly and suddenly vanish away,
And never be met with again!”
Lewis Carroll
The Hunting of the Snark

Key words: angiogenesis, drug resistance, endothelial cells, growth factors

Angiogenesis, the process of new blood vessel formation, plays a central role in both local tumor growth and distant metastasis [1]. Healthy adults require angiogenesis only for wound healing, endometrial proliferation and pregnancy. Thus the inhibition of angiogenesis offers an attractive therapeutic target with little expected toxicity. Based on the low mutation rate of the genetically stable endothelial cells, antiangiogenic therapy was initially touted to be “a treatment resistant to resistance” [2]. Initial xenograft studies supported these theoretical predictions—widespread activity, limited toxicity and no resistance [3]. For a time it was argued that the long-sought oncologic ‘Snark’—disease control if not outright cure—was close at hand.

But could this Snark be a Boojum in disguise? Might the hard-learned lessons of chemotherapy resistance pertain to the novel antiangiogenics as well? Burgeoning laboratory and emerging clinical data suggest that the problem of resistance continues. In support of this contention, we propose the following theoretical and (often) substantiated mechanisms of acquired and de novo resistance to antiangiogenic therapies (Table 1).

Endothelial cell heterogeneity

Initial expectations of widespread activity and limited resistance assumed that ‘normal’ endothelia had been merely usurped by the ‘malignant’ tumor. As normal somatic cells, the endothelia would be incapable of mutating to a resistant phenotype. Moreover, all endothelial cells were presumed similar, if not identical; therefore antiangiogenic agents should be equally effective regardless of the tumor type. However, if endothelial cells are heterogeneous, the potential for selective sensitivity, if not frank resistance, exists.

We need only look to normal embryonic development to find clear evidence of endothelial heterogeneity. For example, endothelia in the brain and testes express high levels of the mdr protein, thereby limiting exposure to potentially harmful xenobiotics [4–6]. Differences in endothelial function become apparent when comparing the results of in vitro studies using different sources of ‘normal’ endothelial cells. Vascular cell adhesion factor-1 (VCAM-1) expression is induced on human umbilical vein endothelial cells (HUVEC) by both tumor necrosis factor alpha (TNF-α) and interleukin-1 alpha (IL-1α), whereas only TNF-α induced VCAM-1 expression on human dermal microvascular endothelial cells (HDMEC) [7]. The differential response was explained by distinct expression patterns of the CXC chemokine and interleukin-8 receptors.

St Croix et al. [8] recently compared the gene expression patterns of vascular endothelial cells derived from normal and malignant colorectal tissues. Of 170 transcripts analyzed, almost half (79) were differentially expressed, including 46 that were elevated at least 10-fold and 33 that were expressed at substantially lower levels in tumor-associated endothelial cells (TEC) compared with normal endothelium. Similar but not identical expression patterns were found in TECs from metastatic lesions and primary tumor sites. The energy-dependent efflux pump, P-glycoprotein (P-gp), is expressed in TECs but not in human umbilical vein endothelial cells [9, 10]. Vincristine with the P-gp antagonist verapamil, but not vincristine alone, inhibits angiogenesis induced by mouse sarcoma 180 cells suggesting P-gp expression in TECs has functional significance [11].

*Correspondence to: Dr K. D. Miller, Indiana Cancer Pavilion, 535 Barnhill Drive RT-473, Indianapolis, IN 46202, USA.
Tel: +1-317-274-0920; Fax: +1-317-274-3646; E-mail: kathmill@inpui.edu

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Table 1. Mechanisms of resistance to antiangiogenic therapy

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Individual differences in endothelial sensitivity to antiangiogenic agents should also be expected. Rohan et al. [12] found up to a 10-fold difference in the response to growth factor-stimulated angiogenesis in the corneal micropocket assay among 12 inbred mouse strains. More importantly, differential sensitivity to angiogenesis inhibitors was seen between mouse strains, with one demonstrating complete resistance to both TNP-470 and thalidomide [12]. Though the genetic factors underlying these differences are not known, variation in sensitivity among patients seems likely as well.

Tumor cell heterogeneity

Invasive cancers commonly express multiple angiogenic factors, some of which may display tissue specificity. A VEGF isoform, endocrine gland-derived vascular endothelial growth factor (EG-VEGF), induces proliferation, migration and fenestration in capillary endothelial cells derived from endocrine glands but has little effect on other endothelial cell types [13]. At least six different proangiogenic factors were identified in each of 64 primary breast tumors studied by Relf et al. [14], with the 121-amino acid isoform of VEGF predominating. Given such redundancy, it seems naive to assume that inhibition of a single factor would produce a sustained clinical effect. Genetic instability of the tumor may result in modulation of both the amount and type of proangiogenic factors expressed [15]. Such modulation may lead to selective outgrowth of a clone resistant to a given antiangiogenic agent.

Production of the same proangiogenic factor does not guarantee the same response to antiangiogenic therapy. Though Wilms’ tumor and neuroblastoma both predominantly produce VEGF, Wilms’ tumor is growth inhibited whereas neuroblastoma is resistant to a VEGF-directed monoclonal antibody [16]. The mechanism underlying this differential sensitivity (e.g. resistance) has not yet been elucidated. More recently, Kerbel [17] have shown that disruption of p53 in tumor cells reduces sensitivity to antiangiogenic metronomic therapy. Chronic hypoxia selects p53 mutant tumor cells resistant to hypoxia-induced apoptosis, leading to a more aggressive phenotype. Indeed, in most long-term xenograft studies of antiangiogenic therapy, tumors eventually progressed (though more slowly than controls) despite continued treatment [18–20].

Hypoxia is a key signal for the induction of angiogenesis. Hypoxia-inducible factors (HIF-1 and HIF-2) are heterodimeric transcription factors consisting of α and β subunits [21–23]. HIF-1α+ tumors have decreased hypoxia-induced VEGF expression and are less vascular but have accelerated growth in vivo compared with HIF-1α− tumors due to decreased hypoxia-induced apoptosis [24]. Yu et al. [25] isolated tumor cells based on their relative proximity to perfused vessels and compared HIF-1α expression and in vivo growth characteristics. In heterogeneous tumors HIF-1α+ cells were located in the perivascular areas and were more much highly dependent on proximity to blood vessels for their growth and survival in vivo than the HIF-1α− cells [26].

Antiangiogenic therapy assumes absolute dependence on angiogenesis and a uniform response to hypoxia, hypoglycemia and waste product accumulation—the physiologic stresses produced by effective inhibition of angiogenesis. Variation in the sensitivity of tumor cells to changing hormonal status, cytotoxic therapy and ionizing radiation are widely accepted and often offered explanations for the failure of such therapies. Should not a variable tolerance for hypoxia, and thus a variable response to antiangiogenic therapy, be expected? If tumor cells can rapidly acquire resistance to doxorubicin [27], why not to hypoxia? Several lines of evidence suggest this possibility. Hypoxia may be chronic due to consumption/diffusion limitations or periodic resulting from transient reductions in tumor blood flow (so-called cyclic hypoxia) [28]. Cyclic hypoxia is quite common (occurring in as many as half of tumor vessels) suggesting that the cancer cell’s natural environment is one of recurring hypoxic insults. Tumor cells by definition must evolve mechanisms to resist such cyclic hypoxia merely to survive. Some cancer cells may remain viable for prolonged periods of hypoxia [29, 30].

Impact of the tumor microenvironment

The complex interaction between tumor cells and the host microenvironment has been recognized for over a century [31, 32]. More recently, Fidler et al. [33] implanted human renal cell carcinoma (HRCC) cells obtained from a surgical specimen into different organs of nude mice. Tumors were then recovered and established in culture. The cell lines each had a unique karyotype, indicating that the local environment of each organ selected for different subpopulations of HRCC. Only those HRCC cells implanted orthotopically metastasized. In an intact-tissue orthotopic transplant model, colorectal lung metastases would only form tumors in athymic mice if implanted in the lung; implants in either the colon or skin did not form tumors [34]. Similarly, human colon cancer cells did not metastasize unless implanted orthotopically in the cecum [35], and injected human prostate cancer cells metastasized preferentially to transplanted human bone rather than transplanted human lung or native mouse bone [36].

The impact of the local tumor microenvironment on angiogenesis and response to antiangiogenic therapy has become increasingly clear. Gohongi et al. [37] implanted a gel impregnated with basic fibroblast growth factor (bFGF) or Mz-ChA-2 tumor in the cranial windows of mice without tumors or mice with subcutaneous or orthotopic cholangiocarcinoma tumors to study angiogenesis and tumor growth at a secondary site. The concentration of transforming growth factor-β1 (TGF-β1) in the
plasma of mice with orthotopic cholangiocarcinoma was 300% higher than that in the plasma of mice without tumors or with subcutaneous tumors. Similarly, angiogenesis in the cranial window was substantially inhibited in mice with orthotopic tumors but only minimally affected by subcutaneous tumors. In a xenograft pancreatic cancer model, orthotopic pancreatic tumors grew faster than ectopic tumors and metastasized in a similar way to advanced human pancreatic cancer. Orthotopic tumors expressed VEGF and maintained vascular density and hyperpermeability during exponential tumor growth. Orthotopic PANC-1 tumors also showed lower leukocyte–endothelial interactions in the early stage of growth compared with subcutaneous PANC-1 tumors [38].

The tumor microenvironment protects the endothelial compartment. Medium conditioned by colon cancer cells increases extracellular signal regulated kinase-1/2 (Erk-1/2) phosphorylation and decreases apoptosis of HUVECs compared with medium conditioned by non-malignant cells [39]. The resistant phenotype can be reproduced in vitro by the addition of VEGF and/or bFGF to HUVEC culture systems. HUVEC antiapoptotic pathways stimulated by VEGF and/or bFGF include (but are by no means limited to) the following: p44 mitogen-activated protein kinase (MAPK), c-jun-NH2-kinase (JNK), phosphoinositide 3-OH kinase (PI-3-kinase), Bcl-2, inhibitors of apoptosis (IAP) and survivin [40–44]. Pericytes invest mature vasculature and provide critical survival signals to vascular endothelial cells. Differences in percyte coverage among tumor types has obvious implications for vessel maturation, survival and sensitivity to antiangiogenic therapies [45].

As many pro- and antiangiogenic factors are contained in or released from the extracellular matrix, differential sensitivity based on site of disease may be anticipated. For example, treatment with the matrix metalloproteinase (MMP) inhibitor batimastat had different effects on tumor progression and growth depending on the site of tumor implantation [46]. Predicting the effect of an individual intervention in such a complicated and interrelated system as the tumor microenvironment is fraught with hazards. The potential for unintended consequences must be kept in mind. For instance, the proteolytic action of the MMPs releases angiostatin from the extracellular matrix, thus MMP inhibition may actually increase angiogenesis by decreasing angiostatin release [47].

The tumor microenvironment affects drug delivery. Pluen et al. [48] studied the diffusion of macromolecules and liposomes in tumors growing in cranial windows (CWs) and dorsal chambers (DCs). For the same tumor types, diffusion of large molecules was significantly faster in CW than in DC tumors. The slower diffusion in DC tumors was associated with a higher density of host stromal cells that synthesize and organize collagen type I. These preclinical findings may seem far removed from the clinical setting at first glance. However, mixed responses (i.e. regressions in lung metastases but growth in liver metastases within the same patient) have been observed in early phase clinical trials of antiangiogenics [49–53]. Though the mechanisms underlying these mixed responses have not been elucidated in the clinic, their frequency argues for the critical role of the tumor microenvironment.

Compensatory responses to treatment

The complex paracrine microenvironment of the tumor provides a natural ‘escape’ from total destruction. As chemotherapy induces tumor cell kill, the production of proangiogenic peptides decreases—leading to regression of the tumor-associated vasculature with increasing tumor hypoxia, stimulating an increase in VEGF production [54, 55]. The increased VEGF production in areas of tumor hypoxia may stimulate brisk angiogenesis, essentially rescuing areas of tumor that are sub-lethally injured. In an in vivo model with rat 13762 mammary carcinomas, treatment with cyclophosphamide resulted in tumor hypoxia with increased VEGF production and increased tumor CD31 staining detectable within 24 h [56]. It seems reasonable to expect VEGF production to increase in response to treatment with the ‘pure’ antiangiogenics as well. Indeed, VEGF levels increased after therapy with doxorubicin and a VEGF receptor tyrosine kinase inhibitor [57].

Tumor growth (or regrowth) may be independent of angiogenesis

Initial tumor growth is not always dependent on angiogenesis as has been presumed; vessel cooption, growth by intussusception, vascular mimicry and vasculogenesis may decrease a tumor’s dependence on classical angiogenesis (sprouting and elongation of existing vessels). The sensitivity of these alternative means of establishing circulation to antiangiogenic therapies has not been studied.

Holash et al. [58] documented that a subset of tumors initially grows by coopting existing host vessels. This coopted host vasculature does not immediately undergo angiogenesis but instead regresses, leading to a secondary avascular tumor and massive tumor cell loss. Ultimately, the remaining tumor is rescued by robust angiogenesis at the tumor margin. Kunkel et al. [59] studied systemic treatment with DC101, a monoclonal antibody against vascular endothelial growth factor receptor (VEGFR) in an orthotopic intracerebral glioma model. Though systemic inhibition of VEGFR-2 blocked angiogenesis and inhibited glioblastoma growth, there was increased cooption of pre-existing cerebral vessels with a distinct growth pattern in the residual tumors. In mice treated with DC101, there was a significant increase in small satellite tumors clustered around, but distinct from, the primary tumor. The satellites contained central vessel cores—coopted vessels.

Intussusceptive microvascular growth refers to vascular network formation by insertion of interstitial tissue columns, called tissue pillars or posts, into the vascular lumen and subsequent growth of these columns, resulting in partitioning of the vessel lumen. Patan et al. [60] used intravital microscopy to observe the growth of the human colon adenocarcinoma (LS174T) in vivo. Both intussusception and endothelial sprouting occurred at the tumor periphery. In the central regions intussusception led to network remodeling and occlusion of vascular segments, interfering with vessel patency and causing heterogenous perfusion and hypoxia thus perpetuating angiogenesis [61]. Interestingly, in mammary tumors of neuT transgenic mice, both sprouting and
intussusceptive angiogenesis was observed simultaneously in the same nodules [62].

Vascular mimicry refers to the unique ability of some aggressive tumor cells to form tubular structures and patterned networks in three-dimensional culture, ‘mimicking’ embryonic vasculogenic networks [63]. Several adhesion factors were exclusively expressed by highly aggressive (vasculogenic) melanoma cells; down-regulation of VE-cadherin expression or restoration of EphA2 ligand binding in the aggressive melanoma cells abrogated their ability to form vasculogenic networks [64, 65]. Multiple vascular cell-associated markers were identified in invasive ovarian cancer cells that lined the vascular structure. Tumor cells lined 7–10% of channels containing red blood cells in patient tumor sections from advanced high-grade ovarian cancers. By comparison, all vascular areas in benign tumors and low-stage cancers were endothelial lined [66, 67].

Postnatal vasculogenesis refers to incorporation of bone marrow-derived endothelial progenitor cells (EPCs) into growing adult vasculature. Using transgenic mice constitutively expressing β-galactosidase under the transcriptional regulation of an endothelial cell-specific promoter (Flk-1/LZ or Tie-2/LZ), Asahara et al. [68] and Hattori et al. [69] identified EPCs in the neovascularature of developing tumors. The role of EPCs was further documented by Lyden et al. [70] using the angiogenic-defective tumor-resistant Id-mutant mice. Recent data suggest that inflammatory breast cancer, a rare but frequently lethal form of the disease, relies almost entirely on vasculogenesis as opposed to angiogenesis, apparently due to the inability of the cancer cells to bind endothelial cells [71].

Pharmacokinetic resistance—the dose and schedule required for antiangiogenic activity is not clinically attainable

Maximal antiangiogenic therapy typically requires prolonged exposure to low drug concentrations, exactly counter to the maximum tolerated doses administered when optimal tumor cell kill is the goal [72]. Three recent reports confirm the importance of dose and schedule. In all three the combination of low, frequent dose chemotherapy plus an agent that specifically targets the endothelial cell compartment controlled tumor growth much more effectively than the cytotoxic agent alone [18, 73, 74].

Dose and schedule are also critical for the antiangiogenics. Constant exposure to low non-cytostatic doses of interferon was more effective in downregulating bFGF expression in the laryngeal cancer cell line HlaC79 than high doses [75]. Interferon-α (10000 units) administered daily was more efficacious in inhibiting the growth of bladder cancer in a murine orthotopic model than the 70000 units given in two or three divided doses over 1 week or as one injection per week [76]. Daily subcutaneous administration of 5000 or 10000 units per day produced maximal reduction in tumor vessel density, bFGF and MMP-9 expression (at both the mRNA and protein levels) and serum levels of bFGF.

The natural inhibitors of angiogenesis, angiotatin and endostatin, are cleared rapidly from the circulation when administered as an intravenous bolus [77, 78]. It is likely that the overall balance of pro- and antiangiogenic factors remains tilted toward angiogenesis for substantial periods with such bolus administration. As expected, the most profound effects in preclinical models maintained constant exposure with continuous infusions [79–81].

Natural history of tumor growth and patient selection

Angiogenesis inhibitors significantly curtail primary tumor growth and establishment of metastases in several pre-clinical minimal disease models; overt shrinkage of large, well established tumors is less common. As such, the most successful clinical application of angiogenesis inhibitors is likely to be in patients with micrometastatic disease. It seems unreasonable to expect inhibition of angiogenesis to eradicate cancer or to produce a clinically meaningful effect in a patient with a kilogram of well-vascularized tumor.

Hahnfeldt et al. [82] have explored a model of tumor growth under angiogenic signaling. This model considers growth of the tumor vasculature to be explicitly time dependent (rather than dependent on tumor volume) and to be under the control of distinct positive and negative signals arising from the tumor. Overall, the model parallels Gompertzian kinetics with tumor growth slowing as tumor size increases. Tumor growth eventually reaches a plateau as the action of stimulators is offset by the increasing production of vascular inhibitors by the primary tumor. Antiangiogenic therapies act to lower this plateau tumor size—hopefully to a level compatible with asymptomatic host survival. Importantly, the final tumor size is dependent only on the balance of positive and negative angiogenic factors and is independent of tumor size at the start of treatment. The model also predicts initial tumor growth with some inhibitors of angiogenesis (particularly angiotatin) before stabilization at the plateau size. This early growth could easily be interpreted (perhaps misinterpreted) as resistance. If this model is correct, physicians and their patients will need to learn to tolerate the prospect of initial disease progression.

Potential means of thwarting resistance to antiangiogenic therapy

The reality of human tumors and initial clinical experience with novel antiangiogenic agents confirms that resistance remains an obstacle. While occasional trials have demonstrated modest evidence of clinical efficacy [83–86], there is currently no suggestion in the clinical literature that antiangiogenic therapies represent the therapeutic ‘Snark’ once hoped.

Acknowledging the persistent problem of resistance does not imply that antiangiogenic therapy will prove fruitless, nor that resistance will befall all patients in all settings. Rather it is meant to suggest that the justified enthusiasm for a novel therapeutic modality should not blind us to the very real challenges ahead. Understanding the potential mechanisms of antiangiogenic resistance suggests several possible means to ameliorate or bypass such resistance (Table 2).
The antiapoptotic effects of VEGF may not be limited to endothelial cells. Neuropilin-1, a receptor important in neuronal guidance, is a newly identified co-receptor for VEGF [94] and is highly expressed by some tumor cells [95–97]. In these tumors, VEGF acts as an antiapoptotic factor, potentially protecting tumor cells against chemotherapeutic agents. It is reasonable to expect that the combination of a chemotherapeutic agent with an agent targeting VEGF will increase the therapeutic efficacy of both the cytotoxic and the antiangiogenic agents.

**Combine multiple antiangiogenic agents**

As tumor progression is associated with expression of increasing numbers of proangiogenic factors, the use of multiple antiangiogenic agents to simultaneously attack this multiple redundant process may thwart resistance to individual agents. This approach is, of course, not unique to antiangiogenic therapy, having previously been used to limit resistance to cytotoxic, antimicrobial and antiviral therapies. The combination of antiangiogenic agents has been tested in preclinical models with success, e.g. interferon and TNP-1470 [98], and angiostatin with endostatin [99]. Major barriers to early use of such combinations in clinical practice will likely be regulatory and commercial rather than scientific.

**Combine antiangiogenic agents with other biologically targeted agents**

Many of the factors regulating angiogenesis are not solely proangiogenic but have other functions as well. For instance, the epidermal growth factor receptor (EGFR) and HER-2 both regulate VEGF in human tumors, and their blockade reduces VEGF production and angiogenesis [100–106]. Given the plethora of indirect influences on angiogenesis, might we be able to utilize the combination of biological agents targeting growth factor receptors such as EGFR and HER-2 as a means of inhibiting angiogenesis? More specifically, might we be able to combine antiangiogenic agents with anti-growth factor receptor agents as a means of overcoming resistance? This strategy was effective in preclinical tumor models [107] and is currently under clinical investigation with combinations of antiangiogenic agents and trastuzumab in patients with HER-2 positive breast cancer.

Conversely, antiangiogenic agents might offer a means of overcoming resistance to growth factor-targeting agents. Recent data from Viloria-Petit et al. [108] suggest that increased production of VEGF represents one mechanism by which tumor cells escape anti-EGFR monoclonal antibody therapy. The combination of a VEGF-targeting agent with an anti-EGFR agent might thereby limit resistance to growth factor receptor therapy.

**Use antiangiogenic therapy as adjuvant therapy**

The use of antiangiogenic therapy as adjuvant therapy is a rare treatment that is more effective for large tumors than for small. Tumor progression, as we have argued above, implies drug resistance for antiangiogenics as for other anticancer agents. One means of thwarting the development of drug resistance associated with

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**Table 2. Means of thwarting resistance to antiangiogenic therapy**

- Use standard therapies with antiangiogenic intent
- Combine multiple antiangiogenic agents
- Combine antiangiogenic agents with standard chemotherapy regimens
- Combine antiangiogenic agents with other biologically targeted agents
- Use antiangiogenic therapy as adjuvant therapy
- Use antiangiogenic therapy as targeted therapy

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**Use standard therapies with antiangiogenic intent**

Chemotherapeutic agents have long been developed based on the concept of maximum tolerated dose, and with the assumption that the cancer cells are the sole—or at least primary—target. Recent preclinical studies call these assumptions into question. Numerous chemotherapeutic agents have antiangiogenic activity, and this activity requires dose levels far lower than those required to kill cancer cells [87]. Chronic low-dose chemotherapy (so-called ‘metronomic therapy’) may be potently antiangiogenic, though this effect seems most pronounced when the chemotherapeutic agent is combined with a specific antiangiogenic agent [18, 88].

Use of chemotherapeutics to target the tumor vasculature has the advantage of using agents that are already commercially available. The disadvantages of this approach are equally real. The optimal ‘antiangiogenic’ dose and schedule for those chemotherapeutic agents with distinct antiangiogenic activity remains unknown. Similarly, if the optimal dose and schedule (concentration × time) for ‘antiangiogenic’ activity differs significantly from the optimal ‘antitumor’ dose and schedule (as is likely), which approach should guide therapy?

**Combine antiangiogenic agents with standard chemotherapy regimens**

A corollary to the use of chemotherapeutic agents as antiangiogenic therapy is to combine chemotherapeutics with antiangiogenic agents. Extensive preclinical data support this approach, with multiple antiangiogenic and chemotherapeutic agents having additive or synergistic combinatorial activity [18, 88–91]. The mechanistic rationale for many of these combinations is poorly understood, and not intuitive as both radiotherapy and chemotherapy depend on an effective blood supply for therapeutic efficacy. A potential explanation may lie in the inherent inefficiency of the tumor vasculature. Antiangiogenic therapy ‘normalizes’ flow initially, resulting in improved tissue oxygenation and increased delivery of cytotoxic agents [92].

Potential interactions between VEGF and chemotherapeutic agents have been extensively examined. VEGF is antiapoptotic for endothelial cells [93] and this survival function may protect tumor endothelial cells from the antiangiogenic effects of commonly used chemotherapeutic agents. For instance, Sweeney et al. [91] demonstrated that VEGF protects endothelial cells against docetaxel, an effect reversed by an anti-VEGF monoclonal antibody.
tumor progression is to treat cancers when they are small rather than large in volume. The adjuvant setting (or similar minimal residual disease setting) is the logical place to accomplish this goal.

The use of antiangiogenics as adjuvant therapy has its own potential barriers. Physicians are frequently loath to use agents in the adjuvant setting until there is evidence of activity in advanced disease. The toxicity of chronic antiangiogenic therapy remains largely unexplored, as is the toxicity of combinations of chemotherapy with antiangiogenic therapy. Though intuitively the impact of angiogenesis inhibition is expected to be greatest in patients with micrometastatic disease, proof of this concept will require commitment of substantial human and financial resources to a randomized adjuvant trial. What pre-requisites should we demand before embarking on such a trial? At the very least, assurance that chronic therapy can be administered safely and with acceptable pharmacokinetics. For instance, we might require that a MMP inhibitor could be given safely for a year with trough serum levels above that required for metalloproteinase inhibition. Such a trial has already been conducted for the MMP inhibitor marimastat in the adjuvant breast cancer setting [109].

Use antiangiogenic therapy as targeted therapy

Antiangiogenic therapy has been used thus far essentially as another form of chemotherapy—that is, antiangiogenics have been used as a general therapy given on a population basis, rather than as a targeted therapy given to patients with a specific molecular phenotype. It is reasonable to ask whether we can call failure to respond to a therapy ‘resistance’ if the target at which the therapy is aimed is not present in the tumor. For instance, while VEGF is an important, perhaps obligatory, component of new blood vessel formation for many cancers, it certainly is not the only proangiogenic factor. Indeed, it is likely to be of little importance to the growth of some individual tumors or even entire classes of tumors. If a patient’s tumor does not express VEGF and therefore fails to respond to an anti-VEGF therapy, is the tumor resistant or is the therapy merely misguided?

This is a practical as well as a semantic issue. As insensitivity due to lack of therapeutic target results in resistance at the patient level, proper targeting is a means of overcoming such resistance. Targeted therapy requires specific molecular targets. Ideally these targets should be biologically relevant (in the sense of being crucial to the tumor’s malignant phenotype), reproducibly measurable and definably correlated with clinical benefit. Examples of molecular targets fulfilling such criteria include estrogen receptor or HER-2 for breast cancer, c-kit for gastrointestinal stromal tumors or bcr-abl for chronic myelogenous leukemia.

At present we are unable to point to any truly targeted antiangiogenic therapy. The multitude of potential targets identified makes such targeting at least potentially realizable. Proangiogenic factors such as VEGF and endothelial markers such as \( \alpha \beta \) integrin are clinically measurable. It is reasonable, if not critical, to require mandatory tissue collection for testing as part of the development of these agents. Though we lack validated assays for most of the antiangiogenic therapeutic targets, the availability of tissues for testing will speed development and validation of appropriate assays.

Conclusion

Early enthusiasm for antiangiogenic therapy, justified by the impressive preclinical data, must give way to clinical reality: resistance continues. Genes are not proteins are not cells are not tissues are not organs are not mice are not patients. With each increase in biological complexity comes increased potential for resistance. This is not to suggest that antiangiogenic therapy is destined to fail or that antiangiogenics will find no place in the therapeutic armamentarium. Quite the contrary, it seems certain that antiangiogenic therapies will be integrated into routine clinical practice. To believe otherwise would be to assume that angiogenesis is both biologically crucial yet therapeutically unimportant, an unlikely paradox. Rather it is to remind us that Francis Bacon’s words still ring true, “Nature to be commanded must first be obeyed”. The Snark is neither captured nor Boojum; the hunt continues. The way forward lies in advancing our knowledge of fundamental cancer biology. We are at the beginning of the revolution, not the end.

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References


