Commentary

Antiangiogenic Tumor Therapy

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INTRODUCTION

The term angiogenesis was first used in 1787 by a British surgeon, Dr. John Hunter, to describe vessels growing in the antlers of reindeer. Since then, angiogenesis has been shown to be involved in embryonic development, tissue regeneration, and chronic inflammatory conditions (1). However, it was arguably Folkman's recognition of the importance of angiogenesis for tumor growth in 1971 (2), combined with the first successful treatment of an angiogenesis-dependent pulmonary hemangioma (a benign tumor) with interferon α -2a, that enabled physicians and scientists to recognize the potential therapeutic benefit of using angiogenesis inhibitors as therapy against cancer (3). Soon after, the first clinical trial of an antiangiogenic agent, TNP-470, began in cancer patients (4). In 1999, the development of antiangiogenic therapies for cancer became a top priority of the U.S. National Cancer Institute (Bethesda, MD, USA).

The research and development of angiogenesis-based medicines represent one of the most heavily funded areas of medical research in history. There are currently more than 200 anticancer agents in various stages of clinical development, including 39 antiangiogenic agents in clinical trials for the treatment of solid tumors. Although the Food and Drug Administration has approved other therapeutic modalities that affect angiogenesis (e.g., Regranex[®], an angiogenesis-stimulating drug for the treatment of diabetic foot ulcers; Ortho-McNeil Pharmaceutical, Raritan, NJ, USA), none have been approved for the treatment of cancer. This report describes the unique aspects of targeting angiogenesis and the challenges associated with the clinical development of this new class of drugs.

A BIOLOGICAL TARGET FOR TUMOR THERAPY

Tumor angiogenesis is dependent on the proliferation and migration of endothelial cells to provide nutrients and oxygen to the tumor (4a). Therefore, tumor vascular endothelial cells have become an important target in the development of antiangiogenic drugs. Targeting cells that support tumor growth offers several unique advantages over conventional cytotoxic chemotherapies. For example, the recruitment of endothelial cells occurs early during angiogenesis (5); therefore, targeting endothelial cells

provides an early opportunity to inhibit neo-vessel formation. Endothelial cells are genetically stable and thus are less likely to accumulate mutations that enable them to acquire a drug-resistant phenotype (6,7). In addition, endothelial cells are easily accessible via the circulation, which allows for the optimal delivery and homogeneous distribution of drugs within the tumor. Furthermore, the collapse of a single capillary within the tumor may amplify an antiangiogenic effect. These potentially therapeutic advantages have led to the strategic development of three major types of angiogenesis inhibitors: direct, indirect, and multiple targets (Figure 1).

Many of the antiangiogenic agents that affect tumors through their vasculature act in a cytostatic or growth-delaying fashion (8,9). These putative inhibitors of angiogenesis act directly on endothelial cells to prevent proliferation or migration in response to pro-angiogenic proteins, such as vascular endothelial growth factor receptor (VEGF), basic fibroblast growth factor receptor (bFGF), and platelet-derived endothelial growth factor- β (PDGF- β) (10,11). These proteins are responsible for stimulating blood vessel growth early in tumorigenesis (12) and are secreted by tumor cells, endothelial cells, or both. Several potential strategies for interrupting the signaling pathways of pro-angiogenic proteins include neutralizing the protein in the microcirculation or preventing the binding of the ligand to its cognate receptor and blocking signal transduction by targeting the intracellular domains of receptor tyrosine kinases (RTKs). The inhibition of signal transduction pathways is likely to induce apoptosis in endothelial cells, subsequently killing surrounding tumor cells (13–15). Other antiangiogenic drugs act indirectly on endothelial cells by targeting tumor cells to prevent the expression of or inhibit the activity of tumor proteins that stimulate angiogenesis. For example, inhibiting the activity of the epidermal growth factor receptor (EGFR) blocks the synthesis of VEGF, bFGF, and transforming growth factor- α (16,17). Perhaps the most encouraging approach to inhibit tumor angiogenesis is to combine the direct and indirect targeting approaches (18). Agents that simultaneously block the secretion of multiple pro-angiogenic factors and receptor activity are likely to have the most optimal therapeutic benefit. All three types of potential antiangiogenic therapies are in clinical trials, and many more molecular variants, such as drugs that inhibit all three cognate receptors of the VEGF receptor (VEGFR), are being developed.

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CLINICAL DEVELOPMENT OF ANTIANGIOGENIC DRUGS

Almost 20 years ago, the first cloning and sequence analysis of a cDNA encoding a cell surface protein, the human EGFR, was chosen as a prototype drug discovery target for cancer (19). A decade later, the first genomic research-based RTK inhibitor, Herceptin® (Genentech, South San Francisco, CA, USA), was approved for breast cancer therapy against ErbB-2/HER2/neu (a proto-oncogene that encodes the production of a ligandless receptor protein) positive tumors (20,21). Genome-based assays are routinely used for patient selection of ErbB2-positive breast cancers (22). The antitumor activity of Herceptin provided proof of principle for therapies that target RTKs, a class of cell surface receptors with intrinsic tyrosine kinase activity (23). Since then, cancer genomics has contributed tremendous progress toward the discovery and development of many small-molecule angiogenesis inhibitors. The most clinically advanced genome-based cancer drug is gefitinib (Iressa®, AstraZeneca, London, UK), which targets the ErbB family of RTKs. Now in Phase III trials, Iressa has demonstrated activity in non-small-cell lung, head and neck, and hormone-resistant prostate cancer (24–27). However, in contrast to the relationship between ErbB-2 expression and Herceptin activity, the efficacy of Iressa is not directly related to the level of EGFR expression (28). Thus, optimizing the efficacy of Iressa in different cancers has been challenging, and the identification of better predictive markers for Iressa activity is imperative to maximize therapeutic bene-

fit. Microarray expression analysis should prove beneficial for identifying potential prognostic markers.

Almost a third of the molecular therapeutics in clinical development target VEGF or its RTK (VEGFR-2). The VEGF RTKs are single-pass transmembrane proteins that affect cell proliferation, differentiation, migration, and metabolism (29,30). Of the three VEGF receptors, VEGFR-2 is exclusively expressed on endothelial cells and plays an essential role in tumor neovascularization (31,32). Thus, drug-targeted therapy against VEGFR-2 has become a central focus in antiangiogenic drug development. In pre-clinical studies, inhibiting the function of VEGFR-2 in endothelial cells demonstrated efficacy against solid tumors and provided optimistic results for clinical investigation (33). However, clinical trials with these agents used as a monotherapy have, overall, demonstrated little efficacy against solid tumors. Among the VEGFR-2 inhibitors, Sugen's SU5416® (South San Francisco, CA, USA) has progressed the furthest in clinical development and is now being studied in combination with conventional cytotoxic agents (34,35).

Other strategies include targeting enzymes that are essential for angiogenesis. The matrix metalloproteinases (MMPs) are a family of proteinases capable of digesting the extracellular matrix, which is a key process necessary for tumor growth and metastasis (36,37). In humans, the overexpression of MMPs has been shown to correlate with metastatic potential and poor prognosis (37,38). Therefore, blocking the activity of MMPs has become an important target to inhibit angiogenesis. Similar to other

novel antiangiogenic agents, clinical trials with these agents have failed to reduce tumor burden in patients with advanced disease (39,40). However, a large number of patients have demonstrated stable disease, following treatment with MMP inhibitors (41,42).

The precise molecular mechanism(s) of many agents with potentially effective antiangiogenic properties remain unknown (e.g., thalidomide). Clinical studies of these unique agents have demonstrated that some patients experience tumor regression or disease stabilization with prolonged therapy (8). Further studies are needed to evaluate these promising angiogenesis inhibitors in newly diagnosed patients and/or at the time of minimal residual disease. Studies suggest that combining antiangiogenic agents with traditional cytotoxic therapies will provide the most therapeutic benefit (43,44). Indeed, a number of antiangiogenic agents are in clinical development either as a monotherapy or multimodality strategy for the treatment of solid tumors (Table 1).

Figure 1. Three major types of antiangiogenic agents in clinical development to treat solid tumors. Direct angiogenesis inhibitors target endothelial cells to prevent them from proliferating, migrating, or avoiding apoptosis in response to pro-angiogenic proteins. Indirect angiogenesis inhibitors target tumor cells and block the secretion or activity of pro-angiogenic proteins that stimulate tumor-associated endothelial cells. Agents that inhibit multiple signal transduction pathways on both tumor-associated endothelial cells and tumor cells may provide the best therapeutic approach for inhibiting angiogenesis in advanced disease.

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UNIQUE CHALLENGES OF ANTIANGIOGENIC THERAPY

Preclinical studies indicate that the cytostatic nature of angiogenesis inhibitors makes them more effective when they are administered at a low, continuous dose to maintain a constant concentration in the circulation. In addition, clinical studies have shown that angiogenesis inhibitors are well tolerated and optimal at concentrations well below the maximum dose studied (39,45). Cytotoxic drugs, in contrast, are usually administered at the maximum tolerated doses, which are determined by the dose-limiting toxicity (46). Traditionally, this paradigm is used to provide an estimate of the active dose range for subsequent clinical trials. However, because there is an uncertain relationship between toxicity and response with angiogenesis inhibitors, the clinical end points used to determine the efficacy of cytotoxic drugs do not apply to antiangiogenic therapy. Thus, determining the optimal biological dose of antiangiogenic agents depends on a different logic than is required for cytotoxic therapy (47). Establishing the optimal dose and schedule to reduce the blood supply to the tumor has been a major clinical challenge (48).

SURROGATE MARKERS OF ANTIANGIOGENIC ACTIVITY

The complexity of monitoring biological responses of angiogenesis inhibitors has accentuated the need to develop surrogate markers to assess antiangiogenic activity. A feasible and routinely used approach is to measure angiogenic protein levels that can be detected in serum, plasma, or urine. The successful interruption of angiogenic pathways might be expected to lead to changes in protein levels that are hypothesized to be direct or indirect targets of the inhibitor. Although several angiogenic factors have been assessed in clinical studies with various angiogenesis inhibitors, most have failed to demonstrate a correlation with clinical response. Interestingly, urine VEGF levels measured before treatment have proven useful as a prognostic indicator for patients with disease stabilization versus progressive disease (35,49). The limitations associated with monitoring angiogenic protein levels include the sensitivity of the assay used to measure the protein. In addition, angiogenic protein levels may be slightly inhibited within the tumor microenvironment so that detecting significant changes in the circulation or urine may be impractical. Furthermore, different tumors may produce varying levels of angiogenic proteins. Additional studies are needed in homogeneous patient populations to determine the value of monitoring angiogenic proteins as surrogate markers of antiangiogenic activity.

A recent strategy for monitoring antiangiogenic activity is by *ex vivo* analysis of isolated peripheral blood cells. Although these methods are still exploratory, using four-color flow cytometry to quantify circulating endothelial cells is encouraging (50). Recent data suggest that angiogenesis is supported by the VEGF-dependent

mobilization of bone marrow-derived circulating endothelial precursors (CEPs) (51). Clinical studies have revealed that angiogenesis inhibitors decrease enough CEPs to the level where a small change (<1%) in the cell populations is detectable by flow cytometry. Investigators have hypothesized that antiangiogenic targeted therapy increases the number of mature circulating endothelial cells (CECs) by causing damage to the endothelium (52). In a Phase I study with ZD6126, a tubulin-binding agent, increases in mature CECs were observed as early as 6 h after treatment in four out of five patients (53). Although this approach is encouraging, validation is necessary to establish a correlation with clinical outcome.

Several noninvasive radiologic techniques that show promise for measuring the effects of antiangiogenic activity include positron emission tomography, magnetic resonance imaging, dynamic computed tomography, and 3-D ultrasound (35,42). It is expected that successful inhibition of angiogenesis might lead to decreased blood flow and/or volume, resulting in decreased vascular permeability (48). Each of these surrogate end points is being measured by all of the aforementioned radiologic methods. Although it is still too early to tell which of these imaging methods will be superior, it is likely that a combination may be required to monitor biological activity and tumor response.

The use of gene expression microarrays can be invaluable in the discovery of diagnostic and prognostic biomarkers of biological response to angiogenesis inhibitors. The identification of subsets of patients who are most likely to benefit from investigational agents is greatly needed, especially during early clinical studies. In addition, microarray technology would be most beneficial to confirm the molecular mechanisms of investigational agents. Although a molecular target may be therapeutically attractive, not all will prove pharmacologically or biologically practicable. Inhibiting the active sites of some enzymes may be limited by structural conformation. Thus, it is important to identify pharmacodynamic biomarkers to confirm that drug-target inhibition has been achieved. Clearly, the use of genomic markers will become increasingly important to enable the selection of patients whose tumors are most likely to respond to molecular therapeutics. Indeed, preclinical and clinical studies with Iressa and Herceptin (and STI571) have provided proof of concept that biological activity and therapeutic benefit occur via hypothesized mechanisms.

LASER-SCANNING CYTOMETRY (LSC) ANALYSIS OF RTK ACTIVITY

Genomics and proteomics should complement each other for a comprehensive understanding of the cell system and biological influence of molecular targeted therapy. The proteome provides a platform for conducting integrated protein analysis. Thus, proteomics is the study of protein properties including post-translational modification, expression, enzymatic regulation, and protein-pro-

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Table 1. Antiangiogenic Agents in Clinical Trials

Agent (Published Results)	Antiangiogenic Target	Phase
Direct Inhibitors		
VEGF Trap	Decoy-soluble receptor for VEGF	I
2-Methoxyestradiol	Induces apoptosis in endothelial cells	I
Carboxyamidotriazole (65)	Inhibitor of calcium influx (endothelium)	I
Combretastatin A4 Phosphate	Anti-Tubulin binding agent	I
EMD 121974, Vitaxin (67), S-247	$\alpha(v)\beta(3)$ integrin antagonists	I
Razoxane (49)	Topoisomerase II inhibitor	II
<u>Phase I</u> : PTK 787 (81), ZK22584 (RTK inhibitors), HuMV833 (MAb) (82)	VEGF receptor antagonist	I/II/III
<u>Phase II</u> : RhuMab (MAb) (83), Angiozyme (ribozyme) (84)		
<u>Phase III</u> : Bevacizumab (MAb) (85,86)		
<u>Phase I</u> : IMC-1C11, ZD6474, AG13736	VEGFR-2 antagonist	I/II/III
<u>Phase II/III</u> : SU5416 (Semaxanib, single) (35) w/ Paclitaxel, w/ Gemcitabine & Cisplatin (34), & w/ Irinotecan & Cisplatin TNP-470 (43)		
	Inhibits endothelial cell proliferation	I
Indirect Inhibitors		
Halofuginone Hydrobromide	Type I collagen inhibitor	I
Interleukin-12 and Interleukin-2 (68)	Induces CD8+ T-cell receptor & $\alpha\beta$ + T cells	I
LY317615 and Capecitabine	Protein kinase C β inhibitor	I
Carboxyamidotriazole (CAI, single) & w/ paclitaxel (65)	Inhibitor of calcium influx (endothelium)	I
<u>Phase I</u> : ABX EGF, CI-1033, PKI-166, EGF-Vaccine, EKB-569, GW2016, ICR-62, EMD 55900 (88,89), CP358 (774), PD153035, AG1478	EGFR	I/II/III
<u>Phase II/III</u> : IMC-C225 (Erbix) (90), ZD1839 (Iressa) (24-27,91), OSI-774 (92)		
<u>Phase III</u> : Erlotinib (Tarceva) (93)		
Squalamine Lactate (MSI-1256F) (76)	Inhibits brush-border isoform NHE3	I
PNU-145156E (99)	Inhibits pro-angiogenic factors	I
Tetrathiomolybdate (45)	Anti-copper agent	I
IM862 (96)	Activate NK cells	I
Multiple Targets		
BAY 12-9566 (single) & Fluorouracil or Doxorubicin (75)	Metalloproteinase Inhibitor-2, -3, -9	I
SU101 (94,95), SU6668, SU11248	PDGFR, VEGFR, bFGF	I
Neovastat (Anti-VEGF) (97,98), Marimastat (40), Prinomastat, BMS-275291, COL-3 (42), MM1270 (41)	MMP inhibitors	I/II/III
Unknown?		
Thalidomide (single) (69-72), or w/ Docetaxel (73), or w/ Cyclophosphamide	Unclear, TNF Antagonist	I
Radiation w/ Thalidomide & Temozolomide (74)	Unclear, TNF Antagonist	I
Tecogalan	Unknown	I
Temozolomide & PEG-Interferon α -2b (89)	Unknown	I
Dimethylxanthenone Acetic Acid (77)	Unclear, induces TNF- α and Nitric Oxide I	
Endostatin (48,60,78-80), Angiostatin	Unknown	I
CC-5013 (66)	Immunomodulatory derivative of thalidomide	I

tein interactions (54). Over the past few years, the field of proteomics has made remarkable advances in understanding the complex interactions between proteins and the biological response to extracellular signals.

Understanding functional proteomics (e.g., phosphorylation of RTKs) has become critically important for the clinical development of molecular targeted therapy. Antiangiogenic drugs that target RTKs are designed to block the intrinsic enzymatic activity that catalyzes the transfer of the γ -phosphate of ATP to tyrosine residues in protein substrates (30). Inhibiting the phosphorylation of these tyrosine residues prevents downstream signaling events,

which affect cellular function (e.g., proliferation, differentiation, migration, or apoptosis) (29). We have developed a quantitative method for monitoring drug-target interactions at the molecular level. Although LSC technology is not high throughput, it has been useful for confirming the molecular mechanisms of (high-content analysis) investigational angiogenesis inhibitors.

The VEGFR-2 has received the most attention as a possible target for antiangiogenic therapy. However, more traditional growth factor receptors also appear to play essential roles in angiogenesis, as introduced earlier for EGF/EGFR and ErbB2. Another growth factor that plays a

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role in angiogenesis is the platelet-derived growth factor (PDGF) (55). PDGF and its cognate receptor (PDGFR) are expressed by a wide variety of solid tumors including glioblastoma, melanoma, gastric, lung, and prostate cancer, in which they are believed to contribute to autocrine growth stimulatory loops that are important for tumor progression (56,57). Recent data suggest that endothelial cells appear to express phosphorylated PDGFR when they are exposed to tumor cells that express PDGF (58). Furthermore, preclinical studies with SU6668, a nonspecific TK inhibitor of VEGFR-2, PDGF- β , and bFGF (with less affinity), have revealed that these RTKs have the potential to induce apoptosis in tumor-associated endothelial cells and tumor cells, significantly reducing tumor burden (59). However, clinical studies with these compounds have been disappointing because of the lack of antitumor activity observed in patients (35). Therefore, it has become critically important to determine whether these small molecules inhibit phosphorylation of the hypothesized RTK target(s) in patient tumors, especially to determine the validity of the RTKs (VEGFR) as potentially effective antiangiogenic targets. Thus, LSC-based technology is capable

of measuring biochemical responses such as changes in the level of phosphorylation of target proteins.

LSC-mediated quantitative analysis is similar to fluorescence-activated cell sorting because it is capable of identifying individual cells within a tumor cross-section based on immunofluorescence staining. The laser-based platform is capable of simultaneously detecting up to five

Figure 2. Effects of SU6668 on multiple RTK targets. Representative patient biopsy obtained after 28 days of SU6668 treatment was processed and immunofluorescently stained (simultaneously) for endothelial cells [CD31, (red)], phosphorylated PDGF- β (green), phosphorylated VEGFR-2 (green), and DNA fragmentation (terminal-deoxynucleotidyl-transferase dUTP nick-end labeling, TUNEL) (green). Images shown are LSC-generated scanned images superimposed and pseudo-colored for visualization of (A) CD31⁺ cells, (B) CD31⁺/phosphorylated-PDGFR- β ⁺, (C) CD31⁺/phosphorylated-VEGFR-2⁺, and (D) CD31⁺/TUNEL⁺.

Figure 3. LSC-mediated quantitative analysis of the effects of SU6668 on phosphorylated-VEGFR-2. Patient biopsies obtained before and 28 days after SU6668 treatment were immunofluorescently stained (see Figure 2) and scanned by LSC to determine the levels of phosphorylated-VEGFR-2 in endothelial cells. The 3-D tissue maps display the location of endothelial cells (bars) within each tissue section and the intensity of phosphorylated-VEGFR-2 (red, high phosphorylation; green/blue, low phosphorylation) (A) before and (B) after SU6668 treatment. In this particular patient, SU6668 treatment slightly decreased phosphorylation of VEGFR-2.

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fluorochromes; therefore, it is extremely powerful for analyzing multiple drug targets (RTKs) and effects on cellular function (e.g., cell death). In our Phase I trial of SU6668, we investigated the inhibitory effects of this small molecule on its putative targets by synchronized immunofluorescent analysis of phosphorylated PDGF- β and VEGFR-2 and whether the inhibitory effects were sufficient to induce apoptosis in endothelial cells (Figure 2). Patient biopsies obtained before and 28 days after daily SU6668 treatment were subsequently analyzed using LSC technology to determine the levels of phosphorylation of the RTKs (Figure 3). Preliminary results indicate that the effects of SU6668 on blocking phosphorylation of PDGF- β and VEGFR-2 were heterogeneous and that the levels of cell death were very low among the patients studied (D.W.

Davis, et. al., manuscript in preparation). In collaboration with other investigators, we have also initiated the analysis of the molecular effects of SU5416 in patient biopsy specimens to determine the validity of targeting VEGFR-2 and to inhibit its activity in solid tumors.

The analysis of surrogate markers in patient biopsies obtained before and at specified time points after therapy has provided new insights for monitoring putative antiangiogenic activity (48,60). Importantly, the ability to measure drug-target effects at the molecular level can provide early proof of whether an antiangiogenic agent has successfully reached its intended target. An important end point of antiangiogenic tumor therapy is assessing apoptosis in endothelial cells (13,15,61). Thus, discerning clear patterns of cell death may be important to optimize the dose and schedule of antiangiogenic agents in Phase I and II studies, especially in the absence of little or no toxicity. LSC-mediated analysis of apoptosis in patient biopsy specimens obtained 48 h after the initiation of the treatment has proven useful for predicting patient response to neoadjuvant therapy for breast cancer (62).

LIMITATIONS AND FUTURE DIRECTIONS

Although antiangiogenic agents have shown great promise in preclinical cancer models, investigators are beginning to realize that developing clinically useful antiangiogenic therapy will be more challenging than originally thought. This likely reflects our limited understanding of tumor vessel biology and how tumor cells crosstalk with tumor-associated endothelial cells. The following issues will have to be considered and investigated. First, we will need to know the redundancy of angiogenic factors. It is obvious that targeting one of potentially many factors will have a limited effect. The lessons we learned from knockout mouse studies apply here. Second, we will need to know what is unique about tumor vessels compared with normal vessels. Many of the factors we target in therapy are also important for normal angiogenesis, limiting the dose that can be applied. There is clear evidence from pathology studies that tumor vessels are different from normal vessels; for example, there are many large vessels in tumors as compared to normal angiogenic processes. Indeed, a transcriptome analysis of vessels from normal and tumor tissues revealed unique gene expression patterns in tumor vessels (63). Another example is offered from studies of human brain tumors. In normal brain, the blood vessels provide a barrier, called the blood-brain barrier, which limits the transport of biological agents from the

Figure 4. The application of genomics and proteomics in the development and optimization of cancer therapeutics. Microarray expression profiling of endothelial cells from normal and tumor tissue can be used to develop novel molecular targets that effect angiogenesis. Genomic and proteomic analyses should complement each other to better understand the biological influence of tumor cells on tumor-associated endothelial cells and the response of the tumor microenvironment to antiangiogenic agents. In addition, these technologies are crucial for the discovery of molecular biomarkers that will become increasingly important for genome-based cancer treatments, especially to determine the optimal biological dose of angiogenesis inhibitors in early clinical studies. Further, combining selective genome-based therapeutics with genome-derived biomarkers has the potential to optimize multimodality therapy and customize cancer treatment in a patient-specific fashion based on the individual patient's genetic fingerprint.

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blood to the brain. However, in brain tumors, this barrier is broken down, and the vessels become much more permeable. Furthermore, our gene expression profiling, coupled with immunohistochemistry analysis, demonstrated that CD5⁺ diffuse large B-cell lymphomas (DLBCL) express CD36 on the large blood vessels, whereas CD5⁺ DLBCLs do not express CD36 on their vessel cells (64). Finally, we will need to know how tumor cells functionally modify endothelial cells, much like we need to know how tumor cells manage to counteract the immune cells often found in tumor tissues. The heterogeneity found in different types of tumors may affect the tumor vasculature in dissimilar ways.

A better understanding of how angiogenesis inhibitors affect their target and the consequential effects on surrounding tumor and stromal cells is necessary for successful translation into the clinic. In addition, it has become clear that, given the multitude of proangiogenic pathways, the successful blockade of angiogenesis will require inhibitors with multiple targets or combinations of agents. Thus, therapeutic agents that target multiple receptors or steps to malignant progression are more likely to demonstrate a clinical benefit against advanced disease. It may therefore be prudent to design drugs that simultaneously target multiple growth factors or receptors. Novel agents that target both the VEGFR and EGFR will begin clinical trials this year. In addition, combining agents that have direct and indirect effects on angiogenesis with conventional cytotoxic modalities may be extremely beneficial for patients with advanced cancer. Nonetheless, it will be critically important to monitor surrogate markers of antiangiogenic activity in patients receiving multimodality therapy and to tailor patient-specific therapy on the basis of the tumor's response over time. The development and validation of new methods to monitor surrogate markers of antiangiogenic activity are essential for optimizing the biological therapeutic benefit, for only then will it be possible to choose the correct dose for this combination. Undeniably, lessons learned from the successful development of Herceptin and Gleevec have shifted the strategy in drug development and cancer therapy. Genomic and proteomic-based technologies will be needed to develop better molecular targets, diagnostic and prognostic markers that can be used to assess the optimal activity of angiogenesis inhibitors (Figure 4).

The development of high-throughput genomic and proteomic technologies has begun to help us acquire some of the information pertaining to the above questions. We expect that the application of genomics in vessel research in the next few years will illustrate a more comprehensive picture of tumor vessel biology. A highly anticipated outcome of this research effort is the identification of specific targets that will result in more effective antiangiogenic therapy.

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