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Forty-Year Journey of Angiogenesis Translational Research

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Abstract

Forty years ago, Judah Folkman predicted that tumor growth is dependent on angiogenesis and that inhibiting this process might be a new strategy for cancer therapy. This hypothesis formed the foundation of a new field of research that represents an excellent example of how a groundbreaking scientific discovery can be translated to yield benefits for patients. Today, antiangiogenic drugs are used to treat human cancers and retinal vascular diseases. Here, we guide readers through 40 years of angiogenesis research and discuss challenges of antiangiogenic therapy.

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INTRODUCTION

In the early 1970s, as a young surgeon who frequently encountered cancer in patients, Judah Folkman observed that tumor tissue was enriched by an extraordinarily high number of blood vessels that were fragile and often hemorrhagic (1). Folkman further noted that tumors remained viable but did not grow when angiogenesis was not observed, which led him to hypothesize that tumors must spur the growth of new blood vessels in the host to support their growth. Indeed, early in vivo experiments demonstrated that tumor cells stimulate endothelial cell (EC) proliferation as well as the sprouting of capillaries from host vessels (1). Conversely, in the absence of this neovascularization, a tumor implant does not grow beyond 2 to 3 mm^3 and enters into a dormant state (2). In 1971, Folkman and colleagues reported the isolation of an angiogenesis-promoting activity called tumor angiogenesis factor (TAF) that induced EC proliferation and angiogenesis in animal models (1).

On the basis of these findings, the research group published a report that proposed that tumor growth depends on angiogenesis (2) and presented several new concepts: (i) malignant cells and ECs within a tumor constitute a highly integrated, growth-interdependent system; (ii) angiogenic factors secreted from tumors stimulate blood vessel growth; (iii) blockade of angiogenesis could lead to tumor dormancy; and (iv) antiangiogenesis represents a potential therapeutic approach against cancer that synergizes with other existing therapies. Specifically, Folkman wrote, “One approach to the initiation of antiangiogenesis would be the production of an antibody against TAF.” The most commonly used antiangiogenic drug (AD) today, bevacizumab, is a humanized monoclonal antibody that neutralizes human vascular endothelial growth factor (VEGF) (3) and was developed based on the principle proposed by Folkman 40 years ago.

MODELING ANGIOGENESIS

In the 1970s, the idea that angiogenesis is rate-limiting for tumor growth was not readily accepted by the scientific community, which believed that a tumor simply co-opted host blood vessels to support its growth, or that new blood vessel formation was a by-product of inflammation unrelated to tumor growth. Then, in 1979, Folkman *et al.* reported the first successful long-term culture of capillary ECs (4); this feat was achieved by supplementing the culture with medium conditioned by cells from a solid tumor, which suggested that tumor cell–derived growth factors are crucial for EC growth and survival. Folkman and co-workers used these ECs to develop the first reproducible in vitro assays to measure EC function, and these assays remain among the most commonly used in vitro models for the identification of new angiogenic stimulators and inhibitors.

Folkman and colleagues were also the first to develop in vivo models of angiogenesis. For example, in the corneal pocket assay, implantation of a piece of a solid tumor into the rabbit cornea enabled study of the tumor’s angiogenic ability in the absence of preexisting blood vessels (5, 6). Advances in imaging technologies, surgical procedures, and chemical materials have made it possible to perform the corneal angiogenesis assay in small rodents, including genetically modified mouse strains (7–9). Auerbach *et al.* (10) and Brem and Folkman (11) also developed the chick chorioallantoic membrane angiogenesis assay, which

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is still used for in vivo screening for both angiogenic and antiangiogenic agents, although this assay is less quantitative than the cornea test owing to the presence of preexisting vessels.

FACTORS CONVERGE

Working with Folkman in 1984, Klagsbrun and Shing purified the first tumor-derived angiogenic factor, which turned out to be identical to the fibroblast growth factor 2 (FGF-2) (12) that had been purified independently by the Gospodarowicz laboratory (13). Earlier, Senger and Dvorak were working to purify factors responsible for vascular hyperpermeability, which is a characteristic of nearly all solid tumors and can lead to accumulation of fluid (ascites) in the pleural, pericardial, and peritoneal cavities. In 1983, these investigators reported purification of vascular permeability factor (VPF), a 38-kD protein from the conditioned medium of a guinea pig liver tumor (14). An antibody to VPF inhibited ascites accumulation induced by the tumor, suggesting that such antibodies might be useful in anticancer therapy.

VPF was later determined to be identical to VEGF, a molecule purified by Ferrara and Henzel in 1989 from the conditioned medium of bovine pituitary follicular cells. VEGF was initially described as a 45-kD protein that specifically induced the growth of ECs (15) and displayed angiogenic activity. Shortly after, Ferrara and colleagues cloned the complementary DNA for VEGF (16); examination of the predicted amino acid sequence revealed that it shared significant similarities with platelet-derived growth factor–A (PDGF-A) and PDGF-B, the latter of which is important in vascular remodeling (17–19). In the same issue of *Science*, Connolly and co-workers showed VPF/VEGF to be a potent angiogenic and vascular permeability factor (20).

The development of quantifiable and reliable angiogenesis assays and the identification and characterization of FGF-2 and VPF/VEGF paved the way for other laboratories to identify more than a dozen additional angiogenic factors from tumor and nontumor tissues (21–26).

HELP FROM WITHIN

The fact that blood vessels in most adult tissues have a very low turnover rate persuaded researchers to hypothesize the existence of endogenous angiogenesis inhibitors that act to counterbalance angiogenic signals that would otherwise trigger persistent vascular growth. Motivated by the notion that anavascular tissue might be enriched for angiogenesis inhibitors, Langer *et al.* partially purified the first of these factors from cartilage and showed that it blocked tumor-induced blood vessel sprouting in the corneal angiogenesis assay (5). The factor also inhibited cancer growth when injected into tumor-bearing animals (27). Moses *et al.* later purified this stifler of neovascularization as an inhibitor of matrix metalloproteinases, thus revealing the factor's underlying mechanism of action (28–30).

The discovery of numerous additional angiogenesis inhibitors followed this initial success (31–34). Several steroids (medroxyprogesterone, dexamethasone, cortisone) were shown to inhibit endothelial activity in vitro and angiogenesis in vivo, and heparin and heparin fragments were found to modulate the angiostatic activity of steroids (35, 36). These

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findings led to the initial clinical success of interferon-α as a treatment for hemangioma (a benign vascular tumor) in infants and newborns (37–39) and represented the first clinical success of antiangiogenic therapy.

From his clinical experience, Folkman noted that removal of a primary tumor sometimes appeared to facilitate metastatic tumor growth, a phenomenon that was familiar to surgeons and oncologists, but of unknown molecular mechanism. Folkman hypothesized that primary tumors produce endogenous angiogenesis blockers that enter the circulation and suppress distant metastatic growth and that removal of the primary tumor eliminates this source of inhibitors, leading to accelerated growth of metastases. In testing this hypothesis, Folkman and colleagues isolated the first tumor-derived angiogenesis inhibitor, angiostatin, and demonstrated its origin as a fragment of plasminogen, the precursor of an enzyme that degrades plasma proteins (40). This discovery validated the endogenous angiogenesis inhibitor hypothesis and heightened interest in angiogenesis research. Using similar approaches and principles, the same research team identified endostatin, a fragment of collagen XVIII, as another potent and EC-specific endogenous angiogenesis inhibitor (41).

The molecular mechanisms that underlie the activity of endogenous angiogenesis inhibitors are not known and are likely to be complex and involve the suppression of several signaling pathways. As a result, pharmaceutical development of these inhibitors as anticancer drugs has been less attractive than other single-target agents. Clinical evaluation of endostatin during early phases of clinical trials for the treatment of neuroendocrine tumors did not demonstrate therapeutic benefits (42); however, the trial was not designed to assess clinical benefits in a large cohort study. Thus, the potential therapeutic value of endostatin and other endogenous angiogenesis inhibitors warrants further investigation.

In support of this notion, a modified version of recombinant endostatin has been successfully developed as an antiangiogenic drug that is routinely combined with chemotherapy for the treatment of cancer patients in China (43). In theory, these endogenous inhibitors might be expected to display more potent antitumor activity than do agents that inhibit a specific growth factor or receptor, because the antiangiogenic factors appear to block a common pathway that governs EC growth. Moreover, because they appear to have a physiological function, endogenous angiogenesis inhibitors may have fewer side effects than exogenous inhibitors (42, 44).

PRECLINICAL PROOF OF CONCEPT

Angiogenesis inhibitors are classified as either direct or indirect, depending on their modes of action (45). In addition to the endogenous inhibitors already described, numerous others —such as thalidomide, integrin inhibitors, and the cell cycle inhibitor TNP-470—act directly on ECs and prevent them from responding to virtually any angiognic factor (28, 29, 46–49). Thalidomide and its related derivative lenali-domide currently are used to treat multiple myeloma (50). Although the anticancer effects of these drugs are not limited to their antiangiogenic activity, researchers suspect that suppression of neovascularization in the bone marrow accounts at least in part for the observed clinical benefits (51).

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Indirect inhibitors block the function of angiogenic agents such as VEGF by targeting growth factor–triggered signaling pathways. The VEGF signaling system can be modulated by (i) small interfering RNAs (siRNAs) that block VEGF production (52), (ii) neutralizing antibodies to VEGF (3), (iii) aptamers (oligonucleotides or peptides) that selectively bind VEGF (53), (iv) neutralizing antibodies to the VEGF receptor (VEGFR) (54), (v) inhibitors of VEGFR tyrosine kinase (TK) activity (55), (vi) inhibitors of the neuron-specific non-TK VEGFR neuropilin (56, 57), and (vii) small molecules that target components of the signaling pathway downstream of the VEGFR (58). Unlike broad-spectrum direct inhibitors, angiogenic factor antagonists most often specifically target a distinct pathway (59–64).

True angiogenesis inhibitors usually display a broad spectrum of activity on various tumors, providing a compelling basis for antiangiogenic cancer therapy. The ideal angiogenesis inhibitor would induce tumor dormancy by reducing the tumor vasculature (65–67). Proof of this concept in preclinical studies encouraged the development of ADs for cancer therapy. Moreover, angiogenesis inhibitors that target distinct angiogenic pathways have been shown to display synergistic antitumor activity in preclinical models (45, 68), and combinations of generic angiogenesis inhibitors with chemotherapeutic agents (69) or radiation therapy (70) also produce synergistic results in animal models of glioblastoma and Lewis lung carcinoma.

CLINICAL PROOF OF CONCEPT

Several ADs have been approved for use in patients by the U.S. Food and Drug Administration (FDA) or by similar authorities outside of the United States. In 2003, thalidomide and bortezomib were approved for the treatment of multiple myeloma (71), and in 2004, the FDA approved bevacizumab, a humanized anti-VEGF antibody, for the treatment of colorectal cancer on the basis of its beneficial effect in combination with traditional cytotoxic chemotherapy (72). This drug was subsequently approved for use, in combination with cytotoxic chemotherapy, in breast, lung, and renal cancers, for which phase 3 clinical trials demonstrated significant improvement in overall survival or delayed tumor progression compared to cytotoxic chemotherapy alone. In a phase 2 trial on recurrent glioblastoma, bevacizumab showed clinical benefit when given as a single agent (73). The use of bevacizumab in oncology stimulated the rapid development by pharmaceutical and biotechnology companies of scores of angiogenesis inhibitors that target VEGF and other angiogenic pathways. Among FDA-approved drugs, bevacizumab and small-molecule inhibitors of the VEGFR TK dominate in terms of clinical use.

However, unlike the results obtained in most preclinical tumor models, bevacizumab does not exhibit marked antitumor effects and survival improvement when delivered as a monotherapy in cancer patients with metastatic disease (73). Recent phase 3 studies in ovarian cancer patients reported some beneficial effects of bevacizumab as a maintenance monotherapy after being used upfront in combination with traditional chemotherapy (74, 75). The clinical benefits of ADs are usually achieved by their addition to existing chemotherapy, likely as a result of their nonoverlapping targets [tumor cells (cytotoxic chemotherapy) and the vasculature (ADs)]. Indeed, several preclinical studies in various animal tumor models, including lung cancer, mammary carcinoma, and sarcoma, have

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uncovered mechanisms behind the additive/synergistic effects of combination therapy (76, 77).

BEYOND CANCER

Although Folkman's original theory was motivated primarily by cancer treatment, he also recognized the relevance of understanding angiogenesis and developing antiangiogenic treatments for ophthalmic diseases characterized by new blood vessel growth. Not surprisingly, VEGF inhibitors work best for conditions in which this growth factor is the principal angiogenic factor, as appears to be the case for a number of proliferative retinopathies, including proliferative diabetic retinopathy, retinopathy of prematurity, branch vein occlusions, and wet age-related macular degeneration (AMD). FDA-approved in 2004, the anti-VEGF aptamer pegaptanib was the first widely used drug for the treatment of AMD (53). Two years later, FDA approved Lucentis (ranibizumab), a fragment of bevacizumab, for the treatment of AMD (78). These agents produce beneficial effects in patients (79). It is anticipated that antiangiogenic therapy will be extended to other indications including obesity and diabetic complications (80–85).

Assuming that VEGF plays a role in the induction of pathological vessels in both tumors and retina, why do these two tissue types respond so differently to the same treatment? One likely explanation is the complexity of the pathologies. In wet AMD, vessel growth and permeability likely is induced by VEGF produced by macrophages or damaged retinal pigment epithelial cells as part of a local response to injury. In contrast, cancers are heterogeneous systems that include tumor, stromal, and inflammatory cells. Moreover, the genetic instability of the tumor cells (and possibly the stromal and vascular cells) probably results in overexpression of a variety of growth factors and their receptors, leading to a redundancy in angiogenic stimulators; this phenomenon appears not to occur in ocular disease.

In addition, the clinical endpoints for cancer versus ocular disease diverge. Whereas survival benefit (usually improvement of overall survival) is commonly used to assess AD efficacy in cancer patients, vision improvement in AMD is the gold standard. In patients with cancer, survival time is determined by a combination of physiological, pathological, and psychological processes. Moreover, cancer patients often suffer from a variety of malignancy-associated systemic disorders and metastatic disease, which have significant impact on survival and quality of life (86). Finally, AD delivery into the eye is straightforward, and a high local concentration of drug can be achieved. Systemic delivery of ADs to tumors is complicated by the tumor's heterogeneous blood supply, which affects drug distribution.

DEFINING THE CHALLENGES

Current ADs produce modest beneficial effects as cancer therapeutics (87–89). As a result of the low-gain and risk balance in several randomized phase 3 trials in breast cancer patients, FDA revoked its approval for the clinical use of bevacizumab in metastatic breast cancer (90). For ADs to become a crucial weapon in our arsenal of cancer treatments, researchers

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must address various complex issues that impede the design of robust antiangiogenic strategies.

Reconciling preclinical and clinical outcomes

Although AD monotherapy improves clinical parameters in preclinical models, patient trials indicate that bevacizumab must be given with traditional chemotherapeutic drugs to have a beneficial effect [with the exception of maintenance monotherapy in ovarian cancer (74)]. This observation raises concerns about the relevance of the commonly used preclinical tumor models.

Indeed, there are important differences between mouse tumor models and cancer patients that might account for divergent responses to ADs (Fig. 1). Often-discussed differences include the vast variability in genetic backgrounds, tumor heterogeneity, and tumor locations in patients relative to mouse models. In addition, the growth rate of frequently used experimental tumors in mice is extremely high, probably making these cells more vulnerable to angiogenesis inhibitors, whereas growth of a similarly sized tumor in humans might take years; these distinct growth rates are likely to reflect differences in vessel growth by the tumor tissues. Also, in experimental tumor models, antiangiogenic therapy is often started at the onset of tumor development, whereas in clinical settings, treatment most often involves patients with advanced metastatic disease. Differences in outcome measures also complicate translation from mouse models, in which the change in tumor size is used as a measure of drug effects, to patients, for whom drug effects are assessed in terms of their survival benefit. And finally, even the largest tumor mass studied in most animal models is significantly smaller than the total tumor mass seen in late-stage cancer patients. Size alone could influence delivery of agents, which could, in turn, significantly limit their efficacy.

Recent preclinical studies in mouse tumor models suggest that antiangiogenic therapy might increase tumor invasiveness and metastasis (91, 92). This paradoxical notion has justifiably raised concern that use of ADs in nonresponsive patients might reduce survival by these mechanisms. However, published clinical data from various trials do support this theory (55, 71, 93), further calling into question the clinical relevance of studies in mouse xenograft models.

More relevant preclinical models include mice that develop spontaneous tumors, the formation of which is followed by a switch to an angiogenic tumor with further progression and metastasis. However, tumor growth in these mouse models is usually driven by overexpression of a particular oncogene or deletion of a tumor suppressor gene, leading to activation or impairment of a specific oncogenic pathway and imbalanced expression of angiogenic factors. This sequence of events may not occur in patients and does not circumvent the problem of human tumor heterogeneity, particularly in the late-stage cancers typically treated in clinical trials. Recapitulation, in a model system, of cancers observed in the clinic may require the development of humanized mice that harbor certain human genes known to promote cancer formation in specific organs.

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Mechanistic insights needed

Scientists have not yet precisely defined the fundamental mechanisms that underlie the clinical benefit of ADs in combination with traditional chemotherapy; however, emerging preclinical and clinical results suggest several possible mechanisms (Fig. 2). First, in both preclinical and clinical settings, anti-VEGF drugs have been reported to induce significant remodeling of tumor blood vessels, leading to a more normalized vasculature (94–96). Because the remodeled vessels induced by AD treatment are less permeable and better perfused than the disorganized and leaky vessels in untreated tumors, the combination of an anti-VEGF agent with standard chemotherapy might result in increased drug delivery to the tumor (69). Second, because chemotherapeutic drugs primarily target tumor cells and ADs target the endothelial compartment, the combination might lead to additive or synergistic antitumor activity, as demonstrated in preclinical tumor models (77, 97). Third, ADs may display as yet undefined off-target effects.

Systemic delivery of ADs to the host may affect both tumor and nontumor vasculatures. In support of this notion, systemic delivery of anti-VEGF agents in tumor-free mice results in significant regression of the microvasculature in several organs (98). If tumor-derived angiogenic factors disrupt nontumor vasculature and thus organ function, ADs may normalize the vasculature of these tissues, improve organ function, and confer survival benefit on the patient. Indeed, in several preclinical models, anti-VEGF agents significantly improve survival without inhibiting tumor growth (70, 99, 100); however, this potential off-tumor mechanism of ADs requires further investigation in cancer patients.

As a fourth possible mechanism, drugs such as the mTOR (mammalian target of rapamycin) inhibitor rapamycin that target both tumor cells and the tumor microenvironment—including stromal and inflammatory cells—might augment the EC-suppressing effects of ADs. Furthermore, treatment of cancer-bearing mice with an antibody to granulocyte colony-stimulating factor (anti–G-CSF) alters the tumor environment by recruitment of bone marrow–derived $CD11b^{+}Gr1^{+}$ myeloid cells, which play a role in enhancing vascular sensitivity to ADs (101). Fifth, anti-VEGF therapies may increase host tolerance to chemotoxicity. Recent studies have demonstrated that both circulating VEGF and traditional chemotherapeutic drugs synergistically suppress bone marrow hematopoiesis in mouse tumor models, leading to early death of the host. However, treatment of these animals with ADs before chemotherapy significantly improves their tolerance to chemotoxicity, resulting in marked survival improvement (99). Finally, other mechanisms that might contribute to the synergism noted in combination therapy include AD-induced (i) tumor blood vessel regression, (ii) prevention of tumor co-opting of vessels from surrounding healthy tissues, and (iii) formation of abnormal nonproductive, rather than robustly perfused, vessels in the tumor microenvironment (89).

Given that broad-spectrum angiogenesis inhibitors, such as TNP-470, angiostatin, and endostatin, target multiple distinct signaling pathways, clinical development of these agents may provide a new opportunity for optimizing combination antiangiogenic therapeutic regimens.

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Timing may be everything

The ideal time frame for treatment of cancer patients with ADs is another open question. ADs do not completely destroy tumor blood vessels, and rapid tumor revascularization after stopping antiangiogenic therapy has been observed in preclinical cancer models (102). Moreover, withdrawal of certain antiangiogenic agents leads to a rebound effect that includes an increase in VEGF concentrations and a decrease in soluble inhibitory VEGFRs (103). One plausible mechanism for rebound revascularization is that ADs induce tumor hypoxia, which in turn up-regulates angiogenic factors such as VEGF, FGFs, and PDGFs (104). AD-induced vessel regression in healthy tissues may also create, in both tumors and normal tissues, a hypoxic environment that could cause an elevation in the amounts of circulating angiogenic factors and thus rebound angiogenesis, although this has not been shown to be the case in AD-treated patients (105).

If long-term AD treatment is determined to be necessary for desired clinical outcomes, these drugs immediately face not only scientific but also economic obstacles because of their high cost. At current prices, most patients could not afford to receive lifelong AD therapy, and insurance plans in general do not cover the cost of AD therapy for an indefinite amount of time. A possible alternative approach to achieve long-term therapy is to implant, in patients, slow-release polymers that are embedded with ADs (87). A polymer-based drug delivery system can be devised to deliver ADs to the local tumor environment so that the required dose might be considerably decreased while still providing an effect similar to systemic delivery. In support of this option, drug-release polymers have been used for the successful treatment of various diseases (for example, the Gliadel wafer for the treatment of glioblastoma) (106–114).

Unraveling and resisting resistance

Antiangiogenesis therapy was based on the idea that ADs target tumor-associated ECs, which, unlike tumor cells, are expected to be genetically stable. Thus, the drug resistance that normally develops over time with conventional cancer therapeutics would not be expected to occur with ADs (115). Clinical findings, however, have challenged this hypothesis, because most cancer patients display intrinsic resistance (that is, they do not respond) to VEGF inhibitor–based ADs. Moreover, a proportion of patients whose tumors initially respond to an AD subsequently exhibit apparent resistance. Although the mechanism that mediates AD resistance remains unknown, it does not seem to be similar to the mechanisms that underlie resistance to tumor-directed drugs (62). Rather, resistance likely arises from compensation by other angiogenic factors (61, 116) and may be less common if more effective ADs are developed that inhibit EC responses to all angiogenic factors.

Predictive biomarkers

One obstacle to the assessment of AD efficacy has been the lack of reliable biomarkers, which would allow clinicians to distinguish between patients who are likely to benefit from AD therapy and non-responders, as well as to facilitate accurate monitoring of therapeutic efficacy, adverse effects, and drug selection (117–119). Candidate biomarkers include urinary metalloproteinases and their complexes (120, 121), amounts of circulating

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angiogenic factors such as VEGF, numbers of circulating ECs, and the extent of side effects such as drug-induced hypertension and skin rash, which have been correlated with clinical benefit. However, these parameters do not predict clinical outcomes. A recent clinical study demonstrated that certain genetic polymorphisms in the *VEGF* and *VEGFR-2* genes correlate with AD-driven beneficial outcomes in patients with metastatic breast cancer (122). If these findings are validated in independent patient populations and for other cancer types, genetic analysis of *VEGF* polymorphisms may help to define reliable biomarkers for this subclass of ADs.

Low-dose antiangiogenic chemotherapy therapy

Because of the troublesome nonspecific cytotoxic effects of traditional chemotherapeutic drugs, Folkman, Kerbel, and colleagues proposed that the tumor microenvironment be subjected to a constant low dose of these agents (123, 124). This so-called “metronomic” approach is based on altering the dose and delivery schedules of standard chemotherapeutic drugs to more continuously target the EC compartment. In some early clinical trials, metronomic chemotherapy was used in combination with ADs at regular doses for the treatment of cancer patients; these early trials have provided promising indications that continuous low-dose treatment with the cytotoxic chemotherapeutic drug cyclophosphamide in combination with ADs improves the rate of clinical benefits, including complete response, partial response, and stable disease (125).

Outlook for Cancer Therapy

ADs of the future need to be more efficacious than the current versions, either alone or in combination with conventional therapies, by targeting multiple angiogenic pathways and producing minimal and clinically manageable adverse effects. Optimization of antiangiogenic therapy requires improved mechanistic understanding of tumor angiogenesis, discovery and validation of reliable biomarkers, identification of molecular mechanisms of drug actions, improved clinically relevant animal models, development of slow-release systems for drug delivery, design of optimal combination therapies, and improved clinical trial design. Designing of optimal clinical trials should consider the diversity of genetic backgrounds of cancer patients, kinetic changes in the tumor environment during cancer progression and treatment, genetic and epigenetic alterations in the expression of angiogenic factors, and the overall state of health of the patients. Thus, clinical trial improvement demands intimate collaborations between clinical oncologists and translational and clinical scientists.

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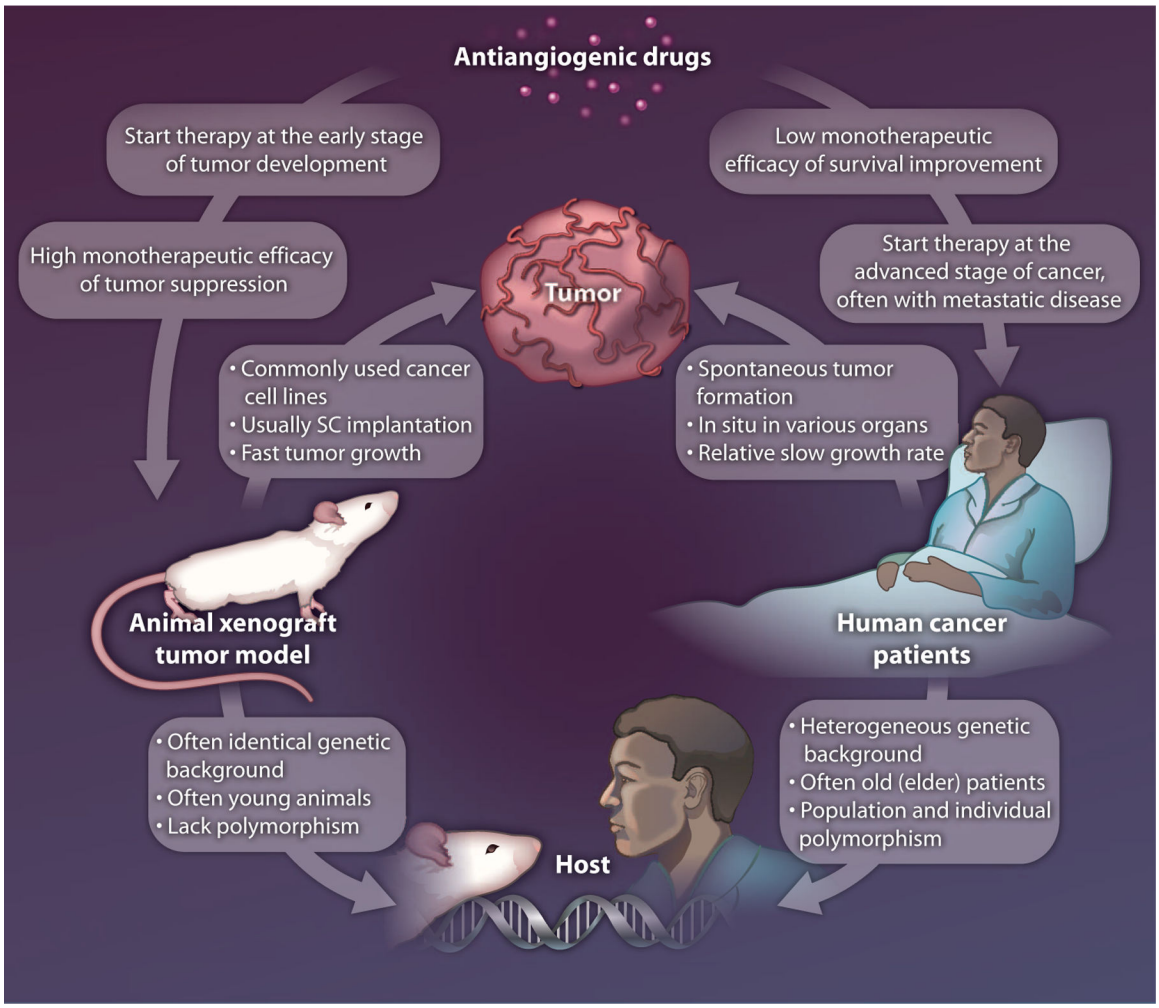

Fig. 1. Not like the other.

Shown are possible bases for differences in response to ADs between preclinical animal models and human patients. S.C., subcutaneous.

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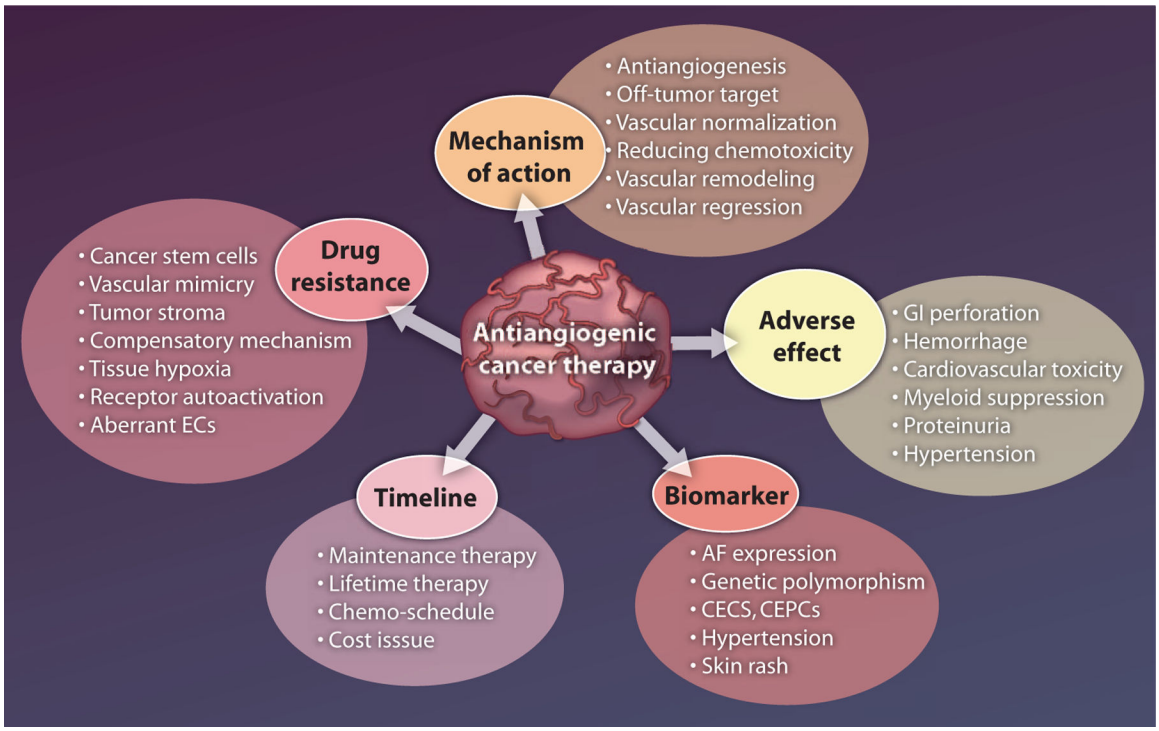

Fig. 2. Constellation of challenges.

Many unanswered questions remain in the realm of antiangiogenic cancer therapy. ECs, endothelial cells; CECs, circulating endothelial cells; CEPCs, circulating endothelial progenitor cells; AF, angiogenic factor; GI, gastrointestinal.