Angiogenesis Inhibitors in Lung Cancer

Edward S. Kim, MD and Roy S. Herbst, MD, PhD

Address
Department of Thoracic/Head and Neck Medical Oncology,
The University of Texas M.D. Anderson Cancer Center,
1515 Holcombe Boulevard, Box 432, Houston, TX 77030, USA.
E-mail: edkim@mdanderson.org

Current Oncology Reports 2002, **4:**325–333
Current Science Inc. ISSN 1523-3790

Lung cancer is a major public health problem and the leading cause of cancer-related death worldwide. Its survival rates have changed little over the past 20 years. The best clinical benefit (*ie*, survival rates) with combination cytotoxic therapies in non–small-cell lung cancer (NSCLC) may have been reached. The need for improved survival rates in NSCLC has driven the development of novel, rationally designed, targeted therapies. Inhibitors of angiogenesis have been developed and are increasingly studied. Potential targets for therapy include inhibitors of vascular endothelial growth factor receptor, endogenous angiogenesis inhibitors, and cyclooxygenase inhibitors. Combining targeted molecules with traditional cytotoxic therapies usually results in lower required chemotherapy doses and fewer, less severe side effects. A number of ongoing randomized studies are underway to evaluate this idea. It is anticipated that these new targeted therapies will play an important role, along with cytotoxic and radiation therapies, in the management of metastatic disease.

Introduction

Lung cancer is a major public health problem and the leading cause of cancer-related death worldwide. In 2001, an estimated 169,500 new cases of lung cancer were diagnosed in the United States, with 157,400 deaths [1]. Non–small-cell lung cancer (NSCLC) accounts for almost 75% of newly diagnosed cases, with the predominant histologic subtypes in the United States being adenocarcinoma and squamous cell carcinoma [2].

A diagnosis of lung cancer represents a particularly poor prognosis often owing to advanced disease at presentation and the lack of efficacious treatment options. Only 13% of the patients in whom lung cancer develops live 5 years or more after the disease process begins. There has been little change in the past 20 years in these dismal figures. Although great progress has been made in development of new anticancer drugs in the past decade, the overall benefit for patients with NSCLC has been marginal in terms of response rate, survival time, and quality of life [3]. Only if the tumor is confined to the chest and is resectable does surgery offer the best chance of long-term survival. Surgery cures 50% to 70% of pathologic stage I disease and about 30% to 40% of pathologic stage II disease [4]. However, only 24% of patients with NSCLC have localized operable stage I or II disease. The majority of patients present initially with either locally advanced unresectable disease (stage IIIA and IIIB, approximately 44%) or metastatic disease (stage IV, about 35%) at diagnosis [5].

It appears that the best clinical benefit (*ie*, survival rates) with combination cytotoxic therapies in NSCLC may have been reached [6]. The need for improved survival rates in NSCLC has driven the development of novel, rationally designed, targeted therapies. Inhibitors of angiogenesis have been developed and are increasingly studied.

Angiogenesis is the process of formation of new blood vessels from preexisting vessels, and it is integral for tumor growth, development (>1 to 2 mm^3), and metastases [7••,8,9]. This multistep process begins when endothelial cells, which line the lumen of the blood vessels, degrade the basement membrane, migrate through the membrane to form a sprout, and then proliferate to extend the new vessel. The process of tumor metastasis involves angiogenesis, adhesion to the endothelial cell basement membrane, proteolytic destruction of the basement membrane, migration to secondary sites, and proliferation at the secondary sites [10]. This five-step process resulting in neoangiogenesis is depicted in Figure 1. For angiogenesis to occur, each of these different steps must occur; hence, there are multiple levels of regulation of this process. Furthermore, negative regulatory factors exist along the way, and their interaction must also be considered.

Tumor angiogenesis is associated with increased incidence of metastases [11], worsening prognosis, and reduced survival time in the setting of NSCLC [12]. The increased frequency of metastases with adenocarcinoma may be attributed to its higher microvessel density, compared with other forms of NSCLC (*ie*, squamous cell carcinoma) [13].

Tumor metastasis has been shown to be a highly selective process consisting of a series of sequential, interrelated steps. Metastatic cells must complete these steps, including angiogenesis, to produce clinically apparent lesions [14]. Vascularization must occur if tumor diameter is to exceed 1 mm^3. The synthesis and secretion of angiogenic factors by tumor and host cells play key roles in establishing a capillary network from the surrounding host tissues. Local

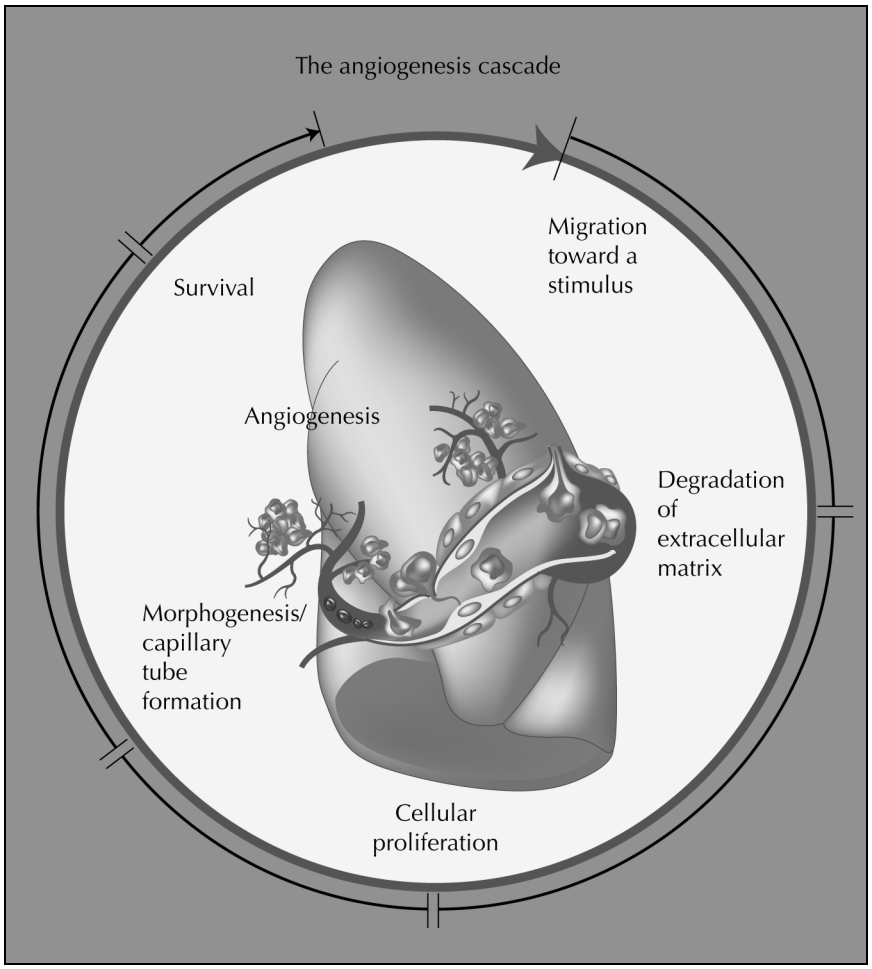

Figure 1. Angiogenesis is a complex process requiring multiple independent steps, as shown. To complete the process, all of the steps must occur.

invasion of the host stroma occurs next as a consequence of the enhanced expression of collagenases. Tumor cells enter easily into the circulation because thin-walled lymphatic channels and venules offer little resistance to their penetration. Tumor cell aggregates detach and embolize into circulation. The tumor cells not destroyed in the circulation then arrest in the capillary beds of organs and adhere to the vessel walls, where extravasation into the parenchyma must occur. Proliferation within the organ parenchyma completes the metastatic process, at which point the lesion must again develop a vascular network (*ie*, angiogenesis) while evading the host immune system.

The growth and metastasis of a neoplasm is also dependent on the formation of adequate vascular support. In lung cancer, the extent of angiogenesis appears to be important when potential survival time is discussed in multiple prospective and retrospective case series [15••,16–18].

Vascular Endothelial Growth Factor–Targeted Agents

An important angiogenesis-regulating process in humans is the interaction of vascular endothelial growth factor (VEGF) with its receptor (VEGFR) [19]. VEGF is a homodimeric protein with at least four isoforms, VEGF-A through VEGF-D.

VEGF is a member of the receptor tyrosine kinase (RTK) family of growth-signal transducing proteins [19]. RTKs are transmembrane proteins and are involved in a number of growth-promoting physiologic processes [20]. They transduce extracellular growth signals (in the form of specific receptor ligands) into intracellular growth responses by initiating various enzymatic cascades through the phosphorylation of tyrosine residues on specific cytosolic proteins. Activated VEGFR phosphorylates several signaling cascade proteins, including phospholipase C, phosphoinositol-3 kinase, and Ras guanosine triphosphate (GTP)-ase activating protein [21].

VEGFR is specifically expressed on the surface of endothelial cells and appears to be regulated largely by hypoxia, as is VEGF [19]. Three isoforms of membrane-bound VEGFR have been identified, and their roles in angiogenesis appear to be distinct [22]. VEGFR-1 (also known as Flt-1 [fms-like tyrosine kinase-1]) has the highest binding affinity for VEGF-A but is capable of generating relatively little kinase activity. VEGFR-2 (also known as KDR [kinase domain region] and Flk-1 [fetal liver kinase-1]) is the isotype most associated with endothelial cell proliferation and chemotaxis. The VEGF receptor Flk-1/KDR has been

demonstrated in mammary, ovarian, and lung tumor tissue and in glioma tissue, indicating roles for VEGF in these forms of cancer and in the identification of this receptor as a potential target [23]. VEGFR-3 (also known as Flt-4) appears primarily to regulate lymphangiogenesis [24].

Monoclonal Antibodies Against the VEGF Protein or Receptor

One approach to the modulation of VEGF-mediated angiogenesis is to utilize antibodies against the VEGF protein itself or the receptor. Antibodies to VEGF inhibited the growth of subcutaneous human xenografts in the nude mouse [9]. Furthermore, antibodies to VEGF reduced human tumor growth and hepatic metastases in a dose- and time-dependent manner and reduced the number of VEGF receptors in mice [25]. A recombinant humanized version of the murine monoclonal antibody (mu MAb) to VEGF (rhuMAb VEGF; Genentech, South San Francisco, CA) has been developed that inhibits endothelial cell proliferation in vitro and in vivo and reduces tumor growth in vivo [26]. Early clinical studies of rhuMAb VEGF produced undetectable serum concentrations of VEGF [27] at doses of 3 mg/kg/wk or more [28]. A phase IB trial combining rhuMAb VEGF with cytotoxic agents revealed no pharmacologic interaction between the antibody and either doxorubicin, carboplatin, and paclitaxel, or 5-fluorouracil (5-FU) and leucovorin (LV). Notable toxic effects possibly related to the drugs were diarrhea (with 5-FU), thrombocytopenia (with carboplatin and paclitaxel), and leukopenia [29].

A report of a phase II, three-arm, multicenter trial was presented at the American Society of Clinical Oncology annual meeting in May 2000. In the study were 99 patients with stage IIIB or IV NSCLC who were randomly assigned to standard therapy with carboplatin and paclitaxel or to one of two experimental arms, either carboplatin and paclitaxel with the anti-VEGF antibody, rhuMAb VEGF, at 7.5 mg/kg, or carboplatin and paclitaxel with rhuMAb VEGF at 15 mg/kg [30]. Because the endpoints of the study were safety, response, and time to tumor progression, the control group was allowed to cross over to the antibody alone upon disease progression. The response rates increased with the antibody by about 20%, and the time to tumor progression was prolonged by about 4 months (4.5 to 7.5 months) in the high-dose antibody group. Of concern was the fact that severe hemoptysis developed in six patients, and four episodes were fatal. In an evaluation of potential risk factors, squamous cell histology and rhuMAb treatment were the only factors associated with hemoptysis. In 2001, analysis of a subset of patients with non–squamous cell histology was performed to ascertain the impact of rhuMab-VEGF on overall response, time to progression, and median length of survival [31]; rhu-Mab-VEGF was given to 53 of 78 patients, and two of them experienced life-threatening hemorrhages. Overall response, time to progression, and length of survival favored this group of patients. This drug has now entered phase III studies in NSCLC in patients with non–squamous cell histology.

Recent results suggest that the addition of rhuMAb to carboplatin and paclitaxel may prolong survival in patients with nonsquamous NSCLC without an excess of toxic effects or deaths [31]. Objective response rates (32% vs 12%) and time to progression (30 vs 17 weeks) were higher in patients in the rhuMAb arm compared with the control group in a randomized phase II trial. The Eastern Cooperative Oncology Group (ECOG) is currently evaluating the effect of adding a 15-mg/kg dose of rhuMab to carboplatin and paclitaxel therapy in patients with advanced nonsquamous NSCLC (ECOG E-4599).

Clinical trials of another recombinant humanized monoclonal antibody, HuMV833 (PDL), are underway. Preliminary results of a phase I dose-finding study of anti-VEGF antibody HuMV833 have been presented [32]. Early safety data suggest that HuMV833 is well tolerated and without attributable grade 3 toxic effects. Furthermore, no hemorrhagic events were observed. Additional studies of this agent in various types of tumors are anticipated.

Monoclonal antibodies also have been developed against the extracellular domain of the VEGFR. For example, DC101 (ImClone Systems, New York, NY) is a rat anti-mouse Flk-1 that competitively blocks VEGF binding that inhibits growth of human ovarian, epidermoid, pancreatic, renal, and glioblastoma tumor xenografts. This process is associated with decreased tumor microvessel density, increased tumor cell apoptosis with decreased proliferation, and extensive tumor necrosis [33–36]. The chimeric antihuman VEGFR antibody IMC-1C11 is currently being evaluated in a phase I study.

VEGFR Tyrosine Kinase Inhibitors

Interruption of VEGF activity can also occur through inhibition of the VEGFR tyrosine kinase by small molecules. One example of such a VEGFR tyrosine kinase inhibitor (TKI) is the quinolone derivative SU5416 (Sugen/Pharmacia, South San Francisco, CA). In vitro, SU5416 has shown activity against VEGF-stimulated proliferation of human endothelial cells. In vivo, the agent inhibited the growth and metastasis of lung, colon, breast, and prostate cancers and melanoma, glioma, and sarcoma xenografts [37–42]. These agents are in early development; no clinical studies have formally evaluated their use in the treatment of NSCLC.

In a phase I dose-ranging trial, SU5416 (4.4 to 190 mg/m^2) was administered intravenously twice weekly to 63 patients with various malignancies. Dose-limiting toxicity (DLT) occurred at the 190-mg/m^2 dose level and consisted of headache, nausea, and projectile vomiting that was reversible within 48 hours [43,44].

A phase IB/IIA trial of SU5416 enrolled 20 male patients with advanced AIDS-associated Kaposi's sarcoma whose disease was stable with antiretroviral therapy. Nine patients showed improvement in disease-related

symptoms (reduced edema or pain, improved mobility, or improved swallowing), and five had objective partial or complete responses of their lesions [45].

SU5416 has also been studied in combination with cytotoxic agents. Twenty-eight patients with untreated metastatic colorectal cancer received SU5416 intravenously at either 85 mg/m^2 or 145 mg/m^2 twice weekly with 5-FU and LV on either the Roswell Park or the Mayo Clinic regimen. The toxic effects experienced were those common to 5-FU and LV therapy. Six patients had objective tumor responses, whereas nine patients experienced stable disease [46].

A randomized phase III trial is ongoing in which SU5416, in combination with the Saltz regimen, is being investigated in patients with advanced colorectal cancer. Interestingly, the combination of SU5416, at a dose of 145 mg/m^2 biweekly, with cisplatin and gemcitabine was not well tolerated, resulting in an unacceptable rate of thromboembolic events [47].

ZD6474 (AstraZeneca, Wilmington, DE) is an oral VEGFR-2 TKI shown to inhibit the growth of prostate cancer xenografts and to induce regressions in preclinical settings. Furthermore, tumor growth was shown to resume with cessation of ZD6474; conversely, tumor regression could be reinduced upon reintroduction of ZD6474 [48]. To date, in an ongoing phase I dose-escalation study, 41 patients with various solid tumors have been treated with ZD6474 [49]. Reported drug-related toxicity has been minimal, with only two National Cancer Institute (NCI) grade 1 (facial flushing, facial rash) and one NCI grade 2 (fatigue) events thus far. No grade 3 or 4 toxicities have been reported. Stable disease has been reported in two patients (gastrointestinal stromal tumor and melanoma) after 56 days of treatment.

SU6668 (Sugen/Pharmacia) is an oral TKI with multiple receptor targets, including not only the VEGF RTK but also the basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) RTKs. In preclinical testing, SU6668 inhibited the growth of various human tumor xenografts in athymic mice. Notably, A431 epidermoid tumor xenografts were eradicated in more than 50% (20 of 39) of the treated mice, and this response was maintained for at least 133 days following cessation of treatment [50].

Clinical evaluation is still in early phases; however, a phase I trial in 68 patients with various advanced tumors demonstrated that SU6668 is well tolerated at a wide range of dose levels (100 to 2400 mg/m^2/d), and only mild to moderate side effects occurred, including nausea, diarrhea, fatigue, and dyspnea [51,52]. Stable disease for more than 4 weeks was observed in 31 of 51 patients, and one patient with a desmoid tumor experienced stable disease for more than 5 months. Data show a minor decline in tumor markers and softening of palpable tumors in several patients. Ongoing studies are being conducted to identify patients who are most likely to respond to SU6668 therapy and to find markers of response.

Endogenous Angiogenesis Inhibitors

Angiogenesis is stimulated by various angiogenic growth factors, including VEGF and bFGF. Tumors also stimulate endogenous angiogenesis inhibitors, including angiostatin, endostatin, and thrombospondin [53,54••]. The angiogenic process is determined by the net balance of angiogenic inducer activity over angiogenic inhibitor activity.

Although multiple clinical trials are evaluating these compounds in the United States, only a few are in phase III development. Both angiostatin and endostatin have been evaluated in preclinical models of lung carcinoma, but clinical studies in NSCLC have not been initiated. TNP-470, a synthetic compound based on fumagillin, a naturally occurring antifungal agent with potent antiangiogenic activity, has demonstrated clinical activity in various solid tumors including NSCLC [55,56]. Other compounds with potential antiangiogenic activity include thalidomide and cyclooxygenase (COX) inhibitors.

Endostatin and Angiostatin

The discovery of both angiostatin and endostatin was based on the observation that the surgical removal of a primary tumor was sometimes associated with the subsequent accelerated growth of metastatic disease [57,58]. Thus, a screening study was conducted to identify endogenous angiogenesis factors that were operative in vivo in suppressing and preventing the angiogenic phenotype. Angiostatin and endostatin are proteolytic cleavage fragments of plasminogen and collagen XVIII, respectively [54••,59].

Endostatin

Endostatin, a 20-kD C-terminal fragment of collagen XVIII produced by hemangioendotheliomas, completely suppresses tumor angiogenesis by blocking the development of blood vessel supply to tumors [54••]. Treatment with endostatin was effective against three types of neoplastic tumors in mice [60] and demonstrated regression of primary tumors to microscopic dimensions in animals. There was no evidence of toxicity in these animal studies. It is possible that the effectiveness of endostatin is dependent on the tissue of origin. Phase I studies with endostatin are in progress [61–64]. Studies using endostatin in combination with other treatment modalities have not yet been reported.

Endostatin has also demonstrated antitumor effects in vivo, resulting in complete regressions in Lewis lung carcinoma, fibrosarcoma (T241), melanoma (B16), and hemangioendothelioma (EOMA) [54••]. Additionally, endostatin was evaluated in a tumor resistance model whereby tumors (Lewis lung carcinoma, melanoma, and fibrosarcoma) were allowed to regrow after initial regression from endostatin therapy [60,65•]. Retreatment of these tumors with endostatin resulted not only in repeated tumor regression after up to six cycles of treatment but also in sustained tumor dormancy and cure in some animals.

In a trial at the University of Texas M.D. Anderson Cancer Center, the primary endpoint was determination of the

optimal biologic dose (OBD). To establish the OBD, multiple surrogate biologic endpoints were incorporated, including serial tumor biopsies, serum sampling for ex vivo bioassays of endothelial cell proliferation, and biologic imaging to quantitate blood flow using ultrasound, dynamic computed tomography, and magnetic resonance imaging. The dose levels explored for endostatin ranged from 15 to 240 mg/m^2/d, by intravenous infusion. The drug was well tolerated, with no DLTs, and exhibited a linear pharmacokinetic profile. Preliminary antitumor effects have been observed in the form of tumor regression and prolonged disease stabilization in a small subset of patients.

Angiostatin

Angiostatin is a cleavage product of plasminogen containing at least three of the kringles of plasminogen. O'Reilly *et al*. [59] demonstrated that angiostatin, derived from Lewis lung carcinoma in which the primary tumor inhibited metastases, also inhibited angiogenesis. Furthermore, systemic therapy with angiostatin limited metastases by inducing a dormant state (defined by a balance of apoptosis and cell proliferation) of the tumor. In a series of 143 patients with NSCLC, 34 (24%) tumors expressed angiostatin. Patients with these tumors lived longer than did patients whose tumors did not express angiostatin (146 vs 77 weeks, P=0.02) [66].

A number of preclinical studies have been conducted to evaluate the role of angiostatin in tumor growth [67,68]. Angiostatin can inhibit growth of three different types of murine primary tumors, even if therapy is initiated when tumors become 2% of body weight [67]. Additionally, no resistance or toxicity has been observed with angiostatin in doses up to 100 mg/kg. Recently, Mauceri *et al*. [68] demonstrated that angiostatin in combination with radiotherapy reduced growth in four tumor types without increasing toxic effects in mice with Lewis lung carcinoma. Tumor volume was reduced to a greater extent using combination therapy than it was with either angiostatin or radiotherapy alone. Preliminary data from the first human trial assessing the safety, pharmacokinetics, and pharmacodynamics of angiostatin have been reported [69]. Use of recombinant human angiostatin appears to be safe, with no treatment-related bleeding or thrombotic events. Moreover, this agent exhibits linear pharmacokinetics and decreases angiogenic growth factor levels (*ie*, bFGF and VEGF).

TNP-470

TNP-470 is a synthetic form of fumagillin, a naturally occurring antiangiogenic compound. This antifungal agent originally was believed to have potent antiangiogenic activity by inhibiting neovascularization [70]. TNP-470 is a potent inhibitor of endothelial cell migration, endothelial cell proliferation, and capillary tube formation [70,71].

Preclinical trials of TNP-470 showed reduced development and metastasis rates of human tumor xenografts in rats. The activity of TNP-470 on tumors and metastases appears to be related to dose, because antitumor effects on HT-1080 cells at the primary site and reduced lymph node metastases in nude athymic mice were observed. Furthermore, TNP-470 inhibited in vitro growth of three human tumor cell lines and four murine tumor cell lines in a dose-dependent manner and also reduced in vivo tumor growth and metastatic spread [72–74].

In preclinical combination studies, TNP-470 with paclitaxel and carboplatin reduced tumor growth and metastasis of NSCLC and breast cancer in mice [75]. In animals with Lewis lung carcinoma, TNP-470 in combination with antibiotics increased the responsiveness of primary tumors and lung metastases to cytotoxic agents. The most effective combination was TNP-470 with minocycline and cyclophosphamide [71,76,77].

In a phase I clinical trial, three of 18 patients with inoperable cervical cancer had disease responses [78]. One patient had complete recovery, and in two patients progressive disease became stable. The antiangiogenic effects of TNP-470 appear to work synergistically with existing cytotoxic agents. In another phase I study, in which TNP-470 with paclitaxel was compared with paclitaxel alone in 32 patients with solid tumors (including NSCLC), the combination appeared to be safe, with myelosuppression and peripheral neuropathy similar in both treatment groups [56]. Notably, 15 of the patients had NSCLC. Of these patients, five (33%) had partial disease responses, including two who had received prior chemotherapy. None of the five patients who had received prior taxane therapy had disease responses. The median survival time for patients was greater than 14 months. The combination of TNP-470 and paclitaxel appeared to be tolerated well and demonstrated encouraging activity. The safety of this combination has been confirmed in another phase I study in patients with advanced tumors [55]. However, another phase I study demonstrated that the combination of TNP-470, paclitaxel, and carboplatin in solid tumors may warrant adjustment of the carboplatin dosage to decrease toxicity [79]. Further evaluation and studies of this combination are needed.

Thalidomide

Thalidomide was first introduced in the 1950s in Germany as an over-the-counter sedative–hypnotic and antiemetic agent for pregnancy. However, thalidomide was taken off the market in the late 1960s because of its association with teratogenicity and phocomelia [80]. Thalidomide was recently (1998) approved by the US Food and Drug Administration for the treatment of erythema nodosum leprosum [81]. The antiangiogenic effects of thalidomide were first documented by D'Amato *et al*. [82], who demonstrated its inhibitory effects on bFGF-induced corneal neovascularization in a rabbit model. Singhal *et al*. [83] reported that thalidomide had significant antitumor activity in patients with refractory multiple myeloma. Thalidomide is currently being evaluated in the treatment of

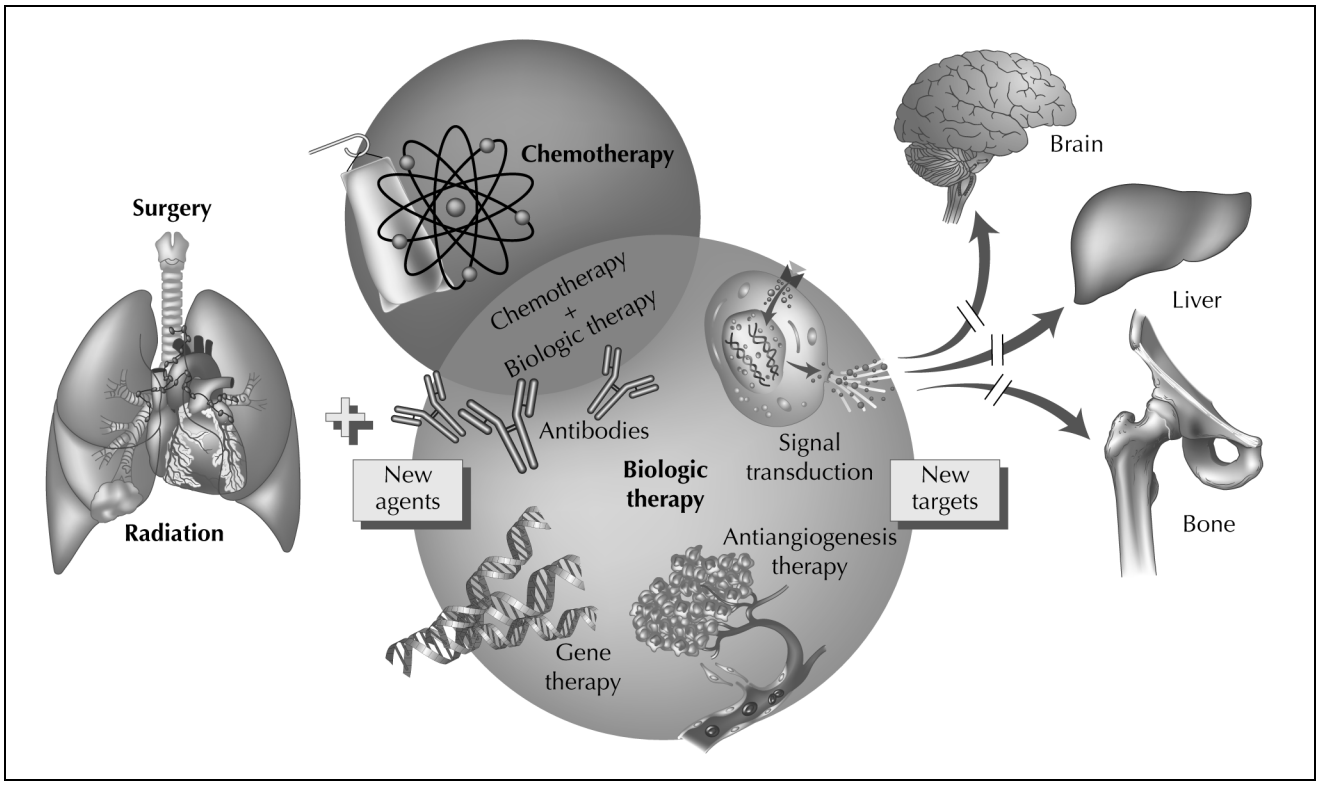

Figure 2. A new paradigm for the use of biologic therapy with chemotherapy for metastatic disease is depicted. Tumor biology has become part of pathologic staging of disease. Accordingly, in addition to receiving conventional chemotherapy, patients will soon routinely be treated with specific small molecules targeted against distinct proteins manifested by the tumor and its blood vessels. Targeting in this way will also prevent new metastases and help to eradicate existing ones.

numerous hematologic and solid malignancies. In the United States, more than 20 current clinical trials include thalidomide in their regimens, alone or in combination with other antineoplastic drugs; some of these trials are in the setting of NSCLC. Thalidomide is highly appealing because it is one of the few putative antiangiogenic agents that is an oral drug, although initial results in single-agent studies have been somewhat disappointing [55,84].

COX-2

Cyclooxygenase, a key enzyme required for prostaglandin synthesis, is transcribed from two distinct genes [85]. COX-1 and COX-2 are enzymes involved in the conversion of arachidonic acid to prostaglandins. COX-1 is expressed constitutively in most tissues, whereas COX-2 is induced by a variety of stimuli. Physiologic functions of COX-1 include platelet thromboxane production, cytoprotection of the stomach, and renal vasodilation. COX-2 has a role in inflammation that has been linked to carcinogenesis, based on its markedly increased expression in 85% to 90% of human colorectal adenocarcinoma. Epidemiologic studies have reported a significant reduction in the risk of colon, breast, and lung cancer in individuals treated with aspirin and COX-2 [86]. Clinical correlates between COX-2 upregulation and poor prognosis have been reported in several cancers, such as carcinoma of the head and neck [87] and lung cancer [88].

A recent study demonstrated overexpression of COX-2 in normal bronchial epithelial cells, type I and II pneumocytes, smooth muscle cells, vascular endothelial cells, and inflammatory mononuclear cells [89]. Substantially higher expression of COX-2 was seen in adenocarcinomas, but only a few small-cell lung cancers and squamous cell carcinomas expressed COX-2. COX-2 expression was upregulated in some premalignant lesions as well. In addition, COX-2 protein expression was detected in precursors of lung carcinomas.

Tsujii *et al.* [90] suggested that COX-2 modulates the production of angiogenic factors by colon cancer cells, thus affecting tumorigenicity. Together, these results suggest that COX-2 may contribute to the development of cancer. Thus, selective blockade of COX-2 may have an important role in cancer prevention and, by extrapolation, cancer treatment—most likely because its effect on prostaglandins may prevent angiogenesis and stimulate immune surveillance and apoptosis [91,92]. Further research on COX-2 inhibitors as therapeutic and preventive agents continues in clinical trials.

Conclusions

Lung cancer causes more deaths than any other type of cancer. This review has shown that angiogenesis is critical to the development of solid tumors; lung cancer is a typical

solid tumor. Hence, the development of approaches to block angiogenesis is of primary importance. A paradigm shift is occurring with the advent of biologic therapies that work against specific biologic pathways involved in malignancies. Biologic agents are developed against specific genes, receptors, and molecules involved in tumor development and are thus expected to have a higher therapeutic index (*ie*, ability to differentiate between normal and malignant cells). Numerous investigators have shown that lung cancer is regulated by angiogenesis and that tumors that have increased microvessel density tend to imply a poorer prognosis [12,93,94]. The identification of angiogenesis as a relevant target of anticancer drugs has resulted in a plethora of new agents, and overall, these compounds are generally less toxic than standard chemotherapy.

The use of both antiproliferative and cytostatic therapies (Fig. 2) may have major implications in transforming a terminal disease into a chronic illness. Combining of targeted molecules with traditional cytotoxic therapies usually results in lower required chemotherapy doses and fewer and less severe side effects. A number of ongoing randomized studies are being conducted to evaluate this idea. It is anticipated that these new targeted therapies will play an important role, along with cytotoxic and radiation therapies, in the management of metastatic disease.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Greenlee RT, Hill-Harmon MB, Murray T, *et al.*: **Cancer statistics.** *CA Cancer J Clin* 2001, **1:**15–36.
2. Mountain CF, Lukeman JM, Hammar SP, *et al.*: **Lung cancer classification: the relationship of disease extent and cell type to survival in a clinical trials population.** *J Surg Oncol* 1987, **35:**147–156.
3. Alberti W, Anderson G, Bartolucci A, *et al.*: **Chemotherapy in non-small cell lung cancer: a meta-analysis using update data on individual patients from 52 randomized clinical trials.** *BMJ* 1995, **311:**899.
4. Mountain CF: **A new international staging system for lung cancer.** *Chest* 1986, **89:**225–233.
5. Bulzebruck H, Bopp R, Drings P, *et al.*: **New aspects in the staging of lung cancer: prospective validations of the International Union Against Cancer TNM classification.** *Cancer* 1992, **70:**1102–1110.
6. Schiller JH, Harrington D, Sandler A, *et al.*: **A randomized phase III trial of four chemotherapy regimens in advanced non-small cell lung cancer (NSCLC) [abstract].** *Proc ASCO* 2000, **19:**1a.
7. •• Folkman J: **What is the evidence that tumors are angiogenesis dependent?** *J Natl Cancer Inst* 1989, **82:**4–6.

A review of angiogenesis and tumor biology.

8. Hori A, Sasada R, Matsutani E, *et al.*: **Suppression of solid tumor growth by immuno-neutralizing monoclonal antibody against human basic fibroblast growth factor.** *Cancer Res* 1991, **51:**6180–6184.
9. Kim KJ, Li B, Winer J, *et al.*: **Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo.** *Nature* 1993, **362:**841–844.
10. Liotta LA: **Molecular biology of metastases: a review of recent approaches.** *Mol Cell Endocrinol* 1995, **110:**205–211.
11. Chodak GW, Haudenschild C, Gittes RF, *et al.*: **Angiogenic activity as a marker of neoplasia and preneoplasia in lesions of the human bladder.** *Ann Surg* 1980, **1:**762–771.
12. Fontanini G, Vignati S, Bigini D, *et al.*: **Epidermal growth factor receptor (EGFr) expression in non-small cell lung carcinomas correlates with metastatic involvement of hilar and mediastinal lymph nodes in the squamous subtype.** *Eur J Cancer* 1995, **31A:**178–183.
13. Sikora J, Slodkowski J, Radomyski A, *et al.*: **Immunohistochemical evaluation of tumour angiogenesis in adenocarcinoma and squamous cell carcinoma of lung.** *Rocz Akad Med Bialymst* 1997, **42(suppl 1):**271–279.
14. Fidler IJ: **Cancer biology: invasion and metastasis.** In *Clinical Oncology,* edn 2. Edited by Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE. St. Louis, MO: Churchill Livingstone; 2000:29–53.
15. •• Fontanini G, Lucchi M, Vignati S, *et al.*: **Angiogenesis as a prognostic indicator of survival in non-small-cell lung carcinoma: a prospective study.** *J Natl Cancer Inst* 1997, **89:**881–886.

Study analyzing angiogenesis and its role in NSCLC.

16. Fontanini G, Vignati S, Boldrini L, *et al.*: **Vascular endothelial growth factor is associated with neovascularization and influences progression of non-small cell lung carcinoma.** *Clin Cancer Res* 1997, **3:**861–865.
17. Macchiarini P, Fontanini G, Dulmet E, *et al.*: **Angiogenesis: an indicator of metastasis in non-small cell lung cancer invading the thoracic inlet.** *Ann Thorac Surg* 1994, **57:**1534–1539.
18. Volm M, Mattern J, Koomagi R: **Expression of platelet-derived endothelial cell growth factor in non-small cell lung carcinomas: relationship to various biological factors.** *Int J Oncol* 1998, **13:**975–979.
19. Ferrara N: **Molecular and biological properties of vascular endothelial growth factor.** *J Mol Med* 1999, **77:**527–543.
20. Millauer B, Wizigmann-Voos S, Schnurch H, *et al.*: **High affinity VEGF binding and developmental expression suggest FLK-1 as a major regulator of vasculogenesis and angiogenesis.** *Cell* 1993, **72:**835–846.
21. Guo D, Jia Q, Song HY, *et al.*: **Vascular endothelial cell growth factor promotes tyrosine phosphorylation of mediators of signal transduction that contain SH2 domains: association with endothelial cell proliferation.** *J Biol Chem* 1995, **270:**6729–6733.
22. Waltenberger J, Claesson-Welsh L, Siegbahn A, *et al.*: **Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor.** *J Biol Chem* 1994, **269:**26988–26995.
23. Millauer B, Longhi MP, Plate KH, *et al.*: **Dominant-negative inhibition of Flk-1 suppresses the growth of many tumor types in vivo.** *Cancer Res 5* 1996, **6:**1615–1620.
24. Kukk E, Lymboussaki A, Taira S, *et al.*: **VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development.** *Development* 1996, **122:**3829–3837.
25. Warren RS, Yuan H, Matli MR, *et al.*: **Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis.** *J Clin Invest* 1ii5, **95:**1789–1797.
26. Presta LG, Chen H, O'Connor SJ, *et al.*: **Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders.** *Cancer Res* 1997, **57:**4593–4599.
27. Margolin K, Gordon MS, Talpaz M, *et al.*: **Phase Ib trial of intravenous (iv) recombinant humanized monoclonal antibody (Mab) to vascular endothelial growth factor (rhuMAb-VEGF) in combination with chemotherapy (ChRx) in patients (pts) with advanced cancer (CA): pharmacologic and long-term safety data [abstract].** *Proc ASCO* 1999, **18:**1678.

28. Gordon MS, Talpaz M, Margolin K, *et al.*: **Phase I trial of recombinant humanized monoclonal anti-vascular endothelial growth factor (anti-VEGF MAb) in patients (pts) with metastatic cancer [abstract].** *Proc ASCO* 1998, **17:**210a.
29. Margolin K, Gordon MS, Holmgren E, *et al.*: **Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data.** *J Clin Oncol* 2001, **19:**851–856.
30. DeVore RF, Fehrenbacher RS, Herbst RS, *et al.*: **A randomized phase II trial comparing rhumab VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) plus carboplatin/paclitaxel (CP) to CP alone in patients with stage IIIB/IV NSCLC [abstract].** *Proc ASCO* 2000, **19:**485a.
31. Johnson DH, Devore R, Kabbinavar F, *et al.*: **Carboplatin (C) + paclitaxel (T) + rhumab-VEGF (AVF) may prolong survival in advanced non-squamous lung cancer [abstract].** *Proc ASCO* 2001, **19:**1256.
32. Jayson GC, Mulatero C, Ranson M, *et al.*: **Anti-VEGF antibody HuMV833: An EORTC biological treatment development group phase I toxicity, pharmacokinetic and pharmacodynamic study [abstract].** *Proc ASCO* 2001, **19:**14.
33. Inoue K, Slaton JW, Davis DW, *et al.*: **Treatment of human metastatic transitional cell carcinoma of the bladder in a murine model with the anti-vascular endothelial growth factor receptor monoclonal antibody DC101 and paclitaxel.** *Clin Cancer Res* 2000, **6:**2635–2643.
34. Rockwell P, Witte L, Hicklin D, *et al.*: **Antitumor activity of anti-flk-1 monoclonal antibodies [abstract].** *Proc Annu Meet Am Assoc Cancer Res* 1997, **38:**266.
35. Prewett M, Huber J, Li Y, *et al.*: **Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors.** *Cancer Res* 1999, **59:**5209–5218.
36. Klement G, Baruchel S, Rak J, *et al.*: **Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity.** *J Clin Invest* 2000, **105:**R15–R24.
37. Fong TA, Shawver LK, Sun L, *et al.*: **SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types.** *Cancer Res* 1999, **59:**99–106.
38. Angelov L, Salhia B, Roncari L, *et al.*: **Inhibition of angiogenesis by blocking activation of the vascular endothelial growth factor receptor 2 leads to decreased growth of neurogenic sarcomas.** *Cancer Res* 1999, **59:**5536–5541.
39. Shaheen RM, Davis DW, Liu W, *et al.*: **Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis.** *Cancer Res* 1999, **59:**5412–5416.
40. Mendel DB, Schreck RE, West DC, *et al.*: **The angiogenesis inhibitor SU5416 has long-lasting effects on vascular endothelial growth factor receptor phosphorylation and function.** *Clin Cancer Res* 2000, **6:**4848–4858.
41. Rosen LS, Kabbinavar F, Rosen P, *et al.*: **Phase I trial of SU5416, a novel angiogenesis inhibitor in patients with advanced malignancies [abstract].** *Proc ASCO* 1998, **17:**843.
42. Vajkoczy P, Thurnher A, Hirth KP, *et al.*: **Measuring VEGF-Flk-1 activity and consequences of VEGF-Flk-1 targeting in vivo using intravital microscopy: clinical applications.** *Oncologist* 2000, **5(suppl 1):**16–19.
43. Rosen L, Mulay M, Mayers A, *et al.*: **Phase I dose-escalating trial of SU5416, a novel angiogenesis inhibitor in patients with advanced malignancies [abstract].** *Proc ASCO* 1999, **18:**161a.
44. Cropp GF, Hannah AL: **SU5416, a molecularly targeted novel anti-angiogenesis drug: clinical pharmacokinetics and safety review [abstract]. Proceedings of the 11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy.** *Clin Cancer Res* 2000, **6(suppl).**
45. Miles S, Arasteh K, Gill P, *et al.*: **A multicenter dose-escalating study of SU5416 in AIDS-related Kaposi's sarcoma [abstract].** *Proc ASCO* 2000, **19:**176a.
46. Rosen PJ, Amado R, Hecht JR, *et al.*: **A phase I/II study of SU5416 in combination with 5-FU/leucovorin in patients with metastatic colorectal cancer [abstract].** *Proc ASCO* 2000, **19:**5d.
47. Giaccone G, Rosen L, Kuene B, *et al.*: **Dose finding study of cisplatin, gemcitabine, and SU5416 in patients with advanced malignancies [abstract]. Proceedings of the 11th NCI EORTC AACR Symposium on New Drugs in Cancer Therapy.** *Clin Cancer Res* 2000, **6(suppl):**263.
48. Wedge SR, Ogilvie DJ, Dukes M, *et al.*: **VEGF receptor tyrosine kinase.** *Proc Annu Meet Am Assoc Cancer Res* 2000, **41:**566.
49. Basser R, Hurwitz H, Barge A, *et al.*: **Phase I pharmacokinetic and biological study of the angiogenesis inhibitor, ZD6474, in patients with solid tumors [abstract].** *Proc ASCO* 2001, **19:**396.
50. Laird AD, Vajkoczy P, Shawver LK, *et al.*: **SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors.** *Cancer Res* 2000, **60:**4152–4160.
51. Rosen LS, Rosen PJ, Kabbinavar F, *et al.*: **Phase I experience with SU6668, a novel multiple receptor tyrosine kinase inhibitor in patients with advanced malignancies [abstract].** *Proc ASCO* 2001, **19:**383.
52. Rosen L, Hannah A, Rosen P, *et al.*: **Phase I experience with oral SU6668, a novel multiple receptor tyrosine kinase inhibitor in patients with advanced malignancies [abstract].** *Clin Cancer Res* 2000, **6(suppl):**458.
53. Chen C, Parangi S, Tolentino MJ, *et al.*: **A strategy to discover circulating angiogenesis inhibitors generated by human tumors.** *Cancer Res* 1995, **55:**4230–4233.
54.•• O'Reilly MS, Boehm T, Shing Y, *et al.*: **Endostatin: an endogenous inhibitor of angiogenesis and tumor growth.** *Cell* 1979, **88:**277–285.

A nice review of an early angiogenic agent, endostatin.

55. Baidas S, Bhargava P, Isaacs C, *et al.*: **Phase I study of the combination of TNP-470 and paclitaxel in patients with advanced cancer [abstract].** *Proc ASCO* 2000, **19:**800.
56. Herbst RS, Tran HT, Madden TL, *et al.*: **Phase I study of the angiogenesis inhibitor TNP-470 (T) in combination with paclitaxel (P) in patients with solid tumors [abstract].** *Proc ASCO* 2000, **19:**707.
57. Sugarbaker E, Thornwaite J, Ketcham A: **Inhibitory effect of a primary tumor on metastasis.** In *Progress in Cancer Research and Therapy.* Edited by Day S, Myers P, *et al*. New York: Raven Press; 1977:227–240.
58. Koike A, Moore GE, Mendoza CB, *et al.*: **Heterologous, homologous, and autologous transplantation of human tumors.** *Cancer* 1963, **16:**1065–1071.
59. O'Reilly M, Holgren L, Shing Y, *et al.*: **Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma.** *Cell* 1994, **79:**315–328.
60. Boehm T, Folkman J, Browder T, *et al.*: **Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance.** *Nature* 1997, **390:**404–407.
61. Fogler WE, Song M, Supko JG, *et al.*: **Recombinant human endostatin demonstrates consistent and predictable pharmacokinetics following intravenous bolus administration to cancer patients [abstract].** *Proc ASCO* 2001, **19:**274.
62. Eder JP, Clark JW, Supko JG, *et al.*: **A phase I pharmacokinetic and pharmacodynamic trial of recombinant human endostatin [abstract].** *Proc ASCO* 2001, **20:**275.
63. Herbst RS, Tran HT, Mullani NA, *et al.*: **Phase I clinical trial of recombinant human endostatin (rHE) in patients (pts) with solid tumors: pharmacokinetic, safety and efficacy analysis using surrogate endpoints of tissue and radiologic response [abstract].** *Proc ASCO* 2001, **20:**9.
64. Thomas JP, Shiller J, Lee F, *et al.*: **A phase I pharmacokinetic and pharmacodynamic study of recombinant human endostatin [abstract].** *Proc ASCO* 2001, **20:**276.

65.• Black WR, Agner RC: **Tumor regression after endostatin therapy.** *Nature* 1998, **391:**450.
A review of endostatin therapy in solid tumors.
66. Volm M, Mattern J, Koomagi R, *et al.*: **Angiostatin expression in non-small cell lung cancer.** *Clin Cancer Res* 2000, **6:**3236–3240.
67. O'Reilly MS, Holmgren L, Chen CC, *et al.*: **Angiostatin induces and sustains dormancy of human primary tumors in mice.** *Nat Med* 1996, **2:**689–692.
68. Mauceri HJ, Hanna NN, Beckett MA, *et al.*: **Combined effects of angiostatin and ionizing radiation in antitumour therapy.** *Nature* 1998, **394:**287–291.
69. Demoraes ED, Fogler WE, Grant D, *et al.*: **Recombinant human angiostatin (rhA): a phase I clinical trial assessing safety, pharmacokinetics (PK) and pharmacodynamics (PD) [abstract].** *Proc ASCO* 2001, **20:**10.
70. Ingber D, Fujita T, Kishimoto S, *et al.*: **Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth.** *Nature* 1990, **348:**555–557.
71. Parangi S, O'Reilly M, Christofori G, *et al.*: **Antiangiogenic therapy of transgenic mice impairs de novo tumor growth.** *Proc Natl Acad Sci U S A* 1996, **93:**2002–2007.
72. Mysliwski A, Szmit E, Szatkowski D, *et al.*: **Suppression of growth of Bomirski Ab melanoma and its metastasis in hamsters by angiogenesis inhibitor TNP-470.** *Anticancer Res* 1998, **18:**441–443.
73. Ohta Y, Watanabe Y, Tabata T, *et al.*: **Inhibition of lymph node metastasis by an anti-angiogenic agent, TNP-470.** *Br J Cancer* 1997, **75:**512–515.
74. Singh Y, Shikata N, Kiyozuka Y, *et al.*: **Inhibition of tumor growth and metastasis by angiogenesis inhibitor TNP-470 on breast cancer cell lines in vitro and in vivo.** *Breast Cancer Res Treat* 1997, **45:**15–27.
75. Herbst RS, Takeuchi H, Teicher BA: **Paclitaxel/carboplatin administration along with antiangiogenic therapy in non-small-cell lung and breast carcinoma models.** *Cancer Chemother Pharmacol* 1998, **41:**497–504.
76. Kakeji Y, Teicher BA: **Preclinical studies of the combination of angiogenic inhibitors with cytotoxic agents.** *Invest New Drugs* 1997, **15:**39–48.
77. Teicher BA, Holden SA, Ara G, *et al.*: **Comparison of several antiangiogenic regimens alone and with cytotoxic therapies in the Lewis lung carcinoma.** *Cancer Chemother Pharmacol* 1996, **38:**169–177.
78. Kudelka AP, Levy T, Verschraegen CF, *et al.*: **A phase I study of TNP-470 administered to patients with advanced squamous cell cancer of the cervix.** *Clin Cancer Res* 1997, **3:**1501–1505.
79. Tran HT, Blumenschein GL, Madden T, *et al.*: **Phase I study of the angiogenesis inhibitor TNP-470 in combination with paclitaxel (P) and carboplatin (Cpt) in patients with solid tumors [abstract].** *Proc ASCO* 2001, **20:**394.
80. Hales BF: **Thalidomide on the comeback trail.** *Nat Med* 1999, **5:**489–490.
81. Calabrese L, Fleischer AB: **Thalidomide: current and potential clinical applications.** *Am J Med* 2000, **108:**487–495.
82. D'Amato RJ, Loughnan MS, Flynn E, *et al.*: **Thalidomide is an inhibitor of angiogenesis [abstract].** *Proc Natl Acad Sci U S A* 1994, **91:**4082–4085.
83. Singhal S, Mehta J, Desikan R, *et al.*: **Antitumor activity of thalidomide in refractory multiple myeloma.** *N Engl J Med* 1999, **341:**1565–1571.
84. Tseng JE, Glisson BS, Khuri FR, *et al.*: **Phase II study of the anti-angiogenesis agent thalidomide in recurrent or metastatic squamous cell carcinoma of the head and neck.** *Cancer* 2002, in press.
85. Shattuck-Brandt RL, Varilek GW, Radhika A, *et al.*: **Cyclooxygenase-2 expression is increased in the stroma of colon carcinomas from IL-10(-/-) mice.** *Gastroenterology* 2000, **18:**337–345.
86. Schreinemachers DM, Everson RB: **Aspirin use and lung, colon, and breast cancer incidence in a prospective study.** *Epidemiology* 1994, **5:**138–146.
87. Chan G, Boyle JO, Yang EK, *et al.*: **Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck.** *Cancer Res* 1999, **59:**991–994.
88. Khuri FR, Wu H, Lee JJ, *et al.*: **Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer.** *Clin Cancer Res* 2001, **4:**861–867.
89. Hida T, Yatabe Y, Achiwa H, *et al.*: **Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically adenocarcinomas.** *Cancer Res* 1998, **58:**3761–3764.
90. Tsujii M, Kawano S, Tsujii S, *et al.*: **Cyclooxygenase regulates angiogenesis induced by colon cancer cells.** *Cell* 1998, **93:**705–716.
91. Tsujii M, Dubois RN: **Alterations in cellular adhesion and apoptosis in epitherlial cells overexpressing prostaglandin endoperoxide synthase 2.** *Cell* 1995, **83:**493–501.
92. Sheng H, Shao J, Morrow JD, *et al.*: **Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells.** *Cancer Res* 1998, **58:**362–366.
93. Giatromanolaki A, Koukourakis M, O'Byrne K, *et al.*: **Prognostic value of angiogenesis in operable non-small cell lung cancer.** *J Pathol* 1996, **179:**80–88.
94. Macchiarini P, Fontanini G, Hardin MJ, *et al.*: **Relation of neovascularisation to metastasis of non-small-cell lung cancer.** *Lancet* 1992, **340:**145–146.