

# Targeting Angiogenesis With Integrative Cancer Therapies

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An integrative approach for managing a patient with cancer should target the multiple biochemical and physiological pathways that support tumor development while minimizing normal tissue toxicity. Angiogenesis is a key process in the promotion of cancer. Many natural health products that inhibit angiogenesis also manifest other anticancer activities. The authors will focus on natural health products (NHPs) that have a high degree of antiangiogenic activity but also describe some of their many other interactions that can inhibit tumor progression and reduce the risk of metastasis. NHPs target various molecular pathways besides angiogenesis, including epidermal growth factor receptor (EGFR), the HER-2/neu gene, the cyclooxygenase-2 enzyme, the NF- $\kappa$ B transcription factor, the protein kinases, Bcl-2 protein, and coagulation pathways. The herbalist has access to hundreds of years of observational data on the anticancer activity of many herbs. Laboratory studies are confirming the knowledge that is already documented in traditional texts. The following herbs are traditionally used for anticancer treatment and are antiangiogenic through multiple interdependent processes that include effects on gene expression, signal processing, and enzyme activities: *Artemisia annua* (Chinese wormwood), *Viscum album* (European mistletoe), *Curcuma longa* (turmeric), *Scutellaria baicalensis* (Chinese skullcap), resveratrol and proanthocyanidin (grape seed extract), *Magnolia officinalis* (Chinese magnolia tree), *Camellia sinensis* (green tea), *Ginkgo biloba*, quercetin, *Poria cocos*, *Zingiber officinale* (ginger), *Panax ginseng*, *Rabdosia rubescens* (rabdosisia), and Chinese destagnation herbs. Quality assurance of appropriate extracts is essential prior to embarking on clinical trials. More data are required on dose response, appropriate combinations, and potential toxicities. Given the multiple effects of these agents, their future use for cancer therapy probably lies in synergistic combinations. During active cancer therapy, they should generally be evaluated in combination with chemotherapy and radiation. In this role, they act as biological response modifiers and adaptogens, potentially enhancing the efficacy of the so-called conventional therapies. Their effectiveness may be increased when multiple agents are used in optimal combinations. New designs for trials to demonstrate activity in human subjects are required. Although controlled trials might be preferred, smaller studies with appropriate end points and surrogate markers for antiangiogenic response could help prioritize agents for the larger resource-intensive phase 3 trials.

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## The Concept of Antiangiogenic Therapies

The induction and promotion of cancer is a multistep process that involves biochemical interactions from the level of the genes, through cell-signaling pathways, intercellular communication mechanisms, supply of nutrients, channels for metastases, and a host immune response. Most of these pathways are involved in the maintenance of homeostasis and environmental adaptation. For example, a wound results in intercellular and intracellular signals that activate the appropriate genes for protein synthesis and cell division to repair the defect. Part of this process is the induction of new blood vessels to supply nutrients and immunocytes. Similar processes are activated by the evolution of cancer. In view of the multitude of redundant interactions, disabling 1 part of the system is unlikely to impede tumor development. A holistic approach targets the biochemical and physiological pathways that support tumor development at various levels. Many natural health products that inhibit angiogenesis also manifest other anticancer activities. We will focus on natural health products that have a high degree of antiangiogenic activity but also describe some of their many other interactions that can inhibit tumor progression and reduce the risk of metastasis. In doing so, we realize that we are taking a reductionist approach that does not reflect the clinical practice of either an herbalist or an integrative physician. In clinical practice, we would advocate a multidimensional approach, realizing that there is synergy between treating the whole person as well as activities at the cell level.

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Cancer progression requires a source of nutrition and oxygen. Tumors that outgrow their oxygen supply cannot form masses more than 1 to 2 mm in size or they develop central necrosis. Neoplasms are genetically plastic and often adapt by switching on genes that result in an increased ability to invade and to metastasize. A critical part of this process is the induction of local small blood vessels, termed *angiogenesis*.<sup>1,2</sup> Tumors do not grow progressively unless they induce a blood supply from the surrounding stroma. Cancers that lack angiogenesis remain dormant. Rapid logarithmic growth follows the acquisition of a blood supply. The tumor angiogenic switch is activated when the balance of angiogenic inhibitors to stimulators is shifted. The process of neovascularization is subtly controlled in normal tissues by a series of endogenous polypeptides that are secreted during growth, healing, and tissue renewal (Table 1). Cancers synthesize or induce some of these polypeptides, especially vascular endothelial growth factor (VEGF) and angiopoietin (APN). These are peptides that are stimulated by hypoxia and result in sprouting of endothelial cords. The cancer induces a profuse but immature network of thin endothelial-lined channels, essential for tumor oxygenation. Although these new vessels allow progressive tumor growth, they are less efficient than the vascular supply of normal tissues. APN normally recruits pericytes and initiates modeling of the vessel wall to more mature forms. However, tumors secrete a relative excess of VEGF, and this results in disorganized and leaky vessels that cause local bleeding and edema.

Antiangiogenic therapy might be less susceptible to development of treatment resistance because it is directed to stromal tissue rather than the genomically unstable tumor cells. Judah Folkman initially proposed the concept of treating cancer by inhibiting the formation of its vasculature.<sup>3</sup> Targeted therapies against new vessel formation have recently been developed. These are monoclonal antibodies that antagonize the formation of new blood vessels. One example is bevacizumab (Avastin). Bevacizumab is a genetically engineered humanized monoclonal IgG antibody that blocks the VEGF receptor in endothelial cells and shuts off the tumor blood supply. It has been shown to extend life for some metastatic colorectal cancer patients by a few months, when used together with chemotherapy.<sup>4</sup> There is preliminary evidence that adding bevacizumab to paclitaxel and carboplatin can improve survival by 2 months for non-squamous-cell lung cancer patients.<sup>5</sup> Although bevacizumab increases survival for some patients, it increases the risk of adverse effects, including leukopenia, diarrhea, and hypertension. There are also major risks for thrombosis, resulting in stroke and myocardial

**Table 1. Endogenous Angiogenic Polypeptides**

Angiogenin (AG) and angiotropin (AT)
Basic fibroblast growth factor (bFGF)
Granulocyte-colony-stimulating factor (G-CSF)
Hepatocyte growth factor (HGF)
Interleukin-8 (Il-8)
Placental growth factor (PGF)
Platelet-derived endothelial cell growth factor (PD-ECGF)
Pleiotrophin (PTN)
Proliferin
Transforming growth factor- $\alpha$ (TGF- $\alpha$ )
Transforming growth factor- $\beta$ (TGF- $\beta$ )
Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )
Vascular endothelial growth factor (VEGF)
Vascular permeability factor (VPF)
Insulin-like growth factor I and II (IGF-I and II)
Cyclooxygenase (COX) and lipoxygenase (LOX)
Nuclear factor- $\kappa$ beta (NF- $\kappa$ B)
Activator protein (AP-1)
Angiopoietin (APN)

infarction, as well as fatal hemorrhage, such as gastrointestinal bleeding or hemoptysis, and visceral perforation.<sup>6</sup> Antiangiogenic therapies may also be combined with radiotherapy to improve local tumor control and to reduce the risk of metastases. During a course of radiotherapy, some tumors increase their angiogenic activity.<sup>7</sup> Combined-modality therapies with antiangiogenic agents induce a normal microvascular bed out of the disorganized tumor vessels. There is a critical time during the antiangiogenic treatment when the VEGF to APN ratio approximates to normal. At that point, pericytes are recruited, the vascular basement membrane adopts a thinner morphology, and tumor oxygenation temporarily increases. This is a favorable time to apply ionizing radiation since it is preferentially lethal to replicating and well-oxygenated cells. The combination of an antiangiogenic agent and radiation therapy is optimally effective if this window of opportunity is exploited, and concerns regarding the induction of metastases have not been confirmed.<sup>8-10</sup>

Single antiangiogenic agents seem to have limited efficacy. Natural health products contain a range of complex organic chemicals that may have synergistic activity. They may inhibit angiogenesis by interacting with multiple pathways, as well as having other activities that can interact with cell signaling, the apoptotic pathway, and the interaction of cancer cells with the immune system. Some antiangiogenic agents also have anticoagulation activity that may also be associated with a reduction of metastases. Heparin is a well-known example of a therapy with both anticoagulation and antiangiogenic activities. Instead of developing multiple monoclonal antibodies to target the various peptides and their receptors, an alternative approach would be to evaluate phytochemicals and some animal-derived chemical derivatives that

influence multiple pathways. The science of pharmacognosy evaluates natural product drugs derived from herbal remedies or phytomedicines. There has been minimal clinical research that evaluates their use as adjuvant therapy to conventional treatment with cytotoxic drugs and radiotherapy. We require formal research on the timing of administration of natural health products with anticancer therapies. Antiangiogenic natural health products may be most effective in impeding cancer recurrence after cytotoxic therapy, encouraging tumors to remain dormant by changing the balance from cell proliferation to cell death by apoptosis.

### The Process of Angiogenesis

Normal angiogenesis is the regulated formation of new blood vessels from existing ones. It is the basis of several physiological processes, such as embryonic development, placenta formation, and wound healing. The tumor can take control of normal processes and deregulate them to its own advantage. Normal formation of new blood vessels consists of stimulation of endothelial cells by angiokinins and specific enzymes, such as matrix metalloproteinase (MMP) and heparinase, that result in the dissolution of the extracellular matrix (ECM). The tight junctions between the endothelial cells are disrupted, and the endothelial cells can then project through the newly created spaces and organize into fresh capillary tubes that grow toward the source of the blood supply.<sup>11,12</sup> Induction of new blood vessels provides the tumor with a survival advantage. The growth and survival of cells are dependent on an adequate supply of oxygen and nutrients and the removal of toxic products. Oxygen can diffuse radially from capillaries for only 150 to 200  $\mu\text{m}$ . When distances exceed this, cell death follows. Thus, the expansion of a tumor mass beyond 1 mm depends on the development of a new blood supply.<sup>13-15</sup> Increasing the density of tumor vasculature raises the probability that it will metastasize. An increased microvascular density or angiogenesis index is a significant indicator of poorer prognosis. Increased vascular density is found not only in solid tumors but also in the bone marrow of patients with acute myeloid leukemia and myeloma.<sup>16,25</sup> The angiogenesis is characterized by oncogene-driven tumor expression of proangiogenic proteins (Table 1).

The formation of new vasculature consists of sequential steps. Endothelial cells must proliferate, migrate, and penetrate host stroma and the ECM. The endothelial cells must also undergo morphogenesis. The process of angiogenesis consists of an activation and resolution phase. Activation requires initial degradation of the basement membrane, followed by

endothelial cell migration, invasion of the surrounding extracellular matrix, endothelial cell proliferation, and capillary lumen formation. Resolution results in the maturation and stabilization of the microvasculature by enclosing the vessel with pericytes, inhibition of endothelial proliferation, basement membrane reconstitution, and formation of gap junctions. The vasculature of many solid tumors is not identical to that in normal tissues.<sup>26</sup> The resolution phase is often incomplete in tumors, resulting in tumor microvessels that are highly irregular and tortuous and only partially lined with endothelium and basement membranes. Arteriovenous shunts and blind ends are common. Failure of resolution may be a consequence of persistent overexpression of angiopoietin-2 in the tumor-associated vasculature. There are differences in cellular composition, permeability, vessel stability, and regulation of growth. The balance between factors that stimulate new blood vessel growth and those that inhibit it determines the vascular density. The inhibitory influence predominates in normal tissues, whereas in tumors many neoplastic cells switch from an angiogenesis-inhibiting to an angiogenesis-stimulating phenotype. This coincides with the loss of the wild-type allele of the p53 tumor suppressor gene and is associated with the reduced production of thrombospondin (TSP-1), a controller of angiogenesis in fibroblasts.<sup>27-32</sup>

The production of VEGF is considered essential for angiogenesis and the migration of cancer cells. A high VEGF expression level is associated with a worse outcome in a wide array of malignancies. VEGF mRNA expression is upregulated by a wide array of oncogenes (including *H-ras* and *K-ras*, *src*, *p53*, and *C-jun*) and growth factors (including epidermal growth factor [EGF], transforming growth factor [TGF]- $\alpha$ , TGF- $\beta$ , insulin-like growth factor-1, and platelet-derived growth factor).<sup>33-39</sup> Table 2 lists some cancer-associated genes implicated in angiogenesis.

### The Angiogenic-Metastatic Pathway as a Target for Anticancer Therapies

The process of cancer metastasis consists of a series of sequential interrelated steps. Each step is rate limited and may be a target for therapy. The outcome of the process is dependent on both the intrinsic properties of the tumor cells and the responses of the host. The balance of these interactions varies between tumors and patients. The major steps in the formation of a metastasis are as follows<sup>40-42</sup>:

1. Transformation of normal cells into tumor cells followed by growth. Initially depends on nutrients supplied by simple diffusion.

**Table 2. Cancer-Associated Genes Implicated in Angiogenesis**

Oncogene	Growth Factors or Cytokine Levels
H-/K-ras	VEGF ↑; TSP-1 ↓; bFGF ↑
src	VEGF ↑
erb2/HER-2	VEGF ↑; TSP-1 ↓
EGFR	VEGF ↑; IL-8 ↑; bFGF ↑
HPV16	VEGF ↑
bcr-abl	VEGF ↑
n-myc/c-myc	VEGF ↑; TSP-1 ↓
p53	VEGF ↑; TSP-1 ↓
C-jun	VEGF ↑; TSP-1 ↓

↑ = increased level; ↓ = decreased level. VEGF = vascular endothelial growth factor; TSP = thrombospondin; bFGF = basic fibroblast growth factor; EGFR = epidermal growth factor receptor; IL = interleukin.

- Extensive vascularization (angiogenesis). This must occur if the tumor mass is to exceed 1 mm in diameter. The production and secretion of proangiogenic factors by tumor cells and host cells play a major role in establishing a capillary network from the surrounding host tissue.
- Local invasion. Tumor cells invade the host stroma through several mechanisms. Thin-walled venules, fragmented arterioles, and lymphatic channels offer little resistance to penetration and provide the most common pathways for tumor cell entry into the circulation.
- Detachment and embolization. Single cells or clumps break away. Most circulating tumor cells are rapidly destroyed. Those that survive must arrest in the capillary beds of distant organs by adhering either to capillary endothelial cells or to the exposed subendothelial basement membrane.
- Extravasation into new host organ or tissue.
- Proliferation within the new host organ or tissue. To continue growing beyond the size of 1 mm in diameter, the micrometastasis must develop a vascular network and evade destruction by host defenses. The cells can then continue to invade blood vessels, enter the circulation, and produce additional metastases.

The growth of many cancers is associated with the absence of the endogenous inhibitors of angiogenesis, such as interferon- $\beta$  (INF- $\beta$ ). INF- $\beta$  is a potent inhibitor of angiogenesis through blocking interleukin (IL)-8, basic fibroblast growth factor (bFGF), and collagenase type V, which are all potent angiogenic factors that aid tumor development and invasiveness. VEGF stimulates the proliferation and migration of endothelial cells and induces the expression of metalloproteinases and plasminogen activity. Overexpression of VEGF in tumor cells enhances tumor growth and metastasis in several animal models by stimulating vascularization.<sup>13,43,50</sup> Some cytotoxic chemotherapy agents are being used at lower than normal doses with the intent of inhibiting angiogenesis and minimizing toxicity.<sup>51,52</sup> This strategy may

permit advanced cancer patients to maintain a better quality of life. The low-dose therapy is termed *metronomic* dosing.<sup>53-55</sup> The metronomic model of conventional cytotoxic chemotherapy suggests that there may also be advantages for administering combinations of phytochemicals that interact with the multistep process of angiogenesis.<sup>56</sup> In other words, targeting the vascular endothelium with continuous low-dose noncytotoxic therapies may maintain tumor control without excessive toxicity. Their potential role for increasing overall survival (but not necessarily disease-free survival) and maintaining quality of life requires evaluation in future clinical trials.

### Role of the Tumor Microenvironment in Mediating the Response to Antiangiogenic Therapy

Individual tumors can display various angiogenic phenotypes because their expression is controlled by a combination of intrinsic factors in the tumor cell and the influence of the host microenvironment.<sup>57</sup> The latter can effect gene expression in tumors growing at different sites. The tumor cells, in turn, can alter the endothelial cell phenotype. Different sites of metastases may express various combinations of angiogenic factors and endothelial cell phenotypes.<sup>58</sup> Interactions among the polypeptide angiogenic factors produced by the tumor are complex, functioning with other factors present in the tumor microenvironment in a dynamic, reciprocal fashion. Therefore, when designing cytokine-targeted antiangiogenic therapies or monoclonal antibodies against angiogenic growth factors, one must also take into account the tumor microenvironment. The efficacy of antiangiogenic compounds will vary between tumors. The more specific the intervention is to 1 domain of the angiogenic pathway, the less likely there will be a beneficial reduction in tumor growth since alternative pathways can compensate. If the angiogenic activity of a tumor is initiated primarily by only 1 factor, then blocking the activity of that 1 factor may provide temporary efficacy. For example, VEGF expression correlates with the metastatic characteristics of human colon cancer, so targeting VEGF alone may be beneficial.<sup>59</sup> However, if several factors mediate the angiogenic activity in a particular tumor, an alternative intervention strategy is required. Natural health products contain a cocktail of biological chemicals that act on multiple pathways that initiate and maintain tumor angiogenesis. In addition, we hypothesize that angiogenesis within the tumor microenvironment may be more sensitive to a cocktail of natural health products administered continuously at relatively low doses, compared to intermittent single-

agent pharmaceutical compounds administered at higher dose levels. In general, tumors contain very immature blood vessels compared to normal tissues that may make them relatively more susceptible to antiangiogenic therapies and thereby allow a therapeutic gain.<sup>60,61</sup>

### Screening Herbs for Antiangiogenic Activity

One of the first isolated antiangiogenic agents was a phytochemical. In 1990, Ingber et al<sup>62</sup> reported the antiangiogenic properties of fumagillin, a secreted antibiotic of the fungus *Aspergillus fumigatus* (Trichocomaceae). Refined fumagillin produces excess toxicity, so analogues of fumagillin were subsequently synthesized. Fumagillin and an analogue labeled TNP-470 are proposed to inhibit angiogenesis by selective inhibition of methionine aminopeptidase type 2 (MetAP-2). However, TNP-470 also demonstrated poor pharmacokinetic behavior and dose-limiting toxicity in clinical trials, and these factors remain obstacles to its use as an anticancer agent. Further modifications of fumagillin have been conducted to develop MetAP-2 inhibitors with desirable pharmacological properties. They have been tested only by in vitro assays, and to date, no clinical trials of these analogues have yet been conducted.<sup>63,64</sup>

Since the angiogenic cascade is a multistep process, numerous assays have been developed to study potential angiogenic activity. Some analyze a single step in the pathway, whereas others test the angiogenic cascade as a whole. The relationship of each assay to clinical activity is poorly defined. Some agents have profound antiangiogenic effects at low doses; others exhibit antiangiogenic activity only at near cytotoxic concentrations. Some agents have activity in one model but none in others. Criteria for antiangiogenic activity should include<sup>52</sup>

- differential cytotoxicity,
- alteration of endothelial cell function,
- critical mechanistic effects, and
- inhibition of angiogenesis in vivo.

Various assays are used to screen natural health products for antiangiogenic activity.<sup>52,65,66</sup> Assays used for screening will be briefly discussed.

#### In Vitro Assays

In vitro assays are designed to recapitulate each of the multiple events that constitute the angiogenic process. Some of them are very specific in analyzing a single event (proliferation, apoptosis, migration, production of proteases), whereas others provide a

more complex picture of the process, involving multiple aspects, cell functions, and interactions with the environment. In vitro assays for the activity of antiangiogenic compounds are usually based on the use of endothelial cells. A critical issue in setting up an in vitro assay is the choice of endothelial cells. Immortalized endothelial cells are sometimes used, as they provide an “unlimited” source of cells. Although these cell lines have the obvious advantages of being easy to grow and relatively stable throughout in vitro passages and among batches, they have usually lost some of the characteristics of endothelial cells, including molecular markers, and exhibit changes in function. The most commonly used endothelial cells are from the human umbilical vein, as the source (the umbilical cord) is easily available and cell isolation is relatively simple. For the same reasons, bovine or murine aortic endothelial cells are often used too, but these come from large vessels, and they have different phenotypic and behavioral characteristics from those of the microvessels that are more likely involved in angiogenesis. Other common sources of microvascular endothelial cells are the skin, brain, adipose tissue, and adrenal gland. Endothelial cells derived from the microvasculature of different tissues/organs are often heterogeneous, imposing a further constraint on the choice of cell model. Ideally, when developing inhibitors of tumor angiogenesis, tumor-derived endothelial cells should be used. However, practical difficulties in their isolation from tumor tissue and maintenance in culture have limited their use in preclinical studies.<sup>67</sup>

The ability to maintain endothelial cells in culture has allowed the study of endothelial cell proliferation, migration, and cellular function. Angiogenic activity may be represented as endothelial cell migration across a Boyden chamber. Compounds with antiangiogenic potential will inhibit the migration. The bovine aortic endothelial cell (BAEC) and the human umbilical vein endothelial cell (HUVEC) assays are established systems. In vitro assays are relatively inexpensive and give more rapid results. However, the ability to inhibit endothelial cell proliferation, migration, and tubule formation in vitro may not necessarily predict in vivo response. In vitro assays are a rapid method for initial screening of large numbers of agents. Definitive conclusions cannot be based on in vitro assays alone.

#### In Vivo Assays

These biological assays are more specific for detecting antiangiogenic activity. The chick embryo chorioallantoic membrane (CAM) model is an extra-embryonic membrane that is commonly used to study agents that influence angiogenesis. An angiogenic re-

sponse occurs 72 to 96 hours after stimulation in the form of increased vessel density around the implant. On the other hand, an angiostatic compound induces the vessels to become less dense around the implant and even disappear. Other systems include animal cornea implantation, disc angiogenesis, Matrigel systems, and tumor xenograft models. The in vivo assays provide a more complete physiologic assessment of angiogenesis but are more time-consuming and expensive.

### **Criteria for Antiangiogenic Activity**

The degree of antiangiogenic activity is dose dependent. Most chemotherapy drugs have antiangiogenic activity when administered at high doses. We are especially interested in compounds that specifically interact and antagonize the steps involved in angiogenesis when administered at low doses. These agents may have relatively low toxicity at low dose and may exhibit a higher therapeutic gain. Most conventional chemotherapy drugs have some degree of antiangiogenic activity as a consequence of their cytotoxic activity. Ideal botanical derivatives would specifically antagonize new vessel formation in tumors, without significant toxicity to normal tissues and without major adverse reactions. The ideal agent would also inhibit tumor cell proliferation through other physiologic pathways, such as influencing intracellular signaling pathways. Multiple levels of antiangiogenic activity may be required to overcome the development of resistance by tumor-associated endothelial cells (TEC). Survival factors, such as the increased secretion of VEGF and bFGF by the tumor cells, activate intracellular pathways that prevent TEC apoptosis. Maximal antiangiogenic activity usually requires prolonged exposure to low concentrations of the active agent. This approach contrasts with the concept of administering maximum-tolerated doses of cytotoxic drugs to maximize tumor cell kill. Some reports have confirmed the utility of combining low, frequent-dose chemotherapy plus an agent that specifically targets the endothelial cell compartment.<sup>53,54</sup> The evidence suggests that an antiangiogenic schedule can be more effective than using high-dose cytotoxic drugs alone. We hypothesize that concomitant scheduling of antiangiogenic botanicals with low, frequent-dose cytotoxic therapies may have biological advantages that can increase therapeutic gain.

### **Natural Health Products That Inhibit Angiogenesis**

Further research is necessary to screen herbs that may be useful antiangiogenic therapies. Table 3 lists natural health products with antiangiogenic activity, and

Table 4 lists herbs and their derivatives that inhibit VEGF.<sup>56</sup> A master herbalist can advise on potential herbal treatments derived from centuries of traditional observations and advanced traditional medical systems, such as traditional Chinese medicine. It will be imperative to develop a new model of modern pharmacology based on traditional pharmacognosy. Our developing knowledge of cancer biology suggests that administering cytotoxic drug therapy at very high doses is not always appropriate. A new approach is to administer lower doses of synergistic organic chemicals. These complexes already exist in myriad botanicals. New laboratory techniques allow more specific assays of activity and enable quality assurance and consistency between batches of botanical preparations to be maintained. This will enable credible clinical trials of antiangiogenic natural health products to be initiated. At the same time, we should not minimize the importance of a holistic approach to managing a patient with cancer. Antiangiogenic therapies form only a small part of a complex management program. Attention to the patient's overall health and ability to mount an immune response are subtle factors that may become more important in tipping the balance toward cancer control.

### **Herbs and Phytochemicals**

#### *Artemisia annua (Chinese Wormwood)*

Artemisinin is the active constituent extracted from the plant *A. annua* L. (Asteraceae). It has been used clinically as an antimalarial drug.<sup>68</sup> More recently, it was shown to be cytotoxic to cancer cells through induction of apoptosis.<sup>69</sup> Artesunate (ART) is a semisynthetic derivative of artemisinin. The in vitro effect of ART was tested on the HUVEC model of angiogenesis. It significantly inhibited angiogenesis in a dose-dependent manner. The inhibition of HUVEC proliferation was greater than the effect on cancer cells, fibroblast cells, and human endometrial cells. This indicates that its antiangiogenic activity is greater than its cytotoxicity. The antiangiogenic effect in vivo was evaluated in nude mice using transplanted human ovarian cancer (HO-891) cells and immunohistochemical staining for microvessel CD31 antigen, VEGF, and the VEGF receptor (KDR/flk-1). Tumor growth was decreased and microvessel density was reduced without any toxicity to the host animals. Artemisinin also lowered the VEGF expression by tumor cells and the KDR/flk-1 expression by endothelial cells.<sup>70</sup> Artemisinin also has anticancer activity through other pathways. It inhibits the activation of nuclear factor  $\kappa$ -B (NF- $\kappa$ B), an important activator protein in cancer development and progression.<sup>71</sup>

**Table 3. Natural Health Products With Potential Direct and Indirect Antiangiogenic Activity**


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Herbs and associated phytochemicals  
*Aloe barbadensis* Mill. (Liliaceae) (aloe vera leaf and pulp extracts)  
*Angelica sinensis* (aqueous extracts)  
*Artemisia annua* (artemisinin)  
*Camellia sinensis* (epigallocatechin)  
*Chrysobalanus icaco* L. (Chrysobalanaceae) (methanol extract)  
*Curcuma longa* (curcumin)  
*Dysoxylum binectariferum* Hook.f. ex Bedd (Meliaceae) (flavopiridol)  
*Flos magnoliae*<sup>a</sup> (magnosalin)  
*Ganoderma lucidum* (triterpenoids)  
*Ginkgo biloba* (ginkgolide B)  
*Glycyrrhiza glabra* L. (Fabaceae) (isoliquiritigenin; glabridin)  
*Hibiscus sabdariffa* (protocatechuic acid)  
*Livistona chinensis* R.Br. (Arecaceae) (aqueous extract from seed)  
*Matricaria chamomilla* L. (Asteraceae) (flavonoids: apigenin, fisetin)  
*Ocimum sanctum* (carnosol; ursolic acid)  
Omega-3 fatty acids (eicosapentaenoic acid, docosahexaenoic acid)  
*Magnolia obovata* Thunb. (Magnoliaceae) (honokiol)  
*Panax ginseng* (saponins: 20(R)- and 20(S)-ginsenoside-Rg3)  
*Polypodium leucatomos* Poir. (Polypodiaceae) (difur)  
*Poria cocos* (1-3- $\alpha$ -D-glucan)  
*Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae) (resveratrol)  
Proanthocyanidin  
Quercetin  
*Rabdosia rubescens* (ponicidin and oridonin)  
*Rosmarinus officinalis* (carnosol and ursolic acid)  
*Scutellaria baicalensis* (baicalin and baicalein)  
*Silybum marianum* (silymarin)  
Soy isoflavones (genistein, daidzein)  
*Tanacetum parthenium* Sch. Bip. (Asteraceae) (parthenolide)  
*Tabebuia avellanedae* Lor. ex Gris. (Bignoniaceae) ( $\beta$ -lapachone)  
*Taxus brevifolia* Nutt. (Taxaceae) (taxoids)  
*Viscum album* (lectins)  
*Zingiber officinale* (6-gingerol)  
Other Chinese herbs (see Table 5)  
Cyclooxygenase-2 antagonists (see Table 6)

Minerals  
Selenium

Animal derived  
Bovine cartilage  
Shark cartilage (water-soluble extract AE-941)  
*Squalus acanthias* (dogfish liver: squalamine)  
Vitamin D (1 $\alpha$ , 25-D3)

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Data are derived from in vitro and in vivo studies cited in the text.  
a. *Magnolia* species.

#### *Viscum album* (European Mistletoe)

One widely used extract of *V. album* L. (Viscaceae) is known as Iscador. It is often used as an anticancer agent in anthroposophical and homeopathic medicine. Laboratory studies show that it is antiangiogenic through downregulation of VEGF, and it induces apoptosis of cancer cells.<sup>72,73</sup> In a mouse model, lung metastases were reduced and survival was increased.<sup>74</sup> A clinical trial in human subjects showed an increase in survival for a variety of cancers, but the study was poorly controlled, and no definitive conclusions can be made.<sup>75</sup> Well-controlled clinical trials of *V. album* derivatives in combination with other anticancer therapies are warranted.

#### *Curcuma longa* (Turmeric)

Curcumin is the most active curcuminoid present in turmeric, *C. longa* L. (Zingiberaceae). It interacts with cancer cells at a number of levels and can enhance the tumoricidal efficacy of cytotoxic chemotherapy and radiotherapy.<sup>76-78</sup> Its anti-invasive effects are partly mediated through the downregulation of MMP-2 and the upregulation of tissue inhibitor of metalloproteinase (TIMP-1).<sup>79</sup> These enzymes are involved in the regulation of tumor cell invasion. Curcumin inhibits the transcription of 2 major angiogenesis factors, VEGF and bFGF.<sup>80</sup> It interacts with VEGF and nitric oxide-mediated angiogenesis in tumors.<sup>81,82</sup> Elevated levels of nitric oxide correlate with tumor growth. Curcumin reduces nitric oxide generation in endothelial cells. CD13/aminopeptidase-N (APN) is a membrane-bound enzyme found in blood vessels undergoing ac-

**Table 4. Herbs and Their Derivatives That Specifically Inhibit Vascular Endothelial Growth Factor and Have Direct Activity Against Angiogenesis**


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<i>Artemisia annua</i> (Chinese wormwood; contains 95% artemisinin and other related terpenes and flavonoids)
<i>Viscum album</i> (European mistletoe; contains mistletoe lectin III [ML3A])
<i>Curcuma longa</i> (turmeric; contains 95% curcumin)
<i>Camellia sinensis</i> (green tea; contains 95% phenols; 50% epigallocatechin)
<i>Vitis vinifera</i> L. (Vitaceae) (grape seed extract; contains 95% proanthocyanidins)
<i>Angelica sinensis</i> (Dong quai; contains 4-hydroxyderricin)
<i>Taxus brevifolia</i> (Pacific yew; contains taxol)
<i>Scutellaria baicalensis</i> (Chinese Baical skullcap; contains 95% baicalin and flavonoids)
<i>Polygonum cuspidatum</i> (Japanese knotweed; contains 20% resveratrol)
<i>Silybum marianum</i> (Milk thistle; contains 80% silymarin [silibin])
<i>Magnolia obovata</i> (contains 90% honokiol)
<i>Zingiber officinale</i> (contains 6-gingerol)
Various Chinese herbs (see Table 5)

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Data are derived from in vitro and in vivo studies cited in the text.

tive angiogenesis. Curcumin binds to APN and blocks its activity, thereby inhibiting angiogenesis and tumor cell invasion.<sup>83,84</sup> Derivatives of curcumin may be developed to target APN, providing a novel approach to reduce neoplastic angiogenesis.<sup>85,86</sup> Curcumin also downregulates the expression of the VEGF and MMP-9 genes that are associated with angiogenesis. Demethoxycurcumin is a structural analogue of curcumin isolated from *Curcuma aromatica* Salisb. (Zingiberaceae). It specifically inhibits the expression of MMP-9.<sup>87</sup> Curcumin can interfere with the activity of both MMP-2 and MMP-9, the basis of the angiogenic switch, thereby reducing the degradation of the extracellular matrix.<sup>88</sup> It also interferes with the release of angiogenic factors that are stored in the extracellular matrix. It inhibits growth factor receptors such as epidermal growth factor receptor (EGFR) and VEGFR and the intracellular signaling tyrosine kinases. This cell-signaling system can promote further angiogenesis through gene activation that increases levels of cyclooxygenase-2 (COX-2), VEGF, IL-8, and the MMPs.<sup>89,91</sup> A phase 1 study of curcumin found no treatment-related toxicity up to 8000 mg/d. Beyond 8000 mg/d, the bulky volume of the drug was unacceptable to the patients. The serum concentration of curcumin usually peaked at 1 to 2 hours after oral intake of curcumin and gradually declined within 12 hours.<sup>92</sup> This study suggested that it may prevent cancer progression. Derivatives of curcumin, such as copper chelates of curcuminoids, may have increased antitumor activity.<sup>86</sup>

#### *Scutellaria baicalensis* (Chinese Skullcap)

Baicalin and baicalein are the main derivatives from the Chinese Skullcap herb, *S. baicalensis* Georgi (Lamiaceae). They are potent antiangiogenic compounds that reduce VEGF, bFGF, 12-lipoxygenase activity, and MMP.<sup>93,94</sup> *S. baicalensis* is one of the herbs found in PC-SPES, a complex of Chinese herbs that may have clinical activity against advanced prostate

cancer.<sup>95-97</sup> However, although a phase 2 trial demonstrated clinical activity in patients with androgen-independent prostate cancer, diethylstilbestrol (DES) and ethinyl estradiol (both known to have potent antiproliferative activity) were detected in various lots of PC-SPES.<sup>98</sup> It is still intriguing that the decline in PSA was greater for PC-SPES that was potentially contaminated with DES than for the comparator group that received DES alone, suggesting some independent activity. Although baicalin and baicalein have multiple anticancer activities in vitro, their clinical activity is not established, and their contribution to any potential therapeutic effect of PC-SPES is unknown.<sup>99</sup>

#### *Resveratrol and Proanthocyanidin*

Resveratrol is a phytoalexin found in grapes and wine. It has antiangiogenic activity demonstrated by its ability to inhibit HUVEC division and to decrease the lytic activity of MMP-2.<sup>100</sup> Resveratrol inhibits VEGF-induced angiogenesis by disruption of reactive oxygen species-dependent Src kinase activation and subsequent VE-cadherin tyrosine phosphorylation.<sup>101,102</sup> Resveratrol inhibits the growth of gliomas in rats by suppressing angiogenesis.<sup>103</sup> Edible berries and grape seed extract contain high concentrations of proanthocyanidin. The latter inhibits tumor necrosis factor (TNF)- $\alpha$ -induced VEGF expression. Feeding proanthocyanidins to mice with tumor xenografts reduces VEGF secretion, which results in reduced intratumoral microvasculature.<sup>104-106</sup> On the other hand, one study showed that grape seed extract may upregulate oxidant-induced VEGF expression, suggesting that proanthocyanidin can induce angiogenesis as part of normal tissue healing.<sup>107</sup>

#### *Magnolia officinalis* (Chinese Magnolia Tree)

The seed cones of *M. officinalis* Rehder & E.H. Wilson (Magnoliaceae) contain substances that inhibit the growth of new blood vessels. Honokiol is the active constituent. It may partly reduce angiogenesis

through the regulation of platelet-derived endothelial cell growth factor and TGF- $\beta$  expression. It also inhibits nitric oxide synthesis and TNF- $\alpha$  expression.<sup>108,109</sup> In animal experiments, it suppresses the proliferation of blood vessel endothelial cells more than other types of cells and thereby reduces tumor growth.<sup>110,111</sup>

#### *Silybum marianum* (Milk Thistle)

Silibinin and silymarin are polyphenolic flavonoids isolated from the fruits or seeds of *S. marianum* (L.) Gaertn. (Asteraceae). In the laboratory, silymarin demonstrates strong activity against a variety of tumors through downregulating VEGF and EGFR.<sup>112,113</sup> Silymarin suppresses VEGF when used as a single agent against human ovarian cancer endothelial cells in vitro.<sup>114</sup>

#### *Camellia sinensis* (Green Tea)

Tea, *C. sinensis* (L.) Kuntze (Theaceae), contains polyphenols and catechins (mainly epigallocatechin-3 gallate [EGCG]).<sup>115</sup> These constituents inhibit MDA-MB231 breast cancer cell and HUVEC proliferation.<sup>116</sup> In addition, they suppress breast cancer xenograft growth and reduce the density of tumor vessels in rodent studies.<sup>117</sup> This is associated with a decrease in VEGF, regulated at the level of transcription. EGCG also suppresses protein kinase C (PKC), another VEGF transcription modulator. Inhibition of VEGF transcription is one of the molecular mechanisms involved in the antiangiogenic effects of green tea that may contribute to its potential use for cancer treatment.<sup>118,119</sup> EGCG may be administered as a powdered extract of green tea. An appropriate dose has been extrapolated from antiangiogenic activity in rodent experiments<sup>120</sup> as well as a phase 1 study in humans.<sup>121</sup> A dose of 1.0 g/m<sup>2</sup> 3 times daily (equivalent to 7-8 Japanese cups [120 mL] 3 times daily) has been recommended. In practice, lower total daily doses of 2 to 4 g of standardized green tea extract (95% polyphenols/60% catechins) are usually prescribed. Each gram of this extract provides 400 to 500 mg of EGCG. The dose-limiting adverse effects are gastrointestinal and neurological effects of caffeine. However, the caffeine may potentiate the antiangiogenic effect of EGCG.<sup>121</sup>

#### *Ginkgo biloba*

*G. biloba* L. (Ginkgoaceae) extract has anticancer effects that are related to its gene-regulatory and antiangiogenic properties. The *G. biloba* extract used in most of the research is EGb 761, which contains about 25% flavonoids (ginkgo-flavone glycosides) and about 5% terpenoids (ginkgolides and bilobalides). The most potent flavonoid is ginkgolide B. This extract inhibits angiogenesis by downregulating VEGF.<sup>122,123</sup>

#### *Quercetin*

Quercetin is a flavone found in apples, onions, raspberries, red grapes, citrus fruit, cherries, broccoli, and leafy greens. It inhibits angiogenesis through multiple mechanisms. These include interaction with the COX-2 and lipoxygenase (LOX)-5 enzymes, the EGF receptor, the HER-2 intracellular signaling pathway, and the NF- $\kappa$ B nuclear transcription protein.<sup>124-128</sup> A prostate cancer xenograft model showed that quercetin could enhance the anticancer effects of tamoxifen through antiangiogenesis.<sup>129</sup>

#### *Poria cocos*

*P. cocos* F.A. Wolff (Coriolaceae) is a mushroom extract that has been traditionally held to have anticancer activity. It inhibits platelet aggregation and appears to be antiangiogenic by downregulating NF- $\kappa$ B.<sup>130-133</sup>

#### *Zingiber officinale* (Ginger)

6-Gingerol, from *Z. officinale* Roscoe (Zingiberaceae), inhibits both the VEGF- and bFGF-induced proliferation of human endothelial cells and causes cell cycle arrest. It also blocks capillary-like tube formation by endothelial cells in response to VEGF and strongly inhibits sprouting of endothelial cells in the rat aorta and mouse cornea in vitro models. In mice receiving injections of B16F10 melanoma cells, intraperitoneal administration of 6-gingerol, at doses less than cytotoxic levels, reduces the number of lung metastases.<sup>134</sup>

#### *Panax ginseng*

The lipophilic constituents of *P. ginseng* C.A. Meyer (Araliaceae) are called saponins (or ginsenosides). These extracts possess anticancer activities in tumors that include antiangiogenesis and induction of tumor cell apoptosis.<sup>135</sup>

#### *Rabdosia rubescens* (Rabdosia)

The herb *R. rubescens* H. Hara (Lamiaceae) is used traditionally to treat cancer and is a constituent of the PC-SPES formula. It contains ponacidin and oridonin, 2 diterpenoids that possess significant antiangiogenic activity.<sup>136</sup>

#### Chinese Medicinal Herbal Extracts

Herbs that are traditionally used in China as anticancer agents have been screened for their antiangiogenic activity.<sup>65</sup> Table 5 lists the most active herbs (exhibiting more than 20% inhibition at 0.2 g/herb/mL), using the CAM and BAEC assays.

#### Copper Antagonists

Some cancers are associated with high serum levels of copper. The role of copper in cancer promotion

**Table 5. Antiangiogenesis Activity of Chinese Medicinal Herbal Extracts (Exhibiting More Than 20% Inhibition at 0.2 g/herb/mL)<sup>65</sup>**

Name	Used Part	% Inhibition (CAM)	% Inhibition (BAEC)
<i>Berberis paraspecta</i> Ahrendt (Berberidaceae)	Root	25	38
<i>Catharanthus roseus</i> G Don (Apocynaceae)	Leaf	27	30
<i>Coptis chinensis</i> Franch (Ranunculaceae)	Rhizome	25	37
<i>Scrophularia ningpoensis</i> Hemsl (Scrophulariaceae)	Root	20	34
<i>Scutellaria baicalensis</i>	Root	27	41
<i>Polygonum cuspidatum</i>	Whole plant	—	28
<i>Taxus chinensis</i> Rehder (Taxaceae)	Bark	—	26

Assays: chick embryo chorioallantoic membrane (CAM) and bovine aortic endothelial cells culture models (BAEC).

through proinflammatory cascades and angiogenesis induction is quite well established.<sup>137</sup> Copper is essential for the function of many angiogenic growth factors. The angiogenic activity of bFGF, VEGF, TNF- $\alpha$ , and IL-1 are copper dependent. Copper chelation with tetrathiomolybdate (TM) is a promising therapy for tumor control.<sup>138,139</sup> Its hypothesized mechanism of action is inhibition of angiogenic cytokines. Unlike some current approaches to antiangiogenic therapy that target single agents, TM inhibits multiple angiogenic cytokines. Part of this effect appears to stem from inhibition of NF- $\kappa$ B that, in turn, controls transcription of many angiogenic factors and other cytokines. Some angiogenic cytokines appear to have separate mechanisms of copper dependence. The inhibition of multiple angiogenic cytokines gives TM the potential to be a more global inhibitor of angiogenesis. Several aromatic herbs, such as *Caryophylli flos*, *Cinnamomi cortex*, *Foeniculi fructus*, and *Zedoariae rhizoma*, inhibit lipid peroxidation or protein oxidative modification by copper.<sup>140</sup> They may have a role to play in antiangiogenesis, but further research is necessary to confirm this.

### Animal Products

#### Shark and Bovine Cartilage

The resistance of cartilage to tumor formation is correlated with its capacity to inhibit the formation of new blood vessels. A number of in vitro and in vivo studies have suggested the existence of antiangiogenic compounds in shark and bovine cartilage.<sup>141</sup> The clinical effectiveness of whole cartilage for the treatment of cancer was not confirmed in a recent phase 3 randomized controlled trial.<sup>142</sup> The main problem is lack of data that correlate bioavailability with pharmacological effects using oral shark cartilage. Unsatisfactory outcome in clinical trials may be secondary to inadequate bioavailability of the active constituents.<sup>143</sup> Bioactive derivatives of shark cartilage are being extracted. AE-941 (Neovastat) is a standardized water-soluble extract that represents less than 5% of the crude cartilage. The biotechnology company Aeterna

developed Neovastat. It is a multifunctional antiangiogenic product that contains several biologically active molecules.<sup>144</sup> The mode of extraction differs from many other preparations and may explain the preservation of its antiangiogenic properties. It is kept frozen until use, to maximally preserve its biological properties. Its antiangiogenic activity may be due to the presence of a metalloproteinase inhibitor, with a preferential inhibition of MMP-2, as well as inhibition of serine elastase, inhibition of VEGF binding to endothelial cells, and the inhibition of tyrosine phosphorylation of the VEGF receptor. It reduces the VEGF-dependent increase in vascular permeability. Paradoxically, shark cartilage extract (including AE-941) also has fibrinolytic activity.<sup>145,146</sup> Nevertheless, fibrinolysis and anticoagulation may also reduce tumor cell metastasis.<sup>147,148</sup> Shark cartilage extracts are pleiotropic, having multiple phenotypic activities. No published phase 3 randomized controlled trials have yet proven the utility of Neovastat for cancer treatment. Aeterna recently announced that development of AE-941 would be focused solely on non-small-cell lung cancer.<sup>149</sup> The MD Anderson Cancer Center's Community Clinical Oncology Program is currently recruiting for a multicenter, double-blind, placebo-controlled phase 3 study of AE-941 in addition to combined modality treatment of locally advanced unresectable non-small-cell lung cancer.<sup>150</sup>

#### Squalus acanthias (dogfish shark)

Squalamine is a cationic steroid isolated from the liver of the dogfish shark, *Squalus acanthias* Linnaeus 1758 (Squalidae).<sup>151</sup> Squalamine significantly blocks VEGF-induced activation of MAP kinase and cell proliferation in human vascular endothelial cells. Squalamine is antiangiogenic for ovarian cancer xenografts and appears to enhance the cytotoxic effects of cisplatin chemotherapy, in an animal xenograft model, independent of HER-2 tumor status. HER-2 overexpression is normally associated with resistance to cisplatin and promotion of tumor angiogenesis.<sup>152</sup> In a phase 2 trial of patients with advanced small-cell lung cancer, squalamine was administered at a dose of 300

mg/m<sup>2</sup> by continuous infusion for 5 days, with paclitaxel and carboplatin given on day 1. Patient survival data and a satisfactory safety profile indicated that the combination should be explored further.<sup>153</sup>

### Multistep Activity of Phytochemical Complexes Derived From Herbs

Botanicals usually act on multiple anticancer targets since they contain a variety of organic chemical complexes. The biochemical signaling pathways of angiogenesis form a complex, interconnected web. Inhibition of one part of this web may result in compensation through another pathway. A potential advantage of phytochemicals is that they may act through multiple pathways and reduce the development of resistance by cancer cells. This model of pharmacognosy recognizes the advantage of administering the whole plant product to maximize activity. Overextraction of a specific chemical constituent may remove this therapeutic gain. The challenge for modern pharmacognosy is to ensure that the optimum mixture of chemical constituents is maintained when purifying the product. Usually, this will require a combination of both chemical and biological assays. Further anticancer properties of some antiangiogenic botanicals will briefly be discussed. Their effects may interact with various biochemical pathways that indirectly influence angiogenesis. Traditional practice has been to combine multiple natural health products, and this may scientifically provide a therapeutic advantage.

#### Targeting Alternative Angiogenesis Pathways

The adipocytokines are polypeptides produced by adipocytes that have autocrine, paracrine, and endocrine activities. They are associated with obesity, hyperinsulinemia, and chronic vascular disease, as well as the development of cancer.<sup>154</sup> The adipocytokines include VEGF, hepatocyte growth factor, leptin, TNF- $\alpha$ , heparin-binding epidermal growth factor, insulin-like growth factor, and IL-6. These can all promote angiogenesis. Curcumin (from turmeric) and EGCG (from green tea) can inhibit APN, a member of the MMP family, that is implicated in the angiogenic switch process.<sup>84,118,119</sup> Curcumin and EGCG can also interfere with the expression of VEGF by suppressing a series of activities that promote angiogenesis. These angiogenic pathways include production of TGF- $\beta$ , COX-2 amplification, EGFR amplification, aberrant Src expression, and the amplification of NF- $\kappa$ B signaling. Curcumin, grape seed extract, and green tea constituents may also interfere with endothelial cell function by inhibiting the engagement of specific integrins.<sup>106,107</sup> These phyto-

chemicals interact at multiple levels to suppress the inflammatory, hyperproliferative, and transformative processes that constitute carcinogenesis.

#### Targeting EGFR (HER-1)

The EGFR is overexpressed in many human tumors. It is associated with more aggressive disease, relative resistance to cytotoxic chemotherapy, and a poorer prognosis. EGFR activity induces angiogenesis.<sup>155</sup> Blockade of the EGFR reduces angiogenesis and cell proliferation.<sup>156</sup> Monoclonal antibodies have been developed to block the receptor or the linked intracellular signaling system.<sup>157-163</sup> EGF stimulates urokinase-type plasminogen activator (uPA) expression that can promote angiogenesis. Both genistein (an isoflavone constituent of soy) and curcumin (a constituent of turmeric) inhibit the effects of EGF.<sup>164</sup> Genistein and curcumin inhibit EGF-stimulated urokinase production and phosphorylation of the EGFR in cell cultures. Both botanicals also inhibit protein tyrosine kinases that could stimulate the enhancement of uPA levels induced by TGF- $\beta$ .<sup>165</sup> Other natural health products that can block activity of the EGFR include resveratrol<sup>127</sup> and quercetin.<sup>120,124,129</sup>

#### Targeting HER-2/neu

The HER-2/neu gene (also known as c-erbB-2) is amplified in more than 30% of patients with breast cancer and is linked to highly aggressive tumors with a poorer prognosis. HER-2 is overexpressed in a significant proportion of patients with other cancer types, including non-small-cell lung cancer, ovarian cancer, prostate cancer, and gastric cancer, in which it may predict a worse outcome.<sup>166-170</sup> HER-2 gene amplification correlates with higher levels of angiogenesis.<sup>171</sup> Herceptin is a drug that inhibits HER-2/neu. It is usually administered adjunctively with cytotoxic chemotherapy. The activity of herceptin may be further enhanced by oleic acid.<sup>172</sup> Emodin, a natural constituent of *Polygonum multiflorum* Thunb. (Polygonaceae) and aloe, inhibits HER-2/neu expression and is toxic against cancer cells but nontoxic for normal cells.<sup>173</sup>

#### Targeting Inflammatory Pathways: COX-2 and NF- $\kappa$ B

Prostaglandins are autacoids derived from arachidonic acid via the COX enzymes. They include prostacyclin, thromboxane, and prostaglandin E, types 1 through 3 (PGE1-3). A role for arachidonic acid-derived prostaglandins in the process of angiogenesis is now established through in vitro assays. PGE2 is a potent inducer of angiogenesis. There is a correlation between COX-2 expression and angiogenesis.<sup>174</sup> Neovascularization is blocked by COX-2 antagonists.<sup>175-180</sup>

The COX-2 and LOX-5 products of n-6 fatty acid metabolism may exert stimulatory effects on cancer progression including angiogenesis. The n-3 fatty acids and some pharmacological inhibitors of eicosanoid biosynthesis antagonize these effects.<sup>181-185</sup> Large amounts of n-3 fatty acids (eicosapentanoic acid and docahexaenoic acid) are found in cold-water fish oils. Licorice contains glycyrrhizic acid and polyphenols that inhibit COX-2, LOX-5, and PKC, as well as downregulating EGF.<sup>186</sup>

NF- $\kappa$ B is a family of closely related protein dimers that bind to a common sequence motif in DNA called the  $\kappa$ B site. The NF- $\kappa$ B inducible transcription factor is increased in tissue inflammation, cell proliferation, and cancers. NF- $\kappa$ B induces the overactivation of COX enzymes and is associated with increased angiogenesis.<sup>187-190</sup> The COX enzymes are expressed in most normal tissues. COX-1 synthesizes noninflammatory prostaglandins, such as PGE1. In contrast, COX-2 is amplified as part of the inflammatory response and produces prostaglandins, such as PGE2, that may induce uncontrolled cell proliferation and carcinogenesis. NF- $\kappa$ B may be amplified by growth factors, including TGF- $\beta$  and bFGF. Besides NF- $\kappa$ B, other transcription factors, such as activator protein (AP)-1 and IL-6, can stimulate COX-2 transcription. AP-1 also promotes the metastatic phase of tumor cells. COX-2-mediated angiogenesis also has a role in the progression of preneoplastic lesions to the invasive phenotype.<sup>191-194</sup> Conventional cancer therapies, such as radiation, surgery, and chemotherapy, may induce COX-2 amplification as part of the inflammatory response.<sup>195</sup> This could reduce therapeutic gain if not prevented. Several phytochemical derivatives are potent inhibitors of NF- $\kappa$ B. These include resveratrol, piceatannol, curcumin, EGCG (green tea), 6-gingerol (ginger), ursolic acid (holy basil), and ginseng.<sup>196-200</sup> Many botanical COX-2-inhibiting agents block the amplified activity of the transcription factor NF- $\kappa$ B without affecting its normal function.

A variety of natural health products can specifically inhibit the COX-2 enzyme and could play a role in reducing tissue toxicity and improving tumor control, when used alongside therapies such as radiotherapy, chemotherapy, and surgery (Table 6).<sup>201</sup> A botanical that protects an organism from the adverse effects of an intervention is termed an *adaptogen*. *P. ginseng* and curcumin are adaptogens that inhibit COX-2 and have antiangiogenic activity derived through the inactivation of NF- $\kappa$ B.<sup>199,202-205</sup>

### Targeting Protein Kinases

Oncogenes that encode protein kinases may contribute to the development of cancer. In normal cells, protein kinases are involved in signals between the cell

**Table 6. Natural Health Products That Inhibit Cyclooxygenase-2 Activity<sup>201</sup>**

Ginger
Aloe vera
Epigallocatechin-3 gallate/green tea
Resveratrol
Licorice
Garlic
Chinese Skullcap
Bilberry
Grape seed extract proanthocyanidins
<i>Panax ginseng</i>
Milk thistle
Fish oils: omega-3 fatty acids (eicosapentanoic acid; docahexaenoic acid)
Green-lipped mussel
Antioxidants: A, C, E, Se, Zn, carotenoids, flavonoids, coenzyme Q10, N-acetylcysteine, lipoic acid
Boswellia
Bromelain
Curcumin
Quercetin

membrane and the nucleus, regulating progression through the cell cycle. Protein kinases control these processes by activating other messenger proteins that can influence the cell proliferation cycle. Mutated kinase genes have been found in a number of malignancies, including chronic myelogenous leukemia and breast and bladder cancers. The mutated kinases can contribute to the development of cancer. Many tumor cells possess protein kinases that are permanently turned on, forcing the cell into constant division. Examples of abnormal kinases are the Abl, Src, and cyclin-dependent kinases. The kinases may be amplified or permanently switched on by mutations in the control regions of their genes. A commonly overproduced kinase in cancer is the receptor for epidermal growth factor. Numerous phytochemicals are reported to interfere with cell signaling and may reverse the adverse effects of protein kinase overactivity. Some botanicals with COX-2 inhibitory activity target the intracellular signaling molecules.<sup>206,207</sup> Inhibition of specific protein kinases suppresses angiogenesis.<sup>208-213</sup>

Carnosol and ursolic acid are compounds found in *Ocimum sanctum* L. (Lamiaceae) (holy basil) and *Rosmarinus officinalis* L. (Lamiaceae) (rosemary).<sup>214</sup> They inhibit the activity of the tyrosine kinases and ornithine decarboxylase.<sup>215</sup> Carnosol also reduces NF- $\kappa$ B<sup>216</sup> and the antiapoptotic protein Bcl-2.<sup>217</sup> Genistein and daidzein (isoflavones found in soy) are specific inhibitors of tyrosine kinases.<sup>218</sup> Many phytochemicals appear to selectively react with the regulatory center of PKC. Curcumin, vitamin E, green tea (catechins) resveratrol, *Ganoderma lucidum* P. Karst (Ganodermataceae), and licorice can inhibit PKC activity.<sup>186,219-221</sup>

### Targeting the Bcl-2 Protein

Bcl-2 is a signaling protein that plays a key role in the process of controlled cell death termed *apoptosis*. Apoptosis is necessary to eliminate aged or damaged cells. Bcl-2 is normally found in the mitochondrial membrane where it regulates the release of cytochrome C. The latter can trigger a series of enzymes (caspases) that lead to cell death.<sup>222-225</sup> High levels of Bcl-2 are associated with most types of human cancer and block the release of cytochrome C. It appears to be a contributor to both inherent and acquired resistance to anticancer treatments. Bcl-2 and p53 regulate VEGF-mediated angiogenesis.<sup>226</sup>

Curcumin and green tea extract inhibit Bcl-2 expression.<sup>227-229</sup> *Scutellaria baicalensis* contains the phenolic compounds baicalin, baicalein, wogonin, and oroxylin. These constituents inhibit Bcl-2 overexpression, as well as COX-2 gene expression and NF- $\kappa$ B activation.<sup>230,231</sup> Hibiscus protocatechuic acid is a phenolic compound isolated from the dried flower of *Hibiscus sabdariffa* L. (Malvaceae). It inhibits Bcl-2 activity.<sup>232,233</sup> Other inhibitors of Bcl-2 include eicosapentanoic acid from fish oil,<sup>234</sup> a lectin extract of *V. album* (mistletoe),<sup>235</sup> 6-gingerol (ginger),<sup>236</sup> grape seed extract,<sup>105</sup> echinocystic acid (a triterpene found in ginseng and other Asiane herbs),<sup>237,238</sup> parthenolide (a sesquiterpene lactone found in Feverfew),<sup>239</sup> and  $\beta$ -lapachone (a quinone obtained from the bark of the lapacho tree).<sup>240-242</sup>

### Targeting Coagulation Pathways Associated With Angiogenesis

In some clinical trials, anticoagulation drugs are associated with a reduction in metastases.<sup>243-245</sup> In Chinese medicine, destagnation herbs are traditionally thought to overcome the blockage of qi and blood. Laboratory evidence now suggests that they may have antiangiogenic and anticoagulation properties.<sup>65,246,247</sup> A randomized placebo-controlled trial from China showed that the addition of "destagnation" herbs (including *Salvia miltiorrhiza* Bunge [Lamiaceae] and *Angelica sinensis* Diels [Apiaceae]) to radiotherapy doubled both the local control and survival rates of patients with nasopharyngeal cancer.<sup>248</sup>

### Conclusion

Angiogenesis involves multiple interdependent processes operating at the molecular level. These include gene expression, signal processing, and enzyme activities. Most antiangiogenic natural health products block new vessel formation at multiple levels. Lack of standardization of screening assays may be an obstacle to defining the most effective products for clinical use. Overextraction of constituents may negate some of

the advantages of potential synergy. Mainly preclinical data exist for most of the naturally derived antiangiogenic agents. Most of the studies of antiangiogenic activity are based on in vitro or animal work, which cannot be readily extrapolated to humans. Phase 1 and 2 studies are required to determine their potential to improve cytotoxic therapies. Despite this, the herbalist has access to hundreds of years of observational data on the anticancer activity of many herbs. Laboratory studies are confirming the knowledge that is already documented in traditional texts.

Quality assurance of appropriate extracts is essential prior to embarking on clinical trials. Since antiangiogenic agents are mainly cytostatic in nature, the usual paradigm for anticancer drug development, in which tumor response in phase 2 trials prompts further development, is not always appropriate. More data are required on dose response, appropriate combinations, and potential toxicities. Given the multiple effects of these agents, their future use for cancer therapy probably lies in synergistic combinations. They may be evaluated alone for the prevention of cancer recurrence following definitive treatment. To be suitable for long-term chronic use, these agents should possess minimal toxicity and should be orally administered. However, angiogenesis is also essential for healing of injuries. Most compounds that inhibit tumor angiogenesis are likely to inhibit physiologic angiogenesis, leading to potential side effects, such as ulceration and bleeding. Studies are required to determine distinguishing features of tumor vessels from normal vessels to enable a therapeutic gain to be achieved. Some of the differences have already been described, but appropriate doses and scheduling of antiangiogenic agents to achieve the optimum therapeutic gain is unclear. During active cancer therapy, they should generally be evaluated in combination with chemotherapy and radiation. In this role, they act as biological response modifiers and adaptogens, potentially enhancing the efficacy of the so-called conventional therapies. The diversity of angiogenic factor expression in different tumors receiving various therapies, combined with the fact that endothelial cells in different tumors are phenotypically distinct, is a major challenge for the development of effective antiangiogenic regimens.<sup>57,249</sup> Their effectiveness may be increased when multiple agents are used in optimal combinations. Surrogate markers, such as angiogenic cytokines, are necessary to predict antiangiogenic response.<sup>250</sup> Circulating levels of FGF-2, VEGF, vascular adhesion molecule (V-CAM-1), endothelial intercellular adhesion molecule (ICAM-1), IGF-1, and cytokines such as IL-8 may correlate with tumor angiogenesis.<sup>251-253</sup> In addition, circulating endothelial

**Table 7. Dose Ranges of Some Phytochemicals Used by an Herbalist for Angiogenesis Inhibition**

<i>Herb/Phytochemical</i>	<i>Preventive Dose, mg/d</i>	<i>Cancer Adjuvant Dose</i>
Turmeric (95% curcumin)	500-1000	1000-2500 mg/3×/d
Green tea (95% phenols; 50% epigallocatechin-3 gallate)	200-500	1000-1200 mg/3×/d
Grape seed extract (95% proanthocyanidin)	100-200	600-1000 mg/d
Japanese knotweed (20% resveratrol)	30-50	300-500 mg/d
Quercetin with bromelain	500-1500	500-1000 mg/3×/d
Holy basil and rosemary (2.37% and 1.5% ursolic acid)	10-20	10-20 mg/3×/d
Silibinin (80% silymarin)	200	Up to 2000 mg/3×/d

Note that these dose ranges have not all been evaluated in clinical pharmacokinetic studies and are not approved by the Food and Drug Administration or Health Canada at this stage. The Natural Health Products Directorate of Health Canada is in the process of registering quality, efficacy, and dosing data on natural health products.

**Table 8. Potential Surrogate Blood Tests for Monitoring Angiogenesis and Its Response to Therapies<sup>250-257</sup>**

Circulating vascular molecules
Vascular endothelial growth factor (VEGF-R1/Flt-1)
Fibroblast growth factor-2 (FGF-2)
Interleukin-8 (IL-8)
Insulin-like growth factor-1 (IGF-1)
Vascular adhesion molecule (V-CAM-1)
Endothelial intercellular adhesion molecule (ICAM-1)
Matrix metalloproteinase (MMP-9)
Circulating cells
Circulating endothelial cells (CEC)
Circulating endothelial cell progenitors (CD34 <sup>+</sup> peripheral blood mononuclear cells)

cells and their progenitors may be a more reliable marker of response to antiangiogenic therapies.<sup>256,257</sup> Noninvasive functional imaging, such as positron emission tomography and functional magnetic resonance imaging, may play a role.<sup>258</sup>

Current laboratory evidence suggests a useful role for natural health products in the treatment of cancer. The input of an herbalist, oncologist, laboratory scientist, and a clinical trials methodologist to the research effort is essential to distill the wealth of traditional knowledge into a modern framework that can be evaluated scientifically. Information on traditional dose levels is important for designing initial phase I clinical trials for safety and maximum tolerated dose (Table 7). However, the traditional model of pharmacognosy may not necessarily use the highest dose. Establishing the maximum tolerated dose in a phase I study may not always be appropriate. Instead, the determination of the biologically active dose that may possess less toxicity may be more relevant. Combinations of whole herbs or constituent phytochemicals at lower doses may be important. In addition, a longer period of exposure to the natural health product may be more effective than a short exposure to the highest possible dose level. New designs for trials to demonstrate activity in human subjects are required. Although controlled trials might be preferred, smaller studies with appropriate end points and surrogate markers for antiangiogenic response could help prioritize agents for the larger resource-intensive phase 3 trials.

Because most of the agents are expected to be cytostatic, it is inappropriate to require the standard criteria of measured tumor response. On the other hand, simply confirming stable disease may be misleading. More research on surrogate markers of antiangiogenic response is obviously necessary prior to directing resources to large-scale clinical trials (Table 8).

A multidisciplinary approach by the herbalist and the oncologist is important for implementing and studying natural health products used for the treatment of patients with cancer. We now have a better understanding of their effects at the molecular level. Nevertheless, our model of therapy always considers treating the whole person rather than just the cancer. Interestingly, the relationship between the proverbial cancer seed and the “soil” is well illustrated by the model of angiogenesis. Manipulation of the “soil” by both antiangiogenesis agents and holistic interventions may tip the balance in favor of increased survival. Introducing these interventions into the clinic through appropriate studies will provide more definitive evidence of efficacy and hopefully improve outcome for many cancer patients.

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