

Antiangiogenesis in prostate cancer

Review Article

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Abbreviations: 2-methoxyestradiol, (2ME); androgen independent prostate cancer, (AIPC); Cyclooxygenases, (COXs); luteinizing-hormone-releasing hormone, (LHRH); Matrix metalloproteinases, (MMPs); multiple myeloma, (MM); National Cancer Institute, (NCI); prostate specific antigen, (PSA); prostatic intraepithelial neoplasia, (PIN); Recombinant humanized anti-VEGF, (RhuMab VEGF); specific cyclin-dependent kinase, (cdk); Vascular endothelial growth factor, (VEGF)

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Summary

Metastatic prostate cancer is the second leading cause of cancer related death. While androgen ablation is an effective initial modality, progression of disease is eventually occurred in majority patients. The benefit of chemotherapy in overall survival is still unclear. Angiogenesis plays a pivotal role for the growth, invasion, and metastasis of prostate cancer. Therefore, antiangiogenesis is a promising new therapeutic modality. Currently, there are more than 20 antiangiogenic agents in various stages of clinical trials. We will discuss current knowledge on controlling tumor angiogenesis and advances in the development of antiangiogenic agents with promising antitumor activity in prostate cancer.

I. Introduction

Prostate cancer is the most common malignancy in American men and the second leading cause of cancer related deaths (29,900 deaths estimated in 2004) (Rini et al, 2001; Jemal et al, 2004). It has been estimated that approximately 20% of men will be diagnosed with prostate cancer. Since the advent of prostate specific antigen (PSA) screening, most patients are found with localized disease. While prostatectomy or radiation treatment is the standard therapy for early-stage prostate cancer, 30-40% of patients will develop recurrent and/or metastatic disease. Androgen ablation with either surgical orchiectomy or the use of luteinizing-hormone-releasing hormone (LHRH) agonists with or without antiandrogens is an effective initial modality for advanced metastatic disease (Figg et al, 1997; Rini et al, 2001). Although a majority of patients with advanced metastatic prostate cancer respond to hormonal therapy for a median of 18-36 months, disease eventually progresses in most patients (Figg et al, 1997; Crawford et al, 1989). The utilization of second line hormonal agents such as corticosteroids, ketoconazole, megestrol acetate, and bicalutamide is generally associated with low response rates in this setting (Goktas et al, 1999; Klotz, 2000). Furthermore, such

responses are generally short duration and have no documented survival benefit.

Chemotherapies have been extensively evaluated in patients with metastatic androgen independent prostate cancer (AIPC) since the 1970s. The initial studies showed low response rates and high toxicities. Recently, however, with the development of new agents targeting prostate cancer both on the cellular and molecular level, promising results have been emerged. The agents, including docetaxel, mitoxantrone, estramustine, vinblastine and etoposide, either as a single agent or as a combination therapy, have showed benefit in clinical response, pain control, and/or quality of life, with estramustine/docetaxel combination showing the most promise (Beedassy et al, 1999; Oh, 2000). However, the benefit in overall survival is still unknown. Therefore, new therapeutic modalities are needed to prevent progression from early-stage to advanced metastatic disease and to improve survival outcomes in patients with advanced APIC.

II. Regulation of angiogenesis

Angiogenesis is the formation of new blood vessels from the pre-existing vascular bed. It is normally suppressed and is activated only transiently during

menstrual cycles and wound healing process (Folkman, 2001). Uncontrolled angiogenesis also occurs in rheumatoid arthritis, diabetic retinopathy, as well as neoplastic process. Angiogenesis is a very complicated process requiring extensive interactions between cells, cytokines, and extracellular matrix components (Folkman, 2001; Liekens et al, 2001). Angiogenic vessel growth is normally regulated by a balance of endogenous stimulators and inhibitors (**Table 1**). The angiogenesis regulators are primarily peptide growth factors, proteinases, or cell adhesion molecules. During angiogenesis, the cooperation and interaction of these regulators leads to endothelial cell proliferation, migration, invasion of the basement membrane, differentiation and capillary-tube formation. Vascular endothelial growth factor (VEGF) plays a key role in normal and abnormal angiogenesis since it stimulates almost every step in the angiogenic process (Folkman, 2001; Liekens et al, 2001). Other factors that have been shown to stimulate angiogenesis include acidic and basic fibroblast growth factor, angiogenin, angiopoietin, E-selectin, fibroblast growth factor-4, hepatocyte growth factor/scatter factor, interleukin-8, placental growth factor, platelet-derived endothelial cell growth factor, platelet-derived growth factor, pleiotropin, proliferin, tumor necrosis factor- and transforming growth factor- , . These endogenous angiogenic stimulators induce new blood vessel formation by either acting on endothelial cells or activating a broad range of other target cells and cell-cell interactions. Endogenous angiogenesis inhibitors include angiostatin, endostatin, thrombospondin-1,-2, antithrombin III, fibronectin, and many others (**Table 1**). The function of these inhibitors is to suppress new vessel formation or to turn off the transient process during physiological angiogenesis.

III. Matrix metalloproteinase and angiogenesis

Angiogenesis ultimately is the culmination of a cascade of many events. Before new blood vessels form, the basement membrane and matrix must be broken down

because these materials ordinarily serve as a supportive matrix and a barrier to endothelial cell migration (Liekens et al, 2001). This process is usually accomplished by the proteolytic activity with different enzymes. Matrix metalloproteinases (MMPs), a family of zinc- and calcium-containing proteolytic enzymes, are the most important enzymes in maintaining extracellular matrix tissue homeostasis and initiating new blood vessel formation (Wojtowicz-Praga et al, 1996; Brown, 1997). MMPs are secreted as precursor zymogens and activated in the extracellular matrix. More than a dozen MMPs have been identified, with MMP2 and MMP9 being particularly important in primary and metastatic tumor growth. These are critical factors in basement membrane degradation to facilitate invasion of malignant cells and angiogenesis (Brown, 1996; Nemeth et al, 2002). Studies have demonstrated that excessive MMP activity and/or overexpression occur in colorectal, lung, gastric, malignant glioblastoma, prostate and many other solid tumors (Curran et al, 1999; Liekens et al, 2001). It also has been shown that there is a good correlation between the level of MMPs and the aggressiveness of the tumors (Parsons et al, 1997).

IV. Angiogenesis and prostate cancer

Angiogenesis plays a pivotal role for the growth, invasion, and metastasis of solid malignant tumors (Folkman, 1990). Since a growing tumor requires an extensive capillary network to provide nutrients, a tumor will not grow beyond a few cubic millimeters without the development of new vessels. These newly formed vessels also provide a disseminating and metastatic route for cancer cells. In 1971, Folkman first proposed that tumor growth and metastasis are an angiogenesis-dependent processes and that inhibition of angiogenesis can be a novel anticancer strategy (Folkman, 1971). This hypothesis has been confirmed by a large body of preclinical and clinical evidence. To initiate new vessel formation, a tumor must acquire an angiogenic phenotype.

Table 1. Some of the most well-known endogenous regulators of angiogenesis

Angiogenesis Stimulators	Angiogenesis Inhibitors
Acidic fibroblast growth factor	Angiostatin
Angiogenin	Antithrombin III (fragment)
Angiopoietin	Canstatin
Basic fibroblast growth factor	Endostatin
E-Selectin	Fibronectin
Fibroblast growth factor (FGF)-4	Interferon and
Hepatocyte growth factor/scatter factor	Maspin
Interleukin-8	Pigment epithelium derived factor (PEDF)
Placental growth factor	Platelet factor-4 (fragment)
Platelet-derived endothelial cell growth factor	Prolactin (fragment)
Platelet-derived growth factor (PDGF)	Thrombospondin-1, 2
Pleiotropin	Tumstatin
Proliferin	Vascular endothelial growth inhibitor
(TGF)- ,	Transforming growth factor
Tumor necrosis factor (TNF)-	Vasostatin
Vascular endothelial growth factor (VEGF)	

Once changed to an angiogenic phenotype, the tumor becomes vascularized and can start to grow exponentially. The transformation to an angiogenic phenotype depends on a net imbalance of positive and negative angiogenic factors in tumor cells (10). New capillary formation can result from the overproduction of stimulators and/or down-regulation of negative modulators. Importantly, data from animal as well as human tissue studies suggest that the acquisition of angiogenic phenotype occurs early in tumor development. For instance, Brem et al, (1978) reported that angiogenic activity is significantly higher in transplanted hyperplastic breast tissues compared with normal breast counterparts in a rabbit model.

Prostate cancer, like other solid tumors, is also angiogenesis dependent. The development of prostate cancer is a multi-step process, advancing from high-grade prostatic intraepithelial neoplasia (PIN) to focal carcinoma, then to invasive carcinoma, and finally to metastatic disease. It is therefore important to target the molecular events that accompany progression of each step. Studies have demonstrated that the expression of angiogenesis stimulating factors such as VEGF, PDGF, and TGF in prostate carcinoma is increased (Bostwick et al, 1998; Jones et al, 1999; Lissbrant et al, 2001). Moreover, it has been shown that there is a progressive increase in angiogenesis as prostate cancer advances through various pathologic stages. Siegal et al reported that microvessel density (MVD) was higher in prostate cancer tissue than in adjacent hyperplastic or benign tissue (Siegal et al, 1995). Also, tumor specimens from patients with clinical prostate cancer have been found to have a remarkably high degree of vascularization compared with autopsy-identified prostate tumors from men without clinical disease (Wakui et al, 1992). Furthermore, studies have demonstrated that the intensity of angiogenesis as measured by MVD is a useful prognostic indicator in prostate cancer. Weidner et al showed that the mean microvessel count among patients with metastatic disease was 76.8 microvessels/field, as compared with 39.2 microvessels/field for those without metastases ($P < 0.0001$) (Weidner et al, 1993). Taken together, these reports indicate that angiogenesis measurement in prostate cancer can be used in predicting both the potential for development of metastatic disease and patient outcome.

V. Antiangiogenesis

The inhibition of angiogenesis, or antiangiogenesis is a promising new therapeutic anticancer modality. Currently, there are more than 20 antiangiogenic agents in various stages of phase I, II, and III clinical trials, and the list of drugs is growing. These agents act at the different steps of the angiogenesis regulatory pathway, and lead to modulation of the process and inhibition of tumor growth (Ellis et al, 2002; Giles 2002). Mechanistically, angiogenesis inhibitors can be subdivided into antagonists of angiogenic stimulators such as VEGF and their receptors, inhibitors of endothelial cell proliferation and/or survival, blockers of extracellular matrix degradation (MMP inhibitors), and drugs with undefined mechanisms (**Table 2**). Even though most of antiangiogenic agents are

in early phases of clinical trials, a few of them appear to be clinically effective (Figg et al, 2001a; Liekens et al, 2001; Ellis et al, 2002; Giles, 2002). Antiangiogenic therapy has advantages over conventional chemotherapy, such as ease of access of drugs to the endothelial cells. Because endothelial cells in a tumor are usually genetically stable, drug resistance is less like to develop with antiangiogenesis therapy. Furthermore, side effects of antiangiogenic agents should be negligible since angiogenesis in adults is restricted. However, because antiangiogenic agents usually simply halt tumor expansion, it is unlikely that angiogenesis inhibitors will work with the same rapidity as cytotoxic agents. In addition, since maximal formation of new blood vessels occurs when minimal tumor burden is present, the best opportunity for antiangiogenic agents to have a therapeutic impact is when there is minimal tumor burden. Minimizing tumor burden can be achieved with concurrent with radiation therapy, hormonal therapy and/or chemotherapy.

The following sections discuss recent advances in the development of antiangiogenic agents that have shown promising antitumor activity in patients with prostate cancer.

A. Thalidomide and its analog

Thalidomide, a glutamic acid derivative, is a potent teratogen that causes dysmelia (stunted limb growth) in humans (Stirling, 2001). It was marketed in Europe as a nonbarbiturate sedative but was withdrawn 30 years ago because of its teratogenic effects. It has been postulated that thalidomide-induced limb defects were secondary to an inhibition of blood vessel growth in the developing fetal limb buds. In 1994, D'Amato et al demonstrated that thalidomide inhibited bFGF-induced angiogenesis (D'Amato, 1994). Bauer et al subsequently determined that a metabolite of thalidomide was responsible for this antiangiogenic activity (Bauer et al, 1998). Thalidomide was later shown to inhibit the growth of V2 carcinoma and Lewis lung carcinoma in animal models by antiangiogenic mechanisms.

These preclinical findings led to clinical testing of thalidomide as an anticancer drug. In recently years, thalidomide has been shown to produce clinical activity in patients with multiple myeloma (MM), glioblastoma multiforme, and prostate cancer (Figg et al, 2001a,b; Stirling, 2001). In our phase II trial conducted at the National Cancer Institute (NCI), 63 metastatic AIPC patients who were heavily pretreated with hormonal and/or chemotherapy were treated with thalidomide. Twenty-seven percent of patients achieved a PSA response (Figg et al, 2001a), and the inhibition of PSA was associated with an improvement of clinical symptoms in majority cases. However, there was no apparent correlation between microvessel counts in pretreatment tissue biopsies and responses to thalidomide in this clinical trial. Similarly, a clear correlation between VEGF and bFGF expression and responses could not be made via assessment of pretreatment biopsy specimens.

Table 2. Angiogenesis inhibitors in prostate cancer.

Drug	Mode of action	Sponsor	Phase	Results
Anti-VEGF ABX	Anti-VEGF	NCI, Genetech	II in AIPC patients	No effects
CC-5013	TNF-	Celgene	I in solid tumor (including prostate cancer)	Pending
Celecoxib	COX2-I	Pharmacia	Phase I trial	Pending
Marimastat	MMP-I	British Biotech	I in stage III/IV	Decrease the rate of rise of PSA
2-ME	?	EntreMed	I in solid tumor (including prostate cancer)	Pending
Prinomastat	MMP-I	Agouron	III in AIPC patients with mitoxantrone/prednisone.	No benefit
SU6416	Anti-VEGF	SUGEN	II in AIPC patients	No effects
Thalidomide	multiple proposed	Celgene	II in AIPC with or without docetaxel	Promising
TNP-470	CDK-I	TAP	Phase III in D0 patients	Pending
			I in solid tumor (including prostate cancer)	No effects

VEGF = vascular endothelial growth factor; TNF- = tissue necrosis factor- ; COX2-I = cyclooxygenase-2 inhibitor; MMP-I = matrix metalloproteinase inhibitor; CDK-I = cyclin dependent kinase inhibitor

In another recent phase II trial of weekly docetaxel with thalidomide in 75 patients with metastatic AIPC (Figg et al, 2001b), 50% of patients receiving docetaxel/thalidomide and 35% of those receiving docetaxel alone had a PSA decrease of at least 50%. While the median overall survival and 18-month survival in docetaxel group were 15.9 months and 47.2%, respectively, the 18-month survival in combination group was 69.3%, and the median overall survival has not been reached in this group (Dahut et al, 2004). This result strongly suggests that the combination of a cytotoxic agent with an angiogenesis inhibitor is a promising area of investigation for prostate cancer management. Thalidomide was well tolerated in vast majority of patients. Constipation, dizziness, edema, fatigue and rebound insomnia after coming off study were the most common side effects. Thrombotic events occurred in the thalidomide/docetaxel combination treatment that can be prevented by prophylactic low molecular weight heparin (Horne et al, 2003).

Thalidomide is now undergoing many clinical trials for the treatment of a wide variety of tumors. At the NCI, a double-blinded randomized phase III study of thalidomide versus placebo in patients with stage D0 androgen dependent prostate cancer was recently initiated. The goal of this study is to determine if thalidomide can improve the efficacy of the LHRH agonist in hormone-responsive patients with a rising PSA after primary definitive therapy (surgery or radiation) for prostate cancer.

CC-5013, -(3-aminophthalimido) glutarimide, is an analogue of thalidomide. *In vitro* studies have shown that CC-5013 is more potent than thalidomide in inhibiting TNF- production and MM cell proliferation (Celgene Corporation, Inc, unpublished data). In the rat aortic ring angiogenesis assay, CC-5013 demonstrated a potent inhibitory effect on microvessel outgrowth (Figg et al, 2002). *In vivo*, CC-5013 showed the inhibitory effects on growth of MM cell line (HS-Sultan). Furthermore, according to preliminary non-clinical and clinical studies conducted to date, CC-5013 appears to lack the sedative and teratogenic activity of thalidomide.

In two phase I studies in MM, a total of 39 patients with relapsed or refractory disease have been treated with CC-5013. Patients received doses ranging from 5 mg to 50 mg daily of CC-5013. It was well tolerated with principal side effects being bone marrow suppression, myalgia, fatigue, headache, constipation, diarrhea, ringing in ears, lightheadedness, and mild elevated LFT and creatinine. In one study conducted at the University of Arkansas, myeloma response was seen at the higher dosages of CC-5013. Four of 15 patients had a greater than 25% reduction (1 patient > 75%) in paraprotein level. Ten of 14 evaluable patients treated at the Dana Farber Cancer Center responded to the drug, including 3 patients with > 50% and 7 patients with 25-50% reduction in paraprotein level. At the NCI, a phase I trial of CC-5013 is currently conducting in patients with solid tumors, including metastatic AIPC, to further study its clinical antitumor activity.

B. Matrix metalloproteinase inhibitors (MMPs)

In recently years, several MMPs inhibitors, such as batimastat, marimastat, prinomastat, and COL-3, have been developed as anticancer drugs and are being actively evaluated in preclinical studies and ongoing clinical trials (Nemunaitis et al, 1998; Macaulay et al, 1999; Heath et al, 2001; Rudek et al, 2001; Ahmann et al, 2002). Batimastat is almost completely insoluble, and consequently, has a very poor bioavailability with oral route. Therefore, the clinical usage of batimastat is limited.

Marimastat has a broad-spectrum inhibitory activity against most of the major MMPs; including MMP2 and MMP9 (Nemunaitis et al, 1998). Marimastat is almost completely absorbed after oral administration with a half-life of approximately 15 hours. It has been evaluated extensively in clinical trials in different solid tumors with promising activity in patients with pancreatic and colorectal cancer. A total of 88 patients with advanced metastatic prostate cancer were evaluated in 6 phase I trials. Marimastat was administered orally for 4 weeks. The therapeutic response was measured by decrease in the rate of rise of serum PSA. In these studies Marimastat was

demonstrated to reduce the rate of rise of serum PSA in a dose-dependent manner (Nemunaitis et al 1998). However, the significance in the change of the PSA slope is unclear. Marimastat has been well tolerated. The principal side effect was dose-related joint pain and stiffness.

Prinomastat is a selective inhibitor of MMP2/MMP3/MMP9. It has been demonstrated that prinomastat inhibits the growth of PC-3 cells in an animal model (Shalinsky et al, 1999). Prinomastat was well tolerated with principal side effect being mild musculoskeletal toxicity in early clinical trials. In a recent phase III trial, 406 patients with chemotherapy-naive AIPC were randomized into mitoxantrone/prednisone with or without prinomastat. No significant difference in PSA response rate, progression-free survival, or overall survival in two groups was observed (Ahmann et al, 2002). While this result showed no benefit by addition of prinomastat to chemotherapy in AIPC, it does not preclude the use of Prinomastat in the treatment of early stage of prostate cancer.

Alendronate, a bisphosphonate and an inhibitor of osteoclastic bone resorption, has been shown to decrease MMP2 and MMP9 secretion in animal models (39). Also, recent studies demonstrated that bisphosphonates have antitumor effect *in vivo* animal systems and promoting apoptosis of tumor cells *in vitro* (Diel et al, 1998; Powles et al, 1998). Stearns et al evaluated the combination of alendronate and paclitaxol on human PC3ML cell bone metastases in SCID mice (Stearns et al, 1996). The pretreatment of SCID mice with alendronate partially blocked the establishment of bone metastases by PC3ML cells and resulted in tumor formation in the peritoneum and other soft tissues. When used separately, alendronate and paclitaxel partially inhibited MMP2 production, but the combination totally blocked protease production and release. Based on these preclinical results, the NCI recently completed a randomized phase II trial of ketoconazole (KT) and alendronate (AL) versus KT in 72 patients with progressive AIPC metastatic to bone. The proportion of patients with a > 50% decline in PSA was similar in the 2 groups (47.2% in KT/AL group vs 44.4% in KT group). However, there was a strong trend toward a prolonged duration of response in KT/AL group compared to ketoconazole group (median, 8.9 months vs 6.3 months, respective; $p=0.055$), and more patients in KT/AL group have not progressed (Liu et al, 2002). This result suggests that alendronate, a potential antiangiogenic agent, improves duration of response in patients with AIPC treated with ketoconazole.

C. TNP-470

TNP-470, a semi-synthetic derivative of fumagillin, was one of the first antiangiogenic compounds to undergo clinical testing (Kruger et al, 2000). Fumagillin is an antibiotic secreted by *Aspergillus fumigatus fresenius* (Ingber et al, 1990). It was subsequently found that fumagillin is a very potent inhibitor of endothelial cell proliferation *in vitro* and tumor-induced angiogenesis *in vivo* (Ingber et al, 1990; Kruger et al, 2000). However, the clinical utility of fumagillin was limited because it caused

profound weight loss in animal studies. Therefore, several synthetic analogues were developed, and among these, TNP-470 has shown the least toxicity with the greatest antiangiogenic activity (Ingber et al 1990; Kusaka et al, 1991). *In vitro* studies revealed that TNP-470 inhibited endothelial cell proliferation in a very low concentration (Kusaka et al, 1994). *In vivo*, TNP-470 has been demonstrated to be a potent antiangiogenic agent in the chick chorioallantoic assay, rat corneal micropocket assay, and in the rat blood vessel organ culture assay (Ingber et al, 1990; Kusaka et al, 1991; Kruger et al, 2000). Furthermore, TNP-470 inhibited the growth of Lewis lung carcinoma, B16 melanoma, and other tumors in animal models (Ingber et al, 1990; Kusaka et al, 1991; O'Reilly et al, 1995). The molecular target of TNP-470 appears to involve transcription inhibition of specific cyclin-dependent kinase (cdk) and cyclin gene family members (Koyama et al, 1996). It might also inhibit cdc2 and cdk2 kinase activation in endothelial cells (Kato et al, 1994).

Several phase I studies of TNP-470 have been completed in patients with Kaposi's sarcoma, renal cell carcinoma, brain cancer, breast cancer, cervical cancer and prostate cancer (Figg et al, 1997; Bhargava et al, 1999; Stadler et al, 1999; Logothetis et al, 2001). These phase I trials often showed that TNP-470 resulted in minor objective responses and was well tolerated. The major dose-limiting toxicities were reversible neurotoxicities, including fatigue, asthenia, nystagmus, diplopia, ataxia, depression and loss of concentration. Interestingly, although antitumor activity was not documented in patients with AIPC, TNP-470 caused a transient increase of serum PSA. It was subsequently found that TNP-470 enhances PSA transcription *in vitro* culture systems (Horti et al, 1999).

D. 2-methoxyestradiol

2-methoxyestradiol (2ME) is a natural metabolite of the endogenous estrogens estradiol-17 and 17-ethynylestradiol (Seegers et al, 1989). In contrast to most estrogens, 2-ME has been shown in preclinical studies to be potentially efficacious in the treatment of cancer. *In vitro*, 2ME has potent antiproliferative activity in many human cancer cell lines, including HeLa cells, Jurkat leukemia cells, and neuroblastoma cells (Seegers et al, 1989, Cushman et al, 1995, and Nakagawa-Yagi et al, 1996). Human breast cancer cell lines are particularly sensitive to the cytotoxic effect of 2-ME irrespective of the estrogen receptor status. *In vivo*, 2-ME has potent activity in primary and metastatic tumor models. Its activity was evident in xenograft models derived from a non-estrogen-dependent human breast tumor cell line (MDA MB-435), MethA sarcoma, B16 melanoma, neuroblastoma, and myeloma (Fotsis et al, 1994, Klauber et al, 1997; Arbiser et al, 1999; Schumacher et al, 2001).

The mechanism of action of 2-ME has not yet been determined, but studies have shown that 2-ME has a potent inhibitory effect on the proliferation of blood-vessel endothelial cells *in vitro* (Fotsis et al, 1994). Additional studies have demonstrated that 2-ME causes apoptosis in cultured arterial endothelial cells and inhibits the

migration of these vascular endothelial cells (Yue et al, 1997). *In vivo*, 2-ME has been shown to be a potent antiangiogenic agent in tumor vasculature studies and many other models (Fotsis et al, 1994; Klauber et al, 1994; Zhu et al, 1998). A phase I clinical trial of 2-ME in metastatic breast cancer patients was recently initiated (Miller et al, 2001). To date 2-ME has been well tolerated, and no dose-limiting toxicity noted. 2-ME treatment did not alter hormonal status in these patients. Ten out fifteen patients had stable disease. At the NCI, we are currently conducting a phase I trial of 2-ME in patients with solid tumors, including metastatic AIPC, to further explore its clinical benefit, biological as well as molecular activities.

VI. VEGF antibody and inhibitors

VEGF and its receptors play a pivotal role in the regulation of angiogenesis (Folkman et al, 2001 and Liekens, 2001). Therefore, inhibition of VEGF and/or its receptor activity can have a potential benefit in cancer treatment. Recombinant humanized anti-VEGF (RhuMab VEGF, bevacizumab, Avastin (Genetech)) is a monoclonal IgG antibody. *In vivo* animal models, it has potent anti-VEGF activity, and suppresses the growth of a broad spectrum of human cancer cell lines (Kim et al, 1993; Warren et al, 1995; Mordenti et al, 1999). Several phase I trials showed that bevacizumab was well tolerated with minimal toxicity. A phase II trial of bevacizumab was conducted in patients with AIPC (Bok et al, 1999). Bevacizumab showed no significant effects on PSA or clinical benefits. Therefore, further studies of this antibody likely will focus on early stage disease, adjuvant treatment, or in combination with other treatment modalities. Dr. Picus reported that a phase II trial combining docetaxel, estramustine and bevacizumab resulted in 79% of patients having a > 50% decrease in PSA and 42% had a partial response. Survival and disease progression have not yet been assessed. (Picus, 2004)

Cetuximab (IMC-C225, anti-EGFR MAb, Erbitux (Imclone, Bristol-Meyers Squibb Oncology)) was studied alone and in combination with paclitaxel in a murine model. Cetuximab alone and in combination significantly decreased growth of the PC-3M-LN4, *in vivo*. A decreased serum concentration of interleukin-8 as well as a decrease in MVD, and tumor cell proliferation and an enhanced of apoptosis were all enhanced by coadministration of paclitaxel (Karahima, 2002).

Semaxanib (SU5416) is a potent VEGF receptor inhibitor. It inhibits VEGF-mediated FLK1 signaling and endothelial cell proliferation *in vitro* culture systems (Millauer et al, 1993). *In vivo*, SU5416 has been demonstrated to inhibit the growth of several type of tumors in animal models (millauer et al, 1996). SU5416 has been tested in phase I studies, in combination with androgen ablation and radiation therapy, and in a phase II study with dexamethasone combination in patients with AIPC. Dose-limiting toxicities in phase I studies consisted headache, fatigue, change in voice, nausea and vomiting, as well as allergic reactions (Cropp et al, 1999). Although SU5416 had promising results in preclinical models, it was withdrawn recently due to its lack of efficacy in clinical

trials.

A. Cyclooxygenases inhibitors

Prostaglandins and their derivatives are signaling lipophilic molecules that regulate many physiologic processes including the inflammatory response, platelet aggression, clot formation, and gastric cyto-protection (Dang et al, 2002). Cyclooxygenases (COXs) are key enzymes in the conversion of arachidonic acid to prostaglandins. There are two isoforms of the COXs. COX-1 is a constitutive enzyme that is present in most normal tissues and is responsible for local prostaglandin synthesis. In contrast, COX-2 is an inducible form that is normally only expressed at a low level in some tissues, such as brain and kidney. COX-2 synthesis is induced by a variety of stimuli, including inflammatory cytokines, growth factors, oncogenes (HER2/neu and Src), tumor promoters and carcinogens (Kosaka et al, 1994; Vadlamudi et al, 1999; Dang et al, 2002). Studies showed that excessive COX-2 overexpression occurs in colorectal, lung, gastric, breast, prostate and many other solid tumors (Eberhart et al, 1994; Ristimaki et al, 1997; Hida et al, 1998; Hwang et al, 1998; Gupta et al, 2000; Dang et al, 2002). Also, accumulating evidence suggests that elevated prostaglandin expression is associated with tumor growth, metastatic potential and recurrence in a variety spectrum of tumor types. Uotila et al showed that the expression of COX-2 in prostate cancer cells is higher compared with normal glandular epithelial of control prostates (Uotila et al, 2001). Although the mechanism is unclear, overexpression of COX-2 may affect different steps in the process of carcinogenesis, such as immune regulation, cell invasion and proliferation, or apoptosis. Recently, studies demonstrated that there is a strong link between COX-2 expression and hypoxia-induced tumor angiogenesis (Liu et al, 1998). Therefore, COX-2 overexpression may increase tumor blood supply and contribute to tumor growth.

In prostate cancer, studies have shown that COX-2 inhibitors could induce apoptosis in prostate cancer cells *in vitro* (Liu et al, 1998). In addition, Celecoxib, an elective COX-2 inhibitor, has been shown to be a potent antitumor and a chemoprevention agent in a DMBA-induced rat mammary tumor model (Alshafie et al, 2000). Furthermore, Kirschenbaum et al reported that the COX-2 inhibitors decrease MVD and angiogenesis in prostate cancer tumor models (Liu et al, 2000). Based upon these preclinical observations, COX-2 inhibitors, the potential antiangiogenesis agents, have been tested as chemoprevention as well as treatment modalities. Several clinical trials reported that Celecoxib and other NSAIDs have chemoprevention effects on intestinal adenomas in patients with familial adenomatous polyposis (FAP) (Waddell, et al, 1983, Hawk et al, 1999; Steinbach et al, 2000). Currently, exisulind, a COX-1/COX-2 inhibitor, is being evaluated in phase I/II trials in prostate cancer patients, either as a single agent or in combination with docetaxel. Also, a neoadjuvant trial is currently conducting in prostate cancer, in which patients are randomized to receive either celecoxib or placebo prior to radical prostatectomy. The results of these trials will help to

determine the future role of COX-2 inhibitors in the treatment and chemoprevention of prostate cancer.

VII. Conclusion

Antiangiogenesis, a new treatment and prevention strategy in patients with prostate cancer and other tumors, is being developed as either monotherapy or a combination therapy. While preclinical models appeared very promising, with the exception of a few agents, early clinical studies of angiogenesis inhibitors in patients with prostate cancer exhibited disappointed results. Most clinical studies reported that no complete angiosuppression or clinical benefit can be obtained. However, it is too early to conclude that they are ineffective since they have been used only in late stage metastatic prostate cancer. In addition, lack of effectiveness in these trials is not surprising since multiple steps and a variety of factors required for angiogenesis, and by inhibiting one factor is insufficient. Therefore, it is possible that synergistic anti-tumor effects can be obtained by targeting of multiple points in the angiogenic cascade, or by combining angiogenesis inhibitors with radiation therapy, hormonal ablation or cytotoxic therapies. It is expected that within the next decade, these combinations will provide new modalities in the treatment of prostate cancer.

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References

- Ahmann, FR, Saad F, Mercier R, et al (2002). Interim results of a phase III study of the matrix metalloproteinase inhibitor Prinomastat in patients having metastatic, hormone refractory prostate cancer (HRPC). *Proc Am Soc Clin Oncol*; abstract 692.
- Alshafie GA, Abou-Issa HM, Seibert K, et al (2000). Chemotherapeutic evaluation of Celecoxib, a cyclooxygenase-2 inhibitor, in a rat mammary tumor model. *Oncol Rep* 7,1377-81.
- Arbiser JL, Panigrathy D, Klauber N, et al (1999). The antiangiogenic agents TNP-470 and 2-methoxyestradiol inhibit the growth of angiosarcoma in mice. *J Am Acad Dermatol* 40, 925-9.
- Bauer KS, Dixon SC, Figg WD (1998). Inhibition of angiogenesis by thalidomide requires metabolic activation, which is species-dependent. *Biochem Pharmacol* 55, 1827-34.
- Beedassy A, Cardi G (1999). Chemotherapy in advanced prostate cancer. *Semin Oncol* 26, 428-38.
- Bhargava P, Marshall JL, Rizvi N, et al (1999). A phase I and pharmacokinetic study of TNP-470 administered weekly to patients with advanced cancer. *Clin Cancer Res* 5, 1989-95.
- Bok R, Corry M, Frohlich M, et al (1999). A phase II trial of humanized monoclonal anti-vascular endothelial growth factor antibody (rhuMAB VEGF) in hormone refractory prostate cancer (HRPC). *Proc Am Soc Clin Oncol* 18, 351a.
- Bostwick DG, Iczkowski KA (1998). Microvessel assay in prostate cancer: prognostic and therapeutic utility. *Semin Urol Oncol* 16, 118-23.
- Brem SS, Jensen HM, Gullino PM (1978). Angiogenesis as a marker of preneoplastic lesions of the human breast. *Cancer* 41, 239-44.
- Brown PD (1997). Matrix metalloproteinase inhibitors in the treatment of cancer. *Med Oncol* 14, 1-10.
- Crawford ED, Eisenberger MA, McCleod DG, et al (1989). A controlled trial of leuprolide with and without flutamide in prostate carcinoma. *N Engl J Med* 321, 419-24.
- Cropp G, Hannah A, Kabbinavar F, et al (1999). Phase I dose-escalating trial of SU5416, a novel angiogenesis inhibitor in patients with advanced malignancies. *Pros ASCO* 18, 618.
- Curran S, Murray GI (1999). Matrix metalloproteinases in tumour invasion and metastasis. *J Pathol* 189, 300-8.
- Cushman M, He HM, Katzenellenbogen JA, et al (1995). Synthesis, antitubulin and antimetabolic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicine binding site. *J Med Chem* 38, 2041-9.
- D'Amato RJ, Loughnan MS, Flynn E, et al (1994). Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci U S A* 91, 4082-5.
- Dahut WL, Gulley JL, Arlen PM, Liu Y, et al. (2004) A randomized phase II trial of docetaxel plus thalidomide in androgen-independent prostate cancer. *J Clin Oncol*. *In press*.
- Dang CT, Shapiro CL, Hudis CA (2002). Potential role of selective COX-2 inhibitors in cancer management. *Oncology* 16, 30-36.
- Diel IJ, Solomayer EF, Costa SD, et al (1998). Reduction in new metastases in breast cancer with adjuvant clodronate treatment. *N Engl J Med* 339, 357-63.
- Eberhart CE, Coffey RJ, Radhika A, et al (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 107, 1183-8.
- Ellis LM, Liu W, Fan F, et al (2002). Synopsis of angiogenesis inhibitors in oncology. *Oncology* 16, 14-22.
- Figg WD, Feuer J, Bauer KS (1997). Management of hormone-sensitive metastatic prostate cancer. *Cancer Practice* 5, 258-263.
- Figg WD, Pluda JM, Lush RM, et al (1997). The pharmacokinetics of TNP-470, a new angiogenesis inhibitor. *Pharmacotherapy* 17, 91-7.
- Figg WD, Dahut W, Duray P, et al (2001). A randomized phase II trial of thalidomide, an angiogenesis inhibitor, in patients with androgen-independent prostate cancer. *Clin Cancer Res* 7, 1888-93.
- Figg WD, Arlen P, Gulley J, et al (2001). A randomized phase II trial of docetaxel (taxotere) plus thalidomide in androgen-independent prostate cancer. *Semin Oncol* 28(4S15), 62-6.
- Figg WD, Kruger EA, Price DK, et al (2002). Inhibition of angiogenesis: treatment options for patients with metastatic prostate cancer. *Invest New Drugs* 20, 183-94.
- Folkman J (1971). Tumor angiogenesis: therapeutic implications. *N Engl J Med* 285, 1182-6.
- Folkman J (1990). What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst.* 82, 4-6.
- Folkman J (2001). Angiogenesis-dependent diseases. *Semin Oncol* 28: 36-42.
- Fotsis T, Zhang Y, Pepper MS, et al (1994). The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature* 368, 237-9.
- Giles FJ (2002). The emerging role of angiogenesis inhibitors in

- hematologic malignancies. **Oncology** 16, 22-29.
- Goktas S, Crawford ED (1999). Optimal hormonal therapy for advanced prostatic carcinoma. **Semin Oncol** 26, 162-73.
- Gupta S, Srivastava M, Ahmad N, et al (2000). Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. **Prostate** 42, 73-8.
- Hawk E, Lubet R, Limburg P (1999). Chemoprevention in hereditary colorectal cancer syndromes. **Cancer** 86, 2551-63.
- Heath EI, O'Reilly S, Humphrey R, et al (2001). Phase I trial of the matrix metalloproteinase inhibitor BAY12-9566 in patients with advanced solid tumors. **Cancer Chemother Pharmacol** 48, 269-74.
- Hida T, Yatabe Y, Achiwa H, et al (1998). Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. **Cancer Res** 58, 3761-4.
- Horne ME 3rd, Figg WD, Arlen P, et al (2003). Increased frequency of venous thromboembolism with the combination of docetaxel and thalidomide in patients with metastatic androgen-independent prostate cancer. **Pharmacotherapy** 23, 315-8.
- Horti J, Dixon SC, Logothetis CJ, et al (1999). Increased transcriptional activity of prostate-specific antigen in the presence of TNP-470, an angiogenesis inhibitor. **Br J Cancer** 79, 1588-93.
- Hwang D, Scollard D, Byrne J, et al (1998). Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. **J Natl Cancer Inst** 90:455-60.
- Ingeber D, Fujita T, Kishimoto S, et al (1990). Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. **Nature** 348, 555-7.
- Jemal A, Thomas A, Murray T, et al (2004). Cancer statistics, 2004. **CA Cancer J Clin** 54, 8-29.
- Jones A, Fujiyama C (1999). Angiogenesis in urological malignancy: prognostic indicator and therapeutic target. **BJU Int** 83, 535-55.
- Karashima T, Sweeney P, Slaton, JW et al (2002). Inhibition of angiogenesis by the anti-epidermal growth factor receptor antibody ImClone C225 in androgen-independent prostate cancer growing orthotopically in nude mice. **Clin Cancer Res** 8, 1253-1264.
- Kato T, Sato K, Kakinuma H, et al (1994). Enhanced suppression of tumor growth by combination of angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (TNP-470) and cytotoxic agents in mice. **Cancer Res** 54, 5143-7.
- Kim KJ, Li B, Winer J, et al (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. **Nature** 362, 841-4.
- Klauber N, Parangi S, Flynn E, et al (1997). Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. **Cancer Res** 57(1), 81-6.
- Klotz L (2000). Hormone therapy for patients with prostate carcinoma. **Cancer** 88: 3009-14.
- Kosaka T, Miyata A, Ihara H, et al (1994). Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. **Eur J Biochem** 221, 889-97.
- Koyama H, Nishizawa Y, Hosoi M, et al (1996). The fumagillin analogue TNP-470 inhibits DNA synthesis of vascular smooth muscle cells stimulated by platelet-derived growth factor and insulin-like growth factor-I. Possible involvement of cyclin-dependent kinase 2. **Circ Res** 79, 757-64.
- Kruger EA, Figg WD (2000). TNP-470: an angiogenesis inhibitor in clinical development for cancer. **Expert Opin Investig Drugs** 9, 1383-96.
- Kusaka M, Sudo K, Fujita T, et al (1991). Potent anti-angiogenic action of AGM-1470: comparison to the fumagillin parent. **Biochem Biophys Res Commun** 174:1070-6.
- Kusaka M, Sudo K, Matsutani E, et al (1994). Cytostatic inhibition of endothelial cell growth by the angiogenesis inhibitor TNP-470 (AGM-1470). **Br J Cancer** 69, 212-6.
- Liekens S, De Clercq E, Neyts J (2001). Angiogenesis: regulators and clinical applications. **Biochem Pharmacol** 61, 253-70.
- Lissbrant IF, Lissbrant E, Damber J, et al (2001). Blood vessels are regulators of growth, diagnostic markers and therapeutic targets in prostate cancer. **Scand J Urol Nephrol** 35, 437-52.
- Liu XH, Yao S, Kirschenbaum A, et al (1998). NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. **Cancer Res** 58, 4245-9.
- Liu XH, Kirschenbaum A, Yao S, et al (1999). Upregulation of vascular endothelial growth factor by cobalt chloride-simulated hypoxia is mediated by persistent induction of cyclooxygenase-2 in a metastatic human prostate cancer cell line. **Clin Exp Metastasis** 17, 687-94.
- Liu XH, Kirschenbaum A, Yao S, et al (2000). Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer in vivo. **J Urol** 164, 820-5.
- Liu Y, Figg WD, Arlen P, et al (2002). A randomized phase II trial of ketoconazole (KT) and alendronate (AL) versus ketoconazole in androgen independent prostate cancer (AIPC). **Proc the third North American symposium on skeletal complications of malignancy**. Abstract D4.
- Logothetis CJ, Wu KK, Finn LD, et al (2001). Phase I trial of the angiogenesis inhibitor TNP-470 for progressive androgen-independent prostate cancer. **Clin Cancer Res** 7, 1198-203.
- Macaulay VM, O'Byrne KJ, Saunders MP, et al (1999). Phase I study of intrapleural batimastat (BB-94), a matrix metalloproteinase inhibitor, in the treatment of malignant pleural effusions. **Clin Cancer Res** 5:513-20.
- Millauer B, Witzmann-Voos S, Schnurch H, et al (1993). High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. **Cell** 72, 835-46.
- Millauer B, Longhi MP, Plate KH, et al (1996). Dominant-negative inhibition of Flk-1 suppresses the growth of many tumor types in vivo. **Cancer Res** 56, 1615-20.
- Miller KD, Haney LG, Pribluda VS, et al (2001). A phase I safety, pharmacokinetic and pharmacodynamic study of 2-methoxyestradiol (2ME2) in patients (Pts) with refractory metastatic breast cancer (MBC). **Proc Am Soc Clin Oncol** 20, Abstract 170.
- Mordenti J, Thomsen K, Licko V, et al (1997). Efficacy and concentration-response of murine anti-VEGF monoclonal antibody in tumor-bearing mice and extrapolation to humans. **Toxicol Pathol** 27, 14-21.
- Nakagawa-Yagi Y, Ogane N, Inoki Y, et al (1996). The endogenous estrogen metabolite 2-methoxyestradiol induces apoptotic neuronal cell death in vitro. **Life Sci** 58, 1461-7.
- Nemeth JA, Yousif R, Herzog M, et al (2002). Matrix metalloproteinase activity, bone matrix turnover, and tumor cell proliferation in prostate cancer bone metastasis. **J Natl Cancer Inst** 94, 17-25.
- Nemunaitis J, Poole C, Primrose J, et al (1998). Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. **Clin Cancer Res** 4, 1101-1109.
- Oh WK (2000). Chemotherapy for patients with advanced prostate carcinoma: a new option for therapy. **Cancer** 88, 3015-21.

- O'Reilly MS, Brem H, Folkman J (1995). Treatment of murine hemangi endotheliomas with the angiogenesis inhibitor AGM-1470. **J Pediatr Surg** 30:325-9.
- Parsons SL, Watson SA, Brown PD, et al (1997). Matrix metalloproteinases. **Br J Surg** 84, 160-6.
- Picus J (2004). Docetaxel/bevacizumab (Avastin) in prostate cancer. Chemotherapy Foundation Symposium XXI, 2004, New York City. **Cancer Investigation** 22 (*In press*). Archived on-line at www.mssm.edu/ctf (Accessed April 2, 2004).
- Powles TJ, McCloskey E, Paterson AH, et al (1998). Oral clodronate and reduction in loss of bone mineral density in women with operable primary breast cancer. **J Natl Cancer Inst** 90, 704-8.
- Rini BI, Small EJ (2001). An update on prostate cancer. **Curr Opin Oncol** 13, 204-11.
- Ristimaki A, Honkanen N, Jankala H, et al (1997). Expression of cyclooxygenase-2 in human gastric carcinoma. **Cancer Res** 57, 1276-80.
- Rudek MA, Figg WD, Dyer V, et al (2001). Phase I clinical trial of oral COL-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. **J Clin Oncol** 19, 584-92.
- Schumacher G, Neuhaus P (2001). The physiological estrogen metabolite 2-methoxyestradiol reduces tumor growth and induces apoptosis in human solid tumors. **J Cancer Res Clin Oncol** 127(7), 405-10.
- Seegers JC, Aveling LM, Can Aswegen CH, et al (1989). The cytotoxic effects of estradiol-17 beta, catecholestradiols and methoxyestradiols on dividing MCF-7 and HeLa cells. **J Steroid Biochem** 32, 797-809.
- Shalinsky DR, Brekken J, Zou H, et al (1999). Broad antitumor and antiangiogenic activities of AG3340, a potent and selective MMP inhibitor undergoing advanced oncology clinical trials. **Ann N Y Acad Sci** 878:236-70.
- Siegal JA, Yu E, Brawer MK (1995). Topography of neovascularity in human prostate carcinoma. **Cancer** 75, 2545-51.
- Stadler WM, Kuzel T, Shapiro C, et al (1999). Multi-institutional study of the angiogenesis inhibitor TNP-470 in metastatic renal carcinoma. **J Clin Oncol** 17, 2541-5.
- Stearns ME, Wang M (1996). Effects of alendronate and taxol on PC-3 ML cell bone metastases in SCID mice. **Invasion Metastasis** 16, 116-31.
- Stearns ME, Wang M (1998). Alendronate blocks metalloproteinase secretion and bone collagen I release by PC-3 ML cells in SCID mice. **Clin Exp Metastasis** 16, 693-702.
- Steinbach G, Lynch PM, Phillips RK, et al (2000). The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. **N Engl J Med** 342, 1946-52.
- Stirling D (2001). Thalidomide: a novel template for anticancer drugs. **Semin Oncol** 28, 602-6.
- Uotila P, Valve E, Martikainen P, et al (2001). Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. **Urol Res** 29, 23-8.
- Vadlamudi R, Mandal M, Adam L, et al (1999). Regulation of cyclooxygenase-2 pathway by HER2 receptor. **Oncogene** 18, 305-14.
- Waddell WR, Loughry RW (1983). Sulindac for polyposis of the colon. **J Surg Oncol** 24, 83-7.
- Wakui S, Furusato M, Itoh T, et al (1992). Tumour angiogenesis in prostatic carcinoma with and without bone marrow metastasis: a morphometric study. **J Pathol** 168, 257-62.
- Warren RS, Yuan H, Matli MR, et al (1995). Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. **J Clin Invest** 95, 1789-97.
- Weidner N, Carroll PR, Flax J, et al (1993). Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. **Am J Pathol** 143, 401-9.
- Wojtowicz-Praga S, Low J, Marshall J, et al (1996). Phase I trial of a novel matrix metalloproteinase inhibitor batimistat (BB-94) in patients with advanced cancer. **Invest New Drug** 14, 193-202.
- Yue TL, Wang X, Loudon CS, et al (1997). 2-Methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis: possible role for stress-activated protein kinase signaling pathway and Fas expression. **Mol Pharmacol** 51(6), 951-62.
- Zhu BT, Conney AH (1998). Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? **Cancer Res** 58(11), 2269-77.

