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Vascular Disrupting Agents

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Abstract It has been well established that a functioning vascular supply is essential for solid tumor growth and metastases. In the absence of a viable vascular network, tumors are unable to grow beyond a few millimeters and therefore remain dormant. Initiation of angiogenesis allows for continued tumor growth and progression. Targeting tumor vasculature, either by inhibiting growth of new tumor blood vessels (antiangiogenic agents) or by destroying the already present tumor vessels (vascular disrupting agents; VDA), is an area of extensive research in the development of new antitumor agents. These two groups differ in their direct physiological target, the type or extent of disease that is likely to be susceptible, and the treatment schedule. VDAs target the established tumor blood vessels, resulting in tumor ischemia and necrosis. These agents show more immediate effects compared to antiangiogenic agents and may have more efficacy against advanced bulky disease. VDAs can be divided into two groups—ligand-bound and small molecule agents. Both VDA groups have demonstrated antitumor effects and tumor core necrosis, but consistently leave a thin rim of viable tumor cells at the periphery of the tumor. More evidence suggests VDAs will have their greatest effect in combination with conventional chemotherapy or other modes of treatment that attack this outer rim of cells. J. Cell. Biochem. 99: 1021–1039, 2006.

Key words: tumor vasculature; tumor endothelium; angiogenesis; vascular targeting; vascular disruption

It has become more apparent that current available therapies for the treatment of most solid tumors are resulting in diminishing returns. A symptomatic solid tumor at presentation usually contains 10^9–10^{11} cells, all of which must be eradicated for a complete cure [Denekamp, 1990]. Even if a particular agent or combination of agents were able to kill 99.9% of the cells, this would only result in a temporary delay of tumor growth [Wilcox, 1966; Skipper, 1971; Denekamp, 1990]. Early development of chemotherapeutic agents focused on broad, nonspecific, antiproliferating targets of tumor cells. The current trend is to develop agents that focus on the subtle differences between malignant and normal cells. Most of these new agents target specific pathways or characteristics of the malignant cell that confer proliferative advantage [Rowinsky, 2002]. Examples of these targeted mechanistic pathways are the signal transduction pathways, the cell cycle, and the apoptotic mechanisms of a cancer cell. Unfortunately, most of these new agents to date have yielded minimal benefits when used as single agents and are usually more effective when used in combination with other agents. However, if the therapy is systemic and is nonselective, which many agents are, the most rapidly dividing normal cells (i.e., bone marrow, hair follicles, and intestinal crypts) will limit the dose that can be given [Denekamp, 1990]. It has been suggested that until we develop agents that will exclusively target tumors alone with no interactions with "normal" cells, these organs will continue to be dose limiting, no matter how unique the approach to damaging the cell cycle [Denekamp, 1990]. Another roadblock is that the genetic instability leading to tumor cell heterogeneity and the ability of tumor cells to use alternative pathways for growth may circumvent the role of these targeted therapies. For all these reasons, it is critical that we continue to seek alternative mechanisms that may be targeted to add to

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our growing armamentarium of anticancer agents.

TUMOR VASCULATURE

The most striking characteristic about solid tumors when compared to normal tissues is the vascular architecture [Carmeliet and Jain, 2000]. In normal tissues, the vasculature is well organized to provide ideal conditions for each cell. In contrast, the growing tumor vasculature is abnormal, chaotic, and inadequate in both structure and function [Carmeliet and Jain, 2000]. This vasculature relies on tubulin alone as the cytoskeleton support, whereas the cytoskeleton of normal tissue is composed of both tubulin and actin. Tumor vasculature also expresses distinct antigens on its cell surface [Carmeliet and Jain, 2000]. Common features of these vessels include dilatation, elongated shapes, blind ends, bulges, leaky sprouts, abrupt changes in diameters, and evidence of vascular compression [Siemann, 2002]. Blood flow in these vessels is sluggish and irregular and may even be via arteriovenous shunts, all of which provide a much poorer nutrient environment than normal cells. This irregularity of vessel density and blood flow leads to altered cellular and biochemical microenvironments resulting in hypoxic areas which are very characteristic of solid tumors [Siemann, 2002]. The poorly nourished, hypoxic environments that result from this altered tumor vasculature limit the effectiveness of both chemotherapy and radiotherapy [Siemann, 2002; Wouters et al., 2002].

Hypoxic cells have been shown to be radioresistant and require at least three times the radiation to kill when compared to well-oxygenated cells [Brown and Le, 2002; Wouters et al., 2002]. Chemotherapeutic drug resistance can also be caused by tumor cell hypoxia. Some agents, such as bleomycin, require oxygen for free radical production in their mechanism of tumor cell killing [Wouters et al., 2002]. Agents which target cell cycle products and require proliferating cells are also inhibited in hypoxic conditions as proliferation is decreased [Siemann, 2002; Wouters et al., 2002]. As a result of the tumor vasculature being so distorted and limited, the effective delivered dose of a chemotherapeutic to hypoxic areas of tumor may be much less than to more oxygenated areas, thus sparing tumor cells that exist away from the blood vessels [Siemann, 2002; Wouters et al., 2002]. Hypoxia has also been shown to promote tumor metastases by inducing expression of proteins involved in the metastatic cascade and by providing selection of a more aggressive phenotype [Siemann, 2002; Wouters et al., 2002]. For example, Graeber et al. [1996] demonstrated that hypoxic tumor cell populations with a high apoptotic index expressed the wild-type tumor suppressor *p53* gene, whereas hypoxic tumor cells with a low apoptotic index (i.e., the more aggressive phenotype) had acquired a mutant *p53* gene. Tumor hypoxia has also been shown to be an independent prognostic indicator of poor outcome in prostate, cervical, and head and neck cancers [Wouters et al., 2002].

While the above qualities of tumor vasculature may be viewed as a hindrance to conventional therapies, the abnormal network and altered cellular phenotype of tumor vasculature provides a unique target for the development of novel antitumor agents. As first suggested by Folkman [1971], attacking the vascular network of tumor cells is an alternative mechanism for the treatment of solid tumors. Angiogenesis, or the formation of new blood vessels from established vessels, is a complex tightly-controlled process that normally occurs in adults only under specific conditions such as wound healing, inflammation, and the development of the corpus luteum in the menstrual cycle [Varner and Cheresh, 1996; van Hinsbergh et al., 1999; Burke and DeNardo, 2001]. In a much less regulated manner, angiogenesis can also occur in certain pathological conditions such as rheumatoid arthritis, psoriasis, diabetic retinopathy, and in the growth of solid tumors [Varner and Cheresh, 1996; Burke and DeNardo, 2001].

It has been established that a tumor 1–2 mm in diameter requires the development of new vasculature to provide oxygen and nutrient support for its continued growth [Folkman, 1999; Anderson et al., 2003], while smaller tumors receive adequate oxygen and nutrients from established capillaries [Remick, 2002]. Anigiogenesis is the result of tumor cell release of a variety of "proangiogenic" factors such as plasminogen activator, metalloproteinases (MMPs), growth factors (e.g., vascular endothelial growth factor (VEGF)), and adhesion molecules, all which stimulate endothelial cell proliferation and migration to form new blood

vessels [Folkman, 1999; Liekens et al., 2001; Anderson et al., 2003]. Folkman proposed that if the development of this vascular supply could be prevented, then all the tumor cells supported by these new vessels could also be inhibited [Folkman, 1971; Burke and DeNardo, 2001]. Increasing knowledge of the molecular mechanisms of angiogenesis has stimulated the development of a variety of antiangiogenic agents to target tumor growth at each step of the process in blood vessel formation. There are multiple preclinical and clinical trials currently ongoing. Results from these studies have suggested that the real power of antiangiogenic therapy may not be as single agents, but rather in combination with radiation therapy, conventional chemotherapy, or other tumor targeting modalities [Burke and DeNardo, 2001].

Although vascular disrupting agents (VDAs), formerly known as vascular targeting agents, have often been grouped with the antiangiogenic agents, the rationale behind this therapy is quite different. VDAs, in contrast to antiangiogenic compounds, are cytotoxic rather than cytostatic [Gaya and Rustin, 2005]. Rather than inhibiting the development of new blood vessels, these agents target the already formed neo-vasculature of actively growing tumors, producing a rapid shutdown of tumor blood vessels. The resulting ischemia can lead to extensive tumor cell death [Anderson et al., 2003]. This concept was first formally proposed by Denekamp in the early 1980s when he demonstrated that physical occlusion of blood supply to tumors implanted in rodents resulted in tumor regression [Denekamp, 1990, 1993; Thorpe et al., 2003]. Since then, there have been numerous studies that suggest these agents may have a unique role in the treatment of solid tumors [Thorpe et al., 2003]. Recently, however, there has been increasing evidence that antiangiogenic compounds may also have a direct effect on existing blood vessels, overlapping with the actions of VDAs. Likewise, VDAs may also be considered as having an antiangiogenic effect, suggesting an even more complex picture than previously thought [Gaya and Rustin, 2005]. This review will focus on VDAs and their development.

VASCULAR DISRUPTING AGENTS (VDAs)

Evidence that the vascular network of a tumor cell plays an important role in tumor growth is not new. In the 18th century, it was observed that some cancer patients who experienced a high fever subsequently had remission of their disease. The later discovery of bacteria by Pasteur provided an opportunity to explore this phenomena. In 1890, the surgeon William Coley administered both live bacterial cultures and sterilized culture supernatants, known later as "Coley's toxins," to patients with inoperable sarcoma. This treatment yielded a cure rate of approximately 10%, which was uncommon during this time period [Starnes, 1994; Baguley and Ching, 2002]. It was not until years later, however, that the mechanisms of these responses were understood. Further research in animals led to the discovery of bacterial cell wall components, exotoxins, and endotoxins, which inhibited blood flow and induced tumor necrosis [MacPherson and North, 1986; Baguley and Ching, 2002]. It was later found that tumor necrosis was not caused by the bacterial components themselves but by the induced host inflammatory cytokine—tumor necrosis factor alpha (TNFα) [Carswell et al., 1975]. These findings suggested the mechanism responsible for the results of Coley's studies. Since then TNFα has been shown in many studies to have antivascular effects in tumors, however clinical trials of TNFα had to wait until the development of recombinant technologies [Carswell et al., 1975; Siemann, 2002]. Unfortunately, clinical trials using recombinant human TNF led to serious side effects when used systemically [Lienard et al., 1992]. More recent studies, however, using isolated limb perfusion and intra-arterial infusion techniques using TNF-based treatment of melanoma and sarcoma have been more successful [Lienard et al., 1992, 1994].

Other studies also support the idea that interruption of blood flow to the tumor causes tissue death. In the early 19th century, it was reported that some solid tumors would regress when their circulation was interrupted either by torsion of the vasculature or by thrombosis of a major feeding vessel [Walsh, 1844; Chaplin and Hill, 2002]. In 1923, after reviewing the evidence on tumor regression, Woglum suggested that damaging the vasculature of a tumor might result in eradication of the tumor [Woglum, 1923; Chaplin and Hill, 2002; Siemann et al., 2002]. However, despite this long interest in the role of tumor vasculature, it has only been recently that there have been

significant strides in the development of agents that target this mechanism.

The current approaches to vascular disruption can be divided into two broad categories; those using biological agents and those using small molecules. Both types of agents produce the same characteristic pattern of widespread tumor necrosis after administration to rodents with solid tumors [Huang et al., 1997; Ching et al., 1999; Blakey et al., 2002a,b; Thorpe et al., 2003]. This necrosis can extend to as much as 95% of the tumor, but a thin rim of viable tumor cells usually survive at the tumor periphery [Huang et al., 1997; Ching et al., 1999; Blakey et al., 2002a,b; Thorpe et al., 2003]. It is thought that these viable cells are obtaining nutrients from unaffected blood vessels supplying normal tissues. A combination of these agents with other modes of treatment that attack this outer rim of cells have been curative [see below, Pedley et al., 1996, 2001, 2002; Siemann and Rojiani, 2002; Thorpe et al., 2003].

LIGAND-DIRECTED VDAs

Biological approaches use gene therapy, antibodies, and peptides to target the tumor endothelium directly. There have been a number of molecules which are upregulated on tumor endothelial cells [Thorpe et al., 2003]. Examples include VEGF receptor (VEGFR), fibronectin ED-B domain, and $\alpha\beta_3$ integrin [Siemann, 2002; Thorpe et al., 2003]. Cell adhesion molecules, such as E-selectin and vascular cell adhesion molecule-1 (VCAM-1) induced by inflammatory mediators are also upregulated in tumors [Thorpe et al., 2003]. Finally, molecules associated with prothrombotic changes occurring on vascular endothelial cells, such as phosphatidylserine, are also increased in tumors [Thorpe et al., 2003]. Targeting any of these molecules could potentially result in a number of scenarios that would result in tumor necrosis. Induction of thrombosis of tumor blood vessels directly, induction of a host driven attack of these tumor vessels, or induction of endothelial shape change which physically blocks tumor vessels are all potential mechanisms which could lead to tumor demise [Thorpe et al., 2003].

Tissue factor (TF), a cell membrane receptor protein normally induced by tissue damage, is an initiator of the extrinsic pathway of the blood coagulation cascade [Hu et al., 2003]. Several groups have examined the ability of the ligand binding domain of this molecule to induce changes in the tumor vasculature once fused to an antibody which targets the tumor endothelium (see Table I). Targeting was achieved with

TABLE I. Summary of Vascular Disrupting Agents in Development*

Compound	Mechanism	Stages of development
Biological VDAs		
Antitissue factor (TF)	Pro-coagulant	Preclinical
Antivascular cell adhesion molecule-1 (VCAM-1)-TF	Pro-coagulant	Preclinical
L19 single chain Fv (scFv) -TF	Pro-coagulant	Preclinical
chTNT-3/tTF	Pro-coagulant	Preclinical
chTV-1/tTF	Pro-coagulant	Preclinical
RGD/tTF	Pro-coagulant	Preclinical
Vascular endothelial growth factor receptor-gelonin	Toxin/antiangiogenesis/vascular remodeling inhibition	Preclinical
Antiendoglin linked to ricin A	Toxin/antiangiogenesis/vascular remodeling inhibition	Preclinical
Anti-TES-23 linked to neocarzinostatin	Toxin/antiangiogenesis/vascular remodeling inhibition	Preclinical
L19 scFV- interleukin 12 (IL-12)	Antibody–cytokine	Preclinical
L19 scFv- TNF-α	Antibody–cytokine	Preclinical
Antiphosphatidylserine (PS)	Naked antibody	Preclinical
Anti-prostate specific membrane antigen	Naked antibody	Phase I
Targeted ATP-Raf gene	Gene therapy	Preclinical
DNA encoding Flk-1 fused to Fas	Gene therapy	Preclinical
Small Molecules VDAs		
CA4P (combretastin A4 phosphate)	Prodrug of CA4	Phase I
DMXAA	Flavinoid	Phase I
ZD6126	Prodrug of N-acetylcolchinol	Phase I
AVE8062A	Combretastin analog	Phase I
Oxi4503 (combretastin A1 phosphate)	Prodrug of CA1	Phase I
ADH-1	Pentapeptide to N-cadherin	Phase I
TZT-1027	Synthetic derivative of dolastatin 10	Preclinical

*Adapted from Thorpe PE, Chaplin DJ, and DC Blakey. Cancer Research 2003; 63: 1141–114 and Gaya, AM and GJS Rustin. Clinical Oncology 2005;17:277–290.

monoclonal antibodies directed against VCAM-1, fibronectin, and MHC class II [Thorpe et al., 2003]. All of these agents were able to induce thrombosis and tumor necrosis in animal models by 72 h [Thorpe et al., 2003]. A study by Hu et al. [2003] examined the action of TF fused to other potential targeting molecules. Three fusion proteins were constructed: chTNT-3/tTF, which targets DNA exposed in degenerative areas of tumors; chTV-1/tTF, which targets fibronectin on the tumor vascular basement membrane; and RGD/tTF, which targets endothelial $\alpha_v\beta_3$ and $\alpha_v\beta_5$ intergrins on tumor vessels. All three agents induced evidence of regional thrombosis and massive necrosis in mice bearing established MAD109 lung and Colon 26 carcinomas [Hu et al., 2003]. Interestingly, chTNT-3/tTF induced clotting in the larger tumor blood vessels whereas chTV-1/tTF induced thrombosis in small and medium vessels of the tumors. Thrombosis caused by RGD/tTF mainly occurred in capillaries and small blood vessels. Furthermore, the most widespread necrosis was seen with those molecules, which caused thrombosis in large and medium vessels [Hu et al., 2003].

There have been several reported studies on the selective targeting of toxins and other cytotoxic agents to tumor vasculature [Siemann, 2002; Veenendaal et al., 2002; Thorpe et al., 2003]. Veenendaal et al. [2002] generated a fusion protein containing VEGF linked to the recombinant plant toxin gelonin. This fusion protein was highly cytotoxic to endothelial cells overexpressing the KDR/Flk-1 receptor, the target of VEGF [Veenendaal et al., 2002]. Human prostate (PC-3) xenografts treated with this fusion protein demonstrated significant damage to the vascular endothelium, vessel thrombosis, extravasation of red blood cell components into the tumor interstitium, and marked tumor regression [Veenendaal et al., 2002]. Significant antitumor effects have also been described for the chemical conjugate of the plant toxin ricin A chain linked to monoclonal antibodies directed to mouse endoglin [Seon et al., 2001]; the cytotoxic molecule neocarzinostatin attached to TES-23, a monoclonal antibody directed against a CD44-related tumor endothelial cell marker [Tsunoda et al., 1999]; and an immunotoxin directed against the VEGF-receptor complex [Thorpe et al., 2003].

While cytokines have demonstrated marked antitumor activity, it is often achieved at the expense of great toxicity. If these cytokines could be specifically targeted to the tumor, toxicity may be decreased. Halin et al. [2003] constructed fusion proteins of interleukin-12 (IL-12) and TNF-α with L19, an antibody fragment specific to the β domain of fibronectin that has been shown to target tumors. This group also constructed a triple fusion protein between IL-12, L19, and TNFα. Results from these studies demonstrate all were active in vitro. Both L19-IL-12 and L19-TNF-α displayed potent antitumor activity separately in vivo. However, neither led to complete eradication of the tumors. The triple protein did not localize to tumors in vivo and had no significant therapeutic effect [Halin et al., 2003]. Most importantly, the combination of both L19-IL-12 and L19-TNF-α displayed synergistic antitumor activity with complete eradication of F9 teratocarcinoma xenografts in mice [Halin et al., 2003].

Direct antibody targeting to a unique antigen associated with the tumor vasculature is also being investigated. An interesting illustration of this is the recent studies of the phospholipid phosphatidylserine (PS). Under normal conditions, PS resides almost exclusively in the inner leaflet of the plasma membrane [Ran and Thorpe, 2002]. This asymmetry can be lost under different physiological and pathological conditions [Ran and Thorpe, 2002]. PS becomes exposed on the surface of tumor endothelium, probably due to exposure to inflammatory cytokines, acidity, hypoxia, and thrombin [Thorpe et al., 2003]. Anti-PS treatment of mice-bearing tumors resulted in tumor suppression and evidence of damage to the tumor vasculature [Ran and Thorpe, 2002; Thorpe et al., 2003]. This antibody also enhances the efficacy of docetaxel against human breast carcinoma cell lines [Huang et al., 2004]. Other antibody-conjugates to phosphatidylethanolamine to target tumor blood vessels are also in development [Gaya and Rustin, 2005].

Another example of a unique tumor associated antigen is prostate specific membrane antigen (PSMA). PSMA is a nonsecreted transmembrane glycoprotein, which is highly expressed by prostate epithelial cells [Siemann, 2002]. More recent immunohistochemical studies, however, demonstrate that PSMA is also expressed by vascular endothelial cells of a variety of solid tumors, but not by normal vascular endothelium or in neoplastic epithelial

cells of non-prostate malignancies [Chang et al., 1999; Chang and Heston, 2002; Milowsky et al., 2002, 2003]. In 2002, Milowsky and colleagues reported on the use of huJ591, an anti-PSMA monoclonal antibody in a small phase I study in patients with refractory solid tumors whose tumor types are known to express PSMA on their neovasculature. Nine patients were treated with either 5 mg (n = 3) or 10 mg (n = 6) of ^{111}indium-labeled huJ591 on a once every 14-day schedule. Indium scanning showed localization of the antibody in 67% of the patients (six of nine patients) [Milowsky et al., 2002]. No objective responses were obtain in this study, however a colon cancer patient did have a 50% decline in carcinoembryonic antigen (CEA) and two patients reported improvement in cancer pain and performance status [Milowsky et al., 2002]. Toxicities reported were infusion related including one patient with grade 3 bronchospasms. Based on these results, the protocol was converted to a weekly times six schedule at 10, 20, 40, and 80 mg/week dose levels. This was followed by a 2-week break with an option for retreatment via 8-week cycles if patients had stable or responding disease [Milowsky et al., 2002]. At the time of the last reporting [2002], six patients had been enrolled in this phase of the study [Milowsky et al., 2002].

Vascular-targeted gene therapies that result in selective apoptosis of tumor endothelial cells are also being studied. Hood et al. [2002] demonstrated that a cationic nanoparticle (NP) coupled to the targeting ligand $\alpha\beta_3$ integrin can deliver genes selectively to blood vessels in tumor bearing mice. Furthermore, he tested the therapeutic efficacy of this approach by generating NPs conjugated to the mutant *Raf*-gene. ATPmu-Raf blocks endothelial signaling and angiogenesis in response to growth factors [Hood et al., 2002; Thorpe et al., 2003]. Injection of this NP into tumor bearing mice resulted in apoptosis of the tumor-associated endothelium and sustained regression of both the primary and metastatic tumors [Hood et al., 2002; Thorpe et al., 2003]. Another study by Carpenito et al. [2002] demonstrated the successful development of adenoviral vectors to deliver Flk-1 (the receptor for VEGF) encoded DNA fused to the proapoptotic protein Fas to the endothelial cells [Carpenito et al., 2002; Thorpe et al., 2003]. When exposed to VEGF, these cells undergo rapid apoptosis [Carpenito et al., 2002]. The results from this study suggest that the delivery of this type of construct to tumor cells in vivo may activate apoptosis. Current studies are ongoing.

The above studies demonstrate many of the molecules currently being studied that target the tumor vasculature. To explore novel receptors on endothelial cells that may be critical in tumor cell proliferation, differentiation, or apoptosis, a variety of genetic screens are currently being devised. The above preclinical studies are very promising, however, and have demonstrated a number of potential mechanisms to be explored in the clinical setting. This is clearly an exciting time in the development of these agents!

SMALL MOLECULE VDAs

Drug-based VDAs have progressed the furthest in development and there are a number of agents currently in clinical trials. Rather than specifically localizing to the tumor endothelium, these agents take advantage of the pathophysiological differences between normal and tumor endothelium to induce selective occlusion of tumor vessels [Thorpe et al., 2003]. A beneficial effect of drug-based vascular disruption was seen as early as 1932 with the tubulin-binding agent colchicine when Dominici first reported oral administration of this agent resulted in the overall improvement of a select group of patients with cancer [Ludford, 1945]. Other studies in 1937 by Oughteson and colleagues demonstrated tumor regression with both intratumoral and subcutaneous injections of colchicine [Ludford, 1945; Thorpe et al., 2003]. When serial biopsies were taken in these studies, significant necrosis in the tumor samples was demonstrated [Ludford, 1945]. In 1939, Brucke and Heuber demonstrated a greater effect when combining intratumoral-injected colchicine and radiation therapy in the treatment of tumors compared to radiation therapy treatment alone [Ludford, 1945]. Finally, Seed and colleagues administered colchicine intramuscularly into solid tumors. Examination of these tumors yielded vascular effects and tumor necrosis. Treatment with colchicines, however, soon fell out of favor because of extreme toxicity [Ludford, 1945; Remick, 2002]. It was not until the late 1980s that the concept of drug-based VDAs resurfaced. Two classes of agents have been identified. The first class includes flavone acetic acid

(FAA) and its derivatives. The second class is comprised of the tubulin-binding agents.

FLAVONOIDS

FAA and LM985

As mentioned previously, it has been shown that TNF-α disrupts tumor vasculature, but its toxicity to normal tissue precludes its use as a systemic agent in humans [Carswell et al., 1975; Thorpe et al., 2003]. One mechanism to increase the antitumor selectivity of TNF-α is to induce its synthesis in tumors in situ [Siemann, 2002; Thorpe et al., 2003]. Flavone-8-acetic acid (FAA) and its ester, FAA ester (LM985), were the first agents reported demonstrating this activity [Thorpe et al., 2003]. FAA was initially synthesized as an anti-inflammatory drug [Atassi et al., 1985; Baguley et al., 1993]. The ester of FAA, NSC 293015 (LM985), emerged from a series of flavonoids from Lyonnaise Indutrielle Pharmaceutique (Lipha) that was found to have antitumor activity during screening at the National Cancer Insitute (NCI) [Corbett et al., 1986; Baguley et al., 1993; Bibby and Double, 1993]. FAA and LM985 were found to be quite cytotoxic both in vitro and in vivo in several murine models. These agents were later found to cause vascular disruption leading to tumor necrosis in mouse tumors indistinguishable from those induced by TNF [Baguley et al., 1993]. The induction of TNFα by FAA and other flavones is further supported by the ability of TNFα antibodies to inhibit FAA-mediated vascular collapse [Mahadevan et al., 1990]. FAA also induced natural killer cell activity and inhibited tumor blood flow [Baguley et al., 1993]. However, the in vivo studies also demonstrated the narrow plasma concentration threshold between activity and toxicity—activity was not observed until drug levels close to its maximum tolerated dose (MTD). Furthermore, these animal studies suggested that the duration of drug exposure is crucial in determining activity as well as side effects [Kerr et al., 1986].

On the basis of the early studies, LM985 was chosen for phase I clinical trial [Kerr et al., 1986]. This drug was administered IV to 26 patients on an every 21-day schedule with doses ranging from 10 mg/m^2 to 1,500 mg/m^2. Dose-limiting toxicity was identified as acute reversible hypotension, which occurred during drug transfusion. At higher doses, mild sedation was seen. No evidence of leukopenia, alopecia, hepatic toxicity, or renal toxicity was observed [Kerr et al., 1986]. Twenty patients were evaluable with only 1 patient with colorectal cancer having stable disease. No other responses were observed [Kerr et al., 1986]. Pharmacokinetics studies yielded the rapid degradation of LM985 to FAA, LM975. In the above animal studies, FAA was active but did not have the cardiovascular side effects seen in rats [Kerr et al., 1986]. From these studies, it was decided that FAA or LM975 would proceed to phase I studies [Kerr et al., 1987]. A phase I trial was performed in a total of 54 patients using multiple schedules of either 1-, 3-, and 6-h infusion on a weekly basis for a minimum of 3 weeks. Dose-limiting toxicity was seen, including uticarial rash, muscle aches, and flushing with 1 h infusion; hypotension, and flushing with the 3-h infusion; and severe watery diarrhea, hypotension, nausea, vomiting, and cholestatic jaundice with the 6-h infusion of FAA [Kerr et al., 1987]. Most disappointingly, no objective antitumor activity was seen with FAA [Kerr et al., 1987]. From the large amount of data to date on FAA and its ester derivative, the most likely explanations for the discrepancies in activity between mouse and human seems to be related to the differences in either the ability of the immune system to respond to FAA or the ability for only murine models to induce TNF-α upon exposure to FAA [Bibby and Double, 1993; Thorpe et al., 2003]. FAA was unable to induce TNF-α in human mononuclear cell lines in contrast to its activity in murine models [Galbraith et al., 2002]. The possibility that there is an inability of these agents to achieve equivalent doses in humans without significant toxicity has also been proposed [Galbraith et al., 2002].

DMXAA

Another more promising flavone derivative is 5,6-dimethylxanthenone-4 acetic acid (DMXAA; NSC 640488). DMXAA was synthesized in an analog development program and found to have even greater activity and a 12-fold higher dose potency than FAA [Rewcastle et al., 1991; Baguley and Ching, 2002]. It has been demonstrated to have antitumor activity in a wide variety of murine tumors and also induces extensive tumor necrosis of human tumor xenografts in nude mice [Pedley et al., 2001]. Like FAA and its ester, this agent appears to function indirectly through the induction of

various immune system modulators, such as nitric oxide (NO), serotonin, and TNFα [Baguley et al., 2003; Siemann, 2002]. In contrast to FAA and its ester, DMXAA appears to overcome the species difference and is able to induce TNFα in both murine and human tumor model systems [Ching et al., 1994; Philpott et al., 1997; Jameson et al., 2003]. DMXAA was also shown to increase the plasma concentration of serotonin and its principal metabolite, 5-HIAA [Baguley and Ching, 2002]. Earlier studies demonstrated that the addition of 5HT receptor antagonists such as cyporheptadine, prevented the induction of tumor necrosis by TNF [Manda et al., 1988]. Co-administration of cyporheptadine also delayed induction of tumor necrosis by DMXAA [Baguley et al., 1993]. These data suggest that there may be a cooperative effect between serotonin and TNF in the induction of tumor necrosis. As either serotonin or 5-HIAA can be easily measured in serum plasma, each may provide a potential surrogate marker for antivascular agents and their effects [Baguley and Ching, 2002]. Preliminary data suggest that the serotonin metabolite 5HIAA correlates with DMXAA induced blood flow changes seen by the administration of DMXAA in both mouse models and in early human clinical trials [Kelland et al., 2005]. NO is also upregulated in mice that were treated with DMXAA [Baguley and Ching, 2002]. This molecule appears to be linked to the actions of both serotonin and TNFα and may provide another potential marker for antivascular effects [Baguley and Ching, 2002].

Preclinical studies of DMXAA were typically characterized by a steep dose response curve with significant activity still occurring only at doses close to the MTD [Remick, 2002; Siemann, 2002]. Dose-limiting toxicity in mice was consistent with hypotension and was not strictly related to TNF production [Philpott et al., 1995]. While DMXAA proceeded into clinical trials because of its favorable profile, further studies in animal tumor models have demonstrated synergistic interactions between DMXAA and radiotherapy [Wilson et al., 1998], chemotherapy, particularly taxanes and cisplatin [Pruijn et al., 1997; Horsman et al., 1999; Wilson and Baguley, 2000; Siemann, 2002; Siim et al., 2003], bioreductive cytotoxic drugs [Cliffe et al., 1994; Lash et al., 1998], radioimmunotherapy [Pedley et al., 1996], antibody-directed enzyme prodrug therapy [ADEPT-Pedley et al., 1999], thalidomide [Cao et al., 1999], and immunotherapy [Kanwar et al., 2001]. These studies further support the introduction of DMXAA into clinical trials, either as a single agent or in combination with other treatments.

Clinical trials with DMXAA are ongoing. Results of a phase I trial were reported by Jameson et al. [2003]. Sixty-three patients received DMXAA as a 20-min infusion every 3 weeks with doses ranging from 6 mg/m^2 to 4,900 mg/m^2. DMXAA was well tolerated at lower doses with no drug-related myelosuppression. At doses of 4,900 mg/m^2, however, rapidly reversible dose-limiting toxicities of confusion, tremor, slurred speech, visual disturbances, anxiety, urinary incontinence, and possible left ventricular failure were observed [Jameson et al., 2003]. Furthermore, transient prolongation of the QT interval was observed in all 13 patients evaluated at 2,000 mg/m^2 and above. The MTD was established at 3,700 mg/m^2. A patient with metastatic cervical carcinoma achieved an unconfirmed partial response at 1,100 mg/m^2, but progressed after 8 cycles. No progression was observed in 22 patients at dose levels from 6 mg/m^2 to 3,700 mg/m^2 and this duration exceeded 12 weeks in 5 patients. A confirmation phase I study by McKeage et al. [2005] was performed using a crossover design with 15 patients allocated randomly to receive 6 sequential doses of DMXAA at doses between 300 mg/m^2 and 3,000 mg/m^2 given weekly as a 20-min infusion. This study yielded 1,200 mg/m^2 as the selected dose for phase II studies. In general, this agent was well-tolerated with transient dose-dependent QTc prolongation, increased blood pressure, and visual disturbances seen at the two highest levels [McKeage et al., 2005].

This group also reported the effects on tumor microcirculation as assessed by dynamic contrast-enhanced magnetic resonance imagining (DCE-MRI) [Galbraith et al., 2002; McKeage et al., 2005]. DCE-MRI is a radiological technique, which allows for the detection of blood flow in the tumor. This technique utilizes the basic principles of standard MRI with the addition of a contrast agent, often the paramagnetic metal gadolinium [Remick, 2002]. The addition of a contrast allows the delineation of blood and surrounding tissue [Remick, 2002]. Sixteen patients treated with DMXAA from 500 mg/m^2 to 4,900 mg/m^2 had DCE-MRI examinations before and after treatment [Galbraith et al.,

2002]. Tumor kinetic parameters [gradient, enhancement, and AUC (first 90 s of the curve)] that correlate to the absolute blood flow were measured. There was significant reduction in all parameters at both 24 and 1,032 h after treatment initiation [Galbraith et al., 2002]. Minimal reduction was observed at the 4-h time point [Galbraith et al., 2002]. In contrast, while there were some significant reductions of these parameters in normal muscle tissue, these reductions were not observed at the 1,032-h timepoint [Galbraith et al., 2002]. Together with preclinical results, these data suggest DMXAA is an effective agent for the reduction of tumor blood flow with minimal effect on normal tissue. To further explore the potential efficacy of DMXAA, combination studies with taxanes and platins are currently underway [McKeage et al., 2005].

TUBULIN-BINDING AGENTS

Historically, there have been reports of a number of tubulin-binding agents that destabilize the tubulin cytoskeleton leading to a disruption of the tumor vasculature. These agents include colchicine, vincristine, and vinblastine. These agents have been found to be antitumorigenic but only at doses close to their MTD [Thorpe et al., 2003]. Development of second-generation tubulin depolymerizing agents which disrupt the tumor vasculature at doses well below their MTD have allowed this mechanism to be reinvestigated [Thorpe et al., 2003]. In vitro experiments demonstrate that these new generation tubulin-binding agents induce cell shape changes in proliferating cells but not quiescent endothelium [Siemann, 2002]. It has been shown that the proliferative index of tumor endothelial cells may be 50- to 100-fold higher than in normal tissue, thereby enhancing tumor endothelial cells as the primary target [Siemann, 2002]. Also, as mentioned above, tumor endothelial cells rely on a cytoskeleton primarily of tubulin to maintain their shape, whereas mature endothelial cells rely on both tubulin and actin [Siemann, 2002]. The proposed mechanism of action of the tubulin-binding agent involves the agent entering the blood vessel and initiating the depolymerization of the tubulin cytoskeleton. This tubulin depolymerization causes endothelial cells to lose their shape, round up, and detach. These detached, irregularly shaped cells occlude the vessel and decrease blood flow. This causes the tumor cells farthest away from the vessel to become hypoxic and die. As the cytoskeleton continues to collapse, this process catapults and massive cell death occurs [Siemann, 2002]. However, a thin rim of viable cells usually survives, as these cells are receiving nutrients from unaffected vessels. These cells are often sufficient enough to repopulate the tumor, suggesting that multiple doses of the VDAs are required or that the best mode of action of VDAs would be in combination with other cytotoxic regimens. Several of the tubulin-binding agents and their actions will be discussed.

Combretastatin A4 Phosphate (CA4P)

CA4P is a water-soluble prodrug of combretastatin A4 (CA4) which was isolated from the root bark of the South African bush willow (*Combretum caffrum*) after it was learned that extracts of this tree had been used by the Zulus as herbal remedies and as paint for spears [El-Zayat et al., 1993; Dorr et al., 1996]. CA4P is converted to CA4 by nonspecific endogenous phosphatases that are present in plasma and on endothelial cells [Rustin et al., 2003]. It has a structure that is similar to colchicine and exerts its actions by binding to the colchicine-binding site on tubulin. Unlike colchicine and vinblastine, however, it has been shown that the endothelial cell shape change induced by CA4 can be reversed on drug removal of CA4P in vitro, suggesting less toxicity of this agent [Gaya and Rustin, 2005]. CA4P has a broad range of cytotoxicity against several tumor lines and is effective in mouse xenograft models [McGown and Fox, 1990; El-Zayat et al., 1993; Dorr et al., 1996; Horsman et al., 1998]. In vitro studies indicate that a short exposure of quiescent endothelial cells to CA4P resulted in long-term cytotoxic effects that become apparent when the cells are stimulated to proliferate [Dark et al., 1997]. In initial animal studies, it was demonstrated that CA4 induces immediate selective shutdown of the tumor vasculature and subsequent tumor necrosis through induction of endothelial cell apoptosis [Dark et al., 1997; Iyer et al., 1998; Malcontenti-Wilson et al., 2001]. The peak effect on tumor blood flow was seen at 6 h post treatment and could be sustained as long as 24 h, depending on the dose administered to these animals [Iyer et al., 1998; Malcontenti-Wilson et al., 2001]. In an ex vivo isolated tumor perfusion system, CA4P caused a

greater than threefold increase in tumor vascular resistance but no change in vascular resistance in a similarly isolated limb perfusion [Dark et al., 1997; Rustin et al., 2003]. In P22 carcinosarcomas in rats, a 100-fold decrease in blood flow rate was observed at 6 h with a much smaller reduction in blood flow in the spleen, skin, skeletal muscle, and brain. No changes in blood flow were observed in the heart, kidney, and small intestine [Tozer et al., 1999]. Additional in vivo studies using CaNT tumors in mice demonstrated that a single dose of 100 mg/kg caused 93% reduction in tumor vascular volume with massive hemorrhagic necrosis [Dark et al., 1997; Rustin et al., 2003]. As with the other VDAs, however, a rim of viable cells remained at the periphery and these cells were sufficient enough to repopulate the tumor, so that this single dose did not result in any significant growth delays [Chaplin et al., 1999]. Toxicity studies using mice, rats, and dogs were performed by Oxigene [Dowlati et al., 2002; Rustin et al., 2003]. Regimens of single dose, daily times 5 and weekly times 4 were investigated [Rustin et al., 2003]. The organ systems affected by CA4P were within the gastrointestinal tract, with only minor effects on the liver, bone marrow, or renal function. Transient weakness in the hind limbs and bradycardia was noted in the dogs at 25 mg/m^2 [Dowlati et al., 2002; Rustin et al., 2003]. The single dose MTD was 360 mg/m^2 in rats and 100 mg/m^2 for dogs [Malcontenti-Wilson et al., 2001; Dowlati et al., 2002; Rustin et al., 2003].

Further studies in animal tumor models have suggested that the most effective treatment utilizing CA4P may be in combination with other cytotoxic treatments. In a colorectal xenograft mouse model, the combination of CA4P with either cisplatin or radiation causes significant synergistic growth delay, tumor regression, and cell death with no tumor recurrence [Pedley et al., 2001; Chaplin and Hill, 2002]. This synergistic response of CA4P and cisplatin was also demonstrated in experimental rodent sarcoma (KHT sarcoma) as well as in breast (SKBR3) and ovarian (OW-1) xenografts [Siemann, 2002]. Interestingly, these results also emphasized the importance of the sequence of drug administration. The greatest effects were achieved when C4AP was administered 1–3 h after cisplatin [Siemann, 2002]. Studies have also demonstrated CA4P can enhance the effects of tumor response to hyperthermia [Horsman et al., 1998; Murata et al., 2002], radiation [Horsman et al., 1998], mild thermoradiotherapy [Murata et al., 2002] and radioimmunotherapy [Pedley et al., 2002]. An interesting study by Davis et al. [2002] described a combination treatment of CA4P with a series of NO inhibitors. CA4P was active against a low-NO-producing tumor (Cant) but showed minimal activity against a high-NO-producing tumor in mice. The addition of NO inhibitors to the treatment regimen resulted in enhanced activity in the Cant tumor and made Sass sensitive to the action of CA4P [Davis et al., 2002]. From these results, it appears that NO may protect the tumors from the action of CA4P, perhaps by inducing vasorelaxation or by effecting cytochrome c oxidase leading to increase survival under conditions of low oxygen [Davis et al., 2002; Thorpe et al., 2003].

The first phase I clinical trial of CA4P was reported by Dowlati et al. [2002]. Twenty-five patients with advanced solid tumor malignancies were given CA4P in escalating doses of 18–90 mg/m^2, initially as a 10-min and subsequently as a 60-min infusion at 3-week intervals [Dowlati et al., 2002]. A main side effect was tumor pain, which occurred in 10% of the cycles given. Tumor pain resulted in four episodes of DLT at dosages $>$60 mg/m^2, including two episodes of acute coronary syndrome. This pain was reported 2–5 h post treatment and responded to narcotic therapy. Other reported grade 3 or greater toxicities include pulmonary toxicity, cardiac ischemia, and nausea. There was no significant myelotoxicity, stomatitis, or alopecia. A significant decrease in tumor blood flow by DCE-MRI was observed in six of seven patients treated with 60 mg/m^2. There was also a significant increase in plasma levels of soluble intercellular adhesion molecule (ICAM) at both 1 and 24 h after treatment [Dowlati et al., 2002]. Increasing levels of this agent may indicate vascular injury [Remick, 2002]. Pharmacokinetics revealed rapid dephosphorylation of CA4P to CA4 with a short plasma half-life of approximately 30 min. There was one complete response in a patient with refractory metastatic anaplastic thyroid cancer (receiving 60 mg/m^2 via the 10-min infusion protocol) who remained disease-free for greater than 30 months [Dowlati et al., 2002]; Remick, 2002]. Two patients, one with colon cancer, one with medullary thyroid cancer, experienced stable disease for over 19 and 12 months, respectively

[Dowlati et al., 2002]. These patients received multiple doses in a decreasing schedule 90–50 mg/m^2 and 60–50 mg/m^2, respectively. These dose reductions were done for safety reasons due to the cardiac side effects encountered in other patients. One patient with non-small cell lung cancer receiving 36 mg/m^2 had a transient 34% reduction in measured disease after two cycles of therapy, but progressed after four cycles of therapy. Finally, a patient with metastatic renal cancer received eight cycles of 18 mg/m^2 and had stable disease for 6 months [Dowlati et al., 2002].

Another phase I trial of weekly CA4P by Rustin et al. [2003] was reported. Thirty-four patients received 10-min weekly infusion of CA4P for 3 weeks followed by a week gap, with intrapatient dose escalation. The starting dose was 5 mg/m^2 and dose escalation was accomplished by doubling until grade 2 toxicities were reported (dose ranges were 5–114 mg/m^2) [Rustin et al., 2003]. As in the previous phase I study described, CA4P was rapidly converted to CA4, which was further metabolized to the glucoronide [Rustin et al., 2003]. The only toxicity reported related to drug dose up to 40 mg/m^2 was tumor pain. DLT was reversible ataxia at 114 mg/m^2, vasovagal syncope and motor neuropathy at 88 mg/m^2, and fatal ischemia in previously irradiated bowel at 52 mg/m^2. Other drug-related grade 2 or higher toxicities seen in more than one patient were pain, lymphopenia, fatigue, anemia, diarrhea, hypertension, hypotension, vomiting, visual disturbances, and dyspnea [Rustin et al., 2003]. In this study, there were no complete or partial responses. One patient with liver metastases from adrenocortical carcinoma had short-lived improvement of her metastases but then progressed [Rustin et al., 2003]. In a complementary study reported by this group, Galbraith and colleagues examined the blood flow by DCE-MRI on a subset of the above-described patients (18 patients). Significant reductions were seen in the K^{trans} constant, a parameter indirectly relating to blood flow, in 6 of 16 patients at 4 and 24 h after treatment with CA4P. No significant changes in reference organs were observed [Galbraith et al., 2003]. This was further supported by a third study from Rustin et al. [2003] using this same patient cohort. Positron emission tomography (PET) was used to measure the effects of CA4P on tumor and normal tissue perfusion and blood volume [Anderson et al., 2003]. Tissue perfusion was measured using oxygen-15 (^{15}O)-labeled water and blood volume was measured using ^{15}O-labeled carbon monoxide. Scans were performed immediately before, 30 min and 24 h after treatment with CA4P infusion at a doses ranging from 5 mg/m^2 to 114 mg/m^2. PET data was obtained on 13 patients. Significant dose-dependent reductions were seen in tumor perfusion and tumor blood volume 30 min after CA4P administration. By 24 h, however, there was tumor vascular recovery. Borderline significant changes in perfusion and blood flow was observed in both the spleen and kidney at 30 min with no significant changes observed at 24 h [Anderson et al., 2003].

A trial performed at the University of Pennsylvania administered 10-min infusions of CA4P daily for 5 days every 3 weeks [Stevenson et al., 2003]. Thirty-seven patients received 133 cycles with doses ranging from 6 mg/m^2 to 75 mg/m^2, with the recommended phase II dose determined to be 52 mg/m^2 [Stevenson et al., 2003]. DLTs were tumor pain at 75 mg/m^2, reversible sensorimotor neuropathy, syncope, and dyspnea. A partial response was observed in a patient with metastatic sarcoma. DCE-MRI analysis of tumor blood flow was similar to previously reported studies. These data demonstrate that CA4P has an effect on tumor vasculature and blood flow at doses that were tolerated by patients and provide evidence to base further clinical trials on.

ZD6126

ZD6126 is a phosphate prodrug of the tubulin-binding agent ZD6126 phenol (N-acetylcolchinol), an inhibitor of microtubule polymerization [Blakey et al., 2002a]. It was developed for its tubulin-binding properties and its ability to induce vascular damage in tumors [Blakey et al., 2002b]. In vitro, at noncytotoxic doses, ZD6126 rapidly (<40 min) disrupts the tubulin cytoskeleton resulting in pronounced changes in cell morphology in proliferating endothelial cells but not in quiescent cells [Blakey et al., 2002a,b]. In vivo intravenous infusion of 20 mg/kg of ZD6126 in rats was rapidly broken down to ZD6126 phenol which has a half-life of approximately 1 h [Blakely et al., 2002a] Peak plasma levels were well above the concentrations required to induce changes in the endothelial cell in vitro. [Blakey et al., 2002a]. Extensive tumor necrosis was observed in the rodent

Hras5 tumor model at doses of either 20 mg/kg or 50 mg/kg. ZD6126 was well tolerated if given as an IV bolus but had increased toxicity if the same dose was given as a 24-h infusion via a subcutaneous mini pump [Blakey et al., 2002a]. When a range of normal tissue was examined 4 days after bolus dose of 50 mg/kg of ZD6126, the only significant toxicities observed were bone marrow atrophy and osteocyte necrosis [Blakey et al., 2002a]. In contrast, the 24-h infusion of ZD6126 was not tolerated at 50 mg/kg. The 24-h infusion of 20 mg/kg of ZD6126 resulted in significant bone marrow atrophy, hepatocyte degeneration, duodenal atrophy, and myocardial degeneration [Blakey et al., 2002a]. In another report, Blakey et al. [2002b] examined the effects of ZD6126 in a variety of mouse xenografts, including human lung (Calu-6), colorectal (LoVo and HT-29), prostate (PC-3), ovarian (SKOV-3), and breast (MDA-MB-231) tumors. ZD6126 was shown to cause rapid effects on tumor endothelium leading to cell retraction, exposure of the basal lamina, and subsequent loss of endothelial cells [Blakey et al., 2002b]. This led to thrombosis and vessel occlusion, resulting in tumor necrosis 24 h after administration of ZD6126 [Blakey et al., 2002a]. These responses were seen with concentrations well below the MTD [Blakey et al., 2002a]. A single dose of 200 mg/kg of ZD6126 resulted in significant growth inhibition in Calu-6 and LoVo tumors [Blakey et al., 2002a]. This inhibition was increased when a lower dose (100 mg/kg) was given in five daily doses. Histological analysis of these tumors, however, yielded the same pattern of viable tumor cells surrounding the periphery that subsequently resulted in tumor regrowth [Blakey et al., 2002a].

Combining ZD6126 with cisplatin, taxol, or radiation to eliminate the viable tumor rim has been shown to enhance the activity of ZD6126 [Blakey et al., 2002b]. In the above described Calu-6 xenograft studies, the combination of ZD6126 with cisplatin resulted in tumor regression and prolonged growth delay compared to results obtained with either agent alone [Blakey et al., 2002b]. In another set of studies, Siemann et al. [2002] demonstrated an enhanced antitumor activity of ZD6126 when combined with cisplatin in experimental rodent (KHT sarcoma) and human renal (Caki-1) tumor models. These experiments further demonstrate more potent activity of these combined agents if the VDA (ZD6126) is administered within a few hours of the chemotherapeutic drug (cisplatin) [Siemann et al., 2002]. Further studies by Siemann et al. [2002] demonstrate increased antitumor efficacy and enhanced radiation response in large, advanced tumors in both rodent and human tumor models when ZD6126 was injected intraperitoneally into animals bearing tumors larger than 1.0 g [Siemann et al., 2002, 2005]. Other studies demonstrate an additive antitumor effect when ZD6126 is combined with the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 (Iressa) in the A549 human non-small cell lung cancer xenograft model [Raben et al., 2004]. The addition of both of these agents also potentiated the antitumor effects of radiotherapy in this animal model [Raben et al., 2004].

The preliminary results of several clinical trials with ZD6126 have been reported [DelProposto et al., 2002; Gadgeel et al., 2002; Radema et al., 2002; Evelhoch et al., 2004]. Gadgeel et al. [2002] reported preliminary results of 27 patients treated with escalating doses of ZD6126 (5–112 mg/m^2). ZD6126 was administered as a 10-min single dose IV infusion every 21 days until disease progression of disease or withdrawal [Gadgeel et al., 2002]. Adverse events noted in greater than 10% of the patients were pain (n = 14), anorexia (n = 3), constipation (n = 6), dyspnea (n = 5), fatigue (n = 5), headache (n = 3), nausea (n = 4), and vomiting. One patient had asymptomatic, reversible grade 2 ischemic changes on EKG and grade 3 elevation in troponin I with subsequent demonstration of coronary artery disease [Gadgeel et al., 2002]. There was no apparent relationship between the incidence of these effects and dose of ZD6126 [Gadgeel et al., 2002]. In this study, ZD6126 was quickly converted to the ZD6126-phenol, which had a $t_{1/2}$ of 2–3 h [Gadgeel et al., 2002].

Radema et al. [2002] reported preliminary data from an ongoing Phase I dose escalation study in which ZD6126 was given as a 10 min IV infusion weekly. The administered dose escalated from 5 mg/m^2 to 7 mg/m^2 weekly for 4 weeks. Twelve patients with refractory solid tumors were enrolled. Grade 2 or greater adverse events included hypokalemia (n = 8) and a transient, reversible decrease in ejection fraction (n = 2). Two patients with active brain metastases not suspected at trial entry developed symptoms of increased intracranial pressure (somnolence, nausea, vomiting, and dizziness) shortly after infusion [Radema et al., 2002].

In terms of efficacy, Radema et al. [2002] reported vascular damage in four of five patients 4–6 h after infusion of ZD6126, as indicated by the twofold elevation of circulating tumor cells used as a surrogate marker [Remick, 2002]. Using the same patient cohort as reported by Gadgeel, DelProposto et al. [2002] used DCE-MRI to delineate blood flow in six patients before and 6 h, 24 h, and 18–21 days after single doses of ZD6126 (56–112 mg/m^2) at 3-week intervals. Overall tumor blood flow decreased by 16–87% 6 h post treatment compared with baseline in five of six patients [DelProposto et al., 2002]. Decreases in blood flow were more apparent toward the core of the tumor. Within 21 days, tumor blood flow had returned to baseline in all but two patients (treated at 56 mg/m^2 and 80 mg/m^2, respectively) [DelProposto et al., 2002]. Evelhoch et al. [2004] reported updated results from this study in 2004. DCE-MRI was performed on nine patients 24–72 h before and 6 h after ZD6126 administration. Patients received single a single IV dose of 56–112 mg/m^2 of ZD6126 [Evelhoch et al., 2004]. At doses of 80 mg/m^2 and higher, IAUC (the initial area under the gadolinium diethylenetraiminepentaacetate uptake versus time curve—a value which reflects blood flow, vascular permeability, and the fraction of interstitial space) was reduced in all tumors studied by 36–72% [Evelhoch et al., 2004]. There was a significant trend of increasing reduction with increasing exposure to drug [Evelhoch et al., 2004].

Other VDAs

AVE8062A is a water-soluble, synthetic derivative of combretastatin A4 [Ohsumi et al., 1999; Remick, 2002]. This molecule inhibits microtubule polymerization and elicits potent cytotoxic activity in both endothelial and tumor cells in vitro at IC_{50} values of approximately 30 nM [Tolcher et al., 2003]. This agent induced necrosis within 24 h of treatment in 35 of 40 mouse tumor models when given by IV injection (60–80 mg/kg) [Lejeune et al., 2002]. The MX-1 human mammary adenocarcinoma model was used to examine the response rate of AVE8062A with a variety of doses and schedules. Both partial and complete responses were observed [Lejeune et al., 2002]. As with the other tubulin-binding agents, AVE8062A appears to have the most effect against large tumors (i.e., >500 mg at the start of therapy) [Lejeune et al., 2002; Remick, 2002]. In the dog, AVE8062 is rapidly converted to its active metabolite RPR258063, which at high doses can induce myocardial necrosis/fibrosis at concentrations equivalent to 865 ng/ml in man [Sessa et al., 2005]. Based on the above studies, this agent entered clinical trials. A phase I study was reported administering AVE8062A as a 30-min IV infusion once weekly for 3 weeks every 28 days [Tolcher et al., 2003]. An accelerated dose scheme was used with single patient dose levels. At the time of reporting, nine patients had received doses of 4.5–30 mg/m^2 [Tolcher et al., 2003]. Asymptomatic systolic hypotension at highest dose range was reported. This hypotension was without CPK, troponin I, or ECG changes [Tolcher et al., 2003]. Preliminary evidence of decreased blood flow by DCE-MRI was observed at the 15.5 mg/m^2 dose level. Other administration levels have recently been reported, along with an update of the above trial [Sessa et al., 2005]. Doses of AVE8062A were given in the following schedules: every 21 days, daily times five, and weekly [Sessa et al., 2005]. In the daily times, five and weekly administrations, the occurrence of four potentially drug-related vascular events (myocardial ischemia, transient asymptomatic hypotension, transient cerebral ischemia, and asymptomatic ventricular tachycardia) led to a voluntary interruption of all trials. In one patient who experienced myocardial ischemia, the C_{max} of RPR258063 was the highest determined and close to the free drug level at which cardiac damage was observed in dog [Sessa et al., 2005]. No vascular event was observed in the single 30-min infusion given IV every 21 days dosing up to a 22.5 mg/m^2 dose, therefore this trial was resumed after tightening eligibility criteria and increasing cardiovascular monitoring [Sessa et al., 2005]. Patients were monitored with continuous 24 h ECG Holter, Holter continual ambulatory blood pressure monitoring, serial CPK levels, troponin levels, ECG, ventriculographies, and echocardiograms. DCE-MRIs were performed for select patients pre treatment and 4 h post infusion. Twenty-three patients were evaluated at dose levels from 6 mg/m^2 to 22 mg/m^2. No DLTs were reported [Sessa et al., 2005]. Grade I diarrhea, rash, and nausea were reported in three patients. No significant modifications of the vascular parameters have been reported [Sessa et al., 2005]. The RPR258063 C_{max} values did not exceed 300 ng/ml. This study is currently ongoing.

ABT-751 is an oral antimitotic agent that binds to the colchicine site of β-tubulin [Hande et al., 2003]. It has demonstrated antitumor activity in human xenograft models, including colon, lung, breast, gastric, and nasopharyngeal cancers [Hande et al., 2003]. In the rodent 9-L glioma tumor model, ABT-751 (30 mg/kg) produced a 30% decrease in tumor blood flow 1-h post infusion using DCE-MRI [Luo et al., 2002]. No effects were seen on mean arterial pressure or heart rate [Luo et al., 2002]. Pharmacokinetics and safety results from a Phase I trial of ABT-751 given daily 7× every 3 weeks were reported [Hande et al., 2003]. Doses were either once or twice daily. Dose-limiting toxicities of neuropathy (peripheral neuropathy and ileus) were seen in two of six subjects at a dose of 300 mg once daily. Maximum tolerated dose (MTD) for this study was 250 mg/day for the once daily schedule. The MTD of the twice daily scheduled dose has not yet been reported. Grade 2 toxicities observed included peripheral neuropathy, constipation, fatigue, myalgias, anemia, nausea, and vomiting. No myelosuppression was observed. Pharmacokinetic studies demonstrated that this agent is rapidly absorbed and eliminated with clearance via glucoronidation and sulfonation [Hande et al., 2003]. A second phase one study has been reported with ABT-751 in the treatment of patients with refractory hematological malignancies [Yee et al., 2005]. Thirty-two patients were treated daily for 7 days every 21 days with doses between 100 mg/m^2 and 150 mg/m^2 or orally daily for 21 days every 28 days with doses between 125 mg/m^2 and 200 mg/m^2. DLT consisted of ileus in one patient at 200 mg/m^2. One patient with relapsed acute myelogenous leukemia achieved a complete remission that was sustained for 2 months. Four other patients had transient hematological improvements. The recommended phase II dose in hematological malignancies was reported to be 175 mg/m^2 daily orally for 21 days every 4 weeks [Yee et al., 2005].

Exherin TM (ADH-1), a novel angiolytic vascular targeting agent, is a cyclic pentapeptide (CHAVC) which competitively inhibits N-cadherin-mediated endothelial cell, pericyte, and tumor cell binding [Jonker et al., 2004]. This molecule has been shown to cause highly-specific tumor angiolysis in multiple murine and xenograft models [Jonker et al., 2004]. The safety and pharmacokinetics from the first phase I trial has recently been reported [Jonker et al., 2005]. Thirty-three patients with refractory solid tumors received 54 cycles of ADH-1 by bolus injections at 8 dose escalations from 50 mg/m^2 to 840 mg/m^2. These patients were stratified by N-cadherin status of their tumors. MTD was not reached. Most common toxicities were nausea, fatigue, hot flushes, pain at sites of tumor, post infusion chest discomfort, and dysguesia. Mean half-life increases from 1.4 h below 300 mg/m^2 to 2.9 h at higher doses were observed. Three patients with N-Cad+/unknown showed antitumor activity. A patient with chemorefractory N-Cad + squamous cell carcinoma of the esophagus showed partial response sustained for six cycles of treatment [Jonker et al., 2005]. No activity has been demonstrated with N-Cad negative patients [Jonker et al., 2005]. This study is currently ongoing.

OXi4503 (Oxigene, Inc., Boston, MA) is a prodrug of combretastatin A1 (CA1). This agent shows comparable effects to CA4 in vitro, however it appears at least 10 times more potent in vivo when tumor vasculature shutdown is used as an endpoint [Hill et al., 2002]. Single-dose studies indicate the MTD in mice is similar to CA4P. Pre clinical studies have shown prolonged inhibition of tumor growth in some tumor models [Hill et al., 2002]. OXi4503 is currently in Phase I clinical trials.

Dolastatin 10 was first isolated from an Indian Ocean mollusk, *Dolabella, auricularia*, and is a tubulin-binding agent. This compound has been shown to have antitumor activity against a variety of murine tumors and human xenografts [Schoffski et al., 2004]. A synthetic derivative, TZT-1027, has been developed in Japan and has also been shown to have potent antitumor activity [Kobayashi et al., 1997]. The first clinical phase I study was initiated in 1994 in Japan and others are currently ongoing [Schoffski et al., 2004; deJonge et al., 2005]. The major toxicity appears to be neutropenia in most studies [Schoffski et al., 2004; deJonge et al., 2005]. Phase II studies are currently underway [deJonge et al., 2005].

CONCLUSION

Preclinical and clinical studies of a variety of agents have clearly established that the concept of vascular disruption has greatly advanced since the 1980s. The target remains either the

endothelial cells lining the tumor vessel or a protein product that has an effect on these endothelial cells. There has been thought that VDAs confer certain advantages over other chemotherapeutic agents. First, stopping blood flow in one vessel of a tumor has the potential to affect thousands of tumor cells dependent on that vessel for nutrients and waste removal. These agents also do not kill endothelial cells, but merely change their shape or initiate thrombus formation [Chaplin and Hill, 2002]. As the endothelial cells are adjacent to the blood stream, agents can easily access their target [Chaplin and Hill, 2002]. There is no need for infiltration of the agent into the tumor [Chaplin and Hill, 2002; Thorpe et al., 2003]. Endothelial cells are unlikely to become resistant to these agents as it is unusual for nontransformed cells to acquire genetic mutation. Furthermore, as these agents affect blood flow, one also has a surrogate marker of biological activity that can be measured in the clinic [Thorpe et al., 2003]. Finally, it has been demonstrated that greater than 99% of tumor cells can be killed in vivo during a 2-h period of ischemia [Chaplin and Hill, 2002]. This suggests that only temporary effects on tumor vasculature may be sufficient and these agents should be given on an intermittent basis concurrent with conventional therapies to enhance the effectiveness of existing and emerging cancer therapies [Thorpe et al., 2003].

Most studies described above demonstrated marked enhancement of antitumor activity when VDAs were administered within a few hours after the administration of conventional chemotherapy or radiotherapy. This may be due to a "trapping" of the chemotherapy agent in tumor tissue once the VDA has been administered—this remains to be seen. In contrast, it appears that VDAs may be less effective if administered before conventional chemotherapy agents. This may be due to the transient reduction in tumor blood flow making it less likely that cytotoxic agents are delivered to their target organs [Siemann et al., 2004]. Perhaps giving the conventional agent after the VDA allows greater delivery to the viable tumor rim because of the now limited tumor vasculature. Some have also hypothesized that there could be an increase in the normal vasculature to the viable rim. In the clinical setting, VDAs have exhibited the ability to produce significant reduction in tumor blood flow at acceptable doses. It will be important to see the efficacy of VDAs in combination with conventional cytotoxic approaches as VDAs advance to phase II trials.

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