

## Review

# Vascular Targeting Agents as Cancer Therapeutics

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### Abstract

Vascular targeting agents (VTAs) for the treatment of cancer are designed to cause a rapid and selective shutdown of the blood vessels of tumors. Unlike antiangiogenic drugs that inhibit the formation of new vessels, VTAs occlude the pre-existing blood vessels of tumors to cause tumor cell death from ischemia and extensive hemorrhagic necrosis. Tumor selectivity is conferred by differences in the pathophysiology of tumor *versus* normal tissue vessels (*e.g.*, increased proliferation and fragility, and up-regulated proteins). VTAs can kill indirectly the tumor cells that are resistant to conventional antiproliferative cancer therapies, *i.e.*, cells in areas distant from blood vessels where drug penetration is poor, and hypoxia can lead to radiation and drug resistance. VTAs are expected to show the greatest therapeutic benefit as part of combined modality regimens. Preclinical studies have shown VTA-induced enhancement of the effects of conventional chemotherapeutic agents, radiation, hyperthermia, radioimmunotherapy, and antiangiogenic agents. There are broadly two types of VTAs, small molecules and ligand-based, which are grouped together, because they both cause acute vascular shutdown in tumors leading to massive necrosis. The small molecules include the microtubulin destabilizing drugs, combretastatin A-4 disodium phosphate, ZD6126, AVE8062, and Oxi 4503, and the flavonoid, DMXAA. Ligand-based VTAs use antibodies, peptides, or growth factors that bind selectively to tumor *versus* normal vessels to target tumors with agents that occlude blood vessels. The ligand-based VTAs include fusion proteins (*e.g.*, vascular endothelial growth factor linked to the plant toxin gelonin), immunotoxins (*e.g.*, monoclonal antibodies to endoglin conjugated to ricin A), antibodies linked to cytokines, liposomally encapsulated drugs, and gene therapy approaches. Com-

bretastatin A-4 disodium phosphate, ZD6126, AVE8062, and DMXAA are undergoing clinical evaluation. Phase I monotherapy studies have shown that the agents are tolerated with some demonstration of single agent efficacy. Because efficacy is expected when the agents are used with conventional chemotherapeutic drugs or radiation, the results of Phase II combination studies are eagerly awaited.

### Introduction

The concepts behind vascular targeting agents (VTAs) as cancer therapeutics were described by Juliana Denekamp in the early 1980s (1, 2). The observation that the physical obstruction of the blood vessels of solid tumors led to tumor regressions in mice led to the proposal that VTAs might be created that pharmacologically cause occlusion of tumor vessels (1, 2). The proposal was later validated when it was shown that a toxin targeted by an antibody specific for tumor blood vessels caused tumor regressions in mice (3–5) and that antitubulin drugs have inherent VTA activity (6, 7). Destruction of the endothelium of solid tumors results in the death of tumor cells from lack of oxygen and nutrients (Fig. 1) leading to the occlusion of blood-transporting vessels as well as the capillary sprouts. This halts blood flow in most of the vessels in the tumor, resulting in the widespread necrosis of established tumors (Fig. 2). VTAs differ conceptually from antiangiogenic agents, which prevent the process of new blood vessel formation from existing vessels. VTAs can be more active in large *versus* small experimental tumors (8, 9). VTAs produce a characteristic pattern of widespread central necrosis in experimental tumors, which can extend to as much as 95% of the tumor (Fig. 2). However, a thin rim of viable tumor cells on the periphery of the tumor usually survives, which later regrows. VTAs are most effective against vessels in the interior of the tumor, possibly because the high interstitial pressure in these regions contributes to vascular collapse. In contrast, many direct-acting antitumor therapies are most effective against the rapidly dividing tumor cells in the well-oxygenated periphery of the tumor. Angiogenesis inhibitors are also most effective against tumor cells in the tumor periphery where angiogenesis occurs most vigorously (10). Combining VTAs with antiproliferative antitumor therapies or angiogenesis inhibitors can lead to additive or synergistic activity in experimental solid tumors (11–13).

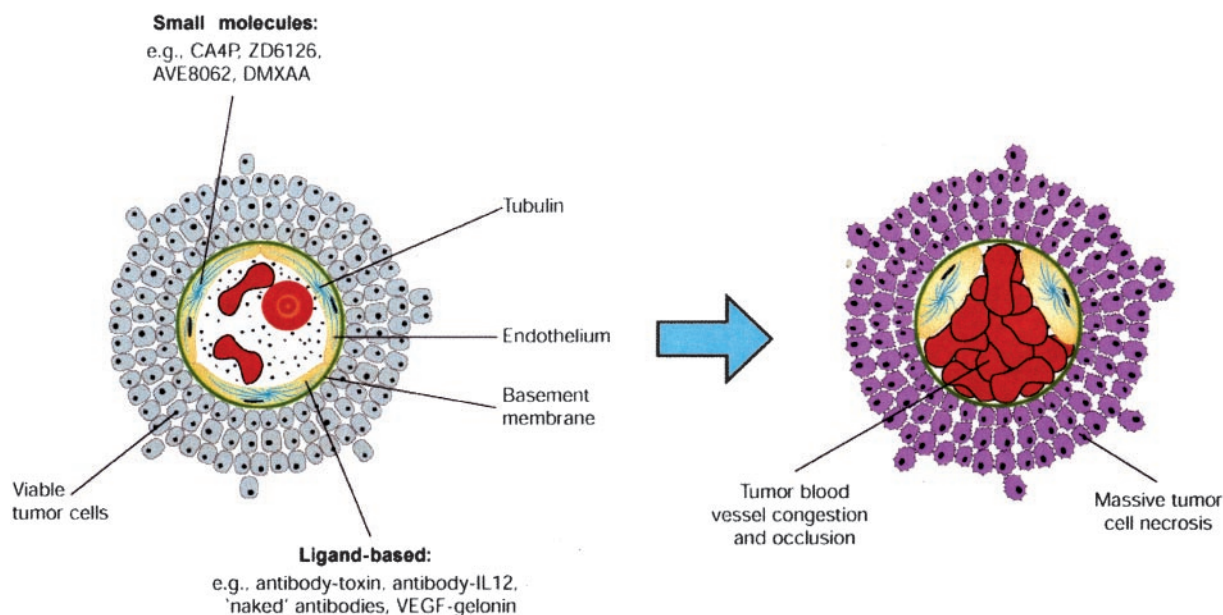
VTAs can be broadly divided into two types, small molecule VTAs and ligand-directed VTAs. Small molecule VTAs do not localize selectively to tumor vessels but exploit pathophysiological differences between tumor and normal tissue endothelium to achieve selective occlusion of tumor vessels. These differences in tumor compared with normal tissue endothelial cells include their increased proliferation, permeability, and reliance on a tubulin cytoskeleton to maintain cell shape (1, 14, 15). In contrast, ligand-based VTAs use a targeting ligand to achieve selectivity of binding to and occluding tumor vasculature. The two types are grouped together because they both

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*Fig. 1* The mechanism of action of vascular targeting agent (VTA) approaches. VTAs exploit differences between tumor and normal tissue blood vessels, cause the selective and rapid occlusion of tumor vasculature, and lead to massive tumor cell necrosis. There are broadly two types of VTAs. The small molecules include combretastatin A-4 disodium phosphate (CA4P), ZD6126, AVE8062, and DMXAA. Ligand-based VTAs use antibodies, peptides, or growth factors to target tumor endothelial cells with agents that occlude blood vessels.

cause acute vascular collapse in tumors, which leads to massive central necrosis (16).

### Small Molecule VTAs

#### Small Molecule Microtubule Destabilizing Agents.

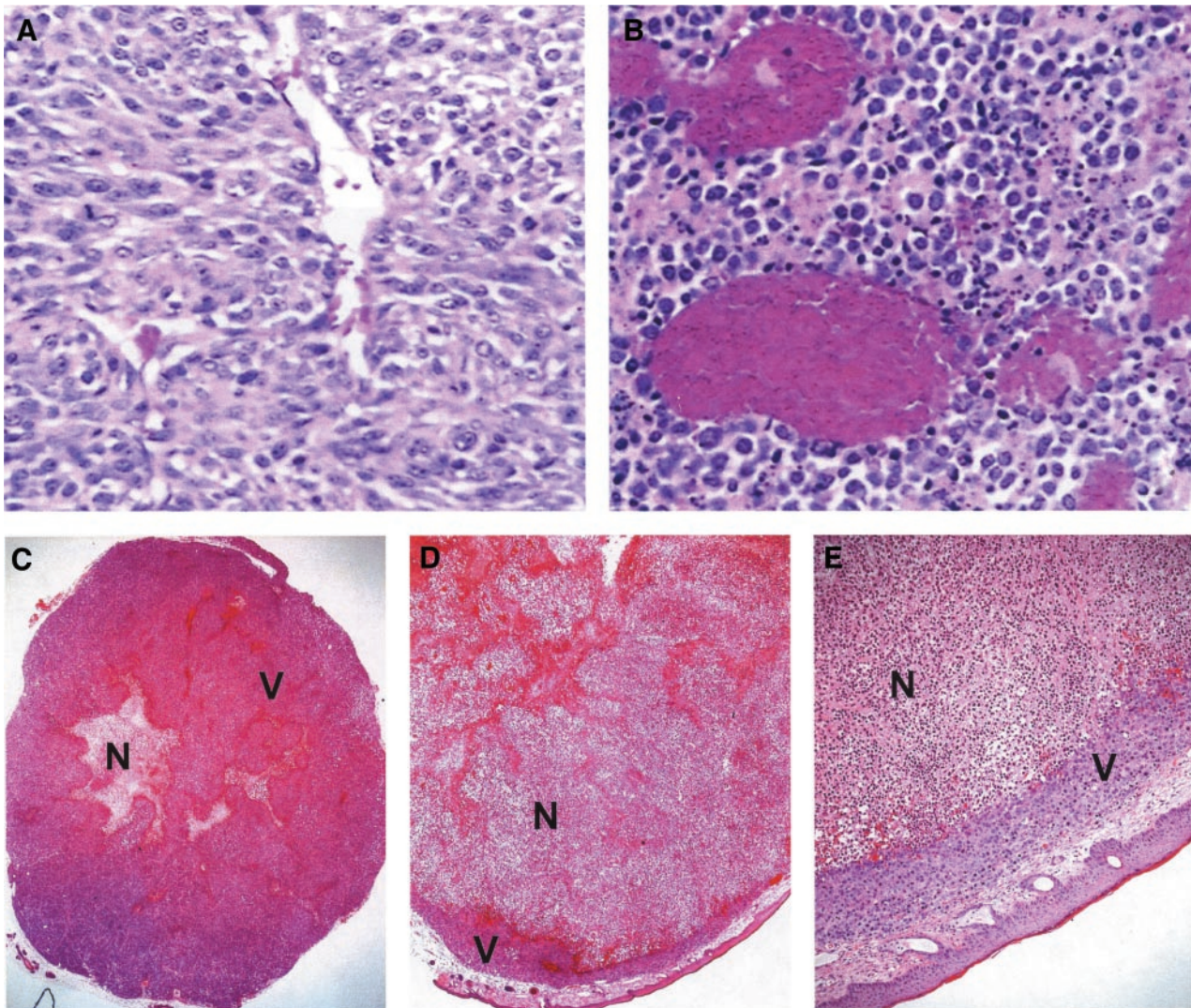
Table 1 lists the small molecule VTAs undergoing preclinical and/or clinical evaluation. The small molecule VTAs studied to date are either microtubule destabilizing agents or cytokine inducers. The strategy behind microtubule destabilizing agents is to disrupt rapidly proliferating and immature tumor endothelial cells based on their reliance on a tubulin cytoskeleton to maintain their cell shape. Tubulin-binding agents have both antimetabolic and antivascular effects that lead to inhibition of spindle formation (mitotic arrest) and reduced tumor blood flow, respectively (17). In many cases antivascular activity is only seen close to the MTD, and direct tumor cell cytotoxicity via mitotic arrest is the dominant mechanism of action. Thus, the early tubulin-binding agents studied, colchicine, vincristine, and vinblastine, have only a narrow therapeutic window (6, 18). Combretastatin A-4 was the first small molecule VTA shown to have antivascular effects at doses below the maximum tolerated dose (19).

**Combretastatin A-4 Disodium Phosphate.** Combretastatin A-4, originally isolated from the South African *Combretum caffrum* tree, is a tubulin-binding agent that resembles colchicine in structure. It inhibits tubulin polymerization by binding to a different site from colchicine on the tubulin molecule (20). The limited water solubility of combretastatin A-4 and complicated drug formation led to the synthesis of water-soluble prodrugs (21), and the CA4P prodrug (Oxigene, Boston, MA) has subsequently undergone extensive preclinical evalua-

tion (22). The soluble prodrug is cleaved to its natural form by endogenous phosphatases. In experimental tumors CA4P causes rapid, selective, and extensive vascular damage resulting in hemorrhagic necrosis within 1 h of treatment and subsequent tumor growth delay (19, 23–25). Tumor blood flow reduction is rapid, can drop to <5% of the starting value 1 h after drug administration, and is accompanied by an increase in vascular permeability (26). In experimental animals, effects on tumors are greater than effects on normal tissues (27). Noncytotoxic concentrations result in microtubule depolymerization and the disorganization of F-actin and  $\beta$ -tubulin in endothelial cells (28), and changes in endothelial cell shape (29). Cytoskeletal alterations in endothelial cells have been attributed to Rho/Rho-kinase activation, which leads to phosphorylation of myosin light chain, actin-myosin contractility, assembly of stress fibers, and formation of focal adhesions. Endothelial contraction and retraction may cause an increase in vascular resistance and obstruction of tumor blood flow (30). CA4P-induced reductions in vascular volume are augmented by nitric oxide synthase inhibitors, suggesting that nitric oxide is involved in the mech-

*Table 1* Small molecule vascular targeting agents in clinical or preclinical development

Common name	Description
CA4P	Combretastatin A-4 disodium phosphate
Oxi 4503	Combretastatin A-1 disodium phosphate
AVE8062	Combretastatin A-4 prodrug, formerly AC-7700
TZT1027	Synthetic derivative of dolastatin 10
ZD6126	Phosphate prodrug of <i>N</i> -acetylcolcholin
DMXAA	Flavonoid



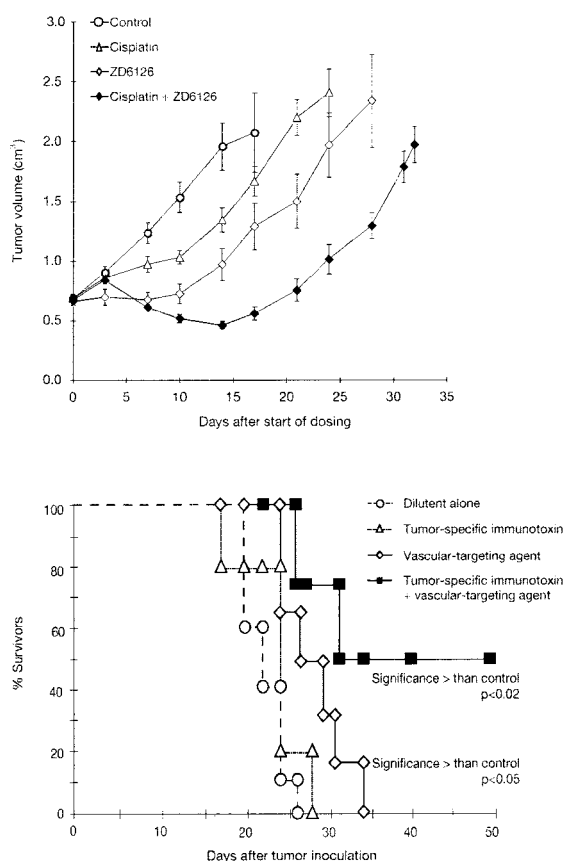
**Fig. 2** The typical tumor vessel congestion and massive tumor necrosis seen after administration of VTAs to animal models. H&E sections of a rat fibroblast tumor, FE8, 1 h after injection of (A) saline or (B) tissue factor targeted to the angiogenesis marker fibronectin ED-B domain [scFv(L19)(TF)]. Thrombosis of tumor vessels is evident in B. Low magnification views of H&E sections of a human lung cancer model, Calu-6, 24 h after injection of tumor-bearing mice with (C) vehicle or (D and E) 200 mg/kg ZD6126. The majority of the tumor is viable in C, whereas almost the entire core of the tumor is necrotic in D. The typical rim of viable tumor cells in the tumor periphery is visible in (E). N = necrotic tumor; V = viable tumor. A and B reprinted with permission (90). C, D, and E reprinted with permission (60).

anism of action of the drug (31). In experimental tumor models CA4P enhances the effects of radiation (32–36), hyperthermia (37–39), 5-fluorouracil (40), cisplatin (12, 33, 41), doxorubicin (42), and radioimmunotherapy (43, 44).

**Oxi 4503, AVE8062, and TZT-1027.** Other combretastatin derivatives are being synthesized and evaluated as potential antivascular agents. The sodium phosphate prodrug of combretastatin A-1 was selected for detailed experimental studies (45). The new compound has been reported to have more potent antivascular and antitumor effects than CA4P (46), is designated Oxi 4503 (Oxigene), and has been identified as a candidate for preclinical development (47). AVE8062 (Aventis Pharma, Paris, France; formerly AC-7700) is a synthetic water-soluble combretastatin A4 derivative that causes shape changes

in proliferating endothelial cells, the rapid shutdown of tumor blood flow, and extensive necrosis in experimental tumor models (17, 48–53). Dolastatin 10 is a natural product isolated from the marine mollusk *Dolabella auricularia* and a tubulin-binding agent that has antivascular activity (54). Like the combretastatins, dolastatins differ from *Vinca* alkaloids in their site of interaction with tubulin. A synthetic derivative of dolastatin 10, designated TZT-1027/Sonidotin (Teikoku Hormone Mfg. Co., Tokyo, Japan), has potent antitumor activity (55, 56) and is being developed in Japan (57).

**ZD6126.** ZD6126 (AstraZeneca, Macclesfield, United Kingdom) is a phosphate prodrug of the tubulin-binding agent *N*-acetylcolchicinol that inhibits microtubule polymerization. ZD6126 disrupts the tubulin cytoskeleton of endothelial cells



**Fig. 3** Vascular targeting agents (VTAs) are effective in combined modality regimens. Growth delay of human lung tumor xenografts treated with cisplatin and ZD6126 alone or in combination (*top*). Reprinted with permission (60). Also, survival of neuroblastoma-bearing mice treated with an immunotoxin and an antitumor vascular endothelial cell immunotoxin alone or in combination (*bottom*). Data from Burrows and Thorpe (5).

leading to endothelial cell detachment at noncytotoxic concentrations (58, 59). *In vivo*, a well-tolerated dose of ZD6126 was shown to cause tumor endothelial cell retraction, exposure of basal lamina in endothelia, and extensive endothelial cell loss (60). Rapid reductions in tumor blood flow (61) and vascular volume (62) are seen. ZD6126 causes massive necrosis in experimental tumor models, has activity in a range of tumor xenograft models (60, 63, 64), and inhibited the metastatic progression of pulmonary metastases from human lung adenocarcinomas in nude mice (65). ZD6126 enhances significantly the antitumor efficacy of cisplatin (Fig. 3; Refs. 60, 64), paclitaxel (62), gemcitabine (66), and radiation (67). ZD6126 has also been studied in combination with ZD6474, an antiangiogenic agent that is a vascular endothelial growth factor (VEGF) receptor (KDR) tyrosine kinase inhibitor. A greater than additive effect was seen suggesting that a combination of VTA and antiangiogenic approaches may have potential in combined modality cancer therapeutic strategies (11). As peripheral neuropathy is a major dose-limiting toxicity associated with antimicrotubule agents such as taxanes and *Vinca* alkaloids, studies have examined the potential for ZD6126 to exacerbate the neurotox-

icity of coadministered agents. Studies in animals showed that chronic intermittent dosing of ZD6126 was well tolerated with no evidence of peripheral neuropathy or aggravation of the neurotoxicity of paclitaxel (68, 69).

#### Small Molecule Cytokine Inducers: FAA and DMXAA.

FAA is a synthetic flavonoid that showed impressive activity toward experimental tumors but was toxic in cancer patients (70). The antivascular effects of FAA in experimental tumors are mediated via the release of tumor necrosis factor  $\alpha$  from activated mouse macrophages (71, 72). The lack of clinical activity of FAA led to the development of the FAA analogue DMXAA (Auckland Cancer Society Research Centre; Ref. 73). DMXAA has shown activity in experimental tumors resulting in necrosis (74). Preclinical studies have shown a selective and significant dose-dependent reduction in tumor perfusion in mice (75). The production of tumor necrosis factor  $\alpha$  is important for the mechanism of action of DMXAA, but it can induce vascular endothelial cell apoptosis in tumors, independent of tumor necrosis factor  $\alpha$  induction (76). The recent observation that DMXAA has activity in tumors growing in tumor necrosis factor receptor-1 knockout mice (77) suggests that the antitumor effects of DMXAA can be mediated via other cytokines or vasoactive factors. Circumstantial evidence suggests that DMXAA may stimulate phosphorylation of inhibitor of nuclear factor  $\kappa$ B, leading to a burst of nuclear factor  $\kappa$ B-mediated gene transcription (78). The spectrum of cytokines and chemokines produced in response to DMXAA is consistent with the involvement of nuclear factor  $\kappa$ B. It is possible that nuclear factor  $\kappa$ B transcription products change the organization of the cytoskeleton of vascular endothelial cells leading to changes in cell shape. Amplification of DMXAA activity by second signals present in the tumor microenvironment may explain its selectivity for tumor vasculature (79). Other studies have implicated the induction of IFN-inducible protein 10 (80), serotonin (81), and nitric oxide (82) in the antitumor effects of DMXAA. DMXAA has also been shown to augment the antitumor effects of melphalan (83), cisplatin (12), cyclophosphamide (12), paclitaxel (13) radioimmunotherapy (84), radiation (36, 74), immunotherapy (85), and hyperthermia (37, 86).

#### Ligand-Directed VTA

The strategy behind ligand-directed approaches is to use ligands that bind selectively to components of tumor blood vessels to target agents that occlude those vessels. Ligand-directed VTAs, therefore, are composed of targeting and effector moieties that are linked together, usually via chemical cross-linkers or peptide bonds. The targeting moiety is usually an antibody or a peptide directed against a marker that is selectively up-regulated on tumor *versus* normal tissue endothelial cells (Table 2). Growth factors that recognize receptors that are overexpressed on tumor vessels can also be used. There are a number of molecules that are up-regulated on tumor *versus* normal tissue vessels. Examples include molecules involved in angiogenesis and vascular remodeling; cell adhesion molecules induced by inflammatory mediators [*e.g.*, interleukin (IL)-1], which are released by tumor cells and tumor-infiltrating normal cells; and molecules associated with prothrombotic changes that occur on tumor vascular endothelium. Table 3 lists the effector

Table 2 Tumor vessel markers for ligand-directed vascular targeting agents

Class	Examples
Angiogenesis/vascular remodeling	VEGF <sup>a</sup> receptors VEGF:receptor complexes $\alpha_v\beta_3$ integrin PSMA CD44-related antigen (TES-23) Fibronectin ED-B domain Collagen IV HUIV26 epitope Endoglin Endosialin MMP2, MMP9
Cell adhesion	VCAM-1 E-selectin
Prothrombotic change	Phosphatidylserine Tissue factor
Infiltrating leukocyte-acquired	Eosinophil peroxidase

<sup>a</sup> VEGF, vascular endothelial growth factor; PSMA, prostate-specific membrane antigen; MMP, matrix metalloproteinase; VCAM, vascular cell adhesion molecule.

moieties being studied. The effector moieties can induce thrombosis directly, kill endothelial cells to cause thrombosis indirectly, redirect host defenses to attack the tumor vessels, or cause shape changes in endothelial cells that then physically block tumor vessels.

The earliest studies in this area involved the development of a model system to investigate the antibody-directed targeting of mouse tumor endothelium. The model involved cytokine gene transfection of tumor cells to induce the selective expression of an experimental marker (MHC class II) on tumor vascular endothelium (3). The tumor cells were grown in nude mice, and the i.v. injection of an antitumor endothelial cell immunotoxin (a ricin-conjugated antibody against the MHC class II antigen) caused complete occlusion of the tumor vasculature and dramatic regressions of large solid tumors (5). Subsequent studies with an immunotoxin directed against the endothelial proliferation/activation marker, endoglin, demonstrated that it is possible to exploit differences in antigen expression to create agents selective for dividing endothelial cells (87).

In several laboratories, the extracellular domain of the human coagulation-inducing protein, tissue factor, has been targeted to tumor vessels to induce specific tumor vessel thrombosis. The extracellular domain of tissue factor is not a coagulant while free in the blood circulation but becomes a powerful and specific coagulant once targeted by a targeting ligand to tumor vasculature. Specific targeting of tissue factor to tumor vessels has been accomplished with antibodies and peptides directed against a variety of tumor vessel markers, including MHC class II (88), the cell adhesion molecule VCAM-1 (89), the ED-B domain of fibronectin (90), and prostate-specific membrane antigen (91). In all of these studies, the VTA homed selectively to tumor vessels and rapidly induced thrombosis. Within a few hours, vessels throughout the tumor were packed with platelet aggregate, erythrocytes, and fibrin. By 24 h, tumor cells showed pyknotic changes that became progressively more marked, and by 72 h, the entire central region of the tumors had degenerated into amorphous debris.

Another successful strategy has been to use human VEGF-A to target toxins to tumor vessels. Fusion proteins and chemical conjugates of VEGF and diphtheria toxin (92, 93) or gelonin (94) induced regressions of tumors in mice. The selectivity for tumor vessels was attributed partly to the up-regulation of VEGF receptors on tumor vessels and partly to the finding that activated/proliferating endothelial cells in tumors endocytose the fusion protein via a route that leads to cytotoxicity. Vessels in normal tissues express low levels of receptor and are resistant. Matsuno *et al.* (95) have described a chemical conjugate of ricin A-chain linked to monoclonal antibodies to mouse endoglin. Treatment of mice bearing established MCF7 breast tumor xenografts induced lasting complete tumor regressions in the majority of the mice. Tsunoda *et al.* (96) described a conjugate of the cytotoxic agent neocarzinostatin and a monoclonal antibody (TES-23) directed against a CD44-related tumor endothelial cell marker. Administration of the conjugate to mice and rats bearing various types of solid tumors had marked antitumor effects.

The antitumor activity of cytokines can be enhanced by targeting them to the extracellular matrix surrounding tumor vessels. IL-2 and IL-12 are cytokines with potent immunostimulatory activity. Halin *et al.* (97) and Carnemolla *et al.* (98) prepared fusion proteins consisting of IL-2 or IL-12 fused to the L19 scFv directed against the ED-B domain of fibronectin, an extracellular matrix marker of angiogenic vessels. These proteins had marked activity toward aggressive murine tumors and metastases in the lungs. The residual small tumor masses seen in treated mice were infiltrated with lymphocytes, macrophages, and natural killer cells. L19 scFv recognizes the human fibronectin ED-B domain as well as that of other species, making it a prime candidate for clinical trials.

Immunoliposomes represent another area for the potential development of ligand-based VTAs. Liposomes are attractive for targeting drugs and other effectors to tumor vasculature because they can carry large payloads and because their size restricts access to extravascular normal tissues. Marty *et al.* (99) encapsulated a cytotoxic drug (an arabinofuranosylcytosine derivative) in polyethyleneglycol-modified liposomes coated with

Table 3 Effectors for ligand-directed vascular targeting agents

Class	Examples
Coagulation-inducing proteins	Tissue factor
Toxins	Diphtheria toxin Ricin Gelonin
Cytotoxic agents	Doxorubicin Neocarzinostatin
Cytokines	Interleukin-2 Interleukin-12 Tumor necrosis factor- $\alpha$
Apoptosis-induction	<i>RAF-1</i> gene Mitochondrial-membrane disrupting peptide
Radioisotopes	Iodine-131 Actinium-225 Bismuth-213
Liposomally encapsulated effectors	Arabinofuranosylcytosine derivative

L19 scFv. Mice treated with the targeted liposomes showed marked reductions in tumor growth.

**Gene Therapy Approaches.** There are a number of gene therapy approaches for targeting the tumor vasculature. Retroviruses have been engineered so that they can be coated with an antibody (e.g., anti-VEGF Flk1/KDR receptor) for the selective delivery of genes to tumor endothelium (100). Another intriguing approach is to create adenoviruses that selectively replicate in and lyse dividing endothelial cells. The combined use of regulatory elements controlling two independent markers of tumor endothelium, Flk-1 and endoglin, gave synergistic effects on targeting specificity *in vitro* (101). A recent strategy for attaining specific effects on tumor vessels is to use tumor cell-specific cytotoxic T lymphocytes to deliver a retrovirus containing a gene encoding a VEGF-toxin fusion protein to tumor cells. The VEGF-toxin synthesized by the tumor cells is expected to destroy the adjacent tumor endothelium, but not angiogenic vessels in normal tissues distant from the tumor (102).

#### The Search for New Targeting and Effector Moieties.

Several new techniques are being used to find markers that could aid the development of targeting moieties with increased tumor specificity over those currently available. Luminal endothelial cell plasma membranes have been physically isolated from various normal tissues and lungs bearing nodules derived from a mammary adenocarcinoma cell line. A caveolar protein that was up-regulated specifically in the tumor endothelium was identified, used to generate a monoclonal antibody, and the antibody shown to accumulate specifically in the vasculature of a tumor (103). Serial analysis of gene expression is also being used to identify tumor endothelial markers (104, 105). In the latter work, endothelial cells from dispersed human normal and malignant colorectal tissue were enriched and purified using endothelial cell markers. Serial analysis of gene expression libraries were generated and compared with libraries from other tissues. Of >170 transcripts expressed predominantly in the endothelium, 79 were differentially expressed, including 46 that were specifically elevated in tumor-associated endothelium, most of unknown function. Many were expressed in many tumor types. Another promising new technique is *in vivo* phage display, where vast numbers of phage, each expressing a different peptide, are injected into animals (106) or terminally ill patients (107). A short time later, samples of tumor and various normal tissues are removed and phage that have localized to the endothelium are recovered. Peptides that confer specific binding to tumor endothelium are being identified. There is optimism that these, and even more sophisticated techniques that may be developed, will permit the identification of tumor vessel markers that improve on our current ability to discriminate between tumor and normal tissue vessels.

All of the markers studied to date are up-regulated on vessels in sites of inflammation, tissue remodeling, or physiological angiogenesis, consistent with Dvorak's concept that "tumors are wounds that do not heal" (108). In the absence of a perfect marker it is important to ensure that the cross-reactivity of a VTA with normal and pathological nonmalignant tissue is tolerated. There are several reasons to believe that this is the case. First, the level of expression of a marker in normal or inflamed endothelia may be below the threshold level for a

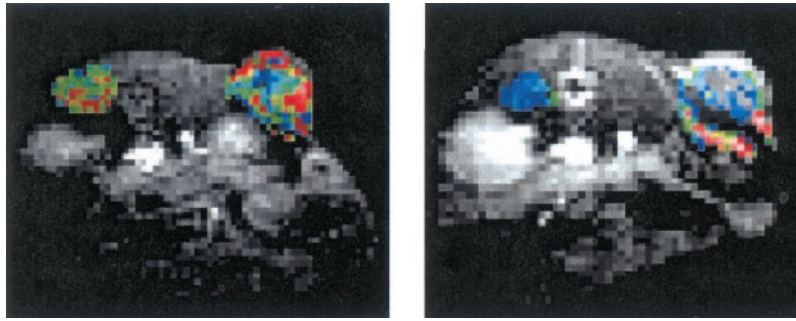
destructive response. Second, the internalization route of an immunoconjugate may differ in a proliferating tumor *versus* a quiescent normal tissue endothelial cell, rendering the latter refractory to the cytotoxic moiety. Third, thrombosis induced by a tissue factor-based VTA requires coincident expression of phosphatidylserine in addition to the target antigen. Tumor vessels express phosphatidylserine, whereas quiescent, normal tissue endothelia do not (109, 110); hence, normal tissue endothelia are resistant to tissue factor-based VTAs, even if they express the target molecule. Treatment with ligand-based VTAs causes little or no toxicity at therapeutic doses in animals, suggesting that such experimental therapies are worthy of exploration as potential clinical treatments.

In addition to targeting moiety specificity for tumor endothelium, another important issue with ligand-based VTAs is marker heterogeneity. As pointed out by Kerbel *et al.* (111), tumors can modulate the markers they induce on the adjacent endothelia, giving rise to heterogeneity in the expression of tumor vessel markers. It might be possible to use combinations of VTAs or bispecific VTAs that recognize two differently regulated tumor vessel markers. The latter approach would not only reduce the influence of marker heterogeneity but also might increase tumor specificity, because endothelial cells in normal pathological tissues might express one, but not the other marker.

There is also interest in identifying novel effectors. Human effectors, such as interleukins and coagulant proteins, have the advantage of low inherent immunogenicity. Naked antibodies that recruit host effectors (complement, ADCC) to attack tumor endothelium have the advantage of simplicity. Antibodies that target phosphatidylserine on tumor endothelium produce antitumor effects in mice, probably by recruiting macrophages to attack the vasculature (112). Different cytotoxic agents and apoptosis-inducers could be investigated. For example, a radioimmunotherapy strategy being investigated involves the vascular targeting of radiation using  $^{213}\text{Bi}$ -radiolabeled monoclonal antibodies (113). It would also be of interest to explore ligand-directed targeting of antiangiogenic agents or even small molecule VTAs as a means of improving tumor specificity and minimizing toxicity.

#### Noninvasive Imaging and Surrogate Markers of VTA Effects

The side effects associated with conventional antiproliferative antitumor agents are used in Phase I dose-finding studies as a guide to determining maximum tolerated doses. However, VTAs are expected to be active at doses below their maximum tolerated dose. In addition, the objective tumor responses seen with antiproliferative agents might not be obtained with anti-vascular compounds that are expected to be effective in combined modality treatments. This has spurred research into alternative methods for assessing the antitumor effects of VTAs, and of particular interest in this area is the use of noninvasive imaging (114). The most commonly used approach is dynamic contrast enhanced magnetic resonance imaging (DCE-MRI). The paramagnetic contrast agent, gadopentetate dimeglumine, is injected i.v. as a rapid bolus and, as it passes through the tissues, it diffuses out of the bloodstream and into the extravascular



**Fig. 4** The typical Vascular targeting agent (VTA)-induced reduction in contrast agent enhancement measured as the integrated area under the curve (IAUC) using dynamic contrast enhanced magnetic resonance imaging. A mouse bearing a s.c. C38 colon adenocarcinoma in the flank was imaged before (*left*) and 24 h after (*right*) treatment with ZD6126. Transaxial images through the abdomen are shown, with the dorsal surface orientated upwards and the abdominal cavity downwards in the figure. The tumor is visible as the colored area in the *top right* of the figure. The colored area on the *top left* is paravertebral muscle. The color scale for IAUC increases from blue to green to yellow to red. ZD6126 treatment led to a reduction in IAUC. With thanks to Zach DelProposto (Wayne State University, Detroit, MI) and Jeff Evelhoch (Pfizer, Inc., Ann Arbor, MI) for supplying the figure.

extracellular space. The signal intensity increases as the concentration of gadopentetate dimeglumine in the extravascular space increases. Changes in signal intensity with time, or contrast enhancement, are recorded in the tumor and normal tissues. The level of contrast enhancement seen in the tumor reflects vascular permeability, interstitial space, and perfusion. Reduction in contrast enhancement is observed in tumors in VTA-treated animals (Fig. 4), as expected of drugs that cut off tumor blood vessels. These effects are visible within hours of drug treatment, can be drug dose-dependent (115, 116), and have been shown to correlate with tumor response to treatment (117). DCE-MRI is reproducible in human tumors (118, 119) and has been used in Phase I trials to demonstrate that CA4P, ZD6126, and DMXAA have antivascular activity in tumors in humans (120–123).

There are other imaging approaches with potential for assessing the antitumor effects of VTAs. Blood oxygen level-dependent MRI, measuring changes in the paramagnetism of hemoglobin as a result of oxygenation (124, 125), has been suggested as an alternative to DCE-MRI. Tumor blood oxygenation tension can be measured using  $^{19}\text{F}$  MRI (126). Near infrared spectroscopy can measure changes in oxy- and deoxy-hemoglobin levels, and has been used to assess rapid changes in the oxygen saturation and volume of blood vessels (126, 127). Magnetic resonance spectroscopy has been used to measure changes in tumor energy status as a reflection of changes in blood flow and tumor necrosis (23, 128, 129). High frequency Doppler ultrasound (61), dynamic computed tomography (130), and positron emission tomography (131, 132) are also being studied. The feasibility of using positron emission tomography measurement of perfusion to assess VTA-induced changes in the vasculature of human tumors was reported recently (133).

There is also interest in finding surrogate markers for the clinical effects of VTAs. Plasma levels of the naturally occurring vasoactive substance, serotonin, are increased after DMXAA administration to tumor-bearing animals (81). Because serotonin is unstable it is not suitable for use as a surrogate marker; however, a metabolite (5-hydroxyindoleacetic acid) also accumulates in plasma and is suitable (134). Analysis

of plasma from patients treated with DMXAA in a Phase I trial showed a DMXAA-induced elevation of plasma levels of the metabolite, suggesting the potential of the approach to monitor the effects of DMXAA in cancer patients (135). Another approach might be to measure the levels of circulating endothelial cells, which in a Phase I trial were shown to increase  $\sim 2$ -fold 4–6 h after ZD6126 administration (136).

### Clinical Studies

CA4P, AVE8062, ZD6126, and DMXAA are being evaluated in patients with advanced solid tumors. Table 4 summarizes the toxicity seen in the first Phase I trials. In a Phase I pharmacokinetic study of CA4P given as a single-dose *i.v.* schedule every 3 weeks, the dose-limiting toxicities were tumor pain, acute coronary syndrome, and shortness of breath (123). A significant decline in tumor blood flow was measured by DCE-MRI. A patient with an anaplastic thyroid cancer had a complete response and was alive 30 months after treatment. In another trial, CA4P was administered weekly for 2 weeks followed by 1 week of rest (137). The drug was well tolerated, the most common toxicities were cardiovascular, and the dose-limiting toxicities included reversible ataxia, vasovagal syncope, and motor neuropathy. A patient with a liver metastases from an adrenocortical carcinoma had an  $\sim 50\%$  decrease in the product of four marker lesions after three, four, and five cycles of treatment (138). DCE-MRI showed a CA4P-induced reduction in contrast agent enhancement in 6 of 16 patients treated at  $\geq 52$   $\text{mg}/\text{m}^2$ , measured 4 and 16 h after treatment (121). No reduction in muscle or kidney enhancement was seen. In another study, CA4P-induced antivascular effects were also assessed using positron emission tomography measurements of perfusion (133). Significant reductions in tumor perfusion were measured 30 min after CA4P administration, with evidence of recovery by 24 h and no significant changes in spleen or kidney. The preliminary data are also available from another Phase I trial where CA4P was given in combination with carboplatin to patients with advanced cancer (138). The combination was

Table 4 Phase I studies

Vascular targeting agent	Schedule	Toxicity	Reference
CA4P	18–90 mg/m <sup>2</sup> q3 wks	Minimal cumulative side effects. DLTs <sup>a</sup> were tumor pain, acute coronary syndrome and shortness of breath.	Dowlati (123)
	5–114 mg/m <sup>2</sup> d1, 7, 14 q28 days	Most common toxicity was cardiovascular. DLTs included reversible ataxia, vasovagal syncope and motor neuropathy.	Rustin (137)
	Carboplatin with 27–45 mg/m <sup>2</sup> CA4P d1 q21 days	Myelosuppression consistent with carboplatin treatment. Also, fatigue, tumor pain, tingling in the extremities.	Bilenker (138)
AVE8062 ZD6126	4.5–30 mg/m <sup>2</sup> wkly	Asymptomatic systolic hypotension.	Tolcher (139)
	5/7 mg/m <sup>2</sup> wkly for 4 wks	Adverse events included anemia, constipation, hypokalemia, hyperkalemia, fatigue, edema.	Radema (136)
DMXAA	5–112 mg/m <sup>2</sup> q3wks	Adverse events included anorexia, constipation, dyspnea, fatigue, headache, nausea, pain, vomiting.	Gadgeel (140)
	6–4900 mg/m <sup>2</sup> q3wks	DLTs were acute neurological (e.g., slurred speech, anxiety, visual disturbance).	Jameson (142)
	6–4900 mg/m <sup>2</sup> wkly	DLTs were neurological (urinary incontinence, visual disturbance, anxiety).	Rustin (141)

<sup>a</sup> DLT, dose-limiting toxicity.

tolerated with DCE-MRI measurements of a reduction in tumor blood flow 4–6 h after treatment.

The combretastatin analogue AVE8062 is undergoing Phase I evaluation in patients with advanced malignancies. Preliminary results indicate that it is feasible to achieve plasma levels of AVE8062 at which antivasular activity has been observed in preclinical models (139).

Preliminary results have also been reported for two Phase I dose escalation studies of ZD6126 (136, 140). In one of these studies (140), ZD6126 was given to 29 patients as a 10-min, single dose iv infusion every 3 weeks. The drug was well tolerated in most patients at doses up to and including 80 mg/m<sup>2</sup>. Adverse events noted in >10% of patients included anorexia, constipation, dyspnea, fatigue, headache, nausea, pain, and vomiting. The use of dynamic contrast-enhanced MRI in this study showed that tumor perfusion is reduced by ZD6126. In the second study (136), ZD6126 was given to 18 patients on a weekly schedule. Adverse events, which occurred in >15% of patients, were anemia, constipation, hypokalemia, hyperkalemia, fatigue, and edema. An increase in circulating endothelial cells was seen 4–8 h after ZD6126 infusion in 5 of 8 patients, which provides additional evidence of the biological activity of ZD6126 in patients with malignant disease.

The results of two Phase I studies of DMXAA have been described recently involving administration weekly (141) or every 3 weeks (142). Dose-limiting toxicities were neurological in both studies (Table 4). There were unconfirmed partial responses in a patient with a locally recurrent melanoma (141) and another with a metastatic cervical carcinoma (142). DCE-MRI was carried out as part of the two Phase I studies, and 9 of 16 patients had significant reductions in tumor contrast agent enhancement measured 24 h after the administration of DMXAA (122).

These early Phase I trials show that VTAs can be given to patients with advanced cancers and lack the hematological toxicity associated with many anticancer agents. Noninvasive imaging approaches have shown VTA-induced reductions in pa-

rameters related to tumor blood flow, which are consistent with the antivasular effects seen in preclinical models. CA4P, ZD6126, and DMXAA are now being evaluated in the Phase II setting.

### Summary and Future Directions

The potential of vascular targeting as a cancer therapeutic approach has been firmly established in experimental studies. The VTAs all lead to rapid reductions in tumor blood flow and extensive necrosis in experimental tumors. They have also been shown to be more effective in large rather than small tumors. Tumor stabilizations are commonly seen in preclinical models, and in combination with antiproliferative modalities lasting tumor regressions are obtained. The preliminary demonstration of tolerability in humans supports the continued development of vascular targeting as a novel cancer therapeutic approach. The tolerability profiles seen and the demonstration of monotherapy efficacy are exciting for the future development of combined modality regimens.

Future directions lie in understanding the molecular basis of the mechanism of action of the drugs currently undergoing clinical evaluation. Increased understanding of the cell signaling processes involved might aid the development of second-generation small molecule VTAs with enhanced specificity for tumor endothelium. Advances in genomics are revolutionizing the discovery of endothelial markers and should improve the tumor selectivity of ligand-based approaches. There is a need to examine the efficacy of ligand-based approaches in clinical trials and a need also for the development of methods that can be used in pharmacodynamic studies in humans. A number of noninvasive imaging methods are being evaluated, and over the next few years these should be validated and standardized for routine clinical use. Increased understanding of the pathophysiological end points being imaged is required to enable the selection of the appropriate method to use with different VTAs.



Clearly additional clinical development of VTAs is warranted and, as activity is likely in many solid tumors, future trials will need to evaluate combinations and scheduling to determine the best regimens for different tumor types.

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