# Vascular Disruptive Agents for the Treatment of Cancer

Tim Meyer
Editor

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# Development of Vascular Disrupting Agents

**Graeme J. Dougherty and David J. Chaplin**

**Abstract** The majority of the cancer therapies in use today target the malignant cell population. In broad terms, specificity is achieved by exploiting intrinsic differences between normal cells and tumor cells with respect to various key processes including proliferative activity, DNA repair and responsiveness to apoptotic stimuli. Although progress continues to be made, it remains the case that chemotherapy alone is rarely curative. Thus, in recent years increased interest has focused on alternative strategies that instead target various normal cell types upon which the survival and growth of a tumor depends. In this chapter we explore the historical events that lead to development of vascular disrupting therapies and discuss the major approaches currently employed to selectively destroy the neovasculature of solid tumors.

## 1 Introduction

For largely historical reasons, the majority of the cancer therapies in use today directly target the malignant cell population. Specificity is achieved by exploiting intrinsic differences between normal cells and tumor cells with respect to various key processes including proliferative activity, DNA repair, responsiveness to apoptotic stimuli and so on. While new tumor-directed therapies targeting novel pathways continue to be developed, it remains the case that chemotherapy alone is rarely curative. Thus, in recent years increased interest has focused on alternative strategies that instead target various normal cell types upon which the survival and growth of a tumor depends (Lorusso and Ruegg 2008; Mbeunkui and Johann 2009). Although a number of such approaches have been explored (Anton and Glod 2009; Dickens and Jubinsky 2009; Hanna et al. 2009; Kiaris et al. 2008; Ma and Adjei 2009; Zhang 2008), perhaps the most dramatic progress has been made in the area of vascular-directed therapies (Heath and Bicknell 2009).

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As is the case for normal tissues, the growth of a tumor requires the provision of adequate levels of oxygen and nutrients and the removal of waste products generated in the course of metabolic activity (Cao 2009). Since the vascular system plays an essential role in each of these processes (Nikitenko 2009), it follows that approaches that compromise blood flow may provide therapeutic benefit (Siemann and Horsman 2009).

Tumors generally arise from a single cell that has undergone a number of genetic events that allow escape from the normal growth control mechanisms that operate within a tissue. Initially, the growing tumor receives sufficient oxygen and nutrients simply by diffusion from nearby blood vessels. However, as the mass increases in size, a point is quickly reached whereby consumption by cells closer to a vessel prevents more distant cells from receiving sufficient oxygen and nutrients to maintain viability, restricting further expansion and resulting in a tumor remaining localized (Fig. 1) (Bertout et al. 2008). For a tumor to continue to grow and ultimately metastasize to distant tissue sites, it is necessary that it trigger the production of new blood vessels (Fig. 1) (Bertout et al. 2008). This process, which is known as angiogenesis, is controlled by a large number of soluble mediators released by tumor cells and/or various tumor-associated normal cell types including macrophages and fibroblasts (Bertout et al. 2008). Working together in a hierarchical fashion, these so called "angiogenic factors" trigger the proliferation of endothelial cells in nearby vessels and coordinate the complex series of cell–cell and cell–matrix interactions that ultimately give rise to new tumor-associated blood vessels. Unlike in normal tissues, the aberrant and/or disregulated nature of the angiogenic process that occurs within tumors generates a structurally and functionally abnormal vasculature that is often described as "chaotic" (Cao 2009).

As understanding of the molecular events involved in the regulation of angiogenesis has increased, the possibility that the process might serve as a target for the development of novel cancer therapies, has gained support. Two distinct but potentially complimentary strategies have emerged. By far the greatest effort has focused on so-called "anti-angiogenic therapies." As first advocated by the late Professor M. Judah Folkman (Klagsbrun and Moses 2008), the goal of such treatments is to inhibit

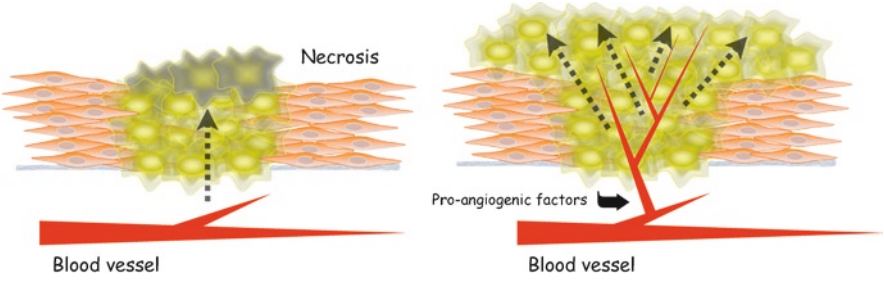


**Fig. 1** Requirement for angiogenesis in tumor progression. As oxygen is consumed as it diffuses through tissue, cells more than ~150 μm from the nearest blood vessel receive insufficient supply to maintain their viability. Thus, in order for a tumor to continue to grow, it must induce the formation of new blood vessels. Tumors that fail to do so do not progress and remain localized

the angiogenic process so as to prevent the *formation* of new blood vessels (Ribatti 2009). Approaches targeting the pro-angiogenic cytokine vascular endothelial cell growth factor (VEGF) have shown the most promise (Fig. 2). Bevacizumab (Avastin), a humanized antibody directed against VEGF, was the first rationally-designed anti-angiogenic agent to be granted approval by the FDA, initially as a first line treatment for metastatic colorectal cancer in combination with fluorouracil-based chemotherapy (Rhee and Hoff 2005; Chase 2008; Grothey and Ellis 2008; Ribatti 2009). A number of small molecule tyrosine kinase inhibitors that block the signal transduction events induced upon the interaction of VEGF with its cognate receptor have also been developed (Wakelee and Schiller 2005; Baka et al. 2006). Examples include SU11248 (Sunitinib), BAY-43-9006 (Sorafenib/Nexavar) and ZD6474 (Zactima) (Fig. 2).

Although anti-angiogenic therapies are clearly of benefit in certain advanced malignancies, there are potential drawbacks with this approach that may limit its usefulness in other settings. Most importantly, while it is evident anti-angiogenic therapies not only prevent the formation of new blood vessels, but can induce the regression or normalization of the tumor-associated neovasculature (Heath and Bicknell 2009; Fukumura and Jain 2007; Huang and Chen 2008), the agents may need to be administered continuously over an extended period of time in order to produce a durable response. Indeed, there is evidence from both human and animal studies to suggest that vessels rapidly regrow once therapy is stopped (Mancuso et al. 2006). More worryingly, it has long been appreciated that since most anti-angiogenic agents including bevacizumab target a single pathway (e.g. VEGF), other angiogenic factors may simply take over in the presence of a specific inhibitor (Kuhn et al. 2006; Ruegg

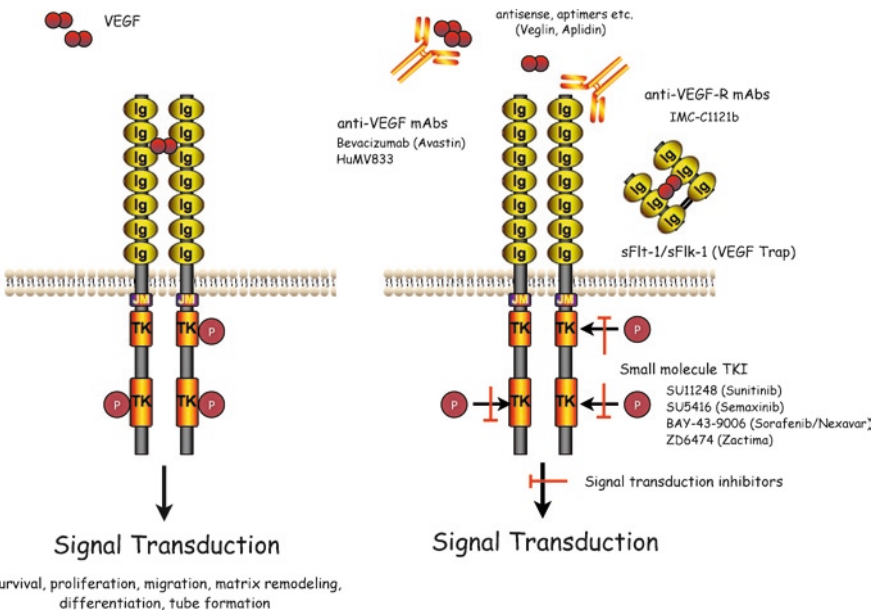


**Fig. 2** Anti-angiogenic therapies targeting the VEGF pathway

and Mutter 2007). Indeed, the results of some mouse experiments suggest that angiogenic inhibitors targeting the VEGF pathway may trigger an adaptive response within a tumor and/or the host that may inadvertently result in enhanced invasiveness and metastatic potential (Paez-Ribes et al. 2009; Ebos et al. 2009). Such findings may be highly relevant with respect to several ongoing studies in which anti-angiogenic agents are being evaluated in an adjuvant and/or neoadjuvant setting in patients with earlier stage disease. In this regard, it may be telling that a recently completed Phase III study (NSABP C-08) in which patients with stage II or III colorectal cancer were assigned to receive FOLFOX chemotherapy with or without bevacizumab following surgery failed to demonstrate improved disease free survival in the arm receiving anti-angiogenic therapy (Wolmark et al. 2009).

It is for these and other reasons that efforts have been made to explore alternative vascular targeting strategies that involve not simply preventing angiogenesis but rather specifically disrupting the existing abnormal vasculature that is found within a tumor so as to prevent the delivery of the oxygen and nutrients required to maintain tumor cell viability (Siemann and Horsman 2009). Originally championed by the late Professor Juliana Denekamp (Fig. 3) (Denekamp 1982, 1984, 1990, 1991, 1993; Denekamp et al. 1983, 1998), this approach has gained acceptance in recent years with the development of several small molecule Vascular Disrupting Agents (VDAs) that have been shown to induce vascular shutdown and anti-tumor responses at well tolerated doses in the clinic (Cai 2007; LoRusso et al. 2008; Rehman and Rustin 2008; Siemann et al. 2009).

Vascular disrupting strategies offer a number of advantages over approaches that directly target tumor cells. With conventional chemotherapeutic agents, eradication of even a small tumor mass with a volume of around 1 $cm^3$ requires that an effective dose of the drug in question be delivered to each of up to $10^9$ cells. Poor and/or intermittent perfusion resulting from the abnormal nature of tumor vasculature, high interstitial pressure and other physiologic considerations conspire to make this a challenging

**Fig. 3** Professor Juliana Denekamp (1943–2001)

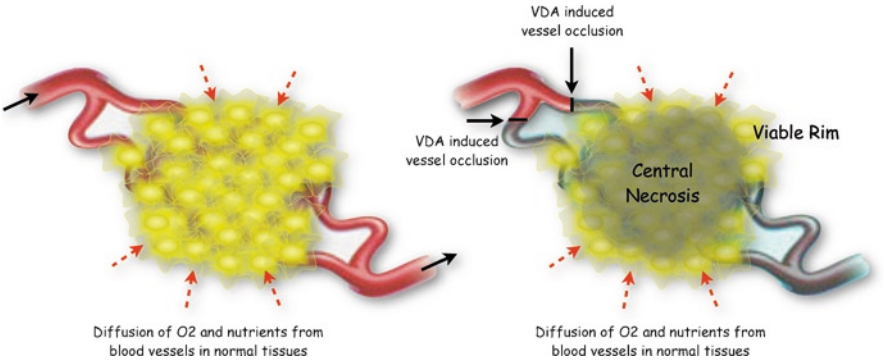


**Fig. 4** Anti-tumor activity of vascular disrupting agents (VDAs). Vessel occlusion resulting from the selective action of VDAs on tumor-associated endothelial cells blocks blood flow and prevents the delivery of required oxygen and nutrients to a tumor mass. Not only are tumor cells in the direct vicinity of the point of damage killed but also all downstream cells supplied by that vessel. Thus VDAs typically produce massive necrosis particularly within central regions of a treated tumor. Cells around the periphery survive as they receive sufficient oxygen and nutrients to maintain viability by diffusion from vessels in surrounding normal tissues. In the absence of additional treatment, cells in this so called "viable rim" can repopulate necrotic regions allowing tumor growth to resume

objective. In contrast, for VDAs, the cells being targeted (i.e. endothelial cells lining tumor-associated blood vessels) are in direct contact with the circulation and thus easily accessible to intravenously administered agents. Moreover, it is not even necessary to kill endothelial cells in order to mediate an effect, as any change in their shape or function, even if temporary, which interferes with blood flow, may be effective. Most importantly, as blood vessels are effectively pipelines through which oxygen and nutrients are carried to, and the toxic waste products of metabolism removed from, a tumor mass it follows that damage at any one point that obstructs blood flow will result in the death not only of cells in the direct vicinity of the point of damage but also all downstream cells supplied by that vessel segment (Fig. 4). Thus even limited damage to the tumor vasculature may result in the death of many thousands of tumor cells if blood flow is shutdown for an adequate period of time.

## 2 Early Studies Supporting the Development of Vascular Disrupting Cancer Therapies

### *2.1 Testicular Torsion*

The discovery that transient disruption of vascular function can cause rapid tissue death came from studies involving various normal tissues. For example, testicular torsion, in which the spermatic cord carrying the blood supply to a testicle becomes twisted, reducing or abolishing blood flow and leading, if untreated, to atrophy or

loss of the affected testicle, was first described in the medical literature by the London surgeon John Hunter in 1776 (Noske et al. 1998). However, it was not until the later half of the nineteenth century that it was appreciated based on animal studies that it was a reduction in blood flow triggered by torsion that was responsible for the resultant hemorrhagic infarction (Follin 1852; Miflet 1879). It is of interest from these studies that gross tissue damage was only evident after a few hours of ischemia (Enderlen 1896; Hellner 1933).

## *2.2 William Henry Woglom*

By the mid 1800s there were occasional apocryphal reports that tumors too sometimes regressed if their blood supply was compromised as a result of torsion of the vascular pedicle or thrombosis of a major feeding vessel (Walshe 1844). However, the therapeutic potential of vascular disrupting strategies seems to have remained largely unrealized until a seminal paper from William Henry Woglom, published in 1923 (Woglom 1923). It is obvious from comments made in this publication, that Woglam understood not only the causal relationship between vessel thrombosis and tumor regression, but more importantly, the unique opportunity that this relationship presented with respect to the development of novel therapies. Clearly, he also appreciated the challenge, when he noted that "the problem of treatment would be to find some agent capable of thrombosing the vessels of a tumor and no others." Most perceptively, he also outlined a potential problem with vascular disrupting therapies when he stated that "even though all the vessels of a tumor could be thrombosed, there would often remain single cells or small groups of cells invading the surrounding tissue and supported, not by the blood-vessels of the neoplasm from which they escaped, but by the fluids imbibed from the normal tissues about them." As discussed below, it is precisely such a mechanism that is believed to explain the characteristic persistence of a so-called "viable rim" around the periphery of a tumor after treatment with a VDA.

## *2.3 Tumor Clamping Studies*

As indicated above, Juliana Denekamp and her colleagues at the CRC Gray Laboratory in the UK played an instrumental role in advancing the concept of vascular disrupting therapy and in providing an experimental basis for the rational development of effective small molecule therapeutics. Of key importance were a series of studies in which ischemia was induced by applying D-shaped metal clamps across the base of transplantable subcutaneous murine tumors (Denekamp et al. 1983). As one would expect from the studies on vessel torsion described above, the extent of tumor cell death was directly proportional to the duration of clamping. Temperature was also important with a much reduced rate of cell death observed for a given period of ischemia if tumors were allowed to cool during treatment (Chaplin and Horsman 1994b). Generally speaking, if tumors were maintained at

37°C greater than 99% of cells were killed if blood flow was interrupted for 2 h (Chaplin and Horsman 1994a, b). However, up to 15 h of vessel occlusion was necessary if the subsequent regrowth of treated tumors was to be prevented (Denekamp et al. 1983). In contrast, studies with C3H mammary tumors indicated that a 6 h period of ischemia was sufficient to cure three of seven treated tumors maintained at 37°C (Chaplin and Horsman 1994a, b). Similar results were also obtained using the CaNT tumor model (Parkins et al. 1994). Together with the work on testicular torsion, these studies demonstrate the potent impact of ischemia on tumor cell survival and suggest that shutting off the blood supply to a tumor for just a few hours may be sufficient to cause extensive cell death and necrosis.

## *2.4 Coley's Toxins*

There have been reports dating back to at least the beginning of the eighteenth century suggesting a causal relationship between infection, particularly bacterial infection, and cancer regression (Hoption Cann et al. 2002). Over the years, various attempts have been made to develop treatments that exploit this relationship. Among the best known early proponents of such an strategy was the New York surgeon Dr. William B. Coley (Hoption Cann et al. 2003). His interest was apparently triggered by his frustration over the poor prognosis of sarcoma patients in his care. In reviewing the associated medical records he became aware of the case of an apparently terminal patient who staged a remarkable recovery after suffering two episodes of erysipelas, associated with infection with the bacterium *Streptococcus pyogenes* (McCarthy 2006). Although this relationship had been noted by others (Busch 1866; Gresser 1987), Coley was among the first to deliberately inoculate cancer patients with bacterial preparations in an effort to induce a therapeutic response (Coley 1891). "Coley's Toxins," a mixture of killed *S. pyogenes* and *Serratia marcescens* (Coley 1914), has been evaluated in numerous clinical trials and although the results were at best mixed, the occasional response served to stimulate interest in this area. Subsequent analysis of the active components in Coley's Toxins suggested a key role for lipopolysaccharide (LPS) (Shear et al. 1943). Importantly from the perspective of vascular disruption, early studies in mice demonstrated that purified LPS can induce the collapse of tumor capillaries producing a pattern of hemorrhagic necrosis, particularly within central regions of a tumor, characteristic of that seen subsequently with small molecule VDAs (Shear 1944; Algire et al. 1952). Similar results were obtained with other non-specific bacterial immunostimulants including *Corynebacterium parvum* and bacillus Calmette-Guérin (BCG).

An important step in the understanding of the mechanisms involved in this effect came with the finding that LPS and can induce the production of various pro-inflammatory cytokines including one that is now known as tumor necrosis factor-alpha (TNF-α) (Carswell et al. 1975; Flick and Gifford 1986). As its name suggests, TNF-α can, in the absence of other factors, induce the collapse of tumor vessels triggering a necrotic response (Carswell et al. 1975; Flick and Gifford 1986). Although

TNF-α is clearly pleiotropic and has both positive and negative effects on endothelial cells (Pober 1987; Balkwill 1989), this finding served to validate vasculature as a target in cancer therapy. It is unfortunate that the systemic toxicity of TNF-α prevents its use as a vascular disrupting agent (Hundsberger et al. 2008). It should also be remembered that bacteria and their products can potentially affect the viability and function of endothelial cells through other mechanisms. For example, it has recently been shown that platelets activated by bacterial endotoxin, induced endothelial cells to produce reactive oxygen species that triggered apoptotic death through a caspase 8- and caspase 9-dependent process (Kuckleburg et al. 2008). Findings such as this help explain the endothelial damage associated with infection with certain bacterial species including *Haemophilus somnus* (Kuckleburg et al. 2008). Whether such bacteria have therapeutic potential in the treatment of cancer remains largely unexplored.

## 3 Vascular Disrupting Therapies Employing High Molecular Weight Agents

The physical obstacles that contrive to limit the efficacy of antibodies, peptides and other large high molecular weight reagents in cancer treatment are far less important in the context of vascular targeted anti-angiogenic and vascular disrupting therapies as the cells being targeted (i.e. endothelial cells) are in direct contact with the circulation and are thus readily accessible to intravenously administered agents (Thorpe et al. 2003). The possibility that limited damage to the vasculature may produce a large downstream effect is an additional benefit.

A number of determinants differentially expressed on the surface of tumor-associated vascular endothelial cells have been identified (Folkman 1999; Thorpe and Ran 2002; Enback and Laakkonen 2007) and in some cases antibodies or other molecules directed against these structures have been shown to express vascular disrupting activity of sufficient magnitude to impact on tumor grown in experimental systems (Thorpe 2004).

### *3.1 Engineered Ligands*

Ligands that interact with receptors that are induced or activated at sites of active angiogenesis can be engineered so as induce endothelial cell death or other changes upon binding. Examples include a fusion between the pro-angiogenic cytokine VEGF and the toxin gelonin, which acts as a potent inhibitor of protein synthesis ($VEGF_{121}$/rGel) (Veenendaal et al. 2002). Studies have shown that the purified homodimeric fusion protein selectively killed proliferating endothelial cells that overexpress the VEGF receptor Flk-1/KDR with an IC50 in the low nM range. Non-dividing endothelial cells were relatively resistant. In a prostate tumor model, $VEGF_{121}$/rGel caused thrombotic damage to tumor vessels, induced hemorrhagic necrosis and reduced

tumor volume (Veenendaal et al. 2002). The growth of MDA-MB-231 breast tumor metastases in SCID mice was similarly inhibited by treatment with $VEGF_{121}$/rGel (Ran et al. 2005b). As one might expect, the lung colonies that did grow in treated animals tended to be smaller and their vascularity was substantially reduced relative to controls (Ran et al. 2005b). The growth of orthotopic human bladder cancer xenografts was also inhibited by this treatment (Mohamedali et al. 2005).

## *3.2 Antibody-Based Approaches*

Encouraging results have been obtained using a number of antibodies and antibody fragments directed against proteins or other molecules that are upregulated or differentially expressed on tumor-associated blood vessels (Pasqualini and Arap 2002). Targets include receptors that bind various angiogenic factors, adhesion proteins that mediate the cell–cell and cell–matrix interactions involved in the formation of new blood vessels and lectins and other molecules induced in response to the plethora of pro-inflammatory cytokines and other stimuli produced within the tumor microenvironment (Thorpe 2004).

While antibody binding alone could potentially cause vessel occlusion as the result, for example, of complement activation, most strategies that have been explored so far have utilized immunoconjugates of one type or another. L19, a humanized scFv antibody fragment specific for the oncofetal ED-B domain of fibronectin fused to the extracellular domain of tissue factor can trigger clotting and block nutritive blood flow after being bound by immature and/or proliferating endothelial cells. The same antibody fragment has also been used with some success to target radioisotopes (Demartis et al. 2001) and various cytokines including TNF-α (Borsi et al. 2003; Balza et al. 2006), IFN-γ (Borsi et al. 2003), IL-12 (Gafner et al. 2006) and IL-15 and GM-CSF (Kaspar et al. 2007) to the tumor vasculature. Tissue factor can also produce vessel occlusion, tumor necrosis and tumor growth delay if localized to tumor vasculature using an antibody to the adhesion protein VCAM-1 (Ran et al. 1998; Dienst et al. 2005).

Recently, much interest in the area of antibody-mediated vascular disrupting therapy has focused on the targeting potential of anionic phospholipids. Although normally found only on the internal (i.e. cytoplasmic) surface of the plasma membrane, negatively charged phospholipids, including most notably phosphatidylserine (PS), are exposed on the outer surface of injured, activated and apoptotic cells. Unexpectedly, PS is also present on the luminal surface on a large proportion of apparently viable endothelial cells in tumor vessels (Ran et al. 2002). While the precise signals responsible for this effect remain to be determined, inflammatory cytokines, thrombin, acidity and periods of hypoxia and reoxygenation all trigger the surface expression of PS on endothelial cells in vitro (Ran and Thorpe 2002). Injury induced by exposure to reactive oxygen intermediates may be key (Ran and Thorpe 2002). Systemic administration of a monoclonal antibody designated 3G4 that specifically binds to surface-expressed PS in the presence of the plasma protein beta-2-glycoprotein 1 (Luster et al. 2006),

produced extensive vascular damage and a resultant reduction in vascular density and functional vascular volume in a number of murine tumor models (Ran et al. 2005a). Evidence suggests that monocyte-mediated antibody-dependent cytotoxic mechanisms may be involved in this effect (Ran et al. 2005a). While tumor growth was substantially reduced, normal tissues were unaffected (Ran et al. 2005a). Additional inhibition of tumor growth was obtained when anti-PS antibodies were combined with conventional cytotoxic agents including docetaxel (Huang et al. 2005) and gemcitabine (Beck et al. 2006). Radiation therapy also enhanced the vascular disrupting and anti-tumor activity of anti-PS antibodies (He et al. 2007). In this later case, there is evidence that exposure to radiation increases the expression of PS on the surface of endothelial cells in tumor vessels, which in turn improves the efficiency of antibody-dependent cell-mediated cytotoxicity (He et al. 2007).

The therapeutic potential of bavituximab, a chimeric version of the anti-PS monoclonal antibody 2aG4, is currently being investigated in three Phase II trials. Two are focused on advanced breast cancer and employ bavituximab in combination with docetaxel or paclitaxel and carboplatin respectively. In the third, bavituximab is being evaluated in combination with paclitaxel and carboplatin for the treatment of advanced non-small cell lung cancers. A Phase I trial of bavituximab monotherapy is also currently underway.

## *3.3 Gene Therapy*

While there are significant practical and regulatory obstacles to the commercial development of molecular approaches to cancer treatment, the exquisite targeting specificity that can be achieved through the use of such techniques has served to stimulate interest in this potentially important area (Edelstein et al. 2004).

A key factor that has limited the more widespread adoption of molecular therapies designed to target the malignant cell population is the relatively poor transduction efficiencies that can be achieved using currently available viral and non-viral vectors (Kouraklis 1999; Kesmodel and Spitz 2003; Dass and Choong 2006; Arnberg 2009). It is partly for this reason that vascular directed gene therapy approaches are so attractive, as there are grounds to believe that even modest damage to tumor vasculature may cause the death of substantial numbers of tumor cells if the gene being expressed results in vessel occlusion thereby preventing the delivery of essential oxygen and/or nutrients to the tumor site (Dougherty et al. 2004; Liu and Deisseroth 2006). Thus, in contrast to other forms of cancer gene therapy it does not seem entirely unreasonable to expect that dramatic anti-tumor effects may be produced even if the therapeutic gene in question is expressed only transiently in subset of endothelial cells in a tumor-associated vessel (Dougherty et al. 2004).

Among the genes that might prove useful in the context of vascular disrupting molecular therapies are those encoding the bacterial toxins *Pseudomonas* exotoxin A and diphtheria toxin, both of which posses potent ADP ribosyltransferase activity and can thus kill endothelial and other cell types by attacking elongation factor 2 and

inhibiting protein synthesis. Genes that encode enzymes that activate various prodrugs or which sensitize endothelial cells to the cytotoxic effects of ionizing radiation and/or chemotherapeutic agents all have their supporters. Genes that activate complement or induce coagulation are also attractive. Rather more speculatively, the results of studies employing small molecule tubulin-deploymerizing agents, as described below, suggest that some thought might be given to the therapeutic potential of genes that can alter the shape and/or adhesive properties of endothelial cells.

Finally, we have advocated a functional targeting strategy that employs genes that are activated to produce an effect (i.e. endothelial cell death) only when triggered by signals that are uniquely present within the tumor microenvironment. One example of this approach involves a chimeric protein in which the extracellular domain of the VEGF receptor Flk-1/KDR is fused in frame to the cytoplasmic death domain of the pro-apoptotic protein Fas (Carpenito et al. 2002; Dougherty and Dougherty 2009). Rather than triggering the growth promoting signals that are normally transduced when Flk-1/KDR binds the angiogenic cytokine VEGF, the chimeric Flk-1/Fas protein instead triggers apoptotic cell death when expressed in endothelial cells (Carpenito et al. 2002). Since the induction of apoptosis requires oligomerization of the chimeric receptor (Carpenito et al. 2002), death only occurs at sites where VEGF is present at a reasonably high level. This ensures that endothelial cells within the tumor microenvironment are selectively killed even if the therapeutic gene is widely expressed.

Endothelial cells can be readily transduced with viral and non-viral vectors both in vitro and in vivo (Nabel et al. 1991; Baker et al. 2005). Differential transduction of endothelial cells lining tumor-associated vessels is more challenging but is necessary if normal tissue damage is to be avoided (Baker et al. 2005). Approaches in which peptides are incorporated into viral receptors in order to redirect or restrict infection to cells expressing a particular differentially expressed counter-receptor have proven effective in endothelial cell targeting (Krasnykh et al. 1998; Cowan et al. 2003; Nicklin et al. 2004; White et al. 2004; Parker et al. 2005; Hajitou et al. 2006; Work et al. 2006; White et al. 2008). Although cell surface structures involved in angiogenesis or induced on endothelial cells in response to signals produced within the tumor microenvironment can be targeted, recently developed phage display techniques allow the identification of defined peptides that bind specifically to tumor-associated endothelial cells without any knowledge of the nature of the structures with which they interact (Nicklin et al. 2004).

Additional control of therapeutic genes in order to ensure that they act only on endothelial cells in tumor vessels can be achieved by placing their expression under the control of an appropriate promoter and/or enhancer element. Sequences upstream of a number of endothelial cell-specific genes have been cloned and several tested for their ability to drive gene expression within tumor-associated endothelial cells (Graulich et al. 1999; Jager et al. 1999; Nicklin et al. 2001; Dancer et al. 2003; De Palma et al. 2003; Greenberger et al. 2004; Work et al. 2004; Dong and Nor 2009; Hodish et al. 2009). The results so far have been encouraging. Screening strategies that permit the isolation of entirely synthetic regulatory elements that possess a desired level of specificity and activity have also proven fruitful in the context of endothelial cell targeting and are likely to grow in importance (Dai et al. 2004).

# 4 Small Molecule Vascular Disrupting Agents

## *4.1 Metals and Metalloids*

The induction of a massive necrotic response within a few hours of drug administration is a defining feature of agents that mediate their anti-tumor effects via vascular damage. Various metals and metalloids that have been used in cancer therapy over the years produce such an effect and in some cases this activity has been attributed to induced changes in blood flow. Thus early studies on lead colloids noted not only the rapid regression of large tumor masses (Fitzwilliams 1927) but related this activity to the thrombosis of tumor vessels (Mottram 1923; Wood 1926). Certain arsenic compounds, too, appear to induce both vascular damage and rapid tumor necrosis although such effects are generally only observed when these compounds are used at, or close to, their maximum tolerated dose (MTD) (Leiter et al. 1952). Of the compounds tested, trivalent arsenicals were among the most effective. More recent animal studies have confirmed that arsenic trioxide, which is employed primarily in the treatment of promyelocytic leukemia, has dramatic effects on blood flow in a number of solid tumor models (Lew et al. 1999; Griffin et al. 2003). It may be relevant in connection with the studies on colchicine described below, that arsenic trioxide has been shown to inhibit GTP-induced polymerization of monomeric tubulin and microtubule formation (Li and Broome 1999). Given their mechanism of action (Lew et al. 1999; Griffin et al. 2003) further rational development of these compounds as vascular disrupting agents may be warranted.

## *4.2 Flavonoids/Xanthenones*

Several investigators have demonstrated that the flavonoid Flavone Acetic Acid (FAA) can reduce tumor blood flow and trigger hemorrhagic necrosis in animal tumor models. The proposed mechanism of action has been attributed to the ability of FAA to trigger the local production of TNF-α by tumor-associated macrophages and/or other tumor-associated host cell types (Baguley 2001). Although a number of trials were initiated, the absence of convincing responses when used as a monotherapy ultimately caused clinical development to be discontinued. While there may be other reasons, the lack of an obvious effect of FAA in the clinic was attributed to the fact that, unlike the situation in rodents, the compound was a only a weak inducer of TNF-α by human cells (Philpott et al. 2001). However, we are now aware that because of the "viable rim effect" VDAs are unlikely to be very effective when used as a monotherapy and in the absence of tumor blood flow data it may have been premature to list this agent as an ineffective VDA in the clinic.

These studies nevertheless served to stimulate interest in finding structurally related compounds that retain activity in humans (Aitken et al. 1998; Pinto et al. 2005).

The most promising agent identified to date is 5,6-dimethylxanthenone 4-acetic acid (DMXAA) (Philpott et al. 1997, 2001; Baguley 2003). DMXAA performed well in certain animal models (Seshadri et al. 2007) both as a single agent and in combination with other treatments and has demonstrated evidence of blood flow reduction in the clinic (Zhou et al. 2002; Baguley and Wilson 2002; Baguley 2003; McKeage 2008; Rehman and Rustin 2008). Acquired by Antisoma, the compound has recently been licensed to Novartis AG and is now referred to as ASA404. In Phase II studies, ASA404 was evaluated in combination with conventional chemotherapy in the treatment of lung, prostate and ovarian cancers (McKeage et al. 2008, 2009). Phase III trials in lung cancer are currently underway and a breast cancer trial is planned (Rehman and Rustin 2008).

### *4.3 N-Cadherin Antagonists*

Adhesive interactions between endothelial cells play an essential role in maintaining the functional integrity of blood vessels (Blaschuk and Rowlands 2000; Vestweber et al. 2009; Gavard 2009; London et al. 2009). The cell surface structures that mediate such interactions are thus obvious targets for therapy (Blaschuk and Rowlands 2000; Lu et al. 2009; Alghisi et al. 2009). In this regard, a cyclic peptide termed ADH-1 or Exherin that blocks the homotypic binding of N-cadherin molecules has been shown to trigger blood flow reductions and hemorrhage within animal tumors (Kelland 2007; Li et al. 2007; Mariotti et al. 2007). Phase Ib/II and Phase II trials of ADH-1 monotherapy have already been completed and combination studies are ongoing (Perotti et al. 2009).

### *4.4 Colchicine*

Colchicine is an tricyclic alkaloid originally extracted from the Autumn crocus (Meadow saffron) *Colchicum autumnale*. While *Colchicum* preparations have been employed since at least Roman times as a treatment for gout, the use of colchicine as an anti-cancer agent has a more recent history. Among the key early studies were those of Eric and Margaret Boyland at the Chester Beatty Research Institute in London. Working with both transplantable and spontaneous chemically-induced tumors they demonstrated that intraperitoneal injection of colchicine could induce hemorrhagic necrosis similar to that produced by bacterial extracts (Boyland and Boyland 1937, 1940). They noted, however, that such effects only occurred at, or very close to, MTD.

Further work on the mechanism of action of colchicine on tumor tissue was carried out by Ludford (Ludford 1948). These important studies provided clear evidence that the anti-tumor activity of colchicine could be attributed mostly to vascular damage that preferentially affected newly formed tumor vessels. Again, it was

noted that these effects only occurred at doses that resulted in the death of significant numbers of treated animals (Ludford 1948).

Concerns over the low therapeutic index of colchicine, did not, at least initially, discourage its evaluation in the treatment of human tumors. In a study carried out by Seed et al. published in 1940 (Seed et al. 1940) two of four patients with large advanced carcinomas that received high doses of colchicine exhibited evidence of "rapid (tumor) degeneration" that occurred within a few days of treatment. Emphasizing the systemic toxicity of the doses of colchicine used in this study, the other two patients could not be evaluated as they died from the effects of colchicine poisoning! Interestingly, in the two patients that did survive, tumor control was only temporary, presumably because malignant cells surviving toward the periphery of the tumor mass rapidly repopulated necrotic regions re-establishing tumor growth. It is now appreciated that this presentation is typical of that seen with newer less toxic VDAs (Chaplin and Hill 2002; Davis et al. 2002; West and Price 2004; Gaya and Rustin 2005; Chaplin et al. 2006; Pilat and Lorusso 2006).

Other more recently discovered tubulin depolymerizing agents used in cancer therapy including podophyllotoxin (Leiter et al. 1950) and the vinca alkaloids vinblastine and vincristine (Baguley et al. 1991; Hill et al. 1994) also disrupt tumor vasculature and induce rapid hemorrhagic necrosis but as with colchicine do so only at doses near MTD.

## *4.5 Novel Vascular Disrupting Tubulin Depolymerizing Agents*

The encouraging results obtained with colchicine and related compounds motivated the search for novel tubulin depolymerizing agents that have vascular disrupting activity at doses well below MTD. These studies were facilitated by the development of a simple perfusion assay involving intravenous injection of the fluorochrome Hoechst 33342 that permitted the effect of drug treatment on tumor blood flow to be rapidly and quantitatively determined (Chaplin et al. 1987). Although most compounds possessed anti-vascular activity only when administered at near toxic doses, several agents were identified that had dramatic effects on tumor blood flow at doses as low as 1/10th MTD (Chaplin et al. 1996; Dark et al. 1997).

One of the first agents identified in this way, was the soluble phosphate prodrug of combretastatin A4 (CA4P), a compound isolated initially from the bark of the South African "bushwillow" *Combretum caffrum* by Pettit and colleagues in the early 1980s (Dark et al. 1997; Pettit et al. 1989; el-Zayat et al. 1993).

The active moiety CA4, released upon dephosphorylation of CA4P, binds rapidly to β-tubulin, at or near the site recognized by colchicine ($kd = 0.4 \pm 0.06\ \mu M$). It can competitively inhibit colchicine binding with a Ki of 0.14 μM and shares with colchicine the ability to prevent tubulin polymerization. Where it differs from colchicine is with respect to dissociation rate. While colchicine dissociates from tubulin with a half-life of approximately 405 min at 37°C, CA4 has a half-life of only 3.6 min. It is this characteristic of CA4 that in part explains the absence of the

toxicities commonly associated with tubulin-directed anti-mitotic agents when the compound is administered in vivo.

Similar functional screening approaches have been used by other groups to identify additional agents that can disrupt tumor blood flow and induce a necrotic response. Of those that have progressed furthest in human trials, most, including ZD6126 (Angiogene), MN029/Denibulin (Medicinova), AVE8062E (Sanofi Aventis), NPI-2358 (Nereus) and BNC-105 (Bionomics) also bind to and disrupt tubulin (Hinnen and Eskens 2007; Cai 2007; Lippert, 2007). A number of other tubulin-binding agents that were originally identified on the basis of their anti-mitotic activity have subsequently been tested for vascular effects. Examples include MPC6827 (Azixa, a brain-penetrating anti-mitotic from Myriad), ABT751 (Abott, an oral anti-mitotic), LP261 (an oral anti-mitotic from Locus), CYT997 (Cytopia) and EPC2407 (Epicept). Although it is not unexpected that certain of these agents will, like vinblastine, vincristine and colchicine, possess tumor-selective VDA activity, it remains to be determined whether the doses required to achieve such effects are sufficiently below MTD so as to permit them to be used in this manner.

The question of specificity is obviously key to the success of VDAs. When administered in vivo, VDAs appear to cause the immature endothelial cells lining the structurally abnormal blood vessels that supply a growing tumor mass to round up and detach from the basement membrane (Blakey et al. 2002b). Intravascular coagulation is subsequently induced resulting in vessel blockage and the slowing or cessation of nutritive blood flow (Blakey et al. 2002b). Without adequate oxygen and nutrients, cells soon die and a massive necrotic response results particularly within central regions of a treated tumor mass (Blakey et al. 2002b). In part, the selective destruction of tumor vasculature can be attributed to the reliance of endothelial cells in newly formed or immature vessels on a tubulin cytoskeleton for the maintenance of their elongated shape, while in more mature non-proliferating endothelial cells this function is largely supplanted by actin (Gotlieb 1990; Galbraith et al. 2001; Lee and Gotlieb 2005). CA4P has also been demonstrated to disrupt adhesive interactions between endothelial cells mediated by the vascular endothelial (VE)-cadherin/β-catenin complex (Vincent et al. 2005). The presence of smooth muscle cells, a characteristic feature of normal tissue vasculature, inhibits this disruption (Vincent et al. 2005). The targeting of recently formed endothelial cells in immature or abnormal vessels which lack a full complement of smooth muscle or pericyte support is thus believed to be responsible in large part for the specificity of tubulin binding VDAs.

It has been suggested that an early consequence of endothelial cell shape change is an increase in vascular permeability. Clearly, if rapid changes in endothelial cell morphology and detachment do occur in vivo, exposure of the basement membrane and a physical narrowing of the vessel lumen will contribute to the reduction in capillary blood flow, increasing vascular resistance as well as inducing hemorrhage and coagulation. The sensitivity of the immature tumor vasculature to CA4P probably relates to not only structural differences between newly formed and mature endothelial cells and the absence or presence of support cells but also to characteristics of the tumor microcirculation such as high

interstitial fluid pressure, pro-coagulant status, vessel tortuosity and heterogeneous blood flow distribution.

Pharmacokinetic considerations are also likely to be important. Thus, in contrast to established cytotoxic agents such as vinblastine or colchicine that bind to and destabilize tubulin, or microtubule-stabilizing cytotoxins such as paclitaxel and docetaxel, the depolymerizing activity of VDAs is rapidly reversible (Blakey et al. 2002b; Chaplin and Hill 2002). As the compounds also have a relatively short plasma elimination half-life following intravenous administration (Blakey et al. 2002a; Dowlati et al. 2002; Beerepoot et al. 2006), effects on the shape and adhesive properties of immature tubulin-dependent endothelial cells are achieved without the toxicities commonly associated with the use of tubulin-directed anti-mitotic drugs (Beerepoot et al. 2006; LoRusso et al. 2008).

Although almost all the focus on the development of VDAs has centered on solid tumor indications, CA4P has recently been shown to elicit significant anti-tumor activity against orthotopically implanted leukemia when used as a single agent (Petit et al. 2008). This activity is believed to result from the ability of CA4P to alter the adherence and attachment of leukemic cells which exist in treatment resistant stromal niches (Petit et al. 2008). It is probable that, as with the effects on immature endothelial cells, alterations in both cell shape and adhesion molecule expression and/or function trigger this release. These results offer the possibility that tubulin binding VDAs may have a role in the treatment of chemotherapy resistant leukemias (Petit et al. 2008; Fang et al. 2008; Xu et al. 2008; Billard et al. 2008).

## 5 Combining VDAs with Other Therapies

As Woglom predicted almost 100 years ago (Woglom 1923), a characteristic of VDA therapy, is the persistence around the periphery of a treated tumor of a layer of viable cells that survive because they obtain the oxygen and nutrients necessary to remain viable, by diffusion from unaffected mature vessels present in surrounding non-malignant tissues (Chaplin and Hill 2002; Davis et al. 2002). In the absence of further treatment, this so called “viable rim” can serve as a reservoir from which malignant cells can invade and repopulate the necrotic central regions of a treated tumor (Chaplin and Hill 2002; Davis et al. 2002). It is for this reason that VDAs are generally most effective when used in combination with conventional cytotoxic agents or radiation therapy that kill the comparatively well-oxygenated and mitotically active cells remaining within the viable rim (Thorpe 2004; Siemann et al. 2004; Siemann and Horsman 2004; Siemann and Shi 2004).

As repopulation of the necrotic regions produced within a tumor as a result of VDA treatment is dependent upon revascularization, it follows that combining small molecule VDA approaches with anti-angiogenic therapies may provide another way of slowing or preventing tumor regrowth. VEGF, upregulated in response to hypoxic stimuli, is a key regulator of revascularization following vascular shutdown (Ferrario et al. 2000) and therapies that target this particular pro-angiogenic pathway

have proven effective when used in conjunction with VDAs in pre-clinical studies (Siemann and Shi 2004; Shi and Siemann 2005; Siemann and Shi 2008). This strategy has now moved into clinical testing.

VDA treatment also appears to stimulate the release of circulating endothelial cell progenitor cells (CEPs) from the bone marrow and their recruitment to the tumor (Shaked et al. 2006). It has been suggested that such cells may contribute to both new vessel formation and the rapid "recanulisation" of recently blocked sections of vessels following VDA treatment (Shaked et al. 2006). Interestingly, recent evidence suggests that anti-angiogenic therapies and approaches that target local angiogenic responses can inhibit the VDA-induced release of CEPs (Shaked et al. 2006).

## 6 Clinical Experience with VDAs

Small molecule VDAs first entered clinical testing over 10 years ago and three agents are now in Phase III clinical trials. Table 1 lists the current clinical status of small molecule VDAs. As the clinical experience with VDAs has been the subject of several recent reviews (Siemann et al. 2009; Chaplin et al. 2006; Siemann and Chaplin 2007) and is covered in another chapter in this book, only a brief summary of findings will be discussed here. The main finding from Phase I studies are that these agents are able to induce blood flow reductions in a range of solid tumors. Surprisingly given their mode of action which, in the absence of a cytotoxic component, spares a viable rim of tumor cells, a number of objective tumor responses were seen when these agents were administered as monotherapy. However, Phase II studies have focused on combinations with conventional cytotoxic/antiproliferative chemotherapy with a particular focus on platinum and taxane based treatment regimes. These combinations have been well tolerated, as would be expected from their non overlapping toxicity profiles. Encouraging signs of anti-tumor activity in these trials have led to the initiation of ongoing Phase III trials in lung, sarcoma and anaplastic thyroid.

As discussed above the potential of combining VDAs with anti-angiogenic treatments is receiving increased attention. The encouraging preclinical data obtained to date has led to the completion of a Phase I trial using CA4P in combination with bevacizumab. This trial demonstrates that the combination is well tolerated and in turn has led to an ongoing Phase II trial in Stage IIIb/IV NSCLC where CA4P is added to the approved treatment of bevacizumab with carboplatin and paclitaxel.

One of the most common side effects seen in the clinic, certainly with VDAs which act through depolymerization of tubulin, is transient hypertension (Rustin et al. 2003; Zweifel et al. 2009). Microtubules help resist constriction of smooth muscle cells and thus their depolymerization may make vessels more sensitive to vasoconstriction. The use of anti-hypertensives such as nitrates and calcium channel blockers has been shown to eliminate the blood pressure effects of VDAs both in animals and in patients (Gould et al. 2007; Zweifel et al. 2009). The importance of this finding is that if left uncontrolled acute hypertensive episodes can, in the presence of underlying cardiovascular disease, lead to cardiac toxicity (LoRusso