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The Unique Characteristics of Tumor Vasculature and Preclinical Evidence for its Selective Disruption by Tumor-Vascular Disrupting Agents

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Abstract

The vasculature of solid tumors is fundamentally different from that of normal vasculature and offers a unique target for anti-cancer therapy. Direct vascular-targeting with Tumor-Vascular Disrupting Agents (Tumor-VDAs) is distinctly different from anti-angiogenic strategies, and offers a complementary approach to standard therapies. Tumor-VDAs therefore have significant potential when combined with chemotherapy, radiotherapy, and angiogenesis-inhibiting agents. Preclinical studies with the different Tumor-VDA classes have demonstrated key tumor-selective anti-vascular and anti-tumor effects.

Keywords

Tumor-vascular disrupting agents; Tumor-VDAs; ASA404; Tubulin-binding agents; CA4P; Tumor vasculature; Anti-vascular therapy

Tumor Vasculature

The blood supply to the normal tissues of the body is maintained by an orderly and efficient vascular network. Blood vessels are regulated by the metabolic demand-driven balance of pro-angiogenic and anti-angiogenic molecular factors and a systematic network of lymphatic vessels which drain fluid and waste metabolic products from the interstitium. The resulting microarchitecture of normal vascular networks is hierarchically organized, with mature vessels that are evenly distributed to allow adequate perfusion of oxygen and other nutrients to all cells (Figure 1A).

In tumors, the aggressive growth of the neoplastic cell population and associated overexpression of pro-angiogenic factors leads to the development of disorganized blood vessel networks that are fundamentally different from normal vasculature. Tumor vasculature is typified by aberrant structural dynamics and vessels that are immature, tortuous, and hyperpermeable. The complex tumor vasculature is typically a disorganized

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Conflict of Interest Statement

The author is a member of the Scientific Advisory Board of OXiGENE, Inc.

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labyrinth of vessels with a lack of conventional blood vessel hierarchy in which arterioles, capillaries, and venules are not clearly identifiable (Figure 1B).¹ Blood vessels are of inconsistent diameter and uneven shape (arrowed) with abnormal bulges and blind ends (circled), arteriolar–venous shunts, and plasma channels lacking red blood cells.^{1–3} Similarly, the accompanying lymphatic vessels are dilated, leaky and discontinuous leading to dilated fluid-engorged vessels.^{4,5}

Functionally, the ability of the tumor vasculature to deliver nutrients via blood vessels and remove waste products via the lymphatic system is drastically diminished. Tumor vessels are more permeable than normal vessels; their immature nature means they are poorly invested with smooth muscle cells and may have a discontinuous endothelial cell lining with an abnormal basement membrane.^{6,7} Increased vessel permeability results in aberrant osmotic forces, leading to accumulation of vascular contents and elevated interstitial fluid pressure.^{8,9} Geometric resistance caused by irregular vessel shape and diameter leads to impaired blood flow, consequently there is often an inadequate oxygen supply to tumor cells with micro-regional hypoxia.^{8–11} These consequences of high structural heterogeneity and uneven flow can readily be demonstrated by computer visualizations of normal and tumor vascular networks. Reductions in calculated oxygen tension (pO_2) in areas of geometric resistance to blood flow and vessel blind ends are clearly identified (Figure 1C–D).¹²

The abnormal characteristics of tumor vasculature lead to aberrant micro-environmental conditions that obstruct traditional therapeutic anti-cancer strategies.⁹ Microregional hypoxia can result in resistance to both radiotherapy¹³ and chemotherapy.¹⁴ Nonetheless, the unique features of tumor vasculature compared with that of normal tissues also present an opportunity for selective therapeutic intervention.

Selective Targeting of the Tumor Vasculature

Targeting the angiogenesis-driven sprouting of new vessels,^{6,15} has seen a revolution in anti-cancer drug development in the past decade. The observation that tumors cannot grow beyond a size of approximately 2 mm³ without the support of neovascularization¹⁶ has led to the clinical development of a plethora of angiogenesis-inhibiting agents (AIAs) that target vascular endothelial growth factor (VEGF) and its receptor (VEGFR).^{17–19} Ongoing anti-angiogenic drug development is also evaluating the potential benefits of targeting a number of other pro-angiogenic pathways, including those involving basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), placental growth factor (PlGF), insulin-like growth factor (IGF), mammalian target of rapamycin (mTOR), and histone deacetylases.^{20–25}

A number of other approaches have sought to target tumor endothelial cells. These include the use of peptides, as well as antibodies directed toward tumor endothelial cell-specific antigens, to deliver bound endothelial cell-damaging agents.^{26–28} Gene therapy with endothelial cell-specific promoters has also been evaluated.²⁹ A number of endothelial cell-specific vectors based on gene promoters are now known but clinical progress has not been documented.^{28,30–32}

An alternative therapeutic approach that directly targets already established tumor vasculature has resulted in the evolution of a novel class of agents known as Tumor-Vascular Disrupting Agents (Tumor-VDAs). Tumor-VDAs selectively disrupt the immature and rapidly (>20 times faster than normal)^{33,34} proliferating endothelial cells of established tumor vasculature either by direct apoptotic effects or by effects related to endothelial cell reliance on a tubulin cytoskeleton to maintain cell shape. These agents aim to arrest the blood flow in tumors, with the resulting ischemia leading to a cascade of secondary tumor

cell death in the central part of tumors.^{26,35,36} A clear division between Tumor-VDA and anti-angiogenic therapies has now been established (Table 1).

Tumor-VDA: Comparison with AIAs

AIAs and Tumor-VDA differ in three key respects: their physiologic target, the type or extent of disease that is likely to be susceptible, and the treatment scheduling.³⁷ Since AIAs are cytostatic in nature, and designed to inhibit the progressive development of tumor neovasculature, they are likely to be inherently tailored toward the targeting of early-stage disease or newly developing metastases.³⁷ The usual course of administration of AIAs is thus one of chronic exposure, where protracted administration or exposure restrains revascularization following initial inhibition, and results in disease stabilization rather than tumor shrinkage.^{20,38–40} In contrast, Tumor-VDA exert a more immediate damaging effect on existing tumor vasculature, and are therefore suited to acute administration, requiring a shorter period of drug exposure. Tumor-VDA lead to the collapse of existing tumor vasculature and secondary tumor cell death, with evidence for a superior effect on bulky disease.^{41,42}

Preclinical studies have not established tumor necrosis as a predominant effect with AIAs, although there is clinical magnetic resonance imaging (MRI) and pathological evidence with some agents.^{43,44} Tumor-VDA, on the other hand, are distinctive in their propensity for causing extensive centrally situated tumor necrosis.^{36,45–53} These key differences are conceptually illustrated in Figure 2.^{54,55}

Both classes of agents have found utility in combination with standard therapies, but for different reasons. Tumor-VDA may be complimentary to radiotherapy and chemotherapy because they predominantly target the tumor core, a region of the tumor typically resistant to conventional anti-cancer therapies. AIAs on the other hand, selectively reduce immature vessel numbers, which may lead to normalization of the peripheral tumor vasculature and thus improved delivery of systemically administered chemotherapy.⁵⁶

A prime target for AIAs is VEGF, and although VEGF is over-expressed by most solid tumors, it is also essential for the development of normal blood vessels. The wide expression of VEGF and its receptors in normal tissues therefore means that normal vascular networks may be affected. The degree of this inhibition is dependent upon the specificity of the inhibitor type. Preclinical studies in mice have shown that VEGF inhibitors may cause both the apoptosis of endothelial cells and regression of normal capillaries in various organs.^{57,58} Vascular effects that occur as a result of systemic VEGF inhibition include hypertension, proteinuria^{59,60} and impaired wound healing.⁶¹ A more selective targeting of fundamental structural differences between normal and tumor vasculature would potentially be of significant clinical therapeutic benefit. Tumor-VDA seek to exploit these differences while minimizing concurrent effects on normal vasculature.

Classes of Tumor-VDA and their Mechanisms of Action

There are currently two classes of Tumor-VDA (Table 2). The tubulin-depolymerizing Tumor-VDA comprise a large and diverse group of compounds that bind to the colchicine binding site of tubulin.^{62–64} These small-molecules are usually either stilbenes of the combretastatin family or heterocyclic compounds. Lead agents of this class include combretastatin A-4 phosphate (CA4P, fosbretabulin),^{45,65,66} a serine-linked amino-derivative – AVE8062,⁴⁸ and the combretastatin A-1 derivative OXi4503.⁶⁷ Other Tumor-VDA that also bind at the colchicine site include the *N*-acetyl colchinol ZD6126, the dolastatin-10 analogue TZT-1027 and other heterocyclic compounds such as MPC-6827, MN-029, NPI-2358 and ABT-751.^{50,68–70} In all cases, binding of these agents to tubulin

causes microtubule depolymerization, cytoskeletal rearrangements and activation of actin stress fibers in endothelial cells, leading to changes in cell morphology (Figure 3).^{47,51,53,66,71–73}

Importantly, these agents selectively disrupt the cytoskeleton of proliferating endothelial cells.⁷¹ Both *in vitro* and *in vivo* studies in mice with the archetypal tubulin-binding Tumor-VDA, CA4P have demonstrated that the drug selectively induces regression of unstable tumor neovessels,^{74–76} in part by disruption of the signaling pathway of the endothelial cell-specific junctional protein, VE-cadherin.⁶⁶ Activation of Rho signaling has been implicated in microtubule disruption and vessel collapse using selective inhibitors of Rho kinase to attenuate tubulin-dependent Tumor-VDA activity.⁷⁷ The net result of these effects is a rounding up and surface blebbing of endothelial cells, together with increased vessel permeability and inhibition of blood flow.^{71,72,76,77} Rho-mediated active vasoconstriction and red cell stacking leads to further flow stagnation and vessel blockage.^{71,72,76} Normal vasculature (including smooth muscle cells) with a lower endothelial proliferation index and greater maturity, remains unaffected by tubulin-binding Tumor-VDAs (Figure 4).^{78,79}

Flavonoid Tumor-VDAs have a tubulin-independent mechanism of action that results in both direct and indirect antivascular activity. This class is led by ASA404 (vadimezan, 5,6-dimethylxanthenone-4-acetic acid/DMXAA), an analog of flavone acetic acid. Direct disruption of the tumor vasculature by flavonoid Tumor-VDAs may be due to induction of apoptosis in tumor blood vessel endothelial cells. This effect has been detected within 30 minutes of administration in animal models (Figure 5).^{80,81} A large and early influx of neutrophils into subcutaneous Colon 38 tumors occurs following ASA404 treatment, and neutrophils have therefore been suggested as mediators of the drug's rapid anti-vascular effects.⁸² Activated neutrophils are strongly implicated in endothelial cell damage and killing during inflammation.⁸³ Increased myeloperoxidase activity, which is indicative of neutrophil activity, has also been reported following treatment with the tubulin-binding Tumor VDA CA4P in murine sarcomas.⁸⁴

Endothelial cell death leads to exposure of the basement membrane, rupture of tumor blood vessels, and extravasation of erythrocytes into the surrounding tissues.^{80,81,85} Flavonoid Tumor-VDA-induced vascular damage leads to platelet accumulation within the damaged vessels, triggering the release of the vasoconstrictor 5-hydroxytryptamine (5-HT, serotonin), detected as its liver metabolite 5-hydroxyindole-3-acetic acid (5-HIAA).^{86–88} This direct disruption of the tumor vasculature leads to a rapid inhibition of tumor blood flow.^{80,81,85,87} Preclinical studies have revealed that flavonoid Tumor-VDAs can also indirectly affect the tumor vasculature by stimulating the production of cytokines such as tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6), macrophage inflammatory 1 α (MIP 1- α), interferon- γ , and chemokines such as interferon-inducible protein-10.^{81,82,87,89–93} Induction of these cytokines may also amplify the initial influx of neutrophils, providing sustained anti-vascular action.⁸² Evidence supporting the role of TNF- α in inducing vascular collapse is provided by the significant reductions in antivascular activity in TNF^{-/-} or TNF receptor^{-/-} (TNFR^{-/-}) knockout mice.^{80,90}

***In Situ* Effects of Tumor-VDA Therapy**

Tumor-VDAs have now been studied in a wide variety of preclinical tumor models, including transplanted and spontaneous rodent tumors, orthotopically transplanted tumors, and human tumor xenografts.^{28,72,75,94} Profound disruption of the tumor blood vessel network has been noted—effects include vascular shutdown, reductions in tumor blood flow, vessel permeability changes, and loss of patent blood vessels. Within minutes of Tumor-VDA treatment, tumor perfusion begins to be compromised. The suppression of tumor blood

flow by both flavonoid and tubulin-binding Tumor-VDA is rapid, dose dependent, and typically sustained for 24–48 hours, with maximal vessel shutdown and permeability changes occurring within 1–6 hours.^{36,47,50,74,80,81,91,95–103} In contrast, such extensive blood flow effects have not been seen in normal tissues.^{29,35} However, since these assessment endpoints are not practical in the clinic, efforts to monitor the effects of Tumor-VDA treatments utilizing non-invasive methods that could be applied in such a setting have begun. MRI studies in an orthotopic model of human head and neck cancer treated with the flavonoid Tumor-VDA ASA404 showed a marked decrease in enhancement within the tumor after contrast imaging, indicative of treatment-induced reduction in vascular perfusion 24 hours after infusion, together with hypo-intense regions within the tumor, indicating tumor hemorrhage, and no observable effects on surrounding tissues (Figure 6).¹⁰⁴ In a study of a tubulin-binding Tumor-VDA, changes in tumor perfusion and tumor necrotic fraction after CA4P treatment were compared in the same individual animals.¹⁰⁵ The results demonstrated that tumor perfusion as observed by MRI strongly correlated with tumor necrosis (Figure 7). Dynamic contrast-enhanced (DCE)-MRI measurements in patients also demonstrated specific changes in tumor perfusion after Tumor-VDA treatment,^{106–108} but these have as yet not been linked to a defined treatment outcome.

The impact of vascular disruption by Tumor-VDA treatments on tumor tissue has been readily demonstrated both by histologic assessments and measures of secondary cell death due to ischemia; two factors that are closely correlated.^{42,52,99,109} Typically, these show extensive, dose-dependent necrosis that can extend to within a few cell layers from the margin of the tumors (Figures 4 and 8).^{28,75,76,94} Histologic evidence for tumor necrosis induced by both flavonoid Tumor-VDA (ASA404^{89,92}) and tubulin-binding Tumor-VDA (ZD6126,^{52,53,109} CA4P,^{36,45,46} AVE8062,^{47,48} OXi4503,^{49,67} MN-029⁵⁰ and TZT-1027⁵¹) has been reported in numerous preclinical tumor models. Importantly, vascular damage resulting from tubulin-binding Tumor-VDA has been confined to tumor blood vessel networks (Figure 4). Similarly, immunostaining and histologic analyses have highlighted the selective nature of ASA404-induced vascular damage and necrosis in these preclinical studies, showing no toxicity in normal salivary gland, heart, liver and skeletal muscle tissues.¹⁰⁴

Blood pressure may be elevated by tumor blood vessel-directed anti-cancer treatments such as anti-angiogenic therapies^{108,110–112} and Tumor-VDA.^{108,113} In mice and rats, tubulin-binding Tumor-VDA can induce hypertension^{114,115} similar to that seen in humans.^{108,113} For example, treating tumor-bearing mice with a 100 mg/kg dose of CA4P raises mean arterial pressure to approximately 130 mm Hg within 1 hour of treatment before returning to near normal 3–4 hours later (Figure 9). Several strategies to counteract tubulin-binding Tumor-VDA-associated hypertension have been investigated preclinically. In mice, administering the vasodilator hydralazine (0.2 mg/kg) just prior to CA4P treatment inhibited the rise in blood pressure seen after CA4P exposure (127 ± 6 mm Hg) to pretreatment values (103 ± 6 mm Hg) (Siemann *et al.*, unpublished results). In rats, infusions of the calcium channel blocker diltiazem and of the vasodilator nitroglycerin resulted in near complete blockage of CA4P-induced hypertension¹¹⁵ and co-administration of the channel blocker atenolol and the beta blocker nifedipine effectively inhibited transient hypertension induced by the tubulin-binding Tumor-VDA ZD6126.¹¹⁴ Gould *et al.* further noted that in susceptible strains of rats tubulin-binding Tumor-VDA-induced blood pressure elevation could lead to detectable cardiac damage; a result that could be prevented by inhibiting the hypertensive response.¹¹⁴ Taken together, these preclinical investigations suggest that treatment with anti-hypertensive agents may prove clinically valuable to prevent potential cardiovascular side effects of Tumor-VDA. Perhaps most importantly, the anti-tumor efficacy of the tubulin-binding Tumor-VDA was still maintained in the presence of anti-hypertensive medications.¹¹⁴ Non-dose-limiting hypertension in patients given the flavonoid

Tumor-VDA ASA404 has only been seen at doses approaching the maximum tolerated dose in Phase I clinical trials,^{116,117} and was not observed in Phase II trials.^{118,119} Nonetheless, monitoring and controlling hypertension as well as excluding patients with a history of cardiovascular disease will be a key element in the Phase II/III protocols with both flavonoid and tubulin-binding Tumor-VDAs as it has been with the anti-angiogenic therapeutics bevacizumab and sorafenib.¹¹¹

The propensity of both classes of Tumor-VDAs to induce necrosis in the poorly perfused core regions of tumors leaving a thin layer of viable cells at the periphery is well documented (Figures 4 and 8).^{98,120–122} This residual rim of viable neoplastic cells is generally believed to survive because these cells derive their nutritional support from vasculature in the adjacent normal tissue which is unaffected by Tumor-VDA treatment.⁷⁵ Recent studies have used spectral imaging of tumor microvessel hemoglobin saturation with mouse window chamber tumors to measure the real-time response of tumors to Tumor VDA treatments. These studies have revealed not only transient vessel collapse with time-dependent oxygenation changes followed by recovery but also extensive vascular remodeling and neovascularization of the tumor rim (Figure 10).¹²³ Thus despite the extensive blood flow shutdown and central tumor necrosis observed with Tumor-VDAs, the surviving 'viable rim' can act as a source of tumor regrowth. Consequently, only repeated multiple-dose treatments with such agents impact tumor growth significantly^{52,75,94,124} and Tumor-VDA treatments alone are unlikely to eradicate the tumor mass. Nonetheless, the destruction of large tumor areas, particularly in the central regions and areas typically most resistant to radiation and chemotherapy, is clearly highly beneficial and desirable. Tumor-VDAs are therefore likely to be of greatest utility when applied in a combined modality setting with conventional anti-cancer therapies.

Combination of Tumor-VDAs with Other Therapies

1. Radiotherapy

The cellular response to radiation has long been known to be strongly dependent upon oxygen concentration.¹²⁵ Since Tumor-VDAs eliminate large portions of oxygen-deficient hypoxic cells from solid tumors, the combination of such agents with radiotherapy is logical. Indeed, it has now been well established that combining localized radiotherapy with various Tumor-VDAs results in significantly enhanced tumor cell killing and tumor growth inhibition compared with radiotherapy alone.^{42,74,94,120,126–128} Figure 11 illustrates the reduction in clonogenic cell survival in murine KHT sarcomas treated with increasing single doses of radiation administered in combination with ASA404 (A)¹²⁶ or OXi4503 (B).^{74,79,94} Enhancement of radiation damage has also been reported for other tubulin-binding Tumor-VDAs including ABT-751, CA4P, MN-029 and TZT-1027.^{42,74,94,127,128} In these studies the Tumor-VDA is typically administered 1–3 hours post radiation treatment – thus avoiding any possible negative effects on radiation efficacy that would arise if the Tumor-VDA treatment rendered some tumor cells hypoxic at the time of irradiation by inducing transient reductions in tumor blood flow.^{74,94} In the case of ASA404, the addition of hypoxia-selective bioreductive drugs such as tirapazamine and CI-1010 further enhanced the tumor response to ASA404 plus radiation, suggesting ASA404 treatment did not entirely eliminate the population of hypoxic cells affecting radiation response.⁹⁸

Clinically most radiotherapy is delivered using daily fractionated dose treatments, therefore the incorporation of Tumor-VDA exposures into such a setting has also been evaluated. In the case of the tubulin-binding Tumor-VDAs CA4P and ZD6126, the drug was administered after the last radiation fraction at the end of each week of treatment. This resulted in a significantly enhanced tumor response to fractionated radiotherapy.^{35,42} Studies combining the flavonoid Tumor-VDA ASA404 with fractionated radiotherapy also reported improved

treatment outcomes.¹²⁰ Interestingly, when ASA404 was utilized it was administered successfully during the course of fractionated radiation.¹²⁰ Importantly, Tumor-VDA have shown neither significant effects on the radiation response of early-responding normal tissue such as skin,^{120,126,129} nor any effects on late-responding normal tissues such as bladder and lung.¹³⁰ Taken together, these findings support the notion that combining Tumor-VDA with radiotherapy may yield a therapeutic benefit.

2. Chemotherapy

Preclinical studies on Tumor-VDA combined with various chemotherapeutic agents have demonstrated improved anti-tumor activity compared with chemotherapy alone. Enhanced therapeutic interactions with the flavonoid Tumor-VDA ASA404 in combination with a number of different cytotoxic agents have been reported in the MDAH-MCa-4 mouse mammary tumor; most notably taxanes (Figure 12).^{102,131,132} Studies with paclitaxel in human non-small cell lung cancer (NSCLC) xenografts have also shown synergistic activity, as well as tumor cures.^{131,133} In contrast, no tumor cures were observed when either agent was used alone.¹³³ Marked potentiation of docetaxel by ASA404 has also been observed in preclinical studies in human prostate cancer xenografts, resulting in a 43% cure rate with no significant increase in host toxicity.¹³⁴ An additive or synergistic effect and thinning of the viable rim has been demonstrated with tubulin-binding Tumor-VDA such as ZD6126⁵² and CA4P^{29,102,135} when combined with various chemotherapeutic agents. Particular efficacy was noted for CA4P in combination with paclitaxel and manumycin A or carboplatin in anaplastic thyroid mouse xenografts.¹³⁶ The related drug AVE8062 in combination with docetaxel significantly prolonged survival in HeyA8-injected mice.⁴⁸

The improved treatment response to chemotherapy upon addition of Tumor-VDA has been attributed to the elimination of those poorly perfused regions of the tumor that are either inaccessible for effective drug delivery or resistant to chemotherapeutic agents due to their proliferation status.^{29,52,74,102,132,135,137} Blood flow reductions caused by vascular disruption may also lead to drug entrapment and an improved response through increased tumor exposure to the drug.^{102,136–138} As with radiotherapy, the schedule of administration of chemotherapeutic agents and Tumor-VDA is critical since rapid vascular disruption may render tumor cells inaccessible to chemotherapy.^{102,139} Preclinical studies with the flavonoid Tumor-VDA ASA404 suggest that a chemotherapeutic agent should be given either before or shortly after Tumor-VDA administration to avoid compromised delivery.¹³² Scheduling studies with tubulin-binding Tumor-VDA indicate that administering the chemotherapy a few hours before may be optimal.^{102,109} When the tubulin-binding Tumor-VDA ZD6126 was combined with a microtubule stabilizing drug, maximum benefit was obtained when the Tumor-VDA was given 72 hours after taxane treatment.¹⁴⁰ Importantly, the inclusion of the antivascular agents did not increase bone marrow stem cell toxicity associated with these anti-cancer drugs, thus giving rise to a therapeutic gain.¹⁰²

Nitric oxide generation has been shown to protect tumor vasculature against Tumor-VDA-induced injury through anti-neutrophil action.⁸⁴ Tumor-VDA have therefore also been investigated in combination with nitric oxide synthase inhibitors. Repeated dosing of *N*-nitro-*L*-arginine with CA4P produced significantly enhanced growth delay in p22, CaNT and mouse mammary tumors. Nitric oxide synthase inhibitors may therefore have utility in combination with other Tumor-VDA in development.^{141,142}

Demonstrating improved tumor responses through the combination of Tumor-VDA and chemotherapy will only be of benefit if such a combined modality treatment does not enhance the response of critical normal tissues. Results from preclinical investigations addressing this question indicate the enhancements in anti-tumor efficacy generally occur without any significant increase in host toxicity.^{29,52,98,102,132,138,139} Data on

chemotherapeutic agent-specific side effects are more limited but the absence of enhanced bone marrow toxicity is encouraging.¹⁰²

3. AIAs

Vascular-targeted therapies have shown impressive anti-tumor effects in preclinical tumor models, and recent clinical observations are encouraging. Nevertheless, the complexity of pathways available for neovascularization implies that impairing only a single aspect of angiogenesis with AIAs will probably not suffice, while Tumor-VDA will not be able to eliminate pockets of tumor cells with a nutritional supply derived from blood vessels in the surrounding normal tissues. A logical extension in vascular targeting is therefore the application of anti-angiogenic and vascular disrupting therapies in concert. Since both the initiation of new vessel formation and the integrity of the existing blood vessel network are critical to tumor growth and survival, such a double assault on the tumor vasculature should hold considerable promise. In view of their disparate modes of action, the combined application of AIAs and Tumor-VDA is likely to lead to complimentary anti-tumor effects.³⁷ This possibility has been supported by observations in preclinical tumor models. For example, the combination of VEGFR2-associated tyrosine kinase inhibition (ZD6474) and Tumor-VDA therapy (ZD6126) was found to lead to marked improvements in treatment outcomes even in tumors demonstrating only a modest response to single-agent therapy.^{143,144} Studies in which the anti-VEGF antibody bevacizumab was combined with the tubulin-binding Tumor-VDA CA4P or OXi4503 to treat human clear cell renal carcinoma xenografts showed that when two vascular-targeted therapies were combined, a significantly greater tumor response could be attained compared with that achieved with single agent treatments (Figure 13).¹⁴⁵ Enhanced anti-tumor activity has also been reported for the flavonoid Tumor-VDA ASA404 in combination with bevacizumab in lung and colon cancer xenografts.^{146,147}

Conclusions, Clinical Status, and Future Perspective

The direct vascular-targeted approach to anti-cancer drug development offers a complementary approach to both standard chemotherapy and other targeted therapies. A wealth of preclinical data has provided proof of concept for selective disruption of established tumor vasculature. Decreases in vascular perfusion and even tumor shrinkage have been observed by techniques such as DCE-MRI, together with immunostaining and histologic evidence for selective and extensive tumor necrosis. These studies have demonstrated the efficacy of Tumor-VDA in various tumor types, however, because microvessels can acquire organ-specific specialization in response to local tissue-derived signals types,¹⁴⁸ it is conceivable that there may be some differences in the response to such agents depending on the tumor site of origin. Importantly the preclinical investigations have concluded that Tumor-VDA hold significant potential when combined with other therapies, most notably taxane chemotherapy, radiotherapy, and anti-angiogenic drugs. Selectivity in a clinical setting has been demonstrated by MRI techniques, and a number of Tumor-VDA have now been evaluated in Phase I and II clinical trials (Table 2). In Phase II trials ASA404 resulted in an apparent 5 month survival advantage in NSCLC patients when administered in combination with cytotoxic drugs.^{118,119} These observations led to two Phase III clinical trials investigating ASA404 in combination with taxane-based chemotherapy for first-line (ATTRACT-1) or second-line (ATTRACT-2) treatment of NSCLC.¹⁴⁹ The former, which combined paclitaxel (200 mg/kg), carboplatin (AUC 6) and ASA404 (1800 mg/m²) was halted when the planned interim analysis showed little prospect of demonstrating a survival benefit with ASA404 in this setting. The ATTRACT-2 trial for the second-line treatment of patients with non-small cell lung cancer (doxorubicin, 75 mg/m² and ASA404, 1800 mg/m²) is ongoing. Following Phase II clinical trial evidence of potential clinical benefit¹⁵⁰ the

tubulin-binding Tumor-VDA, CA4P (fosbretabulin, 60 mg/m²) is currently being studied in a Phase II trial (FALCON) in combination with bevacizumab (15 mg/kg), carboplatin (AUC 6) and paclitaxel (200 mg/m²) as first-line treatment of advanced NSCLC. A Phase III trial (FACT) in anaplastic thyroid cancer is comparing the effects of carboplatin (AUC 6) and paclitaxel (200 mg/m²) with carboplatin (AUC 6) and paclitaxel (200 mg/m²) plus CA4P (60 mg/m²).¹⁵¹ These pivotal trials will determine the future potential of Tumor-VDA in cancer treatment.

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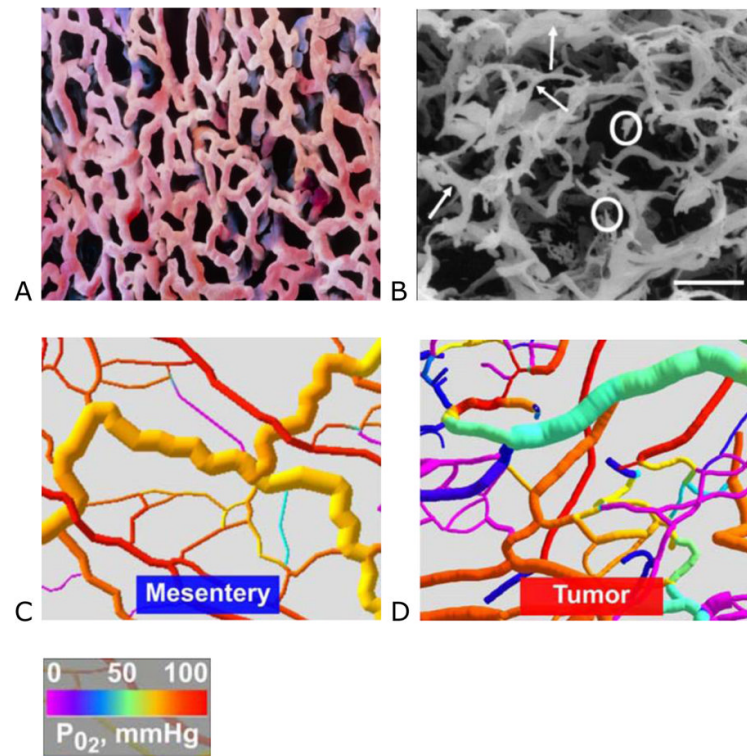


Figure 1.

Panel A: scanning electron microscopy (SEM) image of a microvascular cast from normal lung tissue (Prof. P. Motta, G. Macchiarelli University, La Sapienza, Rome. Science Photo Library) and Panel B: An SEM of a human sigmoidal adenocarcinoma (bar = 100 μm), showing blind ends (circled) and abnormal bulges (arrowed) (used with permission from Macmillan Publishers Ltd: *Br J Cancer* 2001; **84**:1354–1362. Copyright 2001).¹ Panels C and D: Computer visualization of mesenteric and tumor vascular networks, color coded for pO_2 showing poorer oxygenation in areas of restricted flow and blind endings (used with permission).¹²

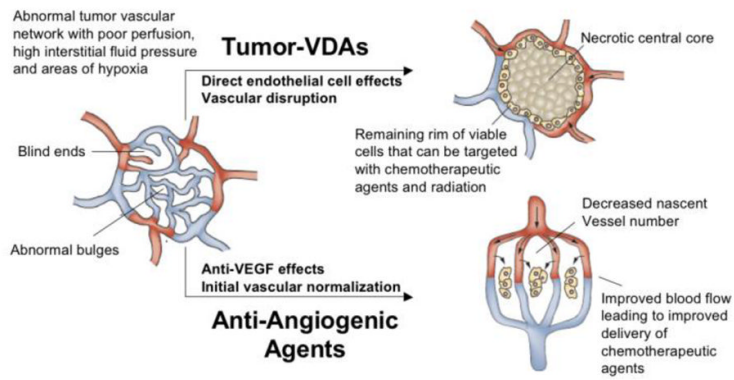


Figure 2.

Diagram illustrating the different preclinical effects of angiogenesis-inhibiting agents (AIAs) and Tumor-Vascular Disrupting Agents (Tumor-VDAs) on abnormal tumor blood vessels. Treatment with AIAs leads to vessel normalization, allowing efficient delivery of chemotherapeutic agents and increased oxygenation to aid radiotherapy. In contrast, Tumor-VDA treatment leads to vascular disruption and extensive central necrosis, leaving a thin rim of surviving viable cells that can be targeted with standard therapies (adapted and redrawn with permission from Macmillan Publishers Ltd: *Nat Rev Clin Oncol* 2009; **6**:395–404. Copyright 2009).^{54,55}

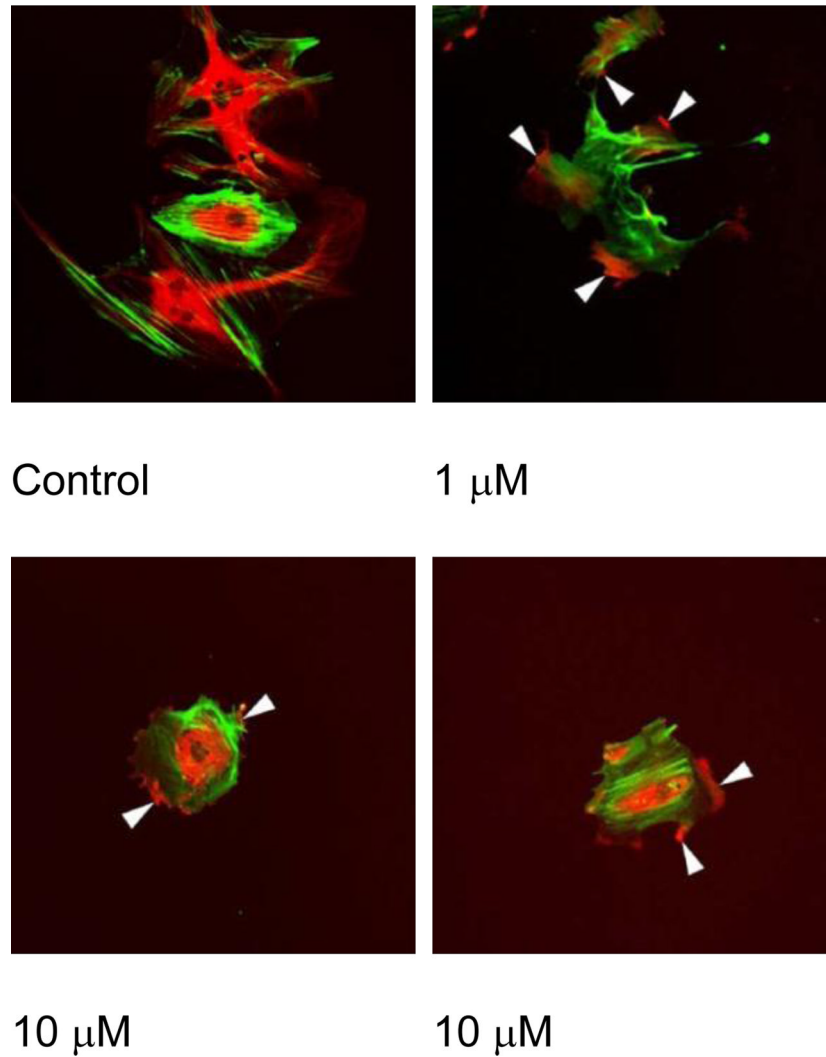


Figure 3. Effect of CA4P treatment on endothelial cell shape.⁷³ Proliferating endothelial cells were untreated or treated with either 1 or 10 μM CA4P for a period of 2 h prior to staining microtubules (red, rotamine) and actin (green, fluorescein phalloidin). Arrows indicate the condensation of microtubules and rounding of endothelial cells (used with permission).⁷³

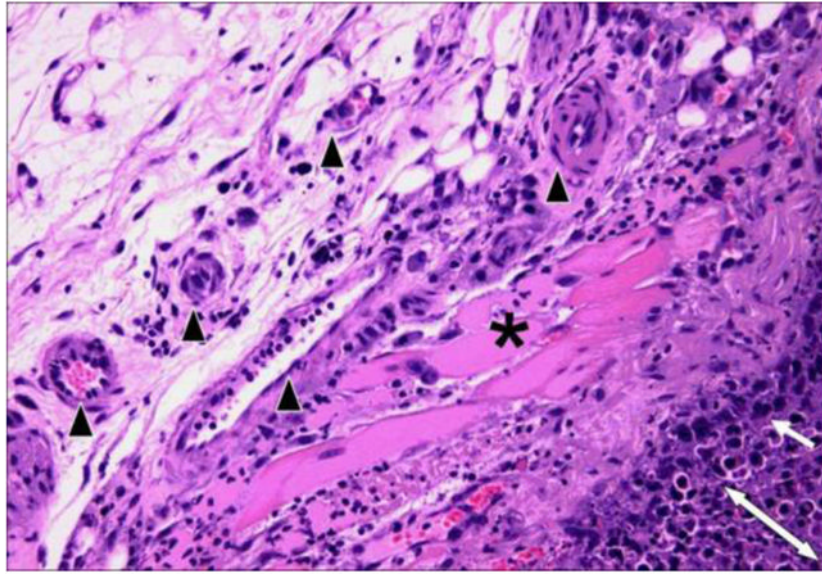


Figure 4. Hematoxylin and eosin stained section (at x 200 magnification) of KHT sarcoma treated with OXi4503 (25 mg/kg). The post-treatment necrotic tumor is defined by the double-headed arrow while the two- or three-cell layer of the viable tumor rim is shown with a single-headed arrow. Preserved muscle (*) and blood vessels (black arrowheads) are clearly evident in the surrounding, unaffected tissue (Siemann DW, unpublished results).

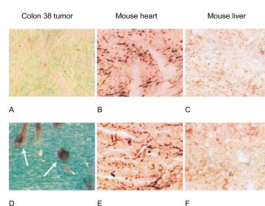
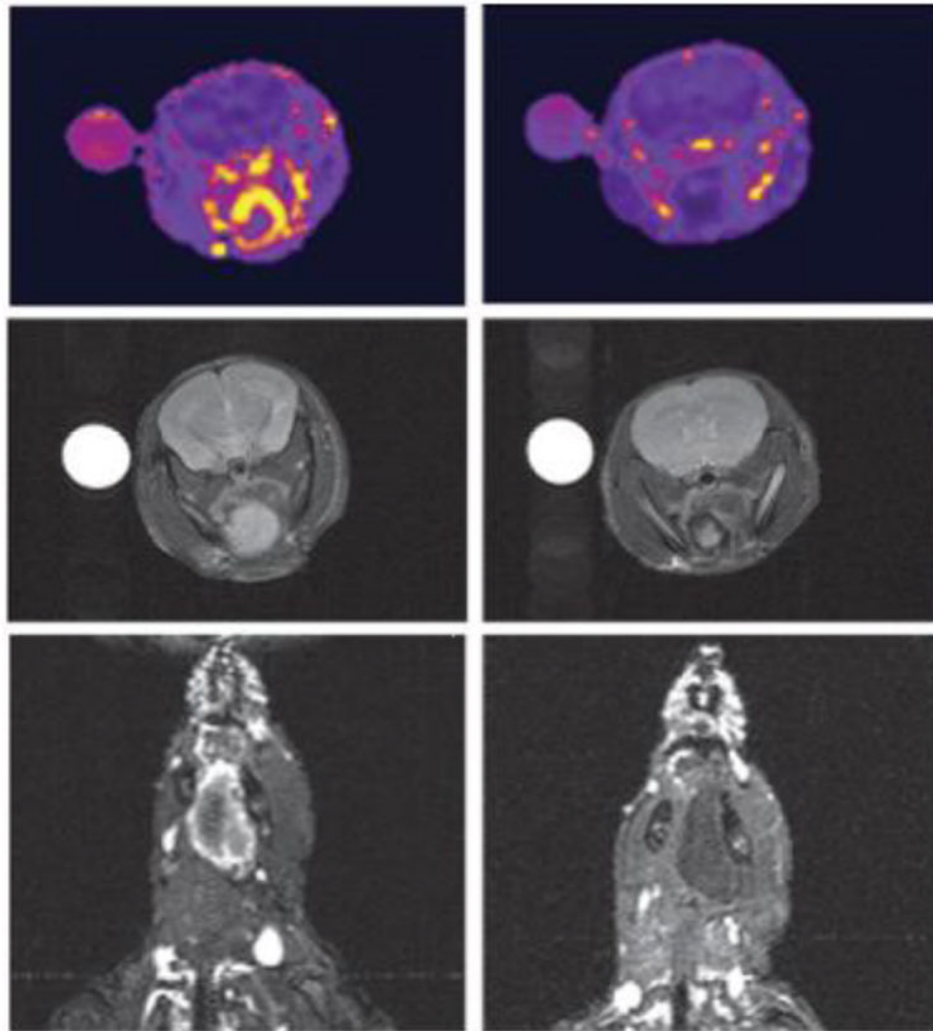


Figure 5. Selective induction of tumor vascular endothelial cell apoptosis (arrows) in the Colon 38 tumor (A,D) compared with mouse heart (B,E) and mouse liver (C,F). Untreated (upper panels) and ASA404-treated (25 mg/kg, 3h) (lower panels). C57B1/6 mice were stained for TdT-mediated dUTP nick-end labeling (TUNEL) with alkaline phosphatase (used with permission from Macmillan Publishers Ltd: *Br J Cancer* 2004; **90**:906–910. Copyright 2004).⁸⁰



Control ASA404-treated

Figure 6.

Contrast enhanced magnetic resonance imaging (MRI) images in an orthotopic head and neck cancer mouse model showing lack of enhancement in the ASA404-treated tumor indicating reduced vascular perfusion (top and bottom panels), and dark, hypo-intense regions in the tumor but not in the surrounding area (middle panels), suggesting selective tumor vascular hemorrhage (used with permission).¹⁰⁴

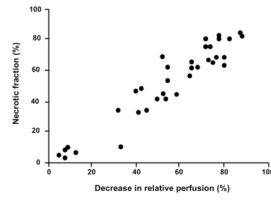


Figure 7.

Comparison of the decrease in relative perfusion and necrotic fraction of KHT tumors in individual mice treated with CA4P.¹⁰⁵ Dynamic contrast-enhanced magnetic resonance imaging measurements and histological analysis of tumor necrotic fraction were made on the same animal (data points represent individual animals); $r=0.89$, $p<0.00001$ (used with permission).¹⁰⁵

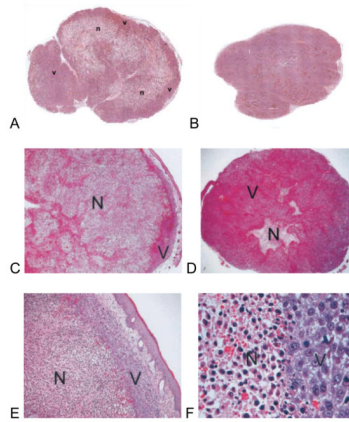


Figure 8.

Comparison of the necrotic effects of a flavonoid Tumor-VDA (ASA404) and a tubulin-binding Tumor-VDA (ZD6126). Panel A: hematoxylin and eosin stained section of a G3H prolactinoma 24 hours post-treatment with ASA404 (350 mg/kg) Grade 4, extensive necrosis (n=necrotic tissue, v=viable tissue) and Panel B: control – Grade 1, no necrosis.⁹² Panel C: stained section of a Calu-6 lung cancer xenograft (x 16 magnification) post-treatment with ZD6126 (200 mg/kg) and Panel D: corresponding vehicle-treated control (N=necrotic tissue, V=viable tissue). Panels E (x 100) and F (x 400): magnifications showing a thin viable rim of cells remaining, and contrasting the necrotic and viable cells (used with permission).⁵²

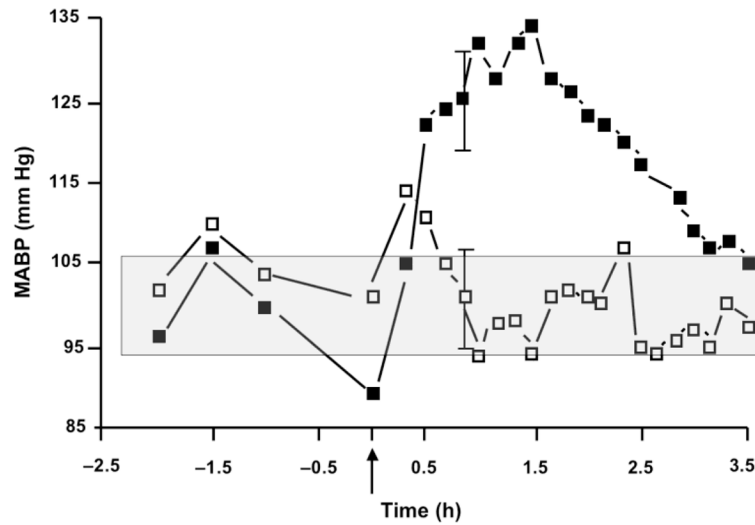


Figure 9. Blood pressure measurements in mice made using thoracic aortic implantation of pressure-sensing catheters combined with the subcutaneous placement of transmitter bodies and monitoring by placing their cages on top of the telemetry receiver pads for data collection. Data are mean (\pm SD) arterial blood pressure (MABP) measurements in groups of 8 control (\square) or CA4P-treated (100 mg/kg) (\blacksquare) C3H mice; shaded area indicates normal range of MABP (Siemann DW, unpublished results).

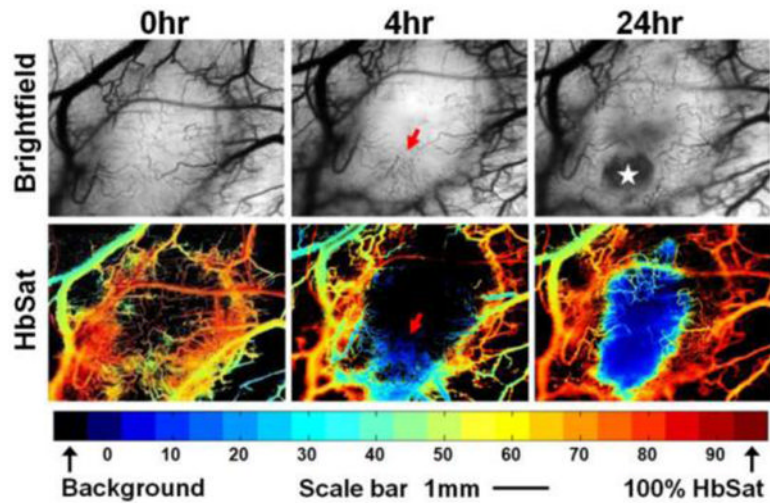


Figure 10.

Typical OXi4503 treatment progression in a 4T1 tumor. The brightfield images (upper panels) and HbSat maps (lower panels) show structural alterations in the vasculature through the course of a single treatment. The oxygenation levels in the HbSat maps are color coded as indicated by the colorbar. Arrows highlight the disintegrating vasculature, while the star indicates the avascular regions created by the OXi4503 treatment (redrawn with kind permission from Springer Science+Business Media: *Oncol Rep* 2010; **23**:685–692. Wankhede M, *et al.* Figure 1. Copyright 2010).¹²³

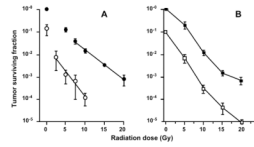


Figure 11.

Enhancement of radiation damage by ASA404 (A) and OXi4503 (B) in the murine KHT sarcoma model, assessed by clonogenic cell survival assay: radiation alone (closed symbols) and radiation plus ASA404 or OXi4503 (open symbols) (Panel A redrawn with kind permission from Springer Science+Business Media: *Radiat Res* 2001; **156**:503–509. Murata R, *et al.* Figure 3. Copyright 2001).^{79,126}

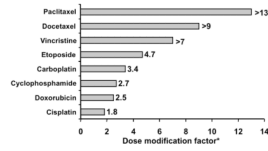


Figure 12. Interaction of ASA404 with chemotherapy. The dose modification factor was defined as the ratio of the effect with ASA404 plus cytotoxic agent versus cytotoxic agent alone (adapted with kind permission from Springer Science+Business Media: *Cancer Chemother Pharmacol* 2003; **51**:43–52. Siim BG, *et al.* Table 1. Copyright 2003).¹³²

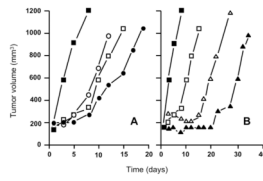


Figure 13.

Response of Caki-1 tumors to bevacizumab (2 mg/kg, twice a week for 2 weeks), CA4P (A) or OXi4503 (B) (100 mg/kg or 25 mg/kg respectively, three times a week for 2 weeks) or the combination of an angiogenesis-inhibiting agent and Tumor-Vascular Disrupting Agent (median tumor responses of groups of 8–10 mice). Controls (■), bevacizumab (□), CA4P (○), OXi4503 (△), bevacizumab + CA4P (●), bevacizumab + OXi4503 (▲) (redrawn with permission).¹⁴⁵

Table 1

Key preclinical differences between the Tumor-Vascular Disrupting Agents (Tumor-VDAs) and anti-angiogenic drug classes.

Tumor-VDAs	Anti-angiogenic agents
Administered acutely	Administered chronically
Disrupt the established tumor vasculature	Inhibit neovascularization
Cause vessel occlusion and inhibition of blood flow	Induce vascular normalization with initial improved tumor blood flow
Cause extensive tumor necrosis	Prevent or limit tumor growth
Particularly active against large tumor masses, causing extensive central necrosis	Particularly active in peripheral tumor locations where nascent vessels are more predominant

Table 2

Current clinical status of Tumor-Vascular Disrupting Agents (Tumor-VDAs) in development (ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; HRMPC, hormone refractory metastatic prostate cancer; MBC, metastatic breast cancer; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma).

Tumor-VDA	Company	Stage of clinical development
Flavonoid		
ASA404 (vadimezan)	Novartis	Phase III (NSCLC), Phase II (HER2 –ve MBC; planned)
Tubulin binding		
CA4P (fosbretabulin)	OXiGENE	Phase II/III (anaplastic thyroid cancer), Phase II (NSCLC)
AVE8062 (ombrabulin)	Sanofi-Aventis	Phase III (sarcoma), Phase I (NSCLC)
NPI-2358 (plinabulin)	Nereus	Phase II (NSCLC)
ABT-751 (E7010)	Abbott	Phase II (pediatric ALL, NSCLC, breast, colorectal, HRMPC, neuroblastoma, RCC)
TZT-1027 (soblidotin)	Daiichi	Phase II (sarcoma)
CYT997	Cytopia	Phase II (multiple myeloma)
Dolastatin 10	–	Phase II (RCC, sarcoma, pancreatic, HRMPC, liver/bile duct/gall bladder, lymphoma, CLL)
ZD6126	Angiogene	Phase II
MPC-6827	Myriad	Phase II (melanoma, glioblastoma)
OXi4503 (CA41P)	OXiGENE	Phase I
EPC2407 (crinobulin)	EpiCept	Phase I
MN-029	Medicinova	Phase I
BNC105	Bionomics	Phase I