Published in final edited form as:

Expert Opin Investig Drugs. 2009 February ; 18(2): 189–197. doi:10.1517/13543780802691068.

A REVIEW AND UPDATE OF THE CURRENT STATUS OF THE VASCULATURE DISABLING AGENT COMBRETASTATIN-A4 PHOSPHATE (CA4P)

Dietmar W. Siemann, PhD^{1,*}, David J. Chaplin, PhD², and Patricia A. Walicke, MD, PhD³

¹Professor and Associate Chair, University of Florida, Department of Radiation Oncology, 2000 SW Archer Road, Gainesville, FL 32610, USA

²Head of Research and Development & Chief Scientific Officer, OXiGENE, Inc, 230 Third Avenue, Waltham, MA 02451, USA

³Vice President & Chief Medical Officer, OXiGENE, Inc, 230 Third Avenue, Waltham, MA 02451, USA

Abstract

Vascular disrupting strategies impair a tumor's blood vessel network which is essential for tumor progression and metastasis. Vascular disrupting agents cause a rapid and selective vascular shutdown in tumors to produce extensive secondary neoplastic cell death due to ischemia. A lead agent in this therapeutic strategy is the tubulin depolymerizing agent combretastatin-A4 phosphate (CA4P). Used alone CA4P induces extensive necrosis in a wide variety of preclinical cancer models and significant blood flow reductions in the patient tumors. Preclinical and clinical data further indicate that CA4P can effectively be combined with chemotherapy or radiotherapy. Finally, the potential of combining VDAs with antiangiogenic therapies has shown considerable promise in preclinical models and such combinations are now beginning to be evaluated in patients.

Keywords

Vascular disrupting agents; CA4P (ZybrestatTM; fosbretabulin); antiangiogenic therapy; tubulin depolymerization

1.0 Introduction

ZybrestatTM, fosbretabulin (USAN), Combretastatin A4 phosphate (CA4P), is a novel vascular disrupting agent (VDA), which induces acute reductions in tumor blood flow. Evidence for vascular disrupting activity was first obtained in rodent cancer models and has subsequently been established to occur in solid tumors in humans.

Combretastatin A4 phosphate (CA4P) is the water-soluble prodrug of Combretastatin A4 (CA4) which was originally isolated from the African tree *Combretum Caffrum* [1]. CA4 is a potent inhibitor of tubulin assembly [2] but it is not its anti-mitotic effects that are of greatest interest. Rather it is its ability to selectively compromise the vascular network in tumors that has advanced this agent into the clinical arena.

* to whom correspondence should be addressed: Tel: 352-265-0287, Fax: 352-265-0759, siemadw@ufl.edu.

The mechanism responsible for the selective vascular damage observed in tumors following administration of CA4P has not been completely elucidated [3]. However, studies have shown that it can have dramatic effects on the three dimensional shape of newly formed endothelial cells, with little or no effect on quiescent endothelial cells [4]. The reason newly formed endothelial cells are more sensitive than more mature cells, is thought to be that the latter have a more highly developed actin cytoskeleton which maintains the cell shape despite depolymerization of the tubulin cytoskeleton [5]. CA4P has also been demonstrated to disrupt cell-cell contacts between endothelial cells mediated by the vascular endothelial (VE)-cadherin/ β -catenin complex [6]. The presence of smooth muscle cells, a characteristic feature of normal tissue vasculature, inhibits this disruption [6]. The targeting of recently formed endothelial cells in immature or abnormal vessels which lack a full complement of smooth muscle or pericyte support is believed to be responsible for the drug specificity.

It has been suggested that an early consequence of endothelial cell shape change is an increase in vascular permeability [7]. 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 differences between newly formed and mature endothelial cells and the 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.

2.0 Preclinical Evaluation

2.1 CA4P Activity as a Single Agent

2.1.1 Tubulin Binding Activity—Combretastatin A4 mono tris phosphate (CA4P.tris) (Figure 1) is a salt form of the pro-drug called CA4P which is activated when it is dephosphorylated into CA4. CA4P does not bind to tubulin, but CA4 is a known tubulin binding agent and has been demonstrated to bind at or near the colchicine binding site of β -tubulin ($K_d = 0.40 \pm 0.06 \mu\text{M}$) [2,8]. CA4 competitively inhibits the binding of colchicine to tubulin (K_i of $0.14 \mu\text{M}$) and, like colchicine, inhibits the polymerization of tubulin (IC_{50} $2.4 \pm 1.4 \mu\text{M}$). CA4 binds rapidly to tubulin, in contrast to colchicine, which binds in a relatively slow and temperature-dependent manner. However, CA4 dissociates from tubulin over 100 times faster than colchicine, with a half-life of 3.6 relative to 405 minutes at 37°C [9]. Notably, the trans-isomer of CA4 does not significantly compete with colchicine in binding tubulin, nor does it interfere with tubulin assembly (IC_{50} $50 \mu\text{M}$)

2.1.2. Vascular Effects—A number of studies have demonstrated the rapid and selective effects of CA4P on tumor vascular function [10–14]. These studies have established that blood flow reductions occur in transplanted and spontaneous rodent tumors and in xenografted human tumors. Blood flow effects can be measured within 10–20 minutes of CA4P administration, a time frame that closely mimics the *in vitro* effects on endothelial cell shape. These blood flow effects persist for hours, although in some tumors restitution of flow can be observed 24 hours following treatment. Since it is known that dramatic tumor cell kill is induced within a few hours of cutting off the blood supply [15] even short periods of blood flow reduction are expected to induce a significant reduction in tumor cell burden.

2.1.3 CA4P and Cardiovascular Adverse Effects—As might be anticipated for a vascular active agent, the adverse event profile of CA4P along with other VDAs includes events of hypertension and cardiac ischemia [16]. In rodents, hypertension post-CA4P administration has been linked to mild smooth muscle contraction which causes an increase in peripheral vascular resistance [16]. In rats with preexisting hypertension and underlying

cardiac changes the increase in blood pressure after administration is greater and is associated in a proportion of animals with an increase in cardiac strain (i.e. elevation in cardiac enzymes). Both the augmented hypertension and cardiac strain seen with CA4P and other tubulin binding VDAs can be prevented by treatment with calcium channel blockers [17]. Controlling hypertension does not influence the blood flow effects of these agents in tumors, consistent with the fact that the two effects are mediated through two distinct cell types [16,17].

2.1.4 Effects on Tumor Histology—It has been established that administration of CA4P to tumor bearing animals results in rapid and extensive changes in tumor histology consistent with necrosis [10,18]. This histological change occurs a few hours following the reductions in tumor perfusion. The necrosis is located in the central regions of the tumor with a characteristic viable rim of a few cell layers adjacent to the normal tissue surrounding the tumor cell mass (Figure 2).

2.1.5 Tumor Response to CA4P—Measurements of clonogenic tumor cell survival in tumors excised following CA4P treatment have shown that extensive tumor cell kill is induced [13,14,19]. Indeed these studies show that 24 hours after a dose of 100 mg/kg, tumor cell survival may be reduced by 90–99%. Despite the extensive cell kill observed following the vascular shutdown achieved with CA4P, significant growth retardation is rarely seen. This is because the residual areas of tumor tissue, believed to survive VDA treatment because their nutritional support is derived from vasculature in the adjacent normal tissue [14], can act as a source of tumor regrowth.

2.1.6 Effect in Leukemia models—CA4P has recently been shown to elicit significant anti tumor activity against orthotopically implanted leukemia [20]. 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.

2.2 CA4P in Combined Modality Settings

2.2.1 CA4P plus Conventional Anticancer Therapies—One approach to eliminate the tumor cells surviving VDA treatment is to combine them with standard anticancer therapies such as radiotherapy or chemotherapy [14,16]. Cells comprising this viable rim of tumor tissue are likely to be in a state of high proliferation and excellent nutrition. These factors, coupled with their accessibility to systemically administered agents, make the surviving tumor cells susceptible to killing by radiation and anticancer drugs. Conversely, VDAs cause the destruction of large areas of the interior of tumors and show excellent activity against large bulky disease [22] that is typically resistant to conventional anticancer therapies.

The concept of combining CA4P with radiotherapy and chemotherapy has been extensively tested in preclinical settings using a variety of rodent tumors and human tumor xenografts. In general, marked enhancements in antitumor activities are observed when CA4P and radiation or chemotherapy are combined (for review see [23]. These enhanced responses were noted over a wide range of single and fractionated dose radiotherapy schedules [13,18,22,24–27] as well as a variety of chemotherapeutic agents [13,19,27–30]. Importantly, in preclinical investigations of combinations of CA4P with anticancer drugs or radiotherapy improved antitumor efficacy has been achieved in the absence of concomitant enhancement of chemotherapeutic agent specific side effects or increased radiation response of normal tissues [19,24,31] indicating that the combination of CA4P with radiotherapy or chemotherapy can lead to a therapeutic benefit.

The pathophysiological effects of CA4P have also been exploited to improve tumor response to heat [32] and radioimmunotherapy [33]. In either case, the inclusion of CA4P in the treatment protocol resulted in significantly enhanced tumor responses. As to the mechanism responsible, there is evidence for both improved tumor heating [24] and entrapment of the radioactive antibody following CA4P-induced vessel collapse [33].

2.2.2 Combining CA4P with Other Vascular Directed Therapies—Vascular directed therapies have shown impressive antitumor effects in preclinical tumor models and recent clinical observations are encouraging. Still, achieving tumor cures in patients with either anti-angiogenic agents (AIs) or VDA monotherapy is likely to be extremely difficult. In the case of the former, the complexity of pathways available for neovascularization implies that disrupting only a single aspect of angiogenesis probably will not suffice [34]. VDAs, on the other hand, by the very nature of their highly selective mechanism of damaging tumor blood vessels, will not be able to eliminate those pockets of tumor cells whose nutritional supply is derived from blood vessels in the surrounding normal tissues [14]. A logical extension in vascular targeting is the application of these therapies in concert. AIs and VDAs differ not only in their mode of action but also in their likely therapeutic application [3]. The objective of antiangiogenic therapies is to interfere with new vessel formation thereby preventing tumor growth and limiting metastatic potential. VDAs compromise established tumor vasculature and have the potential to destroy tumor masses as well as preventing progression. Since both the initiation of new vessel formation and the integrity of the existing blood vessel network are critical to a tumor's growth and survival, such a double assault on the tumor vasculature is likely to be a highly complimentary treatment strategy.

Preclinical data support the rationale to combine AIs and VDAs to enhance antitumor efficacy [35]. Such a strategy was recently investigated by combining CA4P and bevacizumab in the treatment of a model of human clear cell renal cell carcinoma [36]. The results showed that the CA4P-bevacizumab combination led to a significant enhancement of the tumor response beyond that achieved with either vascular targeting therapy alone (Figure 3). A likely explanation for these observations is that while CA4P significantly reduced the viable tumor mass, bevacizumab impaired subsequent tumor regrowth by interfering with the re-establishment of the tumor blood vessel network. From a therapeutic perspective, the enhanced tumor responses resulting from the combination of bevacizumab and CA4P provides an experimental basis for the consideration of such a treatment strategy in the clinic.

3.0 Clinical Experience with CA4P

Clinical experience with CA4P consists of 18 completed and on-going clinical trials in oncology and ophthalmology which together comprise over 350 patients. These studies have included assessment of CA4P monotherapy, as well as combinations with standard chemotherapy, radiation therapy, and anti-angiogenic therapy. CA4P has been demonstrated to decrease tumor blood flow in 7 of 7 Phase 1 and 2 studies, employing perfusion CT, DCE-MRI, DWI or PET scanning as investigative techniques. There is suggestive evidence for clinical benefit in ATC and platinum resistant ovarian cancer, although formal studies are in progress.

3.1 CA4P Monotherapy

3.1.1 Phase 1 and 2 Experience—CA4P monotherapy was most extensively evaluated in three Phase 1 clinical trials in patients with refractory solid tumors [37–39]. Ninety-six subjects were treated with dose levels of CA4P ranging from 5 – 114 mg/m². Inhibition of tumor blood flow was demonstrated in the majority of tumors using either DCE-MRI

[37,38] or (^{15}O)–labeled water PET scan [40], with relatively little effect on systemic blood flow. Although the principal goal of these studies was safety, clinical benefit was noted (Table 1). One subject with anaplastic thyroid cancer (ATC) experienced a complete response and probable cure, remaining disease free off treatment for greater than 5 years. One partial response was seen in a patient with fibrosarcoma and stable disease was observed in 31 (32%) subjects, in some cases lasting for more than one year.

Subsequent CA4P monotherapy has been employed in a Phase 2 study in ATC, another Phase 1 study in refractory tumors, and in ophthalmology studies. An interim report of the ATC study noted evidence suggestive of clinical benefit with progression free interval of 3 months in 28% of patients [41]. The safety profile observed in these subsequent studies has been consistent with that discussed above for the first three Phase 1 oncology studies.

3.1.2 CA4P Adverse Effects—Although the frequency of administration differed, all three Phase 1 trials determined that the maximal tolerated dose was approximately 60 to 68 mg/m^2 ; therefore safety data have been combined for a preliminary analysis. Overall CA4P monotherapy was well tolerated with most adverse events being mild to moderate in intensity. The most frequent (i.e. reported as in 20% subjects) CA4P-related adverse events were, in descending order of frequency: nausea, headache, tumor pain, fatigue, vomiting, sinus tachycardia, paresthesia, diarrhea, sweating and transient hypertension. There was little if any hematological, hepatic or renal toxicity, as reflected by relatively few changes in laboratory parameters. Most CA4P-related adverse events were of relatively brief duration, occurring and resolving on the day of or following drug infusion. Since these were open-label studies, the true relationship of common events such as nausea and headache to CA4P remains to be fully established.

Severe or serious adverse events were only reported in patients who received dose levels 50 mg/m^2 . In these higher dose levels, the only CA4P-related Grade 3 or 4 adverse events seen in more than 1 subject were tumor pain, dyspnea, hypoxia and syncope. Sixteen (16.7%) subjects experienced a serious adverse event related to CA4P, of which the most common were respiratory distress, tumor pain, and asymptomatic Grade 1 QTc prolongation. Two subjects experienced cardiac ischemia, one reversible and one leading to infarction [37,42].

3.2 CA4P in Combined Modality Settings

3.2.1 CA4P plus Conventional Anticancer Therapies—CA4P has been examined in combination with palliative radiation therapy in a Phase 1 study enrolling patients with prostate, non-small cell lung (NSCLC) and head and neck squamous cell (HNSCC) cancers. An interim report showed that addition of CA4P significantly suppressed tumor blood flow as measured by perfusion CT scan. A maximal tolerable dose (MTD) for the combination of radiation therapy and CA4P was not reached. Safety events were similar to those reported in Phase 1 monotherapy studies with tumor pain and mild, self-limiting cardiovascular changes reported [44].

CA4P has been examined in combination with carboplatin, paclitaxel or both. Administration of CA4P and carboplatin on the same day interfered with metabolism of carboplatin and led to increased bone marrow toxicity [45]. When carboplatin and CA4P are given on adjacent days, the increased marrow toxicity is not observed [43,46]. Overall the safety profile of CA4P in the presence of carboplatin and/or paclitaxel appears to be comparable to that observed as monotherapy. VDA activity of CA4P was confirmed by DCE-MRI [45]. Mean change at 24 hours in all refractory solid tumors was about 32%, but changes in two ATC cases were particularly striking at 73 and 79% [46].

Consistent with predictions from preclinical studies, tumor response rates have been somewhat higher in Phase 1 and 2 studies with chemotherapy combinations (Table 1). In a study of 13 subjects with refractory solid tumors receiving triple therapy, the majority had evidence of potential clinical benefit, with 3 (23%) PR and 6 (46%) SD. A Phase 1 b study of varying combinations of carboplatin, paclitaxel and CA4P in refractory solid tumors showed 4 / 27 (15%) PR by RECIST criteria [43]. However a number of patients with refractory ovarian cancer happened to participate in this trial and, using CGIC criteria, 7/17 (41%) were PR. This observation led to a Phase 2 single arm, open label study of CA4P, carboplatin and paclitaxel in platinum resistant ovarian cancer. An interim report from that on-going study showed a confirmed PR rate of 29% in this highly refractory population [47]. Finally, observations of the effectiveness of chemotherapy combined with CA4P have led to an on-going multinational Phase 3 study comparing the effects of carboplatin/paclitaxel to carboplatin/paclitaxel + CA4P in ATC, an exceptionally aggressive and therapy resistant form of thyroid cancer.

3.2.2 CA4P plus Other Antivascular Therapies—A Phase 1 study has recently been completed with the combination of bevacizumab and CA4P in subjects with refractory solid tumors. Dose levels were bevacizumab at 10 mg/kg every 2 weeks, and CA4P 45, 54 or 63 mg/m² every 2 weeks. No MTD was defined for the combination [48].

Overall CA4P and bevacizumab were well tolerated. Drug related grade 3 and 4 related adverse events were headache, abdominal pain, exacerbation of tumor ulceration and hemorrhage. The hemorrhage occurred in a patient with a prior history of hemorrhage into her angiosarcoma; hemorrhage had not been previously reported for CA4P alone or with chemotherapy. One additional serious adverse event of Grade 2 atrial fibrillation was attributed to CA4P. No Grade 3/4 hematologic or biochemical events were ascribed to study medication.

Observations using DCE-MRI were consistent with predictions from preclinical studies. Following a first dose of CA4P, mean K_{trans} decreased 14.9%. Neovascularization commenced and by three days, K_{trans} was returning towards baseline. A second administration of CA4P decreased K_{trans} by 23.3%. This time bevacizumab was administered following the MRI. Three days later, K_{trans} was 32.3% below baseline. The results are consistent with the prediction that by preventing angiogenesis, bevacizumab would enhance effects of CA4P.

Best tumor response for these 15 subjects with refractory tumors was 9 (60%) SD, 4 (27%) PD and 2 (13%) were not evaluable. Among the stable patients, observed time to progression was 116 to 173 days, and one subject had continued SD at 414 days [48]. The observation of prolonged SD was suggestive of clinical benefit and led to initiation of a Phase 2 study in NSCLC comparing bevacizumab + chemotherapy to bevacizumab + chemotherapy + CA4P [48].

3.3 CA4P and Cardiovascular Safety

A review of serious adverse events in the more than 350 patients treated to date with CA4P revealed two instances of myocardial infarction and two of symptomatic ischemia with ECG changes, suggesting an event rate of around 1–2%. This event rate is comparable to that reported for anti-angiogenic agents [49]. Myocardial ischemia and ECG changes occur in temporal association with post- infusion hypertension. CA4P-related hypertension tends to peak by 1–2 hours after infusion and resolves by around 2–6 hours. On average, the increase in systolic blood pressure is around 5–17 mm Hg, though may be higher, up to 60 mm Hg, in some individuals [43,44]. Subjects with symptomatic cardiac ischemia have been in this subset with a large increase in systolic blood pressure.

Transient asymptomatic ECG changes such as T wave inversion or ST segment depression have been reported in some additional subjects. Myocardial infarction has been excluded by lack of elevation in cardiac enzymes such as troponin. Interpretation of such findings can be difficult due to potential contributions from technical artefacts. Review by cardiologists with expertise in oncological cardiology is critical for a thorough assessment.

Basic research has demonstrated that CA4P increases vascular resistance and blood pressure through mild contraction of vascular smooth muscle cells, an effect which is unrelated to its VDA activity. The observation that nitrates can reverse CA4P-induced hypertension has been confirmed in humans. Additionally some individuals who have experienced hypertension or ECG changes following CA4P have subsequently received prophylactic calcium channel blockers with apparent control of symptoms. Specific guidelines for management of hypertension or cardiac ischemia are included in all on-going clinical trials. These interventions would be anticipated to be cardioprotective, given both nonclinical and clinical observations linking post-CA4P hypertension and cardiac ischemia.

By an unrelated and less well characterized mechanism, CA4P causes mild to moderate asymptomatic QTc prolongation. In small cohorts, the increase appears to peak at approximately 3 hours post infusion with mean prolongation of approximately 15 to 30 msec [42,43,44]. Although several serious adverse events of QTc prolongation were reported in one early study [37], these were all Grade 1 severity on NCI Common Toxicity Criteria. Review of approximately 75 subjects in early studies [37, 39, 45] by Dr. Joel Morganroth indicated that, on average, QTc prolongation was less than 20 msec and that there were no Grade 3 events (i.e. 500 msec) [reports on file; OXiGENE]. No ventricular arrhythmias associated with QT prolongation have not been reported with CA4P.

The most common arrhythmias observed after CA4P are sinus bradycardia and sinus tachycardia, which may be secondary to changes in peripheral resistance. Bradycardia occurs first and subsequently followed by tachycardia, which occurs contemporaneously with time of onset of QTc prolongation. As originally noted by Cooney et al [42], the increase in heart rate may “fortuitously afford protection against proarrhythmia associated with prolongation of ventricular repolarization.” Meticulous attention to electrolyte management, particularly potassium and magnesium levels, also is critical for minimization of QT changes. One clinical trial has examined cardiac output following CA4P administration. Approximately a 10% decrease was observed 30 minutes after infusion with recovery at 24 hours. The change in cardiac output was asymptomatic and attributed to increased peripheral vascular resistance [40]. Serious adverse events suggestive of congestive heart failure have not been reported in any clinical trial with CA4P.

4.0 Conclusions

The appeal of vascular directed anticancer therapy lies not only in the recognition of the critical function of the vasculature in a tumor's growth and spread but also in the realization that by targeting a component distinct from that targeted by cytotoxic agents there exists great potential for complementary therapeutic activity. Rapid developments in recent years have now resulted in the identification of a variety of potential targets and a large number of investigational drugs. In the case of VDAs, the tubulin depolymerizing drug CA4P is the most widely studied of a group of compounds that induce hemorrhagic necrosis in tumors. Preclinical evidence has demonstrated not only that CA4P treatment leads to extensive tumor necrosis but the application of this agent in concert with radiotherapy and anticancer drugs, as well as other vessel directed therapies, can lead to markedly enhanced tumor responses. These concepts are now being actively explored in the clinic.

5.0 Expert Opinion

Until recently most cancer therapies were designed to target the proliferation and survival of cancer cells directly. However the knowledge that blood vessel growth plays an integral part in both the continued growth and survival of the tumor cell mass has led to the development of novel treatment strategies. Targeting the growth factor and signalling pathways involved in endothelial cell proliferation has led to the development and approval of several antiangiogenic therapies including the anti VEGF antibody bevacizumab and the tyrosine kinase inhibitors sorafenib and sunitinib. The primary goal of these agents is to suppress the growth of new vessels in tumors. CA4P represents a lead compound in a separate group of agents known as VDAs which unlike the antiangiogenic approaches are designed to selectively and rapidly compromise the function of abnormal tumor neovasculature. CA4P is a reversible inhibitor of tubulin polymerisation with a relatively short plasma half-life. These properties minimise the antiproliferative effects of the tubulin depolymerisation and enable other effects of destabilising of this structural protein to be manifested. A key role of microtubules is to maintain the shape and attachment of newly formed endothelial cells which lack the support cells such as pericytes, a feature common in disease associated neovasculature. CA4P's effects on endothelial cell detachment also appear to be mediated at least in part through the adhesion protein VE cadherin. The disruption of the shape and attachment of just a few endothelial cells will have a profound impact on the blood flow in an entire blood vessel. Such rapid and selective effects on blood flow have been shown to occur in a wide variety of solid tumor masses in both animals and humans. Interestingly the effects on cells shape and attachment may also have a role in the treatment of leukaemias by initiating the release of leukemic cells from treatment resistant stromal niches.

It is also now believed that tubulin depolymerisation underlies one of the most common side effects of CA4P in the clinic, transient hypertension. Microtubules help resist constriction of smooth muscle cells and thus their depolymerisation will make vessels more sensitive to vasoconstriction. The use of antihypertensives such as nitrates and calcium channel blockers has been shown to eliminate the blood pressure effects of CA4P both in animals and in patients. The importance of this is that if left uncontrolled acute hypertensive episodes can in the presence of underlying cardiovascular disease lead to cardiac toxicity. Active monitoring and management of hypertension should be an ongoing part of clinical application of VDAs. It is possible that routine prophylaxis with a calcium channel blocker could become part of the treatment for some patients receiving VDAs. It should be noted that hypertension is short lived, lasting for just a few hours after drug administration, so hypertension control is only required for this period of time.

The lack of significant single agent activity of CA4P in animal tumor models is attributed to the regrowth from a peripheral rim of viable tumor cells supported by the surrounding normal tissue vasculature. This regrowth occurs rapidly and involves not only proliferation of the cancer cells themselves but also rapid revascularisation. This finding has driven the clinical development of using CA4P in combination with conventional cytotoxic chemotherapy as well as antiangiogenic treatment strategies. Potentially the use of CA4P will be complimentary to a wide range of existing therapies because it preferentially eliminates large areas of the central poorly vascularised parts of solid tumors known to be resistant to most conventional anticancer treatments.

References

1. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendall D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. *Experientia*. 1989; 45:209–211. [PubMed: 2920809]

2. Lin CM, Singh SB, Chu PS, Dempcy RO, et al. Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin: a structure-activity study. *Mol Pharmacol*. 1988; 34:200–208. [PubMed: 3412321]
3. Siemann DW, Bibby MC, Dark GG, Dicker AP, Eskens FA, Horsman MR, Marme D, LoRusso PM. Differentiation and definition of vascular-targeted therapies. *Clin Cancer Res*. 2005; 11:416–420. [PubMed: 15701823]
4. Galbraith SM, Chaplin DJ, Lee F, Stratford MR, Locke RJ, Vojnovic B, Tozer GM. Effects of combretastatin A4 phosphate on endothelial cell morphology in vitro and relationship to tumour vascular targeting activity in vivo. *Anticancer Res*. 2001; 21:93–102. [PubMed: 11299795]
5. Chaplin DJ, Dougherty GJ. Tumour vasculature as a target for cancer therapy. *Brit J Cancer*. 1999; 80:57–64. [PubMed: 10466764]
6. Vincent L, Kermani P, Young LM, Cheng J, Zhang F, Shido K, Lam G, Bompais-Vincent H, Zhu Z, Hicklin DJ, Bohlen P, Chaplin DJ, May C, Rafii S. Combretastatin A4 phosphate induces rapid regression of tumor neovessels and growth through interference with vascular endothelial-cadherin signaling. *J Clin Invest*. 2005; 115:2992–3006. [PubMed: 16224539]
7. Tozer GM, Prise VE, Wilson J, Locke RJ, Vojnovic B, Stratford MR, Dennis MF, Chaplin DJ. Combretastatin A-4 phosphate as a tumor vascular-targeting agent: early effects in tumors and normal tissues. *Cancer Res*. 1999; 59:1626–1634. [PubMed: 10197639]
8. Woods JA, Hadfield JA, Pettit GR, Fox BW, McGown AT. The interaction with tubulin of a series of stilbenes based on combretastatin A-4. *Brit J Cancer*. 1995; 71:705–711. [PubMed: 7710932]
9. Lin CM, Ho HH, Pettit GR, Hamel E. Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin. *Biochemistry*. 1989; 28:6984–6991. [PubMed: 2819042]
10. Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ. Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. *Cancer Res*. 1997; 57:1829–1834. [PubMed: 9157969]
11. Beaugerard DA, Thelwall PE, Chaplin DJ, Hill SA, Adams GE, Brindle KE. MRI and MRS of combretastatin A4 prodrug-induced disruption of tumor perfusion and energetic status. *Brit J Cancer*. 1998; 77:1761–1767. [PubMed: 9667644]
12. Horsman MR, Ehrnrooth E, Ladekarl M, Overgaard J. The effect of combretastatin A-4 disodium phosphate in a C3H mouse mammary carcinoma and a variety of murine spontaneous tumours. *Int J Radiat Oncol Biol Phys*. 1998; 42:895–898. [PubMed: 9845117]
13. Chaplin DJ, Pettit GR, Hill SA. Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. *Anticancer Res*. 1999; 19:189–195. [PubMed: 10226542]
14. Siemann DW, Chaplin DJ, Horsman MR. Vascular-targeting therapies for treatment of malignant disease. *Cancer*. 2004; 100:2491–2499. [PubMed: 15197790]
15. Chaplin DJ, Horsman MR. The influence of tumour temperature on ischemia-induced cell death: potential implications for the evaluation of vascular mediated therapies. *Radiotherapy and Oncology*. 1994; 30:59–65. [PubMed: 8153381]
16. Siemann DW, Chaplin DJ. An update on the clinical development of drugs to disable tumor vasculature. *Expert Opinion Drug Discovery*. 2007; 2:1–11.
17. Gould S, Westwood FR, Curwen JO, et al. Effect of pretreatment with atenolol and nifedipene on ZD6126-induced cardiac toxicity in rats. *J Natl. Cancer Institute*. 2007; 99:1724–1728.
18. Li L, Rojiani A, Siemann DW. Targeting the tumor vasculature with combretastatin A-4 disodium phosphate: effects on radiation therapy. *Int J Radiat Oncol Biol Phys*. 1998; 42:899–903. [PubMed: 9845118]
19. Siemann DW, Mercer E, Lepler SE, Rojiani AM. Vascular targeting agents enhance chemotherapeutic agent activities in solid tumor therapy. *International Journal of Cancer*. 2002; 99:1–6.
20. Petit I, Karajannis MA, Vincent L, et al. The microtubule-targeting agent CA4P regresses leukemic xenografts by disrupting interaction with vascular cells and mitochondrial –dependent cell death. *Blood*. 2008; 111:1951–1961. [PubMed: 18024794]
21. Chaplin DJ, Hill SA. The development of combretastatin A4 phosphate as a vascular targeting agent. *Int J Radiat Oncol Biol Phys*. 2002; 54:1491–1496. [PubMed: 12459376]

22. Siemann DW, Shi W. Targeting the tumor blood vessel network to enhance the efficacy of radiation therapy. *Seminars in Radiation Oncology*. 2003; 13:53–61. [PubMed: 12520464]
23. Horsman MR, Siemann DW. Pathophysiologic effects of vascular-targeting agents and the implications for combination with conventional therapies. *Cancer Res*. 2006; 66:11520–11539. [PubMed: 17178843]
24. Murata R, Siemann DW, Overgaard J, Horsman MR. Interaction between combretastatin A4 disodium phosphate and radiation in murine tumours. *Radiother Oncol*. 2001; 60:155–161. [PubMed: 11439210]
25. Ahmed B, Landuyt W, Griffioen AW, van Oosterom A, van den BW, Lambin P. In vivo antitumour effect of combretastatin A-4 phosphate added to fractionated irradiation. *Anticancer Res*. 2006; 26:307–310. [PubMed: 16475711]
26. Murata R, Overgaard J, Horsman MR. Combining combretastatin A-4 disodium phosphate and radiation in a fractionated schedule to improve local tumour control in mice. *Radiother Oncol*. 2000; 56:S98.
27. Li L, Rojiani AM, Siemann DW. Preclinical evaluations of therapies combining the vascular targeting agent combretastatin A-4 disodium phosphate and conventional anticancer therapies in the treatment of Kaposi's sarcoma. *Acta Oncologica*. 2002; 41:91–97. [PubMed: 11990525]
28. Horsman MR, Murata R, Breidahl T, Nielsen FU, Maxwell RJ, Stodkilde-Jorgensen H, Overgaard J. Combretastatins novel vascular targeting drugs for improving anti-cancer therapy. Combretastatins and conventional therapy. *Adv Exp Med Biol*. 2000; 476:311–323. [PubMed: 10949676]
29. Grosios K, Loadman PM, Swaine DJ, Pettit GR, Bibby MC. Combination chemotherapy with combretastatin A-4 phosphate and 5-fluorouracil in an experimental murine colon adenocarcinoma. *Anticancer Res*. 2000; 20:229–233. [PubMed: 10769660]
30. Wildiers H, Ahmed B, Guetens G, et al. Combretastatin A-4 phosphate enhances CPT-11 activity independently of the administration sequence. *Eur J Cancer*. 2004; 40:284–290. [PubMed: 14728944]
31. Horsman MR, Murata R, Overgaard J. Combination studies with combretastatin and radiation: effects in early and late responding normal tissues. *Radiother Oncol*. 2002; 64:S50.
32. Murata R, Overgaard J, Horsman MR. Combretastatin A-4 disodium phosphate: a vascular targeting agent that improves that improves the anti-tumor effects of hyperthermia, radiation, and mild thermoradiotherapy. *Int J Radiat Oncol Biol Phys*. 2001; 51:1018–1024. [PubMed: 11704326]
33. Pedley RB, Hill SA, Boxer GM, Flynn AA, Boden R, Watson R, Dearling J, Chaplin DJ, Begent RH. Eradication of colorectal xenografts by combined radioimmunotherapy and combretastatin a-4 3-O-phosphate. *Cancer Res*. 2001; 61:4716–4722. [PubMed: 11406542]
34. Ellis LM, Takahashi Y, Liu W, Shaheen RM. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. *Oncologist*. 2000; 5:11–15. [PubMed: 10804085]
35. Siemann DW, Shi W. Efficacy of combined antiangiogenic and vascular disrupting agents in treatment of solid tumors. *Int J Radiat Oncol Biol Phys*. 2004; 60:1233–1240. [PubMed: 15519796]
36. Siemann DW, Shi W. Dual targeting of tumor vasculature: Combining antiangiogenic (Avastin) and vascular disrupting (CA4P or OXi4503) agent therapy. *Anticancer Res*. 2008; 28:2027–2032. [PubMed: 18751370]
37. Dowlati A, Robertson K, Cooney M, Petros WP, Stratford M, Jesberger J, Rafie N, Overmoyer B, Makkar V, Stambler B, Taylor A, Waas J, Lewin JS, McCrae KR, Remick SC. A phase I pharmacokinetic and translational study of the novel vascular targeting agent combretastatin a-4 phosphate on a single-dose intravenous schedule in patients with advanced cancer. *Cancer Res*. 2002; 62:3408–3416. [PubMed: 12067983]
38. Stevenson JP, Rosen M, Sun W, Gallagher M, Haller DG, Vaughn D, Giantonio B, Zimmer R, Petros WP, Stratford M, Chaplin D, Young SL, Schnall M, O'Dwyer PJ. Phase I trial of the antivascular agent combretastatin A4 phosphate on a 5-day schedule to patients with cancer: magnetic resonance imaging evidence for altered tumor blood flow. *J Clin Oncol*. 2003; 21:4428–4438. [PubMed: 14645433]

39. Rustin GJ, Galbraith SM, Anderson H, Stratford M, Folkes LK, Sena L, Gumbrell L, Price PM. Phase I clinical trial of weekly combretastatin A4 phosphate: clinical and pharmacokinetic results. *J Clin Oncol*. 2003; 21:2815–2822. [PubMed: 12807934]
40. Anderson HL, Yap JT, Miller MP, Robbins A, Jones T, Price PM. Assessment of pharmacodynamic vascular response in a phase I trial of combretastatin A4 phosphate. *J Clin Oncol*. 2003; 21:2823–2830. [PubMed: 12807935]
41. Cooney M, Savvides P, Agarwala S, et al. Phase II study of combretastatin A4 phosphate (CA4P) in subjects with advanced anaplastic thyroid carcinoma (ATC). American Society of Clinical Oncology Annual Meeting. 2006 Abstract 5580.
42. Cooney M, Radiovoyevitch T, Dowlati A, et al. Cardiovascular safety profile of Combretastatin A4 Phosphate in a single-dose phase I study in patients with advanced cancer. *Clin Cancer Res*. 2004; 10:96–100. [PubMed: 14734457]
43. Rustin GJ, Nathan PD, Boxhall J, Saunders L, Ganesan TS, Shreeves GE. A Phase Ib trial of combretastatin A-4 phosphate (CA4P) in combination with carboplatin or paclitaxel chemotherapy in patients with advanced cancer. *J Clin Oncol*. 2005; 23:3013. (Abstract).
44. Ng Q, Goh V, Carnell D, et al. Phase Ib trial of Combretastatin A4 Phosphate (CA4P) in combination with radiotherapy (RT): Initial clinical results. American Society of Clinical Oncology Annual Meeting. 2005 Abstract 3117.
45. Bilenker J, Flaherty K, Rosen M, et al. Phase I trial of combretastatin a-4 phosphate with carboplatin. *Clin Cancer Res*. 2005; 11:1527–1533. [PubMed: 15746056]
46. Akerley W, Schabel M, Morrell G, et al. A randomized phase 2 trial of combretastatin A4 phosphate (CA4P) in combination with paclitaxel and carboplatin to evaluate safety and efficacy in subjects with advanced imageable malignancies. American Society of Clinical Oncology Annual Meeting. 2007 Abstract 14060.
47. Rustin G, Jayson G, Reed N, et al. Fosbretabulin (Combretastatin A-4 Phosphate [CA4P]) Carboplatin and Paclitaxel is active in patients with platinum resistant ovarian cancer. International Gynecologic Cancer Society Meeting. 2008 abstract 315.
48. Nathan P, Judson I, Padhani A, et al. A phase I study of the safety, tolerability and antitumor activity of escalating doses of Combretastatin A4 Phosphate (CA4P) given in combination with bevacizumab to subjects with advanced solid tumors – final results. American Society of Clinical Oncology Annual Meeting. 2008 Abstract 3504.
49. Jones R, Ewer M. Cardiac and cardiovascular toxicity of nonanthracycline anticancer drugs. *Expert Rev Anticancer Ther*. 2006; 6:1249–1269. [PubMed: 17020459]

Figure 1.
Molecular structure of CA4P.tris

Figure 2. Histological assessment of CaNT tumours that were evaluated at various times after treatment with a 100 mg/kg dose of CA4P. a) Untreated control, b) – d) 1, 3, and 4 days post CA4P exposure, respectively. Figure was modified from (21).

Figure 3. Effect of CA4P treatment alone or in combination with Avastin on renal cell carcinoma (Caki-1) xenograft growth. CA4P (100 mg/kg) was given Mondays, Wednesdays, and Fridays for 2 weeks. Avastin treatment (2 mg/kg) was administered daily (Monday through Friday) for 2 weeks. Data shown are a) the growth of the median tumors and b) the median, 75th, and 90th percentiles of responses of tumors treated at a starting size of 200 mm³. Results in mice treated with combination therapy were significantly greater than those obtained in mice treated only with a single therapy ($p < 0.05$, Wilcoxon rank sum test). Figure was modified from (36).

Table 1

Clinical Response in CA4P Studies

Indication	Regimen	Responses	Reference
Refractory solid tumors (n=25)	monotherapy	1 CR (4%) 11 SD (44%)	37
Refractory solid tumors (n=34)	monotherapy	4 SD (12%)	39
Refractory solid tumors (n=37)	monotherapy	1 PR (3%) 16 SD (43%)	38
ATC (n=21, interim)	monotherapy	6 SD (29%)	41
Refractory solid tumors (n=16)	Combination with carboplatin	4 SD (25%)	45
Refractory solid tumors (n=13)	Combination with carboplatin + paclitaxel	3 PR (23%) 6 SD (46%)	46
Refractory solid tumors (n=27, interim)	Combination with carboplatin and/or paclitaxel	4 PR (15%) 17 SD (63%)	43
Platinum resistant ovarian cancer (n = 23, interim)	Combination with carboplatin and paclitaxel	6 confirmed PR (26%) 3 unconfirmed PR (13%) (CGIC criteria)	47