

Targeting Established Tumor Vasculature: A Novel Approach to Cancer Treatment

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Abstract: The selective targeting of established tumor vasculature represents an attractive new anticancer drug strategy, distinct from inhibiting angiogenesis. This is based on the concept that, in contrast to targeting individual tumor cells, the killing of relatively few vascular endothelial cells could result in the death of a large area of tumor (from lack of oxygen and nutrients), drug delivery to vasculature is less challenging than to large solid tumors (which may harbor regions of hypoxia) and, moreover, cells that comprise vasculature (such as endothelial cells) are more genetically stable than tumor cells and hence less likely to acquire changes causing drug resistance. There is accumulating evidence that there are inherent differences in the vasculature of tumors, both morphologic and biochemical, in comparison to normal organs, thus providing a rational basis for this approach. Vascular disrupting agents (VDAs) are now being tested clinically; several are also in late preclinical development. A major class of small molecule VDA is those targeting tubulin, e.g., combretastatin A4 phosphate (CA4P), ZD6126 and AVE8062A. Another distinct non-tubulin based compound, the flavonoid 5,6-dimethyl xanthenone 4-acetic acid (DMXAA, AS1404) induces direct apoptosis of endothelial cells and secondary induction of various vasoactive agents (such as serotonin and tumor necrosis factor). These agents have all completed Phase I clinical evaluation; dose-limiting toxicities are generally non-overlapping with conventional cytotoxics; there has been evidence of efficacy. A common theme, now being pursued clinically, is that VDAs are expected to show maximum therapeutic benefit when used, intermittently rather than chronically and in combination with either conventional cytotoxics (such as platins or taxanes) or radiotherapy. Thereby, complementary kill of the central compartment of tumors (by VDAs) and the proliferating, well oxygenated, periphery (by cytotoxics or radiotherapy), is predicted. Non-invasive imaging techniques such as dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) have proven useful in the clinical monitoring of VDAs. A variety of additional small molecule anti-tubulin agents, N-cadherin inhibitors and antibody-based products (e.g., delivering effectors such as tissue factor to tumor blood vessels) are in earlier stages of development. The vascular targeting field is entering a particularly exciting phase; the next 1-2 years will be crucial in establishing clinical proof of principle for this approach.

Keywords: Vascular, targeting, combretastatins, DMXAA, tumor blood flow.

1. INTRODUCTION

The past decade has seen a revolution in anticancer drug development. The fields of genomics and proteomics have generated a plethora of new cancer -specific or -selective targets, leading to a paradigm shift in cancer drug development away from relatively poorly selective cytotoxics to a new era of “molecularly targeted therapeutics”. Recently approved agents such as imatinib (Gleevec), trastuzumab (Herceptin), gefitinib (Iressa) and cetuximab (Erbix) already bear witness to the clinical success of this strategy.

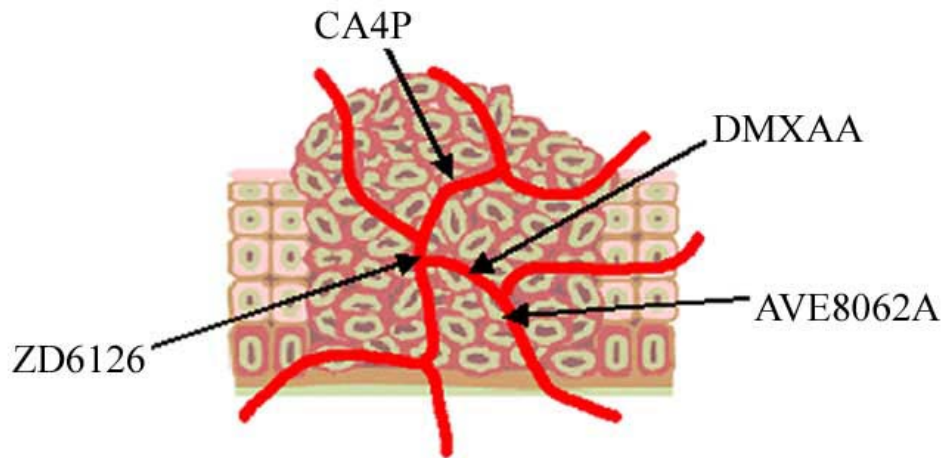
Attention has largely been given to targeting recognised “hallmarks” of cancer as described by Hanahan and Weinberg [1] in terms of six basic acquired properties: (1) self-sufficiency in growth signals; (2) insensitivity to antigrowth signals; (3) evading cell death or apoptosis; (4) limitless replicative potential; (5) sustained tumor blood vessel formation (angiogenesis); (6) tumor invasion and

metastasis. The idea that solid tumors require a functioning network of blood vessels in order to sustain growth through provision of oxygen and nutrients and also to remove toxic by-products of cellular metabolism, has been voiced for over 50 years [2]. The impetus to target angiogenesis, the formation of new blood vessels from the endothelium of existing vasculature, arose from pivotal observations by Folkman in the early 1970s [3]. It is now widely recognised that for any tumor to grow beyond a volume of 1-2mm³ requires a so-called angiogenic switch leading to the formation of new blood vessels (neovascularization) [4]. Over the subsequent years key molecules in this process were identified, such as vascular endothelial growth factor (VEGF) and its receptors, culminating in the recent reporting of clinical proof of principle in colorectal cancer of targeting VEGF with the humanised monoclonal antibody bevacizumab (Avastin) [5]. Other small molecule inhibitors of VEGF receptors are in clinical development (e.g., SU11248, PTK787/ZK22854).

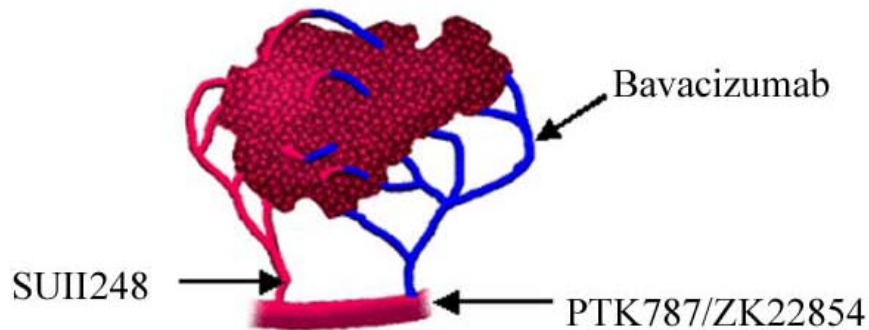
An alternative complementary strategy to anti-angiogenics, that of targeting established tumor vasculature (Fig. 1), was first suggested as long ago as the 1920s [6] and then more recently by Denekamp in the early 1980s

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VDA's



Larger, solid tumors - established blood vessels
 Major effect on central part of tumor
 Causes vessel occlusion and necrosis



Smaller, solid tumors - new blood vessels
 Major effect on tumor periphery
 Inhibits endothelial proliferation and migration

Fig. (1). Vascular disrupting agents versus angiogenesis inhibitors.

[7]. This has led subsequently to the field of vascular targeting for the treatment of cancer, the subject of this mini-review, where the design is to cause a rapid and selective shutdown of tumor blood vessels.

2. TARGETING ESTABLISHED TUMOR VASCULATURE; DREAM OR REALITY?

Conceptually, as suggested many years ago by Denekamp, the ability to selectively target established tumor vasculature is a very appealing strategy. First, one may postulate that, in contrast to targeting individual

cancer cells where every cell generally needs to be killed, the killing of relatively few vascular endothelial cells could result in the death of a large area of tumor via widespread central necrosis. This should be applicable across a wide variety of solid tumors and be independent of histological sub-type. Preclinical "proof of principle" for this strategy was provided via physical obstruction of the blood vessels of solid tumors in mice where tumor regressions were reported [8]. Second, from a pharmacological standpoint, drug delivery to cellular targets lining blood vessels is more readily achievable than the necessity to hit targets within areas of tumor distant from capillaries; this is

particularly relevant to antibody-based VDAs (see below). Third, acquired drug resistance, a feature all too commonly seen with cytotoxic cancer drugs, may be less of a clinical problem with VDAs as the target vascular endothelial cells are comparably genetically stable versus tumor cells. This has been demonstrated preclinically using the angiogenesis inhibitor, Endostatin [9]. Finally, since physiological blood vessel formation is limited primarily to wound healing and menstruation, anti-vascular targeting may be relatively non-toxic to non-tumor tissue.

A fundamental criterion that must be satisfied if vasculature targeting is to be successful in the clinic is that tumor vasculature (which is made up of endothelial cells, pericytes and basement membrane) provides specific or at

least selective targets with respect to normal organ vasculature. Over recent years a large body of cellular and molecular evidence suggests that, indeed, this is the case. First, the vasculature in tumors is proliferating (rate of 50-1000 fold higher) and relatively immature in comparison to that in normal tissues and morphologically complex, chaotic and seemingly disorganised [10] for a review and [11] for an example of a morphological comparison of a human colon cancer and corresponding normal tissue). Also in tumors there is often increased vascular permeability (leakiness) leading to reduced overall blood flow. In recent studies using endothelial cells isolated from human colorectal cancer biopsies, 46 of 170 transcripts expressed mainly in endothelial cells, were significantly elevated above levels in corresponding normal tissues [12]. These include VEGFRs, cell adhesion molecules such as E-selectin, α -integrins and VCAM, CD105/endoglin, the ED-B domain of fibronectin, and phosphatidylserine ([13] for a review). Abnormalities have also been reported in pericytes on tumor blood vessels and endothelial sprouts, including α -smooth muscle actin immunoreactivity [14].

New solid tumor selective luminal endothelial cell surface proteins, such as annexin A1, continue to be described providing additional opportunities for improved imaging or therapeutics (e.g., via radioimmunotherapy) [15].

3. VDAs UNDERGOING CLINICAL DEVELOPMENT

At present, there are two distinct classes of small molecule VDAs undergoing clinical development; those directed at tubulin (e.g., CA4P, ZD6126 and AVE8062) and synthetic flavonoids (e.g., DMXAA/AS1404). Interestingly, many early cancer treatments now appear to have mediated at least part of their anti-tumor effects through vascular targeting; these include bacterial products (so called Coley's toxins) [16], probably via the induction of tumor necrosis factor (TNF) (see below) and lead colloids, probably *via* inducing thrombosis in tumor blood vessels [17].

3.1. Combretastatins, CA4P, AVE8062 (AC7700)

Following the studies of Denekamp and colleagues in mice, a search began for chemicals that could selectively shut down tumor blood flow. It was shown that the tubulin binding (destabilising) agents, vinblastine and vincristine and particularly colchicine appeared to shut down tumor blood flow in mouse tumors, but only at doses close to maximum tolerated [18]. Indeed, clinical studies with colchicine in the 1930s had reported haemorrhagic necrosis in tumors but toxicity precluded its further development [19]. It was then shown by Chaplin and colleagues that another tubulin destabilising natural product isolated from the South African bush willow tree by Pettit and colleagues, named combretastatin, caused extensive tumor vascular damage and necrosis in various transplanted mouse tumors but importantly, at relatively non toxic doses [20]. The development of a more pharmaceutically tractable water soluble prodrug, disodium combretastatin A-4 3-0-phosphate (CA4P, Oxigene Inc,

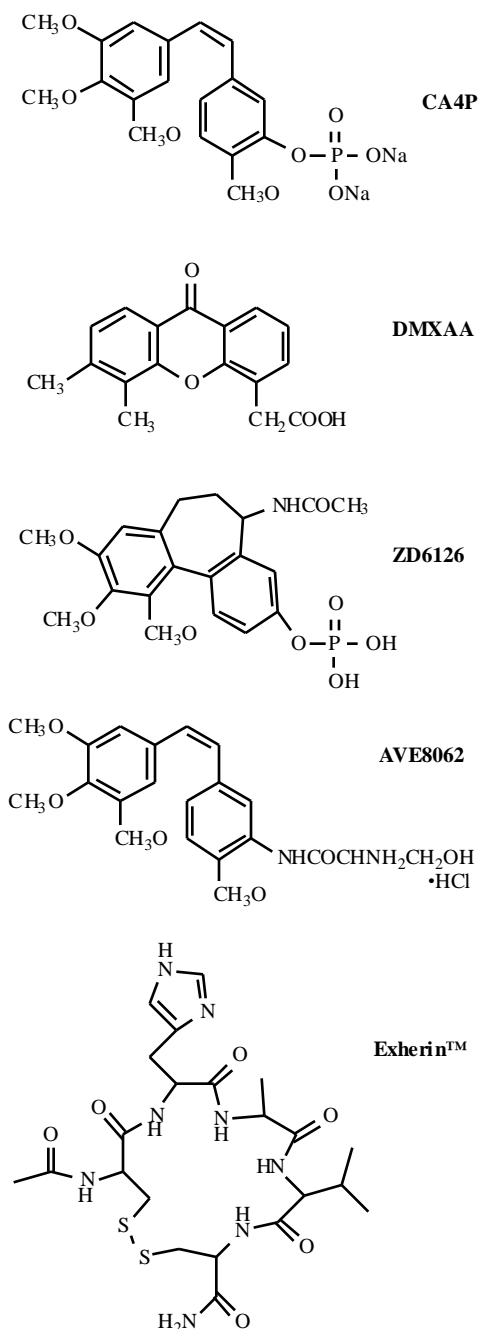


Fig. (2). Structures of VDAs in clinical development.

Fig. 2 for structure), then allowed for the clinical development of this class of molecule.

In parallel, numerous preclinical studies in mouse and rat tumor models demonstrated that CA4P induced a rapid shutdown in tumor blood flow (to almost undetectable levels by 6 hours post administration) whereas blood flow to normal tissues was much less affected [21;22]. The mechanism of action of CA4P causing vascular shutdown in tumors is consistent with affecting the shape of newly formed endothelial cells (with little or no effect on quiescent cells) [23;24]. This appears to occur through Rho kinase mediated reorganisation of the actin cytoskeleton after microtubule breakdown leading to membrane blebbing [25]. A consistent theme with these preclinical studies (and also reported with DMXAA and ZD6126, see below) is that single agent (and single dose) administration, while causing massive central necrosis of tumors, often leaves a narrow viable rim of tumor tissue (e.g., [20]). This is widely thought to reflect the fact that tumor cells on the edge of tumors obtain oxygen and nutrients from normal blood vessels which are less responsive to VDAs. This has led to a widely held view in the vascular targeting field, and supported by preclinical efficacy data (see [24] and [26] for reviews of CA4P) that the optimal clinical use of VDAs is in combination with other treatment modalities such as cytotoxic chemotherapy or radiotherapy.

3.1.1. Clinical Studies with CA4P

Phase I studies with CA4P began in 1998; three trials using different schedules have now been reported [27-29] Table 1). Dose-limiting toxicities (see Table 1) are generally distinct from those of conventional cytotoxic chemotherapeutics. The cardiovascular safety profile of CA4P has been evaluated using a single dose (from 18 to 90mg/m²) in 25 patients; the drug prolongs the QTc

interval and it has been advised that future trials have eligibility guidelines preventing its use in patients with known coronary heart disease until further clinical experience of the drug is built up [30]. There has been evidence of antitumor efficacy; a patient with anaplastic thyroid cancer in the single dose every 3 weeks trial had a complete response [28], one patient at 68mg/m² in the weekly trial had improvement in liver metastases of adrenocortical carcinoma [29], a partial response in a patient with metastatic soft tissue sarcoma was seen in the daily for 5 days trial [27]. A notable and valuable parallel development in the vascular targeting field has been the use of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to provide a non-invasive measure of vascular effects in tumors [31]. Herein it was shown that CA4P has antivascular activity in tumors in man (and in rat tumors) at well tolerated doses (significant reduction in tumor K^{trans} in 6/16 patients treated at 52mg/m² or above), but with no significant changes in kidney or muscle. In addition, positron emission tomography (PET) has been successfully used in patients receiving CA4P to provide an alternative pharmacodynamic marker of changes in tumor perfusion; significant dose-dependent reductions in tumor perfusion 30 minutes after CA4P administration were reported [32]. Numerous phase Ib combination trials with radiotherapy, platins and taxanes, and radioimmunotherapy and a phase II monotherapy trial in thyroid cancer are currently ongoing (Table 1).

3.1.2. AVE8062A

AVE8062A (Aventis Pharma), like CA4P, is a water-soluble analog of combretastatin A4 (see Fig. 2 for structure) which causes a dramatic shutdown in tumor blood flow in many preclinical tumor models leading to necrosis [33]. As with CA4P (and DMXAA and ZD6126) *in vivo* synergy in preclinical models between cytotoxic agents (cisplatin, carboplatin, vinorelbine, doxorubicin)

Table 1. Clinical Studies with CA4P

A) Phase I			
Schedule	Dose limiting toxicity	MTD	Reference
Daily for 5 days, q 3 weeks	Cardiopulmonary (syncope, dyspnea, hypoxia)	52mg/m ²	27
Single, q 3 weeks	Acute coronary syndrome, tumor pain	60mg/m ²	28
Weekly for 3 weeks	Tumor pain, reversible ataxia, vasovagal syncope, ischemia	88-114mg/m ²	29
B) Ongoing Phase I/II			
Ovarian cancer plus carboplatin and paclitaxel (CA4P given 18-24h before chemotherapy)			
Head and neck, lung, prostate with radiotherapy (after 5 daily 2Gy fractions)			
Cervical cancer with radiotherapy and cisplatin			
Phase I with radioimmunotherapy (¹³¹ I A5B7 anti-CEA antibody)			
Phase II monotherapy in thyroid cancer			
Ischemic retinopathy			

and AVE8062A has been observed. It is currently undergoing Phase I evaluation although no details have yet been published.

3.2. Agents Related to Colchicine

ZD6126 (Astrazeneca) is an analogue of colchicine (N-acetylcolchinol-O-phosphate, Fig. 2 for structure) and has recently completed Phase I trials using 3 different schedules (daily for 5 days, weekly and every 3 weeks). Similar to the above tubulin-binding agents, ZD6126 was shown to induce extensive necrosis in preclinical solid tumors, including human tumor xenografts, but a viable rim remained after single-dose administration and minimal growth delay was observed. Multiple administrations (daily for 5 days for 2 or 3 weeks) conferred significant tumor growth delay and the activity of paclitaxel, cisplatin and radiation therapy was enhanced by ZD6126 [34-36]. ZD6126 possesses a wide therapeutic index in preclinical studies with antitumor efficacy observed at doses 8-16 fold lower than maximum tolerated [35]. Both preclinically in rat and murine tumors [37;38] and in human cancers [39], evidence of blood flow reduction has been observed using DCE-MRI (by measuring IAUC; initial area under the gadolinium diethylenetriaminepentaacetate uptake versus time curve). In patients at doses of 80mg/m² and higher, ZD6126 treatment caused a reduction in blood flow to 36-72% from the baseline level in all tumors studied (n=8, 6 patients) while no drug induced changes in muscle or spleen were observed [39].

3.3. Flavonoids, DMXAA

The interest in this distinct non-tubulin class of VDA began in the mid 1980s with the unexpected discovery of the antitumor properties of flavone acetic acid (FAA), a molecule originally synthesised as a non-steroidal anti-inflammatory agent by Lyonnaise Industrielle Pharmaceutique. FAA was active, even curative, against a wide variety of preclinical solid tumor models [40]; effects appeared to be related to the production of TNF [41]. Initial Phase I studies used an ester prodrug of FAA (LM985); the dose-limiting toxicity was acute reversible hypotension occurring during drug infusion, at doses of 1500mg/m² [42]. Later Phase I studies used FAA itself (LM975), again hypotension was dose-limiting but at the substantially higher dose of 10g/m² [43]. None of these trials, including a Phase II in non-small cell lung cancer, showed evidence of efficacy [44]. One Phase I trial of FAA (4.8g/m² given on days 1,8 and 15) in combination 2-4 days after recombinant interleukin-2 was performed; while there was one complete and two partial responses lasting 20+, 17+ and 15+ months, severe (Grade 3 or 4) hypotension after the 3rd dose of FAA was observed and no increase in tumor necrosis was seen in 15 biopsies taken from 10 patients after therapy [45]. This led to the clinical abandonment of FAA prior to any other combination studies being performed, on the premise that FAA does not elicit similar anti-vascular effects in humans as had been seen in many murine tumors [46].

A synthetic program to identify a more potent inducer of TNF was initiated by Denny and Baguley and

colleagues at the University of Auckland, New Zealand, and resulted in DMXAA (Fig. 2 for structure [47] for a review). Encouragingly, DMXAA was shown to induce TNF mRNA in both murine and human cells (whereas FAA only induced TNF in murine cells) [48]. However, the mechanism of action of DMXAA is more complex than simply induction of TNF. Data support an early direct effect on endothelial cells (possibly through involvement of the nuclear factor B although the exact target remains to be identified) leading to rapid apoptosis (within 1 hour). This is followed by indirect effects involving the release of vasoactive agents within tumor tissue, such as serotonin (from platelets), and TNF, possibly other cytokines and, even later, nitric oxide from host cells ([47] for a review). A comparison of blood perfusion effects in a murine tumor (C3H) for CA4P and DMXAA showed that blood flow reduction with CA4P was more rapid (maximum effect only 1 hour after administration for CA4P versus 6 hours for DMXAA) but more transient as perfusion had returned to normal 24 hours later with CA4P but not with DMXAA [22].

DMXAA has shown single agent antitumor activity, including cures in some models such as the syngeneic colon 38, especially when administered as a loading dose of 25mg/kg followed by two supplementary "maintenance" doses of 5mg/kg 4 and 8 hours later [49]. As with CA4P, DMXAA has shown at least additive, even synergistic, antitumor effects in combination with a variety of treatment modalities such as radiation [50], radioimmunotherapy [51] and hyperthermia [52]. Marked potentiation was observed with a variety of chemotherapeutic drugs using a syngeneic mouse mammary tumor model [53]. The therapeutic gain was most striking with paclitaxel (dose modification factor, the gradient of dose response curve with versus without DMXAA, of >13) and docetaxel (>9) but was also apparent for vincristine (>7), etoposide (4.7), carboplatin (3.4) cyclophosphamide (2.7), doxorubicin (2.5) and cisplatin (1.8) [53]. The sequence of administration of DMXAA and paclitaxel was important, while synergy was seen if paclitaxel was given up to 4 hours before or up to 2 hours after DMXAA, the potentiation was lost if paclitaxel was given 4 hours after DMXAA [53]. The synergistic effect of paclitaxel and DMXAA has recently been confirmed using a human tumor xenograft model of non-small cell lung cancer (Fig. 3). At least in mice, the therapeutic index for DMXAA is rather narrow; typically vascular targeting/tumor necrosis effects are seen at doses (>15mg/kg) which are relatively close to maximum tolerated (approx 25mg/kg). This, however, does not appear to be the case in man (see below).

3.3.1. Clinical Studies with DMXAA

DMXAA (AS1404, Antisoma) entered clinical trials under the auspices of the United Kingdom Cancer Research Campaign in 1996 in the UK (weekly schedule) [54] and in New Zealand (3-weekly schedule) [55], both using a 20 minute intravenous infusion. In the UK study, 46 patients received DMXAA over a dose range of 6 to 4900mg/m²; dose-limiting toxicities were urinary incontinence, visual disturbance (as was reported for FAA) and anxiety and thus were unlike that of conventional chemotherapy. There was

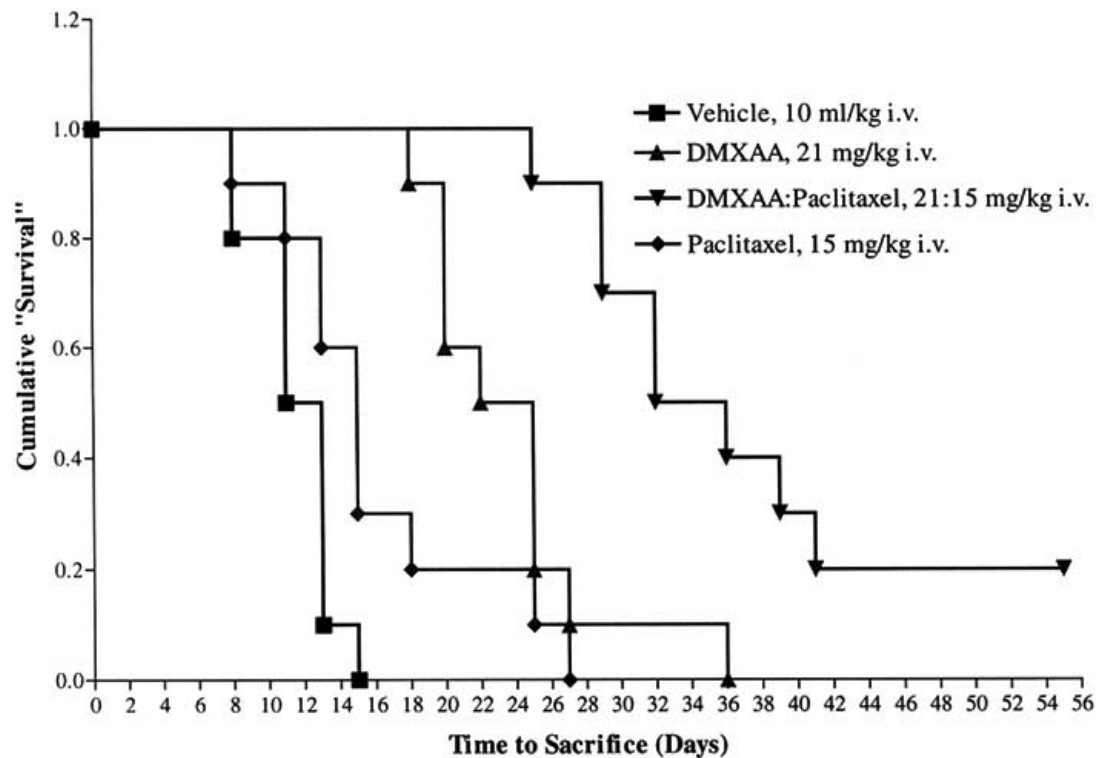


Fig. (3). Potentiation of the antitumor activity of paclitaxel by DMXAA in a human non small cell lung cancer xenograft; drugs were administered on days 0, 4 and 8 after tumors had become established.

one unconfirmed partial response at the 1300mg/m² dose level in a patient with melanoma. The New Zealand study reported very similar findings with rapidly reversible toxicities of confusion, tremor, slurred speech, visual disturbance, anxiety, urinary incontinence and possible left ventricular failure seen at the 4900mg/m² dose level. There was a transient prolongation of cardiac QTc interval observed in 13 patients administered doses of 2000mg/m² and above. A patient with metastatic cervical carcinoma achieved an unconfirmed partial response at 1100mg/m².

Dose-dependent plasma increases in the serotonin metabolite 5-hydroxyindoleacetic acid (5HIAA) were observed at doses above 650mg/m² thus providing a possible pharmacodynamic marker of vascular targeting with this drug. In addition, DCE-MRI studies in patients treated from 500 to 4900mg/m² showed that 9/16 patients had significant reductions in DCE-MRI parameters related to tumor blood flow 24 hours after the 1st dose of DMXAA and 8/11 patients had reductions of up to 66% 24 hours after the last dose [56]. Interestingly, these effects occurred over a wide dose-range suggesting that the vascular targeting properties of DMXAA in tumors may occur at doses considerably below those that cause significant toxicities. No significant effects were seen in muscle 24 hours after the last dose. Also, tumor endothelial cell apoptosis was seen (by TUNEL staining) in a breast cancer biopsy (1 out of 3) taken 3 and 24 hours after infusion of 3.1g/m² [57].

In summary, DMXAA was well-tolerated with both schedules of administration; the lack of anti-proliferative or myelosuppressive toxicities along with considerations of:

- complementary tumor killing (i.e., DMXAA targeting central regions; cytotoxic chemotherapy or radiotherapy preferentially targeting the well oxygenated outer rim)
- the possibility of pharmacokinetic “trapping” of cytotoxic drugs in the tumor (i.e., scheduling the VDA to trap cytotoxics or bioreductive drugs in the tumor)

Strongly suggest that future combination therapy trials are evaluated. Such trials, particularly involving taxanes, have now begun.

4. NEW APPROACHES

Following the clinical lead provided by CA4P and DMXAA, today, there are a number of additional agents and approaches undergoing preclinical or early clinical development. Within new classes of small molecules, of interest are cyclic peptide-based inhibitors of the adhesion protein N-cadherin (Exherin; N-acetyl-L-cysteinyl-L-histidyl-L-alanyl-L-valyl; Adherex technologies, Fig. 2 for structure) [58]. This approach targets N-cadherin mediated endothelial cell-pericyte binding in tumor blood vessels; single intravenous doses of 50 to 281mg/m² have so far been studied. No toxicity limiting further dose escalation was identified; hints of efficacy were reported in 2 patients using DCE-MRI.

Further derivatives of tubulin-targeting agents are also close to Phase I. These include OXI4503 (Oxigene Inc), the disphosphate prodrug of combretastatin A1, which has demonstrated efficacy (including complete regressions) as a single agent used at 25mg/kg in a preclinical subcutaneous

xenograft model of human breast cancer [59] and has been shown to enhance the efficacy of carboplatin and paclitaxel. Effects appear to be mediated by induction of tumor vascular collapse following early endothelial cell apoptosis [60]. In addition, Phase I trials are expected to begin soon with NPI-2358 (Nereus Pharmaceuticals) a novel diketopiperazine structurally unrelated to colchicine and MN-029 (MediciNova) (methyl 6-[(4-([2S]-2-aminopropanoyl)-amino)phenyl]thio]-1H-benzimidazol-2-ylcarbamate hydrochloride). Other tubulin-binding agents in early clinical trial such as the dolastatin 10 derivative (TZT-1027/Sonidotin; Teikoku Hormone Mfg Co) in clinical development in Japan and ABT-751 (Abbot laboratories) may also exert biological effects via targeting vasculature.

One may also be able to exploit tumor vasculature as a means to aid selective drug delivery of conventional cytotoxics such as paclitaxel or camptothecin by incorporation into cationic liposomes (e.g., MBT-026 containing paclitaxel, Munich Biotech which is now undergoing Phase II studies). This approach is proposed to work by exploiting anionic sites on the surface of angiogenic vascular endothelial cells in tumors, possibly via overexpression of the negatively charged lipid phosphatidylserine [61].

There also remains significant interest in using a ligand-based biological targeting approach to selectively deliver a variety of effector molecules to tumor vasculature [62]. Promising proof of principle has been demonstrated in preclinical tumor models for this approach using a variety of vascular targeting antibodies chemically linked or recombinantly fused to effector molecules; for example, a fragment of the blood coagulation inducing protein, tissue factor, recombinantly linked to an antibody fragment (ScFv) which recognises the neovasculature marker ED-B fibronectin [63]. More recently, another humanised IgG to ED-B fibronectin (hBC1) has been recombinantly fused to the cytokine interleukin 12 and shown to be active in preclinical models [64]. VEGF has also been used to target toxins, such as the plant glycosidase gelonin, to tumors in mice [65]. Gelonin, unlike some other toxins such as ricin A chain, does not seem to generate capillary leak syndrome and therefore may be clinically applicable in this context. A naked monoclonal antibody, 3G4, to phosphatidylserine has been shown, in preclinical models, to localise to tumor endothelium and enhance the efficacy of docetaxel [66].

5. SUMMARY

Approximately 20 years on from its beginnings, the field of vascular targeting is now at a particularly exciting evolutionary stage. Pivotal Phase Ib/II combination trials with CA4P have begun, combination trials with DMXAA began during H2 of 2004. Additional anti-tubulin based molecules to CA4P are at earlier stages of late preclinical or early clinical development; other novel approaches to vascular targeting are being evaluated. Already in these early phase clinical trials (e.g., with CA4P, DMXAA and ZD6126) tumor based anti-vascular effects have been observed using DCE-MRI. The majority of the preclinical efficacy data support the clinical use of VDAs

intermittently and in combination with other modalities such as cytotoxic chemotherapy and radiotherapy (already being pursued in the clinic), anti-angiogenics, hyperthermia and photodynamic therapy. Moreover, the toxicities thus far observed with VDAs are generally non-overlapping with those associated with cytotoxics or radiotherapy. Further clinical study is required to determine the optimal scheduling of VDAs in combination with these other modalities. Also, it appears that, in contrast to conventional cytotoxics, some VDAs such as DMXAA, may be optimally used at doses well below toxic doses. The clinical development of biologics based on vascular targeting (e.g., monoclonal antibodies to tumor endothelial targets as delivery vehicles for effectors such as tissue factor, diphtheria toxin or gelonin) has been relatively delayed in comparison to the above small molecule approaches despite compelling preclinical data. This probably reflects the relative increase in complexity in producing such products, either as chemical conjugates or fusion proteins, for clinical study. The field is at an especially exciting phase; it is hoped, as with the recent establishment of clinical proof of principle with the angiogenesis inhibitor, bevacizumab, that VDAs will also enter the armamentarium available to future oncologists.

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