Pathophysiologic Effects of Vascular-Targeting Agents and the Implications for Combination with Conventional Therapies

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Abstract

A functional vascular supply is critical for the continued growth and development of solid tumors. It also plays a major role in metastatic spread of tumor cells. This importance has led to the concept of targeting the vasculature of the tumor as a form of cancer therapy. Two major types of vascular-targeting agent (VTA) have now emerged: those that prevent the angiogenic development of the neovasculature of the tumor and those that specifically damage the already established tumor vascular supply. When used alone neither approach readily leads to tumor control, and so, for VTAs to be most successful in the clinic they will need to be combined with more conventional therapies. However, by affecting the tumor vascular supply, these VTAs should induce pathophysiologic changes in variables, such as blood flow, pH, and oxygenation. Such changes could have negative or positive influences on the tumor response to more conventional therapies. This review aims to discuss the pathophysiologic changes induced by VTAs and the implications of these effects on the potential use of VTAs in combined modality therapy. (Cancer Res 2006; 66(24): 11520-39)

Introduction

The development of a functional blood supply is essential to most solid tumors (1, 2). Typically, tumors can only grow to a few millimeters in size before the tumor cells begin to exist beyond the diffusion distance of oxygen and other essential nutrients. Further growth and development of the tumor then requires the formation of a vascular system to meet the demands of the growing tumor mass. The neovasculature that arises does so from the normal host vessels by the process of angiogenesis (3, 4) and not only provides tumor cells with oxygen and nutrients necessary for survival but is also the principal vehicle for metastatic spread (5). Furthermore, the tumor vascular supply influences the microenvironmental conditions within tumors and as such has a significant effect on the tumor response to therapy (6, 7). The importance of the tumor neovasculature thus makes it a potential therapeutic target.

Targeting the tumor vascular supply for cancer therapy is not a new concept. Indeed, the deliberate use of bacterial infections in cancer treatment in the 1800s involved an effect that was at least partially mediated through vascular targeting (8). Furthermore, as early as the 1920s, the concept of specifically targeting tumor vasculature as a potential therapy was proposed (9). However, it is only more recently that the use of vascular-targeting agents (VTA) has become established as a viable possibility (10, 11). Two major approaches have now emerged. One is based on controlling the blood vessel development by inhibiting the angiogenesis process [angiogenesis-inhibiting agents (AIA)], and the other involves compromising the function of the already existing blood vessels [vascular-disrupting agents (VDA)]. It is now becoming increasingly clear that vascular effects are involved in the action of other therapies, including certain types of chemotherapy, radiotherapy, and inhibitors of epidermal growth factor receptor or cyclooxygenase-2 (12–14), but because the vasculature is not the principal target of these other therapies, they will not be discussed in this review. Instead, we will restrict our comments to those therapies that are specifically designed to target tumor endothelium with little direct effect on the neoplastic cell population.

Regardless of the VTA approach used, it is unlikely that any VTA on its own will lead to tumor control, and the clinical potential of such agents will most likely be realized when combined with more conventional therapies. However, the tumor vascular supply can play a critical role in determining tumor response to such conventional treatments. The tumor blood supply will influence the delivery of blood-borne agents, including chemotherapeutic drugs, antibodies, and gene therapy approaches. It also controls the tumor microenvironment, and this is known to influence tumor response to drugs, radiation, and heat. Because VTAs affect tumor vascularity, they most likely will induce pathophysiologic changes that can have both a negative or a positive influence on the therapy with which it is combined. The aim of this review is to summarize the pathophysiologic changes caused by VTAs and discuss the implications of these changes when using VTAs in a combined modality setting.

Vascular-Targeting Approaches

Although AIAs and VDAs both target the tumor vascular supply, they are two distinct approaches (15). AIAs are designed to prevent further development of the tumor neovascular network. Numerous agents capable of inhibiting new blood vessel formation have been identified, and each affects at least one of the several important stages of angiogenesis. The primary targets are the angiogenic factors, which play the most significant role in neovascularization (16). These are secreted by the tumor cells and are up-regulated by various environmental factors, such as hypoxia, loss of tumor suppressor gene function, and oncogene activation (16, 17). Of these angiogenic factors, the most potent and specific is vascular endothelial growth factor (VEGF), which not only is crucial for endothelial cell proliferation and blood vessel formation but also induces significant vascular permeability and plays a key role in endothelial cell survival signaling in newly formed vessels (16, 17). VEGF has been targeted by a variety of strategies (18–21), including monoclonal antibodies [e.g., bevacizumab (Avastin) and DC101], inhibitors of endothelial cell receptor-associated tyrosine kinase

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activity (e.g., SU5474, SU6668, ZD6474, and PTK787/ZK 222584), and antisense. Other approaches, including those targeting basement membrane degradation, endothelial cell migration, endothelial cell proliferation, and tube formation, have also been actively considered (18–21). Many of these antiangiogenic therapies are currently under clinical evaluation (18, 20, 22).

VDAs are agents that cause direct damage to the already established tumor endothelium (10, 23–26). These include physical treatments, such as hyperthermia or photodynamic therapy (PDT), which have been well documented to induce direct tumor cell killing and an indirect effect through the induction of vascular damage (10, 23). They also include biological response modifiers or cytokines, such as tumor necrosis factor (TNF) and interleukins; certain established chemotherapeutic drugs, such as *Vinca* alkaloids and arsenic trioxide (ATO); and various ligand-based approaches that use antibodies, peptides, or growth factors that can selectively bind to tumor vessels (10, 23–26). But more commonly, VDAs involve the use of small-molecule drugs (26), of which there are two major classes of agents. The first includes flavone acetic acid (FAA) and its derivative 5,6-dimethylxanthenone-4-acetic acid (DMXAA), which have a complex mechanism of action that is poorly understood, but their main effect on vascular endothelial cells is thought to involve a cascade of direct and indirect effects, the latter involving the induction of cytokines, especially TNF-α, leading to the induction of hemorrhagic necrosis (27, 28). A second group includes the tubulin-binding agents combretastatin A-4 disodium phosphate (CA4P), the phosphate prodrug of *N*-acetyl-colchinol (ZD6126), AVE8062, NPI2358, MN-029, and OXi4503 (26, 28). These tubulin-depolymerizing agents are primarily believed to selectively disrupt the cytoskeleton of proliferating endothelial cells, resulting in endothelial cell shape changes and subsequent thrombus formation and vascular collapse (28). Because they preferentially target dividing endothelial cells, this accounts for their tumor specificity. Both types of small molecular drugs have been shown to have potent antivascular and antitumor efficacy in a wide variety of preclinical models, and the lead agents are undergoing clinical evaluation (24).

Because AIAs and VDAs induce vascular effects by very different mechanisms, their antitumor activity and optimal application will be very different. Figure 1 illustrates the major differences. Generally, AIAs are given as a chronic administration and essentially slow tumor development (26). There are examples where tumor growth can be completely inhibited or the treatment of established tumors can result in tumor regression, but these tend to be exceptions rather than the norm (29–31). Consequently, AIAs are probably best suited for early-stage or metastatic disease.

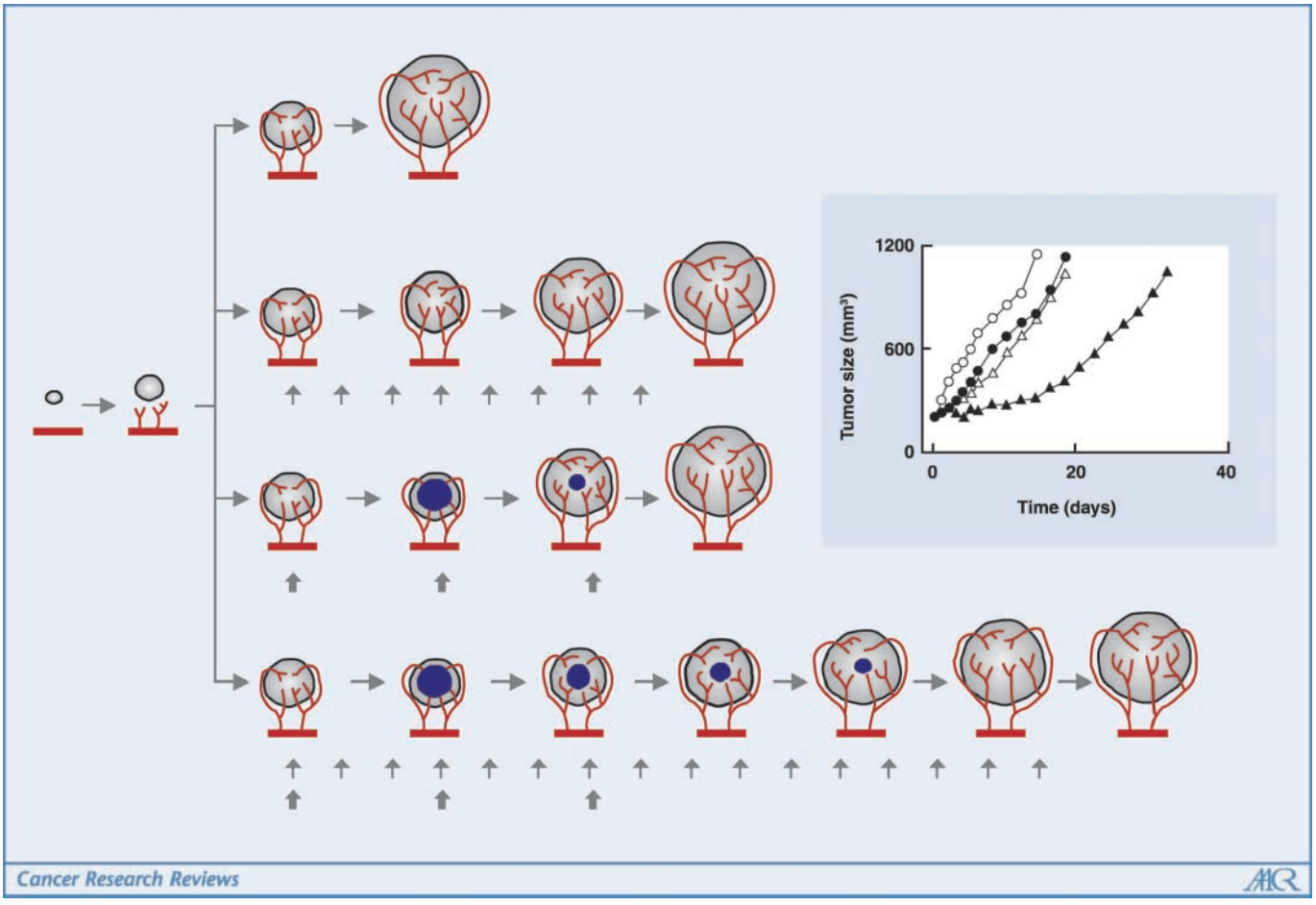

Figure 1. Schematic representation of the effects of AIAs and VDAs on tumors. *Gray,* tumors; *red,* normal and neovasculature; *blue,* induced necrosis. Tumor growth only occurs after they have established their own functional blood supply by angiogenesis. This growth can be inhibited using either AIAs (↑) or VDAs (⬆), alone or in combination, given under optimal conditions. *Inset,* actual growth data for the human ovarian carcinoma OW1 grown s.c. on the flanks of nude mice and treated when at 200 mm^3 in size. Animals were treated with vehicle control (○), ZD6474 given p.o. on days 1 to 5 at a dose of 25 mg/kg (●), ZD6126 injected i.p. on days 1, 3, and 5 at a dose of 100 mg/kg (△), or a combination of both ZD6474 and ZD6126 (▲). Results are mean values for eight mice per group and are taken from ref. 36.

With VDAs, the administration is of a more acute type to induce substantial vascular shutdown (26). Antitumor effects should also be possible with lower doses given over a prolonged period, but that would probably increase the risk of normal tissue vessel damage and defeat the potential benefit. Following treatment with VDAs, tumor shrinkage has been observed, but this seems to be tumor and drug dependent and although significant it is generally modest and thus tumor growth is only temporarily delayed (26, 32). There is good evidence that VDAs have a superior effect on bulky disease (33–35). Also illustrated in Fig. 1 is the effect one observes when AIAs and VDAs are combined. In terms of antitumor activity, the inhibition of tumor growth with the combination is greater than the effects from either treatment alone, and this has been shown with several different combinations of AIAs and VDAs (36–41). Still, irrespective of whether the VTAs are given alone or in combination, for tumor control to be obtained these agents typically must be combined with more conventional therapies.

Pathophysiologic Effects of VTAs

Angiogenesis inhibitors. When compared with the normal tissue vessels from which it arises, the tumor vascular supply is very different (6, 7). It is very primitive in nature, morphologically and functionally abnormal, and typically unable to keep pace with the rapidly growing tumor cell mass. Consequently, the neovascular network fails to meet the demands of the tumor cells for oxygen and nutrients, and this failure results in the development of oxygen-deficient, nutrient-deprived, and highly acidic conditions within the tumor (6, 7). Surprisingly, clonogenic cells can survive in such adverse environmental conditions and are known to play a major role in influencing tumor response to therapy (42, 43) and malignant progression (44, 45).

The inhibition of tumor growth induced by treatment with AIAs is generally associated with a reduced vascular density. This has been shown for anti-VEGF monoclonal antibodies (46, 47), tyrosine kinase inhibitors (29, 31, 47–49), and nonspecific inhibitors, such as endostatin (50, 51), arginine deiminase (47), and anginex (52). However, it is not a universal finding and there are examples where vascular density remained unchanged (52–55) and even one example where it increased (56) despite tumor growth being inhibited. In those situations where vascular density is reduced, it would be expected to increase the adverse microenvironmental conditions within the tumor. Indeed, as listed in Table 1, studies with several different AIAs have reported a decrease in tumor oxygenation status, measured using a variety of conventional end points, such as the Eppendorf polarographic oxygen electrode (47, 52), hypoxic markers (57), or classic radiation response assays (58, 59). Two studies did not measure tumor oxygenation directly but found a decrease in tumor blood perfusion (60, 61), and such changes would have been expected to decrease tumor oxygenation as was seen with anginex (52). However, a reduced oxygenation status with AIA treatment is clearly not a universal phenomenon. Some studies reported no change in tumor oxygenation (46, 47, 49, 55, 62). Using endostatin in a well-vascularized MCa35 mammary carcinoma, this lack of effect was not surprising because the AIA also had no apparent influence on tumor vasculature or growth (55). But for the anti-VEGF monoclonal antibodies in the LS1747 colon adenocarcinoma (46) and 54A small cell lung cancer (62), tumor growth inhibition was reported, and in the LS1747 tumor, this was associated with a decrease in the number of tumor vessels (46). Furthermore, with arginine deiminase, the lack of any effect on oxygenation status of the WAC2 neuroblastoma was observed, despite the AIA decreasing tumor vascular density and perfusion and inhibiting tumor growth (47).

AIA-induced improvements in tumor oxygenation have also been reported (46, 50, 55, 56, 63–67). In one study using the anti-VEGF monoclonal antibody DC101 in a Shionogi mammary carcinoma grown in a window chamber, it was shown that, as untreated tumors grew, vascular density increased whereas oxygenation status decreased (50). Treatment with DC101 every 3 days for a total of 18 days resulted in a general decrease in vascular density and corresponding decrease in oxygenation. It was only at the end of the treatment period that vascular density and oxygenation began to increase. Interestingly, tumor oxygen consumption was unchanged in control tumors and remained relatively constant throughout the initial period of treatment with DC101, only increasing toward the end of treatment. Tumor oxygenation was also increased by an anti-VEGF monoclonal antibody in U87 glioblastomas (46). However, no change was observed when using a clamped tumor growth delay assay, leading to the authors questioning the significance of the oxygenation changes. Similar results were also reported for thrombospondin in a D-12 human melanoma using tumor growth delay (64), but a clear reduction in hypoxia was seen using the classic paired survival curve assay, suggesting that AIAs can decrease tumor hypoxia but that they have other effects that can sometimes mask the improvements in tumor oxygenation. One study that clearly showed an improvement in oxygenation status during treatment with an AIA was that of Winkler et al. (65). Using the hypoxic cell marker pimonidazole, they found a significant improvement in oxygenation of a human glioblastoma xenograft grown orthotopically in the mouse brain during treatment with DC101. This improvement corresponded to a transient period of stabilization of the tumor vessels, in which less mature vessels are destroyed and other vessels are stabilized by the recruitment of pericytes. This stabilization period has been termed the "normalization window" (68). Interestingly, additional studies with the angiogenic inhibitor thalidomide and measuring oxygenation in murine FSaII fibrosarcomas and TLT liver tumors using electron paramagnetic resonance oximetry reported a similar transient window of improved oxygenation (66, 67). The apparent transient nature of this effect would suggest that unless the timing of oxygenation measurement is optimal then the improvement in oxygenation by AIAs could be missed and this could account for the lack of effect in five of the studies in Table 1. But it certainly does not account for those studies showing a decrease in oxygenation. Moreover, the study by Winkler et al. (65) showed that, 2 days after treating animals with DC101 (3×40 mg/kg), tumors were still significantly better oxygenated, yet in the study by Fenton et al. (57), using the same DC101 drug and a similar schedule (3×45 mg/kg), the tumors 2 days after treatment were significantly more hypoxic. These findings suggest that the normalization effect may not be universally observed or, at the very least, a tumor-dependent phenomenon.

Another major pathophysiologic effect of AIAs that has often been reported involves changes in interstitial fluid pressure (IFP). In general, tumor IFP is high, although it is normally lower at the tumor periphery (69, 70). Following treatment with AIAs, IFP drops (46, 66, 71, 72). With vascular density decreasing then, the most likely explanation for a decrease in IFP would be a decrease in the number of tumor cells, and indeed, there is evidence that treatment with AIAs can lead to tumor cell killing (47, 73). However, other

Table 1. Effect of AIAs on tumor oxygenation status

Inhibitor	Tumor type	Assay*	Oxygenation †	Reference
Suramin	DLD-2 human colon	Radiation (SF)	Decreased	(58)
TNP-470	C3H mammary carcinoma	Radiation (TC)	Decreased	(59)
DC101	WAC2 neuroblastoma	Eppendorf (pO_2)	Decreased	(47)
DC101	MCa4 mammary carcinoma	Hypoxic marker (EF5)	Decreased	(57)
DC101	MCa35 mammary carcinoma	Hypoxic marker (EF5)	Decreased	(57)
Anginex	SCK mammary carcinoma	Eppendorf (pO_2)/perfusion (Rb)	Decreased	(52)
SU6668	FSaII + SCK + CFPAC tumors	Perfusion (Rb)	"Decreased"	(60)
ZD6474	CaLu 6 NSCLC	Perfusion (H.33342)	"Decreased"	(61)
Anti-VEGF antibody	LS1747 colon adenocarcinoma	Eppendorf (pO_2)	No change	(46)
DC101	54A SCLC + U87 glioblastoma	Eppendorf (pO_2)/radiation (TG)	No change	(62)
Arginine deiminase	WAC2 neuroblastoma	Eppendorf (pO_2)	No change	(47)
Endostatin	MCa35 mammary carcinoma	Hypoxic marker (EF5)	No change	(55)
SU5416	E106 human glioblastoma	Hypoxic marker (PIMO)	No change	(49)
TNP-470	9L rat gliosarcoma	Eppendorf (pO_2)	Increased	(63)
Suramin	E106 human glioblastoma	Hypoxic marker (PIMO)	Increased	(56)
Anti-VEGF antibody	U87 glioblastoma	Eppendorf (pO_2)	Increased	(46)
Anti-VEGF antibody	Shionogi mammary carcinoma	Phosphorous quenching	Increased	(50)
Endostatin	MCa4 mammary carcinoma	Hypoxic marker (EF5)	Increased	(55)
Thrombospondin	D-12 human melanoma	Radiation (SF/TG)	Increased	(64)
DC101	U87 glioma	Hypoxic marker (PIMO)	Increased	(65)
Thalidomide	FSaII fibrosarcoma	EPR oximetry	Increased	(66)
Thalidomide	TLT mouse liver tumor	EPR oximetry	Increased	(67)

Abbreviations: SF, paired survival curve; TC, clamped tumor control; TG, clamped tumor growth; EF5, pentafluorinated derivative of etanidazole; PIMO, pimonidazole; EPR, electronic paramagnetic resonance; Rb, RbCl uptake; H.33342, Hoechst 33342 staining; NSCLC, non–small cell lung cancer.

* Assays were radiation response measured using the paired survival curve, clamped tumor control, or clamped tumor growth; Eppendorf polarographic oxygen electrode; hypoxic markers, pentafluorinated derivative of etanidazole, or pimonidazole; phosphorous quenching imaging; electronic paramagnetic resonance oximetry; and blood perfusion measured using either RbCl uptake or Hoechst 33342 staining.

† Tumor oxygenation was increased, unchanged (no change), or decreased (the use of the term "Decrease" indicates that tumor oxygenation was not measured directly but what was to be expected based on the perfusion change obtained).

mechanisms may also be involved. One study with PTK787/ZK 222584, a specific inhibitor of the VEGF receptor tyrosine kinases, showed using dynamic contrast-enhanced magnetic resonance imaging in a murine renal cell carcinoma that this inhibitor could decrease vessel permeability and such an effect would be expected to reduce IFP (48).

Vascular-disrupting agents. The effects of VDAs on tumor pathophysiology are less controversial. As a consequence of inducing vascular damage, blood perfusion is reduced, and this has been reported for TNF (74, 75), *Vinca* alkaloids (76–78), ATO (79, 80), FAA (76, 81, 82), DMXAA (83–85), CA4P (84–87), ZD6126 (88–90), AVE8062 (91, 92), OXi4503 (93–96), and MN-029 (97). This is illustrated in Fig. 2 using examples from the two major classes of small-molecule drugs, in which perfusion in one tumor model was measured using the RbCl uptake technique. Typically, the reductions in tumor perfusion occur rapidly, often being detected within minutes after administering the VDA and achieving maximal shutdown within 1 to 6 hours (79–87, 89–91, 93–97). The actual degree and duration of the vascular shutdown are dependent on drug type, drug dose, and tumor model. For example, in a KHT sarcoma, a CA4P dose of 100 mg/kg will produce a >80% maximal decrease in perfusion (85, 98) that shows only a partial recovery at 24 hours (85), but in a C3H mammary carcinoma, this same dose only produces a 25% decrease and maximal reductions are only observed when the drug dose is increased to 250 mg/kg (98), and at both doses perfusion fully recovers by 24 hours (84). With DMXAA, a 20 mg/kg dose will produce a maximal >70% decrease in perfusion in both the KHT sarcoma (85) and C3H mammary carcinoma (99), and this reduction is maintained in both models for at least 24 hours after treatment (85, 99). These differences may reflect different mechanisms by which the VDAs induce vascular effects. How each VDA produces the effect is not clear, but two basic mechanisms have been proposed (28). The first involves a direct effect on endothelial cells, which induces effects, such as rounding up, blebbing, and apoptosis, all of which can lead to vessel blockage (28, 100, 101). Such changes have been detected *in vitro* but are more difficult to show *in vivo* (102). Alternatively, there is a more indirect effect mediated through an increase in vessel permeability, which may still be endothelial cell related. Increases in vessel permeability have been shown both *in vivo* and even clinically (103–109) and should decrease blood viscosity and increase IFP; the former will decrease flow and thus clotting is more likely, whereas the latter will increase the likelihood of vessel collapse. Actual measurements of IFP, primarily made using the wick-in-needle technique, generally show a decrease in IFP after treatment with VDAs (91, 110, 111). These decreases are time and drug dose dependent and begin to occur rapidly after drug injection; for CA4P in a C3H mouse mammary carcinoma[3] and its

[3] Ley, Horsman, and Kristjansen, unpublished observation.

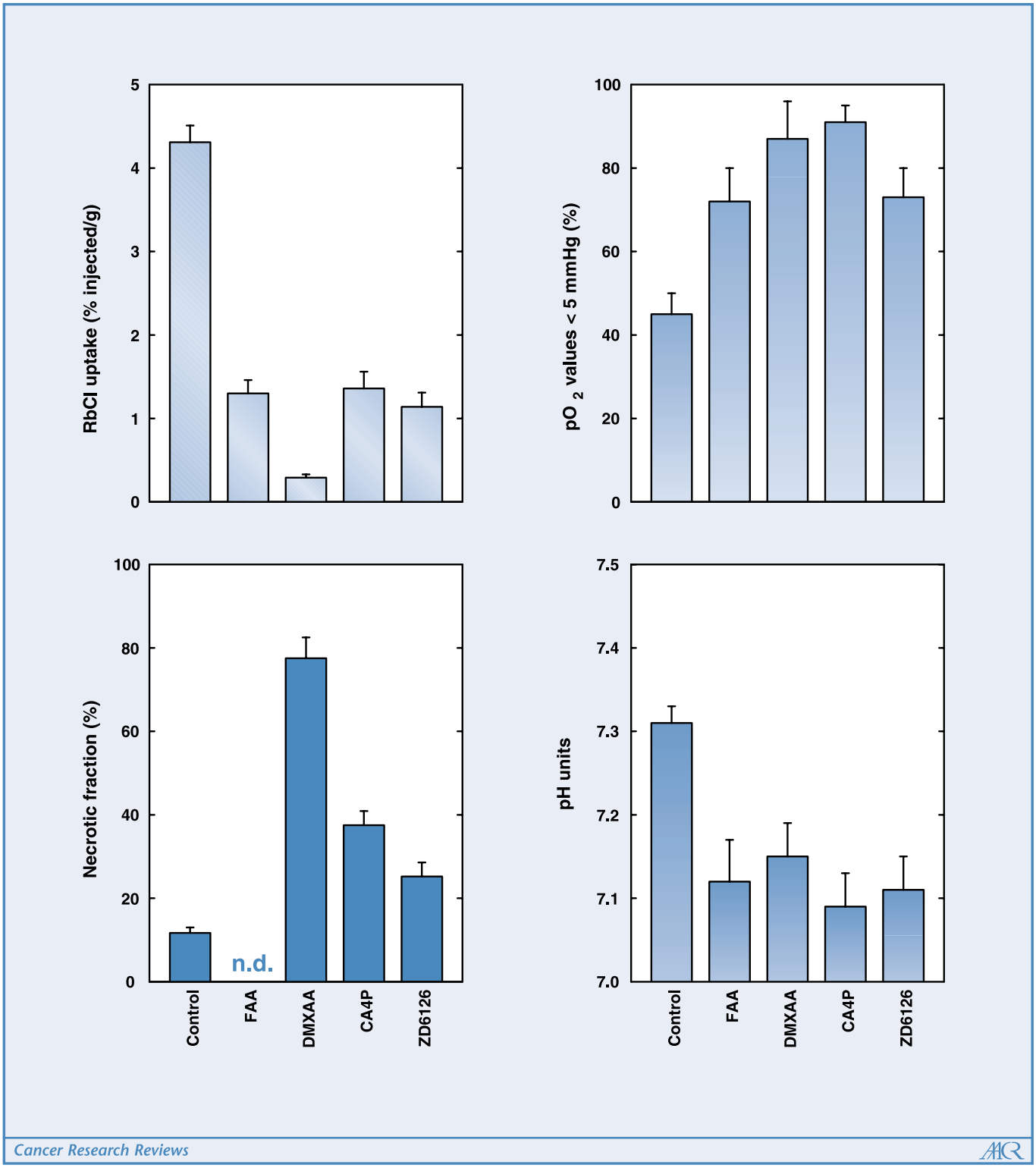

Figure 2. Pathophysiologic effects of treating a C3H mouse mammary carcinoma with VDAs. Tumors were grown in the right rear foot of female CDF1 mice and treated when at 200 mm^3 in size. Animals were given either no treatment (controls) or a single i.p. injection of FAA (150 mg/kg), DMXAA (20 mg/kg), CA4P (250 mg/kg), or ZD6126 (200 mg/kg). Results show relative changes in perfusion (RbCl uptake; percentage injected/gram tumor), necrotic fraction (percentage determined from histologic analysis), hypoxia (percent pO_2 values < 5 mmHg as measured with an Eppendorf electrode), or tumor pH (estimated from ^{31}P MRS measurements). Measurements of perfusion, hypoxia, and pH were made either 1 hour (CA4P and ZD6126) or 3 hours (FAA and DMXAA) after injection. Necrotic fraction was determined after 24 hours after giving all VDAs. *Columns,* mean (n = 6–8); *bars,* SE. Taken from refs. 81, 90, 98, 99, 125 and unpublished observations. n.d., not done.

analogue AVE8062 in a LY80 rat tumor model (91), such decreases were seen within 15 minutes. Interestingly, these changes in IFP occurred either at the same time (91) or actually followed (111)[3] rather than preceded the VDA-induced decrease in tumor blood perfusion. One study actually reported no effect of CA4P on IFP in a BT_4An rat tumor model and an increase with vinblastine (112). However, those measurements were made 3 hours after treatment and the study with ZD6126 in KHT sarcomas showed a decrease in IFP at 1 hour after injection, but at 3 hours, IFP had returned or even exceeded pretreatment levels followed by a progressive decrease reaching ~25% of control values by 72 hours after treatment (111), again confirming the importance of timing. Recently, Vincent et al. (113) showed the selective disruption of the molecular engagement of the endothelial cell junction protein vascular endothelial-cadherin following VDA treatment, providing another possible factor involved in tumor vessel disruption by this class of agents. Regardless of the mechanism(s), the ultimate effect of the vascular shutdown is ischemia and cell death, reflected by an increase in tumor necrosis. This has been observed with ATO (79, 105), FAA (114), DMXAA (83, 99), CA4P (86, 98, 115, 116), ZD6126 (88, 90, 102), OXi4503 (94, 96), and MN-029 (97). The effect of some of these VDAs on necrosis in a C3H mammary carcinoma is also illustrated in Fig. 2 and shows that even within one tumor model the effects of VDAs can be highly variable, an effect that is probably related to the severity of the vascular collapse.

The VDA-induced reductions in functional tumor vasculature will also be reflected in changes in other pathophysiologic variables, and some of these are illustrated in Fig. 2. Most solid animal and human tumors contain varying degrees of hypoxia (6, 117). Following treatment with VDAs, tumor oxygenation status decreases even further (78, 118–122). This is clearly associated with the increased necrosis, but studies using ^{19}F magnetic resonance imaging oximetry suggest that there is a decrease in oxygenation status even in viable tissue (122). Recent measurements of blood flow changes in the tissue that survives VDA treatment support this notion.[4] Tumor pH is another pathophysiologic factor affected by VDAs. The consensus opinion is that intracellular pH of tumor cells is generally maintained within the range typically found in normal cells, whereas extracellular pH tends to be acidic (6, 123). Treatment with VDAs results in a significant and rapid decrease in extracellular pH (124). However, VDAs can also decrease intracellular pH (125–127), and this latter effect is also illustrated in Fig. 2.

Combining VTAs with Other Therapies

Radiation therapy. Combining AIAs with radiation is a logical step. Tumor progression is a major reason for radiotherapy failure and so by inhibiting such progression one should be able to improve radiation response. Numerous preclinical studies have investigated the potential of combining AIAs and radiation, and these studies are summarized in Table 2. The radiation treatments have involved both single and fractionated schedules. For single radiation treatments, there are clear differences in the total doses given, whereas in the fractionated studies, not only do the total doses vary considerably, there are also large differences both in the number of fractions given and the time over which the doses were delivered. The AIAs evaluated include both nonspecific as well as targeted molecules. Here, the lack of standardization is even more obvious. This is true not only for the drug doses and treatment times used but also for the different combination schedules applied with radiation. These include administering the inhibitor during the radiation treatment (54, 59, 66, 128–135), before starting the irradiation (46, 51, 53, 58, 60), after completing the radiation (47, 72, 136), or a combination of before, during, and/or after irradiation (49, 52, 57, 61, 62, 64, 65, 72, 73, 128, 129, 135, 137–143). Such differences make broad generalizations very difficult. However, there is one aspect on which all but a few agree and that is that the combination of AIA and radiation is superior to either treatment alone. Several studies even suggest that this combination gives a result that is greater than an additive response. But without detailed analysis (144, 145), such conclusions may be extremely tenuous. Still, even an additive outcome would have a major benefit provided similar enhancements are not observed in critical normal tissues. Although additional data are clearly needed, one study that did investigate this issue (Table 2; ref. 62) reported no change in radiation-induced skin damage by DC101.

The response of any cell type to radiation is strongly dependent on oxygen concentration (146, 147), and because AIAs have been shown to improve the oxygenation status of tumors (46, 50, 55, 56, 63–67), the potential for a greater than additive effect when AIAs and radiation are combined clearly exists. However, those studies that have investigated the time dependency of this AIA-induced improvement in tumor oxygenation reported that the window of opportunity to exploit this possibility was short (65–67). Indeed, when combined with radiation, the only time synergy was observed was when the radiation was administered at the time of maximal reduction in tumor hypoxia (65). Irradiating immediately before, or after, this period only resulted in an additive response to the AIA and radiation treatment, although hypoxia was still significantly reduced at these times. This suggests that changes in oxygenation may not be the only factor involved, and unless it is possible to accurately predict the window of opportunity for each drug and tumor type, the potential for exploiting oxygenation modification by AIAs remains minimal. At the same time, there is clear evidence that AIAs can also induce hypoxia in tumors (47, 52, 57–61), and such an effect has the potential to reduce the efficacy of the radiation treatment, a result that has also been reported (58, 59). Such a negative effect is a major concern when trying to translate the preclinical studies into clinical trials, and to avoid such a potential problem, it would seem prudent to administer AIAs after radiation.

Several studies have actually investigated the importance of sequencing between radiation and AIAs especially with tyrosine kinase inhibitors. One study using A451 tumors gave a single radiation treatment, either 1 day before or 1 day after the start of a 3-week treatment with SU11657, and found that the latter schedule was superior (72). An additional study using ZD6474 in a fractionated drug and radiation treatment schedule over a 2-week period showed that the combination effect was additive and independent of whether the drug was given before (neoadjuvant), during (concomitant), or after (adjuvant) the radiation treatment (141). Similarly, the same drug given concurrently with radiation in another tumor model was only additive, but when an adjuvant schedule was used, a larger enhancement was obtained (61). An adjuvant schedule was also found to be superior to neoadjuvant or

[4] Salmon and Siemann, unpublished observation.

Table 2. Preclinical tumor studies combining AIAs with radiation

Inhibitor	Tumor type	Radiation schedule	AIA treatment*	Reference
Suramin	DLD-2 human colon carcinoma	1 × 5–25 Gy, day 6 [†]	50 mg/kg/d, i.p., days 0–6 [†]	(58)
TNP-470	Lewis lung carcinoma	5 × 3 Gy, days 7–11 [‡]	30 mg/kg/d, s.c., days 4, 6, 8, 10, 12, 14, 16, and 18 [‡]	(73)
	C3H mammary carcinoma	5 × 10–30 Gy, days 0–4, or 10 × 3.65–14.5 Gy, days 0–4 and 7–11 [†]	100 mg/kg/d, s.c., days 0 + 3; 7 + 10; 0, 3, 7, and 10; 0, 4, 7, and 10; or 7, 10, 14, and 17 [†]	(59)
	U87 human glioblastoma	1 × 10 Gy, day 15 [‡]	6.7 mg/kg/d, s.c., days 8–15 [‡]	(53)
Angiostatin	Lewis lung carcinoma	2 × 20 Gy, days 0 + 1 [†]	25 mg/kg/d, i.p., days 0 + 1 or 0–13 [†]	(128, 129)
	Lewis lung carcinoma	2 × 20 Gy, days 0 + 1 [†]	25 mg/kg/d, i.p., days 0–14 [†]	(137)
	D54 human glioblastoma	6 × 5 Gy, daily [†]	6 × 25 mg/kg, i.p., with radiation [†]	(137)
	SQ-20B squamous cell carcinoma	10 × 5 Gy, daily [†]	10 × 25 mg/kg, i.p., with radiation [†]	(137)
	PC3 prostate adenocarcinoma	8 × 5 Gy, daily [†]	8 × 25 mg/kg, i.p., with radiation [†]	(137)
	Rat C6 glioma	3 × 7.5 Gy, days 0, 2, and 4 [†]	AdK3, i.t., days 1, 3, and 5 [†,§]	(130)
	SCK mammary carcinoma	2 × 10 Gy, days 0 + 1 [†]	25 or 50 mg/kg/d, i.p., days −1, 0, and 1 [†]	(52)
Endostatin	SQ-20B squamous cell carcinoma	10 × 5 Gy, days 0–3, 7–10, and 14 + 15 [†]	2.5 mg/kg/d, i.p., days 0–3, 7–10, and 14 + 15 [†]	(131)
	Lewis lung carcinoma	3 × 15 Gy, days 1–3 [†]	2.5 mg/kg/d, i.p., days 0–3 [†]	(131)
	HT29 colorectal carcinoma	1 × 10 Gy, days 0 [†]	rAAV, i.m., 6 wk before tumor implant [‖]	(51)
Arginine deiminase	WAC2 neuroblastoma	1 × 6 or 12 Gy, day 0 [†]	5 mg/kg/d, i.p., days 0–4 [†]	(47)
Thrombospondin	D-12 human melanoma	1 × 10 Gy, day 14 [‡]	50 μg, i.p., days 13 + 14 or 3×/wk starting day 15 [‡]	(64)
Thalidomide	FSaII fibrosarcoma	1 × 20 Gy, on days 0, 2, and 4 [†]	200 mg/kg/d, i.p., days 0, 0 + 1, 0–3, or 1 + 2 [†]	(66)
Anginex	MA 148 ovarian carcinoma	4 × 5 Gy, days 2, 9, 16, and 23 [†]	10 mg/kg/d for 28 days, s.c. pump [†,¶]	(52)
	SCK mammary carcinoma	1 × 10 Gy, days 0 + 1 [†], or 1 × 25 Gy, day 2 [†]	20 mg/kg, i.p., days −1, 0, and 1 [†], or 20 mg/kg/d for 14 d, s.c. pump [†,¶]	(52)
Anti-VEGF antibody	Lewis lung carcinoma	2 × 20 Gy, days 0 + 1 [†]	10 μg/d, i.p., days 0 + 1 [†]	(132)
	SQ-20B squamous cell carcinoma	4 × 10 Gy, days 0–3 [†]	10 μg/d, i.p., days 0–3 [†]	(132)
	Seg-1 esophageal adenocarcinoma	4 × 5 Gy, days 0–3 [†]	10 μg/d, i.p., days 0–3 [†]	(132)
	U87 glioblastoma	8 × 5 Gy, days 0, 1, 4, 5, 7, 8, 11, and 12 [†]	10 μg/d, i.p., days 0, 1, 4, 5, 7, 8, 11, and 12 [†]	(132)
	U87 glioblastoma	1 × 20–30 Gy, day 11 [†]	100 μg/d, i.p., days 0, 2, 4, 6, 8, and 10 [†]	(46)
	LS1747 colon adenocarcinoma	1 × 20–30 Gy, day 11 [†]	100 μg/d, i.p., days 0, 2, 4, 6, 8, and 10 [†]	(46)
	Seg-1 esophageal adenocarcinoma	4 × 5 Gy, days 0–3 [†]	5 or 25 μg/kg/d, i.p., days 0–3 [†]	(133)
	U87 glioblastoma	8 × 5 Gy, days 0–3 and 7–10 [†]	5 or 25 μg/kg/d, i.p., days 0–3 [†]	(133)
DC101	54A small cell lung cancer	5 × 5–24 Gy, days 1–5 [†]	20 or 40 mg/kg/d, i.p., days 0, 3, 6, 9, 12, and 15 [†]	(62)
	U87 glioblastoma	5 × 5–24 Gy, days 1–5 [†]	20 or 40 mg/kg/d, i.p., days 0, 3, 6, 9, 12, and 15 [†]	(62)
	WAC2 neuroblastoma	1 × 6 or 12 Gy, day 0 [†]	6.5 mg/kg/d, i.p., days 0–4 [†]	(47)
	U87 glioma	3 × 7 Gy, days −9 to −7, −2 to 0, 1–3, 4–6, or 7–9 [†]	40 mg/kg/d, i.p., days 0, 3, and 6 [†]	(65)
	MCa4 mammary carcinoma	5 × 6 Gy, days 4–8 [‡]	45 mg/kg, i.p., every 3 d starting day 4 [‡]	(57)
	MCa35 mammary carcinoma	5 × 6 Gy, days 4–8 [‡]	45 mg/kg, i.p., every 3 d starting day 4 [‡]	(57)
SU5416	GL261 murine glioblastoma	8 × 3 Gy, days 0–3 [†,**]	0.7 mg/d, i.p., days 0, 4, 7, and 11 [†]	(138)
	SCCVII murine carcinoma	5 × 2 Gy, days 0–4 [†]	25 mg/kg, i.p., daily from day 0 [†]	(139)
	WAC2 neuroblastoma	1 × 6 Gy, day 0 [†]	25 mg/kg/d, i.p., days 0–4 [†]	(47)
	E106 glioblastoma	1 × 10 or 20 Gy, day 1 [†]	75 mg/kg/d, i.p., days 0–13 [†]	(49)
SU6668	SCK mammary carcinoma	1 × 15 Gy, day 1 [†]	100 mg/kg/d, i.p., days 0 + 1 [†]	(60)
	SCCVII murine carcinoma	5 × 2 Gy, days 0–4 [†]	75 mg/kg, p.o., daily from day 0 [†]	(139)
	Lewis lung carcinoma	7 × 3 Gy, days 0–6 [†]	1.5 mg/kg/d, i.p., days 0, 2, 4, and 6 [†]	(134)
	GL261 murine carcinoma	7 × 3 Gy, days 0–6 [†]	1.5 mg/kg/d, i.p., days 0, 2, 4, and 6 [†]	(134)

(*Continued on the following page*)

Table 2. Preclinical tumor studies combining AIAs with radiation (Cont'd)

Inhibitor	Tumor type	Radiation schedule	AIA treatment*	Reference
SU11248	Lewis lung carcinoma	7 × 3 Gy, days 0–7 †	40 mg/kg/d, i.p., days 0–7 †	(135)
	GL261 murine glioblastoma	7 × 3 Gy, days 0–7 † (only 6 × 3 Gy in the maintenance study)	40 mg/kg/d, i.p., days 0–7, † or maintenance (40 mg/kg/d on days 0–7 plus 20 mg/kg/d on days 7–16 + 21–30) †	(135)
SU11657	A431 carcinoma	1 × 7.5 Gy, days 0 or 1 †	100 mg/kg, s.c., 3×/wk for 3 wk from day 0 or 1 †	(72)
PTK787/ZK 222584	SW480 colon carcinoma	4 × 3 Gy, days 0–3 †	100 mg/kg/d, p.o., days 0–3 †	(54)
	FaDu squamous cell carcinoma	15 × 2 Gy, starting day 0 †	50 mg/kg/d, p.o., days −18 to −1, 0–15, or 16–45 †	(140)
	UT-SCC-14 squamous cell carcinoma	15 × 2 Gy, starting day 0 †	50 mg/kg/d, p.o., days −18 to −1, 0–15, or 16–45 †	(140)
	FaDu squamous cell carcinoma	30 fractions in 6 wk (days 0–32) †	50 mg/kg, p.o., 2×/d from days 33 to 75 †	(136)
	UT-SCC-14 squamous cell carcinoma	30 fractions in 6 wk (days 0–32) †	50 mg/kg, p.o., 2×/d from days 33 to 75 †	(136)
ZD6474	HT29 colorectal carcinoma	1 × 10 Gy on day 0 or 10 × 2 Gy, days 0–4 and 7–11 †	10 × 25 mg/kg for 2 wk, p.o., after, during, or before radiation †	(141)
	D54 glioblastoma	4 × 2 Gy, days 1, 3, 8, and 10 †	75 mg/kg/d, i.p., days 0–4, 7–11, and 14–18 †	(142)
	CaLu 6 NSCLC	3 × 2 Gy, days 0–2 †	25 or 50 mg/kg daily, p.o., started either before (day 0) or after (day 2) radiation †	(61)
Metastat	B16F10 melanoma	2 × 12.5 Gy, day 3 †,**	2.5 μg/kg/d, i.t., days 2–6 †	(143)

*AIAs were given i.p., s.c., p.o., intratumorally (i.t.), i.m., or not disclosed.
†AIAs and radiation were given on various days when tumors had reached a specific size (day 0).
‡AIAs and radiation were given on various days after tumor implantation (day 0).
§Angiostatin was in the form of an angiostatin–expressing adenovirus, given as an i.t. injection of 5×10^9 plaque–forming units (pfu).
‖Endostatin was produced by an endostatin–expressing adenovirus, given as an i.m. injection of 1×10^8 to 1×10^9 viral vectors.
¶AIA given continuously from a s.c. implanted osmotic pump.
**Radiation given twice daily.

concomitant administration of PTK787/ZK 222584 and radiation in head and neck tumors (140). This benefit of the adjuvant schedule was suggested to be a consequence of the "tumor bed effect" (136). This is a well-known phenomenon in which tumor growth can be delayed by implanting tumors into an area that had been previously treated with high-dose radiation (148, 149). Zips et al. (150) later showed that the growth of FaDu tumors, which were normally unresponsive to a daily dose of 50 mg/kg PTK787/ZK 222584, could be inhibited by this treatment when tumors were grown in a preirradiated bed. They suggested that tumors vascularized by radiation-damaged vessels were far more sensitive to AIA treatment. More recent studies indicate that tumor endothelial cells may be an important target for radiation damage (13) and this could lead to a potential "target interaction" for AIAs and radiation. Indeed, it has been shown *in vivo* that the decrease in microvessel density (MVD) seen after treatment with AIAs can be further enhanced when radiation is also administered (49, 72, 130, 131, 137, 138). Additional *in vitro* studies using various types of cultured endothelial cells confirm a direct interaction between AIAs and radiation (54, 72, 130, 131, 134, 137, 138), an effect that may be mediated through an increase in apoptosis (72, 138). Probably, the most significant finding from these *in vitro* studies was that, when AIAs and radiation were combined, an enhanced response was seen regardless of whether a neoadjuvant (54, 72, 134, 137, 138), concomitant (138, 151), or adjuvant (130, 133, 142) schedule was used. Interestingly, the administration of VEGF could actually rescue human umbilical vascular endothelial cells from the radiation-mediated cell death (133, 151). This interaction between AIA and radiation was sometimes seen in tumor cells (72, 142), but not in all cases (130, 137), suggesting an endothelial cell–specific effect that could be exploited.

Treating tumors with VDAs produces an effect, which even when severe is typically restricted to the central part of the tumor, leaving a rim of viable tumor cells at the periphery (83, 86, 89, 115, 152). This is presumably because the tumor rim receives its nutritional support from nearby normal tissue blood vessels, which are generally unaffected by the VDA treatment (34, 153). Those tumor cells surviving in the periphery are also probably better oxygenated than the more central ones and as such would be expected to be more sensitive to radiation therapy. This suggests a logical rationale for combining VDAs with radiation. Such a combination has been the focus of numerous preclinical studies, and these are summarized in Table 3. All these studies have been done in rodent tumor models and involved both single-dose radiation/drug treatments as well as fractionated schedules. Several studies investigated the importance of timing and sequence between the

VDA and radiation treatment (89, 98, 154, 155). By far, the greatest antitumor activity was observed when the VDA was administered within a few hours after irradiating. With such a schedule, there was an indication that the effect was greater than a simple additive response to each agent alone, suggesting some sort of interaction between the two treatments rather than the VDA and radiation killing two different cell populations. Precisely how the VDAs and radiation might interact is not clear. Recent studies suggest that tumor vasculature may also be an important target for radiation damage (13), and it is possible that some form of interaction at the level of the endothelial cells is occurring, perhaps through the VDA increasing the extent of radiation-induced apoptosis as has been shown *in vitro* with TNF and radiation (156).

It has also been shown that injecting mice with the VDA and then irradiating typically has little or no benefit, and in some situations, there was an indication that the combined effect was less than an additive response to each agent alone (89, 98, 154, 155). This suggested that the vascular shutdown induced by the VDA may have rendered some tumor cells hypoxic at the time of irradiation and that those same cells later reoxygenated and survived. Further support for this concept comes from studies in which the combined effect of DMXAA and radiation could be

Table 3. Preclinical tumor studies combining VDAs with radiation

VDA	Tumor type	Radiation schedule*	VDA treatment †	Reference
TNF	MCA-K mammary carcinoma	1 × 19–55 Gy	5 μg, i.v., daily for 7 d stating 3 h after irradiation	(226)
	MCA-K mammary carcinoma	10 × 3–5 Gy (1 fx/d for 10 d)	2 μg, i.v., 3 h after each irradiation	(227)
	SQ-20B squamous cell carcinoma	4 × 5 Gy (4 fx/wk)	1–5 × 10^7 pfu Ad.Egr–TNF, i.t., timing with radiation not stated	(228)
	KHT sarcoma	1 × 15 Gy	2.5 × 10^5 units/kg, i.v., 0–16 h before irradiation	(190)
ATO	HeLa xenografts	1 × 7.5 Gy	3 × 20 mg/kg, n.s., timing with radiation not stated	(229)
	Meth-A tumors	1 × 30 Gy; 2–4 × 12 Gy (1 fx/wk)	10 mg/kg, i.p., 1 h after each irradiation	(230)
	9L glioma	1 × 25 Gy	8 mg/kg, i.p., various times before/after irradiation	(105)
FAA	KHT sarcoma	1 × 15 Gy	200 mg/kg, i.p., 0–18 h before irradiation	(190)
	C3H mammary carcinoma	1 × 30–70 Gy	150 mg/kg, i.p., 1 h after irradiation	(213)
DMXAA	RIF-1 fibrosarcoma	1 × 5–30 Gy	80 μmol/kg, i.p., 5 min after irradiation	(154)
	MDAH-MCa4 mammary carcinoma	1 × 20 Gy; 8 × 2.5 Gy (8 fx in 4 d)	75–80 μmol/kg, i.p., various times before/after irradiation	(154)
	C3H mammary carcinoma	1 × 5–20 Gy	5–20 mg/kg, i.p., various times before/after irradiation	(155)
	KHT sarcoma	1 × 2.5–20 Gy	5–17.5 mg/kg, i.p., various times before/after irradiation	(155)
CA4P	KHT sarcoma	1 × 5–20 Gy	10–100 mg/kg, i.p., 1 h after irradiation	(152)
	KHT sarcoma	1 × 10 Gy	100 mg/kg, i.p., various times before/after irradiation	(98)
	Carcinoma NT	8 × 5 Gy (4 fx/wk)	100 mg/kg, i.p., given 24 h after fractions 4 + 8	(153)
	Rhabdomyosarcoma	1 × 8 Gy	25 mg/kg, i.p., 24 h after irradiation	(231)
	Rhabdomyosarcoma	5 × 3 Gy (5 fx/wk)	25 mg/kg, i.p., 24 h after last irradiation	(157)
	C3H mammary carcinoma	1 × 25–70 Gy	100–250 mg/kg, i.p., various times before/after irradiation	(98)
	C3H mammary carcinoma	10 × 4–8 Gy (5 fx/wk)	250 mg/kg, i.p., 30 min after the 5th and 10th irradiations	(158)
	Kaposi's sarcoma	1 × 5–25 Gy	100 mg/kg, i.p., 1 h after irradiation	(196)
ZD6126	KHT sarcoma	1 × 5–25 Gy; 10 × 2.5 Gy (5 fx/wk)	150 mg/kg, i.p., various times before/after irradiation	(35, 89)
	C3H mammary carcinoma	1 × 5–20 Gy	200 mg/kg, i.p., 1 h after irradiation	(90)
	A549 NSCLC	4 × 4 Gy (2 fx/wk for 2 wk)	150 mg/kg, i.p., 24 h after irradiations 2 + 4	(232)
	U87 glioblastoma	1 × 10 Gy; 3 × 5–7.5 Gy (on days 0, 2, and 4)	150 mg/kg, i.p., various times before/after each irradiation	(119)
OXi4503	KHT sarcoma	1 × 5–20 Gy	10 mg/kg, i.p., 1 h after irradiation	(233)
MN-029	KHT sarcomas	1 × 5–25 Gy	100 mg/kg, i.p., 1 h after irradiation	(97)

Abbreviations: fx, fractionated; n.s., not stated.
*Radiation was given either as a single treatment or in a fractionated schedule.
†VDAs were given i.p., i.v., i.t., or not stated.

enhanced by including the hypoxia-selective bioreductive drug tirapazamine in the treatment schedule (154). The reduced effect obtained when giving the VDA before radiation has important clinical applications in which fractionated radiation schedules are generally used. To avoid any possible complications, the optimal approach would probably involve giving the VDA after the last radiation treatment each week in a conventional fractionated schedule. Using such an approach, several preclinical studies have shown a benefit of combining VDAs and fractionated radiation (89, 153, 157, 158). Giving the VDA more often could still be beneficial provided there is sufficient time for any induced hypoxia to disappear before the next radiation treatment is applied. Indeed, one study using DMXAA and radiation showed that VDAs could be administered more often than once a week during a fractionated schedule without loss of benefit (154). However, whether this holds true for all VDAs and tumor types is not known, and this critical issue of hypoxia induction by VDAs and the possible consequences of such an induction clearly need further investigation.

Improving tumor response to therapy by combining VDAs and radiation will only be of benefit if such a combination does not enhance the response of critical normal tissues to the same degree. This is an aspect that has not been investigated in great detail. However, the preclinical results that have been obtained from the limited studies that have been done are encouraging. Using normal tissues that show an early response to radiation damage, such as skin (98, 154, 155), or late responding bladder and lung (159), no enhancement of radiation damage was observed. This is perhaps not entirely surprising because, although VDAs can induce some vascular shutdown in skin, the effects are small compared with that seen in tumors (84, 87), and no reductions in blood flow have been found in bladder and lung (84).

Chemotherapy. Numerous studies have also investigated the potential combination of VTAs with chemotherapeutic drugs. Those studies involving AIAs are summarized in Table 4. Not listed in this table are those studies that combined AIAs with low-dose "metronomic" chemotherapy (160–163); the latter being a modified chemotherapeutic regime so that the chemotherapeutic drug itself has antiangiogenic properties, and as such, these studies represent the combination of AIAs rather than true combination of AIAs with chemotherapy. As can be seen in Table 4, the various permutations for combinations of AIAs with chemotherapeutic drugs are extensive. The schedules used are also highly variable, although typically these studies involved giving the AIAs and chemotherapeutic drugs over the same time period. What is clear is that the majority of studies reported an increased benefit of the combination approach, although in a few examples no additional benefit was found (164–166).

Whether the increased effect of combining AIAs with drugs results in a response that is greater than additive is uncertain. However, there are reasons why a greater than additive response may be possible. These involve both nonpathophysiologic as well as pathophysiologic mediated effects. The combination of AIAs and chemotherapy can increase apoptosis. This has been seen in tumors *in vivo* (167–175) as well as with tumor and endothelial cells *in vitro* (72, 176, 177); this latter effect probably explains the decrease in MVD seen after such combination therapy (72, 165, 167, 172, 174, 178–180). A decrease in MVD might be expected to decrease drug delivery, and in fact, two studies have shown that treatment with TNP-470 can reduce the uptake of temozolomide in rat glioma models (181, 182). But such an effect would have a negative effect on the combination of AIAs and chemotherapeutic drugs, and this is clearly not seen with any of the studies listed in Table 4. Other pathophysiologic changes induced by AIAs, which could influence tumor response when AIAs and chemotherapy are combined, include tumor oxygenation and pH, both of which are critical factors in determining the activity of certain drugs (183–188). For example, bleomycin, cisplatin, 5-fluorouracil (5-FU), and methotrexate are more cytotoxic toward well-oxygenated cells in tumors (186, 188), although this dependency is not always seen *in vitro* (183, 184); bioreductive drugs are more effective under hypoxic conditions (183, 186, 187); and alkylating agents generally work better in hypoxia-related acidic conditions (185, 187). Changes in tumor pH following AIA treatment have not been reported, so the role this factor plays in the combination studies is unclear. Tumor oxygenation effects of AIAs have been investigated (Table 1), but the controversy surrounding the effects of AIAs on tumor oxygenation status makes it almost impossible to state whether this factor is responsible for the improved response observed when specific AIAs and drugs are combined. Nevertheless, in one study, the ability of thalidomide to enhance the antitumor effect of cyclophosphamide was shown to correlate with the maximal increase in tumor oxygenation by thalidomide (67). This was not the result of a decrease in hypoxia *per se* but rather correlated with the period of "normalization" of the tumor vasculature, and this resulted in an increased uptake of the cyclophosphamide into the tumor. Another pathophysiologic effect of AIAs that probably plays an important role is IFP. As previously mentioned, AIAs decrease IFP (46, 66, 71, 72), and it has been suggested that such a drop in IFP can result in an induced hydrostatic pressure gradient across tumor vasculature, which would enhance the tumor penetration of large molecules, thus resulting in an increase in drug uptake (71).

Whatever the explanation for the improvements in antitumor response when AIAs and chemotherapy drugs are combined, there is clear interest in developing clinical studies with such combinations (22). To date, the most convincing clinical study showing the potential benefit of combining AIAs and chemotherapy comes from the phase III trial combining the anti-VEGF monoclonal antibody bevacizumab with irinotecan, fluorouracil, and leucovorin (IFL) in previously untreated metastatic colorectal cancer (189). In that study, patients were randomized to receive IFL plus bevacizumab or IFL and placebo, and the results showed that the addition of bevacizumab to the chemotherapy regime significantly improved survival.

The combination of VDAs and chemotherapy has also been investigated, and these studies are summarized in Table 5. For most of these combinations, the enhanced response is most likely attributable to the VDA and cytotoxic drugs targeting two distinct cell populations. By destroying the more central part of the tumor, VDA treatments eliminate cells in those areas that are less well vascularized and where the delivery of systemically administered chemotherapeutic drugs is limited. The cells in these areas are also oxygen deficient, are at low pH, and exhibit reduced proliferation, all of which can reduce the effectiveness of many chemotherapeutic drugs (183–188). Conversely, such drugs are more likely to kill cells in the viable rim of tumor tissue that survive the VDA treatment because of the better vascularization, improved oxygenation, pH, and proliferation status in those areas. However, this is not the situation with all combinations because the VDAs can improve the effectiveness of both bioreductive drugs, which are preferentially toxic to hypoxic cells (77, 83, 85, 190–193), and

Table 4. Combination studies in tumors using AIAs and chemotherapeutic agents

AIA	Chemotherapy	Tumor type	Treatment schedule*	Reference
TNP-470	Cisplatin	Lewis lung, EMT6	AIA (30 mg/kg, s.c., alternate days from 4 to 18) drug (10 mg/kg, i.p., day 7) †	(73, 234)
	Cisplatin	B16 melanoma, Lewis lung carcinoma	AIA (20 mg/kg, s.c., as 6 or 7 treatments between days 5 and 21) drug (4 mg/kg, i.p., on day 5 or 10) †	(235)
	Cisplatin	S-SLM osteosarcoma	AIA (10 mg/kg/wk, days 7–21) ‡ drug (2.5 mg/kg, i.p., day 21 or 24) †	(236)
	Cisplatin	HPC-3H4 human pancreatic carcinoma	AIA (90 mg/kg, s.c., alternate days for 4 wk starting day 1) drug (0.25 mg/kg, i.p., days 1–5) †	(237)
	Cisplatin	S-SLM rat osteosarcoma	AIA (2.5 mg/kg/wk, s.c., days 7–21) ‡ drug (1.25 mg/kg, i.v., day 21 or 24) †	(238)
	Cisplatin	CNE-2 nasopharyngeal	AIA (20 mg/kg, s.c. alternate days 4–12) drug (4 mg/kg, i.p., day 4) †	(164)
	Melphalan	Lewis lung carcinoma	AIA (30 mg/kg, s.c., alternate days from 4 to 18) drug (10 mg/kg, i.p., day 7) †	(73)
	Cyclophosphamide	Lewis lung, EMT6	AIA (30 mg/kg, s.c., alternate days from 4 to 18) drug (150 mg/kg, i.p., days 7, 9, and 11) †	(73, 239, 240)
	BCNU	Lewis lung carcinoma	AIA (30 mg/kg, s.c., alternate days from 4 to 18) drug (15 mg/kg, i.p., days 7, 9, and 11) †	(73)
	BCNU	9L glioblastoma	AIA (25 mg/kg, s.c., alternate days from 4 to 18) drug (15 mg/kg, i.p., days 7, 9, and 11) †	(63)
	Adriamycin	B16 melanoma, Lewis lung carcinoma	AIA (20 mg/kg, s.c., as 6 or 7 treatments between days 5 and 21) drug (2.5 mg/kg, i.p., on same days as the AIA) †	(235)
	Adriamycin	9L glioblastoma	AIA (25 mg/kg, s.c., alternate days from 4 to 18) drug (1.75 mg/kg, i.p., days 7–11) †	(63)
	Adriamycin	Lewis lung carcinoma	AIA (30 mg/kg, s.c., alternate days from 4 to 18) drug (1.75 mg/kg, i.p., days 7–11) †	(239, 240)
	Mitomycin C	B16 melanoma, Lewis lung carcinoma	AIA (15–75 mg/kg, s.c., as 2, 5, 6, or 7 treatments between days 3 and 21) drug (0.5–2.5 mg/kg, i.p., on same days as the AIA) †	(235)
	5-FU	B16 melanoma	AIA (75 mg/kg, s.c., days 3 + 5) drug (70 mg/kg, i.p., days 3 + 5) †	(235)
	5-FU	CNE-2 nasopharyngeal	AIA (20 mg/kg, s.c. alternate days 4–12) drug (60 mg/kg, s.c., days 4 + 6) †	(164)
	Etoposide	ISOS-1 angiosarcoma	AIA (30 mg/kg, s.c., 3×/wk from days 7 to 28) drug (5 mg/kg, i.p., days 7–11) †	(241)
	Prednisolone	ISOS-1 angiosarcoma	AIA (30 mg/kg, s.c., 3×/wk from days 7 to 28) drug (10 mg/kg, i.p., days 7–28) †	(241)
	Docetaxel	253J B-V human bladder carcinoma	AIA (15 mg/kg, s.c., daily for 4 wk starting day 3 or 21) drug (20 mg/kg, i.p., days 3 + 10, 17 + 24, 21 + 28, or 35 + 42) †	(167)
	Gemcitabine	KoTTC-1 human bladder cancer	AIA (15 mg/kg/d, s.c., days 7–28) drug (60 mg/kg, i.p., 1×/wk from days 7 to 28) †	(242)
	Gemcitabine	SW1990 human pancreatic carcinoma	AIA (30 mg/kg, s.c., alternate days for 8 wk starting day 7) drug (50 mg/kg, i.p., days 0, 3, 6, and 9) †	(178)
Suramin	Cyclophosphamide	Ehrlich carcinoma	AIA (10 mg/kg, i.v., day 0 or 1) drug (90 mg/kg, s.c., day 0) §	(243)
	Cyclophosphamide	Lewis lung carcinoma	AIA (20 mg/kg, i.p., days 4–18) drug (150 mg/kg, i.p., days 7, 9, and 11) †	(239, 240)
	Adriamycin	Ehrlich carcinoma	AIA (10 mg/kg, i.v., day 0) drug (8 mg/kg, i.v., day 0) §	(243)
	Adriamycin	Lewis lung carcinoma	AIA (20 mg/kg, i.p., days 4–18) drug (1.75 mg/kg, i.p., days 7–11) †	(239, 240)
	Cisplatin	Human ovarian carcinoma	AIA (5–10 mg/kg, i.p., 1×/wk for 10 wk starting day 7) drug (2 mg/kg, i.p., as with the AIA) †	(244)
	Paclitaxel	PC3-LN prostate, MCF-7 breast carcinoma	AIA (10 mg/kg, i.v., 2×/wk for 3 wk) drug (15 mg/kg, i.v., as with the AIA) §	(168, 169)

(*Continued on the following page*)

Table 4. Combination studies in tumors using AIAs and chemotherapeutic agents (Cont'd)

AIA	Chemotherapy	Tumor type	Treatment schedule*	Reference
	Doxorubicin	Human PC3 prostate carcinoma	AIA (10 mg/kg, i.v., 2×/wk for 3 wk) drug (5 mg/kg, i.v., as with the AIA)§	(170)
	Mitomycin C	RT4 bladder tumors	AIA (10 mg/kg, i.v., on days 0, 4, and 8 or i.p., 2×/wk for 3 wk) drug (3 mg/kg,, same schedule as for AIA)§	(171)
Angiostatin	Cyclophosphamide	Lewis lung carcinoma	AIA (5 mg/kg, i.p., days 3–9) drug (75 mg/kg, i.p., days 4–7) †	(245)
	Doxorubicin	C26 colon carcinoma	AIA (10–100 mg/kg, daily from days 0 to 12) ‡ drug (10 mg/kg, i.v., day 3) †	(246)
Endostatin	Cyclophosphamide	Non–Hodgkin's lymphoma	AIA (50 μg, s.c., days 15–19 + 25–29) drug (75 mg/kg, i.p., days 3, 5, and 7) †	(247)
	Doxorubicin	C26 colon carcinoma	AIA (500 μg/d, s.c., days 0–12) drug (10 mg/kg, i.v., day 3) †	(246)
	Carboplatin	Human testicular tumor	AIA (10 mg/kg/d from days 15–28) ‡ drug (30 mg/kg, i.p., days 14 + 21) †	(248)
	Carboplatin	Human ovarian epithelial carcinoma	AIA (rAAV, i.m., day 2)‖ drug (32.5 mg/kg, s.c., every 3 d for 10 doses starting day 7) †	(249)
	Gemcitabine	A549 lung adenocarcinoma	AIA (Ad-hEndo, i.t., days 7 + 10 and 14 + 16)‖ drug (125 mg/kg, i.p., days 9, 12, 15, and 18) †	(172)
Thalidomide	Cyclophosphamide	Colon 38 tumors	AIA (1–100 mg/kg, i.p., on day 0) drug (220 mg/kg, i.p., on day 0)§	(250)
	Cyclophosphamide	TLT mouse liver tumors	AIA (200 mg/kg, i.p., 2 or 4 daily doses) drug (50 mg/kg, i.p., once before, during, or after the AIA)§	(67)
	Paclitaxel	LS174T + HT29 colorectal carcinoma	AIA (100 mg/kg, i.p., days 0–20) drug (5 mg/kg, i.p., days 0–4, 7–11, 14–18)§	(165)
	Dacarbazine	518 A2 human melanoma	AIA (400 mg/kg, i.p., days 1–23) drug (80 mg/kg, i.p., days 12–16) †	(173)
Anginex	Carboplatin	MA148 human ovarian epithelial carcinoma	AIA (10 mg/kg, days 7–35) ‡ drug (32.5 mg/kg, i.p., every 3 d from 7–35) †	(251)
Thrombospondin	Carboplatin	Human testicular tumor	AIA (20 mg/kg/d from days 15–28) ‡ drug (30 mg/kg, i.p., days 14 + 21) †	(248)
	Irinotecan	HT29 colorectal adenocarcinoma	AIA (10 or 20 mg/kg, i.p., on 5 consecutive days for 4 wk) drug (125 or 150 mg/kg, i.p., 1×/wk for 4 wk)§	(252)
	Gemcitabine	AsPC-1 pancreatic cancer	AIA (3 mg/kg, i.p., days 7–28) drug (150 mg/kg, i.p., 2×/wk between days 7 and 28) †	(166)
DC101	Paclitaxel	253J B-V human bladder carcinoma	AIA (1 mg, i.p., 2×/wk for 4 wk starting day 21) drug (10 mg/kg, i.p., 1×/wk for 2 wk starting day 21) †	(174)
Anti-VEGF antibody	Doxorubicin	MCF-7 spheroids	AIA (200 μg, i.p., 2×/wk) drug (5 mg/kg, i.v., 1x/wk) †	(180)
	Topotecan	SK-NEP-1 Wilms' tumor	AIA (100 μg, i.p., 2×/wk for 5 wk) drug (0.36 mg/kg, i.p., days 7–11, 14–18, 28–32, and 35–39) †	(253)
	Paclitaxel	CWR22R prostate	AIA (5 mg/kg, i.p., 2×/wk for 4 wk) drug (6.25 mg/kg, s.c., 5×/wk for 3 wk)§	(179)
	Paclitaxel	OVCAR3 tumors	AIA (5 μg/g, i.p., 2×/wk for 6 wk) drug (20 μg/g, i.p., 2× to 3×/wk for 6 wk)§	(175)
	CPT-11	HT29 colon cancer	AIA (200 μg, i.p., days 0 + 4) drug (100 mg/kg, i.p., day 7)§	(254)
	Carboplatin	MA148 human epithelial ovarian carcinoma	AIA (2 mg, i.p., every 3 d for 10 treatments starting day 10) drug (32.5 mg/kg, i.p., every 3 d for 5 treatments starting day 10) †	(176)
SU6668	Paclitaxel	HOC79 human ovarian carcinoma	AIA (200 mg/kg, p.o., 3 or 5 cycles of 5 daily treatments) drug (6 mg/kg, i.v., every 2 d for 10 treatments or 20 mg/kg, i.v., 3 or 5 cycles 1×/wk) all started day 7 †	(255)
SU5416	Gemcitabine	MIA PaCa-2 pancreatic tumor	AIA (2×/wk i.p. injections of 25 mg/kg for 28 d or 50 mg/kg for 14 d starting day 1) drug (120 mg/kg, i.p., days 1, 4, 7, and 11) †	(177)
SU11657	Pemetrexed	A431 carcinoma	AIA (100 mg/kg, i.p., days 0, 2, 4, 7, 9, 11, 14, 16, and 18) drug (150 mg/kg, i.p. days 0–3)§	(72)

*AIAs and drugs given i.p., i.v., s.c., p.o., i.m., or i.t.

†AIAs and drugs were given on various days after tumor implantation (day 0).

‡AIA given continuously from a s.c. implanted osmotic pump.

§AIAs and drugs were given on various days when tumors had reached a specific size (day 0).

‖Endostatin was produced by an endostatin–expressing adenovirus, given as an i.m. or i.t. injection of 1×10^9 viral particles.

Table 5. Combination studies in tumors using VDAs and chemotherapeutic agents

VDA	Chemotherapy drug	Tumor type	Treatment schedule*	Reference
FAA	Chlorambucil	CaNT	VDA (150–200 mg/kg, i.p.) up to 1 h before/after drug (15–30 mg/kg, i.p.)	(194)
	Doxorubicin	B16 melanoma	VDA (1–2 × 150 mg/kg, i.v.) 1 h after drug (1–2 × 5 mg/kg, i.p.)	(256)
	Vinblastine	CaNT	VDA (150 mg/kg, i.p.) various times before/after drug (5 mg/kg, i.p.)	(76)
	Mitomycin C	CaNT	VDA (150–200 mg/kg, i.p.) up to 24 h before/after drug (3–5 mg/kg, i.p.)	(191)
	Tirapazamine	SCCVII	VDA (200 mg/kg, i.p.) various times before/after drug (0.05–0.2 mmol/kg, i.p.)	(114)
	Tirapazamine	KHT, RIF-1	VDA (200 mg/kg, i.p.) 15 min before or after drug (50 mg/kg, i.p.)	(190)
	Tirapazamine	MDAH-MCa4 mammary carcinoma	VDA (700 μmol/kg, i.p.) simultaneously with drug (0–300 μmol/kg, i.p.)	(192)
	Tirapazamine	CaNT	VDA (150 mg/kg, i.p.) immediately after drug (50 mg/kg, i.p.)	(77)
	RSU-1069	KHT, RIF-1	VDA (200 mg/kg, i.p.) 15 min after drug (80 mg/kg, i.p.)	(190)
	Mitomycin C	KHT, RIF-1	VDA (200 mg/kg, i.p.) 15 min after drug (5 mg/kg, i.p.)	(190)
DMXAA	Cisplatin	KHT, SKBR3, OW-1	VDA (5–17.5 mg/kg, i.p.) various times before/after drug (2.5–30 mg/kg, i.p.)	(85)
	Cisplatin	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–42.1 μmol/kg, i.p.)	(257)
	Carboplatin	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–316 μmol/kg, i.p.)	(257)
	Cyclophosphamide	KHT, SKBR3	VDA (17.5 mg/kg, i.p.) 1 h after drug (12.5–75 mg/kg, i.p.)	(85)
	Cyclophosphamide	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–716 μmol/kg, i.p.)	(257)
	Melphalan	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) various times before/after drug (34 μmol/kg, i.p.)	(195)
	5-FU	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–1780 μmol/kg, i.p.)	(257)
	Etoposide	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–75 μmol/kg, i.p.)	(257)
	Vincristine	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–1.0 μmol/kg, i.p.)	(257)
	Docetaxel	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–23.7 μmol/kg, i.p.)	(257)
	Paclitaxel	MDAH-MCa4 mammary carcinoma	VDA (60–80 μmol/kg, i.p.) various times before/after drug (0–31.6 μmol/kg, i.p.)	(257)
	Doxorubicin	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (0–23.7 μmol/kg, i.p.)	(257)
	Tirapazamine	MDAH-MCa4 mammary carcinoma	VDA (65–70 μmol/kg, i.p.) various times before/after drug (0–300 μmol/kg, i.p.)	(192)
	Tirapazamine	MDAH-MCa4 mammary carcinoma	VDA (20–90 μmol/kg, i.p.) simultaneously with drug (200 μmol/kg, i.p.)	(83)
	SN 23862	MDAH-MCa4 mammary carcinoma	VDA (65 μmol/kg, i.p.) various times before/after drug (200 μmol/kg, i.p.)	(192)
	SN 23816	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (300 μmol/kg, i.p.)	(83)
	AQ4N	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) 1 h before drug (450 μmol/kg, i.p.)	(193)
	CI-1010	MDAH-MCa4 mammary carcinoma	VDA (80 μmol/kg, i.p.) simultaneously with drug (940 μmol/kg, i.p.)	(83)
CA4P	Cisplatin	CaNT adenocarcinoma	VDA (100 mg/kg, i.p.) 15 min or 24 h after drug (5 mg/kg, i.p.)	(153)
	Cisplatin	C3H mammary carcinoma	VDA (250 mg/kg, i.p.) 1 h after drug (2–8 mg/kg, i.p.)	(258)
	Cisplatin	KHT, SKBR3, OW-1	VDA (10–100 mg/kg, i.p.) various times before/after drug (2.5–30 mg/kg, i.p.)	(85)
	Cisplatin	Kaposi's sarcoma	VDA (100 mg/kg, i.p.) 1 h after drug (5–20 mg/kg, i.p.)	(196)
	Cyclophosphamide	KHT, SKBR3	VDA (100 mg/kg, i.p.) 1 h after drug (12.5–100 mg/kg, i.p.)	(85)
	5-FU	MAC 29	VDA (100–125 mg/kg, i.p.) 20 min after drug (125 mg/kg, i.p.)	(197)

(*Continued on the following page*)

Table 5. Combination studies in tumors using VDAs and chemotherapeutic agents (Cont'd)

VDA	Chemotherapy drug	Tumor type	Treatment schedule*	Reference
	Vinblastine	Kaposi's sarcoma	VDA (100 mg/kg, i.p.) 1 h after drug (1.25–15 mg/kg, i.p.)	(196)
	Irinotecan	Rhabdomyosarcoma	VDA (25 mg/kg, i.p.) up to 1 h before/after drug (45 mg/kg, i.p.)	(259)
AVE8062	Cisplatin	Murine colon 26, S180, M109	VDA (10–80 mg/kg, i.v.) days 7, 11, and 15 simultaneously with drug (2.5–5 mg/kg, i.v.)	(199)
	Cisplatin	Human LX1, LS180	VDA (20 mg/kg, i.v.) days 11, 15, and 19 (LX1) or 10, 14, and 18 (LS180) simultaneously with drug (5 mg/kg, i.v.)	(199)
ZD6126	Cisplatin	KHT, Caki-1	VDA (10–150 mg/kg, i.p.) various times before/after drug (2.5–20 mg/kg, i.p.)	(198)
	Cisplatin	CaLu 6	VDA (100 mg/kg, i.p.) daily for 5 d starting 24 h after drug (4 mg/kg, i.p.)	(102)
	Cisplatin	PC14PE6 lung adenocarcinoma	VDA i.p. at 200 mg/kg (1 h) or 100 mg/kg (days 0–20) after drug (6 mg/kg, i.v.)	(260)
	Paclitaxel	FaDu squamous cell carcinoma	VDA (125 mg/kg, i.p.) 15 min after drug (15 mg/kg, i.p.)	(88)
OXi4503	Cisplatin	MAC 29 colon adenocarcinoma	VDA (100 mg/kg, i.p.) 20 min after drug (6 mg/kg, i.p.)	(261)
MN-029	Cisplatin	KHT sarcoma	VDA (100 mg/kg, i.p.) 1 h after drug (2.5–10 mg/kg, i.p.)	(97)

*VDAs and chemotherapy drugs were given either i.p. or i.v.

various drugs that show increased efficacy against cells at low pH (194, 195). In those situations, the enhanced response is probably due to these drugs killing those cells that are made hypoxic following the vascular shutdown induced by the VDA treatment yet had survived this insult. What is clear is that timing and sequencing of the VDAs and chemotherapeutic agents have major effects on the effectiveness of such combinations. Generally, the greatest enhancements are seen when the VDAs are administered within a few hours after giving the chemotherapeutic drug (83, 102, 153, 192, 195–198). Most studies show that at this time the enhancement is equivalent to a simple additive response, so it is unlikely that the vascular shutdown by the VDA traps the drug in the tumor. Indeed, in one study with CA4P and 5-FU, an enhanced tumor response was seen with the combination, yet pharmacokinetic analysis showed no effect of CA4P on tumor levels of 5-FU up to 4 hours after treatment, and at longer time intervals, there was actually an increase in drug clearance (197). However, a VDA-mediated increase in drug uptake in tumors has also been reported (199). In contrast, injecting VDAs immediately before chemotherapy often results in a loss of benefit (85, 153, 195), and this can probably be attributed to the reductions in blood flow by the VDA impairing the delivery of the chemotherapeutic drug to the tumor.

Even if the combined VDA-chemotherapy treatment yields only an additive tumor response, such an outcome should still result in a therapeutic benefit because the pathophysiologic effects of VDAs that lead to the enhanced tumor response do not occur to the same extent in normal tissues (84, 87). Indeed, several preclinical studies have now shown improved antitumor effects without concomitant increases in either host toxicity (83, 153, 192, 195–198) or chemotherapy agent specific normal tissue damage (85, 198).

Other therapies. There are several other less conventional therapies with which VTAs have been combined and enhanced tumor response observed and in which the pathophysiologic effects of VTAs clearly play a significant role in the enhancement. A review of the pathophysiologic effects of VTAs and their implications for therapy would not be complete without some reference to these less conventional approaches. Principal among these is hyperthermia. The response of tumors to heat treatment is strongly dependent on tumor pathophysiology. Blood flow, being one of the major means by which heat is dissipated from tissues, will affect the ability to heat tumors. Generally, the lower the rate of blood flow, the easier it is to heat (200, 201). The tumor microenvironment also plays an important role in influencing the tumor response to heat. Several studies have shown that cells incubated under the oxygen-deprived and highly acidic adverse conditions, such as those often found in tumors (6, 7), are more sensitive to the cytotoxic action of heat (202, 203). This suggests that treatments that can modify tumor pathophysiology should be capable of changing heat sensitivity, and this has been shown using clamping (204, 205) or physiologic modifiers of blood flow (206–208).

Very few studies have investigated the potential of combining AIAs with hyperthermia. One study using the metalloproteinase inhibitor batimastat failed to show any enhancement of heat damage (209). An improved response to heat was observed with the synthetic analogue of fumagillin, TNP-470 (210, 211). However, because the effect was temperature dependent and the treatment schedule involved giving TNP-470 after heating, the enhancement was attributed to the AIA inhibiting angiogenesis that occurred following heat-induced vascular damage rather than due to any AIA-induced pathophysiologic changes (210, 211). Numerous studies have examined the combination of VDAs and heat. The VDAs include TNF (74, 75), ATO (80, 212), vinblastine (78), FAA (81, 124, 213), DMXAA (99), and CA4P (78, 214, 215). All resulted in an enhancement of the heat response. This outcome was time and schedule dependent, with the maximum response generally observed if the heat was started 1 to 6 hours after VDA administration (78, 80, 81, 99, 124, 212, 214, 215), corresponding to the maximal reduction in blood flow in those studies. As to the exact mechanism responsible for this enhancement, there is evidence for both an improved tumor heating (78, 124, 214) and a decrease in tumor pH (124–127).

On its own, hyperthermia has no role to play in the curative treatment of cancer and its clinical potential lies in its use as an adjuvant to other more conventional modalities, especially radiation (216). Indeed, several randomized clinical studies have shown the benefit of combining radiation and heat (217). Preclinical studies have now also shown that the effect of this thermoradiotherapy can be significantly improved by including FAA (32, 213), DMXAA (32, 218), or CA4P (32, 215) in the radiation and heat schedule. These VDAs were always administered after irradiating and before heating so as to exploit the pathophysiologic changes that could enhance both treatments.

Another "physical treatment" in which the tumor microenvironment influences response is PDT. PDT involves the administration of a photosensitizing agent and its subsequent activation by light, and this reaction is strongly dependent on oxygen concentration (219). Combining PDT with agents that have the potential to improve oxygen delivery is clearly a valid approach, and several studies have now shown that, when various tyrosine kinase inhibitors are combined with PDT, a significant improvement in tumor response can be obtained (220, 221). However, in those studies, the AIAs were always given after the PDT treatment, so the enhancement obtained was not the result of any pathophysiologic effect of the AIAs. Like hyperthermia, PDT not only kills tumor cells through a direct cytotoxic effect but can also have an indirect effect mediated through the induction of vascular damage (10, 23); thus, the results may have reflected an inhibition of angiogenesis following this vascular damage.

The pathophysiologic effects of VDAs have also been exploited to improve tumor response to radioimmunotherapy (222, 223) and both antibody-directed (ADEPT) and clostridia-directed (CDEPT) prodrug therapy (224, 225). The radioimmunotherapy studies have been done with DMXAA (222) and CA4P (223). Both VDAs were administered 48 hours after injecting the radioactive antibodies to allow sufficient time for maximal tumor accumulation of the latter before the VDA-induced inhibition of tumor blood flow occurred. With both VDAs, a substantial improved response was observed with the combination treatment. Although this may have simply been the result of independent actions of each treatment, the VDA killing hypoxic cells in the center of the tumor and the radioactive antibody killing cells in the more radiosensitive tumor rim, the results also suggested enhancement of the radioimmunotherapy response by entrapment of the radioactive antibody following VDA-induced vessel collapse (222, 223). Improved tumor response has also been observed for the combinations of ADEPT plus DMXAA (224) and CDEPT with DMXAA or ZD6126 (225). Timing and sequencing were important factors influencing response, and under optimal conditions, substantial VDA-induced entrapment of the ADEPT or CEDPT moieties was found (224, 225).

Summary and Conclusions

Although VTAs on their own may elicit significant antitumor effects, their greatest use likely lies in their combination with more conventional therapies. However, the very nature of their mode of action will result in pathophysiologic changes that can influence these other therapies, in both a positive and a negative fashion, and thus, timing and sequencing become critical factors. When AIAs are combined with radiation, the opinion often expressed is that AIAs improve tumor oxygenation and that this can be exploited to enhance radiation response. But this is clearly too simple a generalization. AIAs can also decrease tumor oxygenation, and even under conditions where improvements in oxygenation are seen, only a narrow window of opportunity exists to exploit this effect. Clearly, unless it is possible to accurately and reliably predict the effects on tumor oxygenation for each AIA, each tumor type, and likely each individual tumor, then it would seem prudent to select a schedule that avoids any potentially negative consequences. For VDAs, the situation is clearer in that they induce hypoxia, and giving the VDA after irradiation would seem to be the optimal approach. With chemotherapy, VDAs should be administered in a fashion that minimizes the effects on blood flow to avoid affecting the delivery of the chemotherapeutic agent. Again, this would argue for giving the VDAs after the conventional anticancer drug. Indeed, shutting down blood flow after the chemotherapeutic agent has entered the tumor would still allow for other VDA-induced pathophysiologic changes to be exploited.

These findings suggest that spatial separation between the VTAs and other modalities offers the best strategy for maximizing antitumor effects while minimizing the possibility of reductions in the efficacy of the conventional therapy. Preclinical studies indicate that, when this is done, additive antitumor effects are usually observed. Such a response in the tumor can lead to a substantial therapeutic benefit, although further investigations of normal tissue side effects under these treatment conditions are needed. Overall, the pathophysiologic effects of VTAs need to be considered when the combination of VTAs with other cancer therapies is planned. Based on the results reported in a large body of preclinical investigations, significant improvements in treatment outcome in clinical studies combining VTAs and conventional anticancer therapies are anticipated.

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References

1. Brem S, Brem H, Folkman J, Finkelstein D, Patz A. Prolonged tumor dormancy by prevention of neovascularization in the vitreous. Cancer Res 1976;36:2807–12.
2. Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue? Cancer Res 1986;46:467–73.
3. Hahnfeldt P, Panigrahy D, Folkman J, Hlatky L. Tumor development under angiogenic signaling: a dynamic theory of tumor growth, treatment response, and postvascular dormancy. Cancer Res 1999;59:4770–5.
4. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401–10.
5. Stoeltzing O, Ellis LM. The role of microvasculature in metastasis formation. In: Siemann DW, editor. Vascular-targeted therapies in oncology. Chichester: John Wiley & Sons, Ltd.; 2006. p.31–62.
6. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 1989;49:6449–65.
7. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. Semin Radiat Oncol 2004;14:198–206.
8. Coley Nauts H, Fowler GA, Bogatko FH. A review of the influence of bacterial infection and bacterial products (Coley's toxins) on malignant tumours in man. Acta Med Scand 1953;274:29–97.
9. Woglem WH. A critique of tumour resistance. J Cancer Res 1923;7:283–311.
10. Denekamp J, Hill S. Angiogenic attack as a therapeutic strategy for cancer. Radiother Oncol 1991;Suppl. 20:103–12.
11. Denekamp J. The tumour microcirculation as a target in cancer therapy: a clearer perspective. Eur J Clin Invest 1999;29:733–6.
12. Kerbel RS, Kamen BA. The anti-angiogenic basis of

metronomic chemotherapy. Nat Rev Cancer 2004;4:423–36.
13. Garcia-Barros M, Paris F, Cordon-Cardo C, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155–9.
14. Wachsberger P, Burd R, Dicker AP. Tumor response to ionising radiation combined with antiangiogenesis or vascular targeting agents: exploring mechanisms on interaction. Clin Cancer Res 2003;9:1957–71.
15. Siemann DW, Bibby MC, Dark GG, et al. Differentiation and definition of vascular-targeted therapies. Clin Cancer Res 2005;11:416–20.
16. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
17. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 2003;9:677–84.
18. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2002;2:727–39.
19. Marmé D. The impact of anti-angiogenic agents on cancer therapy. J Cancer Res Clin Oncol 2003;129:607–20.
20. Eskens FA. Angiogenesis inhibitors in clinical development; where are we now and where are we going? Br J Cancer 2004;90:1–7.
21. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. Nature 2005;438:967–74.
22. National Cancer Institute Web site: http://www.cancer.gov/clinicaltrials.
23. Horsman MR, Overgaard J. Thermal radiosensitization in animal tumors: the potential for therapeutic gain. In: Urano M, Douple E, editors. Hyperthermia and oncology, vol. 2. Utrecht: VSP; 1985. p.113–45.
24. Chaplin DJ, Dougherty GJ. Tumour vasculature as a target for cancer therapy. Br J Cancer 1999;80:57–64.
25. Thorpe PE. Vascular targeting agents as cancer therapeutics. Clin Cancer Res 2004;10:415–27.
26. Siemann DW, Chaplin DJ, Horsman MR. Vascular targeting therapies for treatment of malignant disease. Cancer 2004;100:2491–9.
27. Baguley BC. Antivascular therapy of cancer: DMXAA. Lancet Oncol 2003;4:141–8.
28. Tozer GM, Kanthou C, Baguley BC. Disrupting tumour blood vessels. Nat Rev Cancer 2005;5:423–35.
29. Laird AD, Vajkoczy P, Shawver LK, et al. SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. Cancer Res 2000;60:4152–60.
30. O'Reilly MS, Holmgren L, Chn C, Folkman J. Angiostatin induces and sustains dormancy of primary tumors in mice. Nat Med 1996;2:689–92.
31. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signalling, angiogenesis, and tumor growth following oral administration. Cancer Res 2002;62:4645–55.
32. Horsman MR, Murata R. Combination of vascular targeting agents with thermal or radiation therapy. Int J Radiat Oncol Biol Phys 2002;54:1518–23.
33. Landuyt W, Verdoes O, Darius DO, et al. Vascular targeting of solid tumours: a major "inverse" volume-response relationship following combretastatin A-4 phosphate treatment of rat rhabdomyosarcomas. Eur J Cancer 2000;36:1833–43.
34. Siemann DW. Vascular targeting agents. Horizons Cancer Ther 2002;3:4–15.
35. Siemann DW, Rojiani AM. The vascular disrupting agent ZD6126 shows increased antitumor efficacy and enhanced radiation response in large, advanced tumors. Int J Radiat Oncol Biol Phys 2005;62:846–53.
36. Shi W, Siemann DW. Targeting the tumor vasculature: enhancing antitumor efficacy through combination treatment with ZD6126 and ZD6474. In Vivo 2005;19:1045–50.
37. Ching LM, Xu ZF, Gummer BH, Palmer BD, Joseph WR, Baguley BC. Effect of thalidomide on tumour necrosis factor production and anti-tumour activity induced by 5,6-dimethylxanthenone-4-acetic acid. Br J Cancer 1995;72:339–43.
38. Ching LM, Browne WL, Tchernegovski R, Gregory T, Baguley BC, Palmer BD. Interaction of thalidomide, phthalimide analogues of thalidomide and pentoxifylline with the anti-tumour agent 5,6-dimethylxanthenone-4-acetic acid: concomitant reduction of serum tumour necrosis factor-α and enhancement of anti-tumour activity. Br J Cancer 1998;78:336–43.
39. Cao Z, Joseph WR, Browne WL, et al. Thalidomide increases both intra-tumoural tumour necrosis factor-α production and anti-tumour activity in response to 5,6-dimethylxanthenone-4-acetic acid. Br J Cancer 1999;80:716–23.
40. Mysliwski A, Bigda J, Koszalka P, Szmit E. Synergistic effect of the angiogenesis inhibitor TNP-470 and tumor necrosis factor (TNF) on Bomirski Ab melanoma in hamsters. Anticancer Res 2000;20:4643–7.
41. Landuyt W, Ahmed B, Nuyts S, et al. *In vivo* antitumor effect of vascular targeting combined with either ionizing radiation or anti-angiogenesis treatment. Int J Radiat Oncol Biol Phys 2001;49:443–50.
42. Nordsmark N, Overgaard M, Overgaard J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother Oncol 1996;41:31–9.
43. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. Radiother Oncol 1999;53:113–7.
44. Hoeckel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56:4509–15.
45. Nordsmark N, Alsner J, Keller J, et al. Hypoxia in human soft tissue sarcomas: adverse impact on survival and no association with p53 mutations. Br J Cancer 2001;84:1070–5.
46. Lee C-G, Heijn M, di Tomaso E, et al. Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res 2000;60:5565–70.
47. Gong H, Pöttgen C, Stüben G, Havers W, Stuschke M, Schweigerer L. Arginine deiminase and other anti-angiogenic agents inhibit unfavorable neuroblastoma growth: potentiation by irradiation. Int J Cancer 2003;106:723–8.
48. Drevs J, Müller-Driver R, Wittig C, et al. PTK787/ZK 222584, a specific vascular endothelial growth factor-receptor tyrosine kinase inhibitor, affects the anatomy of the tumor vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging. Cancer Res 2002;62:4015–22.
49. Schuuring J, Bussink J, Bernsen HJJA, Peeters W, van der Kogel AJ. Irradiation combined with SU5416: microvascular changes and growth delay in a human xenograft glioblastoma tumor line. Int J Radiat Oncol Biol Phys 2005;61:529–34.
50. Hansen-Algenstaedt N, Stoll BR, Padera TP, et al. Tumor oxygenation in hormone-dependent tumors during vascular endothelial growth factor receptor-2-blockade, hormone ablation, and chemotherapy. Cancer Res 2000;60:4556–60.
51. Shi W, Teschendorf C, Muzyczka N, Siemann DW. Gene therapy delivery of endostatin enhances the treatment efficacy of radiation. Radiother Oncol 2003;66:1–9.
52. Dings RPM, Williams BW, Song CW, Griffioen AW, Mayo KH, Griffin RJ. Anginex synergizes with radiation therapy to inhibit tumor growth by radiosensitizing endothelial cells. Int J Cancer 2005;115:312–9.
53. Lund EL, Bastholm L, Kristjansen PEG. Therapeutic synergy of TNP-470 and ionizing radiation: effects on tumor growth, vessel morphology, and angiogenesis in human glioblastoma multiforme xenografts. Clin Cancer Res 2000;6:971–8.
54. Hess C, Vuong V, Hegyi I, et al. Effect of VEGF receptor inhibitor PTK787/ZK222548 combined with ionizing radiation on endothelial cells and tumour growth. Br J Cancer 2001;85:2010–6.
55. Fenton BM, Paoni SF, Grimwood BG, Ding I. Disparate effects of endostatin on tumor vascular perfusion and hypoxia in two murine mammary carcinomas. Int J Radiat Oncol Biol Phys 2003;57:1038–46.
56. Bernsen HJJA, Rijken PFJW, Peters JPW, et al. Suramin treatment of human glioma xenografts; effects on tumor vasculature and oxygenation status. J Neuro-oncol 1999;44:129–36.
57. Fenton BM, Paoni SF, Ding I. Pathophysiological effects of vascular endothelial growth factor receptor-2-blocking antibody plus fractionated radiotherapy on murine mammary tumors. Cancer Res 2004;64:5712–9.
58. Leith JT, Papa G, Quaranto L, Michelson S. Modifications of the volumetric growth response and steady-state hypoxic fractions of xenografted DLD-2 human colon carcinomas by administration of basic fibroblast growth factor or suramin. Br J Cancer 1992;66:345–8.
59. Murata R, Nishimura Y, Hiraoka M. An anti-angiogenic agent (TNP-470) inhibited reoxygenation during fractionated radiotherapy of murine mammary carcinoma. Int J Radiat Oncol Biol Phys 1997;37:1107–13.
60. Griffin RJ, Williams BW, Wild R, Cherrington JM, Park H, Song CW. Simultaneous inhibition of the receptor kinase activity of vascular endothelial, fibroblast, and platelet-derived growth factors suppresses tumor growth and enhances tumor radiation response. Cancer Res 2002;62:1702–6.
61. Williams KJ, Telfer BA, Brave S, et al. ZD6474, a potent inhibitor of vascular endothelial growth factor signaling, combined with radiotherapy: schedule-dependent enhancement of antitumor activity. Clin Cancer Res 2004;10:8587–93.
62. Kozin SV, Boucher Y, Hicklin DJ, Bohlen P, Jain RK, Suit HD. Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. Cancer Res 2001;61:39–44.
63. Teicher BA, Holden SA, Ara G, et al. Influence of an anti-angiogenic treatment on 9L gliosarcoma: oxygenation and response to cytotoxic therapy. Int J Cancer 1995;61:732–7.
64. Rofstad EK, Henriksen K, Galappathi K, Mathiesen B. Antiangiogenic treatment with thrombospondin-1 enhances primary tumor radiation response and prevents growth of dormant pulmonary micrometastases after curative radiation therapy in human melanoma xenografts. Cancer Res 2003;63:4055–61.
65. Winkler F, Kozin SV, Tong R, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004;6:553–63.
66. Ansiaux R, Baudelet C, Jordan BF, et al. Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. Clin Cancer Res 2005;11:743–50.
67. Segers J, Di Fazio V, Ansiaux R, et al. Potentiation of cyclophosphamide chemotherapy using the anti-angiogenic drug thalidomide: importance of optimal scheduling to exploit the "normalization" window of the tumor vasculature. Cancer Lett 2006;244:129–35.
68. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 2001;7:987–9.
69. Boucher Y, Baxter LT, Jain RK. Interstitial pressure gradients in tissue-isolated and subcutaneous tumors: implications for therapy. Cancer Res 1990;50:4478–84.
70. Milosevic M, Fyles A, Hedley D, Hill R. The human tumor microenvironment: invasive (needle) measurement of oxygen and interstitial fluid pressure. Semin Radiat Oncol 2004;14:249–58.
71. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res 2004;64:3731–6.
72. Huber PE, Bischof M, Jenne J, et al. Trimodal cancer treatment: beneficial effects of combined antiangiogenesis, radiation, and chemotherapy. Cancer Res 2005;65:3643–55.
73. Teicher BA, Holden SA, Ara G, et al. Potentiation of cytotoxic cancer therapies by TNP-470 alone and with other anti-angiogenic agents. Int J Cancer 1994;57:920–5.
74. Kallinowski F, Moehle R, Vaupel P. Substantial enhancement of tumor hyperthermic response by tumor necrosis factor. In: Sugahara T, Saito M, editors. Hyperthermic oncology, vol. 1. London: Taylor and Francis; 1989. p.258–9.
75. Lin JC, Park HJ, Song CW. Combined treatment of IL-α and TNF-α potentiates the antitumour effect of hyperthermia. Int J Hyperthermia 1996;12:335–44.
76. Hill SA, Sampson LE, Chaplin DJ. Anti-vascular approaches to solid tumour therapy: evaluation of

vinblastine and flavone acetic acid. Int J Cancer 1995;63: 119–23.
77. Chaplin DJ, Pettit GR, Parkins CS, Hill SA. Antivascular approaches to solid tumour therapy: evaluation of tubulin binding agents. Br J Cancer 1996;74:S86–8.
78. Eikesdal HP, Bjerkvig R, Dahl O. Vinblastine and hyperthermia target the neovasculature in BT_4AN rat gliomas: therapeutic implications of the vascular phenotype. Int J Radiat Oncol Biol Phys 2001;51: 535–44.
79. Lew YS, Brown SL, Griffin RJ, Song CW, Kim JH. Arsenic trioxide causes selective necrosis in solid murine tumors by vascular shutdown. Cancer Res 1999;59:6033–7.
80. Griffin RJ, Lee SH, Rood KL, et al. Use of arsenic trioxide as an antivascular and thermosensitizing agent in solid tumors. Neoplasia 2000;2:555–60.
81. Horsman MR, Sampson LE, Chaplin DJ, Overgaard J. The *in vivo* interaction between flavone acetic acid and hyperthermia. Int J Hyperthermia 1996;12:779–89.
82. Bibby M, Double JA, Loadman PM, Duke CV. Reduction of tumor blood flow by flavone acetic acid: a possible component of therapy. J Natl Cancer Inst 1989;81:216–20.
83. Lash CJ, Li AE, Rutland M, Baguley BC, Zwi LJ, Wilson WR. Enhancement of the anti-tumour effects of the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) by the combination with 5-hydroxytryptamine and bioreductive drugs. Br J Cancer 1998;78:439–45.
84. Murata R, Overgaard J, Horsman MR. Comparative effects of combretastatin A-4 disodium phosphate and 5,6-dimethylxanthenone-4-acetic acid on blood perfusion in a murine tumour and normal tissues. Int J Radiat Biol 2001;77:195–204.
85. Siemann DW, Mercer E, Lepler S, Rojiani AM. Vascular targeting agents enhance chemotherapeutic agent activities in solid tumor therapy. Int J Cancer 2002;99:1–6.
86. Dark GD, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ. Combretastatin A-4, an agent that displays potent and selective toxicity towards tumor vasculature. Cancer Res 1997;57:1829–34.
87. Tozer GM, Prise VE, Wilson J, et al. Combretastatin A-4 phosphate as a tumor vascular-targeting agent: early effects in tumors and normal tissues. Cancer Res 1999;59:1626–34.
88. Davis PD, Dougherty GJ, Blakey DC, et al. ZD6126: a novel vascular-targeting agent that causes selective destruction of tumor vasculature. Cancer Res 2002;62: 7247–53.
89. Siemann DW, Rojiani AM. Enhancement of radiation therapy by the novel vascular targeting agent ZD6126. Int J Radiat Oncol Biol Phys 2002;53:164–71.
90. Horsman MR, Murata R. Vascular targeting effects of ZD6126 in a C3H mouse mammary carcinoma and the enhancement of radiation response. Int J Radiat Oncol Biol Phys 2003;57:1047–55.
91. Hori K, Saito S. Microvascular mechanisms by which the combretastatin A-4 derivative AC7770 (AVE8062) induces tumour blood flow stasis. Br J Cancer 2003;89: 1334–44.
92. Hori K, Saito S, Sato Y, et al. Differential relationship between changes in tumour size and microcirculatory functions induced by therapy with antivascular drug and cytotoxic drugs: implications for the evaluation of therapeutic efficacy of AC7700 (AVE8062). Eur J Cancer 2003;39:1957–66.
93. Hill SA, Tozer GM, Pettit GR, Chaplin DJ. Preclinical evaluation of the antitumour activity of the novel vascular targeting agent Oxi 4503. Anticancer Res 2002;22:1453–8.
94. Howell SE, Cooper PA, Thompson MJ, et al. Anti-tumor and anti-vascular effects of the novel tubulin-binding agent combretastatin A-1 phosphate. Anticancer Res 2002;22:3933–40.
95. Hua J, Sheng Y, Pinney KG. Oxi4503, a novel vascular targeting agent: effects on blood flow and antitumor activity in comparison to combretastatin A-4 phosphate. Anticancer Res 2003;23:1433–40.
96. Sheng Y, Hua J, Pinney KG, et al. Combretastatin family member Oxi4503 induces tumor vascular collapse through the induction of endothelial apoptosis. Int J Cancer 2004;111:604–10.
97. Shi W, Siemann DW. Preclinical studies of the novel vascular disrupting agent MN-029. Anticancer Res 2005; 25:3899–904.
98. Murata R, Siemann DW, Overgaard J, Horsman MR. Interaction between combretastatin A-4 disodium phosphate and radiation in murine tumours. Radiother Oncol 2001;60:155–61.
99. Murata R, Overgaard J, Horsman MR. Potentiation of the anti-tumor effect of hyperthermia by combining with the vascular targeting agent 5,6-dimethylxanthenone-4-acetic acid. Int J Hyperthermia 2001;17: 508–19.
100. Galbraith SM, Chaplin DJ, Lee F, et al. 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.
101. Kanthou C, Tozer GM. The tumor vascular targeting agent combretastatin A-4 phosphate induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells. Blood 2002;99: 2060–9.
102. Blakey DC, Westwood FR, Walker M, et al. Antitumor activity of the novel vascular targeting agent ZD6126 in a panel of tumor models. Clin Cancer Res 2002;8:1974–83.
103. Ferrero E, Villa A, Ferrero ME, et al. Tumor necrosis factor α-induced vascular leakage involves PECAM1 phosphorylation. Cancer Res 1996;56:3211–5.
104. Tozer GM, Prise VE, Wilson J, et al. Mechanisms associated with tumor vascular shut-down induced by combretastatin A-4 phosphate: intravital microscopy and measurement of vascular permeability. Cancer Res 2001;61:6413–22.
105. Kim JH, Lew YS, Kolozsvary A, Ryu S, Brown SL. Arsenic trioxide enhances radiation response of 9L glioma in the rat brain. Radiat Res 2003;160:662–6.
106. Robinson SP, McIntyre DJO, Checkley D, et al. Tumour dose response to the antivascular agent ZD6126 assessed by magnetic resonance imaging. Br J Cancer 2003;88:1592–7.
107. Galbraith SM, Maxwell RJ, Lodge MA, et al. Combretastatin A4 phosphate has tumor antivascular activity in rat and man as demonstrated by dynamic magnetic resonance imaging. J Clin Oncol 2003;21: 2831–42.
108. Evelhoch JL, LoRusso PM, He Z, et al. Magnetic resonance imaging measurements of the response of murine and human tumors to the vascular-targeting agent ZD6126. Clin Cancer Res 2004;10:3650–7.
109. Zhao L, Ching L-M, Kestell P, Kelland LR, Baguley BC. Mechanisms of tumor vascular shutdown induced by 5,6-dimethylxanthenone-4-acetic acid (DMXAA): increased tumor vascular permeability. Int J Cancer 2005;116:322–6.
110. Ferretti S, Allegrini PR, O'Reilly T, et al. Patupilone induced vascular disruption in orthotopic rodent tumor models detected by magnetic resonance imaging and interstitial fluid pressure. Clin Cancer Res 2005;11: 7773–84.
111. Skliarenko JV, Lunt SJ, Gordon ML, Vitkin A, Milosevic M, Hill RP. Effects of the vascular disrupting agent ZD6126 on interstitial fluid pressure and cell survival in tumours. Cancer Res 2006;66:2074–80.
112. Eikesdal HP, Landuyt W, Dahl O. The influence of combretastatin A-4 and vinblastine on interstitial fluid pressure in BT_4An rat gliomas. Cancer Lett 2002;178: 209–17.
113. Vincent L, Kermani P, Young LM, et al. Combretastatin A4 phosphate induces rapid regression of tumor neovessels and growth through interference with vascular endothelial-cadherin signalling. J Clin Invest 2005;115:2992–3006.
114. Sun J-R, Brown JM. Enhancement of the antitumor effect of flavone acetic acid by the bioreductive cytotoxic drug SR 4233 in a murine carcinoma. Cancer Res 1989;49:5664–70.
115. Grosios K, Holwell SE, McGown AT, Pettie GR, Bibby MC. *In vivo* and *in vitro* evaluation of combretastatin A-4 and its sodium phosphate prodrug. Br J Cancer 1999;81:1318–27.
116. Chaplin DJ, Hill SA. The development of combretastatin A4 phosphate as a vascular targeting agent. Int J Radiat Oncol Biol Phys 2002;54:1491–6.
117. Moulder JE, Rockwell S. Hypoxic fractions in solid tumors. Int J Radiat Oncol Biol Phys 1984;10: 695–712.
118. 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 tumors. Int J Radiat Oncol Biol Phys 1998;42:895–8.
119. Wachsberger PR, Bird R, Marero N, et al. Effect of the tumor vascular-damaging agent, ZD6126, on the radioresponse of U87 glioblastoma. Clin Cancer Res 2005;11:835–42.
120. Eikesdal HP, Bjerkvig R, Raleigh JA, Mella O, Dahl O. Tumor vasculature is targeted by the combination of combretastatin A-4 and hyperthermia. Radiother Oncol 2001;61:313–20.
121. El-Emir E, Boxer GM, Petrie IA, et al. Tumour parameters affected by combretastatin A-4 phosphate therapy in a human colorectal xenograft model in nude mice. Eur J Cancer 2005;41:799–806.
122. Zhao D, Jiang L, Hahn EW, Mason RP. Tumor physiologic response to combretastatin A4 phosphate assessed by MRI. Int J Radiat Oncol Biol Phys 2005;62: 872–80.
123. Griffiths JR. Are cancer cells acidic? Br J Cancer 1991;64:425–7.
124. Sakaguchi Y, Maehara Y, Baba H, Kusumoto T, Sugimachi K, Newman RA. Flavone acetic acid increases the antitumor effect of hyperthermia in mice. Cancer Res 1992;52:3306–9.
125. Breidahl T, Nielsen FU, Stodkilde-Jorgensen H, Maxwell RJ, Horsman MR. The effects of the vascular disrupting agents combretastatin A-4 disodium phosphate, 5,6-dimethylxanthenone-4-acetic acid, and ZD6126 in a murine tumour: a comparative assessment using MRI and MRS. Acta Oncol 2006;45:306–16.
126. Maxwell RJ, Nielsen FU, Breidahl T, Stødkilde-Jørgensen H, Horsman MR. Effects of combretastatin on murine tumours monitored by ^{31}P MRS, ^{1}H MRS, and ^{1}H MRI. Int J Radiat Oncol Biol Phys 1998;42: 891–4.
127. Beauregard DA, Thelwall PE, Chaplin DJ, Hill SA, Adams GE, Brindle KM. Magnetic resonance imaging and spectroscopy of combretastatin A4 prodrug-induced disruption of tumour perfusion and energetic status. Br J Cancer 1998;77:1761–7.
128. Gorski DH, Mauceri HJ, Salloum RM, et al. Potentiation of the antitumor effect of ionizing radiation by brief concomitant exposures to angiostatin. Cancer Res 1998;58:5686–9.
129. Gorski DH, Mauceri HJ, Salloum RM, Halpern A, Seetharam S, Weichselbaum RR. Prolonged treatment with angiostatin reduces metastatic burden during radiation therapy. Cancer Res 2003;63:308–11.
130. Griscelli F, Li H, Cheong C, et al. Combined effects of radiotherapy and angiostatin gene therapy in glioma tumor model. Proc Natl Acad Sci U S A 2000; 97:6698–703.
131. Hanna NN, Seetharam S, Mauceri HJ, et al. Antitumor interaction of short-course endostatin and ionizing radiation. Cancer J 2000;6:287–93.
132. Gorski DH, Beckett MA, Jaskowiak NT, et al. Blockade of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res 1999;59:3374–8.
133. Guptra VK, Jaskowiak NT, Beckett MA, et al. Vascular endothelial growth factor enhances endothelial cell survival and tumor radioresistance. Cancer J 2002;8: 47–54.
134. Lu B, Geng L, Musiek A, et al. Broad spectrum receptor tyrosine kinase inhibitor, SU6668, sensitizes radiation via targeting survival pathway of vascular endothelium. Int J Radiat Oncol Biol Phys 2004;58: 844–50.
135. Schueneman AA, Himmelfarb E, Geng L, et al. SU11248 maintenance therapy prevents tumor regrowth after fractionated irradiation of murine tumor models. Cancer Res 2003;63:4009–16.
136. Zips D, Hessel F, Krause M, et al. Impact of adjuvant inhibition of vascular endothelial growth factor receptor tyrosine kinases on tumor growth delay and local tumor control after fractionated irradiation in human squamous cell carcinomas in nude mice. Int J Radiat Oncol Biol Phys 2005;61:908–14.

137. Mauceri HJ, Hanna NN, Beckett MA, et al. Combined effects of angiostatin and ionizing radiation in antitumor therapy. Nature 1998;394:287–91.

138. Geng L, Donnelly E, McMahon G, et al. Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. Cancer Res 2001;61:2413–9.

139. Ning S, Laird D, Cherrington JM, Knox SJ. The antiangiogenic agents SU5416 and SU6668 increase antitumor effects of fractionated irradiation. Radiat Res 2002;157:45–51.

140. Zips D, Krause M, Hessel F, et al. Experimental study on different combination schedules of VEGF-receptor inhibitor PTK787/ZK222584 and fractionated irradiation. Anticancer Res 2003;23:3869–76.

141. Brazelle WD, Shi W, Siemann DW. VEGF associated tyrosine kinase inhibition increases the tumor response to single and fractionated dose radiotherapy. Int J Radiat Oncol Biol Phys 2006;65:836–41.

142. Damiano V, Melisi D, Bianco C, et al. Cooperative antitumor effect of multitargeted kinase inhibitor ZD6474 and ionizing radiation in glioblastoma. Clin Cancer Res 2005;11:5639–44.

143. Kaliski A, Maggiorella L, Cengel KA, et al. Angiogenesis and tumor growth inhibition by a matrix metalloproteinase inhibitor targeting radiation-induced invasion. Mol Cancer Ther 2005;4:1717–28.

144. Steel GG, Peckham MJ. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys 1979;5: 85–91.

145. Steel GG. Terminology in the description of drug-radiation interactions. Int J Radiat Oncol Biol Phys 1979; 5:1145–50.

146. Gray LH, Conger AD, Ebert M, et al. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 1953;26:638–48.

147. Horsman MR, Overgaard J. The oxygen effect and tumour microenvironment. In: Steel GG, editor. Basic clinical radiobiology for radiation oncologists. 3rd ed. London: Edward Arnold; 2002. p.158–68.

148. Stenstrom KW, Vermund H, Mosser DG, Marvin JF. Effects of roentgen irradiation on the tumor bed. I. The inhibiting action of local pretransplantation roentgen irradiation (1500 r_{α}) on the growth of mouse mammary carcinoma. Radiat Res 1955;2:180–91.

149. Hewitt HB, Blake ER. The growth of transplanted murine tumours in pre-irradiated sites. Br J Cancer 1968;22:808–24.

150. Zips D, Eicheler W, Geyer P, et al. Enhanced susceptibility of irradiated tumor vessels to vascular endothelial growth factor receptor tyrosine kinase inhibition. Cancer Res 2005;65:5374–9.

151. Abdollahi A, Lipson KE, Han X, et al. SU5416 and SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor production and amplify the direct anti-endothelial action of radiation *in vitro*. Cancer Res 2003;63:3755–63.

152. 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.

153. Chaplin DJ, Pettit GR, Hill SA. Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. Anticancer Res 1999;19: 189–96.

154. Wilson WW, Li AE, Cowan D, Siim BG. Enhancement of tumor radiation response by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid. Int J Radiat Oncol Biol Phys 1998;42:905–8.

155. Murata R, Siemann DW, Overgaard J, Horsman MR. Improved tumor response by combining radiation and the vascular damaging drug 5,6-dimethylxanthenone-4-acetic acid. Radiat Res 2001;156:503–9.

156. Kimura K, Bowen C, Spiegel S, Gelmann EP. Tumor necrosis factor-α sensitizes prostate cancer cells to γ-irradiation-induced apoptosis. Cancer Res 1999;59: 1606–14.

157. Ahmed B, Landuyt W, Griffioen AW, van Oosterom A, van den Bogaert W, Lambin P. *In vivo* antitumour effect of combretastatin A-4 phosphate added to fractionated radiation. Anticancer Res 2006; 26:307–10.

158. 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.

159. Horsman MR, Murata R, Overgaard J. Combination studies with combretastatin and radiation: effects in early and late responding tissues. Radiother Oncol 2002; 64:S50.

160. Browder T, Butterfield CE, Kraling BM, et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 2000;60:1878–86.

161. Klement G, Huang P, Mayer B, et al. Differences in therapeutic indexes of combination metronomic chemotherapy and an anti-VEGFR-2 antibody in multidrug-resistant human breast cancer xenografts. Clin Cancer Res 2002;8:221–32.

162. Zhang L, Yu D, Hicklin DJ, Hannay JAF, Ellis LM, Pollock RE. Combined anti-fetal liver kinase 1 monoclonal antibody and continuous low-dose doxorubicin inhibits angiogenesis and growth of human soft tissue sarcoma xenografts by induction of endothelial cell apoptosis. Cancer Res 2002;62:2034–42.

163. Yap R, Veliceasa D, Emmenegger U, et al. Metronomic low-dose chemotherapy boosts CD95-dependent antiangiogenic effect of the thrombospondin peptide ABT-510: a complementation antiangiogenic strategy. Clin Cancer Res 2005;11:6678–85.

164. Qian CN, Min HQ, Lin HL, Hong MH. Combination of angiogenesis inhibitor TNP-470 with cytotoxic drugs in experimental therapy of nasopharyngeal carcinoma. Ann Otol Rhinol Laryngol 2000;109:641–5.

165. Fujii T, Tachibana M, Dhar DK, et al. Combination therapy with paclitaxel and thalidomide inhibits angiogenesis and growth of human colon cancer xenograft in mice. Anticancer Res 2003;23:2405–12.

166. Zhang X, Galardi E, Duquette M, Lawler J, Parangi S. Antiangiogenic treatment with three thrombospondin-1 type 1 repeats versus gemcitabine in an orthotopic human pancreatic cancer model. Clin Cancer Res 2005; 11:5622–30.

167. Inoue K, Chikazawa M, Fukata S, Yoshikawa C, Shuin T. Docetaxel enhances the therapeutic effect of the angiogenesis inhibitor TNP-470 (AGM-1470) in metastatic human transitional cell carcinoma. Clin Cancer Res 2003;9:886–99.

168. Song S, Wientjes MG, Walsh C, Au JL-S. Nontoxic doses of suramin enhance activity of paclitaxel against lung metastases. Cancer Res 2001;61:6145–50.

169. Song S, Yu B, Wei Y, Wientjes MG, Au JL-S. Low-dose suramin enhanced paclitaxel activity in chemotherapy-naïve and paclitaxel-pretreated human breast xenograft tumors. Clin Cancer Res 2004;10:6058–65.

170. Zhang Y, Song S, Yang F, Au JL-S, Wientjes MG. Nontoxic doses of suramin enhance activity of doxorubicin in prostate tumors. J Pharmacol Exp Ther 2001; 299:426–33.

171. Xin Y, Lyness G, Chen D, Song S, Wientjes MG, Au JL-S. Low dose suramin as a chemosensitizer of bladder cancer to mitomycin C. J Urol 2005;174:322–7.

172. Wu Y, Yang L, Hu B, et al. Synergistic anti-tumor effect of recombinant human endostatin adenovirus combined with gemcitabine. Anticancer Drugs 2005;16: 551–7.

173. Heere-Ress E, Boehm J, Thallinger C, et al. Thalidomide enhances the anti-tumor activity of standard chemotherapy in a human melanoma xenotransplatation model. J Invest Dermatol 2005;125: 201–6.

174. Inoue K, Slaton JW, Davis DW, et al. Treatment of human metastatic transitional cell carcinoma of the bladder in a murine model with the anti-vascular endothelial growth factor receptor monoclonal antibody DC101 and paclitaxel. Clin Cancer Res 2000;6:2635–43.

175. Hu L, Hofmann J, Zaloudek C, Ferrara N, Hamilton T, Jaffe RB. Vascular endothelial growth factor immunoneutralization plus paclitaxel markedly reduces tumor burden and ascites in athymic mouse model of ovarian cancer. Am J Pathol 2002;161:1917–24.

176. Wild R, Dings RPM, Subramanian I, Ramakrishnan S. Carboplatin selectively induces the VEGF stress response in endothelial cells: potentiation of antitumor activity by combination treatment with antibody to VEGF. Int J Cancer 2004;110:343–51.

177. Bocci G, Danesi R, Marangoni G, et al. Antiangiogenic versus cytotoxic therapeutic approaches to human pancreas cancer: an experimental study with a vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor and gemcitabine. Eur J Pharmacol 2004;498: 9–18.

178. Jia L, Zhang M-H, Yuan S-Z, Huang W-G. Anti-angiogenic therapy for human pancreatic carcinoma xenografts in nude mice. World J Gastroenterol 2005;11: 447–50.

179. Fox WD, Higgins B, Maiese KM, et al. Antibody to vascular endothelial growth factor slows growth of an androgen-independent xenograft model of prostate cancer. Clin Cancer Res 2002;8:3226–31.

180. Börgstrom P, Gold DP, Hillan KJ, Ferrara N. Importance of VEGF for breast cancer angiogenesis *in vivo*: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin. Anticancer Res 1999;19:4203–14.

181. Devineni D, Klein-Szanto A, Gallo JM. Uptake of temozolomide in a rat glioma model in the presence and absence of the angiogenesis inhibitor TNP-470. Cancer Res 1996;56:1983–7.

182. Ma J, Pulfer S, Li S, Chu J, Reed K, Gallo JM. Pharmacodynamic-mediated reduction of temozolomide tumor concentrations by the angiogenesis inhibitor TNP-470. Cancer Res 2001;61:5491–8.

183. Teicher BA, Lazo JS, Sartorelli AC. Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. Cancer Res 1981; 41:73–81.

184. Tannock I, Guttman P. Response of Chinese hamster ovary cells to anti-cancer drugs under aerobic and hypoxic conditions. Br J Cancer 1981;43:245–8.

185. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumour pH to the treatment of malignant disease. Radiother Oncol 1984;2:343–66.

186. Grau C, Overgaard J. Effect of cancer chemotherapy on the hypoxic fraction of a solid tumor measured using a local tumor control assay. Radiother Oncol 1988;13: 301–9.

187. Durand RE. The influence of microenvironmental factors on the activity of radiation and drugs. Int J Radiat Oncol Biol Phys 1991;20:253–8.

188. Grau C, Overgaard J. Effect of etoposide, carmustine, vincristine, 5-fluorouracil, or methotrexate on radiobiologically oxic and hypoxic cells in a C3H mouse mammary carcinoma. Cancer Chemother Pharmacol 1992;30:277–80.

189. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.

190. Edwards HS, Bremner JCM, Stratford IJ. Induction of tumour hypoxia by FAA and TNF: interaction with bioreductive drugs. Int J Radiat Biol 1991;60:373–7.

191. Parkins CS, Denekamp J, Chaplin DJ. Enhancement of mitomycin-C cytotoxicity by combination with flavone acetic acid in a murine tumour. Anticancer Res 1993;13:1437–42.

192. Cliffe S, Taylor ML, Rutland M, Baguley BC, Hill RP, Wilson WR. Combining bioreductive drugs (SR 4233 or SN 23862) with the vasoactive agents flavone acetic acid or 5,6-dimethylxanthenone acetic acid. Int J Radiat Oncol Biol Phys 1994;29:373–7.

193. Wilson WR, Denny WA, Pullen SM, et al. Tertiary amine N-oxides as bioreductive drugs: DACA N-oxide, nitracrine N-oxide, and AQ4N. Br J Cancer 1996;74: S43–7.

194. Parkins CS, Chadwick JA, Chaplin DJ. Enhancement of chlorambucil cytotoxicity by combination with flavone acetic acid in a murine tumour. Anticancer Res 1994;14:1603–8.

195. Pruijn FB, van Daalen M, Holford NHG, Wilson WR. Mechanisms of enhancement of the antitumour activity of melphalan by the tumour-blood-flow inhibitor 5,6-dimethylxanthenone-4-acetic acid. Cancer Chemother Pharmacol 1997;39:541–6.

196. 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 Oncol 2002;41:91–7.

197. 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;20:229–34.

198. Siemann DW, Rojiani AM. Antitumor efficacy of conventional anticancer drugs is enhanced by the vascular targeting agent ZD6126. Int J Radiat Oncol Biol Phys 2002;54:1512–7.

199. Morinaga Y, Suga Y, Ehara S, Harada K, Nihei Y, Suzuki M. Combination effect of AC-7700, a novel combretastatin A-4 derivative, and cisplatin against murine and human tumors *in vivo*. Cancer Sci 2003;94: 200–4.

200. Jain RK, Grantham FH, Gullino PM. Blood flow and heat transfer in Walker 256 mammary carcinoma. J Natl Cancer Inst 1979;62:927–33.

201. Patterson J, Strang R. The role of blood flow in hyperthermia. Int J Radiat Oncol Biol Phys 1979;5: 235–41.

202. Overgaard J, Bichel P. The influence of hypoxia and acidity on the hyperthermic response of malignant cells *in vitro*. Radiology 1977;123:511–4.

203. Gerweck LE, Nygaard TG, Burlett M. Response of cells to hyperthermia under acute and chronic hypoxic conditions. Cancer Res 1979;39:966–72.

204. Thrall DE, Gillette EL, Dewey WC. Effect of heat and ionizing radiation on normal and neoplastic tissue of the C3H mouse. Radiat Res 1975;63:363–77.

205. Hill SA, Denekamp J. The effect of vascular occlusion on the thermal sensitization of a mouse tumor. Br J Radiol 1978;51:997–1002.

206. Horsman MR, Christensen KL, Overgaard J. Hydralazine-induced enhancement of hyperthermic damage in a C3H mammary carcinoma *in vivo*. Int J Hyperthermia 1989;5:123–36.

207. Prescott DM, Samulski TV, Dewhirst MW, et al. Use of nitroprusside to increase tissue temperature during local hyperthermia in normal and tumor-bearing dogs. Int J Radiat Oncol Biol Phys 1992;23:377–85.

208. Urano M, Montoya V, Booth A. Effect of hyperglycemia on the thermal response of murine normal and tumor tissue. Cancer Res 1983;43:453–5.

209. Eikesdal HP, Bjorkhaug ST, Dahl O. Hyperthermia exhibits anti-vascular activity in the BT_4An rat glioma: lack of interaction with the angiogenesis inhibitor batimastat. Int J Hyperthermia 2002;18:141–52.

210. Yano T, Tanase M, Watanabe A, et al. Enhancement effect of an anti-angiogenic agent, TNP-470, on hyperthermia-induced growth suppression of human esophageal and gastric cancers transplantable to nude mice. Anticancer Res 1995;15:1355–8.

211. Nishimura Y, Murata R, Hiraoka M. Combined effects of an angiogenesis inhibitor (TNP-470) and hyperthermia. Br J Cancer 1996;73:270–4.

212. Griffin RJ, Monzen H, Williams BW, Park H, Lee SH, Song CW. Arsenic trioxide induces selective tumour vascular damage via oxidative stress and increases thermosensitivity of tumours. Int J Hyperthermia 2003; 19:575–89.

213. Horsman MR, Murata R, Overgaard J. Improving local tumor control by combining vascular targeting drugs, mild hyperthermia, and radiation. Acta Oncol 2001;40:497–503.

214. Eikesdal HP, Schem BC, Mella O, Dahl O. The new tubulin inhibitor combretastatin A-4 enhances thermal damage in the BT_4An rat glioma. Int J Radiat Oncol Biol Phys 2000;46:645–52.

215. Murata R, Overgaard J, Horsman MR. Combretastatin A-4 disodium phosphate: a vascular targeting agent that improves the anti-tumor effects of hyperthermia, radiation, and mild thermoradiotherapy. Int J Radiat Oncol Biol Phys 2001;51:1018–24.

216. Overgaard J. Rationale and problems in the design of clinical trials. In Overgaard J, editor. Hyperthermic oncology, vol. 2. London: Taylor and Francis; 1985. p.325–38.

217. Horsman MR, Overgaard J. Overcoming tumour radioresistance resulting from hypoxia. In: Steel GG, editor. Basic clinical radiobiology for radiation oncologists. 3rd ed. London: Edward Arnold; 2002. p.169–81.

218. Murata R, Horsman MR. Tumour-specific enhancement of thermoradiotherapy at mild temperatures by the vascular targeting agent 5,6-dimethylxanthenone-4-acetic acid. Int J Hyperthermia 2004;20:393–404.

219. Henderson BW, Miller AC. Effects of scavengers of reactive oxygen and radical species on cell survival following photodynamic treatment *in vitro*: comparison to ionizing radiation. Radiat Res 1986;108:196–205.

220. Dimitroff CJ, Klohs W, Sharma A, et al. Anti-angiogenic activity of selected receptor tyrosine kinase inhibitors, PD1666285 and PD173074: implications for combination treatment with photodynamic therapy. Invest New Drugs 1999;17:121–5.

221. Zhou Q, Olivo M, Lye KYK, Moore S, Sharma A, Chowbay B. Enhancing the therapeutic responsiveness of photodynamic therapy with the antiangiogenic agents SU5416 and SU6668 in murine nasopharyngeal carcinoma models. Cancer Chemother Pharmacol 2005; 56:569–77.

222. Pedley RB, Boden JA, Boden R, et al. Ablation of colorectal xenografts with combined radioimmunotherapy and tumor blood flow-modifying agents. Cancer Res 1996;56:3293–300.

223. Pedley RB, Hill SA, Boxer GM, et al. Eradication of colorectal xenografts by combined radioimmunotherapy and combretastatin A-4 3-*O*-phosphate. Cancer Res 2001;61:4716–22.

224. Pedley RB, Sharma SK, Boxer GM, et al. Enhancement of antibody-directed enzyme prodrug therapy in colorectal xenografts by an antivascular agent. Cancer Res 1999;59:3998–4003.

225. Ahn G-O, Brown JM. Vasculature-disrupting strategies combined with bacterial spores targeting hypoxic regions of solid tumors. In: Siemann DW, editor. Vascular-targeted therapies in oncology. Chichester: John Wiley & Sons, Ltd.; 2006. p.261–76.

226. Sersa G, Willingham V, Milas L. Anti-tumor effects of tumor necrosis factor alone or combined with radiotherapy. Int J Cancer 1988;42:129–34.

227. Nishiguchi I, Willingham V, Milas L. Tumor necrosis factor as an adjunct to fractionated radiotherapy in the treatment of murine tumors. Int J Radiat Oncol Biol Phys 1990;18:555–8.

228. Mauceri HJ, Hanna NN, Wayne JD, Hallahan DE, Hellman S, Weichselbaum RR. Tumor necrosis factor α (TNF-α) gene therapy targeted by ionizing radiation selectively damages tumor vasculature. Cancer Res 1996; 56:4311–4.

229. Chun YJ, Park IC, Park MJ, et al. Enhancement of radiation response in human cervical cancer cells *in vitro* and *in vivo* by arsenic trioxide (As_2O_3). FEBS Lett 2002;519:195–200.

230. Lew YS, Kolozsvary A, Brown SL, Kim JH. Synergistic interaction with arsenic trioxide and fractionated radiation in locally advanced murine tumor. Cancer Res 2002;62:4202–5.

231. Landuyt W, Ahmed B, Nuyts S, et al. *In vivo* antitumor effect of vascular targeting combined with ionising radiation or anti-angiogenesis treatment. Int J Radiat Oncol Biol Phys 2001;49:443–50.

232. Raben D, Bianco C, Damiano V, et al. Antitumor activity of ZD6126, a novel vascular-targeting agent, is enhanced when combined with ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor, and potentiates the effects of radiation in a human non-small cell lung cancer xenograft model. Mol Cancer Ther 2004;3:977–83.

233. Shi W, Horsman MR, Siemann DW. Combined modality approaches using vasculature-disrupting agents. In: Siemann DW, editor. Vascular-targeted therapies in oncology. Chichester: John Wiley & Sons, Ltd.; 2006. p.123–36.

234. Teicher BA, Holden SA, Dupuis NP, et al. Potentiation of cytotoxic therapies by TNP-470 and minocycline in mice bearing EMT-6 mammary carcinoma. Breast Cancer Res Treat 1995;36:227–36.

235. Kato T, Sato K, Kakinuma H, Matsuda Y. Enhanced suppression of tumor growth by combination of angiogenesis inhibitor *O*-(chloroacetyl-carbamoyl)fumagillol (TNP-470) and cytotoxic agents in mice. Cancer Res 1994;54:5143–7.

236. Morishita T, Mii Y, Miyauchi Y, et al. Efficacy of CDDP and AGM-1470 chemotherapy against lung metastasis in rat osteosarcoma depends on the timing of combined administration. Jpn J Clin Oncol 1997;27: 236–9.

237. Shishido T, Yasoshima T, Denno R, Muyaiya M, Sato N, Hirata K. Inhibition of liver metastasis of pancreatic carcinoma by angiogenesis inhibitor TNP-470 in combination with cisplatin. Jpn J Cancer Res 1998;89:963–9.

238. Morishita T, Miyauchi Y, Mii Y, et al. Delay in administration of CDDP until completion of AGM-1740 treatment enhances antimetastatic and antitumor effects. Clin Exp Metastasis 1999;17:15–8.

239. Teicher BA, Holden SA, Ara G, Korbut T, Menon K. Comparison of several antiangiogenic regimens alone and with cytotoxic therapies in the Lewis lung carcinoma. Cancer Chemother Pharmacol 1996;38: 169–77.

240. Kakeji Y, Teicher BA. Preclinical studies of the combination of angiogenic inhibitors with cytotoxic agents. Invest New Drugs 1997;15:39–48.

241. Ma G, Masuzawa M, Hamada Y, et al. Treatment of murine angiosarcoma with etoposide, TNP-470, and prednisolone. J Dermatol Sci 2000;24:126–33.

242. Muramaki M, Miyake H, Hara I, Kawabata G, Kamidono S. Synergistic inhibition of tumor growth and metastasis by combined treatment with TNP-470 and gemcitabine in a human bladder cancer KOTCC-1 model. J Urol 2004;172:1485–9.

243. Osswald H, Youssef M. Suramin enhancement of the chemotherapeutic actions of cyclophosphamide or adriamycin of intramuscularly-implanted Ehrlich carcinoma. Cancer Lett 1979;6:337–43.

244. Kikuchi Y, Hirata J, Hisano A, Tode T, Kita T, Nagata I. Complete inhibition of human ovarian cancer xenografts in nude mice by suramin and *cis*-diammine-dichloroplatinum(II). Gynecol Oncol 1995;58:11–5.

245. Mauceri HJ, Seetharam S, Bechett MA, et al. Angiostatin potentiates cyclophosphamide treatment of metastatic disease. Cancer Chemother Pharmacol 2002;50:412–8.

246. te Velde EA, Vogten JM, Gebbink MFGB, van Gorp JM, Voest EE, Borel Rinkes IHM. Enhanced antitumour efficacy by combining conventional chemotherapy with angiostatin or endostatin in a liver metastasis model. Br J Surg 2002;89:1302–9.

247. Bertolini F, Fusetti L, Mancuso P, et al. Endostatin, an antiangiogenic drug, induces tumor stabilization after chemotherapy or anti-CD20 therapy in a NOD/SCID mouse model of human high-grade non-Hodgkin lymphoma. Blood 2000;96:282–7.

248. Abraham D, Abri S, Hofmann M, Höltl W, Aharinejad S. Low dose carboplatin combined with angiostatic agents prevents metastasis in human testicular germ cell tumor xenografts. J Urol 2003;170: 1388–93.

249. Subramanian IV, Nguyen TMB, Truskinovsky AM, Tolar J, Blazar BR, Ramakrishnan S. Adeno-associated virus-mediated delivery of a mutant endostatin in combination with carboplatin treatment inhibits orthotopic growth of ovarian cancer and improves long-term survival. Cancer Res 2006;66:4319–28.

250. Ding Q, Kestell P, Baguley BC, et al. Potentiation of the antitumour effect of cyclophosphamide in mice by thalidomide. Cancer Chemother Pharmacol 2002;50: 186–92.

251. Dings RPM, Yokoyama Y, Ramakrishnan S, Griffioen AW, Mayo KH. The designed angiostatic peptide anginex synergistically improves chemotherapy and antiangiogenesis therapy with angiostatin. Cancer Res 2003;63: 382–5.

252. Allegrini G, Goulette FA, Darnowski JW, Calabresi P. Thrombospondin-1 plus irinotecan: a novel anti-angiogenic-chemotherapeutic combination that inhibits the growth of advanced human colon tumor xenografts in mice. Cancer Chemother Pharmacol 2004;53:261–6.

253. Soffer SZ, Moore JT, Kim E, et al. Combination antiangiogenic therapy: increased efficacy in a murine model of Wilms tumor. J Pediatr Surg 2001;36:1177–81.

254. Wildiers H, Guetens G, De Boeck G, et al. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. Br J Cancer 2003;88: 1979–86.

255. Garofolo A, Naumova E, Manenti L, et al. The combination of the tyrosine kinase receptor inhibitor SU6668 with paclitaxel affects ascites formation and

tumor spread in ovarian carcinoma xenografts growing orthotopically. Clin Cancer Res 2003;9:3476–85.
256. D'Alessandro N, Borsellino N. *In vivo* effects of tumor necrosis factor-α or flavone acetic acid in combination with doxorubicin on multidrug-resistant B16 melanoma. Anticancer Drugs 1996;7:281–7.
257. Siim BG, Lee AE, Shalal-Zwain S, Pruijn FB, McKeage MJ, Wilson WR. Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Cancer Chemother Pharmacol 2003; 51:43–52.
258. Horsman MR, Murata R, Breidahl T, et al. Combretastatins novel vascular targeting drugs for improving anti-cancer therapy. Adv Exp Med Biol 2000;476:311–23.
259. 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;40:284–90.
260. Goto H, Yano S, Matsumori Y, Ogawa H, Blakey DC, Sone S. Sensitization of tumor-associated endothelial cell apoptosis by the novel vascular-targeting agent ZD6126 in combination with cisplatin. Clin Cancer Res 2004;10:7671–6.
261. Shnyder SD, Cooper PA, Pettit GR, Lippert JW III, Bibby MC. Combretastatin A-1 phosphate potentiates the antitumour activity of cisplatin in a murine adenocarcinoma model. Anticancer Res 2003; 23:1619–24.