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

Michael R. Horsman and Dietmar W. Siemann

1Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark and 2Department of Radiation Oncology, University of Florida, Gainesville, Florida

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 neovascularature 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 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 neovascularature 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.

(Am J Pathol 2006; 169(4): 1485-95)

Vascular-Targeting Approaches

Although AIs and VDs both target the tumor vascular supply, they are two distinct approaches (15). AIs 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

doi:10.1158/0008-5472.CAN-06-2848

Requests for reprints: Michael R. Horsman, Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus C, Denmark. Phone: 45-8949822; Fax: 45-86197109; E-mail: mike@oncology.dk.

©2006 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-06-2848

Cancer Res 2006; 66: (24). December 15, 2006 11520 www.aacrjournals.org
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-dimethylxanthone-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 prodru of N-acetyl-colchinol (ZD6126), AVE8062, NPI2358, MN-029, and OX14503 (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 mm³ 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.
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 AIA 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 AIs have reported a decrease in tumor oxygenation status, measured using a variety of conventional endpoints, 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 MCAa55 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 AIs 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 AIs 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 AIs 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 AIs, 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 AIs can lead to tumor cell killing (47, 73). However, another
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 pathophysioloogy 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 and its

---

**Table 1. Effect of VDAs on tumor oxygenation status**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Tumor type</th>
<th>Assay*</th>
<th>Oxygenation ?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suramin</td>
<td>DLD-2 human colon</td>
<td>Radiation (SF)</td>
<td>Decreased</td>
<td>(58)</td>
</tr>
<tr>
<td>TNP-470</td>
<td>C3H mammary carcinoma</td>
<td>Radiation (TC)</td>
<td>Decreased</td>
<td>(59)</td>
</tr>
<tr>
<td>DC101</td>
<td>WAC2 neuroblastoma</td>
<td>Eppendorf (pO2)</td>
<td>Decreased</td>
<td>(47)</td>
</tr>
<tr>
<td>DC101</td>
<td>MCA4 mammary carcinoma</td>
<td>Hypoxic marker (EF5)</td>
<td>Decreased</td>
<td>(57)</td>
</tr>
<tr>
<td>DC101</td>
<td>MCA35 mammary carcinoma</td>
<td>Hypoxic marker (EF5)</td>
<td>Decreased</td>
<td>(57)</td>
</tr>
<tr>
<td>Anginex</td>
<td>SCK mammary carcinoma</td>
<td>Eppendorf (pO2)/perfusion (Rb)</td>
<td>Decreased</td>
<td>(52)</td>
</tr>
<tr>
<td>SU6668</td>
<td>FSAI + SCK + CFAC tumors</td>
<td>Perfusion (Rb)</td>
<td>&quot;Decreased&quot;</td>
<td>(60)</td>
</tr>
<tr>
<td>ZD6474</td>
<td>Calu 6 NSCLC</td>
<td>Perfusion (H33342)</td>
<td>&quot;Decreased&quot;</td>
<td>(61)</td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>LS1474 colon adenocarcinoma</td>
<td>Eppendorf (pO2)</td>
<td>No change</td>
<td>(46)</td>
</tr>
<tr>
<td>DC101</td>
<td>5A SCLC + U87 glioblastoma</td>
<td>Eppendorf (pO2)/radiation (TG)</td>
<td>No change</td>
<td>(62)</td>
</tr>
<tr>
<td>Arginine deiminase</td>
<td>WAC2 neuroblastoma</td>
<td>Eppendorf (pO2)</td>
<td>No change</td>
<td>(47)</td>
</tr>
<tr>
<td>Endostatin</td>
<td>MCA35 mammary carcinoma</td>
<td>Hypoxic marker (EF5)</td>
<td>No change</td>
<td>(55)</td>
</tr>
<tr>
<td>SU5416</td>
<td>E106 human glioblastoma</td>
<td>Hypoxic marker (PIMO)</td>
<td>No change</td>
<td>(49)</td>
</tr>
<tr>
<td>TNP-470</td>
<td>9L rat gliosarcoma</td>
<td>Eppendorf (pO2)</td>
<td>Increased</td>
<td>(63)</td>
</tr>
<tr>
<td>Suramin</td>
<td>E106 human glioblastoma</td>
<td>Hypoxic marker (PIMO)</td>
<td>Increased</td>
<td>(56)</td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>U87 glioblastoma</td>
<td>Eppendorf (pO2)</td>
<td>Increased</td>
<td>(46)</td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>Shionogi mammary carcinoma</td>
<td>Phosphorous quenching</td>
<td>Increased</td>
<td>(50)</td>
</tr>
<tr>
<td>Endostatin</td>
<td>MCA4 mammary carcinoma</td>
<td>Hypoxic marker (EF5)</td>
<td>Increased</td>
<td>(55)</td>
</tr>
<tr>
<td>DC101</td>
<td>U87 glioma</td>
<td>Hypoxic marker (PIMO)</td>
<td>Increased</td>
<td>(65)</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>FSAl fibrosarcoma</td>
<td>EPR oximetry</td>
<td>Increased</td>
<td>(66)</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>TLT mouse liver tumor</td>
<td>EPR oximetry</td>
<td>Increased</td>
<td>(67)</td>
</tr>
</tbody>
</table>

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).

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 mm³ 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 pO₂ values < 5 mmHg as measured with an Eppendorf electrode), or tumor pH (estimated from 31P 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) rather than preceded the VDA-induced decrease in tumor blood perfusion. One study actually reported no effect of CA4P on IFP in a B16.AAn 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. $^1$Tumor pH is another pathophysiological 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

$^1$Salmon and Siemann, unpublished observation.
Table 2. Preclinical tumor studies combining AIAs with radiation

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Tumor type</th>
<th>Radiation schedule</th>
<th>AIA treatment*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suramin</td>
<td>DLD-2 human colon carcinoma</td>
<td>1 × 5–25 Gy, day 6 ³</td>
<td>50 mg/kg/d, i.p., days 0–6 ³</td>
<td>(58)</td>
</tr>
<tr>
<td>TNP-470</td>
<td>Lewis lung carcinoma</td>
<td>5 × 3 Gy, days 7–11 ¢</td>
<td>30 mg/kg/d, s.c., days 4, 6, 8, 10, 12, 14, 16, and 18 ¢</td>
<td>(73)</td>
</tr>
<tr>
<td>C3H mammary carcinoma</td>
<td>5 × 10–30 Gy, days 0–4, or 10 × 3.65–14.5 Gy, days 0–4 and 7–11 ¢</td>
<td>100 mg/kg/d, s.c., days 0 + 3; 7 + 10; 0, 3, 7, and 10; 0, 4, 7, and 10; 0, 7, 10, 14, and 17 ¢</td>
<td>(59)</td>
<td></td>
</tr>
<tr>
<td>U87 human glioblastoma</td>
<td>1 × 10 Gy, day 15 ¢</td>
<td>6.7 mg/kg/d, s.c., days 8–15 ¢</td>
<td>(53)</td>
<td></td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Lewis lung carcinoma</td>
<td>2 × 20 Gy, days 0 + 1 ¢</td>
<td>25 mg/kg/d, i.p., days 0 + 1 or 0 –3 ³</td>
<td>(128, 129)</td>
</tr>
<tr>
<td>Lewis lung carcinoma</td>
<td>2 × 20 Gy, days 0 + 1 ¢</td>
<td>25 mg/kg/d, i.p., days 0–14 ¢</td>
<td>(137)</td>
<td></td>
</tr>
<tr>
<td>D54 human glioblastoma</td>
<td>6 × 5 Gy, daily ¢</td>
<td>6 × 25 mg/kg, i.p., with radiation °</td>
<td>(137)</td>
<td></td>
</tr>
<tr>
<td>SQ-20B squamous cell carcinoma</td>
<td>10 × 5 Gy, daily ¢</td>
<td>10 × 25 mg/kg, i.p., with radiation °</td>
<td>(137)</td>
<td></td>
</tr>
<tr>
<td>PC3 prostate adenocarcinoma</td>
<td>8 × 5 Gy, daily ¢</td>
<td>8 × 25 mg/kg, i.p., with radiation °</td>
<td>(137)</td>
<td></td>
</tr>
<tr>
<td>Rat C6 glioma</td>
<td>3 × 7.5 Gy, days 0, 2, and 4 ¦</td>
<td>AdK3, i.t., days 1, 3, and 5 ¦</td>
<td>(130)</td>
<td></td>
</tr>
<tr>
<td>SCK mammary carcinoma</td>
<td>2 × 10 Gy, days 0 + 1 ¢</td>
<td>25 or 50 mg/kg/d, i.p., days 1–0, and 1 °</td>
<td>(52)</td>
<td></td>
</tr>
<tr>
<td>Endostatin</td>
<td>SQ-20B squamous cell carcinoma</td>
<td>10 × 5 Gy, days 0–3, 7–10, and 14 + 15 ¢</td>
<td>2.5 mg/kg/d, i.p., days 0–3, 7–10, and 14 + 15 ¢</td>
<td>(131)</td>
</tr>
<tr>
<td>Lewis lung carcinoma</td>
<td>3 × 15 Gy, days 1–3 ¦</td>
<td>2.5 mg/kg/d, i.p., days 0–3 ¦</td>
<td>(131)</td>
<td></td>
</tr>
<tr>
<td>HT29 colorectal carcinoma</td>
<td>1 × 10 Gy, days 0 ¦</td>
<td>rAAV, i.m., 6 wk before tumor implant ¦</td>
<td>(51)</td>
<td></td>
</tr>
<tr>
<td>Arginine deiminase</td>
<td>1 × 6 or 12 Gy, day 0 ¦</td>
<td>5 mg/kg, i.p., days 0–14 ¦</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Thrombospondin</td>
<td>D-12 human melanoma</td>
<td>1 × 10 Gy, day 14 ¦</td>
<td>50 μg, i.p., days 13 + 14 or 3 ×/wk starting day 15 ¦</td>
<td>(64)</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>FSaII fibrosarcoma</td>
<td>1 × 20 Gy, on days 0, 2, and 4 ¦</td>
<td>200 mg/kg/d, i.p., days 0, 0 + 1, 0–3, or 1 + 2 °</td>
<td>(66)</td>
</tr>
<tr>
<td>Anginex</td>
<td>MA 148 ovarian carcinoma</td>
<td>4 × 5 Gy, days 2, 9, 16, and 23 °</td>
<td>10 mg/kg/d for 28 days, s.c. pump °, 7 ×</td>
<td>(52)</td>
</tr>
<tr>
<td>SCK mammary carcinoma</td>
<td>1 × 10 Gy, days 0 + 1 ¢, or 1 × 25 Gy, day 2 ¢</td>
<td>20 mg/kg, i.p., days 1, 0, and 1 °</td>
<td>(52)</td>
<td></td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>Lewis lung carcinoma</td>
<td>2 × 20 Gy, days 0 + 1 ¢</td>
<td>10 μg/d, i.p., days 0 + 1 °</td>
<td>(132)</td>
</tr>
<tr>
<td>SQ-20B squamous cell carcinoma</td>
<td>4 × 10 Gy, days 0–3 ¦</td>
<td>10 μg/d, i.p., days 0–3 °</td>
<td>(132)</td>
<td></td>
</tr>
<tr>
<td>Seg-1 esophageal adenocarcinoma</td>
<td>4 × 5 Gy, days 0–3 ¦</td>
<td>10 μg/d, i.p., days 0–3 ¦</td>
<td>(132)</td>
<td></td>
</tr>
<tr>
<td>U87 glioblastoma</td>
<td>8 × 5 Gy, days 0, 1, 4, 5, 7, 8, 11, and 12 ¦</td>
<td>10 μg/d, i.p., days 0, 1, 4, 5, 7, 8, 11, and 12 ¦</td>
<td>(132)</td>
<td></td>
</tr>
<tr>
<td>U87 glioblastoma</td>
<td>1 × 20–30 Gy, day 11 ¦</td>
<td>100 μg/d, i.p., days 0, 2, 4, 6, 8, and 10 °</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>LS1747 colon adenocarcinoma</td>
<td>1 × 20–30 Gy, day 11 ¦</td>
<td>100 μg/d, i.p., days 0, 2, 4, 6, 8, and 10 °</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>Seg-1 esophageal adenocarcinoma</td>
<td>4 × 5 Gy, days 0–3 °</td>
<td>5 or 25 mg/kg/d, i.p., days 0–3 °</td>
<td>(133)</td>
<td></td>
</tr>
<tr>
<td>U87 glioblastoma</td>
<td>8 × 5 Gy, days 0–3 and 7–10 ¦</td>
<td>5 or 25 mg/kg/d, i.p., days 0–3 ¦</td>
<td>(133)</td>
<td></td>
</tr>
<tr>
<td>DC101</td>
<td>54A small cell lung cancer</td>
<td>5 × 5–24 Gy, days 1–5 ³</td>
<td>20 or 40 mg/kg/d, i.p., days 0, 3, 6, 9, 12, and 15 °</td>
<td>(62)</td>
</tr>
<tr>
<td>U87 glioblastoma</td>
<td>5 × 5–24 Gy, days 1–5 ³</td>
<td>20 or 40 mg/kg/d, i.p., days 0, 3, 6, 9, 12, and 15 °</td>
<td>(62)</td>
<td></td>
</tr>
<tr>
<td>WAC2 neuroblastoma</td>
<td>1 × 6 or 12 Gy, day 0 °</td>
<td>6.5 mg/kg/d, i.p., days 0–4 °</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>U87 glioma</td>
<td>3 × 7 Gy, days −9 to −7, −2 to 0, 1–3, 4–6, or 7–9 °</td>
<td>40 mg/kg/d, i.p., days 0, 3, and 6 °</td>
<td>(65)</td>
<td></td>
</tr>
<tr>
<td>MCA4 mammary carcinoma</td>
<td>5 × 6 Gy, days 4–8 °</td>
<td>45 mg/kg, i.p., every 3 d starting day 4 °</td>
<td>(57)</td>
<td></td>
</tr>
<tr>
<td>MCA35 mammary carcinoma</td>
<td>5 × 6 Gy, days 4–8 °</td>
<td>45 mg/kg, i.p., every 3 d starting day 4 °</td>
<td>(57)</td>
<td></td>
</tr>
<tr>
<td>SU5416</td>
<td>GL261 murine glioblastoma</td>
<td>8 × 3 Gy, days 0–3 ³, ⁴, ⁵, ⁶</td>
<td>0.7 mg/d, i.p., days 0, 4, 7, and 11 °</td>
<td>(138)</td>
</tr>
<tr>
<td>SCCVII murine carcinoma</td>
<td>5 × 2 Gy, days 0–4 ³</td>
<td>25 mg/kg, i.p., daily from day 0 ³</td>
<td>(139)</td>
<td></td>
</tr>
<tr>
<td>WAC2 neuroblastoma</td>
<td>1 × 6 Gy, day 0 °</td>
<td>25 mg/kg/d, i.p., days 0–4 °</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>E106 glioblastoma</td>
<td>1 × 10 or 20 Gy, day 1 ³</td>
<td>75 mg/kg/d, i.p., days 0–13 ³</td>
<td>(49)</td>
<td></td>
</tr>
<tr>
<td>SU/6668</td>
<td>SCK mammary carcinoma</td>
<td>1 × 15 Gy, day 1 ³</td>
<td>100 mg/kg/d, i.p., days 0 + 1 °</td>
<td>(60)</td>
</tr>
<tr>
<td>SCCVII murine carcinoma</td>
<td>5 × 2 Gy, days 0–4 ³</td>
<td>75 mg/kg, p.o., daily from day 0 °</td>
<td>(139)</td>
<td></td>
</tr>
<tr>
<td>Lewis lung carcinoma</td>
<td>7 × 3 Gy, days 0–6 °</td>
<td>1.5 mg/kg/d, i.p., days 0, 2, 4, and 6 °</td>
<td>(134)</td>
<td></td>
</tr>
<tr>
<td>GL261 murine carcinoma</td>
<td>7 × 3 Gy, days 0–6 °</td>
<td>1.5 mg/kg/d, i.p., days 0, 2, 4, and 6 °</td>
<td>(134)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
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 models and involved both single-dose radiation/drug 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

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

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

*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 $5 \times 10^9$ plaque–forming units (pfu).

|| Endostatin was produced by an endostatin–expressing adenovirus, given as an i.m. injection of $1 \times 10^9$ viral vectors.

*AIAs given continuously from a s.c. implanted osmotic pump.

†Radiation given twice daily.
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

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

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.L, 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 AIAAs and chemotherapeutic agents

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

(Continued on the following page)
<table>
<thead>
<tr>
<th>AIA</th>
<th>Chemotherapy</th>
<th>Tumor type</th>
<th>Treatment schedule*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>Human PC3 prostate</td>
<td>carcinoma</td>
<td>AIA (10 mg/kg, i.v., 2×/wk for 3 wk drug) (5 mg/kg, i.v., as with the AIA)</td>
<td>(170)</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>RT4 bladder tumors</td>
<td></td>
<td>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)</td>
<td>(171)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Cyclophosphamide</td>
<td>Lewis lung carcinoma</td>
<td>AIA (5 mg/kg, i.p., days 3–9 drug (75 mg/kg, i.p., days 4–7)</td>
<td>(245)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>C26 colon carcinoma</td>
<td></td>
<td>AIA (10–100 mg/kg, daily from days 0 to 12)</td>
<td>(246)</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Cyclophosphamide</td>
<td>Non–Hodgkin's lymphoma</td>
<td>AIA (50 µg, s.c., days 15–19 + 25–29) drug (75 mg/kg, i.p., days 3, 5, and 7)</td>
<td>(247)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>C26 colon carcinoma</td>
<td></td>
<td>AIA (500 µg/d, s.c., days 0–12) drug (10 mg/kg, i.v., day 3)</td>
<td>(246)</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Human testicular tumor</td>
<td></td>
<td>AIA (10 mg/kg/d from days 15–28) drug (30 mg/kg, i.p., days 14 + 21)</td>
<td>(248)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Cyclophosphamide</td>
<td>Colon 38 tumors</td>
<td>AIA (1–100 mg/kg, i.p., on day 0) drug (220 mg/kg, i.p., on day 0)</td>
<td>(250)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>MA148 human epithelial carcinoma</td>
<td></td>
<td>AIA (10 mg/kg, days 7–35) drug (32.5 mg/kg, i.p., every 3 d from 7–35)</td>
<td>(251)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Carboplatin</td>
<td>Human testicular tumor</td>
<td>AIA (20 mg/kg/d from days 15–28) drug (125 or 150 mg/kg/i.p., 1×/wk for 4 wk)</td>
<td>(252)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>MA148 human epithelial carcinoma</td>
<td></td>
<td>AIA (10 mg/kg, days 7–35) drug (32.5 mg/kg, i.p., every 3 d from 7–35)</td>
<td>(253)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Carboplatin</td>
<td>Human testicular tumor</td>
<td>AIA (20 mg/kg/d from days 15–28) drug (125 or 150 mg/kg/i.p., 1×/wk for 4 wk)</td>
<td>(254)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Carboplatin</td>
<td>Human testicular tumor</td>
<td>AIA (20 mg/kg/d from days 15–28) drug (125 or 150 mg/kg/i.p., 1×/wk for 4 wk)</td>
<td>(255)</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>AsPC-1 pancreatic cancer</td>
<td></td>
<td>AIA (3 mg/kg, i.p., days 7–28) drug (150 mg/kg/i.p., 2×/wk between days 7 and 28)</td>
<td>(245)</td>
</tr>
<tr>
<td>DC101</td>
<td>Paclitaxel</td>
<td>253J B-V human bladder</td>
<td>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)</td>
<td>(246)</td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>Dovorubicin</td>
<td>MCF-7 spheroids</td>
<td>AIA (200 µg, i.p., 2×/wk (5 mg/kg, i.v., 1x/wk)</td>
<td>(247)</td>
</tr>
<tr>
<td>Topotecan</td>
<td>SK-NEP-1 Wilms' tumor</td>
<td></td>
<td>AIA (100 µg, i.p., 2×/wk for 5 wk drug (30 mg/kg, i.p., days 1–23) drug (30 mg/kg, i.p., days 12–16)</td>
<td>(248)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>CWR22R prostate</td>
<td></td>
<td>AIA (5 mg/kg, i.v., 2×/wk for 4 wk drug (6.25 mg/kg, s.c., 5×/wk for 3 wk)</td>
<td>(249)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>OVCAR3 tumors</td>
<td></td>
<td>AIA (5 µg, i.p., 2×/wk for 6 wk drug (20 µg, i.p., 2× to 3×/wk for 6 wk)</td>
<td>(250)</td>
</tr>
<tr>
<td>CPT-11</td>
<td>HT29 colon cancer</td>
<td></td>
<td>AIA (200 µg, i.p., days 0 + 4) drug (100 mg/kg, i.p., day 7)</td>
<td>(251)</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>MA148 human epithelial carcinoma</td>
<td></td>
<td>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)</td>
<td>(252)</td>
</tr>
<tr>
<td>SU6668</td>
<td>Paclitaxel</td>
<td>HOC79 human ovarian</td>
<td>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 1 drug (120 mg/kg, i.p., days 1, 4, 7, and 11)</td>
<td>(253)</td>
</tr>
<tr>
<td>SU5416</td>
<td>Gemcitabine</td>
<td>MIA PaCa-2 pancreatic tumor</td>
<td>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)</td>
<td>(254)</td>
</tr>
<tr>
<td>SU11657</td>
<td>Pemetrexed</td>
<td>A431 carcinoma</td>
<td>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)</td>
<td>(255)</td>
</tr>
</tbody>
</table>

*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).  
‡AIAs 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 × 10⁹ viral particles.
<table>
<thead>
<tr>
<th>VDA</th>
<th>Chemotherapy drug</th>
<th>Tumor type</th>
<th>Treatment schedule*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAA</td>
<td>Chlorambucil</td>
<td>CaNT</td>
<td>VDA (150–200 mg/kg, i.p.) up to 1 h before/after drug (15–30 mg/kg, i.p.)</td>
<td>(194)</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>B16 melanoma</td>
<td>VDA (1–2 × 150 mg/kg, i.v.) 1 h after drug (1–2 × 5 mg/kg, i.p.)</td>
<td>(256)</td>
</tr>
<tr>
<td></td>
<td>Vinblastine</td>
<td>CaNT</td>
<td>VDA (150 mg/kg, i.p.) various times before/after drug (5 mg/kg, i.p.)</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C</td>
<td>CaNT</td>
<td>VDA (150–200 mg/kg, i.p.) up to 24 h before/after drug (3–5 mg/kg, i.p.)</td>
<td>(191)</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine</td>
<td>SCCVII</td>
<td>VDA (200 mg/kg, i.p.) various times before/after drug (0.05–0.2 mmol/kg, i.p.)</td>
<td>(114)</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine</td>
<td>KHT, RIF-1</td>
<td>VDA (200 mg/kg, i.p.) 15 min before or after drug (50 mg/kg, i.p.)</td>
<td>(190)</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine</td>
<td>MDAH-MCa4</td>
<td>VDA (700 μmol/kg, i.p.) simultaneously with drug (0–300 μmol/kg, i.p.)</td>
<td>(192)</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine</td>
<td>RSU-1069</td>
<td>VDA (200 mg/kg, i.p.) 15 min after drug (80 mg/kg, i.p.)</td>
<td>(190)</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C</td>
<td>KHT, RIF-1</td>
<td>VDA (200 mg/kg, i.p.) 15 min after drug (5 mg/kg, i.p.)</td>
<td>(190)</td>
</tr>
<tr>
<td>DMXAA</td>
<td>Cisplatin</td>
<td>KHT, SKBR3, OW-1</td>
<td>VDA (5–17.5 mg/kg, i.p.) various times before/after drug (2.5–30 mg/kg, i.p.)</td>
<td>(85)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–421 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Carboplatin</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–316 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>KHT, SKBR3</td>
<td>VDA (17.5 mg/kg, i.p.) 1 h after drug (12.5–75 mg/kg, i.p.)</td>
<td>(85)</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–716 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Melphalan</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) various times before/after drug (34 μmol/kg, i.p.)</td>
<td>(195)</td>
</tr>
<tr>
<td></td>
<td>5-FU</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–1780 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Etoposide</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–75 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Vincristine</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–1.0 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–237 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel</td>
<td>MDAH-MCa4</td>
<td>VDA (60–80 μmol/kg, i.p.) various times before/after drug (0–316 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (0–237 μmol/kg, i.p.)</td>
<td>(257)</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine</td>
<td>MDAH-MCa4</td>
<td>VDA (65–70 μmol/kg, i.p.) various times before/after drug (0–300 μmol/kg, i.p.)</td>
<td>(192)</td>
</tr>
<tr>
<td></td>
<td>SN 23862</td>
<td>MDAH-MCa4</td>
<td>VDA (65 μmol/kg, i.p.) various times before/after drug (200 μmol/kg, i.p.)</td>
<td>(192)</td>
</tr>
<tr>
<td></td>
<td>SN 23816</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (300 μmol/kg, i.p.)</td>
<td>(83)</td>
</tr>
<tr>
<td></td>
<td>AQ4N</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) 1 h before drug (450 μmol/kg, i.p.)</td>
<td>(193)</td>
</tr>
<tr>
<td></td>
<td>CI-1010</td>
<td>MDAH-MCa4</td>
<td>VDA (80 μmol/kg, i.p.) simultaneously with drug (940 μmol/kg, i.p.)</td>
<td>(83)</td>
</tr>
<tr>
<td></td>
<td>CA4P</td>
<td>MDAH-MCa4</td>
<td>VDA (100 mg/kg, i.p.) 15 min or 24 h after drug (5 mg/kg, i.p.)</td>
<td>(153)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>CaNT adenocarcinoma</td>
<td>VDA (250 mg/kg, i.p.) 1 h after drug (2–8 mg/kg, i.p.)</td>
<td>(258)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>C3H mammary carcinoma</td>
<td>VDA (10–100 mg/kg, i.p.) various times before/after drug (2.5–30 mg/kg, i.p.)</td>
<td>(85)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>KHT, SKBR3, OW-1</td>
<td>VDA (100 mg/kg, i.p.) 1 h after drug (5–20 mg/kg, i.p.)</td>
<td>(196)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>Kaposi's sarcoma</td>
<td>VDA (100 mg/kg, i.p.) 1 h after drug (125 mg/kg, i.p.)</td>
<td>(85)</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide</td>
<td>KHT, SKBR3</td>
<td>VDA (100–125 mg/kg, i.p.) 20 min after drug (125 mg/kg, i.p.)</td>
<td>(197)</td>
</tr>
</tbody>
</table>

(Continued on the following page)
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 chemotherapeutic drug 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 VDAs have been combined and enhanced tumor response observed and in which the pathophysiologic effects of VDAs clearly play a significant role in the enhancement. A review of the pathophysiologic effects of VDAs 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 TNP (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).

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

<table>
<thead>
<tr>
<th>VDA</th>
<th>Chemotherapy drug</th>
<th>Tumor type</th>
<th>Treatment schedule</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVE8062</td>
<td>Cisplatin</td>
<td>Murine colon 26, S180, M109</td>
<td>VDA (10–80 mg/kg, i.v.) days 7, 11, and 15 simultaneously with drug (2.5–5 mg/kg, i.v.)</td>
<td>(199)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDA (10–100 mg/kg, i.v.) daily for 5 d starting 24 h after drug (4 mg/kg, i.p.)</td>
<td>(102)</td>
</tr>
<tr>
<td>ZD6126</td>
<td>Cisplatin</td>
<td>KHT, Caki-1</td>
<td>VDA (10–150 mg/kg, i.p.) various times before/after drug (2.5–20 mg/kg, i.p.)</td>
<td>(198)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDA (100 mg/kg, i.p.) at 200 mg/kg (1 h) or 100 mg/kg (days 0–20) after drug (6 mg/kg, i.v.)</td>
<td>(260)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDA (125 mg/kg, i.p.) 15 min after drug (15 mg/kg, i.p.)</td>
<td>(88)</td>
</tr>
<tr>
<td>OXi4503</td>
<td>Paclitaxel</td>
<td>FaDu squamous cell carcinoma</td>
<td>VDA (100 mg/kg, i.p.) 20 min after drug (6 mg/kg, i.p.)</td>
<td>(261)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VDA (100 mg/kg, i.p.) 1 h after drug (2.5–10 mg/kg, i.p.)</td>
<td>(97)</td>
</tr>
</tbody>
</table>

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

Acknowledgments

Received 8/1/2006; accepted 11/6/2006.

Grant support: Danish Cancer Society and the U.S. National Cancer Institute (USPHS grants CA 84408 and CA 89655).

References

12. Kerbel RS, Kamen BA. The anti-angiogenic basis of
39. Cao Z, Joseph WR, Browne WL, et al. Thalidomide-induced antitumor effects are dependent on the timing of the vascular normalization step, the production and anti-tumor activity in response to 5-


152. Stenstrom KW, Vermund H, Mosser DG, Marvin JF.
153. Horsman MR, Overgaard J. The oxygen effect and
138. Abdollahi A, Lipson KE, Han X, et al. SU5416 and
155. Wilson WW, Li AE, Cowan D, Siim BG. Enhance-
154. Fujii T, Tachibana M, Dhar DK, et al. Combination
159. Inoue K, Slaton JW, Davis DW, et al. Treatment of
164. Devineni D, Klein-Szanto A, Gallo JM. Uptake of
temozolomide in prostate tumors. J Pharmacol Exp Ther 2001;
201–6.
194. Parkins CS, Chadwick JA, Chaplin DJ. Enhancement
of melphalan by the tumour-blood-flow inhibitor 5,6-
189. Edwards HS, Bremner JCM, Stratford IJ. Induction
of chlorambucil cytotoxicity in a human melanoma xenograft
172. Inoue K, Slaton JW, Davis DW, et al. Treatment of
treatment with VEGF-neutralizing antibody bevacizumab plus
combination of VEGF-neutralizing antibody bevacizumab and
eradiation therapy for patients with advanced non-small-cell lung
combination of VEGF-neutralizing antibody bevacizumab and
eradiation therapy for patients with advanced non-small-cell lung
combination of VEGF-neutralizing antibody bevacizumab and
eradiation therapy for patients with advanced non-small-cell lung
amic therapies: opportunities and challenges in kidney cancer.


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

Michael R. Horsman and Dietmar W. Siemann


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/24/11520

Cited articles
This article cites 249 articles, 85 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/24/11520.full.html#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
/content/66/24/11520.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.