Design of Clinical Trials of Radiation Combined with Antiangiogenic Therapy

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Key Words. Tumor angiogenesis • Radiotherapy • Antiangiogenic therapy • Hypoxia • Clinical trials

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Discuss the impact of tumor angiogenesis and hypoxia on the outcome of radiation therapy.
2. List the classes of antiangiogenic agents that are in clinical development.
3. Identify mechanisms by which antiangiogenic therapy can enhance radiation efficacy.

ABSTRACT

Clinical trials showing longer survival when chemotherapy is combined with antiangiogenic agents (AAs) have led to growing interest in designing combined modality protocols that exploit abnormalities in tumor vasculature. Approved agents include bevacizumab, a recombinant monoclonal antibody that binds to vascular endothelial growth factor, and two small molecule multitargeted tyrosine kinase inhibitors of angiogenesis (SU11248 and BAY-43–9006) that have been approved for therapy of renal cancer. Targeting tumor vasculature has a strong biological rationale in radiation therapy, and preclinical studies consistently show an increase in radiosensitization with combined treatment. Preclinical studies indicate that excessive damage to tumor vasculature can result in radioresistance in some situations, and early clinical data suggest that treatment sequencing may be important when combining AAs with radiation. Radiation itself appears to antagonize any hypoxia that can be induced by long-term administration of AAs. The optimal biological doses of AAs with radiotherapy are unknown, and surrogate markers of efficacy remain to be validated. Early clinical trials should therefore include studies designed to identify mechanisms of interaction and increases in tumor hypoxia. This review highlights preclinical and early clinical data that are relevant for clinical trial design. Optimal radiation planning and delivery is required to minimize the volume of irradiated normal organs and to establish safe dose-volume parameters for phase II–III clinical trials. The Oncologist 2007;12:465–477

Disclosure of potential conflicts of interest is found at the end of this article.

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TUMOR ANGIOGENESIS

Radiation is an important modality in the treatment of cancer as nearly 50% or more of all cancer patients undergo radiotherapy as part of their treatment [1]. Curative radiotherapy can be applied as a single treatment modality, it can be used prior to surgery to increase the likelihood of a complete surgical excision, or it can be administered after surgery to destroy microscopic cancer cells that were not surgically removed. Radiation-induced cell death is usually attributed to DNA damage to tumor cells or triggered through non-DNA pathways, which in turn induces cell death by apoptosis and/or necrosis. Oxygen is a potent radiosensitizer, and its interaction with radicals formed by radiation induces DNA damage. Hypoxia contributes to radiation resistance, with cells irradiated in air being about three times more radiosensitive than those irradiated under conditions of severe hypoxia [2]. The view that stem cells and clonogens are primary targets for tissue response to radiation has been challenged by the finding that expression of damage is linked to radiation-induced endothelial apoptosis in tissue microvasculature [3].

Tumor angiogenesis is the process leading to the formation of blood vessels within a tumor, and it plays a key role in cancer cell survival, local tumor growth, and the development of distant metastases [4, 5]. The term “angiogenic switch” refers to the transition of a tumor from the “avascular” or “prevascular” phase to the “proangiogenic” phase, with increased growth and metastatic potential. New tumor vessels are often structurally and functionally abnormal, as a result, in part, of tortuous and leaky vasculature, leading to increased interstitial pressure, tissue hypoxia, and further stimulation of angiogenesis. Pericytes are cells that provide support for the endothelial cells (ECs) and their association with tumor ECs is poorly defined. Cytoplasmic projections of pericytes in normal tissue interact with ECs, whereas they can extend deep into tumor tissue [6]. Pericyte recruitment and coverage in human tumor vasculature is often very heterogeneous.

Growth factors and growth inhibitors regulate blood vessel development. In contrast to the quiescent endothelium in normal tissue, tumor ECs proliferate up to 40 times more frequently and preferentially overexpress the cell-surface molecules integrin-α/β, E-selectin, endoglin, endostatin, and vascular endothelial growth factor receptor (VEGFRs), all of which stimulate endothelium adhesion and migration. Most biological effects of VEGF are mediated via two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, but specific VEGF isoforms also bind neuroplins (NP)-1 and NP-2, non–tyrosine kinase receptors originally identified as receptors for semaphorins, which are polypeptides with essential roles in neuronal patterning [7, 8]. VEGF acts as a survival factor for ECs and its withdrawal can cause vascular damage [9]. In addition, VEGF is a vasodilator involved in the synthesis and/or release of nitric oxide [10]. VEGFR-1 and VEGFR-2 are found on vascular ECs and VEGF binding to cell surface receptors stimulates EC proliferation, migration, and survival [11, 12]. Endothelium in normal tissues resides primarily in a resting state with low expression of VEGFR-2, thereby remaining relatively insensitive to VEGFR-2 blockade. Epidermal growth factor receptor (EGFR) signaling is also linked to angiogenic mechanisms [13], and treatment with anti-EGFR agents produces an antitumor effect that is partly a result of inhibition of secretion of various paracrine angiogenic growth factors.

Tumors respond to radiation by secreting cytokines that inhibit apoptosis in ECs, including a major angiogenesis regulator, hypoxia-inducible factor (HIF)-1α [14]. HIF-1α is a transcriptional factor that is activated when mammalian cells experience hypoxia, and it regulates a number of processes, including promoting ATP metabolism, proliferation, and p53 activation [15]. By stimulating EC survival, HIF-1 also promotes tumor radiation resistance and the net effect of HIF-1 blockade on tumor radioresponsiveness is highly dependent on treatment sequencing. Under conditions of hypoxia, HIF-1α dimerizes with HIF-1β, and the complex translocates to the nucleus and binds to the VEGF promoter, leading to increased VEGF transcription [16].

IMPACT OF HYPOXIA ON RADIOTHERAPY OUTCOME

In human tumors, hypoxia is strongly associated with a diminished therapeutic response to radiation and with malignant progression in tumors of the head and neck, uterine cervix, and sarcomas [17–20]. HIF-1α overexpression is associated with increased microvessel density and/or VEGF expression in a large number of tumor types, including non-small cell lung cancer (NSCLC), astrocytoma, and colon and esophageal tumors, and HIF-1α expression levels have correlated with a greater risk for mortality in some tumors [21]. In preclinical studies, primary tumors recurring after inadequate radiation therapy showed increased metastatic propensity, which was associated with an increase in the fraction of hypoxic cells and also with hypoxia-induced upregulation of metastasis-promoting gene products [22]. Possible mechanisms that may account for increased metastasis are hypoxia-induced neoangiogenesis facilitating hematogenous spread and the promotion of lymphangiogenesis that facilitates tumor invasion. In summary, the pivotal role of tumor hypoxia and neoangiogenesis in determining radiation response serves to emphasize...
the clinical potential of combining radiation with antiangiogenic agents (AAs).

**Agents Targeting Tumor Angiogenesis**

Tumors can successfully grow up to 1–2 mm in size without blood vessel formation [4]. Single-agent activity of AA therapy is unlikely to eradicate solid tumors [16]. As such, optimal therapeutic gains for the use of AAs are expected to arise in combination with cytotoxic therapies. VEGF appears to be expressed as the principal proangiogenic factor for early-stage cancers [23], but tumor progression is often accompanied by altered expression of a range of proangiogenic factors [24–26]. Agents directed toward inhibition of tumor angiogenesis and for therapeutic vascular targeting have been classified as the following.

**Antiangiogenic Agents (AAs)**

AAs aim to inhibit the growth of new blood vessels in tumors. Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA) is a humanized monoclonal antibody that acts by binding and neutralizing VEGF, which is a ligand with a central role in signaling pathways controlling tumor blood vessel development and survival [11, 27, 28]. In patients with rectal carcinoma, a single infusion of bevacizumab decreased tumor perfusion, vascular volume, microvascular density, interstitial fluid pressure (IFP), and circulating ECs and progenitor cells [29]. These findings suggest that anti-VEGF therapy has a direct antivascular effect in human tumors. In contrast, small molecule tyrosine kinase inhibitors (TKIs) prevent activation of VEGFRs, thus inhibiting downstream signaling pathways rather than binding to VEGF directly. Serial magnetic resonance imaging (MRI) studies in recurrent human glioblastomas treated with AZD2171, a potent oral, pan-VEGFR TKI with activity against platelet-derived growth factor (PDGF) receptors and c-Kit, revealed that tumor vessel size decreases within 24 hours, a change that persisted for at least 28 days [30]. The vascular changes in that study correlated with reductions in vasogenic brain edema. Many small molecule TKIs also show activity against EGFR or c-Kit, which may produce direct antitumor effects. For example, ZD6474 is an orally available inhibitor of key pathways including VEGFR-dependent tumor angiogenesis and EGFR-dependent tumor cell proliferation and survival.

**Vascular Damaging (or Disrupting) Agents**

Vascular damaging (or disrupting) agents (VDAs) cause a rapid shutdown of the established tumor vasculature, thereby leading to secondary tumor-cell death. Although there are differences between antiangiogenic and antivascular strategies, including their administration schedules, individual agents might show both antiangiogenic and antivascular effects. A VDA that destroys tumor vasculature and improves vascular permeability is the cytokine tumor necrosis factor, which is delivered using isolated limb perfusion in locally advanced melanomas and sarcomas of the limbs [31]. The microtubule-destabilizing agents, including combretastatins and ZD6126, and drugs related to 5,6-dimethylxanthenone-4-acetic acid (DMXAA) are the two main groups of VDAs that are currently in clinical development [32]. The VDAs that are in development produce a much greater blood flow reduction in tumors than in normal tissues, and possible reasons for this include factors such as high vascular permeability, high IFP, and heterogeneous blood-flow rates.

**Mixed Inhibitors**

Mixed inhibitors, including agents such as EGFR inhibitors or neutralizing agents and cytotoxic anticancer agents, target both tumor ECs and malignant cells. Similarly, the combination of AAs with VDAs can result in complementary antitumor effects. Treatment of tumor-bearing mice with VDAs leads to an acute mobilization of circulating endothelial progenitor cells (CEPs), which home to the viable tumor rim that characteristically remains after such therapy [33]. The administration of AAs disrupted the spike in CEPs and resulted in marked reductions in tumor rim size and blood flow as well as enhanced VDA antitumor activity.

A randomized study in patients with recurrent or advanced NSCLC assigned to chemotherapy with or without bevacizumab reported a median survival time of 12.3 months in the chemotherapy plus bevacizumab group, as compared with 10.3 months in the chemotherapy-alone group [34]. The median progression-free survival (PFS) times in the two groups were significantly different at 6.2 and 4.5 months, respectively, as were the corresponding response rates of 35% and 15% (p < .001). Bevacizumab is also registered for use in combination with 5-fluourouracil (5-FU)–based chemotherapy for first-line treatment of patients with metastatic colorectal carcinoma in the U.S. and Europe. A pivotal study in patients receiving bevacizumab in combination with irinotecan, 5-FU, and leucovorin (IFL) provided evidence of the value of antiangiogenic therapy in improving patient survival over chemotherapy alone [35]. The synergistic activity of AAs with chemotherapy is postulated to be a result of the paradox of antiangiogenic therapy, a term that refers to the transient increases in tumor blood flow observed with such agents [5]. Bevacizumab administered every 3 weeks with IFL chemotherapy may have resulted in a fortuitous coincidence with the vascular “nor-
malization window.” The increased blood flow allows for greater drug penetration, and the reduction in hypoxia improves the efficacy of cytotoxic therapies including radiation. The longer survival time with combined chemotherapy and AAs may also be a result of the prevention of rapid tumor cell repopulation after chemotherapy and augmentation of the antivascular effects of chemotherapy [36]. In contrast, sustained and aggressive AA therapy may damage tumor vasculature and impede blood flow and drug access and lead to increased hypoxia.

In contrast to the positive results seen with the every-3-weeks administration of bevacizumab, a trial evaluating continuous administration of the small molecule anti-VEGF inhibitor PTK787/ZK in colorectal cancer reported a higher progression-free survival (PFS) rate, but no difference in overall survival (OS) [37]. A subgroup analysis revealed higher PFS and OS rates in patients with high serum lactate dehydrogenase (LDH) levels. Previous work has linked high LDH levels to an upregulated HIF pathway and an aggressive phenotype in colorectal adenocarcinomas [38]. Other postulated causes for the observed differences in survival between these two AAs in colorectal cancer are (a) the ability of antibody-based therapies to target cell surface antigens, thereby rendering tumor cells more vulnerable to attack by the immune system; (b) the ability of small molecule inhibitors with antiangiogenic properties to impair pericyte-associated PDGF, which in turn appears to be required for vascular normalization; (c) suboptimal potency, dosing, and/or pharmacokinetics; and (d) a reduced effect in the heavily pretreated patients included in the PTK787/ZK study, who may have had heterogeneous vasculature with angiogenesis governed by a wider range of proangiogenic factors [24–26].

The findings with these two classes of AA in a single disease entity (colorectal cancer) underscore the need to better understand the mechanisms of action so as to optimize the design of clinical trials of radiotherapy with these agents. To maximize the synergistic effects of combined antiangiogenic and cytotoxic therapy, development of non-invasive biomarkers of drug penetration and tumor oxygenation is important. In the absence of surrogate biomarkers of vascular normalization that may allow for the effects of AA therapy to be tracked, ongoing clinical trials are largely designed with the dual targeting of tumor cells and blood vessels in mind. However, the possibility that higher tumor cell kill could be achieved with appropriately timed (and possibly lower) doses of an AA in combination with chemotherapy and radiotherapy is appealing, because it could reduce treatment toxicity and costs, as opposed to the current “hit and miss” approaches.

RATIONAL FOR COMBINING AAS WITH RADIATION

1) Following radiotherapy or chemoradiotherapy, a substantial proportion of patients fail to achieve long-term local tumor control. Hypoxia or HIF-1 expression is associated with a lower radiation response and malignant progression in tumors of the uterine cervix, head and neck, and sarcomas [21].

2) Preclinical studies have demonstrated enhanced radiation-induced cell kill when AA therapy is combined with radiotherapy [39–41]. The normalization of tumor vasculature by an anti–VEGFR-2 antibody creates a time period of increased oxygenation, during which enhanced radiation-induced regression is observed [42]. Serial MRI in patients treated with AZD2171 reported the reversible normalization of blood vessels in human tumors [30], which suggests that the timing of chemotherapy and radiation during these windows would lead to more effective therapy.

3) During chemoradiotherapy, the access to tumors of cytotoxins and monoclonal antibodies can be impeded by abnormal tumor vasculature and high IFP. Targeting tumor vasculature improves the delivery of cytotoxic drugs and leads to greater tumor cell kill [5]. Supportive clinical data have emerged in human glioblastoma, in which decreases in vascular permeability and vasogenic edema were observed during treatment with AZD2171 [30].

4) Radiation itself induces transient tumor hypoxia, which in turn stimulates VEGF production and VEGFR-2 expression [43–45]. VEGF protects ECs from radiation-induced cytotoxicity [44–46] and vasculature damage decreases tumor cell survival [3]. Irradiation also upregulates the nitric oxide pathway in ECs, which leads to phenotypic changes that promote tumor angiogenesis [47]. Radiation-induced VEGF may also serve as a paracrine proliferative stimulus that promotes out-of-field growth in human tumors [48].

5) Combined AAs and chemoradiotherapy can eradicate smaller metastases in their prevascular phase, for example, in peritumoral lymph nodes. In experimental models, anti-VEGF therapy was most effective when started early, at day 7 after tumor cell inoculation [49]. The survival benefits observed in trials of bevacizumab and chemotherapy suggest that it is logical to incorporate the same agents into earlier stages of colorectal, lung, and breast cancer with radiotherapy.

6) Combined AAs and radiotherapy may optimize tumor control because single-agent objective response rates with AAs are generally 10% or less [16], and a gradual loss of activity may be seen with monotherapy [50–52]. This finding is consistent with the adaptive ability of tumor cells
in using multiple pathways for angiogenic signaling [53]. Tumors can call on these secondary pathways when the primary pathway is inhibited.

7) Treatment-induced hypoxia by inhibitors of angiogenesis may be minimized when combined with a cytotoxic treatment modality. Continuous blockade of the VEGF pathway can eventually lead to vascular collapse and regions of poorly vascularized tumor, and induced hypoxia may result in malignant progression with increased metastatic potential [54]. Combined VEGF inhibition during fractionated irradiation antagonized the increase in hypoxia, and resulted in extended tumor growth delay and tumor cell apoptosis [55].

8) Combined AAs and radiotherapy may be effective against tumor stem cells, which have self-renewing potential, quiescence of cell cycling, relative resistance to growth factors, and an ability to differentiate into diverse tumor components [56]. These stem cells can show prolonged dormancy of up to 1 year [57] and lead to resistance to cytotoxic chemotherapy. Stem cell–like tumor cells can also be a crucial source of key angiogenic factors in cancers and proangiogenic effects of stem cell–like glioma cells on ECs were abolished by bevacizumab [58].

DETERMINING OPTIMAL BIOLOGICAL DOSES: THE NEED FOR BIOMARKERS

The optimal biological dose (OBD) of molecular targeted agents is defined as the lowest dose level showing optimal biological activity and no dose-limiting toxicity. In traditional phase I trials, the maximum tolerated dose (MTD) is derived based upon dose escalation that is usually toxicity guided, and this may not necessarily coincide with the most efficacious dose. The MTD of bevacizumab in humans is around 20 mg/kg, because of severe migraine headaches [59], but the OBD of AA therapy combined with chemotherapy is unclear [60]. Establishing an OBD for an AA is made difficult by the fact that these agents are generally cytostatic in nature, making tumor shrinkage in clinical trials an insensitive measure of efficacy [61]. The OBD in this setting may not be an absolute value and may be context specific, varying depending on the tumor type, coadministered chemotherapy and/or radiotherapy, and pharmacogenetic background of the patient.

An example in which the OBD is unclear is with the monoclonal antibody bevacizumab, which, despite having a well-defined target, has no parameters predictive for efficacy [62, 63]. A phase III trial using bevacizumab, at a 10-mg/kg dose, and 5-FU, leucovorin, and oxaliplatin (FOLFOX4) chemotherapy in the second-line treatment of colorectal cancer showed tumor activity [64]. However, in a subsequent randomized phase II trial of the drug in patients with metastatic colorectal carcinoma, a 5-mg/kg dose (but not the 10-mg/kg dose) was effective in prolonging survival [65]. This raises the question of whether or not the 5-mg/kg dose, if it had been used, would have been just as effective, particularly as more toxicity was seen at the higher dose of bevacizumab. Similarly, discontinuing AA therapy in patients who do not show a measurable response may adversely affect their survival [61]. These findings point to the need for biomarkers to guide anti-VEGF monotherapy and combination therapy, especially to guide patient selection and protocol design and to serve as early surrogate measures of benefit and development of treatment resistance.

Examples of biomarkers that have been evaluated in clinical trials are summarized in Table 2, and this topic has been the subject of recent reviews [66, 67]. The properties of an ideal surrogate marker [60] include being: (a) noninvasive and repetitively available with minimal clinical consequences, (b) affordable and robust, (c) reasonably sensitive and quantitative, (d) highly reproducible, (e) as specific as possible for tumor-associated angiogenesis, and (d) correlated closely with antiangiogenic (and antitumor) efficacy in different tumor stages, preferentially in a broad range of different neoplasias and under various therapies.

The use of tissue biomarkers to select efficacious antiangiogenic drugs is still in an exploratory stage and validation to objectively assess the relevance of such biomarkers is awaited. Structured strategies for biomarker development in clinical trials of AA therapies should be a priority, particularly as the high cost of such targeted agents may result in a major burden for health care systems. The approach of assessing several variables concurrently in clinical trials in order to provide a comprehensive picture of the antiangiogenic activity can provide useful data [30]. Data obtained in this manner will allow the OBD to be estimated more quickly and used for guiding further clinical development.

DRUG SEQUENCING AND RADIATION ENHANCEMENT

Preclinical studies have shown that the timing of AAs relative to radiotherapy may be crucial for all classes of AA [68, 69]. However, the limitations of using animal models for AA research must be appreciated [70]. Differences between human and animal tumor models include the following: (a) murine tumors are fast growing with high EC growth rates, (b) murine tumors have poorer pericyte coverage, and (c) treatment results are influenced by varying tumor burden and sequencing AAs. An important aspect of the proposed vascular normalization model of VEGF blockade is that continuous blockade eventually leads to vascular collapse and regions of poorly vascularized tumor,
leading to malignant progression with increased metastatic potential. Changes consistent with the reversibility of “vascular normalization” have been observed in patients with glioblastoma after 28 days of treatment with AZD2171 [30], indicating that identification of the beginning and end of the vascular normalization process may be crucial for optimizing combined treatments.

Preclinical data showing the importance of sequencing are most consistent for VDAs, which cause extensive tumor necrosis with a typical rim of surviving tumor cells [32]. One such agent, ZD6126, showed maximal tumor cell kill when administered 24 hours prior to radiation or 1 hour or more after radiation, but was less effective if given 1 hour prior to radiation [71]. In the U87 tumor model, ZD6126 given 1 hour before radiation led to less tumor growth delay compared with that of radiation alone, and this protective effect was explained by acute ZD6126-induced tumor hypoxia [68].

Similar data are available for monoclonal antibodies. In two different human tumor xenografts, the combined effect of radiation and a single injection of VEGFR-2–specific monoclonal antibody DC101 administered concurrently were not significantly different from additive in terms of growth delay [42]. However, when radiotherapy was administered 4–6 days after DC101 treatment, a synergistic increase in tumor growth delay was observed, coinciding with the time of maximum tumor oxygenation (days 5–8). Similarly, vessel coverage by pericytes increased markedly between days 2 and 5, and fell again by day 8, peaking on day 2, when the diameter of tumor vessels had decreased significantly. Comparable data for bevacizumab reported the most potent tumor response for combined treatment when the drug followed radiotherapy [72].

Sequencing data on small molecule TKIs of VEGFR are less clear. ZD6474 (Zactima™; AstraZeneca Pharmaceuticals, Wilmington, DE) is one such agent with additional activity against EGFR tyrosine kinase. In two xenograft models of lung cancer, concurrent administration of ZD6474 plus radiation showed greater antitumor effects than with radiation therapy alone [73]. Tumor perfusion was significantly reduced after three initial doses of ZD6474 versus control, suggesting that impaired reoxygenation between fractions of radiotherapy in the concurrent protocol may be a possible basis for the schedule-dependent radiation enhancement observed. In the above-mentioned study, the administration of ZD6474 after radiotherapy was more effective than concurrent administration of the two modalities. However, in a setting that combined a 2-week

<table>
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<th>Table 1. Drugs referred to in this review</th>
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<tr>
<td><strong>Drug</strong></td>
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<tr>
<td>AZD2171</td>
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<tr>
<td>Bevacizumab</td>
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<td>Bay-43–9006 (sorafenib)</td>
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<td>PTK787/ZK (vatalanib)</td>
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<td>Rapamycin</td>
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<td>SU5416</td>
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<td>SU6668</td>
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<td>SU11248 (sunitinib)</td>
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<td>VEGF-Trap</td>
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<tr>
<td>ZD6474 (Zactima)</td>
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<tr>
<td>Combretastatin A4P</td>
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<td>DMXAA (5,6-dimethylxanthenone-4-acetic acid, AS1404)</td>
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<tr>
<td>Tumor necrosis factor</td>
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<td>ZD6126</td>
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Abbreviations: EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; mTOR, mammalian target of rapamycin; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.
course of fractionated radiotherapy with ZD6474 treatment, the enhancement in the tumor response was independent of the sequencing of the two therapies [74]. Important differences between the two studies included the tumor model (colorectal carcinoma, HT29, versus NSCLC, Calu-6) and fractionation schedule (2 weeks as opposed to 3 daily fractions of 2 Gy) used. Other TKIs with antiangiogenic effects, such as SU5416 (a VEGFR inhibitor) and SU6668 (an inhibitor of VEGF, fibroblast growth factor, and PDGF receptors), also enhance the antitumor effects of fractionated irradiation independent of drug sequencing [75]. Another such inhibitor, SU11248, enhanced the radiation effect when combined during radiation, and it also prevented tumor regrowth with maintenance therapy after completion of radiation [76].

AA-induced hypoxia can also be important for rapamycin, an agent that reduces VEGF production by tumor cells and inhibits VEGF-induced proliferation in ECs. Rapamycin combined with radiation in tumor-bearing glioblastoma xenografts caused no further increase in tumor growth delay when compared with radiation alone, a finding related to vessel thrombosis post–rapamycin treatment leading to elevated tumor hypoxia [77]. The authors concluded that thrombosis and tumor hypoxia may be confounding factors limiting the effectiveness of rapamycin in combination with radiotherapy.

A point of concern in radiotherapy is whether the long-term administration of AAs during a 5–6 week course of radiotherapy can lead to relative radiation resistance because of radiation-induced VEGF expression and tumor hypoxia. Preclinical data on VEGF tyrosine kinase inhibition with PTK787 during fractionated irradiation found that radiation antagonized the increase in hypoxia, and resulted in extended tumor growth delay and tumor cell apoptosis [55]. Similarly, the expected vascular shutdown with the VDA combretastatin A4P was instead replaced by an early increase in vascular permeability in patients with prostate cancer undergoing combined treatment [78]. Hence, the

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**Table 2. Candidate biomarkers for antiangiogenic therapy**

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<th>Biomarker</th>
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<td>Tumor biopsy material to determine MVD in samples with antibodies against endothelial markers (such as CD34, CD31, and vWf) and counted by microscopy.</td>
<td>Small biopsy samples might not reflect the MVD of an entire tumor because of tumor tissue heterogeneity. MVD has not been shown to be correlated with patient response in clinical trials of bevacizumab.</td>
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<tr>
<td>Circulating levels of factors that promote or suppress angiogenesis including VEGF, basic fibroblast growth factor, hepatocyte growth factor, and interleukin 8.</td>
<td>Increase in levels of circulating VEGF and/or the VEGF family member placental growth factor, and concurrent decrease in levels of soluble VEGF receptor 2 observed during therapy with the tyrosine kinase inhibitor sunitinib in renal cell cancer. No growth factor has yet been validated for predicting response to AA therapies. Clinical use may be complicated by the fact that platelets can contain and may release many such factors.</td>
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<td>A subpopulation of CECs shows a progenitor-like phenotype (CEPs) and has a key role in promoting cancer vasculogenesis and in late stages of cancer development. Cell-surface marker–based techniques are the most commonly used means of quantifying CECs.</td>
<td>CEC kinetics and viability found to correlate with clinical outcomes in some clinical patients treated with AA approaches. Increase in CEPs may occur early in the course of therapy and require sampling at multiple time points for interpretation of clinical data. Significant individual variability seen in patients, making relative changes more informative. Process of identifying and quantifying cell populations is not uniform, which in turn may account for conflicting clinical reports. Identification of nonviable CECs or mature hematopoietic cells important.</td>
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<td>Functional imaging to measure changes in the tumor vessels themselves or the effect of such therapy on the tumor cells. Significant changes in vascular function have been detected using PET, DCE-MRI, perfusion CT, and Doppler sonography [113, 115].</td>
<td>There is particular interest in using (DCE-MRI), which is able to offer insights into blood flow, microvessel permeability and size, tissue oxygenation, and metabolism. Expensive instrumentation is usually available in a limited number of institutions; lack of standard protocols for measurement of vascular parameters; test–retest repeatability and rationale for timing in clinical studies often unclear [115].</td>
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For details see [30, 66, 67].

Abbreviations: AA, antiangiogenic; CEC, circulating endothelial cell; CT, computed tomography; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; MVD, microvessel density; PET, positron emission tomography; VEGF, vascular endothelial growth factor; vWf, von Willebrand factor.
risk for treatment-induced hypoxia by AAs appears to be reduced when used in combination with radiotherapy. In the absence of reliable noninvasive markers for tumor hypoxia and radioresistance, we suggest that careful review of all preclinical data as well as the incorporation of well-designed biomarker studies are necessary in early-phase clinical trials.

### Radiation-Induced Normal Tissue Toxicity

Studies of AAs in colorectal cancer, breast cancer, and NSCLC are the focus of clinical interest, and the potential for an increase in collateral normal organ toxicity is of potential concern. Radiation injury can be classified as acute, consequential, or late effects, according to the time before the appearance of symptoms [79]. The underlying molecular and cellular processes in acute and late effects are complex and lead to a range of events, making the definitions more operational than mechanistic [80]. Early symptoms may not manifest in some organs that eventually develop late injury, such as the kidney, and trauma or surgery months or years after irradiation can precipitate acute breakdown of tissue that had been functioning normally.

The bowel is a radiosensitive organ, and grade 3 and 4 acute and late radiation enteritis occurs in 27% and 14% of patients, respectively, after treatment with preoperative chemoradiotherapy [81]. Late bowel toxicity manifests as diarrhea, bowel stricture, perforation, or hemorrhage and may also be irreversible. The tolerance dose, which is defined as the bowel dose that gives a 5% risk of late toxicity at 5 years, is estimated to be around 45–50 Gy [82], and irradiation of larger volumes is associated with increased toxicity [83]. When patients with lung cancer are treated with

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<tr>
<th>Table 3. Approaches for design of phase I–II trials of antiangiogenic therapy and radiotherapy</th>
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<td><strong>Design of experimental arm</strong></td>
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<tr>
<td>Induction RT or CT-RT, followed by maintenance AA (alone or in combination with CT). (Note: Consider randomization between short- versus long-term AA maintenance in a subsequent phase II study.)</td>
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<td>A short course of induction AA, followed by RT or CT-RT, followed by maintenance AA.</td>
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<td>Induction CT with AA, followed by RT alone, followed by maintenance AA.</td>
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<td>RT combined with escalating doses of concurrent AA.</td>
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<td>CT-RT coadministered with escalating doses of concurrent AA.</td>
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<tr>
<td>CT-RT plus escalating doses of concurrent AA, followed by maintenance AA (randomization between short- versus long-term AA maintenance).</td>
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Trial information accessed on October 1, 2006, at http://www.cancer.gov/search/ResultsClinicalTrialsAdvanced. Abbreviations: 5-FU, 5-fluorouracil; AA, antiangiogenic agent; CT, chemotherapy; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; OBD, optimal biological dose; RT, radiotherapy; SWOG, Southwest Oncology Group.

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Abbreviations: 5-FU, 5-fluorouracil; AA, antiangiogenic agent; CT, chemotherapy; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; OBD, optimal biological dose; RT, radiotherapy; SWOG, Southwest Oncology Group.
concurrent chemoradiotherapy, high-grade esophagitis manifests in 33%–41% of patients [84]. However, acute esophagitis is generally self-limiting and late toxicity to the esophagus is uncommon. Radiation pneumonitis correlates with the volume of normal lung irradiated, and symptoms usually manifest within 6 months post-treatment [85]. Late complications have been reported in >5% of patients after high-dose chemoradiotherapy, and nonpulmonary events include esophageal strictures and cardiac complications [86]. Patients with Hodgkin’s disease have a higher risk for cardiovascular mortality 5 years after the lower doses of radiotherapy that are used for lung tumors [87]. Asymptomatic patients who received mediastinal doses of 35 Gy have also been found to have a high prevalence of diastolic dysfunction, which was in turn associated with stress-induced ischemia and a poorer survival [88].

**Potential for Increased Radiation-Induced Toxicity**

Potential for increases in toxicity when AAs are combined with radiotherapy are suggested by the following data.

Myocardial toxicity: Myocardial damage and/or a reduction in ejection fraction rates have been reported with AAs, including sunitinib [89] and imatinib [90]. Dose-limiting acute coronary syndromes have been reported with VDAs such as combretastatin A4P [91], and myocardial necrosis has been reported with ZD6126 [92]. Patients treated with bevacizumab have a twofold higher incidence of transient ischemic attacks, angina, myocardial infarction, and stroke [93]. More thrombotic and embolic events were also seen with the administration of the VEGF inhibitor PTK787/ZK in patients with colorectal cancer [37].

Parenchymal lung damage: Patients with lung cancer often have coexisting chronic obstructive pulmonary disease (COPD). VEGF expression is increased in the pulmonary arteries of smokers and patients with moderate COPD [94, 95], and reduction of VEGF levels in induced sputum correlates with increases in the severity of COPD [96]. In rats, the inhibition of VEGFRs with the inhibitor SU5416 causes alveolar cell apoptosis and emphysema [97]. Furthermore, mutant mice with PDGF deficiency develop lung emphysema that is associated with a loss of alveolar myofibroblasts as the disease progresses [98]. However, both stromal cells and ECs can respond to ionizing radiation by expressing various cytokines, including PDGF and transforming growth factor-β, both of which appear to have a role in radiation pneumonitis and fibrosis [99]. As such, AAs that also block the effect of PDGF may potentially reduce radiation fibrosis.

Damage to bronchial cartilage: The administration of VEGF-Trap for 10 days in 4-, 8-, and 16-week-old mice reduced the number of capillaries in the tracheal mucosa by 39%, 28%, and 14%, respectively [100]. Nonhealing erosions of the nasal septum have been reported in patients treated with bevacizumab [101]. In an early-stage clinical trial, bevacizumab-treated patients with lung cancer had a higher risk for fatal pulmonary hemorrhage [102]. Early data suggested that patients with squamous cell carcinoma could be at higher risk for hemorrhage, and this histological type was subsequently excluded from a phase III trial in NSCLC that showed a survival benefit for chemotherapy combined with bevacizumab [34]. In the follow-up study, rates of clinically significant bleeding were higher with bevacizumab (4.4% and 0.7%, p < .001), and of 15 treatment-related deaths in the chemotherapy plus bevacizumab group, pulmonary hemorrhage was the cause in five patients.

Bowel toxicity: Administration of anti–VEGFR-2 antibodies 1 month following whole-body irradiation resulted in fatal bowel toxicity in 20% of mice [103]. Two cases of acute ischemic colitis and a third with fatal gastrointestinal perforation were reported when bevacizumab was administered to patients 4–16 months after pelvic radiotherapy [104]. Fatal intestinal perforations were observed in 11% of patients, with a further 11% developing bowel obstruction, when bevacizumab was administered to patients with ovarian cancer progressing after prior chemotherapy [105]. Bowel ischemia was also dose limiting in a phase I trial of combretastatin A4P [106].

Neurological damage: VEGF-A has neurotrophic and neuroprotective effects on neuronal and glial cells in culture and in vivo, and can stimulate the proliferation and survival of neural stem cells [7]. VEGF also mediates the angiogenic response to cerebral and peripheral ischemia, and promotes nerve repair following traumatic spinal injury [107].

Preclinical and clinical toxicity data suggest a potential for higher rates of normal tissue toxicity for radiotherapy combined with AAs. This indicates a need to optimize radiotherapy planning and delivery in early-stage clinical trials in order to (a) minimize damage to normal tissues and organs and (b) better estimate the actual radiation doses received by critical organs. Improvements in the quality of radiotherapy can be achieved by measures such as adherence to guidelines for target definition in rectal cancer [108] and for treatment planning and delivery for lung tumors [109]. Data arising from well-designed studies will allow for specific radiotherapy planning criteria to be derived for subsequent phase II–III studies.
DESIGNING TRIALS INTEGRATING AA THERAPY WITH RADIATION

In the absence of validated surrogate biomarkers for patient selection, a reasonable approach for combining AAs with radiotherapy may be to focus on tumor entities in which local control is suboptimal. In addition, tumor entities for which these agents have produced superior survival in the treatment of advanced disease are also logical candidates. Another target group is tumors for which approaches such as optimized radiotherapy planning and delivery, with or without concurrent chemotherapy, are insufficient to reduce local tumor recurrences.

An important issue is whether early studies should first evaluate the combination of AAs with radiotherapy alone, possibly preceded by induction chemotherapy, in order to exclude the possibility of unexpected enhancement of normal organ toxicity. Experience with radiotherapy combined with the thalidomide is illustrative. Thalidomide has antiangiogenic, immunomodulatory, anti proliferative, and proapoptotic properties [110]. The combination of thalidomide (200 mg/day) with low-dose interleukin-2 and radiation therapy resulted in excessive toxicity, including deep venous thrombosis and radiation myelopathy at a dose not expected to cause such problems [111]. Evaluation of a lower dose of thalidomide (50 mg/day) as a potential protector of normal tissues in combination with thoracic radiotherapy and low-dose vinorelbine (5 mg/m² administered 3 times per week) was stopped because of unacceptable toxicities [112]. This experience highlights the need for caution when evaluating new agents with radiotherapy and chemoradiotherapy. However, as concurrent chemoradiotherapy is considered the standard of care for patients with some tumors, a cautious introduction of AAs into such schemes in well-designed trials may be a reasonable approach.

A number of clinical approaches for designing phase I–II trials of AAs and radiotherapy are summarized in Table 3.

CONCLUSIONS

The available data strongly support the introduction of AAs into combined modality treatment schemes that include fractionated radiotherapy. As reliable, noninvasive markers for tumor hypoxia and radioresistance are not available, incorporating translational research into early clinical trials and evaluating changes during combined treatment may enable optimal treatment schedules to be identified. Elucidating the molecular interplay between radiation toxicity to normal organs and AAs is also important to facilitate the design and testing of clinical trial strategies aimed at minimizing toxicity. Strategies for biomarker development in clinical trials of AA therapies should be a priority, particularly as the high cost of such targeted agents may result in a major burden for health care systems.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

S.S. has acted as a consultant for Roche.

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