Radiation enhances both epithelial growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) expression, which are a part of key pathways for tumor progression. Some tumors may not respond well to EGFR inhibitors alone or may develop resistance to EGFR inhibitors. Therefore, drug therapy targeted to VEGF receptors and EGFRs, when combined with radiotherapy (RT), may improve tumor control and provide wider applicability. This article focuses on ZD6474, an inhibitor of EGFR and VEGF receptor signaling in combination with RT. We discuss preclinical and clinical studies with RT and inhibitors of VEGF or EGFR signaling first. We then address issues associated with ZD6474 pharmacokinetic dosing, and scheduling when combined with RT. We also discuss ZD6474 in the context of anti-EGFR therapy resistance. Dual inhibition of EGFR and VEGF receptor signaling pathways shows promise in enhancing RT efficacy. © 2006 Elsevier Inc.

**INTRODUCTION**

Targeted drugs that have been specifically designed to block cellular signaling processes that are vital for the growth and invasion of malignant tumors have stimulated a new era of research in the treatment of cancer. The hope is that tumor-signaling processes can be perturbed to the point at which cancer regresses or becomes dormant or becomes hypersensitive to the killing effects of radiotherapy (RT) or chemotherapy. RT plays a role in upregulating the expression of molecules such as vascular endothelial growth factor-A (VEGF) receptor (VEGFR) and the epidermal growth factor receptor (EGFR), both of which are considered key targets for novel anticancer therapies. Data are accruing from various studies to suggest that inhibitors of VEGFR- and EGFR-dependent signaling may enhance the cytotoxic effects of RT. This article discusses the use of inhibitors of VEGFR and EGFR signaling in combination with RT. We focus on ZD6474, a potent inhibitor of both VEGFR and EGFR tyrosine kinase activity (1). By targeting VEGFR-dependent tumor angiogenesis and EGFR-dependent tumor cell proliferation, ZD6474 offers the potential advantages of inhibiting two key pathways of tumor growth (Fig. 1).

Simultaneous inhibition of both VEGFR and EGFR signaling may present a novel and exciting opportunity to augment the antitumor effects of RT.

**VEGFR-SIGNALING INHIBITORS IN COMBINATION WITH RT**

For >30 years, angiogenesis has been recognized as an essential process for sustaining the growth and development of solid tumors and metastases (2, 3). VEGF is a key proangiogenic factor that stimulates endothelial cell proliferation and migration and promotes endothelial cell survival (2). The VEGFR family comprises three receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR), and VEGFR-3 (Flt-4) (4). Of these, VEGFR-2 is considered the most important receptor for mediating the angiogenic effects of VEGF. Therefore, inhibition of VEGFR-2-mediated tumor angiogenesis has become an important therapeutic goal.

Radiation-dependent increases in VEGF expression have been measured in tumor cells in preclinical studies (5–7), and intensification of angiogenesis during RT has been...
suggested in the clinical setting (8). VEGF is an upstream activator of endothelial nitric oxide synthase (9), and RT has been shown to upregulate the nitric oxide pathway in endothelial cells, leading to phenotypic changes that promote tumor angiogenesis (10). A number of preclinical studies have shown that the therapeutic benefit of ionizing RT may be greatly enhanced when used in combination with inhibitors of VEGFR-2 signaling, including an anti-VEGF antibody (6, 11), VEGF sequestration (12), and VEGFR-2 tyrosine kinase inhibitors (5, 12, 13). Because VEGF acts as an important endothelial survival factor in newly formed vessels, abrogation of VEGF signaling may potentiate the response to RT by inhibiting VEGF-induced protection of endothelial cells against radiation damage (6).

Given that oxygen is a potent radiosensitizer, the combination of ionizing RT and antiangiogenic agents may appear counterintuitive, but recent studies have demonstrated that antiangiogenic agents combined with ionizing RT may result in increased oxygen levels in tumors (11, 14, 15). Lee et al. (11) observed an increase in tumor oxygenation despite a decrease in tumor vascular density induced by anti-VEGF antibody therapy and suggested that this phenomenon was associated with vessel reorganization (11). The concept of vascular normalization leading to an enhanced efficacy of conventional therapies has also been proposed (16, 17). Inhibition of VEGF signaling could, in time, have profound effects on the response to ionizing RT, but the effects of RT on existing and developing vasculature are complex, and the interaction of RT with inhibitors of VEGF and other proangiogenic growth factors is not yet well defined (18).

EGFR SIGNALING INHIBITORS IN COMBINATION WITH RT

Aberrant EGFR tyrosine kinase activity has been reported in a number of human tumors and may contribute to tumor growth and the development of metastases (19). Radiation exposure not only results in activation of EGFR, but also upregulates the downstream MAPK pathway. MAPK signaling in turn is associated with increased expression of other growth factors such as transforming growth factor-α and VEGF (leading to possible paracrine and autocrine angiogenic stimulation) (20).

Several preclinical studies have been conducted with inhibitors of EGFR signaling in combination with RT. Administration of cetuximab (C225), the humanized antibody against EGFR, improved the response to RT in xenograft models of non–small-cell lung cancer (21) and epidermoid carcinoma (22). Similarly, combining the EGFR tyrosine kinase inhibitor gefitinib with RT produced a cooperative antitumor effect in human colon and lung cancer xenografts (23). Furthermore, clonogenic assays revealed a significant radiosensitizing action of gefitinib in bladder cancer cell lines (24). The potential mechanisms underlying the enhanced response to RT in the presence of EGFR signaling inhibition remain to be determined. However, possible explanations include enhanced tumor cell apoptosis and inhibition of EGFR-dependent production of endothelial cell survival factors such as VEGF. Of particular interest are the results from a randomized Phase III trial investigating high-dose RT alone or in combination with cetuximab in advanced head-and-neck cancer (25). Patients who received combination therapy showed a significant prolongation of overall survival compared with those receiving RT alone. These highly promising data will encourage continued clinical evaluation of RT and targeted agents as combination therapy.

ZD6474, AN INHIBITOR OF VEGFR AND EGFR SIGNALING, IN COMBINATION WITH RT

ZD6474 inhibits both VEGFR- and EGFR-mediated intracellular signaling, and its use in combination with RT may provide significant clinical efficacy. Preclinical evidence supporting the combined use of ZD6474 and RT has recently been reported. One study investigated the effects of ZD6474 and RT on human umbilical vein endothelial cell proliferation, as well as on tumor growth in a human non–small-cell lung cancer xenograft model (H226 and A549) (26). In vitro, ZD6474 inhibited human umbilical vein endothelial cell proliferation and induced accumulation of cells in G₁, inhibited the formation of capillary-like networks, and enhanced the radiosensitivity of human umbilical vein endothelial cell in combination with RT. In vivo, the combination of daily oral ZD6474 plus RT produced significantly greater antitumor effects in H226 and A549 tumor xenografts compared with single-agent therapy alone.
Williams et al. (27) assessed the in vivo effect of chronically administered ZD6474 (25 or 50 mg/kg/d), with or without RT (three fractions of 2 Gy on Days 1–3) in a Calu-6 human lung cancer xenograft model. Two schedules were examined: (1) ZD6474 dosing initiated 2 h before the first radiation dose (concurrent schedule), and (2) ZD6474 dosing initiated 30 min after the last radiation dose (sequential schedule). The growth delay induced using the concurrent schedule was significantly greater than that induced by either ZD6474 or RT alone (22 ± 1 vs. 9 ± 1 and 17 ± 2 days, respectively; p = 0.03 vs. RT alone). However, when administered sequentially, the growth delay was markedly enhanced (36 ± 1 day; p <0.001 vs. RT alone or the concurrent schedule). Tumor perfusion (as measured by Hoechst staining) was significantly reduced after three initial doses of ZD6474 vs. control, suggesting that impaired reoxygenation between RT fractions in the concurrent protocol may be a possible basis for the schedule-dependent radiopotentiation observed.

Schedule optimization of ionizing RT and antiangiogenic combination therapy may differ across a variety of tumor types. For example, recent studies by Gustafson et al. (28) of a head-and-neck squamous cell carcinoma xenograft model indicated that concurrent administration of ZD6474 and RT significantly delayed tumor growth and was superior to single modalities or sequential combination therapies.

To mimic more effectively the patterns of growth of non–small-cell lung cancer observed in the clinic, an orthotopic mouse model was used to evaluate the effect of ZD6474 (15 mg/kg/d), RT (4-Gy fractions three times weekly up to 20 Gy), and the combination of ZD6474 plus RT on the growth of the human lung adenocarcinoma NCI-H441 (29). ZD6474 (5 μM) inhibited sublethal damage repair in H441 cells in an in vitro clonogenic assay, and the combination of ZD6474 plus RT produced the greatest suppression of tumor growth, development of metastases, and pleural effusion in vivo. In addition, tumor cell apoptosis was increased, with a significant decrease in microvessel density in the ZD6474 plus RT combination.

The antitumor effects of a range of ZD6474 doses were also examined in a human colorectal cancer xenograft model (HT29), either alone or combined with RT (30). Three different schedules of combination therapy were assessed: ZD6474 before RT, concomitant administration, and ZD6474 after RT. In that model, irrespective of sequencing, combination therapy resulted in a significantly greater growth delay than either RT or ZD6474 treatment alone. More recently, ZD6474 has been reported to potentiate the antitumor activity of RT in vitro and in vivo in a model of human glioblastoma (31); the in vivo effects of combination therapy correlated with a reduction in VEGF expression.

Overall, these results suggest that ZD6474 may be a successful agent combined concurrently and/or sequentially with clinical RT in a variety of tumor types. The scheduling of treatments will continue to be an important issue to ensure optimal efficacy but is likely to only be assessed adequately in the clinical setting.

Pharmacokinetic dosing of ZD6474 in vivo

Initial preclinical studies with ZD6474 in xenograft models have shown activity against a variety of tumor types using doses ranging from 12.5 to 100 mg/kg/d in mouse models (1). In these initial studies, most human tumor xenografts continued to grow, albeit slowly, at doses <50 mg/kg/d. The most marked antitumor effects, including regression, usually required dosing at >50 mg/kg/d. Doses of 20–50 mg/kg/d in mouse models (28) appear to produce plasma drug levels similar to those achieved in Phase I trials with ZD6474 (32). Therefore, preclinical studies that take into account the relationship between human and mouse pharmacokinetics may provide more predictive information on treatment combination efficacy.

The pharmacokinetics of ZD6474 in humans is linear, with a long terminal half-life (at least 100 hours) in both Japanese (33) and Western populations (32). At doses of 50–600 mg/d, the plasma trough levels at steady-state range of 100–1,100 ng/mL, with levels at the recommended 100–300 mg/d dose ranging from approximately 400 to 1,000 ng/mL. Steady-state levels are attained only after 20–30 days of daily drug dosing owing to the long half-life. The terminal half-life of ZD6474 in mice is significantly shorter (~30 h) than that seen in humans. The tissue distribution of ZD6474 is very high, with liver levels approximately 100 times that of plasma after daily oral dosing in mice (34). Overall, the pharmacokinetics of ZD6474 are consistent with the maintenance of therapeutic levels after once-daily treatment, and dosing in animal models can be used to approximate human exposure.

ZD6474—overcoming resistance to EGFR inhibition and use in combination with other targeted antitumor agents

ZD6474 may also be a useful treatment option in the setting of acquired EGFR resistance. Overcoming resistance to EGFR signaling inhibition has been examined in a recent study from Ciardiello et al. (35). GEO human colon cancer xenografts that had developed resistance to inhibitors of EGFR activity (gefitinib or cetuximab) were subsequently exposed to daily dosing of ZD6474. Significant tumor growth inhibition for the entire duration of dosing (up to 150 days) was observed with administration of ZD6474; in contrast, animals bearing gefitinib- or cetuximab-resistant tumors failed to respond when treatment with either EGFR inhibitor was reinitiated after a treatment break. Western blot analysis revealed increases in proangiogenic factors in the resistant GEO colon tumor cell lines, suggesting a potential mechanism underlying the strong antitumor effects of ZD6474 in these experiments (Fig. 2).

An important issue is whether a single agent will be sufficient to result in a prolonged response or disease stabilization. Recent evidence from Siemann et al. (36) showed that combining ZD6474 with ZD6126, a vascular targeting drug that interacts directly with, and destroys, the
rapidly dividing immature tumor vasculature, produces an increased tumor growth delay compared with either agent alone in human renal cell and Kaposi sarcoma xenograft models.

**CONCLUSIONS**

Despite recent controversies surrounding the efficacy of novel agents that target growth-factor signaling, bench-to-bedside triumphs have been reported in combination with chemotherapy (37) and RT (25). The clinical activity of cetuximab combined with RT in patients with locally advanced head-and-neck cancer suggests exciting possibilities ahead (25). To this end, we are now beginning to explore the feasibility of combining molecular-targeted agents with RT in the clinical setting. Willett et al. (38) have also demonstrated responses in patients with locally advanced rectal cancer treated with bevacizumab and RT, with no unanticipated toxicity.

This brief article attempts to provide the rationale for the use of molecular-targeted therapy to improve RT outcomes. We focused on ZD6474 because it inhibits both VEGFR and EGFR signaling pathways. In the next few years, researchers will begin to address crucial questions such as the clinical relevance of this approach. For example, the optimal scheduling of novel agents in combination with RT remains to be determined. Furthermore, the optimal dosing schedule of these agents with RT may differ from one tumor type to another. Some of the questions are whether combination use will allow reduced radiation doses, and hence the potential for lower toxicity; the relevance of hypoxia when using agents that target EGFR and VEGFR signaling; and whether we will discover proteins and genes that predict the response to ZD6474. These and other questions require additional investigations, but it certainly is a fascinating time for radiation oncology. Intensity-modulated RT and sophisticated technology have already taken us to a higher ground, and we are now entering a new area, in which integrating advances in radiation biology with advances in molecular medicine has the potential to provide similar, or greater, gains in cancer management and survival. Targeted anticancer therapies seem likely to be part of a new clinical paradigm for radiation oncologists in the near future.

**REFERENCES**


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