SU11248 Maintenance Therapy Prevents Tumor Regrowth after Fractionated Irradiation of Murine Tumor Models

Aaron J. Schueneman, Eric Himmelfarb, Ling Geng, Jiahua Tan, Edwin Donnelly, Dirk Mendel, Gerald McMahon, and Dennis E. Hallahan

Vanderbilt University School of Medicine, Nashville, Tennessee 37232 [A. J. S., E. H.]; Departments of Radiation Oncology [L. G., J. T., D. E. H.], Radiology [E. D.], and Cancer Biology [D. E. H.], Vanderbilt University School of Medicine, Nashville, Tennessee 37232-5671; and Sugen, Inc., South San Francisco, California 94080 [D. M., G. M.]

INTRODUCTION

Receptor tyrosine kinase activation contributes to cell viability during cytotoxic therapy. The novel broad spectrum receptor tyrosine kinase inhibitor, SU11248, inhibits vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor, c-kit, and fetal liver tyrosine kinase 3. In this study, we maintained SU11248 plasma levels beyond the completion of radiotherapy to determine whether tumor regrowth can be delayed. The angiogenic effects of SU11248 were demonstrated using human umbilical vein endothelial cells in vitro. Apoptosis increased and clonogenic survival decreased when SU11248 was used in combination with radiation from 0 to 6 Gy on endothelial cells. In vivo tumor growth delay was increased in C57B6J mice with Lewis lung carcinoma or glioblastoma multiform (GL261) hind limb tumors. Mice were treated with daily i.p. injections (40 mg/kg) of SU11248 during 7 days of radiation treatment (21 Gy). Combined treatment with SU11248 and radiation significantly reduced tumor volume as compared with either treatment alone. Concomitant reduction in vasculature was confirmed using the dorsal vascular window model. The vascular length established using images taken from a consistent quadrant in the window show the combination therapy was more effective in destroying tumor vasculature than either treatment alone. SU11248 maintenance administration beyond the completion of radiotherapy results in prolongation of tumor control. In summary, SU11248 enhances radiation-induced endothelial cytotoxicity, resulting in tumor vascular destruction and tumor control when combined with fractionated radiotherapy in murine tumor models. Moreover, inhibition of angiogenesis well beyond radiation therapy may be a promising treatment paradigm for refractory human neoplasms.

MATERIALS AND METHODS

Cell Culture. HUVECs were obtained from Clonetics and were maintained in EBM-2 medium supplemented with EGM-2 singlequots (BioWhit-taker). The GL261 cell line was obtained from Dr. Yancey Gillespie (University of Alabama, Birmingham, AL; Ref. 10). GL261 cells were maintained in DMEM with Nutrient Mixture F-12:1:1 (Life Technologies, Inc.) with 7% FCS, 0.5% penicillin-streptomycin, and 1% sodium pyruvate. LLC cells were obtained from American Type Tissue Culture (11) and were maintained in high-glucose (4.5 g) DMEM supplemented with 10% FCS and 1% penicillin-streptomycin. All cells were incubated in a 37°C in a 5% CO2 incubator.

Received 12/18/02; accepted 5/8/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported, in part, by NIH Grants R01-CA58508, R01-CA70937, R01-CA80706, R21-CA89674, CA89888, and the Vanderbilt Lung Cancer SPORE Grant P50-CA90949, Vanderbilt-Ingram Cancer Center Grant CCSG P30-CA68485, and a grant from Sugen Inc.

2 To whom requests for reprints should be addressed, at Department of Radiation Oncology, Vanderbilt University, 1301 22nd Avenue South, B-902 The Vanderbilt Clinic, Nashville, Tennessee 37232-5671. Phone: (615) 343-9244; Fax: (615) 343-3075, E-mail: Dennis.Hallahan@mcmail.vanderbilt.edu.

3 The abbreviations used are: RTK, receptor tyrosine kinase; PDGF, platelet-derived growth factor; Flk-1/KDR, fetal liver kinase-1; PDGF, PDGF receptor; Flk-1, KDR, PDGF receptor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; LLC, Lewis lung carcinoma; HUVEC, human umbilical vein endothelial cell; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; VLD, vascular length density. 
An Eldorado 8 Teletherapy 60 Co Unit (Atomic Energy of Canada Limited) was used to irradiate endothelial cell cultures at a dose rate of 0.84 Gy/min. Delivered dose was verified by use of thermoluminescence detectors. Delivered dose was verified by use of thermoluminescence detectors. The irradiation was performed using a Biologic BRT-200. The delivered dose was verified by use of thermoluminescence detectors. The irradiation was performed using a Biologic BRT-200.

**Viability Assays.** The number of cells undergoing apoptosis was quantified by microscopic analysis of apoptotic nuclei. Cells were then fixed and stained with H&E at 24 h after treatment. H&E was used to stain cell cultures at a dose rate of 0.84 Gy/min. For each radiation dose, five high-power fields (×400 objective) were examined, and the number of apoptotic and total cells was determined. From these numbers, the percentage of apoptotic cells for each group was determined.

Clonogenic survival analysis was performed as we have described previously (2). Briefly, HUVEC culture plates were treated at each radiation dose level with/without 1 μM SU11248 for 1 h before irradiation. After treatment with radiation and/or antagonist, cells were trypsinized, counted by hemocytometer, and subcultured into fresh medium. After 14 days, the cells were fixed with cold methanol and stained with 1% methylene blue. Colonies with >50 cells were counted, and the surviving fraction was determined.

**Western Immunoblots.** HUVECs were treated with 100 nm SU11248 for 1 h and/or irradiated with 6 Gy. HUVECs were washed twice with PBS and lysed buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM Na PP, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml leupeptin) added. Protein concentration was quantified by the Bio-Rad method. Twenty μg of total protein were loaded into each well and separated by 12% SDS-PAGE gel, depending on the size of the target protein being investigated. The proteins were transferred onto nitrocellulose membranes (Hybond ECL; Amersham, Arlington Heights, IL) and probed with antibodies to caspase 3 and caspase 3-cleaved fragment (Cell Signaling).

**Tumor Vascular Window Model.** Life Technologies, Inc., penicillin-streptomycin solution (200 μl) was injected into the hind limb of the mouse before the procedure. The dorsal skin-fold window was a 3-g plastic frame applied to the skin of the mouse and remained attached for the duration of the study. The chamber was screwed together, whereas the epidermis was incised and remained open with a plastic covering. The midline was found along the back, and a clip was placed to hold the skin in position. A template, equivalent to the outer diameter of the chamber, was traced, producing the outline of the incision. A circular cut was made tracing the perimeter (7-mm diameter) of the outline followed by a crisscross cut, thus producing four skin flaps. The epidermis of the four flaps was then removed using a scalpel with an effort to follow the hypodermis superior to the fascia. The area was then trimmed with fine forceps and iris scissors. The template was removed, and the top piece of the chamber was fixed with screws. During surgery, the area was kept moist by applying moist drops of PBS with 1% penicillin/streptomycin solution. The bottom portion of the chamber was put in place, and the top was carefully positioned on the cut side so that the window and the circular incision were fitted. The area was then trimmed at this time. The three screws that held the chamber together were then positioned into the chamber holes and tightened so that the skin was not pinched, thus avoiding diminished circulation.

**Tumor blood vessels developed in the window within 1 week.** We studied the time- and dose-dependent response of tumor blood vessels to radiation using the window model. Vascular windows were created in the skin by making an incision. A circular cut was made tracing the perimeter (7-mm diameter) of the area incised and remained open with a plastic covering.

**Tumor Blood Vessels developed in the window within 1 week.** We studied the time- and dose-dependent response of tumor blood vessels to radiation using the window model. Vascular windows were created in the skin by making an incision. A circular cut was made tracing the perimeter (7-mm diameter) of the area incised and remained open with a plastic covering.

**Tumor Growth Delay Assays.** C57BL/6 mice received s.c. injections in the right thigh with 10^6 viable cells of a murine glioblastoma (GL261) or lung carcinoma (LLC) suspended in 0.2 ml of a 0.6% solution of agarose. Each set of six mice was stratified into four groups on day 1 (SU11248 vehicle control, radiation, SU11248, and SU11248 + radiation) to control for mean tumor volume. An equal number of large- and intermediate-sized tumors were present in each group. Mouse tumors were stratified into groups so that the mean tumor volume of each group was comparable. Mean tumor volumes of LLC were 425 ± 40, 420 ± 40, and 432 ± 45 mm^3 for and for GL261 were 530 ± 50, 510 ± 35, and 535 ± 55 mm^3 for groups of tumors treated with radiation alone, SU11248 alone, or SU11248 and radiation, respectively.

Irradiated mice were immobilized in Lucite chambers, and the entire body was shielded with lead except for the tumor-bearing hind limb. Radiation was administered within 30 min of SU11248 (40 mg/kg) i.p. injection. A total dose of 21 Gy was administered to the appropriate mice in seven fractionated doses of 3 Gy on days 1, 2, 3, 4, 5, 7, and 8, the first dose being administered on day 1. Tumor volumes were measured three times weekly using skin calipers as described previously (12–14). The volumes were calculated from a formula (a × b × c / 2) that was derived from the formula for an ellipsoid (m/3 / 6). Data were calculated as the percentage of original (day 1) tumor volume and graphed as fractional tumor volume ± SE for each treatment group.

For the maintenance study of SU11248, C57BL/6 mice bearing GL261 hind limb tumors were stratified as described above into six groups. Mean tumor volumes were 320 ± 28, 310 ± 23, and 330 ± 29 mm^3 in groups treated with radiation alone, SU11248 alone, or SU11248 and radiation, respectively. Radiation was administered within 30 min of SU11248 (40 mg/kg) i.p. injection. A total dose of 18 Gy was given to the appropriate groups in six fractionated doses of 3 Gy on days 1, 2, 3, 4, 5, and 8. After day 8, three groups received twice daily SU11248 injections (20 mg/kg, 3 h apart) for 7 days (days 9, 10, 11, 12, 15, 16, and 17), and after a 4-day interruption in therapy (days 18–21), they were subsequently maintained for the remainder of the experiment. Tumor volumes were assessed as above.

**Amplitude-modulated Doppler Blood Flow Analysis.** Blood flow within these tumors was quantified by Power Doppler after the third fraction of irradiation. Tumor blood flow was imaged with a 10–5 MHz linear Entos probe attached to a HDI 5000 (probe and HDI 5000 from ATL/Philips, Bothell, WA) as we have described previously (15). Power Doppler sonography images were obtained with the power gain set to 82%. A 20-frame cine loop sweep of the
entire tumor was obtained with the probe perpendicular to the long axis of the lower extremity along the entire length of the tumor. Color area was quantified using HDI lab software (ATL/Philips). This software allows direct evaluation of Power Doppler cineloop raw. The color area was recorded for the entire tumor. Five mice were entered into each treatment group. Values for color area were averaged for each tumor set, and treated groups were compared with controls with the unpaired Student \( t \) test.

Microscopy of Tumor Sections. C57BL6 mice bearing LLC tumors were treated with 3 Gy alone, SU11248 alone, or SU11248 and radiation. Twenty-four h after treatment, tumors were fixed, sectioned, and stained. Phosphorylation of the molecular target for SU11248, PDGFRα, was studied by use of the phospho-specific antibody (Cell Signaling). TUNEL staining was used to identify apoptotic cells (DeadEnd Colorimetric TUNEL System, Promega no. G7130). Sections were counterstained with antibody to the endothelial-specific antigen von Willebrand factor using rabbit anti-VWF, IgG (Dako, Carpinteria, CA). Secondary antibody: peroxidase-labeled goat antirabbit IgG, Dako stained with Vector Nova Red Substrate kit (Vector, Burlingame, CA) Sections were imaged by light microscopy using ×200 under oil emersion.

Statistical Analysis. We used the general linear model (logistic regression analysis) to test for associations between the numbers of apoptotic cells present in culture, clonogenic survival, tumor blood flow, and tumor volumes. We applied the Bonferroni method to adjust the overall significant level equals to 5% for the multiple comparisons in this study. All statistical tests were two-sided, and differences were considered statistically significant for \( P < 0.05 \). SAS software version 8.1 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

RESULTS

RTK inhibitors enhance the cytotoxic effects of ionizing radiation in vascular endothelial cells by the increased activation of the apoptotic pathway (2, 16). To determine whether broad spectrum RTK inhibition enhances the cytotoxic effects of radiation on vascular endothelium, SU11248 was added 30 min before irradiation. HUVECs were treated with vehicle control and either 100 nM SU11248 plus the indicated dose of radiation. The percentage of endothelial cells demonstrating apoptotic nuclei 24 h after treatment was...
Fig. 4. Tumor growth delay analysis. Mice with (A) LLC and (B) GL261 hind limb tumors were treated with i.p. injection of 40 mg/kg SU11248 or vehicle 30 min before each of seven doses of radiation. Tumors were irradiated with 0 or 3 Gy daily for seven treatments (21 Gy total). Therapy was halted after day 8 (arrows). Shown are the means of changes in tumor volumes in five mice in each of the treatment groups (vehicle, SU11248 maintenance main, vehicle + 21 Gy; and SU11248 + 21 Gy). Bars indicate SE: *, P < 0.05. PDGFRβ phosphorylation was studied by use of the phosphospecific antibody on immunohistochemical analysis of tumor sections. C and D show microscopic (×40) photographs of immunohistochemical staining of LLC tumors after i.p. administration of (C) SU11248 or (D) vehicle. Sections were stained for phosphor-PDGFRβ using alkaline phosphatase (blue) stain and counterstained with eosin. Arrows indicate microvasculature that stains positive for phosphor-PDGFRβ.
whether enhanced apoptotic response in endothelial cells treated with SU11248 alone for cells treated with SU11248 alone, 6 Gy alone, or SU11248 followed by 6 Gy. Untreated control cells show 2% apoptotic nuclei as compared with 7 and 8% after treatment with SU11248 or radiation, respectively (P > 0.1). HUVECs treated with SU11248 followed by 6 Gy showed 21% of cells with apoptotic nuclei at 24 h, which was significantly greater than either agent alone (P < 0.02) or untreated control cells (P < 0.001). Caspase activation was studied to verify the role of apoptosis in the interaction between SU11248 and radiation. Fig. 1B shows the Western immunoblot staining for caspase 3. This shows that caspase 3 was not cleaved in endothelial cells after treatment with either SU11248 alone or radiation alone, whereas caspase 3 was cleaved in HUVECs treated with SU11248 followed by irradiation.

Prior studies have shown that VEGF receptor antagonists enhance the therapeutic effects of ionizing radiation (2, 7). To determine whether enhanced apoptotic response in endothelial cells treated with SU11248 results in reduced clonogenic cell survival, HUVECs were subcultured, and colony formation was quantitated. Fig. 2 shows the surviving fraction of HUVECs treated with vehicle or 1 μM SU11248 before the indicated dose of radiation. There was no reduced plating efficiency of HUVECs treated with SU11248 alone. HUVECs treated with SU11248 before irradiation showed a significant reduction in clonogenic survival as compared with radiation alone (∗, P < 0.05).

Growth factors produced by tumors could enhance the viability of tumor vascular endothelium. To determine whether SU11248 enhances radiation-induced destruction of tumor vasculature, SU11248 was administered to mice before irradiation with 3 Gy. Tumor vascular linear density was measured by use of intravital tumor vascular window. Fig. 3 shows the vasculature within LLC tumors implanted into the dorsal skin-fold window in C57BL6 mice. Representative photographs of tumor vasculature before and 48 h after treatment with SU11248, 3 Gy, or SU11248 followed by 3 Gy indicate that RTK inhibition increases tumor vascular destruction as compared with either agent alone. Five mice were treated in each of the treatment groups, and the vascular length density after treatment was quantified. Mean vascular length densities over 4 days are shown as a bar graph. Within 72 h, VLD in tumors was significantly reduced to 8% of that at 0 h (P < 0.01). In comparison, tumors treated with either 3 Gy or SU11248 alone showed an insignificant reduction in VLD to 75 and 84% that of 0 h, respectively. Combined SU11248 and 3 Gy achieved significantly greater reduction in VLD as compared with either agent alone (P < 0.05).

 Destruction of tumor vasculature results in ischemia, which could reduce radiosensitivity of tumors. To determine whether SU11248 enhances tumor growth delay in irradiated tumors, mice bearing LLC and GL261 hind limb tumors were treated with i.p. injection of 40 mg/kg SU11248 or drug vehicle 30 min before each 3-Gy dose of radiation for a total of seven administrations. Both the inhibitor and radiation were discontinued after day 8 (arrows, Fig. 4). The mean fold increase in tumor volumes in five mice in each of the treatment groups (vehicle, SU11248, vehicle + 21 Gy, and SU11248 + 21 Gy) are shown. Time to doubling of LLC tumor size was 5, 6, 8, and 16 days for each group, respectively. Both LLC and GL261 tumors showed a significant increase in tumor growth delay when SU11248 was added before daily 3-Gy fractions as compared with either agent alone (∗, P < 0.05).

The PDGFRβ is one of the molecular targets for SU11248 (9). Phosphorylation of PDGFRβ within tumor tissue, therefore, serves as a biomarker for response to SU11248 in tumor models. PDGFRβ phosphorylation was studied by use of the phospho-specific antibody on immunohistochemical analysis of tumor sections. Fig. 4, C and D, shows immunohistochemical staining of LLC tumors with and without SU11248 administration. This shows that PDGFRβ phosphorylation was detected in the stroma and endothelium of tumors before treatment. At 3 h after SU11248 administration, PDGFR β phosphorylation could not be detected by immunohistochemistry (Fig. 4D). This indicates that SU11248 is biologically active within mouse tumor models after systemic administration.

To determine whether prolonged growth delay correlated with reduction in tumor blood flow, amplitude modulated Power Doppler was used to monitor blood flow. Fig. 5 shows representative images of signal intensity of blood flow in GL261 tumors on day 5 of treatment. Reduced blood flow in tumors treated with SU11248 and radiation correlated with the improved tumor growth delay that was found in Fig. 4. The bar graph shows the average blood flow within GL261 tumors. Tumors treated with SU11248 and radiation approached a significant reduction in blood flow as compared with tumors treated with radiation alone (∗, P < 0.053) and a trend toward significance when compared with SU11248 alone (P = 0.15).

Upon the discontinuation of therapy, tumors rapidly regrow. To determine whether maintenance of SU11248 beyond the completion of radiation improved growth delay, this RTK inhibitor was administered twice daily continuously in groups treated with radiation alone or SU11248 and radiation. Beginning after day 8 (Fig. 6, arrow A), three groups, one drug alone, one radiation + vehicle, and one combined therapy were maintained with twice daily i.p. administrations of 20 mg/kg SU11248 for an additional 7 days. Maintenance treatment (main in Fig. 6) was discontinued after day 17 (arrow B).
and subsequently resumed after 4 days of interruption of SU11248 administration (arrow C). Fig. 6 shows the means of changes in tumor volumes in five mice in each of the treatment groups. Each of the maintenance groups showed significantly delay in growth to 4-fold that of day 0 tumors ($P < 0.05$ in all groups). Reinitiation of SU11248 at day 21 (arrow C) produced a second phase of tumor growth delay.

RTK inhibition could affect either cancer cells or the vascular endothelium within tumor microvasculature. To determine which cell type contributes to radiation sensitization by SU11248, we studied TUNEL staining of tissue sections from tumors treated with radiation, SU11248, or SU11248 followed by irradiation. Fig. 6 shows TUNEL staining of endothelial cells costained for VWF in tumor blood vessels.
sels. Tumors treated with SU11248 alone or radiation alone developed no TUNEL staining, whereas SU11248 followed by irradiation resulted in TUNEL staining positive in endothelial cells (Fig. 6D). We found no tumor cells staining positive with TUNEL stain after treatment with SU11248 and radiation.

**DISCUSSION**

Local and regional recurrences continue to impact survival in many neoplasms, especially malignant gliomas, head and neck cancer, and lung cancer. This study addresses two strategies to improve tumor control after radiotherapy. First, RTK antagonists attenuate signaling through viability pathways in tumor vascular endothelium, resulting in enhancement of cytotoxic effects. Second, a maintenance schedule of antiangiogenic therapy could prevent or delay progression of neoplastic growth.

Although the predominant effect of SU11248 may be at the level of tumor vascular endothelium, PDGFR antagonists could prevent tumor regrowth by direct effect on tumor cells. For example, the PDGFR inhibitor STI571 has been somewhat effective in treatment of solid tumors (17, 18). Several other human cancers may overexpress c-Kit or PDGFR, and clinical trials to evaluate the role of STI571 in the treatment of such cancers are currently ongoing (17). STI571 inhibited PDGF-mediated growth and lead to apoptosis of osteosarcoma cells in vitro by selective inhibition of the PDGFR tyrosine kinase (18). Although SU11248 could prevent tumor growth through inhibition of tumor cell proliferation, in this study, apoptosis (TUNEL staining) was limited to tumor vascular endothelial cells. This indicates that the primary site of interaction between SU11248 and radiation was at the vascular endothelium.

With regard to enhancement of cytotoxicity in tumor vascular endothelium, we found reduced clonogenic survival in irradiated endothelial cells pretreated with SU11248 as compared with radiation alone. The proposed mechanism of interaction between RTK antagonists and cytotoxic therapy is the attenuation of signal transduction through the phosphatidylinositol 3′-kinase/Akt pathway (19). Attenuation of this pathway alters the levels of Bcl gene products, resulting in increased susceptibility to apoptosis (20). This model of diminished cell viability is supported by this study, which shows SU11248 significantly increased radiation-induced apoptosis in vascular endothelial cells pretreated with the broad spectrum RTK antagonist SU11248. Caspase 3 was cleaved only in HUVECs treated with both SU11248 and radiation but not either agent alone. This suggests that RTK inhibition, together with cytotoxic therapy, may be a beneficial combination in cancer therapy. The enhancement in radiation-induced cytotoxicity was also measured by clonogenic assay, which shows a subtle, albeit significant reduction in surviving fraction of endothelial cells. This interaction between SU11248 and radiation was minimized by washing the kinase inhibitor off of cultures after 1-h incubation. Washing and feeding of cell cultures were required because colonies will not form if this VEGF receptor antagonist remains on cells during clonogenic assays. Taken together with positive TUNEL staining in tumor endothelium, these data support the notion that apoptosis is one mode of endothelial cell death when SU11248 is combined with radiation.

Tumors produce growth factors that could contribute to tumor resistance to cytotoxic therapy. Moreover, growth factors that activate RTKs on tumor microvascular endothelium include VEGF, PDGF, as well as others. This study addresses the contribution of RTK signaling to radioresistance. SU11248 blocks signaling from a broad spectrum of receptors, which could be advantageous (9). Several methods have been described to study microvessel density, including fluorescent dyes (21) and vascular staining (22). Two measures of tumor vascular lature (Doppler and Vascular Window) were used to assess the effectiveness of SU11248 at enhancing radiation-induced tumor vascular destruction. Intravital tumor vascular window provided a measure of vascular length density that was significantly reduced when SU11248 was added before irradiation. This finding was supported by amplitude modulated Doppler blood flow measurement, which showed reduced microvascular blood flow when tumors were treated with SU11248 together with radiation as compared with either agent alone. Taken together, these findings suggest that RTK inhibition enhances the therapeutic effect of radiation, in part, through improved tumor vascular destruction. The contribution of tumor vascular destruction on tumor control is additionally supported by previous studies that show antiangiogenic agents enhance radiation-induced tumor control (2, 7, 16, 23–29). Mechanisms of interaction between SU11248 and radiation could involve increased cytotoxicity within vascular endothelium or within tumor cells. Histological assessment of each of these cellular components consisted of TUNEL staining, which showed only vascular endothelial cells undergoing apoptosis while tumor cells showed no response. This finding indicates that the predominant mechanism of interaction between this VEGF receptor antagonist and radiation is within the tumor blood vessels.

Diminished tumor blood flow results in hypoxia, which could contribute to radioresistance (30). The effect of hypoxia could be overcome by the concomitant reduction in nutrients and growth factors achieved by ischemia. To evaluate whether the ischemia induced by SU11248 and radiation has an impact on tumor control, we studied growth delay in hind limb tumors. This showed that destruction of tumor vasculature correlates with improved growth delay as compared with tumors treated with radiation alone. Although hypoxia could have a lessening effect upon the interaction between these agents, there was a significant improvement in tumor growth delay.

Upon completion of treatment with SU11248 and radiation, tumors rapidly regrew. SU11248 was developed as an antiangiogenic agent (9). The study of maintaining SU11248 after the completion of radiation indicates that tumor growth delay can be improved by continuation of this antiangiogenic agent. This maintenance schedule significantly delayed regrowth as compared with tumors receiving no maintenance SU11248. Prior studies have shown that resumption of antiangiogenic agents can again accomplished tumor control (31). Likewise, the resumption of SU11248 additionally delayed tumor regrowth. The clinical significance of this schedule of administration is that ongoing Phase I trials show accumulation of SU11248 necessitating drug holidays. We found that resistance to antiangiogenic therapy does not develop in mice bearing GL261 tumor grafts.

**ACKNOWLEDGMENTS**

We thank Allie Fu and Halina Onishko for technical support.

**REFERENCES**


SU11248 Maintenance Therapy Prevents Tumor Regrowth after Fractionated Irradiation of Murine Tumor Models

Aaron J. Schueneman, Eric Himmelfarb, Ling Geng, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/14/4009

Cited articles
This article cites 31 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/14/4009.full#ref-list-1

Citing articles
This article has been cited by 26 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/63/14/4009.full#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.