HIV Protease Inhibitors Enhance the Efficacy of Irradiation

Kyle C. Cuneo,1 Tianxiang Tu,2 Ling Geng,2 Allie Fu,2 Dennis E. Hallahan,2,3 and Christopher D. Willey2

1Vanderbilt University School of Medicine; Departments of 2Radiation Oncology and 3Cancer Biology, Vanderbilt University, Nashville, Tennessee

Abstract
Tumor vascular endothelium is rather resistant to the cytotoxic effects of radiation. The HIV protease inhibitors (HPI) amprenavir, nelfinavir, and saquinavir have previously been shown to sensitize tumor cells to the cytotoxic effects of radiation. Additionally, this class of drug has been shown to inhibit angiogenesis and tumor cell migration. Therefore, in the current study, we wanted to determine whether HPIs could enhance the effect of radiation on endothelial function. Our study shows that HPIs, particularly nelfinavir, significantly enhance radiation’s effect on human umbilical vein endothelial cells (HUVEC) and tumor vascular endothelium. We show that pretreatment of HUVEC with nelfinavir results in enhanced cytotoxicity, including increased apoptosis, when combined with radiation. Moreover, using several functional assays, we show that combination treatment effectively blocks endothelial cell migration and organization. These findings were accompanied by attenuation of Akt phosphorylation, a known pathway for radioresistance. Last, in vivo analysis of tumor microvasculature destruction showed a more than additive effect for nelfinavir and radiation. This study shows that HPIs can enhance the effect of ionizing radiation on vascular endothelium. Therefore, the Food and Drug Administration–approved drug, nelfinavir, may be an effective radiosensitizer in the clinic. [Cancer Res 2007;67(10):4886–93]

Introduction
HIV protease inhibitors (HPI) mimic endogenous peptides and inhibit the active site of HIV aspartyl protease, a viral enzyme responsible for cleaving the gag-pol polyprotein (1). This class of drug has been very effective in controlling the effects of HIV in patients. Although these molecules were designed to specifically inhibit viral enzymes, this class of drug affects many metabolic processes. Long-term side effects of treatment include hyperbilirubinemia, insulin resistance, dyslipidemia, and bone disease (2).

Recently, HPIs have been shown to have antitumor effects. Kaposi’s sarcoma is an AIDS-defining illness characterized by proliferation of highly vascular tumor nodules (3, 4). Use of HPIs has a well-documented ability to inhibit the growth of these lesions in patients (5). Initially, it was believed that a restoration of immune function was the contributing mechanism; however, studies using non-HIV–infected models have shown that HPIs directly affect tumor cell proliferation and related angiogenesis (6, 7). Further research has shown that HPIs inhibit tumor cell proliferation in non–Hodgkin’s lymphoma (5), multiple myeloma (8), prostate carcinoma (9), breast carcinoma (10), head and neck carcinoma (11), and other forms of cancer. Although the mechanism of action is unknown, this class of drug has been shown to affect a number of molecular targets, including Akt (9, 10, 12), extracellular signal-regulated kinase (Erk; ref. 8), signal transducers and activators of transcription 3 (8, 9), nuclear factor-κB (NF-κB; ref. 13), matrix metalloproteinase (MMP; ref. 14), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF; ref. 15).

In addition to blocking tumor cell proliferation, HPIs enhance the cytotoxic effects of ionizing radiation (12, 16). Gupta et al. (12) showed that amprenavir, nelfinavir, and saquinavir attenuated Akt activation and sensitized cell lines with a constitutively active phosphatidylinositol 3-kinase (PI3K) to the cytotoxic effects of ionizing radiation. Phosphorylation of Akt after radiation is known to induce survival signaling pathways, including the inhibition of GSK-3β and activation of p53 and NF-κB (17). Targeting this pathway has the potential to affect the cellular response to ionizing radiation.

We and others have shown that radiation-induced signal transduction through the PI3K/Akt pathway contributes to endothelial cell viability (18, 19). Furthermore, the inhibition of VEGF receptor signaling affects radiation-induced destruction of tumor vasculature by blocking PI3K/Akt signaling (20–22). Most recently, nelfinavir has been shown to down-regulate VEGF expression in tumor cells via hypoxia-inducible factor 1α to contribute to radiation sensitivity (23). Therefore, because HPIs have been shown to have antiangiogenic properties (6, 11) and attenuate Akt activation in tumor cells, we decided to investigate them within irradiated vasculature. In the current study, we examined the effect of HPIs on irradiated vascular endothelial cells and the ability of these inhibitors to enhance the effects of ionizing radiation on endothelial function.

Materials and Methods
Cell culture. Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics and maintained in EBM-2 medium supplemented with EGM-2 MV Singlequots (Cambrex). Lewis lung carcinoma cells were obtained from American Type Tissue Culture and maintained in high glucose (4.5 g) DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. All cell lines were incubated at 37°C in a 5% CO2 incubator. Only cell passages 3 to 5 were used.

Amprenavir, nelfinavir, and saquinavir were obtained from the Vanderbilt University Medical Center pharmacy and stored in the dark at 4°C. Amprenavir and saquinavir gel capsules were punctured and diluted to 10 μmol/L in ethanol. Nelfinavir pills were crushed and diluted to 5 μmol/L in ethanol. Drugs were administered to cells 30 to 60 min or 18 h before irradiation. A Mark-1 irradiator 137Cs (JL Shepard and Associates) was used to irradiate HUVEC cultures at a dose rate of 1.897 Gy/min.

Cell proliferation assay. Passage 3 to 5 HUVECs were grown to 70% confluency, suspended, and subcultured into a 96-well plate at a concentration of 5,000 cells per well. The following day, cells were treated
with the inhibitors at several dilutions. Twenty-four or 48 h later, 10,000 cells were counted by an observer who was blinded to the experimental conditions for each of the cultures. The percentage of cells dividing the dose (Gy) for radiation alone by the dose for radiation plus nelfinavir (normalized for plating efficiency of nelfinavir) for which a reduction in absorbance was seen after treatment with 50 μM nelfinavir, 40 μM saquinavir, and 100 μM ampranavir. Twenty-four hours later, cells were stained with WST-1 reagent and absorbance was analyzed by a fluorescent plate reader. Figure 1A shows the effect of various concentrations of HPIs on endothelial cell proliferation. A 50% reduction in absorbance was seen after treatment with 5 μM/L nelfinavir, 40 μM/L saquinavir, and 100 μM/L ampranavir. Treatment with >10 μM/L nelfinavir resulted in minimal cell proliferation. The effect of HPIs on endothelial cell proliferation was verified using manual cell counts 48 h after treatment (data not shown). Therefore, nelfinavir was selected as the best inhibitor.

Nelfinavir sensitizes endothelial cells to the cytotoxic effects of ionizing radiation. To determine whether nelfinavir can sensitize endothelial cells to the cytotoxic effects of ionizing radiation, HUVECs were plated onto fibronectin-lined culture dishes. HUVECs were treated with or without nelfinavir, incubated for 60 min, and then irradiated at 3 Gy. Cells were incubated for 24 h then stained with DAPI. Fifty high-power fields were scanned in a fluorescence microscope. Experiments were done at least thrice to ensure reproducibility.

Confluent survival. Passage 3 to 5 HUVECs were grown to 70% to 80% confluence. Cells were washed with PBS, suspended with trypsin, and adjusted to specific densities for each condition. The cells were then plated on fibronectin-coated plates (BD Biosciences) and allowed to attach for 4 h. Inhibitors were added at 1:1,000 dilution to both chambers, and the plates were irradiated 60 min later. The mean and SE of vascular density within the microscopic field was used to calculate clonogenic survival. The mean and SE were calculated for each treatment group. Nelfinavir (30 mg/kg) was administered by p.o. gavage 200 μl of top layer of cells (nonmigrated cells) was removed with a cotton swab. The insert chambers were washed with PBS, fixed in methanol, and stained with DAPI. Five high-power fields from each sample were counted by fluorescent microscopy.

Endothelial closure assay. Passage 3 to 5 HUVECs were grown to 70% to 80% confluence. Four parallel wounds were created on each plate using a 200 μl pipette tip. Inhibitors were added at a 1:1,000 dilution and irradiated 60 min later by 3 Gy. Twelve or 24 h later, plates were fixed with 70% ethanol and stained with 1% methylene blue. Photographs were taken and relative cell density was calculated as follows: (number of cells / original wound area) / (number of cells / nonwound area).

Tumor vascular window model. We studied the time- and dose-dependent response of tumor blood vessels to radiation using the window model as previously described (24). Briefly, three mice were studied in each of the treatment groups. Nelfinavir (30 mg/kg) was administered by p.o. gavage 60 min before irradiation. The vascular windows were treated with 2 Gy of superficial X-rays using 80 kVp (Pantak X-ray Generator, East Haven, CT). The window frame was marked with coordinates, which were used to photograph the same microscopic field each day. Tumor blood vessels were quantified using the ImagePro software by determining the vascular length density of blood vessels within the microscopic field. The mean and SE of vascular length density for each treatment group were calculated. All animals used were cared for according to the Institutional Animal Care and Use Committees guidelines.

Tumor immunohistochemistry. Lewis lung carcinoma cells were injected s.c. into the hind limb of C57/BL6 mice. After tumors grew to 200 mm^3 (1-1 week), the mice were treated with five consecutive daily treatments of 30 mg/kg p.o. nelfinavir followed 30 min later by 2 Gy irradiation using an 80 kVp superficial X-ray generator. Twelve hours after the last treatment, mice were sacrificed and tumors were harvested, fixed in paraffin, and sectioned by the Vanderbilt University Immunohistochemistry Core Facility. These sections were stained for anti-CD34 (1:100; Santa Cruz Biotechnology) and anti–von Willebrand factor (1:900; Dakocytomation) as we have done before (25, 26). Microvascular photos were analyzed using ImagePro software to quantify staining.

Statistical analysis. The mean and SE were calculated using Microsoft Excel software. Student's t test was used to determine P values between treatment groups. P values ≤0.05 were considered statistically significant.

Results

Effect of HPIs on endothelial cell proliferation. To determine which HPI shows the greatest effect on endothelial cells, we studied proliferation of endothelial cells in vitro. HUVECs were subcultured in 96-well plates, treated with 0 to 100 μM/L of ampranavir, nelfinavir, and saquinavir. Twenty-four hours later, cells were stained with WST-1 reagent and absorbance was analyzed by a fluorescent plate reader. Figure 1A shows the effect of various concentrations of HPIs on endothelial cell proliferation. A 50% reduction in absorbance was seen after treatment with 5 μM/L nelfinavir, 40 μM/L saquinavir, and 100 μM/L ampranavir. Treatment with >10 μM/L nelfinavir resulted in minimal cell proliferation. The effect of HPIs on endothelial cell proliferation was verified using manual cell counts 48 h after treatment (data not shown). Therefore, nelfinavir was selected as the best inhibitor.

Nelfinavir sensitizes endothelial cells to the cytotoxic effects of ionizing radiation. To determine whether nelfinavir can sensitize endothelial cells to the cytotoxic effects of ionizing radiation, HUVECs were plated onto fibronectin-lined culture dishes. HUVECs were treated with or without nelfinavir, incubated for 60 min, and then irradiated at 3 Gy. Cells were incubated for 24 h then stained with DAPI. Fifty high-power fields were scanned in a fluorescence microscope. Experiments were done at least thrice to ensure reproducibility.
for 18 h was more effective than 1 h; however, both treatments resulted in a statistically significant difference at 2, 4, and 6 Gy (P < 0.05). The dose enhancement ratios for 1 and 18 h nelfinavir were 1.31 and 1.58, respectively. Treatment with amprenavir resulted in no significant change, whereas treatment with saquinavir resulted in no colony formation in nonirradiated and irradiated groups (data not shown).

**Effects of HPIs on radiation-induced signal transduction.** To determine the mechanism of interaction between nelfinavir and radiation, Western blot analysis was done to study the effect of protease inhibition on radiation-induced signal transduction. Because nelfinavir seemed to have the most efficacy in the proliferation assay, we selected nelfinavir for the subsequent assays.

HUVECs were treated with either vehicle control (ethanol) or 5 μmol/L nelfinavir for 18 h followed by sham irradiation or 4 Gy then processed immediately (0 min), 5, 15, and 30 min later. Figure 2A shows immunoblots using phospho-Akt, total Akt, phospho-Erk, total Erk, and actin antibodies for radiation induced phosphorylation of Akt and Erk in HUVEC after 18 h preincubation with nelfinavir. Treatment with nelfinavir effectively attenuated radiation-induced Akt activation after 18 h pretreatment, which correlates with the clonogenic data shown in Fig. 1. Densitometric analysis of three separate experiments (Fig. 2B) shows that nelfinavir attenuated both basal and radiation-induced Akt activation. The effect of nelfinavir on Erk signal transduction was less pronounced. Of note, the phospho-p42Erk (bottom band) has greater density than the p44Erk (top band). This seems to be tissue and species dependent as other groups have shown a similar pattern (27–29).

**HPIs enhance radiation-induced apoptosis.** Vascular endothelium show little apoptosis in response to 4 to 6 Gy but inhibition of Akt signaling enhances radiation-induced apoptosis (19, 30). To determine whether nelfinavir enhances radiation-induced apoptosis, we studied caspase activation and pyknotic nuclei. HUVECs were treated with HPIs for 1 h followed by 4 or 6 Gy. Six hours later, cells were harvested and processed for Western blot analysis.
Figure 3A shows the resulting immunoblot using anti–caspase 3, cleaved caspase-3, and actin antibodies. Treatment with radiation resulted in caspase cleavage at 6 h and treatment with nelfinavir before radiation enhanced this response. Using the same treatment conditions, cell nuclei were stained with DAPI 18 h after irradiation and pyknotic nuclei were quantified by microscopy (Fig. 3B). Treatment with nelfinavir alone resulted in a significant increase in the percentage of apoptotic nuclei compared with controls (P = 0.05), whereas treatment with irradiation alone was not statistically different from controls. When cells were treated with nelfinavir followed by radiation, the level of apoptosis was significantly increased by a factor of 4 for 4 Gy (P = 0.04) and factor of 8 for 6 Gy (P = 0.01) compared with control.

**HPIs and radiation attenuate tubule formation.** To determine the effects of HPIs on physiologic function of endothelial cells, tubule formation in Matrigel was studied. HUVECs were plated onto Matrigel-lined wells, were treated with or without 5 μM nelfinavir, followed by sham irradiation or 3 Gy, and then monitored for tubule formation. Shown are representative photographs (Fig. 4A) and the mean and SE (n = 4) for each treatment condition (Fig. 4B). The use of radiation alone did not have a significant effect on tubule formation. However, use of nelfinavir alone reduced the number of tubules by ~20% (P = 0.05) and combined treatment significantly reduced the number of tubules formed by 50% (P < 0.001). The reduction seen with combined treatment was greater than what would be predicted by an additive effect of either agent alone.

**Effect of HPIs on endothelial cell migration.** To determine the effect of HPIs on endothelial cell migration, we studied migration using both Boyden chambers and endothelial cell closure assays. HUVECs were plated onto an 8 μm pore membrane then treated with nelfinavir and/or radiation. Twenty-four hours later, cells that had migrated across the membrane were fixed and stained. Figure 5A shows the mean and SE (n = 4) of the number of migrated cells per high power field. Use of radiation alone or nelfinavir alone significantly decreased endothelial migration by 25% (P = 0.03) and 15% (P = 0.04), respectively. Combined treatment inhibited endothelial cell migration by 60% compared with control (P < 0.001), suggesting a more than additive effect.

Endothelial cell closure assay was studied in HUVECs grown to 80% confluency on 6-cm plates. A wound area free of cells was created using a 200 μL pipette tip. Plates were then treated with nelfinavir followed 1 h later by 3 Gy. Cells were fixed and stained 12 and 24 h later. Figure 5B shows representative photographs for each treatment condition. The mean relative cell density in the wound area and SE (n = 4) for each treatment condition are shown in Fig. 5C. By 24 h, control plates had 100% closure. Use of radiation alone resulted in a reduced number of cells 24 h after treatment; however, when normalized for this reduction, the amount of closure was comparable with control. Treatment with nelfinavir alone attenuated wound closure at 12 and 24 h by ~50% compared with control plates at the corresponding time points (P < 0.001). Combined treatment with nelfinavir and 3 Gy synergistically attenuated endothelial closure by over 80% at 12 h (P < 0.001) and 75% at 24 h (P < 0.001) compared with controls.

**Effect of nelfinavir on blood vessel destruction in the vascular window model.** To determine the effect of nelfinavir on blood vessel destruction in vivo, we studied both tumor vascular...
window model and density of tumor microvasculature on histologic sections of tumor. The vascular window is a transparent chamber inserted onto the dorsal skin fold of mice allowing for visualization of blood vessel formation and destruction. Mice were fitted with the window chamber and injected with Lewis lung carcinoma cells. Once vessels formed, mice received one treatment of 30 mg/kg nelfinavir via gastric gavage followed 1 h later by 2 Gy. Treatment with nelfinavir alone attenuated blood vessel growth but had a minimal effect of vessel destruction. Compared with either control, combined treatment with radiation and nelfinavir resulted in a lower density of blood vessels at each time point and nearly obliterated visible blood vessels 72 h after treatment. Representative photographs are shown in Fig. 6A. Figure 6B shows the average vascular length density and SE (n = 3) for each treatment condition.

The effect of nelfinavir on blood vessel density was studied in tumor xenografts. Lewis lung carcinoma cells were grown in the hind limb of C57BL6 mice. Once tumors were formed, the mice were treated with five consecutive treatments of nelfinavir and/or 2 Gy fractions of ionizing radiation. The tumors were then harvested and stained with anti-CD34 antibodies. Figure 6 shows representative photographs (C) and the mean and SE (D) for each treatment condition. Use of irradiation or nelfinavir alone significantly reduced the amount vasculature by 50% (P = 0.025) and 20% (P = 0.03) compared with control, respectively. Treatment with both nelfinavir and radiation reduced the level of CD34 staining by over 90% (P = 0.002). These data suggests that nelfinavir attenuates blood vessel formation and this effect is enhanced by radiation.

Discussion

Patients receiving highly active antiretroviral therapy often have serum concentrations of protease inhibitors in the 1 to 10 μmol/L range. In the current study, we analyzed the effect of HPI concentration on endothelial cell proliferation. Nelfinavir was the...
most effective at inhibiting endothelial cell proliferation at these physiologic concentrations, whereas amprenavir and saquinavir required concentrations four to five times higher to have similar effects. These data indicate that nelfinavir effectively attenuates endothelial cell growth and is more effective at concentrations routinely reached in patients than the other protease inhibitors.

Irradiation of endothelium induces signal transduction through the PI3K/Akt and mitogen-activated protein kinase/Erk pathways. Compounds that block these pathways have been shown to be effective radiosensitizers. Previous studies have indicated that HPIs can attenuate Akt (31) and Erk activation (8). Furthermore, a recent report suggests that HPIs have the ability to sensitize tumor cells with constitutively active PI3K signaling to the cytotoxic effects of ionizing radiation (12). We and others have previously shown that the PI3K/Akt pathway is activated in HUVECs after ionizing radiation (18, 22, 30, 32, 33) and compounds that inhibit this pathway sensitize endothelial cells to radiation. In the current study, nelfinavir was found to attenuate the phosphorylation of Akt as well as Erk to a lesser extent after 3 Gy, a clinically relevant dose. The other HPIs were less effective at inhibiting these pathways.

Because the PI3K/Akt pathway is stimulated by radiation and this pathway is active in endothelial cells, we expected to find that nelfinavir sensitizes endothelial cells to radiation. In the clonogenic assays, treatment with nelfinavir for either 1 or 18 h before radiation achieved radiosensitization. Interestingly, treatment with nelfinavir for 18 h produced a greater radiosensitizing effect. The enhancement with increased treatment time suggests that the mechanism could be multifactorial, possibly involving genetic and/or epigenetic changes. Previous studies have attributed the radiosensitizing effects of HPIs to down-regulation of hypoxia-inducible factor 1α and VEGF (23) and an inhibition of proteasome...

Figure 6. Vascular window model and tumor xenograft model. A transparent chamber was inserted onto the dorsal skin fold of C57/BL6 mice to allow for visualization of blood vessels. Lewis lung carcinoma cells were injected into the chamber and once vessels formed (6–8 d), the mice were treated with one p.o. dose of 30 mg/kg nelfinavir followed 1 h later by 2 Gy. Images were taken daily and the density of blood vessels was quantified for each treatment group. A, representative photographs. B, points, mean vascular length density (n = 3) for each treatment group; bars, SE. C, Lewis lung carcinoma cells were implanted into the hind limb of C57/BL6 mice. Once tumors formed, the mice were treated with five consecutive daily treatments of 30 mg/kg nelfinavir and/or 2 Gy fractions. Tumors were harvested and stained for anti-CD34. Microscopic photos of immunohistochemistry. XRT, radiation therapy. D, the mean level of CD34 staining and SE were calculated for each treatment condition. *P < 0.03, **P < 0.001.
function affecting NF-κB activation (16). However, one possible mechanism is that of inhibition of Akt. Several groups have shown nelfinavir-induced Akt inhibition in a variety of cell types, including INS-1 cells (34), prostate cancer cells lines LNCaP and PC3 (9), 3T3-L1 adipocytes (31), L6 myotubes (35), as well as SQ20B and A549 head and neck and lung cancer cell lines, respectively (12, 23). For all of these studies, the effects were seen after 18 h of nelfinavir incubation, which is consistent with our data.

The process of angiogenesis involves invasion, migration, and proliferation (15). HPIs have been shown to attenuate angiogenesis in numerous tumor models. Previous reports have shown that attenuating the P53/Akt pathway enhances the antiangiogenic effect of ionizing radiation (15, 22, 30, 36, 37). In the current study, we examined the effect of HPIs and radiation on endothelial cell function. Three in vitro functional assays were done using low-dose radiation. A dose of 3 Gy alone had minimal effect on endothelial cell function; however, use of HPIs before treatment attenuated tubule formation, cell migration, and wound closure independent of cell death. The effect seen in these studies was greater than what would be predicted by an additive effect of either agent alone. Although nelfinavir was most effective in blocking endothelial cell function, the other protease inhibitors showed similar antiangiogenic and migratory effects (data not shown). Because amprenavir and saquinavir had minimal effects on attenuating radiation-induced Akt phosphorylation (data not shown), the mechanism behind this response is likely multifactorial. Indeed, several reports have shown that HPIs inhibit MMP degradation, alter levels of bFGF and VEGF, and affect activation of NF-κB (23, 38).

To determine efficacy in vivo, two models of vascular formation were studied. The vascular window model is an effective means to quantify the effects of a compound on angiogenesis and blood vessel destruction in vivo. In the current study, a single p.o. dose of nelfinavir followed by 2 Gy obliterated tumor blood vessels within 72 h. Treatment with 2 Gy alone or nelfinavir alone had minimal effect on blood vessel destruction during the same time course. The in vitro studies indicated that nelfinavir in combination with irradiation directly affects endothelial function. Previous reports have further shown the protease inhibitors can attenuate bFGF- and VEGF-induced angiogenesis (15). Both the direct effects on endothelial cells and indirect effects through tumor cells and surrounding connective tissue are likely affecting angiogenesis.

The toxicity profile of this class of drug and the long-term effects on metabolism suggest that HPIs affect many cellular processes of normal tissue. Indeed, HPI-induced insulin resistance, lipodystrophy, and accelerated atherosclerosis have been shown in patients in what is sometimes described as highly active antiretroviral therapy–associated metabolic syndrome. Multiple potential mechanisms have been suggested, including increased free fatty acid production, decreased perilipin, increased intracellular reactive oxygen species, and inhibition of GLUT4, PKC, and Akt (39). As such, there is the possibility of normal tissue toxicity from concomitant nelfinavir and radiation treatment. However, nelfinavir seems to have less normal vascular toxicity compared with other HPIs based on studies in porcine coronary arteries (40). In addition, some of the studies that have shown significant apoptosis and reactive oxygen species production after nelfinavir treatment used doses up to four times higher than what was used in our study (39). In our study, although nelfinavir alone had some biological effects on the endothelial cells, dramatic effects were only seen when used in combination with radiation. The clinical data for radiation treatment in combination with highly active antiretroviral therapy is limited but promising. Retrospective reviews have not only shown better survival outcomes but also less treatment-related toxicity with the addition of highly active antiretroviral therapy to chemoradiation for HIV-positive patients with anal carcinoma (41, 42).

HPIs have over 10 years of safety data. The side effects of these compounds are tolerable and the pharmacokinetics and pharmacodynamics have been well characterized. For these reasons, this class of drug has potential as an antineoplastic agent. In the current study, concentrations of amprenavir, nelfinavir, and saquinavir routinely achieved in HIV patients were used in a HUVEC model and in the in vivo vascular window model. The compounds effectively enhanced the antiangiogenic effect of ionizing radiation and are promising agents for future clinical trials.

Acknowledgments

Received 10/4/2006; revised 1/31/2007; accepted 3/6/2007.

Grant support: U24 DK93637 and P30 CA64885-08 (for immunohistochemical experiments); R01CA64885, B01-CAN9674, B01-CA88076, P50-CA90949, P30-CA64885, Vanderbilt-Ingram Cancer Center, and Ingram Charitable Fund.

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.

References

16. Pajonk F, Himmelsbach J, Riess K, Sommer A, McBr=H. The human immunodeficiency virus (HIV)-1 protease inhibitor saquinavir inhibits protea-
some function and causes apoptosis and radiosensitiza-
17. Mitsiades CS, Mitsiades N, Koutsilieris M. The Akt path-
way: molecular targets for anti-cancer drug devel-
18. Edwards E, Geng L, Tan J, Onishko H, Donnelly E, Hallahan DE. Phosphatidylinositol 3-kinase/Akt signal-
19. Tan J, Hallahan DE. Growth factor-independent activation of protein kinase B contributes to the inherent resistance of vascular endothelium to radia-
23. Pore N, Gupta AK, Cerniglia GJ, et al. Nelfinavir down-regulates hypoxia-inducible factor 1α and VEGF expression and increases tumor oxygenation: implica-
mediated, vasculature-targeted therapy using quantified power Doppler sonography: implications for improve-
30. Geng L, Tan J, Himmelstahl E, et al. A specific antagonist of the p110α catalytic component of phosphatidylinositol 3-kinase, IC486608, enhances radi-
32. Lee CM, Fuhrman CB, Planelles V, et al. Phosphatid-
ylinositol 3-kinase inhibition by LY294002 radiosensi-
37. Kumar P, Benedict R, Urraza F, Fischbach C, Mooney D, Polverini P. Combination treatment signif-
38. Monini P, Sgadari C, Barillari G, Eosoli R. HIV protease inhibitors: antitumor agents with envi-
42. Blazy A, Hennequin C, Gornet JM, et al. Anal carcinomas in HIV-positive patients: high-dose che-
HIV Protease Inhibitors Enhance the Efficacy of Irradiation

Kyle C. Cuneo, Tianxiang Tu, Ling Geng, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/10/4886

Cited articles
This article cites 42 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/10/4886.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/67/10/4886.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.