

# Preclinical Evaluation of a Potent Novel DNA-Dependent Protein Kinase Inhibitor NU7441

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## Abstract

**DNA double-strand breaks (DSB) are the most cytotoxic lesions induced by ionizing radiation and topoisomerase II poisons, such as etoposide and doxorubicin. A major pathway for the repair of DSB is nonhomologous end joining, which requires DNA-dependent protein kinase (DNA-PK) activity. We investigated the therapeutic use of a potent, specific DNA-PK inhibitor (NU7441) in models of human cancer. We measured chemosensitization by NU7441 of topoisomerase II poisons and radiosensitization in cells deficient and proficient in DNA-PK<sub>CS</sub> (V3 and V3-YAC) and p53 wild type (LoVo) and p53 mutant (SW620) human colon cancer cell lines by clonogenic survival assay. Effects of NU7441 on DSB repair and cell cycle arrest were measured by  $\gamma$ H2AX foci and flow cytometry. Tissue distribution of NU7441 and potentiation of etoposide activity were determined in mice bearing SW620 tumors. NU7441 increased the cytotoxicity of ionizing radiation and etoposide in SW620, LoVo, and V3-YAC cells but not in V3 cells, confirming that potentiation was due to DNA-PK inhibition. NU7441 substantially retarded the repair of ionizing radiation-induced and etoposide-induced DSB. NU7441 appreciably increased G<sub>2</sub>-M accumulation induced by ionizing radiation, etoposide, and doxorubicin in both SW620 and LoVo cells. In mice bearing SW620 xenografts, NU7441 concentrations in the tumor necessary for chemopotentialization *in vitro* were maintained for at least 4 hours at nontoxic doses. NU7441 increased etoposide-induced tumor growth delay 2-fold without exacerbating etoposide toxicity to unacceptable levels. In conclusion, NU7441 shows sufficient proof of principle through *in vitro* and *in vivo* chemosensitization and radiosensitization to justify further development of DNA-PK inhibitors for clinical use.** (Cancer Res 2006; 66(10): 5354-62)

## Introduction

Ionizing radiation and the topoisomerase II poisons, such as etoposide and doxorubicin, are agents widely used in the treatment of a variety of human solid malignancies and leukemia (refs. 1–4; <http://www.cancerhelp.org.uk>). The principal cytotoxic lesion induced by these agents is the DNA double-strand break (DSB; ref. 5). DSBs are considered the most lethal type of DNA lesion, and a single DSB may be enough to kill a cell (6–8). DSBs can be caused

endogenously or by exogenous toxins as well as therapeutics, and, if left unrepaired, they will trigger cell cycle arrest and/or cell death. Cells have, of necessity, developed complex mechanisms to repair DSBs, and such repair constitutes a potential mechanism of therapeutic resistance.

In mammalian cells, homologous recombination and nonhomologous end-joining (NHEJ) are the major DSB repair pathways (9, 10). The principal factor determining the choice of mechanism is the cell cycle stage (11, 12). Homologous recombination is a complex error-free pathway, which is dependent on the proximity of the sister chromatid and can therefore only take place in late S and the G<sub>2</sub> phases of the cell cycle. DSB repair is also achieved by means of NHEJ, which brings the broken ends together and rejoins them without reference to a second template. This pathway is, therefore, predominant during G<sub>1</sub> or G<sub>0</sub> phases, but it is also operational at other phases of the cell cycle (13).

The core NHEJ machinery consists of the Ku70/80 heterodimer and the catalytic subunit of DNA-dependent protein kinase (DNA-PK<sub>CS</sub>), which together make the active DNA-PK, XRCC4, ligase IV, and the endonuclease artemis. Ku locates at the break first, recruiting DNA-PK<sub>CS</sub> to bring about synapsis of the ends. Artemis processes the DNA ends, and the final ligation of the juxtaposed ends is accomplished by the XRCC4/ligase IV complex (14–16). DNA-PK has been shown to phosphorylate several cellular proteins *in vivo*, including itself and the variant histone H2AX (15). Cells deficient in components of the NHEJ pathway are defective in DSB repair and highly sensitive to ionizing radiation and topoisomerase II poisons (17–24). Moreover, overexpression of DNA-PK<sub>CS</sub> can accelerate the repair of ionizing radiation-induced, etoposide-induced, and doxorubicin-induced DNA DSBs and confer resistance to these agents (25, 26). DNA-PK may also be responsible for chemoresistance and radioresistance in the clinical setting; studies have shown that DNA-PK protein expression correlates with sensitivity to etoposide in human chronic lymphocytic leukemia samples (27).

Inhibition of NHEJ is therefore an attractive approach to modulating resistance to therapeutically induced DNA DSBs. DNA-PK is a member of the phosphatidylinositol-3 kinase (PI3K)-related protein kinase (PIKK) family of enzymes. Inhibitors of PI3K, such as wortmannin and LY294002, also inhibit DNA-PK in a non-competitive and competitive manner, respectively (28). Both wortmannin and LY294002 have been reported to retard DSB repair and enhance the cytotoxicity of ionizing radiation and etoposide, which has largely been attributed to inhibition of DNA-PK<sub>CS</sub> (29–31). Using LY294002 as a lead, a more potent and specific inhibitor of DNA-PK<sub>CS</sub>, NU7026 (2-(morpholin-4-yl)-benzo[h]chromen-4-one), was developed, which also increased the cytotoxicity of both ionizing radiation and topoisomerase II poisons and retarded DSB repair (24, 32). Further compound elaboration has identified

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NU7441 (2-*N*-morpholino-8-dibenzothiophenyl-chromen-4-one) as a yet more potent and specific inhibitor of DNA-PK, with an IC<sub>50</sub> of only 14 nmol/L and at least 100-fold selectivity for this enzyme compared with other PI3KK family kinases (33, 34).

We report here the preclinical evaluation of NU7441, showing that (a) potentiation of ionizing radiation and etoposide cytotoxicity by NU7441 is due to DNA-PK inhibition using Chinese hamster ovary cells deficient and complemented with DNA-PK<sub>CS</sub>; (b) NU7441 increases the persistence of  $\gamma$ H2AX foci after ionizing radiation-induced or etoposide-induced DNA damage; (c) NU7441 prolongs the G<sub>2</sub>-M arrest and profoundly increases the cytotoxicity induced by ionizing radiation and etoposide in human colon cancer cell lines differing in their p53 status. Furthermore, NU7441 enhances the antitumor activity of etoposide in a human colon cancer xenograft model.

## Materials and Methods

**Chemicals.** NU7441 was kindly provided by Dr. Justin Leahy (Northern Institute for Cancer Research, Chemistry Section, University of Newcastle upon Tyne), and the specific ATM inhibitor KU55933 (35) was provided by Marc Hummersone and Laurent Rigoreau (KuDOS, Horsham, United Kingdom). Etoposide phosphate was purchased from the National Health Service (United Kingdom). All other chemicals were purchased from Sigma (Poole, United Kingdom) unless stated otherwise. NU7441, KU55933, doxorubicin, and etoposide were dissolved in DMSO as 10 mmol/L stocks and stored at  $-20^{\circ}\text{C}$ . All drugs were added to cells such that the final concentration of DMSO was 0.5%, and results were compared with controls incubated with 0.5% DMSO alone.

**Cell lines and culture.** LoVo and SW620 human colon cancer cells were obtained from the American Type Culture Collection (Manassas, VA). V3 (DNA-PK<sub>CS</sub> deficient Chinese hamster ovary cells) and their derivative V3-YAC, transfected with a yeast artificial chromosome (YAC) carrying the human DNA-PK<sub>CS</sub> gene, were a kind gift from Dr. Penny Jeggo (University of Sussex, United Kingdom). All cell lines were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, penicillin (50 units/mL), and streptomycin (50  $\mu\text{g}/\text{mL}$ ) at  $37^{\circ}\text{C}$  in an atmosphere of 5% CO<sub>2</sub> in air. V3-YAC cells were maintained under antibiotic selection to retain the YAC using G-418 sulfate (Invitrogen, Carlsbad, CA) at a final concentration of 500  $\mu\text{g}/\text{mL}$ . Cells were free of *Mycoplasma* contamination. The doubling time of the cells was  $\sim 24$  hours (LoVo and SW620) and 16 hours (V3 and V3-YAC).

**Cytotoxicity and growth inhibition studies.** The effect of NU7441 on cellular survival following exposure to etoposide, doxorubicin, and ionizing radiation was measured in SW620, LoVo, V3, and V3-YAC cells by clonogenic assays. Briefly, we exposed exponentially growing cells in six-well plates or 6-cm dishes to etoposide or doxorubicin with or without NU7441 (0.5 or 1.0  $\mu\text{mol}/\text{L}$ ) for 16 hours. For radiosensitization studies, NU7441 was added to the cells 1 hour before irradiation. V3 and V3-YAC cells were exposed to  $\gamma$ -irradiation (3.1 Gy/min <sup>137</sup>Cesium, Gammacell 1000 Elite, Nordion International Ltd., Ottawa, Ontario, Canada). SW620 and LoVo were exposed to X-irradiation (2.9 Gy/min at 230 kV, 10 mA; Gulmay Medical Ltd., Camberly, United Kingdom) due to the equipment available. After irradiation, the cells were incubated with or without NU7441 for a further 16 hours. Cells were then harvested by trypsinization, counted, and seeded into 10-cm diameter Petri dishes at densities varying from 100 to 100,000 per dish in drug-free medium for colony formation. Colonies were stained with crystal violet after 10 to 14 days and counted with an automated colony counter (ColCount, Oxford Optronics Ltd., Oxford, United Kingdom). The survival reduction factor (SRF) was calculated as the surviving fraction of cells in the absence of NU7441 divided by the surviving fraction of cells in the presence of NU7441 for any given dose or concentration of cytotoxic agent. The dose modification ratio (DMR<sub>90</sub>) is calculated as the concentration/dose of cytotoxic agent required to kill 90% of the cells in the absence of NU7441 divided by the concentration/dose of cytotoxic agent required to kill 90% of the cells in the presence of NU7441.

Cell growth inhibition following 5-day continuous exposure to NU7441 was determined in LoVo and SW620 cells grown in 96-well plates as described previously (36).

**$\gamma$ H2AX focus assay.** DNA DSBs were quantified by H2AX focus formation in SW620 cells grown to 50% to 70% confluence on round No. 1 coverslips in 6-cm dishes. To investigate the effect of NU7441 on ionizing radiation-induced focus formation and loss, we incubated cells with or without 1  $\mu\text{mol}/\text{L}$  NU7441 for 1 hour before irradiation with 2 or 4 Gy X-irradiation (2.9 Gy/min at 230 kV, 10 mA) followed by incubation at  $37^{\circ}\text{C}$  in control medium or that containing 1  $\mu\text{mol}/\text{L}$  NU7441 for up to 4 hours. Similarly, to investigate the effect of NU7441 on etoposide-induced focus formation and loss, cells were incubated in the presence or absence of 1  $\mu\text{mol}/\text{L}$  NU7441 for 1 hour before adding etoposide (1  $\mu\text{mol}/\text{L}$ ) for 1 hour followed by extensive washing (thrice in drug-free medium) and incubation at  $37^{\circ}\text{C}$  in medium with or without 1  $\mu\text{mol}/\text{L}$  NU7441 for up to 24 hours. Coverslips were washed in PBS and fixed in methanol at  $-20^{\circ}\text{C}$  for 5 minutes before washing thrice for 10 minutes each in PBS to rehydrate. Blocking was carried out for 1 to 18 hours at  $4^{\circ}\text{C}$  in KCM buffer [120 mmol/L KCl, 20 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 7.5), 0.5 mmol/L EDTA, 0.1% (v/v) Triton X-100] containing 10% (w/v) dried milk powder and 2% (w/v) bovine serum albumin. The coverslips were incubated with the primary mouse monoclonal antibody for  $\gamma$ -H2AX<sup>ser139</sup> (Upstate Technology, Buckingham, United Kingdom) diluted 1:200 in KCM for 1 hour at room temperature before washing in KCM and incubating for 1 hour at dark room temperature with FITC-conjugated second antibody anti mouse IgG (Molecular Probes, Eugene, OR) diluted 1:200 in KCM. The coverslips were mounted using Vectorshield mounting medium (Vector Laboratories, Burlingame, CA), which contains 1.5  $\mu\text{g}/\text{mL}$  4',6-diamidino-2-phenylindole (DAPI). Images were obtained using an Olympus BH2-RFCA fluorescence microscope fitted with a xenon lamp and a  $\times 40$  objective (DplanApo 40UV Olympus, Tokyo, Japan). Separate 16-bit gray-scale images were recorded for DAPI (420 nm) and FITC (590 nm) using a Hamamatsu ORCA-RT 1024 cooled CCD camera. Image Pro Plus software (Media Cybernetics, Silver Spring, MD) was used for image capture and quantitative image analysis. Subsequent image handling was carried out in Photoshop (Adobe, San Jose, CA). We counted foci in 30 cells, from three different areas per sample, for each exposure.

**Flow cytometric cell cycle analysis.** We treated exponentially growing asynchronous populations of SW620 and LoVo cells (seeded 24 hours previously) with control or 1  $\mu\text{mol}/\text{L}$  NU7441-containing medium for 1 hour before ionizing radiation or adding etoposide or doxorubicin and incubating for a further 16 hours. Cells were then harvested by trypsinization, washed with ice-cold PBS, fixed in 70% ethanol, and stored at  $4^{\circ}\text{C}$ . Cellular DNA was labeled with propidium iodide (400  $\mu\text{g}/\text{mL}$ ) in the presence of the RNase (1 mg/mL; Sigma). Flow cytometry was done on a Becton Dickinson FACScan (Heidelberg, Germany) equipped with an argon ion laser (excitation at 488 nm).

**NU7441 plasma pharmacokinetics following different routes of administration.** All *in vivo* experiments were reviewed and approved by the relevant institutional animal welfare committees and done according to national law. We determined the plasma pharmacokinetics after administering NU7441 *i.v.* at 5 mg/kg in 10% DMSO/10% cyclodextrin in saline or *i.p.* or orally at 10 mg/kg (dissolved at 1 mg/mL in 40% PEG400/saline) to female BALB/c mice. These were the maximum administrable doses by the route used due to the limit of solubility of NU7441. Mice were killed at intervals up to 360 minutes after NU7441 administration; blood was taken and immediately centrifuged, and the plasma fraction was removed and stored at  $-20^{\circ}\text{C}$ .

**NU7441 tissue distribution study.** Female athymic nude mice (CD1 *nu/nu*, Charles River, Wilmington, MA) were maintained and handled in isolators under specific pathogen-free conditions for tissue distribution and efficacy studies. We implanted SW620 colorectal tumor cells ( $1 \times 10^7$  in 50  $\mu\text{L}$  culture medium per animal) *s.c.* into the flanks of the mice and did tissue distribution studies when tumors had reached a size of  $\sim 650 \text{ mm}^3$  (10-14 days after implantation). We administered NU7441 (10 mg/kg *i.p.*) to tumor-bearing female CD-1 nude mice, which were then killed 10, 30, 60, 90, or 240 minutes later, and the blood and tissues were taken for analysis.

Tissues were excised rapidly, wrapped in foil, and snap frozen in liquid nitrogen before storage at  $-80^{\circ}\text{C}$ ; plasma was obtained as above. Before assay, tissue samples were homogenized in PBS [1:3 (w/v)], using a stirrer/mascercator homogenizer (IKA, Werke GmbH and Co., KG., Staufen, Germany), in 10 second bursts, on ice to prevent warming.

**High-performance liquid chromatography analysis of NU7441 in plasma and tissue homogenates.** NU7441 was extracted from plasma and tissue homogenates (50  $\mu\text{L}$ ) using protein precipitation with acetonitrile [1:4 (v/v)] followed by centrifugation ( $5,000 \times g$  at room temperature), evaporation of the supernatant to dryness under nitrogen at  $30^{\circ}\text{C}$ , and reconstitution in high-performance liquid chromatography mobile phase before analysis. NU7441 was separated on a Waters Alliance 2780 separation module (Waters, Watford, United Kingdom) using a Genesis 4- $\mu\text{m}$  C18 column ( $4.6 \times 100$  mm; Jones Chromatography Ltd., Mid Glamorgan, United Kingdom) and a mobile phase of sodium acetate buffer [0.02 mol/L (pH 5)] and acetonitrile [75: 25 (v/v)] at a flow rate of 1 mL/min. Detection was by UV absorption (329 nm; PDA 996 detector, Waters). Plasma samples were quantified using a standard curve, for NU7441, prepared in plasma, which was linear over the range of 0.05 to 10  $\mu\text{g}/\text{mL}$  ( $r^2 = 0.9$ ) with duplicate QA standards (at 0.1, 1, and 10  $\mu\text{g}/\text{mL}$ ). Tissue concentrations were calculated using the method of addition (37) to account for the efficiency of recovery and compensate for intersample variation.

**Antitumor efficacy study.** CD-1 nude mice bearing palpable SW620 colorectal cancer xenografts s.c. ( $\sim 5$  mm  $\times$  5 mm, 8-10 days after implantation,  $n = 5$  per group) were treated with normal saline (control

animals), single agent NU7441 (dissolved in 40% PEG 400 in saline), or etoposide phosphate (11.35 mg/kg in saline) i.p. daily for 5 days. For combinations, NU7441 was given immediately before etoposide phosphate. Etoposide phosphate was given as a water-soluble equivalent to etoposide, the dosage being equivalent to free etoposide at 10 mg/kg.

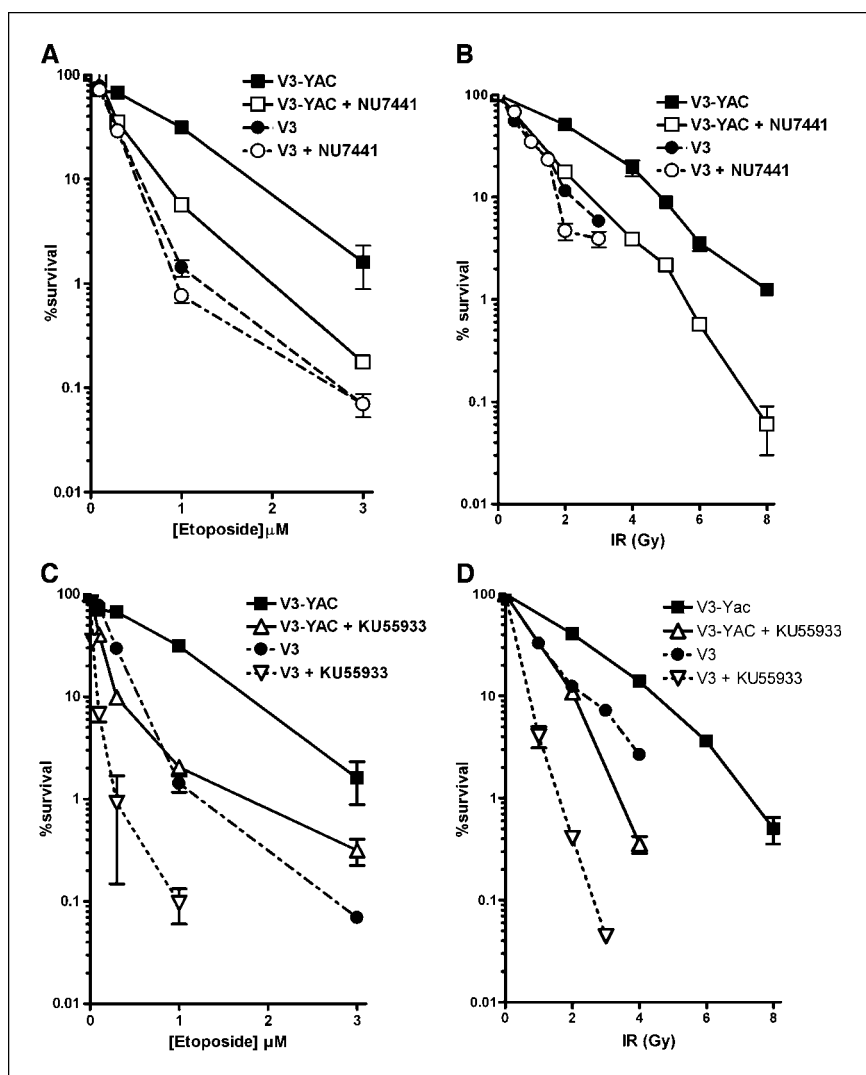
Tumor volume was calculated from two-dimensional electronic caliper (Mitutoyo, Andover, United Kingdom) measurements using the equation  $a^2 \times b/2$ , where  $a$  is the smallest measurement, and  $b$  is the largest measurement. Data are presented as median relative tumor volume (RTV), where the tumor volume on the initial day of treatment (day 0) is assigned an RTV of 1.

**Statistical analysis.** We analyzed data using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). For the *in vitro* studies, the significant differences were determined by Student's  $t$  test (parametric), and for *in vivo* studies, Mann-Whitney nonparametric test was used.

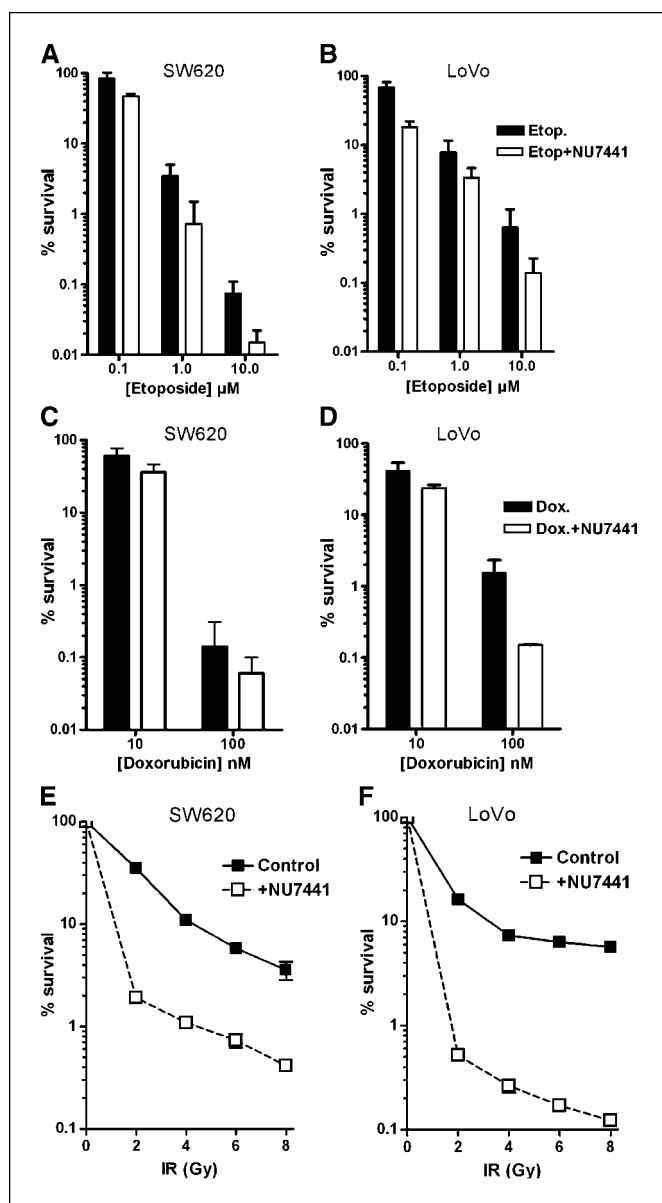
## Results

### Chemopotentiation and radiopotentiality by NU7441 *in vitro*.

The cellular specificity of NU7441 for DNA-PK was studied in V3 and V3-YAC cells, deficient and proficient in DNA-PK<sub>CS</sub>, respectively. V3 cells were  $\sim 2$ -fold more sensitive than V3-YAC cells to ionizing radiation ( $\text{LD}_{90} = 2.4$  and 4.5 Gy in V3 and V3-YAC cells, respectively) and 3-fold more sensitive to etoposide ( $\text{LD}_{90} = 0.6$  and 1.8  $\mu\text{mol}/\text{L}$  in



**Figure 1.** Determination of the cellular specificity of NU7441 for DNA-PK-dependent cell survival after exposure to ionizing radiation (IR) or etoposide. *A*, clonogenic survival of V3 and V3-YAC cells exposed to etoposide alone or in combination with NU7441 (0.5  $\mu\text{mol}/\text{L}$ ) for 16 hours before seeding for colony formation. *B*, V3 and V3-YAC cells exposed to ionizing radiation alone or in the presence of NU7441 (0.5  $\mu\text{mol}/\text{L}$ ) and for further 16 hours to NU7441 before seeding for colony formation. *C*, clonogenic survival of V3 and V3-YAC cells exposed to etoposide alone or in combination with the ATM inhibitor KU55933 (10  $\mu\text{mol}/\text{L}$ ) for 16 hours before seeding for colony formation. *D*, V3 and V3-YAC cells exposed to ionizing radiation alone or in the presence of KU55933 (10  $\mu\text{mol}/\text{L}$ ) and for further 16 hours to KU55933 before seeding for colony formation. Points, mean of triplicate samples from two independent experiments; bars, SD.



**Figure 2.** Sensitization of human colon cancer cells to ionizing radiation (IR) and topoisomerase II poisons by NU7441. Clonogenic survival of SW620 cells (A and C) and LoVo cells (B and D) exposed to etoposide (Etop.; A and B) or doxorubicin (Dox.; C and D) in the presence or absence of NU7441 (1  $\mu\text{mol/L}$ ) for 16 hours before seeding for colony formation in drug-free medium. Solid columns, topoisomerase II poison alone; open columns, topoisomerase II poison + NU7441. Columns, means of three independent experiments; bars, SD. Clonogenic survival of SW620 (E) and LoVo (F) cells irradiated in the absence (■) or presence (□) of 1  $\mu\text{mol/L}$  NU7441 with further incubation in presence or absence of NU7441 for 16 hours before seeding for colony formation in drug-free medium. Data points, mean of three replicates in a single representative experiment; bars, SD.

V3 and V3-YAC cells, respectively). NU7441 alone had no significant effect on the survival of either V3 or V3-YAC cells ( $94 \pm 17\%$  and  $97 \pm 16\%$  survival compared with DMSO controls, respectively). In V3-YAC cells, NU7441 increased the cytotoxicity of ionizing radiation 1.6-fold ( $\text{LD}_{90}$  in the presence of NU7441 = 2.8 Gy) and etoposide 2.3-fold ( $\text{LD}_{90}$  in the presence of NU7441 = 0.8  $\mu\text{mol/L}$ ) but had little effect in V3 cells (Fig. 1A and B). In contrast, the ATM inhibitor KU-55933 increased the cytotoxicity of ionizing radiation

~2-fold in the V3-YAC and 3-fold in V3 cells and etoposide by 6-fold in both cell lines (Fig. 1C and D).

We then investigated chemosensitization and radiosensitization in human colon cancer cells LoVo [p53 wild type (wt)] and SW620 (p53 mutant), selected for their reproducible growth *in vitro* and as xenografts, and because they had previously been used for chemosensitization and radiosensitization studies (34, 38). NU7441 (1  $\mu\text{mol/L}$ ) was not inherently cytotoxic in these cells either (survival of SW620 and LoVo cells was  $93 \pm 17\%$  and  $113 \pm 38\%$  compared with DMSO controls, respectively). However, NU7441 was growth inhibitory in LoVo cells following 5 days of continuous exposure ( $\text{GI}_{50}$  was 0.52  $\mu\text{mol/L}$  and 1  $\mu\text{mol/L}$  reduced growth by 78%). NU7441 did not substantially affect the growth of SW620 cells (8% reduction in cell growth following exposure to 1  $\mu\text{mol/L}$  NU7441; data not shown). As predicted, NU7441 did markedly enhance the cytotoxicity of ionizing radiation, doxorubicin, and etoposide in both cell lines (Fig. 2A-D). In SW620 cells, NU7441 enhanced the cytotoxicity of etoposide 1.8-fold ( $P = 0.058$ ) to 12-fold ( $P = 0.004$ ), depending on the concentration of etoposide, and enhanced doxorubicin cytotoxicity 2- to 3-fold (Fig. 2A and C;  $P = 0.004$  at 10  $\mu\text{mol/L}$  doxorubicin). In LoVo cells, the reduction in cell survival caused by coinubation with NU7441 was 2- to 4-fold for etoposide ( $P < 0.001$  at 0.1  $\mu\text{mol/L}$  etoposide) and 2- to 10-fold for doxorubicin ( $P < 0.01$  at 0.1  $\mu\text{mol/L}$  doxorubicin), depending on the concentration of the cytotoxic drug (Fig. 2B and D). NU7441 caused a sizeable potentiation of ionizing radiation in both cell lines with  $\text{DMR}_{90\text{s}}$  of 3.6 in SW620 cells ( $\text{LD}_{90}$  ionizing radiation alone = 4 Gy, and for ionizing radiation + NU7441 = 1.1 Gy) and 3 in LoVo cells ( $\text{LD}_{90}$  ionizing radiation alone = 3 Gy, and for ionizing radiation + NU7441 = 1 Gy), with SRFs at 2 Gy of 19 and 32 in SW620 and LoVo cells, respectively (Fig. 2E and F).

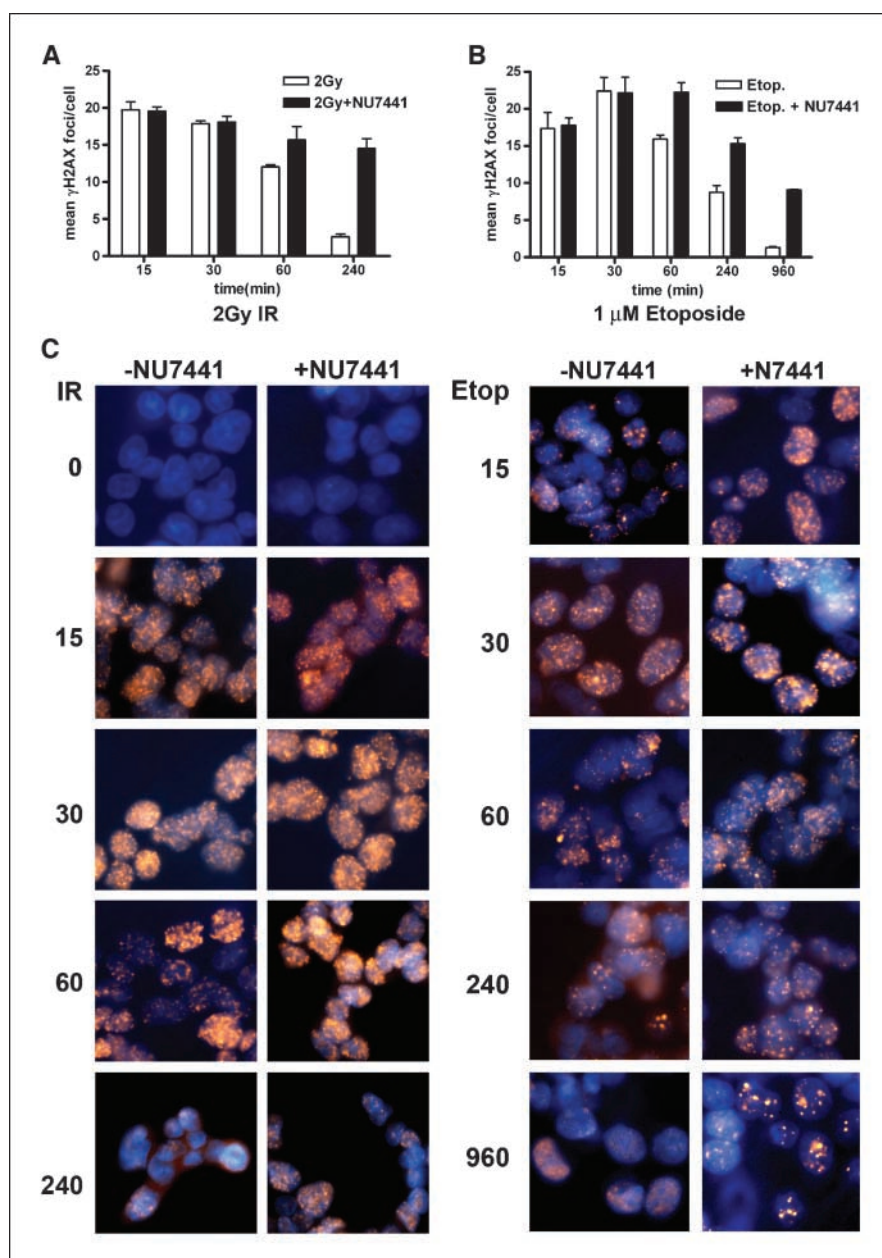
**Effects of NU7441 on  $\gamma\text{H2AX}$  phosphorylation.** To examine the effect of NU7441 on the repair of ionizing radiation-induced and etoposide-induced DNA DSBs, we counted  $\gamma\text{H2AX}$  phosphorylation foci (Fig. 3). There was a very low level of background focus formation of  $0.13 \pm 0.06$  foci per cell in DMSO-treated control cells, which was not increased by NU7441 alone ( $0.13 \pm 0.12$  foci per cell). Exposure to 2 Gy alone induced  $20 \pm 1.9$  foci per cell 15 minutes after irradiation, which declined rapidly, such that by 4 hours, only 13% of foci remained ( $2.6 \pm 0.7$  foci per cell). NU7441 did not affect the level of ionizing radiation-induced focus formation ( $20 \pm 1.0$  foci per cell) but did significantly retard the loss of foci, such that at 4 hours, 74% ( $15 \pm 2.3$  foci per cell  $P = 0.001$  compared with cells irradiated in the absence of NU7441) remained (Fig. 3A and C, left). Similar results were seen after exposure to 4 Gy ionizing radiation (data not shown). After exposure to etoposide, foci accumulated gradually, reaching a peak of  $22 \pm 3.2$  foci per cell 30 minutes after etoposide removal (Fig. 3B and C, right). The rate of loss of foci was slower compared with ionizing radiation-induced foci, and at 4 hours, ~39% ( $8.8 \pm 1.5$  foci per cell) remained. Continuing the incubation for 16 hours after etoposide removal resulted in almost complete loss of foci, and only 5.3% of the peak ( $1.3 \pm 3.6$  foci per cell) remained. As with ionizing radiation-induced foci, NU7441 did not affect etoposide-mediated induction of  $\gamma\text{H2AX}$  foci (peak level at 30 minutes was  $22 \pm 3.6$  foci per cell) but did significantly inhibit loss of foci, such that at 4 and 16 hours after etoposide removal, 69% ( $15 \pm 1.4$  foci per cell  $P = 0.005$  compared with cells treated with etoposide alone) and 41% ( $9.1 \pm 0.21$  foci per cell  $P < 0.001$  compared with cells treated with etoposide alone) remained, respectively.

**Effects of NU7441 on the cell cycle phase distribution.** We investigated the cell cycle phase distribution of asynchronous populations of LoVo and SW620 cells following 16 hours of exposure to 1  $\mu\text{mol/L}$  NU7441, with and without 2 Gy ionizing radiation or coincident 16 hours of exposure to etoposide or doxorubicin, by flow cytometry (Fig. 4). NU7441 alone caused a modest 15% increase in  $G_1$ , with consequent 24% reduction in the S phase in p53 mutant SW620 cells. In the p53 wt LoVo cells, NU7441 caused a more substantial 54% increase in  $G_1$  and 72% decrease in S phase in accordance with its pronounced growth inhibitory effect in this cell line.

The most marked effect of the cytotoxic agents alone and in combination with NU7441 was a  $G_2$ -M phase accumulation. In SW620 cells (Fig. 4A), etoposide, doxorubicin, and ionizing radiation alone caused an 83%, 158%, and 141% increase in  $G_2$ -M phase, respectively, which was accompanied by a decrease in

S phase. In these cells, NU7441 increased the etoposide, doxorubicin, and ionizing radiation-induced  $G_2$ -M arrest by 92%, 21%, and 61%, respectively. A similar but less marked trend was observed in LoVo cells (Fig. 4B).

**Plasma pharmacokinetics of NU7441 following different routes of administration.** The plasma pharmacokinetic variables obtained after administration of NU7441 to BALB/c mice by various routes are given in Table 1. We administered NU7441 at the maximum administrable dose by all routes, which was 5 mg/kg i.v. and 10 mg/kg i.p. and orally. Following i.p. administration, the half-life ( $T_{1/2}$ ) and clearance were similar to those after i.v. injection, and the area under the concentration/time curve after 10 mg/kg i.p. was approximately double that of 5 mg/kg given i.v. The calculated bioavailability for i.p. administration was 100%. Similar pharmacokinetic analysis of NU7441 after oral administration revealed substantial differences compared with i.v. administration,



**Figure 3.**  $\gamma\text{H2AX}$  foci formation and loss in SW620 cells exposed to ionizing radiation (IR) or etoposide (Etop.) in the presence and absence of NU7441. A, average number of foci per cell in cells after exposure to 2 Gy ionizing radiation alone (open columns) or ionizing radiation + 1  $\mu\text{mol/L}$  NU7441 followed by incubation in NU7441 alone-containing medium (solid columns). Columns, mean of 30 nuclei per sample in triplicate; bars, SD. B, average number of foci per cell in cells after exposure for 1 hour to 1  $\mu\text{mol/L}$  etoposide alone (open columns) or etoposide + 1  $\mu\text{mol/L}$  NU7441 followed by incubation in NU7441 alone-containing medium (solid columns). Columns, mean of 30 nuclei per sample in triplicate; bars, SD. C, photomicrographs ( $\times 400$  magnification) of cells exposed to 2 Gy ionizing radiation and allowed to repair in the absence or presence of 1  $\mu\text{mol/L}$  NU7441 (left) or cells exposed to etoposide (1  $\mu\text{mol/L}$ , 1 hour) and allowed to repair in the absence or presence of 1  $\mu\text{mol/L}$  NU7441 (right).

and the calculated bioavailability was only 33%, indicating less than complete absorption by this route. For this reason, we used i.p. administration for all subsequent studies with NU7441.

**Tissue distribution of NU7441 in SW620 bearing mice following 10 mg/kg i.p.** We measured the concentration of NU7441 in plasma, tumor, liver, kidney, spleen, and brain of CD1 nude mice bearing the SW620 human tumor xenograft after i.p. administration of 10 mg/kg NU7441. The concentration of NU7441 in different organs at various time points after i.p. dosing is shown in Fig. 5A, and Table 2 shows the tissue to plasma ratio. NU7441 distributed well to the tissues studied and was retained within them after clearance from the plasma. Importantly, concentrations of NU7441 required for chemopotential and radiopotential *in vitro* (0.5-1  $\mu\text{mol/L}$ ) were maintained in the tumors for at least 4 hours after administration.

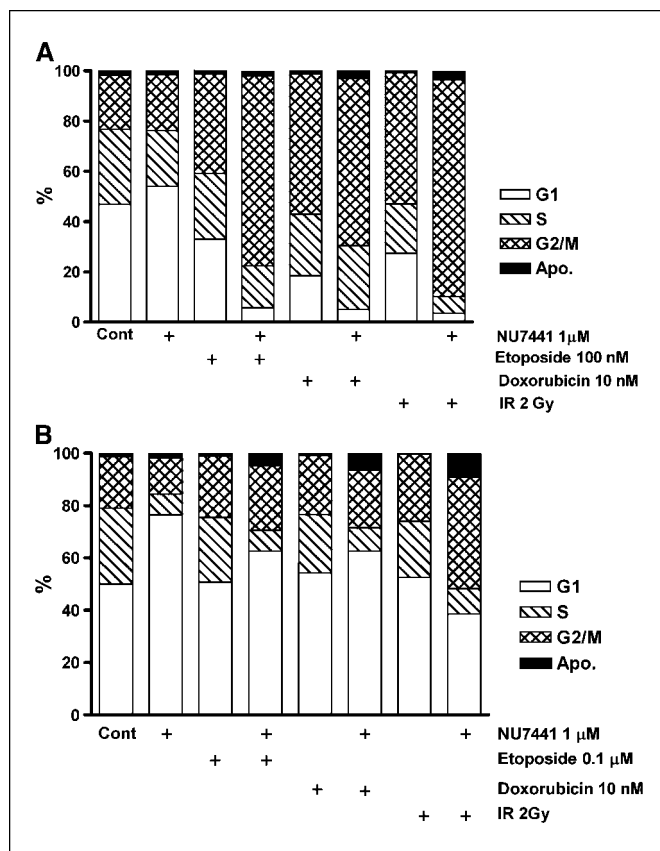
Because of the high levels accumulating in the liver, we were concerned that there may be some toxicity in efficacy studies in combination with etoposide. Toxicity studies were done in female BALB/c mice. We treated mice (five mice per group) with etoposide phosphate at 11.35 mg/kg (equivalent to 10 mg/kg etoposide free base) alone five times daily or etoposide phosphate plus NU7441 10 mg/kg (five times daily). Neither etoposide phosphate alone nor etoposide phosphate plus NU7441 caused any measurable toxicity (the maximum body weight loss being 8% and 12%, respectively).

**Antitumor activity of etoposide and NU7441 in SW620 tumor-bearing mice.** The initial proposed clinical development of DNA-PK inhibitors is chemosensitization, we therefore investigated whether the marked potentiation of etoposide cytotoxicity in human colon cancer cells by NU7441 we observed *in vitro* could be reproduced *in vivo*. We treated mice bearing SW620 tumor xenografts with etoposide phosphate alone and in combination with NU7441 (Fig. 5B). Tumors in control mice reached four times their starting volume (RTV4) at a median time of 5.6 days (i.e., time to RTV4 = 5.6 days). Treatment with etoposide phosphate alone caused a tumor growth delay of 2.7 days (time to RTV4 = 8.3 days), which was extended to 5.4 days (time to RTV4 = 11 days,  $P = 0.0159$  compared with etoposide alone) by coadministration of NU7441. Thus, NU7441 enhanced etoposide phosphate efficacy by 100%.

In this study, neither NU7441 nor etoposide phosphate alone caused any significant toxicity (maximum body weight loss = 6%), and the combination of drugs did not cause unacceptable toxicity (maximum body weight loss = 12%; Fig. 5C).

## Discussion

DNA-PK is a novel target for the development of drugs designed to improve cytotoxic anticancer chemotherapy and radiotherapy. NU7441 represents the most potent and selective member of this new class of drugs to date (34). We verified that DNA-PK is important for cellular survival following genotoxic damage with DSB-inducing agents, and that NU7441 is targeting DNA-PK<sub>CS</sub> using DNA-PK<sub>CS</sub> deficient and complimented cells. That is, the DNA-PK<sub>CS</sub>-deficient V3 cells were more sensitive to ionizing radiation and etoposide than the DNA-PK<sub>CS</sub>-complimented V3-YAC cells, and that NU7441 enhanced the cytotoxicity of both ionizing radiation and etoposide in V3-YAC cells but not the DNA-PK<sub>CS</sub>-deficient V3 cells. Furthermore, the lack of potentiation in the V3 cells was not due to ionizing radiation or etoposide, causing saturating levels of cytotoxicity in these inherently sensitive cells, because the specific



**Figure 4.** Effect of NU7441 on cell cycle distribution following exposure to topoisomerase II poisons and ionizing radiation (IR). Flow cytometric analysis of asynchronous SW620 (A) and LoVo (B) cells exposed to NU7441 (1  $\mu\text{mol/L}$ ) for 16 hours with or without etoposide (100 nmol/L) or doxorubicin (10 nmol/L) or ionizing radiation (2 Gy). Cell cycle phases: open columns, G<sub>1</sub>; hatched columns, S; cross-hatched columns, G<sub>2</sub>-M; solid columns, sub-G<sub>1</sub> (apoptotic) cells. From a single representative experiment.

ATM inhibitor KU55933 was capable of enhancing the cytotoxicity of ionizing radiation and etoposide further.

There is evidence of competition between NHEJ and homologous recombination, and cells lacking components of the NHEJ pathway have been reported to have higher levels of homologous recombination, which may partially compensate for the defect (39-41). However, rather than stimulating homologous recombination activity, DNA-PK inhibitors have been reported to block homologous recombination in a dominant-negative fashion by preventing DNA-PK dissociation from DNA, thereby blocking the access of homologous recombination proteins (42). Similar studies have also suggested that DNA-PK inhibition may also block the access of poly(ADP-ribose) polymerase-1 (PARP-1) to DSB and thus inhibit PARP-1-mediated repair as well (24, 43). The knock-on effect of DNA-PK inhibitors on other DNA repair pathways, as well as NHEJ, may therefore contribute to the pronounced chemosensitization and radiosensitization by NU7441 that we observed in our models of human colon cancer. We found that low, non-cytotoxic concentrations of NU7441 (1  $\mu\text{mol/L}$ ) caused a 3- to 4-fold reduction in the ionizing radiation dose needed to kill 90% of cells, with a substantial 20- to 30-fold reduction in survival at 2 Gy in both cell lines studied. Chemopotentiation studies also showed that these low concentrations of NU7441 were capable of causing a sizeable increase in etoposide-induced cytotoxicity of between 2- and 10-fold. Potentiation of ionizing radiation and

**Table 1.** Plasma pharmacokinetics of NU7441 following different routes of administration

Variable	Route of administration (dose)		
	i.v. (5 mg/kg)	Oral (10 mg/kg)	i.p. (10 mg/kg)
$C_{max}$ ( $\mu\text{g/mL}$ )	1.6*	0.5	2.4
$T_{max}$ (min)	5	30	5
$AUC_{last}$ ( $\mu\text{g/mL min}$ )	76	49	150
$AUC_{inf}$ ( $\mu\text{g/mL min}$ )	78	72	152
$T_{1/2}$ (min)	46	127	53
CL ( $\text{mL/kg/min}$ )	65	140	66
Bioavailability (%)	100	33	100

NOTE:  $C_{max}$  = concentration maximum.  $T_{max}$  = time of  $C_{max}$ .  $T_{1/2}$  = elimination half-life.

Abbreviations: AUC, area under the curve; CL, clearance.

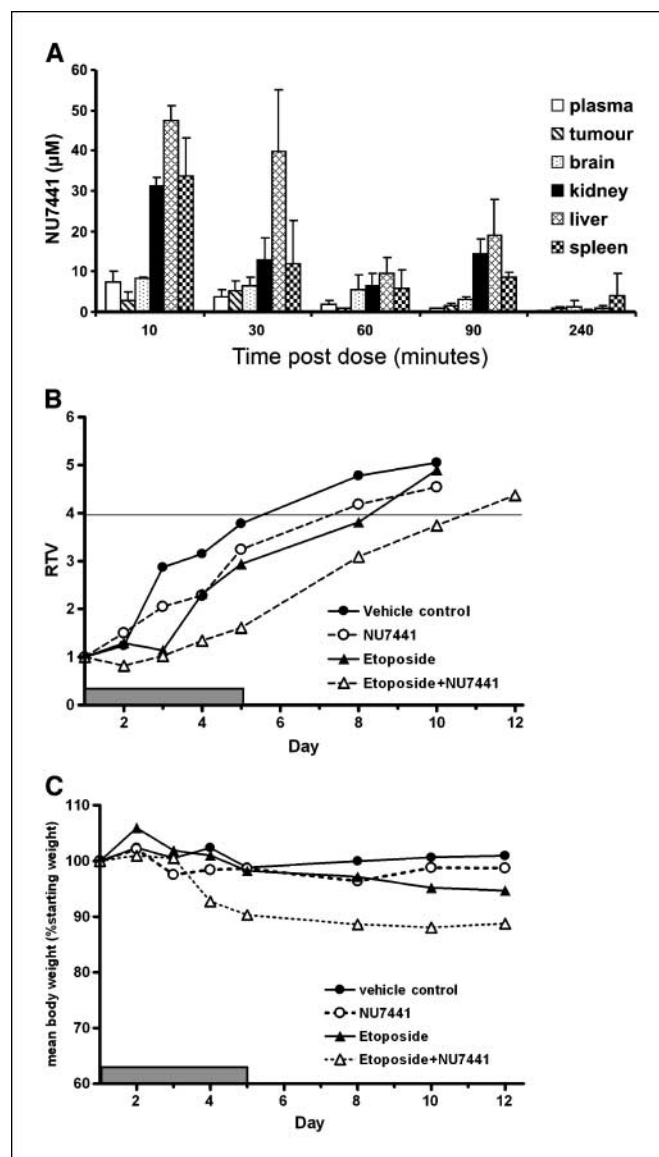
\*Data are calculated from means obtained at 5, 10, 15, 30, 60, 90, 120, 180, 240, and 360 min after administration from three mice per time point.

topoisomerase II poisons was not apparently dependent on the p53 status of the cells. Hitherto, higher concentrations of DNA-PK inhibitor have been required for similar levels of radiopotentialization and chemopotentialization. For example, 20  $\mu\text{mol/L}$  wortmannin and 50  $\mu\text{mol/L}$  LY294002 were required to cause a radiosensitization  $DMR_{90}$  of 2.8 and 1.9, respectively, in SW480 cells (31). In another human colon cancer cell line (HCT116), the novel DNA-PK inhibitor, IC86621 (100  $\mu\text{mol/L}$ ) enhanced the cytotoxicity of ionizing radiation 4-fold and etoposide 15-fold (44). Hence, NU7441 is  $\sim 20$  to  $100\times$  more potent at the cellular level than previously described molecules.

We verified that the radiosensitization and chemosensitization by NU7441 was due to the inhibition of DNA DSB repair by measuring  $\gamma\text{H2AX}$  foci.  $\gamma\text{H2AX}$  foci are generated rapidly at DSBs induced by ionizing radiation and disappear as repair proceeds. For topoisomerase II poisons,  $\gamma\text{H2AX}$  foci are thought to appear as topoisomerase cleavable complexes are processed to frank DSBs and at stalled replication forks. Although H2AX phosphorylation is both ATM and DNA-PK dependent, NU7441 did not affect focus induction by ionizing radiation or etoposide, in agreement with the observed redundancy of ATM and DNA-PK for this process (45). The profound suppression by NU7441 of the rate of focus loss shows its ability to inhibit repair of both ionizing radiation-induced and etoposide-induced DSB. Previous studies show that radiosensitivity and chemosensitivity correlates with persistence of H2AX phosphorylation after exposure to DSB-inducing drugs and ionizing radiation (46, 47). Our data reinforce these observations by showing, for the first time, DNA-PK inhibitor-mediated increase in persistence of  $\gamma\text{H2AX}$  foci is related to an increase in cytotoxicity.

We found that chemosensitization and radiosensitization was accompanied by an increase in the ionizing radiation-induced  $G_2$  arrest, as has been reported with wortmannin (31), and topoisomerase II poison-induced  $G_2$  arrest, as has been reported with NU7026 (32), suggesting that cytotoxin-induced DSBs, persisting in the presence of DNA-PK inhibitor, signal cell cycle arrest at the

$G_2$ -M checkpoint, possibly via ATM. Interestingly, in the p53 wt LoVo cells, NU7441 alone caused  $G_1$  arrest, resulting in cell growth inhibition, but NU7441 did not cause such a marked effect in the p53 mutant SW620 cells. It is tempting to speculate that DNA-PK inhibition can trigger a p53-dependent  $G_1$ -S checkpoint, although



**Figure 5.** Tissue distribution of NU7441, following i.p. administration of 10 mg/kg, and toxicity, and efficacy of NU7441 in combination with etoposide phosphate in SW620-bearing mice. **A**, tissue distribution: levels of NU7441 in various tissues measured up to 4 hours i.p. administration of 10 mg/kg NU7441 to CD1 nude mice bearing the SW620 human tumor xenografts. Columns, mean from three replicate mice per time point in a single experiment; bars, SD. Plasma (open columns), tumor (hatched columns), brain (shaded columns), kidney (solid columns), liver (cross-hatched columns), spleen (checkered columns). **B**, growth of SW620 xenografts is presented as the median RTV. Animals (five per group) were treated with vehicle control (●), NU7441 alone (10 mg/kg daily  $\times$  5, ○), etoposide phosphate alone 11.5 mg/kg (equivalent to etoposide-free drug at 10 mg/kg daily  $\times$  5, ▲), or NU7441 and etoposide phosphate (△). Shaded bar, dosing period. **C**, toxicity evaluation of etoposide and NU7441 in SW620 tumor-bearing nude mice. Points, mean body weight of mice treated as described in (B): vehicle control (●), NU7441 alone (10 mg/kg daily  $\times$  5, ○), etoposide phosphate alone (11.5 mg/kg, equivalent to etoposide-free drug at 10 mg/kg, daily  $\times$  5, ▲), or NU7441 and etoposide phosphate (△). Shaded bar, dosing period.

**Table 2.** Tissue to plasma concentration ratios in SW620 tumor-bearing nude mice

Time (min)	NU7441 tissue to plasma ratio				
	Tumor	Brain	Kidney	Liver	Spleen
15	0.38*	1.1	4.2	6.4	4.6
30	1.4	2.2	3.4	11	3.2
60	0.44	3.2	3.6	5.4	3.2
90	1.5	3	14	19	8.7
240	4.6	6.2	1.0	5	19

\*Data are means of three values obtained from three replicate mice per time point expressed as NU7441 concentration in the tissue divided by NU7441 in the plasma at each time point after i.p. injection of NU7441 at 10 mg/kg.

further studies with isogenically matched cell lines would be needed to verify this supposition.

Whole animal studies show that concentrations of NU7441 required for chemosensitization and radiosensitization *in vitro* were achievable and maintained within the tumor tissue for at least 4 hours after administration of nontoxic doses of NU7441. However, the poor aqueous solubility of this compound precluded the investigation of higher doses that might have led to these levels of the drug being retained within the tumor for longer periods. Nevertheless, based on the promising pharmacokinetics, efficacy studies in combination with etoposide were conducted, and these did indeed show that NU7441 doubled the etoposide-induced tumor growth delay without increasing the toxicity to an unacceptable

degree. Similar results have been reported using the HCT116 human colon cancer xenograft system, where IC86621 caused a 4-fold enhancement of ionizing radiation-induced tumor growth delay and increase in survival. However, this compound required administration at a dose of 1,600 mg/kg to maintain levels above 50  $\mu\text{mol/L}$  (concentration required for potentiation *in vitro*) for 4 hours (44) due its pharmacokinetic properties, limiting its further use.

In conclusion, NU7441 is a potent and specific DNA-PK inhibitor in *in vitro* enzyme inhibition assays; its cellular radiosensitization and chemosensitization are due to DNA-PK inhibition and are accompanied by increased persistence of DSBs and G<sub>2</sub>-M arrest. NU7441 potentiates the activity of radiation, doxorubicin, and etoposide in human tumor cell lines *in vitro* and etoposide in a human tumor xenograft model *in vivo*. Concentrations (*in vitro*) and doses (*in vivo*) of NU7441 required for potentiation are lower than those reported necessary for similar effects using previously reported DNA-PK inhibitors and are not toxic per se, with daily dosing *in vivo* being sufficient to produce potentiation. The limited aqueous solubility and oral bioavailability of NU7441 restrict further development of this compound. However, NU7441 has provided excellent proof of principle *in vitro* and *in vivo* chemosensitization and radiosensitization data to warrant further development of this class of compound for clinical use.

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## References

- Riggs CE. Antitumor antibiotics and related compounds. In: Perry MC, editor. The chemotherapy source book. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 227–52.
- Tolcher AW, Rowinsky EK. DNA topoisomerase II inhibitors. In: Perry MC, editor. The chemotherapy source book. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 278–89.
- Sandler AB. Chemotherapy for small cell lung cancer. *Semin Oncol* 2003;30:9–25.
- Johnson SRD. 2004 ovarian cancer: review of the National Institute for Clinical Excellence (NICE) guidance recommendations. *Cancer Invest* 2004;22:730–42.
- Pommier Y, Leteurtre F, Fesen MR, et al. Cellular determinants of sensitivity and resistance to DNA topoisomerase inhibitors. *Cancer Invest* 1994;12:530–42.
- Ward JF. DNA damage produced by ionising radiation in mammalian cells: identities, mechanism of formation and reparability. *Prog Nucleic Acid Res Mol Biol* 1988; 35:95–125.
- Ward JF. The yield of DNA double-strand breaks produced intracellularly by ionising radiation: a review. *Int J Radiat Biol* 1990;57:1141–50.
- Iliakis G. The role of DNA double strand breaks in ionising radiation-induced killing of eukaryotic cells. *Bioessays* 1991;13:641–8.
- Kanaar R, Hoeijmakers JH, van Ghent DC. Molecular mechanisms of DNA double-strand break repair. *Trends Cell Biol* 1998;8:483–9.
- Jackson SP. Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 2002;23:687–96.
- Takata M, Sasaki MS, Sonoda E, et al. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J* 1998;17:5497–508.
- Yoshida M, Hosoi Y, Miyachi H, et al. Roles of DNA-dependent protein kinase and ATM in cell cycle-dependent radiation sensitivity in human cells. *Int J Radiat Biol* 2002;78:503–12.
- Rothkamm K, Kruger I, Thompson LH, Lobrich M. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol Cell Biol* 2003;23:5706–15.
- Lees-Miller SP, Meek K. Repair of DNA double strand breaks by non-homologous end joining. *Biochimie* 2003; 85:1161–73.
- Collis SJ, DeWeese TL, Jeggo PA, Parker AR. The life and death of DNA-PK. *Oncogene* 2004;24:949–61.
- Weterings E, van Gent DC. The mechanism of non-homologous end-joining: a synopsis of synopsis. *DNA Repair (Amst)* 2004;3:1425–35.
- Smith GCM, Jackson SP. The DNA-dependent protein kinase. *Genes Dev* 1999;13:916–34.
- Jeggo PA, Caldecott K, Pidsley S, Banks GR. Sensitivity of Chinese hamster ovary mutants defective in DNA repair to topoisomerase II inhibitors. *Cancer Res* 1989;49:7057–63.
- Caldecott K, Banks G, Jeggo P. DNA double-strand break repair pathways and cellular tolerance to inhibitors of topoisomerase II. *Oncogene* 1990;50:5778–83.
- Tanaka T, Yamagami T, Oka Y, Nomura T, Sugiyama H. The scid mutation in mice causes defects in the repair system for both double-strand DNA breaks and DNA cross-links. *Mutat Res* 1993;288:277–80.
- Blunt T, Finnie NJ, Taccioli GE, et al. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell* 1995;80:813–23.
- Lees-Miller SP, Godbout R, Chan DW, et al. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science* 1995;267:1183–5.
- Jeggo PA. DNA breakage and repair. *Adv Genet* 1998; 38:185–211.
- Veuger SJ, Curtin NJ, Richardson CJ, Smith GCM, Durkacz BW. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer Res* 2003;63:6008–15.
- Shen H, Schultz M, Kruh GD, Tew KD. Increased expression of the DNA-dependent protein kinase confers resistance to Adriamycin. *Biochim Biophys Acta* 1998;1381:131–8.
- Hansen LT, Lundin C, Helleday T, et al. DNA repair rate and etoposide (VP16) resistance of tumour cell subpopulations derived from a single human small cell lung cancer. *Lung Cancer* 2003;40:157–64.
- Eriksson Å, Levensohn R, Larsson R, Nilsson A. DNA-dependent protein kinase in leukaemia cells and correlation with drug sensitivity. *Anticancer Res* 2002; 22:1787–94.
- Izzard RA, Jackson SP, Smith GCM. Competitive and noncompetitive inhibition of the DNA-dependent protein kinase. *Cancer Res* 1999;59:2581–6.
- Price BD, Youmell M. The PI 3-kinase inhibitor wortmannin sensitizes murine fibroblasts and human tumour cells to radiation and blocks induction of p53 following DNA damage. *Cancer Res* 1996;56:246–50.
- Boulton S, Kyle S, Durkacz BW. Mechanisms of enhancement of cytotoxicity in etoposide and ionising



- radiation-treated cells by the protein kinase inhibitor wortmannin. *Eur J Cancer* 2000;36:535-41.
31. Rosenweig KE, Youmell MB, Palayoor ST, Price BR. Radiosensitization of human tumour cells by the phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G<sub>2</sub>-M delay. *Clin Cancer Res* 1997;3:1149-56.
32. Willmore E, de Caux S, Sunter NJ, et al. A novel DNA-dependent protein kinase inhibitor, NU7026, potentiates the cytotoxicity of topoisomerase II poisons used in the treatment of leukaemia. *Blood* 2004;103:4659-65.
33. Leahy JJJ, Golding BY, Griffin RJ, et al. Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening chromenone libraries. *Bioorg Med Chem Lett* 2004;14:6083-7.
34. Hardcastle IR, Cockroft X, Curtin NJ, et al. Discovery of potent chromen-4-one inhibitors of the DNA-dependent protein kinase (DNA-PK) using a small-molecule library approach. *J Med Chem* 2005;48:7829-46.
35. Hickson I, Zhao Y, Richardson CJ, et al. Identification of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res* 2004;64:9152-9.
36. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990;82:1107-12.
37. Potter GWH. *Analysis of biological molecules: an introduction to principles, instrumentation and techniques*. 1st ed. London: Chapman and Hall; 1995. p. 202.
38. Calabrese CR, Almasy R, Barton S, et al. Preclinical evaluation of a novel poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor, AG14361, with significant anticancer chemo- and radio-sensitization activity. *J Natl Cancer Inst* 2004;96:56-67.
39. Essers J, van Steeg H, de Wit J, et al. Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *EMBO J* 2000;19:1703-10.
40. Allen C, Kurimasa A, Brennemann M, Chen DJ, Nickoloff JA. DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination. *Proc Natl Acad Sci U S A* 2002;99:3758-63.
41. Delacote F, Han M, Starnato TD, Jasin M, Lopez BS. An *xrcc4* defect or Wortmannin stimulates homologous recombination specifically induced by double strand breaks in mammalian cells. *Nucleic Acids Res* 2002;30:3454-63.
42. Allen C, Halbrook J, Nickoloff JA. Interactive competition between homologous recombination and non-homologous end joining. *Mol Cancer Res* 2003;1:913-20.
43. Veuger SJ, Curtin NJ, Smith GCM, Durkacz BW. Use of novel inhibitors of poly(ADP-ribose) polymerase-1 and the DNA-dependent protein kinase to investigate reciprocal regulation of enzyme activity and DNA double and single strand break repair. *Oncogene* 2004;23:7322-9.
44. Kashishian A, Douangpanya H, Clarke D, et al. DNA-dependent protein kinase inhibitors as drug candidates for the treatment of cancer. *Mol Cancer Ther* 2003;2:1257-64.
45. Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionising radiation. *Cancer Res* 2004;64:2390-6.
46. Banath JP, Olive PL. Expression of phosphorylated histone H2AX as a surrogate of cell killing by drugs that create DNA double strand breaks. *Cancer Res* 2003;63:4347-50.
47. Olive PL, Banath JP. Phosphorylation of histone H2AX as a measure of radiosensitivity. *Int J Radiat Oncol Biol Phys* 2004;58:331-5.

## Preclinical Evaluation of a Potent Novel DNA-Dependent Protein Kinase Inhibitor NU7441

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