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ORIGINAL ARTICLE

Chemosensitization and radiosensitization of human lung and colon cancers by antimitotic agent, ABT-751, in athymic murine xenograft models of subcutaneous tumor growth

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Abstract
Purpose ABT-751 is an orally active antimitotic agent that is currently in Phase II clinical trials. This agent binds to the colchicine site on ß-tubulin and inhibits polymerization of microtubules. This disruption of microtubule dynamics leads to a block in the cell cycle at the G_2/M phase, and promotes apoptosis. ABT-751, as a single agent, has antitumor activity against a series of xenograft models including non-small cell lung cancer (NSCLC) and colon cancer. The current studies were conducted to determine whether ABT-751 enhances antitumor activity of standard cytotoxic therapies currently in clinical use.
Methods Efficacy of ABT-751, in combination with cisplatin, 5-FU, and radiation, was evaluated in the Calu-6 NSCLC, HT-29 colon, and HCT-116 colon carcinoma xenograft models, respectively. Tumor-bearing athymic mice were treated with ABT-751 orally once a day at 75 or 100 mg/kg/day on a 5-days-on, 5-days-off schedule for two cycles.
Results Efficacy of ABT-751 at 100 mg/kg/day was tested in combination with cisplatin at its maximum tolerable dose (MTD) (10 mg/kg/day, i.p. x1) in Calu-6 tumor-bearing athymic mice. The percent treated/control (%T/C) tumor volume ratios on day 38 were 35, 37, and 6, and the percent tumor growth delay (%TGD) values were 71, 65, and 188 for cisplatin, ABT-751 and the combination groups, respectively. HT-29 colon tumors were used to test ABT-751 in combination with an MTD of 5-FU, 30 mg/kg/day, i.p., q.d. x5. The %T/C ratios on day 38 were 22, 28, and 5 and the %TGD values were 75, 75, and 150 for 5-FU, ABT-751, and the combination groups, respectively. Treatment of HCT-116 colon carcinoma tumors with ABT-751, concurrent with the radiation treatment, was able to both enhance radiation-induced tumor regression, and delay the time to recurrence and progression. Growth curves allowed calculation of enhancement of radiation-induced growth delay (defined as the additional time required for a treated tumor to reach four times its original size) of 2, 9, and 12 days, for ABT-751 alone, radiation alone, and the combination, respectively.
Conclusion Collectively, these studies demonstrate that ABT-751 enhanced efficacy of standard cytotoxic therapies in a variety of tumor xenograft models, and that enhancement was at least additive in all systems.

Keywords Antimitotic · Microtubules · Colchicine · Chemotherapy · Radiotherapy

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Introduction

Microtubules are major structural components of cells. They have roles in cell shape, cellular polarity, cellular movement, intracellular transport and the segregation of chromosomes during mitosis. The microtubules are

highly regulated. As cells enter mitosis, the interphase microtubules disappear and are replaced with a new network of microtubules that interact with the mitotic spindle [8, 9]. Disruption of these new microtubules leads to cell cycle arrest. These important and highly labile microtubule arrays comprising the mitotic spindle are the principle target of oncologic antimitotic compounds.

Known antimitotic agents fall into three distinct classes. The *vinca* alkaloids (vincristine, vinblastine, and vinorelbine) have importance in the treatments of leukemia, lymphomas, small cell lung cancer, and other malignancies [17]. The taxanes (paclitaxel and docetaxel) are effective in the treatment of breast, lung, ovarian, head and neck, prostate, and gastric and bladder carcinomas [4, 16, 18]. A third class, typified by colchicine, is comprised of a structurally diverse collection of small molecules that are related by the fact that all bind to a common site on tubulin, known as the colchicine site [14, 16]. No representatives of this third class have yet been approved for use in cancer chemotherapy.

These three classes of compounds bind to distinct subunits of tubulin. The *vinca* alkaloids and colchicines—potent inhibitors of microtubule polymerization—work by inhibiting cell proliferation at metaphase during mitosis, while the taxanes stabilize microtubules and block their depolymerization [10]. At low drug concentrations, all three classes can actually stabilize microtubule dynamics without changing microtubule mass thus leading to mitotic block and cell death by apoptosis. The microtubule-targeted agents have been a successful oncology-therapeutic class, and their ability to impact diverse cellular processes makes them attractive for combination therapies using either classical cytotoxic agents as well as molecularly targeted drugs.

ABT-751 (formally E7010, Eisai, Co., Ltd. [12]) is an orally active antimitotic agent that binds to the colchicines site on β-tubulin and inhibits microtubule formation [26]. The interference with normal microtubule dynamics leads to a block in the cell cycle at G2/M, and cell death by apoptosis. In preclinical studies, ABT-751 inhibited the proliferation of several human tumor cell lines in vitro, including those expressing the multidrug resistance (MDR) phenotype. Antitumor activity, independent of the MDR status of the model, also was demonstrated in vivo in syngeneic and human xenograft tumor models of colon, lung, breast, pancreatic, and bladder cancer [12]. In Phase 1 trials, ABT-751 demonstrated rapid absorption and elimination and linear pharmacokinetics across the dose ranges studied [25]. In a pediatric phase 1 study, ABT-751 significantly prolonged time-to-disease progression and had some preliminary evidence of tumor regressions in neuroblastoma [5], while in adult lung cancer, overall survival and progression-free rates compared favorably to historical controls [2].

In the current study, the in vivo activity of ABT-751 alone and in combination with different cytotoxic therapies was evaluated in subcutaneous nude mouse xenograft models to explore the potential for augmenting current cancer therapies with ABT-751. One human lung and two human colon cancer tumors were evaluated with cisplatin, 5-FU, and radiation therapies, respectively. Results showed that ABT-751 or the cytotoxic therapies alone produced growth delay in all the tumor models, and that the combined treatments uniformly produce a significant improvement in tumor growth delay over either agent alone.

Research design and methods

Cell culture

Three different human carcinoma cell lines were used to generate subcutaneous tumors in nude mice—Calu-6 non-small cell lung carcinoma (NSCLC), HT-29 colon carcinoma, and HCT-116 colon carcinoma.

Calu-6 cells and HT-29 were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, nonessential amino acids, sodium pyruvate and sodium bicarbonate. Cells were incubated at 37°C in the presence of 5% CO_2.

Wild-type HCT-116 human colon carcinoma cells were kindly provided by Dr. Bert Vogelstein (Howard Hughes Medical Institute, Johns Hopkins Oncology Center, Baltimore, MD, USA) [7]. HCT-116 cells were maintained in monolayer culture in McCoy's 5A modified medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml). The cells were MAP tested by BioReliance Inc., and found to be negative for murine pathogens, making them suitable for subcutaneous injections into mice.

Drug preparation and administration

ABT-751 was obtained from Abbott Laboratories and the compound was prepared for gavage by dissolving in 4% ethanol/96% dextrose solution (D5W) with 1 eq. 1 N HCl, at appropriate concentrations for 0.2 ml gavage doses.

Tumor propagation and measurement

Calu-6 NSCLC and HT-29 colon carcinoma tumors

Cultured tumor cells were passaged three times as subcutaneous tumors in 5- to 6-week-old CD-1 nu/nu athymic mice (Charles River Laboratories) before inoculation into the test animals. With each passage, fragments of tumors collected from multiple donor animals were combined, minced, and homogenized into single cell suspensions in RPMI 1640 media. Cell suspensions were washed three times to remove debris and other foreign materials. Viable cells were counted by trypan blue exclusion technique using a hemocytometer. Test mice were injected subcutaneously with 0.5 ml of the cell suspension containing 1×10^6 cells. Tumors reached appropriate treatments sizes (200–250 mm^3) in approximately 10 days. Day 38 was chosen for the %T/C comparisons since the vehicles had all mice remaining in the treatment groups prior to reaching their 3 g endpoint of analysis. Tumor growth delay (TGD) afforded by the drug treatments was calculated based on the time, in days, it took for the treated tumors (Ti) to reach 1,000 mm^3 minus the average time it took for the vehicle controls (C). The %TGD was $(Ti-C)/C \times 100\%$. The combined chemotherapy experiments were each conducted once, but the standard cytotoxic monotherapies currently in clinical use were previously evaluated several times in each model thus confirming the model's consistency (data not shown).

HCT-116 colon carcinoma

In preparation for injection, HCT-116 cells were maintained within T175 flasks, under their normal media conditions, as described above. Cells were trypsinized and harvested when they reached 95% confluence. The cell pellets were washed with phosphate-buffered saline twice to remove the residual serum and resuspended in McCoy's 5A modified medium without serum and antibiotics at 5×10^7 cells per ml. Tumor cells were inoculated subcutaneously into the sacral region of 4- to 6-week-old NCR nu/nu athymic female mice (Taconic Laboratories; Germantown, NY, USA) with 0.10 ml of cell suspension containing 5×10^6 cells. The injection vehicle was tissue culture medium without serum. Tumor measurements began 1 week post-injection and continued twice weekly through the course of the experiment. Tumor volumes were calculated by the formula: (length × width × depth). Treatment began when tumor volumes reached the range of 100–300 mm^3, with a median volume of approximately 200 mm^3. The tumor-bearing animals were randomized into the different treatment arms. Baseline body weights were determined at the beginning of the study, just before tumor cells were injected, and twice weekly thereafter. All experimental procedures were done in accordance with a protocol approved by the Georgetown University Animal Care and Use Committee. Relative tumor volumes were plotted for each of the study arms, and the amount of growth delay afforded by the drug and radiation treatments was calculated based on the time it took for the treated tumors to reach four times their original volume minus the average time it took for the untreated controls to reach four times their original volume.

Tumor irradiations

Irradiations of subcutaneous tumors were performed with a Mark I Model 30 irradiator (J.L. Shepherd & Associates; San Fernando, CA, USA) with a ^{137}cesium gamma ray source. The mice were restrained in customized clear plastic holders with lead covers, which shielded all but the sacral region of the mouse. The holders were placed behind a lead barrier to further shield the mouse's body, and four mice were irradiated simultaneously. The dose rate to the tumor (1.5 Gy/min) was calibrated using thermoluminescent dosimetry. The entire time of confinement, including irradiation, was less than 8 min for each mouse. Treatment doses were fractionated over 4 or 5 days.

Results

This study was designed to evaluate the potential of ABT-751, an antimitotic from the colchicine class, to enhance cytotoxic treatment regimens currently in use for common cancers, using a clinically relevant animal model. Subcutaneous growth of human tumors in athymic ("nude") mice is often used as a preclinical model for evaluation of cancer therapies. For this study, we chose three tumor xenograft models with proven utility in assessing three cytotoxic cancer therapies in widespread use—cisplatin, 5-FU, and radiation. We used tumor growth rate, with and without treatment, as a relevant endpoint that would be expected to reflect potential clinical benefit, and we used treatment conditions that mimic actual clinical treatment regimens as closely as possible.

Calu-6 NSCLC and cisplatin

Cisplatin has been used to treat a variety of different cancers, such as NSCLC, where it is often combined

with other drugs, including antimitotics in the taxane class (e.g. paclitaxel, docetaxel), to improve efficacy. Since the taxane antimitotics have already proved a useful adjuvant in cisplatin therapy for NSCLC, we evaluated ABT-751 to determine whether it might also enhance cisplatin treatment.

Tumor cells inoculated into male nude mice on day 0, produced tumors by day 10 with an average size of 233 mm^3, which were then size-matched and placed into the following groups for treatment as indicated.

Group

1. ABT-751—100, 75, and 0 mg/kg/day (p.o., q.d., 5 days on–5 days off ×2)
2. Cisplatin—10 mg/kg/day (ip, q.d. x1)
3. Combination therapy—100/10, 75/10 mg/kg/day (p.o., q.d., 5 days on–5 days off ×2/ i.p., q.d. x1)

Appropriate doses for ABT-751 for oral dosing were determined from an earlier maximum tolerable dose study, where doses were cycled, 5 days on–5 days off for two cycles, to minimize toxicity. In this MTD study, ABT-751 dosed at 100 mg/kg/day for more than seven consecutive days resulted in a greater than 15% body weight loss and severe diarrhea, but was well tolerated when dosed 5 days on–5 days off for 2 cycles (data not shown).

In this Calu-6 xenograft model, ABT-751 administered as a single agent at 100 and 75 mg/kg/day demonstrated significant antitumor activity (Fig. 1) as did a single dose of cisplatin at 10 mg/kg/day. The tumor growth delays were 15.5 and 14.0 days for the 100 and 75 mg/kg/day ABT-751 treatments, respectively, and 17 days for the cisplatin treatment. In combination with cisplatin, ABT-751 demonstrated a dose-dependent enhancement in growth delay. The tumor growth delays were 45 and 38 days for the 100 and 75 mg/kg/day combinations of ABT-751 with cisplatin, respectively. The response was greater than additive for both doses of ABT-751 tested (Table 1) since the %TGD for the combinations (188% for the 100/10 mg/kg/day and 158% for the 75/10 mg/kg/day combination) were greater than the theoretical sum of each agent alone [e.g. 65% (100 mg/kg/day ABT-751) + 71% (Cisplatin) = 136%].

HT-29 colon carcinoma and 5-FU

5-FU is a mainstay in the treatment for colon cancer. It is used at all stages of the disease, and often in combination with other cytotoxic drugs. We have found that the HT-29 human colon carcinoma murine xenograft

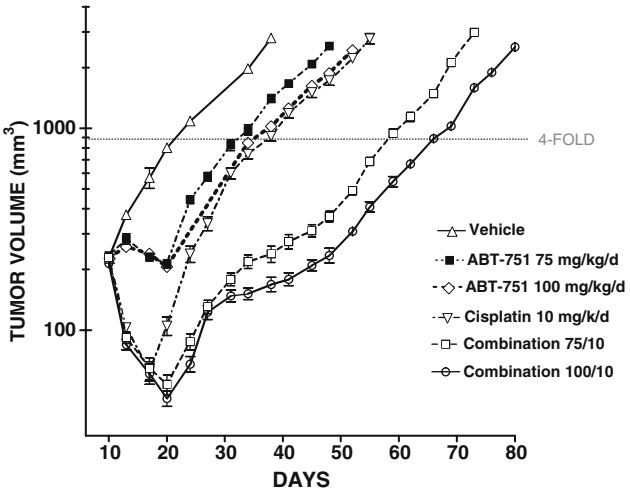

Fig. 1 Efficacy of ABT-751 in combination with cisplatin in the Calu-6 flank xenografts grown in nude mice. Mice were size matched for established tumors (~233 mm^3) and therapy was initiated 10 days post-tumor cell inoculation. ABT-751 was administered at 100 and 75 mg/kg/day, p.o., q.d., on a (5 days on–5 days off) ×2 schedule. A single dose of cisplatin at 10 mg/kg was administered i.p., on day 10. *Horizontal line* shows tumor growth to four times original tumor volume. Means are shown with error bars indicating 1 standard error (SE) of the mean; $n = 10$ mice per treatment group

model to be useful in assessing 5-FU efficacy for colon cancer. In this experiment, we combined 5-FU with ABT-751 and evaluated the effect on HT-29 tumor growth.

Tumor cells inoculated into female nude mice on day 0 produced tumors by day 10 with an average size of 236 mm^3, which were then size matched and placed into the following groups and treated as indicated.

Group

1. ABT-751—100, 75, and 0 mg/kg/day (p.o., q.d., 5 days on–5 days off ×2)
2. 5-FU—30 mg/kg/day (i.p., q.d. x1)
3. Combination therapy—100/30, 75/30, mg/kg/day (p.o., q.d., 5 days on–5 days off ×2/ i.p., q.d. x1)

In the HT-29 colon xenograft model, ABT-751 administered as a single agent at 100 and 75 mg/kg/day on a 5-days-on, 5-days-off schedule for two cycles, demonstrated significant antitumor activity (Fig. 2) similar to the findings in the Calu-6 NSCLC model. The tumor growth delays were 21 and 14 days for the 100 and 75 mg/kg/day ABT-751 treatments, respectively. 5-FU at 30 mg/kg also significantly delayed tumor growth with a tumor growth delay of 21 days. In combination with 5-FU, ABT-751 produced a dose-dependent enhancement in growth delay. The tumor growth delays were 42 and 28 days for the 100 and 75 mg/kg/day

Table 1 In vivo efficacy of ABT-751 alone and in combination with cisplatin in the Calu-6 lung subcutaneous flank xenografts grown in male *nude* mice

Treatment	Dose (mg/kg/day)	Tumor volume[a] day 38	% T/C[b] day 38	TGD[c]	% TGD[d]
ABT-751	100	1,022 ± 48	37***[e]	15.5	65***
	75	1,406 ± 65	50***	14	58***
Vehicle	0	2,799 ± 111			
Cisplatin	10	914 ± 49	35***	17	71***
Vehicle	0	2,624 ± 89			
ABT-751/Cis	100/10	169 ± 14	6***	45	188***[f]
	75/10	239 ± 22	9***	38	158***[f]
Vehicle	0/0	2,759 ± 71			

Tumor cells were inoculated into mice on day 0. Tumors were size matched at ~233 mm^3 on day 10 and therapy was initiated

[a] Mean ± SEM

[b] Ratio of tumor volume for treated versus control (vehicle)

[c] *TGD* tumor growth delay: median time in days for the treated tumors (Ti) to reach a 1,000 mm^3 minus the time for the vehicle controls (*C*). Each vehicle took 24 days to reach 1,000 mm^3

[d] %TGD was $(Ti-C)/C \times 100\%$

[e] *P* values versus vehicle, ***< 0.001

[f] $P < 0.001$ compared to either cisplatin—10 mg/kg/day, ABT-751—100 or 75 mg/kg/day

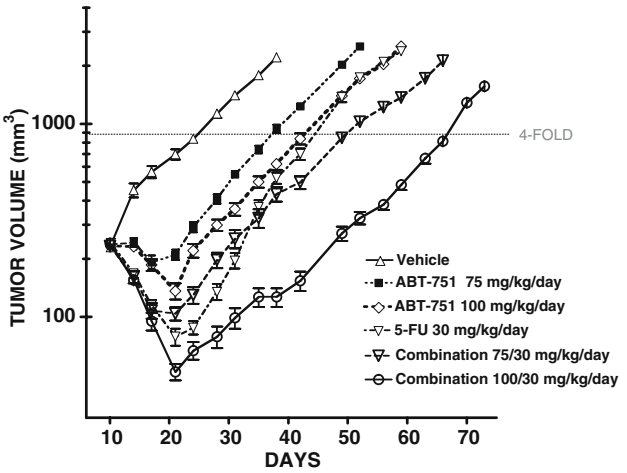

Fig. 2 Effects of ABT-751 in combination with 5-FU in the HT-29 flank xenografts grown in nude mice. Mice were size matched for established tumors (~236 mm^3) and therapy was initiated 10 days post-tumor cell inoculation. ABT-751 was administered at 100 and 75 mg/kg/day, p.o., q.d., on a (5 days on–5 days off) ×2 schedule. 5-FU was administered i.p., q.d. ×5 starting day 10. *Horizontal line* shows tumor growth to four times original tumor volume. Means are shown with error bars indicating 1 SE of the mean; $n = 10$ mice per treatment group

combinations of ABT-751 with 5-FU, respectively. The enhancement appeared to be additive (Table 2) since the %TGD for the combinations (150% for the 100/10 mg/kg/day and 100% for the 75/10 mg/kg/day combination) were approximately the same as the theoretical sum of each agent alone [e.g. 75% (100 mg/kg/day ABT-751) + 75% (5-FU) = 150%].

HCT-116 colon carcinoma and radiation

In dividing cells, ABT-751 produces a block at the G_2/M phase of the cell cycle. Cells in G_2/M are sensitive to radiation. So the possibility exists that ABT-751 might radiosensitize tumors by a mechanism beyond merely its cytotoxic effects. This potential interaction could improve therapeutic responses from combined radiation and ABT-751 therapy further than that which could be achieved by either therapy alone.

HCT-116 is a human colon cancer line that has been very well characterized in terms of its tumor radiation responses, including growth delay, cell cycle arrest, and apoptosis [21, 22, 24]. Additionally, there are isogenetic variants for this tumor line that have been specifically mutated in individual genes that are known to play important roles in cell cycle arrest and apoptosis, including p53, p21, and 14-3-3sigma [1, 3, 22]. The availability of this set of isogenetic cell lines provides a powerful tool for determining mechanistic events underlying therapeutic radiosensitization. In fact, this model was first used to demonstrate that enhancement of radiation-induced apoptosis can be curative [23]. For these reasons, the HCT-116 xenograft model, along with its isogenetic counterparts, has become an extremely valuable tool for tumor radiotherapy studies. Thus, use of the HCT-116 murine xenograft tumor model allowed us to directly address the possible modifying effect of ABT-751 on a well-established radiobiological tumor model.

Fractionated radiation treatment was used because it mimicked actual clinical treatment conditions,

Table 2 In vivo efficacy of ABT-751 alone and in combination with 5-FU in the HT-29 colon subcutaneous flank xenografts grown in male *nude* mice

Compound	Dose (mg/kg/day)	Tumor volume[a] day 38	% T/C[b] day 38	TGD[c]	% TGD[d]
ABT-751	100	619 ± 40	28***[e]	21	75***
	75	942 ± 46	43***	14	50***
	0	2,210 ± 82			
5-FU	30	525 ± 35	22***	21	75***
Vehicle	0	2,399 ± 114			
ABT-751/5-FU	100/30	127 ± 14	5***	42	150***[f]
	75/30	433 ± 38	17***	28	100***[f]
Vehicle	0/0	2,572 ± 125			

Tumor cells were inoculated into mice on day 0. Tumors were size matched at ~236 mm^3 on day 10 and therapy was initiated

[a] Mean ± SEM

[b] Ratio of tumor volume for treated vs. control (vehicle)

[c] TGD: median time in days for the treated tumors (Ti) to reach a 1,000 mm^3 minus the time for the vehicle controls (*C*). Each vehicle took 28 days to reach 1,000 mm^3

[d] %TGD was $(Ti-C)/C \times 100\%$

[e] *P* values versus vehicle, ***< 0.001

[f] $P < 0.001$ compared to either 5-FU—30 mg/kg/day, ABT-751—100 or 75 mg/kg/day

minimized the chances of side effects such as skin ulceration, and it nicely complemented the multi-day treatment regimen defined for ABT-751 in the other xenograft models. The optimal radiation dose was defined as that dose which produced temporary growth delay in all tumors, achieved no cures, and 100% progression within 1–2 weeks. For the HCT-116 model the optimal total dose was in the range of 10–15 Gy (Fig. 3).

To test for radiosensitization by ABT-751, female nude mice were injected with tumor cells and those animals that developed tumors were subdivided into four treatment arms that were either treated or not with the optimal doses of radiation and/or ABT-751 as follows.

Group

1. ABT-751—100 and 0 mg/kg/day (p.o., q.d., 4 days on–4 days off ×2)
2. Radiation—12 Gy (3 Gy/day for four consecutive days)
3. Combination therapy—100 mg/kg/day/12 Gy (radiation was delivered concurrent with the first drug cycle)

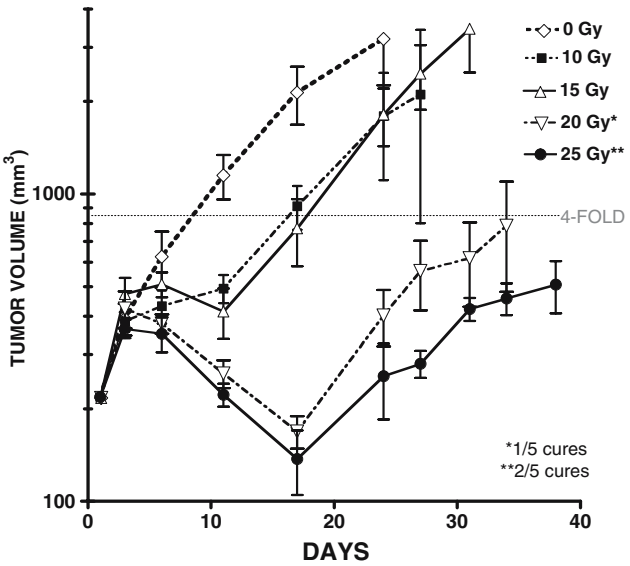

Fig. 3 Radiation dose response curves for relative tumor growth in HCT-116 xenografts. Gamma radiation was delivered to subcutaneous tumors, up to the total dose indicated, over five consecutive daily fractions (days 1 through 5). Tumor volumes for each animal were normalized relative to their starting volume on day 1. *Horizontal line* shows tumor growth to four times original tumor volume. Means are shown with error bars indicating 1 SE of the mean; $n = 5$ mice per treatment group

For the animals receiving combined radiation and ABT-751, radiation was delivered daily on days 1 through 4, between 1 and 5 h after ABT-751 administration. After allowing a 1-week break in treatment, to allow the animals to recover and reduce the toxicity, another 4 days of drug treatment were given with no further radiation. Results of this experiment showed growth delays of 2, 9, and 12 days, for ABT-751, radiation, and radiation/ABT-751, respectively, suggesting an additive effect (Fig. 4). A similar experiment, with a slightly modified dosing schedule, showed growth delays of 5, 13, and 18 days, for ABT-751, radiation, and radiation/ABT-751 (data not shown), thus supporting the basic finding of an additive effect for the combined treatment.

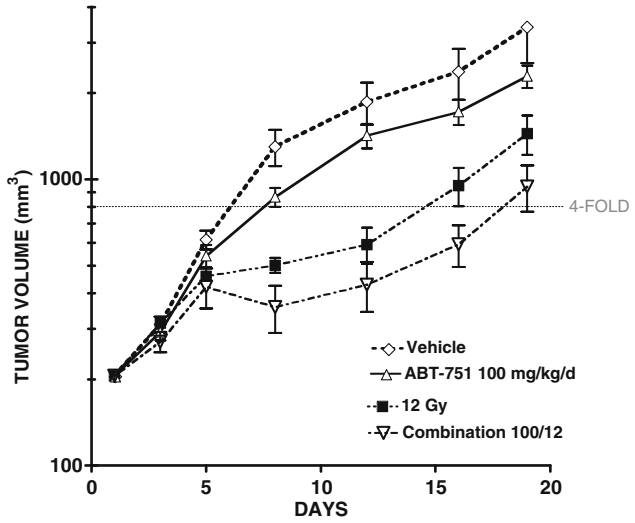

Fig. 4 Combined radiation/ABT-751 dose response curves for relative tumor growth in HCT-116 xenografts. ABT-751 was delivered daily by gavage to animals bearing subcutaneous tumors, at 100 mg/kg/day over four consecutive days (days 1 through 4). Gamma radiation was delivered to subcutaneous tumors at 12 Gy fractionated over four consecutive daily fractions (days 1 through 4). Animals were left untreated for seven consecutive days (days 5 through 11). Then animals in the drug treatment arms received another 4 days of drug treatment at the same daily dose (days 12 through 15). Tumor volumes for each animal were normalized relative to their starting volume on day 1. *Horizontal line* shows tumor growth to four times original tumor volume. Means are shown with error bars indicating 1 SE of the mean; $n = 12$ mice per treatment group

Conclusion

Efficacy of ABT-751 was tested in the Calu-6 NSCLC, HT-29, and HCT-116 colon nude mouse xenograft models. As a single agent, ABT-751 demonstrated efficacy in all three xenograft models. ABT-751, in combination with cisplatin (Calu-6 NSCLC), 5-FU (HT-29), or radiation (HCT-116) showed additive or greater efficacy compared to single agents alone. The results suggest that adding ABT-751 to a cytotoxic treatment regimen may be an effective way to enhance the therapeutic tumor response for a variety of cancers.

In these tumor models, ABT-751 alone significantly inhibited tumor growth, but even at the highest dose tested (100 mg/kg/day) little actual tumor regression was seen. Treatment with ABT-751 concurrent with the cytotoxic treatments was, however, able to both enhance the regression produced by those agents and delay the time to recurrence and progression.

The growth delay findings suggest an additive effect for cytotoxic drugs and ABT-751. Yet, a supra-additive or synergistic response might be achievable by optimizing dose/time treatment regimens. This notion is supported by the fact that ABT-751 stalls cells at the G2/M border [26], which is a phase of the cell cycle where cells are relatively sensitive to DNA damaging agents [11, 13]. ABT-751 dosing regimens designed to enrich the number of G_2/M phase cells in the tumor prior to cytotoxic treatment might greatly enhance the effect. The sequence of cytotoxic drugs in combinations (concurrent vs. sequential) can alter responses, depending upon their pharmacologic mechanisms of action [20]. Altering the ABT-751 schedule needs to be further investigated in synchronized cell model systems, and with other in vivo models.

Another possible mechanism for interaction is through apoptosis. ABT-751 promotes apoptotic cell death, and cytotoxic agents can induce apoptosis in tumors. However, not all cells and tissues are susceptible to apoptosis. ABT-751 may actually enhance apoptosis in tumors that are normally refractory to this mode of death. We have previously shown that increased apoptosis is associated with enhanced therapeutic responses in the HCT-116 xenograft model [21]. Differential cell cycle phase sensitivity to the cytotoxic/apoptotic effects of radiation is well established, with S phase being more resistant and G_2/M more sensitive to radiation [6]. In this regard, it would be interesting to further explore the role of ABT-751 in tumor apoptosis.

The tumor vasculature is another potential target for ABT-751. Combretastatin and colchicine analogs such as ABT-751 have been recently investigated as antivascular agents [10, 15, 19]. These vascular-targeting agents cause microtubules of endothelial cells to depolymerize and die, thus shutting down the tumor vasculature. Thus, ABT-751 may be inhibiting tumor growth in the Calu-6 NSCLC, HT-29, and HCT-116 colon nude mouse xenograft models by targeting not only the tumor cells but also the tumor vasculature. As mentioned above, altering the combination schedules may further enhance the antitumor responses in terms of growth delay.

Growth delay, however, may not be the best endpoint to demonstrate therapeutic interactions for ABT-751. Many cytotoxic therapies would be curative if high enough treatment doses were attainable. However, clinical toxicities associated with the treatment often prevent attainment of curative dose levels. This is true for chemotherapy as well as radiotherapy, where sensitive tissues near the tumor may be in the radiation field. If ABT-751 could lower the curative threshold for cytotoxic therapies, it could greatly enhance the cure rates. Although cure rates were not assessed here, there was some indication for curative potential for ABT-751 in the radiation experiments. One mouse in the combined radiation/drug treatment group had a

complete remission from a relatively large tumor, and was apparently cured (i.e. the tumor did not regrow for 8 weeks beyond the experiment). The combined treatment might have produced a much higher cure rate had we used a slightly higher radiation dose, since we found that radiation alone was curative in this model at higher doses (Fig. 3). Further work with combined cytotoxic/ABT-751 in models with curative endpoints needs to be pursued.

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References

1. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282:1497–1501
2. Carlson DM, Steinberg JL, Gordon GB (2005) Targeting the unmet medical need: the Abbott Laboratories oncology approach. Clin Adv Hematol Oncol 3:703–710
3. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B (1999) 14-3-3s is required to prevent mitotic catastrophe after DNA damage. Nature 401:616–620
4. Taxotere-Patient Information Leaflet [http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction = Search.Label_ApprovalHistory#apphist]
5. Fox E, Adamson PC, Hagey A, Widemann BC, Maris JM, Cohn SL, Cai Y, Medina DM, Meek KA, Balis FM, et al (2005) Phase I trial of oral ABT-751 in pediatric patients: preliminary evidence of activity in neuroblastoma (NBL). J Clin Oncol 23:8527
6. Hall EJ (2000) Radiobiology for the radiologist, 5th edn. Lippincott Williams & Wilkins, New York
7. Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW, Vogelstein B (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol Cell 1:3–11
8. Jordan MA, Wilson L (1998) Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr Opin Cell Biol 10:123–130
9. Jordan MA, Wilson L (1998) Use of drugs to study role of microtubule assembly dynamics in living cells. Methods Enzymol 298:252–276
10. Jordan MA, Wilson L (2004) Microtubules as a target for anticancer drugs. Nat Rev Cancer 4:253–265
11. Kelner MJ, McMorris TC, Rojas RJ, Trani NA, Velasco TR, Estes LA, Suthipinijtham P (2002) Enhanced antitumor activity of irofulven in combination with antimitotic agents. Invest New Drugs 20:271–279
12. Koyanagi N, Nagasu T, Fujita F, Watanabe T, Tsukahara K, Funahashi Y, Eisai, California, Exp. Cancer-Chemother. Res. Lab., University of Osaka, California (1994) In vivo tumor growth inhibition produced by a novel sulfonamide, E7010, against rodent and human tumors. Cancer Res 54:1702–1706
13. Li CJ, Li YZ, Pinto AV, Pardee AB (1999) Potent inhibition of tumor survival in vivo by beta-lapachone plus taxol: combining drugs imposes different artificial checkpoints. Proc Natl Acad Sci USA 96:13369–13374
14. Li Q, Sham HL, Rosenberg SH (1999) Antimitotic agents. Annu Rep Med Chem 34:139–148
15. Luo Y, Mohning KM, Nuss ME, Hradil VP, Joseph IB, Frost DJ, Sham HL, Rosenberg SH, Reinhart GA, Cox BF (2002) A novel anti-tubulin agent (ABT-751) selectively reduces tumor perfusion in a subcutaneous rat tumor model as measured by MRI. In: Proceedings of the American Association for Cancer Research 93rd Annual Meeting, 6–10 April 2002, San Francisco, CA, USA
16. Raynaud FI, Judson IR (1996) Synthetic microtubule inhibitors. Drugs Future 21:65–70
17. Rowinsky EK, Donehower RC (1997) Pharmacology of cancer chemotherapy: antimicrotubule agents. In: DeVita VT, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology. Lippincott-Raven, Philadelphia, pp 467–483
18. Rowinsky EK (1997) The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annu Rev Med 48:353–374
19. Segreti JA, Polakowski JS, Koch KA, Marsh KC, Bauch JL, Rosenberg SH, Sham HL, Cox BF, Reinhart GA (2004) Tumor selective antivascular effects of the novel antimitotic compound ABT-751: an in vivo rat regional hemodynamic study. Cancer Chemother Pharmacol 54:273–281
20. Teicher BA, Alvarez E, Liu P, Lu K, Menon K, Dempsey J, Schultz RM (1999) MTA (LY231514) in combination treatment regimens using human tumor xenografts and the EMT-6 murine mammary carcinoma. Semin Oncol 26:55–62
21. Tian H, Wittmack EK, Jorgensen TJ (2000) $p21^{WAF1/CIP1}$ antisense therapy radiosensitizes human colon cancer by converting growth arrest to apoptosis. Cancer Res 60:679–684
22. Waldman T, Kinzler KW, Vogelstein B (1995) p21 is necessary for the p53-mediated G_1 arrest in human cancer cells. Cancer Res 55:5187–5190
23. Waldman T, Lengauer C, Kinzler KW, Vogelstein B (1996) Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature 381:713–716
24. Waldman T, Zhang Y, Dillehay L, Yu J, Kinzler K, Vogelstein B, Williams J (1997) Cell-cycle arrest versus cell death in cancer therapy. Nature Med 3:1034–1036
25. Yamamoto K, Noda K, Yoshimura A, Fukuoka M, Furuse K, Niitani H (1998) Phase I study of E7010. Cancer Chemother Pharmacol 42:127–134
26. Yoshimatsu K, Yamaguchi A, Yoshino H, Koyanagi N, Kitoh K (1997) Mechanism of action of E7010, an orally active sulfonamide antitumor agent: inhibition of mitosis by binding to the colchicine site of tubulin. Cancer Res 57:3208–3213