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Endothelin Receptor A Blockade Enhances Taxane Effects in Prostate Cancer[1]

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Abstract

Endothelin (ET) 1 is important in the growth of prostate cancer cells through the activation of the endothelin A (ET_A) receptor. ET receptor blockade is a new therapeutic target in treating advanced prostate cancer. This study investigates the impact of the combination of the ET_A antagonist atrasentan (ABT-627) and taxane chemotherapy on prostate cancer cell survival *in vitro* and on the delay of prostate cancer in a xenograft mouse model. *In vitro*, PPC-1 cells transfected with an ET_A-overexpressing vector were treated with ABT-627, paclitaxel/docetaxel, or both. Clonogenic viability and cell death assays were used to determine cell survival and apoptosis, respectively. ABT-627 and docetaxel combination treatment was used *in vivo* to treat mice with established ET_A-overexpressing PPC-1 xenograft tumors, and tumor growth rates were assessed. Cell proliferation and vascularity were determined with Ki-67 and CD31 staining, respectively. Cells treated with combination therapy had significantly fewer viable cells and more programmed cell death than cells given monotherapy. Xenograft tumor growth rates were significantly lower in mice treated with combination therapy than in animals given a single agent. Ki-67 immunostaining demonstrated significantly fewer proliferative cells following combination therapy than following monotherapy. This study demonstrates ABT-627 to have additive antitumor effects when used in combination with taxane drugs both *in vitro* and *in vivo*.

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Introduction

Prostate cancer remains the second leading cause of cancer-related deaths among males in the United States. An average male carries an 18% lifetime risk of developing a clinically apparent disease [1]. If detected early, localized therapy, such as prostatectomy or radiation, can be curative; however, approximately 25% of patients with localized disease experience biochemical recurrence following treatment [2]. Initially, such men are treated with androgen deprivation to induce apoptotic pathways, but the effects are typically short-lived as patients develop androgen-refractory prostate cancer (ARPC), followed by painful bone metastasis and death at a median of 20 months following recurrence [3]. Unfortunately, very little is known about the molecular mechanisms behind advanced prostate cancer and the loss of androgen sensitivity. As such, no curative therapy exists for ARPC.

In the treatment of advanced prostate cancer, one promising target is the potent vasoconstrictor, endothelin (ET) 1. ET peptides, which include ET-1, ET-2, and ET-3, work by binding to endothelin A (ET_A) and endothelin B (ET_B) G-protein–coupled seven-transmembrane receptors. Through interaction with these receptors, ET peptides have been implicated in several cancer-related processes, including nociception, tumor invasion, neovascularization, osteogenesis, mitogenesis, and apoptosis [4,5], in many solid organ malignancies, including prostate cancer [6–14].

Accordingly, investigators have turned their attention to the selective blockade of ET_A receptors as a possible therapeutic approach toward treating advanced prostate cancer. The orally available atrasentan (ABT-627) potently (K_i = 34 pM) and selectively (1862-fold) blocks the ET_A receptor [15]. Although prior studies have demonstrated a significant effect of ABT-627 monotherapy on prostate cancer [16], the ET_A antagonist is likely to find its therapeutic niche as an agent best used in combination with other chemotherapeutics, specifically the cytotoxic taxanes paclitaxel and docetaxel. Currently the most effective Food and Drug Administration–approved

Abbreviations: ABT-627, atrasentan; ARPC, androgen-refractory prostate cancer; ELISA, enzyme-linked immunosorbent assay; ET-1, endothelin-1; ET_A, endothelin A; ET_B, endothelin B; HPLC, high-performance liquid chromatography; PI3-K, phosphatidyinositol 3-kinase; VEGF, vascular endothelial growth factor

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treatment for advanced prostate cancer, the taxanes inhibit microtubular depolymerization, thereby arresting cells in the G_2M phase of the cell cycle [1]. Preliminary xenograft studies demonstrated that paclitaxel + ABT-627 combination therapy significantly reduces tumor growth compared to monotherapy alone [17]. In an ovarian cancer model, Rosano et al. [18] described a significant additive effect of taxane + ABT-627 on the reduction of tumor growth, apoptosis, and angiogenesis both *in vivo* and *in vitro*, further supporting the potential of this combinational therapy. Given these encouraging preliminary results, we set out to further investigate the role of taxane + ABT-627 combination therapy in a prostate cancer model.

Materials and Methods

Cell Lines

Wild-type PPC-1 prostate cells transfected previously with an ET_A-overexpressing vector (pCMV-ET_A) were grown in RPMI 1640 supplemented with 10% fetal bovine serum (GibcoBRL, Grand Island, NY) and 1% penicillin/streptomycin. As negative controls, PPC-1 cells transfected with the pCMV vector alone were grown under the same conditions.

Clonogenic Viability Assay

PPC-1-ET_A and PPC-1-pCMV cells were each plated at a density of 1.0×10^3 cells/well in six-well plates. Cells were allowed to adhere overnight and were then pretreated with either serum-free medium, ET-1 (10^{-7} M), ET-1 (10^{-7} M) + ABT-627 ET_A antagonist (10^{-6} M; Abbott Laboratories, Abbott Park, IL), ET-1 (10^{-7} M) + A192621 ET_B antagonist (10^{-6} M; Abbott Laboratories), or ABT-627 (10^{-6} M) alone for 2 hours. Following pretreatment, serum-free medium, paclitaxel (10^{-7} M), or docetaxel (10^{-7} M) was added to each of the conditions for 24 hours. Afterward, cells were given fresh growth media and allowed to grow under normal conditions. After 4 weeks, cells were washed with serum-free medium, fixed, and stained with 0.1% crystal violet dissolved in 20% methanol for 15 minutes. Plates were rinsed and allowed to dry overnight. Colonies were counted in a blinded fashion by three individuals and averaged; the results were graphed and analyzed using GraphPad Prism3 (San Diego, CA). Clonogenic assay was replicated in three separate experiments for both docetaxel and paclitaxel.

Cell Death Detection

PPC-1-ET_A cells were plated at a density of 2×10^4 cells/well in 100-mm^3 dishes. Cells adhered overnight and were then pretreated with ET-1 (10^{-7}M), serum-free medium, or ABT-627 (10^{-6} M) for 2 hours. Afterward, cells were treated for 24 hours with pretreatment conditions plus either paclitaxel (10^{-7} and 10^{-8} M), docetaxel (10^{-7} and 10^{-8} M), or vehicle. Spectrophotometric enzyme-linked immunosorbent assay (ELISA) was used to quantify histone-associated DNA fragments present in cell lysates, according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Briefly, the standard solution and samples were added to the wells of a 96-well plate coated with a monoclonal antibody. After incubation, the plate was washed, and an enzyme-labeled antibody was added. After further incubation, the plate was washed again and treated with the substrate, and optical density was determined at 405 nm using an absorption spectrophotometer (Benchmark 680; BioRad, Hercules, CA). Apoptosis assays were repeated in three independent experiments.

Xenograft Studies

PPC-1-ET_A cells were grown in culture in the above conditions. When cells had reached 80% confluency, cells were dissociated with trypsin and washed twice in Hank's balanced salt solution (HBSS), and 40 male athymic nu/nu mice (Harlan Laboratories, Indianapolis, IN) received subcutaneous flank injections of 5×10^5 cells per 100 μl of HBSS. When the average tumor size had reached 0.05 cm^3, the mice were given one of four drug treatments: 1) vehicle (ethanol, i.p., every 3 days); 2) ABT-627 (20 mg/kg per day in drinking water); 3) docetaxel (5 mg/kg, i.p., every 3 days); or 4) ABT-627 (20 mg/kg per day in drinking water) + docetaxel (5 mg/kg, i.p., every 3 days). Once the largest tumor burden had reached 2 cm^3, the mice were sacrificed and the tumors were removed, measured, weighed, fixed in 4% paraformaldehyde, and embedded in paraffin for further analysis. Given ET's known role in angiogenesis, serum was also collected for the quantification of vascular endothelial growth factor (VEGF) by ELISA, in accordance with the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Immunohistochemistry

Five-micron sections of paraffin-embedded tumors were quenched in 3% hydrogen peroxide for 15 minutes and stained following citrate-steam antigen retrieval with TEC-3 Ki-67 (M7249; Dako, Caripinteria, CA) and CD31 (SC-1506; Santa Cruz Biotechnology, Santa Cruz, CA) primary antibodies to determine cell proliferation and angiogenesis, respectively. ET_A and ET_B polyclonal antibodies (Abbott Laboratories) were also used to verify ET receptor expression in xenograft tumors. A biotinylated secondary antibody was used, followed by streptavidin-conjugated horseradish peroxidase and 3,3′-diaminobenzidine chromogen (K0690; Dako). Slides were scored by three separate individuals in a blinded fashion.

Tumor Pharmacokinetic Studies

Tumors were removed 12 hours after the administration of docetaxel alone and in combination with ABT-627. Tumor samples were obtained, weighed, frozen in liquid nitrogen, and stored at −80°C until analysis. Tumor samples were processed using a liquid–liquid extraction procedure followed by solid-phase extraction, as previously described [19]. Docetaxel concentrations in tumors were determined by a liquid chromatography–mass spectrometry assay [20].

Statistical Analysis

All experimental data are representative of experiments performed at least in triplicate. For statistical comparisons of

groups, Graph Pad Prism software was used (Graph Pad Software, San Diego, CA). For comparisons of values between groups, Student's *t* test was used. Data are presented as mean ± SE, and the level of significance was set at < .05 for all analyses.

Statistical Modeling

Tumor growth patterns in xenograft mice were assessed using an exponential growth model ($Y = \text{baseline} \times e^{\alpha \times \text{time}}$), with the determination of doubling time for each treatment (Doubling Time = 0.693 / α). This growth pattern served as a template for determining growth parameters by nonlinear mixed-effects modeling (NONMEM). Each treatment group was assessed while preserving within-individual and between-individual variances present in measurements of tumor growth over time. In addition, the mixed-effects modeling approach used every data point to determine growth parameters, both as a mean and as a variance across individuals. Therefore, balance in the groups was not as critical (weighting across individual contributions to this model is based on data contributed, as well as on the informativeness of data to a particular parameter). In mixed-effects modeling, population average and variance were calculated. In the case of an exponential model, population average and variance were based on the population average baseline tumor size (S_0) and a population average growth rate parameter (α). Each treatment group was tested as a covariate in the growth model to examine whether that group exhibited unique growth characteristics. The threshold for determining significance was based on the objective function returned by the NONMEM software program, which is equal to −2 times the log likelihood. The difference between nested models of this objective function follows a chi-square distribution and thus provides a means of statistically comparing growth descriptions across models. The α level for significance was set at .05, corresponding to an objective function change of 3.84 points for 1 *df*.

Results

Combination Therapy Yields Fewer Viable Cells Than Does Monotherapy

Clonogenic studies of PPC-1-ET_A cells demonstrated that treatment with 10^{-7} M paclitaxel significantly decreased cell viability (data not shown) ($P = .0033$). Figure 1*D* illustrates that pretreatment of cells with 10^{-7} M ET-1 counteracted the effects of paclitaxel. The addition of 10^{-6} M ABT-627, an ET_A antagonist, to ET-1 pretreatment blocked the ability of ET-1 to protect cells from paclitaxel, significantly decreasing the average number of colonies ($P = .0438$). Cells pretreated with the ET_B antagonist A192621, along with ET-1, remained viable after paclitaxel administration ($P = .4520$) (data not shown), suggesting that ET-1 mediates its protective effect through the ET_A receptor. This corroborates our earlier findings that ET_B receptor expression is absent in PPC-1 cells through the hypermethylation of ET_B promoter region *EDNRB* [21], and that ET-1 survival signaling works through the ET_A receptor using the phosphatidyinositol 3-kinase (PI3-K) pathway [22,23].

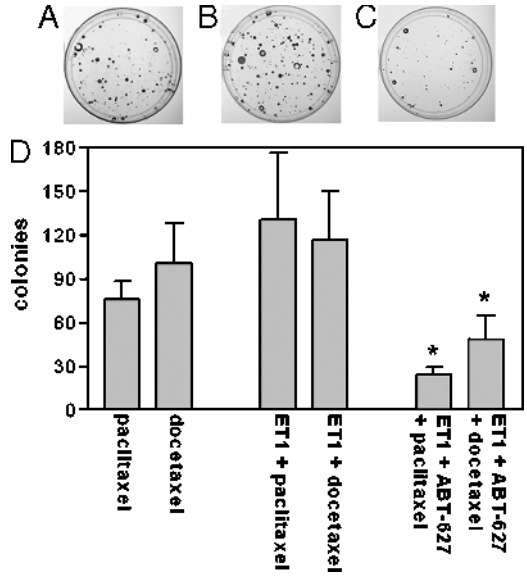

Figure 1. *(A–C) ET-1 + ABT-627 + taxanes combination treatment resulted in significantly fewer viable cells than treatment with taxanes alone. PPC-1 cells transfected with an ET_A-overexpressing vector were pretreated with vehicle, ET-1, ET-1 + ABT-627 ET_A antagonist, or ET-1 + A192621 ET_B antagonist, followed by treatment with paclitaxel or docetaxel. Crystal violet analysis was performed after 3 weeks to determine cell survival after treatments: (A) docetaxel, (B) docetaxel + ET-1, and (C) docetaxel + ABT-627 + ET-1. Results are reported as the mean of the number of colonies ± SE from three independent experiments. (D) ET-1 increased the number of colonies in docetaxel-treated and paclitaxel-treated PPC-1 cells. Blocking the ET_A receptor with ABT-627 significantly reduced cell survival. Results are reported as the mean of the number of colonies ± SE from three independent experiments (P < .05).*

Docetaxel treatment produced similar effects. Crystal violet assessment of untreated and ET-1–treated groups yielded too many colonies to be counted; the number of viable cells was significantly decreased by the administration of 10^{-8} M docetaxel alone. Cells pretreated with the ABT-627 ET_A antagonist exhibited a significant decrease in viability after docetaxel treatment when compared to docetaxel-treated cells preincubated with ET-1 alone ($P = .0338$), illustrating an additive effect of ET_A blockade with docetaxel (Figure 1*D*). Representative images of paclitaxel-treated cell plates are shown above their respective treatment conditions (Figure 1, *A–C*).

The efficacy of ABT-627 in both the presence and the absence of ET-1 pretreatment suggests the presence of an endogenous ET-1 ligand that promotes survival through the ET_A receptor—an observation consistent with our previously published findings that prostate cancer cell lines, including PPC-1, secrete significant levels of mature ET-1 [12]. Additionally, treatment of PPC-1-CMV control cells yielded results similar to those of PPC-1-ET_A (data not shown), suggesting that PPC-1 cells expressed enough endogenous ET_A receptors to yield a protective response.

Combination Therapy Results in More Apoptosis

To determine if the decrease in cell viability occurred through an apoptotic mechanism, we used an ELISA-based

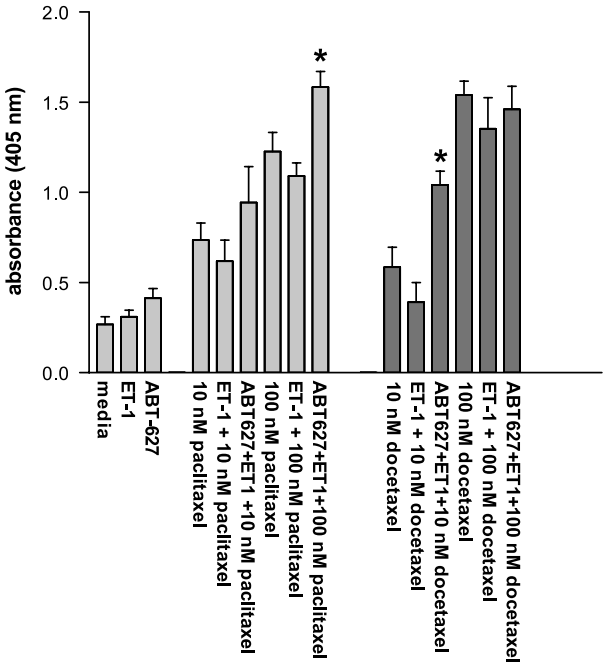

Figure 2. *Combination treatment with ABT-627 + paclitaxel or docetaxel resulted in significantly more cell deaths than treatment with taxane alone. Prostate cancer cells were pretreated with serum-free medium, ET-1, or ABT-627 ET_A antagonist, followed by treatment with vehicle, 10 nM paclitaxel, 100 nM paclitaxel, 10 nM docetaxel, or 100 nM docetaxel. Apoptosis was quantified with a spectrophotometric ELISA-based assay. Results are reported as the mean ± SE from three independent experiments ($P < .05$).*

assay to measure DNA fragmentation in PPC-1-ET_A cells that had undergone various treatments. Treatment with ABT-627 significantly increased the number of apoptotic cells (P = .044) (Figure 2). As monotherapy, administration of 10^{-7} and 10^{-8} M paclitaxel and of 10^{-7} and 10^{-8} M docetaxel also significantly increased cell death in a dose-dependent fashion ($P < .0001$, .0001, .0001, and .045, respectively). Coadministration of 10^{-6} M ABT-627, however, significantly increased the amount of cell death following 10^{-7} M paclitaxel ($P = .0094$) and 10^{-8} M docetaxel ($P = .02$), respectively, over treatment with the corresponding taxane monotherapy. However, this significance of ABT-627 combination therapy was not present following lower-dose paclitaxel or higher-dose docetaxel, suggesting a specific therapeutic window in which combination therapy is most effective. Overall, these *in vitro* results demonstrate an additive effect of ABT-627 and cytotoxic taxane therapy on apoptosis. These data suggest that, for *in vivo* studies, docetaxel can be used at a dose lower than that of paclitaxel in combination with ABT-627 to effectively reduce tumor volume.

Combination Therapy Reduces Xenograft Growth Rates More Than Does Monotherapy

The efficacy of low-dose docetaxel monotherapy *versus* ABT-627 + docetaxel combination therapy was assessed *in vivo* using a xenograft mouse model. Athymic mice bearing established PPC-1-ET_A xenograft tumors were treated with vehicle, ABT-627 alone, docetaxel alone, or ABT-627 + docetaxel. Figure 3 illustrates the tumor volumes of these mice over the treatment period. Mice treated with vehicle had the same tumor burdens as those treated with either ABT-627 or docetaxel monotherapy. However, mice given the combination therapy had significantly smaller tumor volumes than mice in the other treatment groups. Note that, at the end of the experiment, all mice treated with vehicle or ABT-627 remained alive; in contrast, 6 of 9 mice treated with docetaxel and 5 of 10 mice given the combination therapy survived. This was most likely secondary to the toxic effects of drug accumulation following seven doses of docetaxel.

A tumor growth mathematical model was used to more accurately assess tumor growth patterns in xenograft mice (Table 1). Using an exponential growth model, only the combination treatment group displayed a tumor growth rate distinguishable from those of the other groups. The combination treatment group had a tumor doubling time of 8.7 days (12.1% coefficient of variation), whereas the other groups all had a tumor doubling time of 5.8 days (25.8% coefficient of variation). The use of a separate growth rate for the combination treatment group improved the description of the data ($P = .0045$, $\chi^2 = 8$, $df = 1$).

Combination Therapy Significantly Decreases Tumor Cell Proliferation, But Not Vascularity

Xenograft tumors were immunostained with Ki-67 and CD31 antibodies to illustrate cell proliferation and tumor vascularity, respectively. Positive cells were counted and averaged between three individuals. Figure 4*I* illustrates that tumors of mice treated with ABT-627 + docetaxel combination therapy had significantly fewer proliferative cells than tumors of mice treated with either vehicle, ABT-627 alone, or docetaxel alone ($P = .0225$, .0031, and .0457, respectively). Representative images of Ki-67–stained tumors are illustrated in Figure 4, *A*, *C*, *E*, and *G*.

In contrast, CD31 staining illustrated no significant difference between treatment groups (data not shown). Representative images of xenograft tumors stained with CD31 demonstrate no significant difference in tumor vascularity

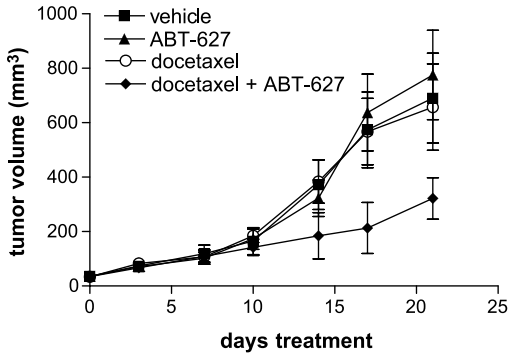

Figure 3. *Mice treated with combination ABT-627 + docetaxel had significantly smaller tumor burdens and growth rates than mice treated with vehicle, ABT-627, or docetaxel alone. ET_A-overexpressing PPC-1 cells were injected as xenograft tumors in athymic mice. Mice with established tumors were treated with vehicle, docetaxel, ABT-627 ET_A antagonist, or a combination of docetaxel + ABT-627. Tumors were measured every 2 days. Results are reported as mean tumor size ± SE.*

Table 1. ET_A Receptor Antagonist and Docetaxel Treatment in Mice Bearing PPC-1 Xenograft Tumors.

Treatment	Baseline Tumor (mm^3)	Final Tumor Volume (mm^3)	Doubling Time (mm^3/day)
Vehicle	63.73	690.5 ± 165.0	5.93
ABT-627	54.58	775.5 ± 164.2	5.36
Docetaxel	55.57	657.4 ± 158.3	5.41
ABT-627 + docetaxel	55.1	321.7 ± 76.1*	8.29*

*Statistically different from all other treatment groups ($P < .05$).

following monotherapy or combination therapy. Serum was also harvested from xenograft mice and analyzed for VEGF levels by ELISA. Similar to CD31 data, ELISA revealed no significant difference in VEGF levels between mice in any of the treatment groups (data not shown). Analysis of ET_A expression in the tumors demonstrated that receptor levels remained high in all treatment groups, suggesting that selection for subpopulations of PPC-1 cells with altered ET_A expression did not contribute to the change in tumor volume with combination therapy (data not shown). ET_B receptor expression was not detectable in prostate tumor cells, whereas endothelial cells of the tumor vasculature were used as internal controls and were positive for ET_B expression in all treatment groups.

Combination Treatment Does Not Significantly Change Tissue Docetaxel Concentration

Given ET's vasoactive properties, LC/MS analysis was performed to determine whether tissue concentrations of docetaxel increased with coadministration of ABT-627. The average tumor concentration of docetaxel in mice treated with docetaxel monotherapy was 110.7 ± 53.26 nM. Mice treated with combination therapy had a slightly decreased tumor docetaxel concentration of 16.33 ± 13.33 nM, but this difference was not significant ($P = .0843$).

Discussion

The normal prostate expresses both ET receptors [24]. Luminal epithelial cells secrete ET-1 [25], which is normally found in greatest concentration within the ejaculate of both intact and vasectomized men [26]. Although its physiological role in the prostate has yet to be determined, ET involvement in neoplastic transformation is becoming evident. ET-1 activation of ET_A receptors localized on prostate stromal cells has antiapoptotic effects. Earlier studies from our laboratory have shown that ET_A/ET-1 receptor–ligand binding correlates with a time-dependent and dose-dependent activation of PI3-K, leading to phosphorylation of AKT and the subsequent inactivation of the proapoptotic protein, Bad [23]. In addition, prostate tumor cell exposure to ET-1 decreases the overall expression of Bad, as well as of the other proapoptotic proteins Bak and Bax. In contrast, activation of the ET_B receptors found within epithelial luminal cells counteracts ET_A by inducing both apoptotic and ET-1 clearance pathways. Studies have shown that increased expression of ET_B may increase apoptosis through an increased expression of the same proapoptotic proteins Bad, Bak, and Bax [23].

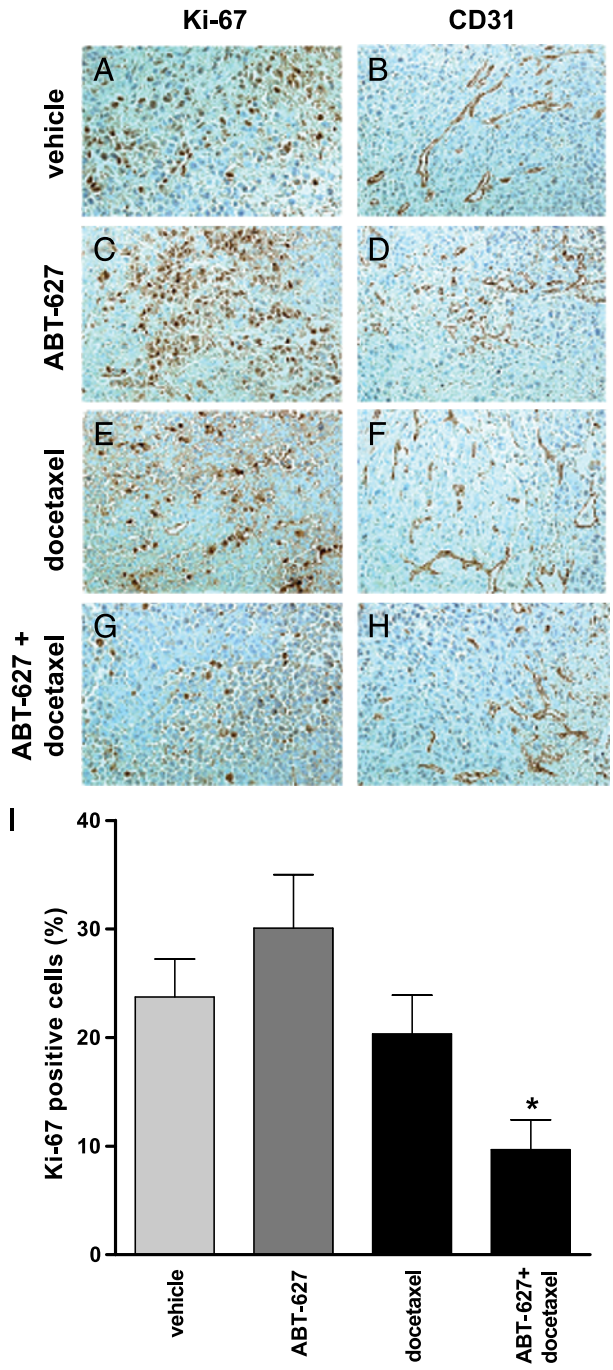

Figure 4. *Mice treated with combination therapy had significantly fewer proliferative cells than those given monotherapy or vehicle alone. Tumor vascularity was not affected. ET_A-overexpressing PPC-1 xenograft tumors were grown in athymic mice treated with vehicle, docetaxel, ABT-627 ET_A, or a combination of docetaxel + ABT-627. After 21 days of treatment, tumors were removed and analyzed by immunohistochemistry for Ki-67 (for the evaluation of cell proliferation) and CD31 (for tumor vasculature analysis) expression. (A and B) Vehicle, (C and D) ABT-627, (E and F) docetaxel, (G and H) ABT-627 + docetaxel, and (I) Ki-67$^+$ cells were quantified in each tumor section. Combination therapy reduced tumor cell proliferation relative to vehicle-treated or monotherapy-treated mice ($P < .05$). In contrast, CD31 staining remained unchanged with treatment.*

ET receptor expression is altered in prostate cancer. Although ET_A receptor expression increases with disease aggressiveness [27], ET_B receptor levels decrease. The decrease in ET_B results, in part, from the hypermethylation of the *EDNRB* promoter region [21]. Hypermethylation correlates with the grade and the stage of prostate cancer [28,29]. *EDNRB* promoter methylation leads to a decrease in ET_B-induced ET-1 clearance. Advanced prostate cancer also corresponds with a decreased activity of the ET-1–cleaving enzyme, neutral endopeptidase 24.11 (NEP) [30]. Given these observations, it is not surprising that patients with ARPC have greater serum ET-1 levels than those with localized disease or than those with no disease [12]. This suggests a prostate cancer model in which the decreased expression of NEP and ET_B results in increased ET-1 levels and uninhibited ET_A activation, culminating in the promotion of carcinogenic pathways.

In addition to its effects on apoptosis, the ET axis has also been implicated in mitogenesis through the activation of the protein kinase C, PI3-K, and MAP kinase signaling pathways [31]. Within a prostate cancer model, ET-1 has been shown to induce the proliferation of prostate cancer cells, especially when used synergistically with polypeptide growth factors such as epidermal growth factor [32]. This mitogenic effect also occurs in osteoblasts—the bone-forming cells responsible for painful bone lesions characteristic of advanced prostate cancer. With approximately 10^4 ET_A receptors per cell, osteoblasts have a surprisingly high density of receptors [33]. *In vitro* studies have shown that ET-1 both induces osteoblastic bone proliferation and inhibits osteoclastic bone resorption; in a murine model, the administration of a selective ET_A receptor antagonist significantly attenuates this effect [34,35]. In addition to the inhibitory potency of ET-1 in osteoclast bone resorption, there is a concentration-dependent reduction in osteoclast motility at nanomolar levels of ligand [36]. ET-1 produced by marrow endothelial cells, as well as prostate tumor cells residing in the bone, will have important roles in the local regulation of osteoclast function. In ovarian cancer models, ET-1 induces VEGF and other angiogenic factors as well, leading to tumor neovascularization [37]; however, our present study did not detect a significant effect of ET_A blockade on tumor vascularity or serum VEGF.

Our data showed that the combination of ET_A antagonism and cytotoxic taxane therapy is significantly more efficacious than treatment with either modality alone. *In vitro*, both paclitaxel and docetaxel, when combined with ABT-627, had additive effects on both decreasing cell viability and increasing apoptosis in PPC-1 cells. *In vivo*, ABT-627 and docetaxel combination therapy significantly reduced the rate of growth of xenograft tumors more than did monotherapy. Furthermore, Ki-67 staining of xenograft tumors illustrated that combination therapy attenuated the number of proliferating cells significantly more than treatment with either docetaxel or ABT-627 alone.

Additive effects can be explained mechanistically; although both drugs activate the proapoptotic proteins of the Bcl-2 family, each has distinct nonoverlapping anticarcinogenic mechanisms. The taxanes primarily inhibit microtubular depolymerization, whereas ABT-627 blocks ET_A-associated proliferative and antiapoptotic pathways [22,23]. Taxanes also work by decreasing Bcl-2 binding to Bax—the same proapoptotic protein inhibited by ET-1 [38].

Given ET's vasoactive properties, it would be reasonable to assume that the increased effectiveness of combination therapy would be due to ABT-627–mediated vascular changes. However, serum VEGF analysis and CD31 vascular endothelium labeling of xenograft tumor demonstrated no differences between mice in the different treatment groups. Additionally, xenograft tumors illustrated no difference in docetaxel concentrations following ET_A blockade. Thus, it appears that ABT-627–mediated enhanced cytotoxicity was not due to changes in blood flow or vascularity. Prior studies have shown ET-1 to increase blood flow in a breast cancer model through the activation of the ET_B receptor [39]; our results suggest that ET_A blockade does not have similar effects in a prostate cancer model. However, in this study, we examined concentrations at a single time point 12 hours after the last of seven docetaxel doses. A pharmacokinetic study to further examine docetaxel distribution in tumors at serial time points with and without ABT-627 would provide a more complete picture of the role of ET_A blockade in reducing tumor growth with combination therapy.

Prostate cancer is characterized by low rates of proliferation, making it resistant to most forms of cytotoxic chemotherapy. Prostate cancer chemotherapy is not curative; in a recent phase III clinical study, the Southwest Oncology Group reported that treatment of ARPC with docetaxel + estramustine increased disease survival by only 3 months in patients who received only mitoxantrone with prednisone [40]. As such, the demand for new treatments remains high. With implicated roles in tumor cell survival, apoptosis, mitogenesis, neovascularization, and osteogenesis, the ET axis is well suited for therapeutic manipulation. Initially designed as an antihypertensive, the ET_A antagonist ABT-627 has a half-life of 25 hours, making it ideally suited for long-term once-daily dosing. Adverse effects are mild and correspond with the drug's vasoactive properties, including headache, rhinitis, and peripheral edema [4].

As monotherapy, ABT-627 has already been well-studied clinically. Following encouraging phase I and II studies [16,41,42], recent phase III clinical trials have focused on the efficacy of ABT-627 in combating ARPC. These studies assessed time to disease progression, prostate-specific antigen progression, bone metabolism markers, and quality of life in prostate cancer patients given ABT-627 *vs* placebo. Although the first completed set of these trials demonstrated that ABT-627 both significantly delayed disease progression and attenuated the increase in bone and tumor markers in the evaluable population, the drug did not significantly delay disease progression in the intent-to-treat population. Although the lack of a significant effect for ABT-627 monotherapy in this first phase III trial was disappointing, a therapeutic effect from treatment clearly exists.

In light of these clinical results, our study supports the hypothesis that ABT-627 may have greater clinical utility

when used in combination with taxane treatment. Ongoing phase I clinical studies are already examining ABT-627 + docetaxel combination therapy in patients with advanced disease. Although the results of these trials have yet to be published, it will be of great interest to see if clinical studies confirm our results. Although our data support the concept that ABT-627 has additive effects on treating advanced prostate cancer with the cytotoxic taxanes, further studies are necessary to optimize the combination to evoke the most effective therapeutic response. Only then will we be able to determine the full therapeutic potential of ET manipulation in the treatment of advanced prostate cancer.

Conclusion

The ET axis is involved in several tumorigenic pathways in prostate cancer. Both *in vitro* and *in vivo*, blockade of the ET_A receptor with ABT-627 demonstrated significant additive effects when used in combination with the cytotoxic taxanes paclitaxel and docetaxel. Given the efficacy, low toxicity profile, bioavailability, and half-life of ABT-627, additional studies should be performed to further delineate the therapeutic role of this promising agent.

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