Timing Is Everything: Preclinical Evidence Supporting Simultaneous Rather Than Sequential Chemohormonal Therapy for Prostate Cancer

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Abstract Purpose: Androgen ablation is the mainstay of systemic therapy for prostate cancer, with cytotoxic therapies reserved for hormone-refractory disease. It is not clear, however, that this is the most appropriate sequence of interventions for this disease. This study addresses the ideal timing of systemic treatments in the Shionogi and LNCaP xenograft models. We explored the hypothesis that stress-induced gene expression changes after chemotherapy can induce a hormone-independent phenotype.

Experimental Design: Three groups of mice bearing either Shionogi or LNCaP xenografts were treated with (a) initial castration and delayed paclitaxel, (b) initial paclitaxel and delayed castration, or (c) simultaneous castration plus paclitaxel. End points were time to tumor progression and time to sacrifice. Microarray and reverse transcription-PCR analyses were carried out to assess changes in gene expression induced by paclitaxel.

Results: Mice receiving simultaneous therapy showed a significant improvement in median time to progression (TTP: Shionogi, 65 versus 38 days, \( P = 0.004 \); LNCaP, 105 versus 70 days, \( P = 0.032 \)) and time to sacrifice (Shionogi, 83 versus 66 days, \( P < 0.014 \)) versus best sequential therapy. A marked lack of response to castration was observed after initial paclitaxel therapy. Gene expression and reverse transcription-PCR studies confirmed that several genes known to play a role in androgen independence were up-regulated in response to paclitaxel exposure.

Conclusions: In laboratory models of prostate cancer, simultaneous androgen deprivation plus paclitaxel is more effective than sequential treatments. These findings provide preclinical proof-of-principle for ongoing clinical trials addressing the role and timing of systemic therapies in prostate cancer.

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in Canadian men. In 2004, it is estimated that 20,100 Canadians will be diagnosed with prostate cancer and that 4,200 will die of this disease (Canadian Cancer Statistics, 2004). Androgen withdrawal is the most effective form of systemic therapy for men with advanced disease, producing symptomatic and/or objective responses in >80% of patients. Unfortunately, androgen-independent progression is inevitable, and development of hormone refractory disease and death occurs within 18 to 24 months in most men (1). The development of hormone refractory disease is marked by an increase in serum prostate-specific antigen in the setting of androgen ablation. The survival associated with the development of hormone-refractory prostate cancer is in the range of 12 to 18 months (2, 3).

The role of systemic chemotherapy for prostate cancer is presently limited to the setting of hormone-refractory prostate cancer. Two recent randomized trials have shown a small but consistent survival advantage for docetaxel-based chemotherapy in this setting (2, 3). These developments, coupled with the observation that hormonal therapies alone have an invariably finite efficacy, have led to further studies aimed at demonstrating the effectiveness of chemotherapy given earlier in the course of the disease and in combination with hormonal therapies.

Whereas the evaluation of therapeutic recipes aimed at better disease control is essential, preclinical data evaluating the optimal timing and combination of androgen withdrawal with cytotoxic chemotherapy for prostate cancer is limited (4). Furthermore, clinical trials in the setting of breast cancer treatment have failed to show any significant improvement in disease free or overall survival for the use of combination chemohormonal/hormonal therapy (5, 6) and have shown a
superiority of sequential treatment when compared with simultaneous therapy (7). The goal of this study was to determine the effect of taxane-based chemotherapy given either precastsaration, concurrent with castration, or post-castration in an animal model of prostate cancer and to characterize chemotherapy-induced changes in gene expression to better understand the biological basis for different responses observed.

Materials and Methods

Tumor cell lines. Shionogi mouse mammary carcinoma was passaged as a xenograft in DMEM with low glucose. LNCaP human prostate carcinoma cells were cultured and maintained in RPMI (Life Technologies, Inc., Gaithersburg, MD) supplemented with 5% heat-inactivated FCS as previously described (8, 9). Cells underwent 4 to 10 passages before mouse inoculation.

Chemotherapeutic agents. For in vivo use, polymeric micellar paclitaxel was generously supplied by Dr. Helen M. Burt (Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada). Paclitaxel was diluted in sterile normal saline to the appropriate concentration in in vitro experiments used stock paclitaxel (Sigma, St. Louis, MO) diluted to the appropriate concentration in 0.2% bovine serum albumin (Sigma) at 48°C. Slides were prehybridized in 5× SSC, 0.01% SDS, 8% DMSO and 0.2% bovine serum albumin (Sigma) at 48°C for 45 minutes, washed in deionized water, dipped in isopropanol, and dried in a centrifuge at 2,000 rpm for 2 minutes. Arrays were hybridized with reverse-transcribed fluorescently labeled (Cy3-or Cy5-dUTP; Amersham-Pharmacia, Piscataway, NJ) cDNA (from 20 μg of total RNA) at 42°C for 16 hours in a hybridization buffer consisting of 50% formamide, 5× SSC, 0.01% SDS, 8 μg bovine serum albumin, 25 μg yeast tRNA, and 20 μg salmon testes DNA. Following stringent washes (1× SSC and 0.1% SDS, then 0.1× SSC), fluorescent images of the slides were acquired using a microarray scanner (ScanArray, Perkin-Elmer, Woodbridge, Ontario, Canada). Signal quality and quantity were assessed using Imagej 5.6 (BioDiscovery, San Diego, CA). Data from Imagej were analyzed using GeneSpring 6.1 software with a per spot and per chip intensity-dependent (LOWESS) normalization (Silicon Genetics, Redwood City, CA) for profiling significant changes in gene expression. After applying a multiple comparison test (Benjamini Hochberg False Discovery Rate), gene expression level changes were considered significant if an expression change of >1.5 or <0.6 relative to baseline was observed.

Total RNA from each treatment sample was compared with control sample on the same chip. Quality and quantity of RNA was assessed with an Agilent 2100 Bioanalyzer (Caliper Technologies Corp., Hopkins, MA). A dye-swap for each pair was done to account for dye bias. Experiments were repeated four times.

Reverse transcription-PCR analysis. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) and reverse transcribed using random hexamers (Perkin-Elmer Applied Biosystems, Branchburg, NJ) and 20 units of Moloney murine leukemia virus reverse transcriptase M-MLV (Invitrogen) in 20 μL of total volume at 25°C for 10 minutes and at 37°C for 60 minutes. Finally, the reaction was stopped by exposure to 95°C for 5 minutes. The resulting first-strand cDNA was used as template for the real-time quantitative-PCR. The Applied Biosystems 5700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA) was used for real-time monitoring of PCR amplification of the cDNA following the Taqman Universal PCR Master Mix protocol (10). The amplification of target cDNA was done using primers and Taqman probes (Nucleic Acid Products Service Unit, University of British Columbia Biotechnology Laboratory, Vancouver, British Columbia, Canada) consisting of the following sequences: Clusterin, 5′-GAGGACCTGACAGGAGCATT-3′ as a forward primer, 5′-CTTGCCCTGGGTGAGCT-3′ as a reverse primer, 5′-VIC-ACCTGCTGTTGCTCGAGCAGT-3′ as the Taqman probe; Human IGF-BP2, forward GCTCCCTGAGACCTCCTACT, reverse ATCTIGC-AGCCTGTAGGTTGCTA, probe ACATGCCCAACTGTGACAGG-CAAGCC; Human Bcl-XL (forward GCCGTCGAGCCCTGCTTGTG, reverse CAGCCGGAGGAATTCGACAGA, probe AACTCTACGCCG- CAGCATGCC; Human Bcl-XL, for primers forward GCCGGACGTGCTTTTGC, reverse CAGCCGGAGGAATTCGACAGA, probe AACTCTACGCCG-CAGCATGCC; Human Bcl-XL primers, forward GCTCCGATT-GTGCCCTTT, forward TCTCCGTCCTCGCTGTTTTCCA, probe ACAGTGCCCCCAGGAGGAGA.

All probes have 5′ VIC and 3′ TAMRA modifications. Relative quantification of gene expression was done using RNA as a control. Ribosomal cDNA was amplified separately on a duplicate set of samples using standard primers and Taqman probe (Perkin-Elmer). The comparative Ct (cycle threshold) method was used for relative quantification of clusterin mRNA.

The principles of the real-time reverse transcription-PCR detection with hydrolysis probes (Taqman) have been previously described (11, 12). Statistical significance was determined by ANOVA model. The Ct value is defined as the cycle number in which the detected fluorescence exceeds the threshold value (11, 12).

Fold difference = 2^-ΔCt (1/clusterin) – Ct (tRNA) – 2^-ΔCt (2/clusterin) – Ct (2/tRNA).

Where Ct (clusterin) and Ct (tRNA) represent the Ct values for the treated samples, respectively. Ct (2/clusterin) and Ct (2/tRNA) represent the Ct values for the untreated samples, respectively.

In vivo growth and treatment assays. All animal procedures were done according to local guidelines on animal care and with appropriate institutional certification.

Shionogi xenografts were generated by s.c. inoculation of 5 × 10^6 Shionogi cells in 0.5 mL of medium at the nape of neck in 30 male DD/ S mice ages 6 to 8 weeks. Bidimensional tumor measurements were taken daily with digital calipers, and volumes were calculated as V = 0.5 × d × b × 0.5236 (where d is the smaller diameter). Once tumors grew to a mean volume of 0.5 cm^3 (roughly equivalent to 1 × 1 cm), mice were divided into three groups of 10. The primary treatment end points for each group were TTP and time to sacrifice. The TTP end point was prospectively defined as the time in days measured from the initiation of treatment to the point in time at which both chemohormonal and hormonal therapy had been given and tumor volumes had again grown to 0.5 cm^3. The three groups of mice were treated as follows:

- Group AA-P: (Castration with second-line paclitaxel) mice were castrated via a scrotal approach. Tumor volumes were measured daily until they again attained a volume of 0.5 cm^3. At that point, treatment with paclitaxel was initiated. Polymeric micellar paclitaxel 0.5 mg was given via tail vein injections daily on days 1 to 4 and 15 to 18. Mice were again assessed on a daily basis until tumors reached 0.5 cm^3 (TTP end point).

- Group P-AA: (Paclitaxel with second-line castration) mice were treated with paclitaxel and assessed daily as described for group A above. At the point of tumor regrowth to 0.5 cm^3 (or within 2 days of the last paclitaxel dose if tumor shrinkage to a volume of <0.5 cm^3 was not achieved), mice were castrated via a scrotal approach. Mice were then assessed on a daily basis until the TTP end point was reached.

- Group C: (Combination treatment) mice were treated with chemotherapy and castration simultaneously using the methods described above. The TTP end point for this group of mice was set as the time to tumor regrowth to 0.5 cm^3 from this initial therapy.

LNCaP xenografts were generated by s.c. inoculation of 1 × 10^6 LNCaP cells were inoculated s.c. with 0.25 mL of Matrigel (Becton

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Dickinson, Bedford, MA) in the flank region of 6- to 8-week-old athymic male mice. Of the injected mice developing tumors (~75%), tumor growth was typically visible 8 to 12 weeks following inoculation. Tumors were allowed to grow until they reached 5 to 10 mm in diameter. The mice were then assigned to one of three treatment groups as described above each consisting of eight mice (i.e., groups AA-P, P-AA, and C). In the sequential treatment groups, the second-line treatment was applied 14 days after the first. The mice were distributed so mean tumor volume at time of randomization was equivalent among the three groups. Study end points were time to androgen-independent growth, tumor volume, and mouse survival. Although the study was completed at 28 weeks following randomization, three mice in the combination therapy group were still alive without evidence of visible tumor at 40 weeks. For the purpose of calculating the mean time to androgen-independent tumor growth, these three mice were assigned a value of 40 weeks.

Statistical analysis. Tumor volumes over time were compared using nonparametric one-way ANOVA. Comparisons between two groups were done using the Student’s t test. Survival analysis was evaluated using Kaplan-Meier curves and log rank tests. P < 0.05 was considered statistically significant. All statistical calculations were done using Graphpad Prism 4.0 (GraphPad Software, Inc., San Diego, CA).

**Results**

Combination therapy (paclitaxel plus androgen deprivation) has additive growth suppressive effects in vitro. To ascertain whether androgen deprivation enhanced the apoptotic effects observed with paclitaxel, LNCaP cells were grown in medium supplemented with fetal bovine serum or CSS. Cells were then exposed to increasing doses of paclitaxel. Cell viability was assessed after 48 hours of paclitaxel exposure. Growth in CSS resulted in significantly increased sensitivity to paclitaxel, reducing the IC_{50} by >2 log, from 2 to 0.01 nmol/L (data not shown).

Simultaneous paclitaxel plus castration suppresses tumor growth more effectively than sequential therapy: Shionogi model. To assess the effects of combined compared with sequential therapy rates on tumor suppression in vivo, intact male mice bearing Shionogi xenografts were divided into treatment groups as described above. Mice treated with simultaneous chemotherapy (group C) had a more pronounced tumor regression and longer time to recurrence than either of the sequentially treated groups (Fig. 1). The mean decrease in Shionogi tumor volume at first nadir was 95.5% for group C versus 94% for AA-P and only 61% for P-AA. Furthermore, mice in the P-AA group experienced only a minimal response to paclitaxel, and tumor volumes subsequently progressed at a rapid rate. As it became apparent that tumors were continuing to progress during the course of chemotherapy, mice in this group were castrated within 2 days of completing paclitaxel treatment. As expected, tumor shrinkage occurred in response to androgen ablation, but the relative magnitude and duration of tumor response was significantly shorter than in the other two treatment groups and from previously reported experiments on tumors that had not been pretreated with chemotherapy before castration (13).

A more rigorous comparison of the other two treatment groups (C versus AA-P) is shown in Fig. 1B. A rapid decline in tumor volume after castration was seen in both groups; however, the trend was to maximal tumor response for the combination group (99.5% versus 94.0%, P = 0.08), and all but one mouse had a complete response to therapy in the C group.

In group AA-P, disease progression requiring the initiation of taxol treatment was seen by week 4. At this point, chemotherapy resulted in only a short-lived decrease in the rate of tumor growth, and by week 6, the difference in relative tumor volumes between these two groups had become significant. In comparison with sequential therapy, the simultaneous group experienced almost a doubling of median TTP (38 days versus 65 days, P < 0.005) as seen in Fig. 2A. Time to sacrifice was also significantly improved (66 versus 83 days, P = 0.01; Fig. 2B). At the termination of this experiment, all mice in the sequential group had been sacrificed, whereas four mice in group C were still alive, one without palpable tumor.

LNCaP model. In the LNCaP model, initial androgen ablation and paclitaxel therapy resulted in the most immediate and largest decrease in tumor volume, similar to what was observed in the Shionogi model (Fig. 3A and B). Immediate paclitaxel administration alone led to tumor volume decreases that were augmented when androgen ablation was added 2 weeks later (P-AA). Immediate androgen ablation followed by androgen ablation and paclitaxel); bars, ± SE. Boxes indicate the time of paclitaxel administration. B, points, mean Shionogi tumor volumes for the two groups with best treatment response; bars, ± SE. The tumor response to combination therapy was more pronounced (nadir reduction in tumor volume 99.5% versus 94%; P = 0.08) and sustained significantly longer than that for sequential treatment. ---AA-P, P-AA; C, Paclitaxel administration.
by paclitaxel (AA-P) resulted in the least decrease in tumor volume although a delayed effect was seen after 3 to 6 weeks. Mean nadir tumor volume reductions were 100% for simultaneous therapy (group C), 95.7% for AA-P, and 89.2% for P-AA (\(P < 0.001\) for AA-P versus C, \(P < 0.001\) for P-AA versus C).

The median time to androgen-independent tumor growth for the P-AA, AA-P, and C groups were 70, 77, and 105 days, respectively (Fig. 4A; \(P = 0.032\)). At the end of the study period, three of eight (37.5%) mice in the simultaneously treated group C were without evidence of visible or palpable tumor, whereas all mice in the P-AA and AA-P groups exhibited androgen-independent growth. At all time points during the 28 week study period, mice in the C group exhibited the smallest mean tumor volume (Fig. 3B; \(P = 0.0026\)). Using Kaplan-Meier survival curves, there was a strong trend towards mice treated with combination therapy having a survival advantage over the other two groups (Fig. 4B; C versus P-AA, \(P = 0.058\), C versus AA-P, \(P = 0.096\)).

**Exposure to paclitaxel in vitro leads to the induction of genes known to play a role in androgen-independent growth.** The markedly attenuated response to castration seen in vivo for mice in group P-AA led us to examine whether primary chemotherapy induced resistance to androgen deprivation. To explore this scenario, LNCaP cells were grown under standard conditions and medium and treated with 1 nmol/L taxol. Total RNA from cells at 0, 24, and 72 hours of taxol exposure was assayed via reverse transcription-PCR for transcription levels of several genes known to have antiapoptotic roles and/or play a role in the development of androgen independence (i.e., bcl-2, bcl-xl, clusterin, Hsp27, and IGFBP-2). Relative RNA expression levels increased over time for all of these genes except IGFBP-2 (Fig. 5). RNA microarray analysis was also undertaken on these samples to identify other potential genes whose expression may have been altered by this cytotoxic stress. As shown in Table 1, 24-hour taxol treatment results in decreased expression of genes such as prostate differentiation factor, which classically play a proapoptotic role. Similarly, up-regulated genes fell into classes normally associated with metastasis, antiapoptosis, and transcription factors involved in cell proliferation.

**Discussion**

Until recently, prostate cancer had been considered a relatively chemotherapy resistant entity. It is now clear however that in the setting of symptomatic hormone-refractory prostate cancer, a palliative benefit exists for mitoxantrone/prednisone (14) and a modest survival benefit has recently been documented for docetaxel-based regimens (2, 3). This has

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Fig. 2. A, Kaplan-Meier analysis of Shionogi TTP. AA-P, androgen ablation followed by paclitaxel; C, combination androgen ablation and paclitaxel. Median TTP was 65 versus 38 days (\(P < 0.005\)). B, Kaplan-Meier analysis of Shionogi survival time. P-AA, paclitaxel followed by androgen ablation; AA-P, androgen ablation followed by paclitaxel; C, combination androgen ablation and paclitaxel. Median survival for group C was 83 versus 66 days for group AA-P (\(P = 0.01\)).

Fig. 3. A, points, mean LNCaP tumor volume of mice in each treatment group during the first 7 weeks of treatment (P-AA, paclitaxel followed by androgen ablation; AA-P, androgen ablation followed by paclitaxel; and C, combination androgen ablation and paclitaxel); bars, ± SE. B, points, mean LNCaP tumor volume following randomization (P-AA, paclitaxel followed by androgen ablation; AA-P, androgen ablation followed by paclitaxel; and C, combination androgen ablation and paclitaxel; \(P = 0.0028\)). Bars, ± SE. AA-P, C; P-AA.
already led to the initiation of several clinical trials aimed at improving upon this benefit through the administration of chemotherapy at earlier stages of the disease process (15), in the neoadjuvant setting with or without androgen ablation (16) and in combination with androgen ablation post-operatively (RTOG 99-02 and SWOG S9921).

A clinical precedent existed, however, that (i) raised questions regarding the sequence of administration of chemohormonal/hormonal therapies and (ii) allowed for concern regarding a potential risk of detriment rather than benefit from the concurrent administration of hormonal and cytotoxic therapies. In the setting of adjuvant breast cancer treatment, several large studies and an EBCTCG analysis have failed to show a benefit of combined ovarian ablation plus chemotherapy (5, 6, 17). It should be noted though that chemotherapy alone results in a significant component of ovarian suppression and this would diminish the magnitude of measurable benefits in this setting. The other concerning evidence against concurrent chemohormonal/hormonal therapy came from the Inter-group study INT 0100, which randomized postmenopausal breast cancer patients to receive CAF chemotherapy and tamoxifen in either a combination or sequential manner. Preliminary results showed a significant disease-free survival advantage for the sequential group (7). We felt it was therefore necessary to fully explore the various sequencing and combinations of chemohormonal/hormonal therapy in preclinical models of prostate cancer.

The Shionogi and LNCaP xenograft models have individual strengths and limitations as preclinical models of prostate cancer (18). The Shionogi model shows robust tumor shrinkage, apoptosis, and development of androgen-independent growth upon castration (18, 19), and the LNCaP model, whereas exhibiting a less reproducible tumor volume response in general, has been widely used in preclinical studies and is a better cell line for in vitro investigations. We therefore elected to examine our hypotheses in both animal models in a collaborative effort, with in vivo Shionogi model studies completed in Vancouver and in vivo LNCaP model studies completed in Chicago. In terms of overall results, both sets of in vivo experiments pointed to the same conclusion that concurrent chemohormonal therapy is superior to either sequential option.

The markedly diminished tumor regression observed upon castration of taxol-treated Shionogi mice can only partly be accounted for by the fact that these tumors had attained a significantly large volume at the time of castration. Previous work in our laboratory has shown that even when Shionogi tumors are allowed to grow to significant volumes (>2 mL), regression in response to castration was still pronounced (~90% volume reduction; ref. 13). In contrast, the mean volume reduction observed in these mice was only 37%, and androgen-independent growth ensued rapidly. One potential explanation for this finding is that paclitaxel treatment induces a stress-response and may result in the up-regulation of genes involved in antiapoptosis and/or androgen independence. It has already been shown, for example, that paclitaxel treatment can induce Bcl-2 protein phosphorylation (20) and that clusterin (a protein known to play a role in the progression to androgen-independent growth) is up-regulated by chemotherapy (21).

Fig. 4. A, Kaplan-Meier analysis of LNCaP TTP. P-AA, paclitaxel followed by androgen ablation; AA-P, androgen ablation followed by paclitaxel; C, combination androgen ablation and paclitaxel. Median TTP was 70 (P-AA) versus 77 (AA-P) versus 105 (C) days (one-way ANOVA, P = 0.032). B, Kaplan-Meier survival curve of combination androgen ablation and paclitaxel (C), paclitaxel followed by androgen ablation (P-AA) and androgen ablation followed by paclitaxel (AA-P); log-rank test: C versus P-AA, P = 0.058; C versus AA-P, P = 0.096; and P-AA versus AA-P, P = 0.5. Death was defined as tumor overgrowth (>2.5 cm in diameter), tumor ulceration, severe tumor-related morbidity requiring euthanasia, or mouse death. ——, P-AA; -=, AA-P; ——, C.

Fig. 5. Effect of paclitaxel exposure on mRNA expression levels of genes involved in apoptosis and/or androgen independence. LNCaP cells were exposed to 1 nmol/L paclitaxel for 0, 24, or 72 hours. mRNA levels for the following genes were then assessed via reverse transcription-PCR: clusterin, bcl-2, bcl-xl, Hsp27, and IGFBP-2. Bars, 1 SD. ■, 0 h; □, 24 h; △, 72 h.
It is not surprising then that reverse transcription-PCR analysis of paclitaxel-treated LNCaP cells showed a significant up-regulation of bcl-2, bcl-x, clusterin, and Hsp27 (Fig. 5). The finding that IGFBP-2 mRNA levels do not increase significantly may reflect the fact that IGF signaling could induce proliferation which would be deleterious in the face of cytotoxic agents. Similarly, mRNA array screening altered expressions in the expression of several other genes implicated in malignant progression. After 24 hours of taxol treatment, the most marked alterations seen were a 2.77-fold decrease in expression levels of prostate differentiation factor which is an antitumorigenic/proapoptotic factor whose expression levels are inversely correlated with Gleason grade and serum prostate-specific antigen (22) and a >3.4-fold increase in expression of caveolin-1 which has been shown related to metastasis and promotion of cell survival in prostate cancer (23). These results lend credence to the hypothesis that chemotherapy of hormone-naive cancers can induce or select for the androgen-independent phenotype.

Potentially confounding this hypothesis, however, is the observation that the initial response to androgen ablation in the LNCaP xenografts was worse than the response to chemotherapy. There are several possible explanations for this. First, LNCaP xenografts are known to exhibit inconsistent changes in tumor volumes in response to castration and changes in apoptosis have not been shown (18). Conversely, chemotherapy does lead to tumor volume decrease and apoptosis in LNCaP xenografts (4). There were also methodologic differences between the two in vivo experiments. In the LNCaP study, the AA-P tumor group had a mean starting tumor volume that was 32% larger than the other two groups. Furthermore, the second sequential treatment was given at 2 weeks following randomization regardless of response to the first treatment. These factors may have affected interim results during the experiment, although it should be noted that in the long run, it was still the AA-P group that had the more marked response (95.7% versus 89.2% shrinkage, P < 0.001).

In conclusion, our results show that the combination of taxane-based chemotherapy and androgen ablation is significantly more effective than the sequential administration of these treatments in the Shionogi and LNCaP tumor model. These findings provide preclinical proof-of-principle for ongoing clinical trials addressing the role and timing of systemic therapies in prostate cancer.

Acknowledgments
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Table 1. Microarray analysis of LNCaP cells treated with paclitaxel shows mRNA expression changes consistent with hormone-resistant phenotype

<table>
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<tr>
<th>Genbank accession no.</th>
<th>Gene name</th>
<th>Change in expression relative to baseline</th>
<th>Range in expression change (n = 4 arrays)</th>
<th>Function</th>
<th>Reference</th>
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<td>S78825</td>
<td>Inhibitor of DNA binding 1, IDI</td>
<td>6.6</td>
<td>2.9-10.6</td>
<td>Antiapoptotic in prostate cancer, induces NF1-B</td>
<td>(23)</td>
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<td>AF084260</td>
<td>Thyroid receptor interacting protein 15</td>
<td>2.5</td>
<td>2.5-5.1</td>
<td>Csn2 subunit (proteasomal subcomplex homologue), involved in p53, IκB degradation</td>
<td>(24)</td>
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<td>S73591</td>
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<td>0.36</td>
<td>0.33-0.39</td>
<td>Tumor suppressor gene, transcriptional repressor</td>
<td>(25)</td>
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<td>AB000584</td>
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<td>0.36</td>
<td>0.33-0.39</td>
<td>Proapoptotic, inversely correlated with Gleason score and prostate-specific antigen</td>
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References
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