Effects of Hormone Deprivation and 2-Methoxyestradiol Combination Therapy on Hormone-Dependent Prostate Cancer In Vivo

Fuminori Sato*,†, Hiroshi Fukuhara† and James P. Basilion*

*Center for Molecular Imaging Research and NFCC—Center for Molecular Analysis and Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; †Department of Urology, Faculty of Medicine, Oita University, Oita, Japan; ‡Department of Urology, Faculty of Medicine, Tokyo University, Tokyo, Japan

Abstract
2-Methoxyestradiol (2-ME) has potent antiproliferative effects on cancer cells. Its utility alone or in combination with other therapies for treating prostate cancer, however, has not been fully explored. Androgen-dependent and independent human prostate cancer cells were examined in vivo for their response to combination therapy. Efficacy was assessed by terminal deoxynucleotide transferase–mediated dUTP nick-end labeling assay and measuring microvessel density (MVD) in excised tumors. Animals harboring hormone-dependent tumors treated with 2-ME alone, androgen deprivation therapy alone, or the combination of the two had a 3.1-fold, 5.3-fold, and 10.1-fold increase in apoptosis, respectively. For hormone-independent tumors, treatment with 2-ME resulted in a 2.43-fold increase in apoptosis and a 73% decrease in MVD. 2-ME was most effective against hormone-dependent tumors in vivo and combination therapy resulted in a significant increase in efficacy compared to no treatment and trended toward greater efficacy than either 2-ME or androgen deprivation alone. Combination therapy should be investigated further as an additional therapeutic option for early prostate cancer.

Keywords: 2-methoxyestradiol, androgen deprivation therapy, androgen-dependent cancer, microvessel density, combination therapy.

Introduction
Cell growth and cell death are highly regulated ongoing cellular processes, the ratio of which is responsible for tissue renewal and homeostasis within an organism. In general, cancer results as a perturbation of this tightly regulated ratio such that cellular growth exceeds death. Therefore, modulation of the ratio by decreasing cell growth, increasing cell death, or both, can stop cancer. For prostate cancer, androgens are the major perturbants of this balance, both stimulating proliferation and inhibiting apoptosis through the androgen receptor. Because removal of hormones both inhibits proliferation and increases apoptosis of prostate cancer, androgen deprivation therapy has been the gold standard for the past 30 years in patients with advanced prostate cancer [1]. Although initially effective at blocking tumor growth, androgen deprivation therapy eventually fails, leading to a uniformly lethal drug-resistant stage of the cancer, called androgen-independent disease [2]. There is some divergence in thought if the hormone-independent form of the disease is selected by hormone deprivation therapy, or is the result of a new mutation. It is clear, however, that a large number of cancers originally classified as androgen-dependent prostate cancer (ADPC) have the ability to change status from androgen-dependent to androgen-independent growth, which is far more difficult to manage [2]. Thus, in the longer term, androgen deprivation therapy is not an effective first-line treatment for prostate cancer, and there exists a need to develop better therapeutic modalities for early-stage prostate cancer.

2-Methoxyestradiol (2-ME) is an endogenous metabolite of 17-β-estradiol reported to have high antiangiogenic activity [3] and to directly inhibit the growth of various cancer cells in vitro [3,4,5] as well as solid tumors in vivo, such as melanoma [6], breast cancer [7], and prostate cancer [7,8]. Both antiangiogenic and antitumor actions of 2-ME suggest that it may well have a tremendous potential for fighting cancer [9,10] and as a first-line therapy for ADPC. Despite these results, neither clinical nor animal trials with this agent have addressed hormone-dependent prostate disease. A recent report suggests that the antitumor action of this molecule is not dependent on the α or β estrogen receptor [11], but that 2-ME may induce apoptosis in cancer cells through a variety of molecular targets including c-Jun N-terminal kinase (JNK) [8,10,12,13].

Address all correspondence to: James P. Basilion, Center for Molecular Imaging Research, Massachusetts General Hospital, Building 149, 13th Street, Room No. 5406, Charlestown, MA 02129. E-mail: basilion@helix.mgh.harvard.edu

Copyright © 2005 Neoplasia Press, Inc. All rights reserved 1522-8002/05/$25.00
DOI 10.1593/neo.05145
Because 2-ME has minimal estrogenic effects, it is expected to have little, if any, of the cardiotoxicity associated with estrogen [14]. In a recent clinical trial of 2-ME for advanced prostate cancer, efficacy was shown without serious adverse effects and little significant toxicity was noted with doses exceeding 1000 mg/body per day [7]. Clinical trials are now underway to test this agent against nontreated locally advanced prostate cancer and against metastatic androgen-independent prostate cancer (AIPC).

One of the important questions not addressed in these trials, however, is if 2-ME, alone or in combination with androgen deprivation therapy, is an effective treatment for ADPC, thereby potentially impacting the later development of hormone-independent cancer. It seems likely that an early combinatorial approach with these agents for ADPC might have increased efficacy because 2-ME and androgen deprivation therapy act on different molecular targets within both the tumor and the vasculature.

Although the antiproliferative and apoptotic effects of 2-ME on human prostate cancer cells have been demonstrated, most of these studies have looked at later time points and prolonged treatment periods. In the work presented here, we have studied the early effects of this treatment on both ADPC and AIPC. We report here that 2-ME treatment induced apoptosis both of ADPC and AIPC, as well as decreased tumor microvessel density (MVD) in vivo. In addition, the combination of 2-ME treatment and androgen deprivation therapy showed an increased efficacy in stimulating apoptosis and decreasing MVD in ADPC. Further long-term studies will be required to address if combination therapy will impact the conversion of ADPC to hormone-independent cancer.

Materials and Methods

Tumor Models

The HONDA xenograft tumor model was originally derived from a metastatic human prostate carcinoma and was kindly provided by Dr. Y. Ito (Gunma University, Maebashi City, Gunma, Japan). The HONDA tumor model has been characterized as an androgen-dependent and prostate-specific antigen (PSA) producing prostate cancer [15,16], and the xenografts undergo rapid tumor regression after castration of the mouse host. HONDA tumors were maintained in vivo by serial passage in nu/nu mice. For experiments, tumors were resected using sterile technique from the flanks of nu/nu mice and minced into 2-mm cubes in serum-free RPMI 1640 medium on ice. Individual tumor cubes were transplanted subcutaneously into the flank of the male nu/nu mice that had been implanted with a 12.5-mg testosterone pellet (Innovative Research of America, Sarasota, FL) 2 days earlier. Supplemental testosterone was used for all studies to normalize the level of circulating androgens and to increase tumor take rates [17,18].

PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA) and were used as a model of AIPC. One million PC-3 cells were resuspended in Matrigel (Becton Dickinson, Bedford, MA) diluted 1:1, and subcutaneously injected into nu/nu mice. PC-3 tumor-bearing mice did not receive testosterone pellets. The mice were housed under standard conditions with food and water ad libitum. Three to 4 weeks after tumor implantation, when the tumor volumes were approximately 600 mm³ (HONDA) and 300 (PC-3) mm³, the mice were allocated into different treatment groups with visual sorting to standardize the average size of tumors within groups.

Mice were grouped as outlined in Figure 1. For HONDA tumors: group 1 was injected with vehicle, 0.2 ml of sterile olive oil (Sigma Chemical, St. Louis, MO), given intraperitoneally daily; group 2 was injected with 2-ME (Sigma Chemical), 20 mg/kg per day, approximately 0.2 ml given intraperitoneally daily; group 3 received androgen deprivation therapy (androgen pellet removal plus surgical castration); and group 4 received combined therapy, 2-ME treatment and androgen deprivation, as described above. The daily dose of 2-ME was based on a previous report showing that 1000 mg/body per day was well tolerated in prostate and breast cancer patients [7]. Groups 1 and 2 received sham surgeries to control for surgical effects on tumor growth and vascularization. In the experiments demonstrated in Figures 3–6, the mean tumor sizes were 642 mm³ on day 0 and 1589 mm³ on day 3 in control animals; 641 mm³ on day 0 and 962 on day 3 in 2-ME–treated animals; 650 mm³ on day 0 and 619 mm³ on day 3 in castrated animals; and 681 mm³ on day 0 and 465 mm³ on day 3 in animals treated with combination therapy.

For studies with PC-3 tumors, only groups 1 and 2 were investigated. For PC-3 human prostate cancer: group 1 was injected with vehicle and group 2 was injected with 2-ME (20 mg/kg per day, approximate total volume of 0.2 ml). For both series of experiments, 2-ME was prepared by dissolving and sonicating it in sterile olive oil at a concentration of 2 mg/ml. Seventy-two hours after initial treatment, all animals were euthanized for analysis. Tumor size was measured by microliter and the tumor volume was calculated as reported previously [19]. All animal experiments were done with the permission of the local ethical committee.

For all studies, no androgen receptor blockade was utilized.

Immunohistochemistry and Terminal Deoxynucleotide Transferase–Mediated dUTP Nick-End Labeling (TUNEL) Assay

Tumors excised from euthanized animals were fixed with 4% paraformaldehyde for 24 to 48 hours at 4°C, and then washed in 0.1 M phosphate-buffered saline (PBS; pH 7.4). The tissues were snap-frozen in TissueTek embedding medium (Sakura, Zoeterwoude, The Netherlands) and cut into 7-μm sections using a CM 1900 Cryotome (Leica Microsystems, Wetzlar, Germany). To reveal the presence of androgen receptor or PSA in HONDA tumors, a rabbit anti-androgen receptor antibody that has cross reactivity with human and mouse receptors and rabbit antihuman PSA antibody were used. Briefly, separate tumor sections were blocked with 10% goat serum and then incubated with either
rabbit antiandrogen receptor antibody (PG-21; Upstate, Lake Placid, NY) (5 μg/ml) or rabbit anti-PSA antibody (Abcam, Cambridge, MA) for 60 minutes at room temperature. After washing thoroughly with PBS, the slides were incubated with Cy3–conjugated goat antirabbit IgG at a 1:100 dilution (Jackson ImmunoResearch, West Grove, PA) for 30 minutes at room temperature, and then the sections were rinsed again in PBS. The visualization of nuclei was developed with 4′,6-diamidino-2-phenylindole (DAPI; Sigma Chemical) for 5 minutes at room temperature. Slides were mounted with a ProLong Antifade Kit (Molecular Probes, Eugene, OR) and cover slips.

As a measure of apoptosis, the tumor sections were assessed for DNA fragmentation by TUNEL assay performed according to the manufacturer’s instructions (ApopTag Plus FITC; Chemicon, Temecula, CA). For HONDA tumors, PSA staining was performed following the TUNEL staining, and nuclei were visualized by counterstaining with DAPI as described above with the following changes: After TUNEL staining, tissue sections were refixed with 4% paraformaldehyde for 10 minutes and washed in PBS. In sections where MVD was assessed, the sections were first stained with an antimouse CD31 antibody (monoclonal, MEC13.3; BD PharMingen, San Diego, CA) and visualized with either Cy2-Goat Antimouse IgG or Cy3 Goat Antimouse IgG staining (Jackson ImmunoResearch) at a 1:100 dilution, and then stained for PSA or androgen receptor as described above. PSA positivity was used to assess the number of cancer cells; CD31, a specific marker for mouse endothelial cells, was used to assess the number of endothelial cells; and DAPI positivity was used to assess the total number of cells viewed.

Fluorescence microscopy was performed using an inverted microscope (Zeiss Axiovert 100 TV, Wetzlar, Germany) fitted with appropriate filter sets (Omega Optical, Brattleboro, VT). Images were acquired using a Photometrics CH250 CCD (Photometrics, Tucson, AZ), with image acquisition, pseudo-color image fusion, and storage controlled by IP LabSpectrum software (Signal Analytics, Vienna, VA).

Quantification of Apoptosis and MVD and Statistical Analysis

To quantify the degree of apoptosis, the total number of TUNEL-positive (apoptotic) cells and PSA-positive (tumor) cells were reported per 1000 PSA-positive (tumor) cells counted at ×400 magnification. The apoptotic index was expressed as the ratio of TUNEL-positive tumor cells to all tumor cells. MVD was also evaluated under ×400 magnification using CD31-stained sections as described earlier [20]. The mean and standard deviation (SD) were obtained, and unpaired Student’s t tests were performed to compare between groups. A P value smaller than .05 was considered to be statistically significant.

Results

Effect of 2-ME on Hormone-Dependent Prostate Cancer

The first step in our studies was to verify that HONDA tumors grown in nude mice continued to express PSA and androgen receptor. For these studies, tumors were grown in vivo, excised, and stained for the expression of each protein. The HONDA tumors expressed intense signals for both PSA and androgen receptor (Figure 2).
We next determined the best time point to assess the efficacy of combining 2-ME and hormone deprivation therapy. One, 3, or 7 days after initial treatment, animals harboring HONDA tumors were euthanized and the tumors were resected for histologic analysis. TUNEL assays indicated that combination therapy resulted in a rapid increase in tumor apoptosis (11.6-fold) within 24 hours, and that this increase remained relatively unchanged for the 7-day observation period (mean tumor sizes for control animals were 139 mm³ on day 0, 193 mm³ on day 1, 301 mm³ on day 3, 453 mm³ on day 7, and 131, 139, 134, and 143 mm³ for the same time points in the treated animals). In light of these results and to avoid tumor reaction against hypoxia or reestablishment of angiogenesis, which might be accelerated 3 days after castration, as reported for the normal prostate gland [21–23], we performed further studies at the 3-day time point.

To investigate in detail the effect of 2-ME on the androgen-dependent HONDA tumors, animals were implanted with tumors and sorted into four groups to compare the effects of the therapies administered independently or in combination (Figure 1).

In animals that were treated with either 2-ME or androgen deprivation therapy alone, tumor growth was slowed but tumors did not regress. In contrast, combination of both treatments resulted in tumor regression within the 3-day observation period (mean tumor sizes for control animals were 139 mm³ on day 0, 193 mm³ on day 1, 301 mm³ on day 3, 453 mm³ on day 7, and 131, 139, 134, and 143 mm³ for the same time points in the treated animals). In light of these results and to avoid tumor reaction against hypoxia or reestablishment of angiogenesis, which might be accelerated 3 days after castration, as reported for the normal prostate gland [21–23], we performed further studies at the 3-day time point.

To investigate in detail the effect of 2-ME on the androgen-dependent HONDA tumors, animals were implanted with tumors and sorted into four groups to compare the effects of the therapies administered independently or in combination (Figure 1).

In animals that were treated with either 2-ME or androgen deprivation therapy alone, tumor growth was slowed but tumors did not regress. In contrast, combination of both treatments resulted in tumor regression within the 3-day observation period (Figure 3). The changes in apoptosis paralleled those for tumor growth (Figure 4). Tumor sections from mice treated with 2-ME had a 3.1-fold increased apoptotic index compared with tumor sections from control mice. Those that underwent androgen deprivation therapy showed an increase in apoptotic index of 5.3-fold compared with control. The largest change, a 10.1-fold increase in apoptotic index, was observed in tumors of mice treated with a combination of 2-ME and androgen deprivation therapy. This degree of apoptosis was also significantly elevated when compared to that achieved with either agent alone (P = .0001 for 2-ME and P = .018 for androgen deprivation). These data suggest that there is a correlation between the degree of apoptosis and the extent to which HONDA tumors stop growing and/or shrink.

Antiangiogenic Effect of 2-ME on Hormone-Dependent Prostate Cancer

To determine the effects of treatment on tumor microvasculature, the HONDA tumors were excised, sectioned, and stained with anti-CD31 antibody (Figure 5). In these studies, 2-ME treatment alone resulted in a 53.3% decrease in MVD compared to tumors from control animals. Hormone deprivation therapy alone resulted in less pronounced decreases in the MVD (31.3%). Simultaneous treatment of the animals with both therapies was not significantly better than either regimen alone, reducing the MVD by only 54.8%. Therefore, the combination therapy with 2-ME and androgen deprivation did not show an obvious advantage for modulating MVD in this hormone-dependent cancer.

Figure 2. Immunohistochemical detection of androgen receptor and PSA in HONDA tumors. (a) The pseudo-color fluorescence images of androgen receptor and CD31 double staining. The fusion images were obtained by overlaying three pseudo-color images. Blue indicates nuclei stained by DAPI; red color indicates Cy3-labeled androgen receptor–positive cells; green color indicates Cy2-labeled CD31-positive cells. Note that the androgen receptor–positive cells show a pink color as a result of overlaying red and blue colors. Please note that the endothelial cells (green-colored) did not stain with antiandrogen receptor antibody, which has cross reactivity for both human and mouse androgen receptors. CD31 is a specific marker for mouse endothelial cells. (b) The pseudo-color fluorescence images of PSA staining. The fusion images were obtained by overlaying two pseudo-color images. Blue color indicates nuclei stained by DAPI; red color indicates Cy3-labeled PSA-positive cells. PSA is a marker for HONDA tumor cells.

Figure 3. Relative changes of HONDA tumor volume 72 hours after treatment. Animals were treated as indicated and the tumor volumes were measured before and after treatment. Each bar shows the percentage of treated tumor volume in comparison with initial tumor volume. Error bars: ± SD. Compared to control animals, the regression rates observed for 2-ME, androgen ablation, and combination therapies were significant (P = .04, P = .0027, and P = .0014, respectively). There were no significant differences among the efficacies of 2-ME, androgen ablation, or combination therapies.
It is important to note that, in several sections of tumors isolated from treated animals, it was apparent that endothelial cells also underwent apoptosis. As seen in Figure 6, when tumor sections from animals treated with combination therapy were stained for CD31 (an endothelial cell marker) and with TUNEL, there were CD31-positive cells that were also positive for apoptosis. Studies of tumors derived from control animals did not show any cells staining for both apoptosis and CD31 (data not shown).

**Effect of 2-ME on Hormone-Independent Prostate Cancer**

We also examined the 2-ME effect on hormone-independent PC-3 prostate cancer using this in vivo model system. Treatment of the animals harboring PC-3 tumors with 2-ME showed a 2.43-fold increase in apoptosis compared with the tumors from control animals with no observed shrinkage in tumor volume (Figure 7). In contrast to these modest changes in apoptosis, treatment with 2-ME resulted in a 73% decrease in MVD compared to tumors growing in control animals (Figure 8).

**Discussion**

In this study, we focused on the early in vivo effects of 2-ME treatment on hormone-dependent (HONDA) and hormone-independent (PC-3) prostate tumor models. Interestingly, treatment of either tumor type in vivo with 2-ME alone had very similar effects on the apoptotic index, increasing it between two-fold and three-fold. There was, however, a much more pronounced decrease in MVD in hormone-independent tumors. Combination therapy was most effective in treating hormone-dependent cancers, significantly increasing the apoptotic index over 10-fold and causing a shrinkage of tumors within 3 days. The studies reported here are a prerequisite first step to begin to understand the mechanism of—and determine if early combination therapy might be more effective than—hormone deprivation therapy alone. Although long-term relapse studies will be necessary to determine the effectiveness of this approach for prostate cancer patients, the results from these studies do support further investigations of a therapeutic strategy that combines hormone deprivation and chemotherapy with 2-ME.

Our studies demonstrate that 2-ME treatment results in an increase in the number of TUNEL-positive cells in HONDA and PC-3 tumors. Bu et al. [8] have reported using another prostate tumor model, Dunning R3327-PAP, and a 14-day administration of 2-ME, a good correlation between tumor shrinkage and an increase in TUNEL-positive cells for tumors treated in vivo. There are several possible mechanisms by which 2-ME can induce apoptosis; however, it seems clear from the work of Bu et al. [8] that 2-ME is not an agonist for the estrogen receptors, suggesting that the

---

*Figure 4. TUNEL staining and apoptotic index of HONDA tumors 72 hours after treatment. Animals were treated as described in Figure 1; tumors were resected and analysed for PSA expression and apoptosis. (a) The fusion images were obtained by overlaying three pseudo-color images. Blue indicates nuclei stained by DAPI; red indicates Cy3-labeled PSA-positive cells; green color indicates FITC-labeled TUNEL-positive cells. (b) Each bar shows the percentage of TUNEL-positive (apoptotic) cells in 1000 PSA-positive (tumor) cells. Error bars: ± SD. AD, AD, androgen deprivation. Compared to control animals, the apoptotic indices observed for 2-ME, androgen ablation, and combination therapies were significant (P < .01, P < .01, and P < .01, respectively). The differences were also significant when several of the treatments were compared: 2-ME versus combination therapy (P < .01); androgen ablation versus combination therapy (P < .05). There was no significant difference between androgen ablation and 2-ME treatments (P = .7).*
Figure 5. Relative changes in MVD in HONDA tumors 72 hours after treatment. Animals harboring HONDA tumors were treated as described in Figure 1 and then stained for PSA and CD31, a marker for mouse endothelial cells. (a) The fusion images were obtained by overlaying three pseudo-color images. Blue color indicates nuclei stained by DAPI; red color indicates Cy3-labeled PSA-positive cells; green color indicates Cy2-labeled CD31-positive vasculature. (b) Each bar shows the percentage of MVD in treated tumors in comparison with that in the control tumor. Error bars: ± SD. AD, androgen deprivation. Compared to control animals, only treatments with 2-ME alone or 2-ME and androgen deprivation resulted in significant reductions in MVD (P < .01 for both). Androgen depletion did not significantly reduce MVD. Similarly, the differences measured between different treatments were not statistically significant (AD vs 2-ME, AD vs 2-ME + AD, and 2-ME vs 2-ME + AD).

Figure 6. Endothelial cells undergo apoptosis after combination therapy. Blue color indicates nuclei stained by DAPI; red color indicates Cy3-labeled CD31-positive (endothelial) cells; green color indicates FITC-labeled TUNEL-positive (apoptosis) cells. The merged image was obtained by overlaying these three pseudo-colors. Arrowheads indicate the cells that showed colocalization with CD31 and TUNEL-positive signals. CD31 is a marker for endothelial cells.
antiproliferative activity of 2-ME must occur through another pathway. One possible explanation for the antiproliferative effects of 2-ME is that it acts to induce apoptosis through the JNK-dependent mitochondrial pathway [8,10,12,13], and possibly by targeting p53 [13,24], the death receptor 5 [25], and microtubules [9].

Figure 7. Changes in tumor volume and apoptotic rate for PC-3 tumors 72 hours after treatment. Animals harboring PC-3 tumors were treated as described in Figure 1. (a) The tumor size was measured using microcalipers and tumor volume calculated as described in Materials and Methods section. Error bars: ± SD. (b) The fusion images were obtained by overlaying two pseudo-color images. Blue indicates nuclei stained by DAPI; green color indicates FITC-labeled TUNEL-positive cells. (c) Animals were treated with control or 2-ME, and the percentage of TUNEL-positive cells per 1000 tumor cells was determined. Error bars: ± SD. PC-3 cells were identified morphologically because they do not express PSA.

Figure 8. Relative changes of MVD in PC-3 tumors 72 hours after treatment. Animals were treated as outlined in Figure 1 and the changes in MVD were measured by staining for CD31, an endothelial cell marker. (a) The fusion images were obtained by overlaying two pseudo-color images. Blue color indicates nuclei stained by DAPI; green color indicates Cy2-labeled CD31-positive endothelial cells. (b) MVD was normalized to control tumors and expressed as a percentage for the treated tumor. Error bars: ± SD. P = .0041.
We were also able to demonstrate that 2-ME treatment has antiangiogenic activities and decreases MVD following in vivo treatment of either tumor type with 2-ME (Figures 5 and 8). The decrease in MVD was greater, however, for hormone-independent tumors. It is evident that angiogenesis plays an important role in the growth and spread of prostate cancer [26,27], and there are several reports demonstrating the antiangiogenesis action of 2-ME [3,9,10]. Mabjeesh et al. [9] have demonstrated that cytotoxic antitumor drugs induce apoptosis in vivo and, like in vitro drug-induced apoptosis, this can be a relatively rapid event [30–34]. Moreover, it has been shown that apoptosis is a good early predictor of in vivo tumor response to therapy [31,34]. Serial biopsies in breast cancer patients have suggested that response to complete therapy correlates with early posttreatment increases in the tumor’s apoptotic index [32]. Mohsin et al. [34] reported that neoadjuvant transstumab treatment for breast cancer patients significantly induced apoptosis within 1 week and correlated with clinical tumor regressions. Mohammed et al. [35] reported that there was a strong association between doubling of the apoptotic index and reduction in urinary bladder tumors. However, no association or correlation was found between initial proliferative index or change in proliferation with treatment and tumor response to therapeutic drug [33–35]. These data demonstrate the importance of conducting further investigations in which long-term studies will determine if combination therapy can be more effective at reducing tumor burden than either of its components alone.

Even under androgen deprivation therapy, 2-ME exerts strong direct killing effects on ADPC cells, suggesting that the combination of 2-ME and androgen deprivation therapy may be a reasonable strategy against ADPC. Potentially, ADPC may not be completely androgen-dependent by nature, and hormone ablation therapy may select for populations of cells containing pre-existing mutations that allow AIPC to emerge. The combination of 2-ME treatment and hormone deprivation therapy could potentially reduce this population of cells and extend the period of regression that most patients enjoy after hormone ablation therapy. This particular combined therapy may potentially also achieve clinically significant results, in combination with surgical treatment or radiation therapy.

The results reported here may be useful in designing future long-term animal relapse studies and eventually clinical trials for prostate cancer. First, the 2-ME treatment may be a promising therapy for AIPC. It is well known that AIPC resists existing chemotherapeutic agents. Here we have demonstrated that AIPC cells show significant sensitivity to 2-ME in vivo. Therefore, 2-ME may be a candidate therapeutic agent for patients with advanced AIPC. Second, the combination therapy of 2-ME and androgen deprivation has potential benefit for patients with ADPC. We feel that the results described here should compel: 1) further examination of 2-ME and hormone deprivation therapy in longer-term animal survival and relapse studies, and 2) eventually, the combined results of these short-term and future long-term studies may be used to inform the design of future clinical trials for ADPC.
Acknowledgements
The authors thank Gail Newton for her helpful suggestions and technical advice.

References