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Pre-Clinical Evaluation of Androgen Receptor Inhibitor Enzalutamide for Prevention of Recurrent Ovarian Tumor Growth

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Abstract

Objectives: To evaluate androgen receptor (AR) expression in a panel of human ovarian cancer cell lines compared to healthy ovarian tissue. Assess the *in vitro* growth inhibitory activity of enzalutamide alone in panel of human ovarian cancer cell lines. Finally, evaluate if enzalutamide would delay/prevent ovarian tumor growth in platinum-sensitive and platinum resistant human ovarian cancer microscopic orthotopic mouse models.

Methods: A selected panel of human ovarian cancer cell lines and healthy human ovarian tissue were evaluated for AR expression by western blot analysis. The growth inhibitory activity of enzalutamide alone was evaluated with standard MTT assays. *In vivo* studies were in a platinum sensitive, TOV-112D-S, and platinum resistant, TOV-112D-R, microscopic tumor (0.5×10^6 cells) orthotopic mouse models treated with enzalutamide 30 mg/kg by mouth once daily for twelve weeks. Steady state concentrations of enzalutamide were determined using a validated UPLC-MS/MS assay.

Results: AR expression was observed in seven of ten human ovarian cancer cell lines. Healthy ovarian tissue did not express the AR. Enzalutamide growth inhibitory activity across the selected panel of human ovarian cancer cell lines was observed. In the TOV-112D-S platinum sensitive microscopic human ovarian cancer mouse model, enzalutamide delayed tumor growth. This did not occur in the platinum resistant microscopic human ovarian cancer mouse model. Therapeutic enzalutamide steady state concentrations were achieved and confirmed in all enzalutamide treatment mice in both the platinum sensitive and platinum resistant mouse models.

Conclusions: This study confirmed AR is readily expressed in human ovarian cancer cell lines but not in healthy (normal) ovarian tissue. The *in vitro* studies suggested enzalutamide had growth inhibitory activity in the "chemo-sensitive" cell line, TOV-112D-S which was confirmed in the *in vivo* microscopic tumor model mouse study that demonstrated prevention/decreased of ovarian tumor growth. Clinical evaluation of enzalutamide for primary prevention of ovarian cancer recurrence is warranted.

Keywords: Ovarian cancer; Androgen receptor; Enzalutamide; Ovarian cancer cell lines

Introduction

Epithelial ovarian cancer accounts for 95 percent of ovarian malignancies and is the second most common gynecological cancer [1]. In 2017 there will be 22,440 cases of women with ovarian cancer and roughly 14,080 women will die of this disease. [2]. The high malignancy rate is due to the insidious onset of nonspecific symptoms resulting in most patients not presenting until the cancer has progressed to stage III-IV disease [3].

The current "gold standard" treatment regimen involves treatment with primary taxane/platinum therapy following cytoreduction and surgical tumor surgery, which has led to modest clinical outcomes [4, 5]. Although the response rate for platinum-based treatment is 60-70%, most advanced ovarian cancer patients relapse within the first two years after completion of primary treatment, with only 30% surviving greater than five years. Chemoresponse assays have validated that treatment resistance to platinum-based drugs is associated with shortened progression free survival for patients [6]. The response rate of platinum-resistance ovarian cancer to second line chemotherapy agents ranges from 20% to 30% with significant side effects that impact quality of life including neurotoxicity, hematologic toxicity, and alopecia [7, 8]. Prevention of recurrence or at minimum extending the platinum-free interval may improve the progression free survival of ovarian cancer patients.

The ovarian surface epithelium (OSE) in proximity to thecal cells, stromal and granuloma cells, which produce androgens, estrogen, and progester one [6]. Researchers believe that ARs may promote proliferation of OSE and thus have a role in the pathogenesis of ovarian cancer [9]. Interestingly, expression of ARs have been demonstrated in ovarian cancers using ligand binding assays. Also, Nodin and colleagues had reported AR expression in approximately 30% of ovarian tumors evaluated while other studies reported that AR is expressed in the majority of ovarian tumors [10-12].

Numerous hormonal therapies for the treatment of ovarian cancer have been evaluated in pre-clinical and clinical studies by investigators over the past few decades [13]. The low toxicity and cost effectiveness associated with hormonal therapies have been accepted as reasonable alternatives

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for the treatment of relapsed disease. Previously, studies demonstrated that letrozole has potential benefit to be used for prevention of recurrence ovarian cancer microscopic tumor models [13]. However, there was some concern that long term use of letrozole may decrease bone integrity and increases the risk of osteoporosis so alternative anticancer hormonal agents should be considered. Since the AR expression in ovarian cancer has been well documented in the literature the AR inhibitor, enzalutamide, is a reasonable candidate to evaluate as an anticancer hormone for prevention of ovarian cancer recurrences. Enzalutamide is well tolerated and has not been reported to impact bone integrity or risk of osteoporosis. The objective of this pre-clinical study was to evaluate AR expression inhuman ovarian cancer cell lines compared to healthy ovarian tissue and screen the potential growth inhibitory activity of enzalutamide in a panel of human ovarian cancer cell lines. Finally, ultimate objective was to evaluate if enzalutamide would delay and/or prevent ovarian tumor (re)growth using both a human ovarian cancer platinum sensitive and platinum resistant microscopic orthotopic mouse models.

Material and Methods

AR expression assay in selected ovarian cancer cell lines

Androgen receptor (AR) expression was determined in a panel of ten selected human ovarian cancer cell lines: TOV-112D, TOV-112D-R, TOV-21G, ES-2, Hey-A8, $SKOV_3$, $SKOV_3$-IP1, $OVCAR_3$, OV-90, $CaOV_3$. All cell lines, except TOV-112D-R, were obtained from American Type Culture Collection (ATCC, Manassas, VA). The HeLa cell line was used as a positive control and CAL-27 as the negative control for AR expression. Briefly, cells were harvested from confluent cultures and protein extracts were prepared by lysing cells on ice in 300-400 µL of NP40 lysis buffer. Pierce Micro BCA Protein Assay Kit (Pierce: Rockford, IL) was used to determine the protein concentration. For each series of protein determinations, a standard curve was constructed with known concentrations of bovine serum albumin(BSA). For direct immunoblotting, 50 µg protein was run on 10% SDS-PAGE gels, transferred to polyvinylidene difluoride (PVDF) membranes and probed with the AR antibodies using manufacturer's protocol (Calbiochem- Novabiochem Co. San Diego, CA). Beta-actin was used to monitor protein loading control. Western blot results were confirmed by ELISA assay to quantify the differences in AR expression.

Growth inhibitory activity of enzalutamide

The standard MTT assay was used for growth inhibition studies. For the single agent study with enzalutamide, 1000 µg/mL stock was used then followed by serial 1:10 dilutions with final concentrations in columns 3-10 being: 1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 µg/mL, respectively. Briefly, 5,000-15,000 cells in 100 µL of each cell line were placed in each well of 96 well plates except first column, which served as the blank. Cells were incubated for 24 hours after initially plated. All cells were grown in media supplemented with 5% charcoal-stripped serum. Second column of wells did not have any drug, which served as control. Stock solutions of drugs were made in cell free media so that the final concentration of DMSO or PEG did not exceed 0.2%. Cells incubated at 37 °C for 72 hours in the presence of enzalutamide. After 72 hours, 25 µL of MTT solution (final concentration of 0.3 mg/mL) was added in each well except blank column and the plates were further incubated at 37°C for another two hours. After centrifugation at 800g for five minutes medium from each well was removed by aspiration and 50 µL DMSO was added. Plates were shaken for two minutes on a plate shaker. The optical density of the resulting solution was measured at 562nm. An empty column of wells served as the blank control and untreated cells served as the untreated control.

Determination of AR expression in normal ovarian tissue

The tissue collection protocol was approved be the University of Texas Health Sciences Center at Houston investigational review board (IRB). After obtaining informed consent from each patient, fresh human ovarian tissue was collected from five patients undergoing oophorectomy procedures for benign conditions at Memorial Hermann Hospital-TMC. Samples were stored in fresh saline/medium within four hours after surgery and then placed in liquid nitrogen until further use. Cells were lysed with protein lysis buffer containing cocktail of protease inhibitor and distilled water, as well as 0.1 % MP40. Proteins were estimated using BCA Protein assay (Pierce: Rockford, IL) and read using Tecan Infinite® 200 PRO series Plate Reader (Seestrasse, Switzerland). Proteins were loaded on SDS-PAGE gel and transferred on PVDF membrane. After blocking with dry milk in PBS-Tween 20, membrane was immunoblotted with primary antibody, washed, then blotted with horseradish peroxidase conjugated secondary antibody (Bio-Rad). Proteins were visualized via chemiluminescence using Kodak *In-Vivo* Imaging Station (Kodak, Rochester, NY).

Effectiveness of enzalutamide in microscopic human ovarian tumor orthotopic mice models

Chemicals and Reagents: Enzalutamide 500 mg was provided by Astella Pharma Global Development Inc. (Kansas City, MO). Enzalutamide solution was prepared at a concentration of 3.75 mg/mL in a mixture of 70:30 water and tween 80 to provide dose of 30 mg/kg for each mouse.

Cell Culture: All *TOV-112D platinum sensitive (TOV-112D-S)* cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA) and platinum resistant *TOV-112D* cells (TOV-112D-R) were grown in lab using continuous exposure to cisplatin over nine months until a ten-fold increase in cisplatin IC_{50} was achieved.

Scheme for the development of cisplatin-resistant TOV-112D cells:

Cytotoxicity assays (MTT) were done to evaluate IC_{50} value of cisplatin for TOV-112D (IC_{50} = 0.32µg/mL). Chemoresistant variants of TOV-112D cells were reproducibly obtained by exposing the cells to 0.3µg/mL (1mM) cisplatin for 72 hours. Hereafter this concentration is referred to as C1x. Drug was removed and surviving cells were rinsed with PBS three times. The cultures were maintained for two to three weeks by regular changes of culture medium, until drug-surviving cells were recovered a normal growth pattern. Then, cisplatin treatment was repeated with increasing concentrations of cisplatin for 72 hours repeatedly over three-week intervals in the TOV-112D cells according to the protocol schematized below: C1x→C1x→C1x→C1x→C5x→C5x→C5x→C10x→C10x→C10x. The chemoresistant variants of TOV-112D (TOV-112D-R) were evaluated for ten times increase in the IC_{50} value for cisplatin. After establishment, the chemoresistant variants were maintained by periodic exposure to C5x every month.Untreated cells were used as control and all experiments were done in triplicate. The cell lines were propagated in a mixture of 1:1 MCDB 105 and medium 199 with 2 mM L-glutamine and Earl's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 10% FBS. All cell lines were grown in 75-cm^2 culture flasks in 5% CO_2 in air at 37°C to 90% confluence. Cell lines used for this study were maintained for less than 15 passages to maintain consistency in cell line characteristics.

Human ovarian cancer microscopic orthotopic mouse models

The University of Texas Health Sciences Center institutional animal care and use committee (IACUC) reviewed and approved the protocol prior to initiating any animal work. For this study, 60 female nude mice that had undergone bilateral oophorectomy prior to study were obtained from Charles River Laboratories Wilmington, MA, USA. All the mice weighed about 18 to 23 grams and were maintained five per cage in specific pathogen free (SPF) barrier room, with a temperature of 22 ± 3°C and 45 ± 3°C RH%. They were provided free access to autoclaved food and reverse osmosis autoclaved water. The experiment procedures and the

handling of the mice were in strict accordance with the guide for the care and use of laboratory animals.

The mice were divided into three groups of ten for each cell line (N=60 for two cell lines). There were three arms of this study: one treatment arm (N=10 each), untreated control arm (N=10) and a vehicle control arm (N=10). The treatment arm received an oral dose (30.0 mg/kg, in 0.2 mL, gastric gavage) of enzalutamide (MV3100) and the vehicle control arm received an oral dose of diluent (70:30 Water and Tween 80). TOV-112D-S and TOV-112D-R cells (0.5×10^6) were dispersed in PBS with 20% matrigel and injected subcutaneously on day zero. Each mousegrew one tumor on the dorsal surface. Tumor measurements were obtained three times per week with electronic calipers (Mitutoyo, Utsunomiya, Japan). Blood was collected by facial vein using 18-gauge needle from the vehicle group and treatment group on day 4, day 28, day 56, day 84 and by cardiac puncture at the end of study after animal euthanized (day 114). Plasma from samples was separated via centrifugation for five minutes and then stored in negative 80°C freezer overnight before analysis. Mice were monitored daily for signs/symptoms of morbidity including but not limited to lethargy, weight loss, anorexia, hunching, etc. Mice were sacrificed via CO_2 inhalation followed by cervical dislocation on day 84 and day 114. Three mice from treatment, vehicle, and untreated groups were sacrificed on Day 84 and tumor collected and frozen at -80°C for future analysis. At the end of the study all the remaining mice were sacrificed. When sacrificed, total tumor burden was determined by macroscopic dissection. Immediately after sacrifice, tumors were surgically removed from all mice and stored at -80°C.

Determination of enzalutamide concentrations

Reagents and materials: Enzalutamide (EZT) and its internal standard EZT –D6 were obtain from Alsachim, (Illkirch-Graffenstaden, France). Ammonium formate were purchased from Sigma-Aldrich (St Louis, MO, USA). LC-MS grade acetonitrile and methanol were from Fisher Scientific (Pittsburg, PA, USA). Sarala from Cerulean (Round Rock, Texas, USA) were used for calibration curve

Sample preparation: Primary stock solutions for EZT and EZT-D6 (1 mg/mL) were made by dilution these compounds in methanol and were stored in glass bottles at -20 °C . To prepare calibration standards 200 µL of SeraFlx was spiked in with EZT to the final concentration of 1000 ng/mL. Other calibration levels of 500, 250, 100, 50 and 25 ng/mL were received by subsequent serial dilution of highest calibrant with SeraFlx. Samples preparation was done by plasma protein precipitation by acetonitrile. Then 200 µL of acetonitrile containing 300ng/mL EZT-D6 was added to 100 µL of mouse plasma and calibration standards. Samples were vigorously mixed for five minutes and centrifuged at 13,500 g for ten minutes. Next, 100 µL of the supernatant was transferred to a high recovery vials (Waters, Milford). An aliquot (10 µL) of this solution was then directly injected into the UPLC-MS/MS system. All samples were run in triplicates. Method validation demonstrate linear dynamic range from 25 to 1000 ng/mL (R^2=0.997), with accuracy between 85 to 100% and precision span from 18 to 6% for different calibration levels. Mouse samples were diluted 1:10 to ensure plasma concentrations fell within range of this calibration curve.

UPLC-MS-MS analysis: Shimadzu Triple Quad 8040 (Columbia, MD, USA) with UPLC system (binary pump LC-20) was used for analysis. Compound separation were done by Restek Raptor 2.7um, 50 x 2.1mm biphenyl column, mobile phase A (water, 2mM ammonium formate, 0.1% formic acid) and mobile phase B (methanol, 2mM ammonium formate, 0.1% formic acid) by followed gradient B: 0 minute (min) - 40%, 1 min - 40%, 3 min - 98%, 4 min – 98% with 1-minute equilibrium and flow rate of 0.6 mL/min. The column and autosampler tray were preserved at 50°C and 4°C, respectively. The elutant was introduced to mass-spectrometer through ESI source at positive mode with following settings: drying gas flow 15 L/min; nebulizer gas flow 2L/min, DL temperature 250°C; heat block temperature, 400 °C. Multiple reaction monitoring (MRM) mode was used for quantification at m/z 465→209 and 380 for EZT and m/z 471→215 for the EZT-D6. Data analysis were done with Lab Solution software (Shimadzu).

Data and Statistical analysis

All experiments were carried out at least in triplicate and repeated if the coefficient of variance was greater than 20%. Final results are described through appropriate summary descriptive statistics (e.g. means, standard deviations and correlation coefficients). A paired t-test was used to evaluate the viability in continuous data as appropriate. Results were considered to be significant when $p > 0.05$. The program GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA, USA) was used to perform the analysis.

Results

Presence of AR

AR expression was observed in seven of the ten human ovarian cancer cell lines: Hey-A8, OV-90, $OVCAR_3$, $SKOV_3$, $SKOV_3$-IP1, TOV112D-S, and TOV112-R. (Figure 1) Of these seven cell lines, all except TOV-112D-S were chemotherapy resistant cell lines. The expression of AR was not observed in the normal ovarian tissue (Figure 2).

Enzalutamide *in vitro* growth inhibition activity

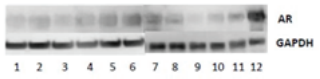
The *in vitro* growth inhibition assays demonstrated enzalutamide activity across the selected panel of AR+ human ovarian cancer cell with highest activity in TOV-112D-S, TOV-112D-R, $SKOV_3$ and $SKOV_3$-IP1 cell lines. However, enzalutamide had greater potential activity in the chemo-sensitive cell line, TOV-112D-S with IC_{50} of 0.4 µM compared to the chemo-resistance cell line with mean IC_{50} of 323 ± 494.9 µM (19.4 to 1322 µM). The enzalutamide growth inhibition data is summarized in (Table 1).

Enzalutamide *in-vivo* study in microscopic orthotopic human ovarian cancer mouse model

Human orthotopic models were used in single agent studies. The enzalutamide steady state plasma concentrations (C_{SS}) were consistent across in both treatment groups 17.2 ± 3 µg/mL (37 µM) for TOV-112D-S group and 15.9 ±1.4 µg/mL (32.7 µM) for the TOV-112D-R group. In the TOV-112D platinum sensitive human ovarian cancer microscopic orthotopic model, enzalutamide significantly delayed tumor growth by 18.5% ($p < 0.03$) compared to the untreated and vehicle controls. However,

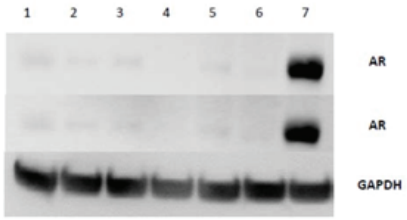
Table 1: IC_{50} values of ovarian cancer cell lines to measure inhibition activity of enzalutamide. Data is summary of 50% growth inhibitory (IC_{50}) activity of enzalutamide in $SKOV_3$, $SKOV_3$-IP1, TOV- 112D(S), TOV-112D (R), Hey A8, OV-90, $OVCAR_3$ cell lines. Enzalutamide growth inhibitory activity across selected panel of AR+ ovarian cancer cell lines was highest in TOV-112D-S, TOV-112D-R, $SKOV_3$, and $SKOV_3$-IP1 cell lines

	$SKOV_3$	$SKOV_3$ - IP1	TOV-112D[S]	TOV-112D[R]	Hey A8	OV-90	$OVCAR_3$
IC_{50} (µM)	103.8	79.9	0.4	19.4	215.3	1322	197.4
IC_{25} (µM)	27.3	2.1	0.2	9.7	13.5	19.4	64.7



1=CAL-27 (-con), 2=CaOV-3, 3=ES-2, 4=Hey A8, 5=OV-90, 6=OVCAR-3, 7=SKOV-3, 8=SKOV3 IP1, 9=TOV-21G, 10=TOV-112D(S), 11=TOV-112D(R), 12=HeLa (+con)

Figure 1: Western blot data for AR expression in ovarian cancer cell lines. Data is a summary of androgen receptor (AR) expression in 11 ovarian cancer cell lines. Hela cell line was used as a positive control and glyceraldehyde 3-phosphate dehydrogenase (GADPH) was used as a biomarker in assay. AR receptor expression was observed in seven of the ten human ovarian cancer cell lines: Hey-A8, OV-90, $OVCAR_3$, $SKOV_3$, $SKOV_3$-IP1, TOV112D, and TOV112-R



1 = negative vehicle control, 2 to 6 = five human normal/healthy ovarian tissue samples, 7 = positive control

Figure 2: Western blot data for AR expression in normal ovarian tissue. Data is a summary of androgen receptor (AR) expression in normal/healthy ovarian tissue samples run in duplicate. Normal samples were compared to negative and positive control and showed no AR expression (AR-)

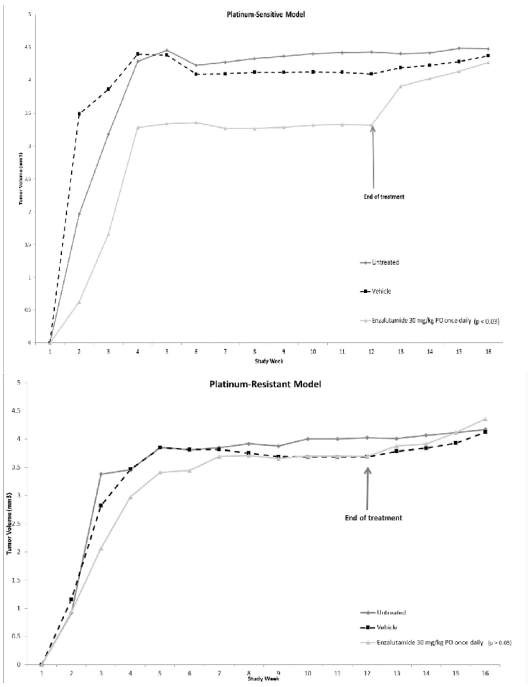


Figure 3: Enzalutamide growth inhibitory activity in human orthotopic mice models. Data is a summary of tumor growth activity in TOV-112D-S and TOV-112D-R platinum sensitive human ovarian cancer orthotopic models. In the TOV-112D-S (platinum sensitive) models, enzalutamide delayed tumor growth. There was no difference in tumor growth in the TOV-112D-R (platinum resistant) models between treated and untreated/vehicle groups

in the TOV-112D-R ovarian cancer microscopic orthotopic models there was no difference observed in tumor growth between enzalutamide compared to untreated or vehicle control (Figure 3).

Discussion

This study confirmed that ovarian cancer cell lines, Hey-A8, OV-90, $OVCAR_3$, $SKOV_3$, $SKOV_3$-IP1, TOV-112D-S and TOV-112D-R, display AR expression consistent with results found in other studies. [8, 12] In the current study, the *in vitro* growth inhibitory activity of enzalutamide was observed in all the ovarian cancer cell lines expressing AR. Not until recently have androgen receptors been utilized as a target for treatment for recurrent ovarian cancer using enzalutamide. [14, 16]. This series of preclinical studies focused on the potential use of the anti-androgen agent, enzalutamide for the treatment of ovarian cancer, which is currently being explored in a phase II clinical studies [16]. In this current study utilized a microscopic tumor model that mimics clinical scenario that we achieve "complete response" with no detectable tumor at completion of adjuvant, first line chemotherapy in over 75% of advanced ovarian cancer patients. However, over 50% will recur within the first two years after achievinga CR to first line treatment [17]. There is a need to identify new options for consolidation that have minimal side effect profiles, reasonable cost and efficacy. The concept of "consolidation" treatment for ovarian cancer over the past decade has ranged from 12 months of paclitaxel which is associated with considerable side effects to 12 months of bevacizumab which is better tolerated however has significant cost. Enzalutamide has limited toxicity, low cost and the convenience of being an oral agent, making it a viable option to be considered for consolidation treatment after completion of primary cytotoxic treatment.

The data from this study confirmed that is there is a rationale mechanism of activity for enzalutamide in ovarian cancer based on expression of AR in ovarian cancer cell lines and even some potential activity in absence of AR expression based on the growth inhibition assays. In the microscopic human ovarian cancer model demonstrated that enzalutamide was effective in prevention of tumor growth. However, the new data from this study suggest the activity of enzalutamide is greater in chemo-sensitive ovarian cancer as observed *in vitro* was consistent and confirmed *in vivo* in the platinum sensitive microscopic human ovarian cancer orthotopic mouse model.

Hormonal therapies have been used to study risk of ovarian cancers in peri-menopausal and menopausal women, and few have yet utilized androgen receptors [11,14,16]. The finding that androgen receptor was expressed in ovarian tumor was consistent with the results of other studies. This suggests there is a need to understand the relationship between AR expression of cancerous ovarian cells and their drug resistance, to potentially help optimize treatment. Of the seven ovarian cancer cell lines studied with AR expression, six are also associated with multi-drug resistance (MDR). This was an interesting observation based on data from Montani and colleagues that reported multidrug resistance protein 4 (MRP4) is expressed in prostate cancer cell line and is correlated with androgen receptor expression [8,17, 18]. The study also observed induction of AR was associated with increase in MDR while anti-androgen agents, such as enzalutamide, decreased MDR expression in metastatic prostate cancer cell lines. MRP4 has also been suggested to be readily expressed in ovarian cancer often associated with unfavorable role in outcomes [19, 20]. While enzalutamide decreases MDR expression and has activity in prostate chemo-resistant cancer, preliminary data presented in this study observed less activity in the ovarian cancer cell lines/tumors with platinum/chemo-resistance. Additional studies are needed to full elucidate what, if any, impact enzalutamide may have on MDR expression in ovarian cancer.

While this is first study to suggest enzalutamide is viable candidate for consolidation treatment for prevention of ovarian cancer recurrences, there were some limitations to this study. While it was beneficial to evaluate AR expression in normal tissue, there was a very limited small sample size in this series hence may not be representative of population. There is always a healthy skepticism if mouse studies will translate to clinical activity since previous preclinical findings have not been confirmed in humans.

In conclusion, enzalutamide is an anti-androgen agent that is well established in the armatarium of agents used for management of metastatic prostate cancer and is emerging as potential viable treatment for triple-negative breast cancer unrelated to expression of estrogen receptor [21, 22]. This study confirmed AR is expressed in ovarian cancer and is potential target for treatment. The *in vitro* studies suggested enzalutamide had activity across the AR the human ovarian cancer platinum sensitive cell line which was also confirmed in the *in vivo* platinum sensitive TOV-112D-S but not in the platinum-resistant TOV-112D-R cell line human ovarian cancer microscopic orthotropic mouse models suggesting the role of enzalutamide is perhaps earlier in treatment of advanced cancer rather than in the recurrent setting after failing cytotoxic chemotherapy regimens. Clinical evaluation of enzalutamide for prevention of ovarian cancer recurrence is warranted.

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Conflicts of Interest

JAS was the principal investigator of the unrestricted research grants from Medivation, Inc and Astellas Pharma Global Development, Inc. received at the UTHealth McGovern Medical School. No other authors have a conflict of interest to declare.

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