AWARD NUMBER: W81XWH-14-1-0154

TITLE: A Novel Therapeutic Modality for Advanced-Stage Prostate Cancer Treatment

PRINCIPAL INVESTIGATOR: Subhash C. Chauhan, PhD

CONTRACTING ORGANIZATION: University of Tennessee, Memphis, TN 38103

REPORT DATE: Dec 2018

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE: Dec 2018	**2. REPORT TYPE:** Final	**3. DATES COVERED:** 22 Sep 2014 - 21 Sep 2018

4. TITLE:

A Novel Therapeutic Modality for Advanced-Stage Prostate Cancer Treatment

5a. CONTRACT NUMBER:

5b. GRANT NUMBER: W81XWH-14-1-0154

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S):

Subhash C. Chauhan

E-Mail:schauha1@uthsc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES):
University of Tennessee Health Science Center (UTHSC),
62S Dunlap Street Rm 300
Memphis, TN, 38103-4903

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT:

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES: None

14. ABSTRACT: Prostate cancer (PrCa) is the second leading cause of cancer death in American men. There is an increasing need to develop effective therapies for advanced stage PrCa due to their limited or no response to androgen ablation therapy. Chemotherapy is an alternative approach for the treatment of advanced stage PrCa. However, the available chemotherapeutic agents used to treat PrCa are non-selective and provide only limited response rate Thus, novel treatment modalities are needed to treat advanced stage PrCa. In this proposal, we intend to develop a novel therapeutic modality for advanced stage metastatic prostate cancer.

There is an urgent need to develop effective therapies for the treatment of advanced stage prostate cancer (PrCa) due to their limited or no response to androgen ablation therapy. In this proposal, we intend to develop a novel therapeutic agent Ormeloxifene (ORM) for the treatment of advanced stage metastatic PrCa. Our results illustrated that ORM treatment effectively inhibited invasion and motility of PrCa cells. Further, we observed that ORM treatment induced the expression of tumor suppressor PKD1 (a modulator of nuclear β-catenin signaling) in PrCa cells. Interestingly, ORM treatment inhibited expression of oncogenic isoform of PKD (PKD3) in PrCa cells. We have also observed that ORM mediated overexpression/activation of PKD1 effectively inhibits metastasis associated protein 1 (MTA1) in PrCa cells. MTA1 has been reported to be very tightly associated with cancer metastasis in various cancer types including PrCa. To further investigate association of ORM with MTA1 suppression, we performed molecular docking studies with MTA1 which illustrated potential binding sites of ORM on MTA1 protein. Considering effective therapeutic index of ORM, we are also making more potent analogues of ORM. These findings suggest that ORM could be a potential therapeutic molecule to inhibit growth of advanced stage PrCa and its metastasis.

15. SUBJECT TERMS:
Prostate Cancer (PrCa), Metastasis, Ormeloxifene (ORM), Wnt/β-catenin signaling, Androgen Receptor (AR), Estrogen Receptor (ER)

16. SECURITY CLASSIFICATION OF: U			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	28	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

INTRODUCTION

1. BACKGROUND

Prostate cancer (PrCa) is the second leading cause of cancer death in American men. There is an increasing need to develop effective therapies for advanced stage PrCa due to their limited or no response to androgen ablation therapy (1). Chemotherapy is an alternative approach for the treatment of advanced stage PrCa. However, the available chemotherapeutic agents used to treat PrCa are non-selective and provide only limited response rate (2). Thus, novel treatment modalities are needed to treat advanced stage PrCa. In addition, precise understanding of molecular pathogenesis of disease is required to develop novel chemotherapeutic modalities for the treatment of advanced stage PrCa. The androgen receptor (AR) is required for PrCa growth at all stages, including androgen-independent tumors in the presence of very low levels of androgens (3). Recent studies are suggested that in addition to androgens/AR, estrogens/estrogen receptors (ER) may also play crucial role in the development and progression of PrCa. It is shown that the co-administration of both testosterone and E2 is required for the initiation of PrCa. Additionally, the interaction between β-catenin and the AR and ER suggests a possible mechanism of cross talk between Wnt and androgen/estrogen signaling pathways (4). Several lines of evidence have shown that β-catenin can act as an AR/ER co-activator and enhance their transcriptional activity (4). Thus targeting β-catenin-AR/ER signaling pathway by novel chemotherapeutics may have strong clinical implications in developing strategies for PrCa treatment (5). In recent studies, a novel tri-phenyl ethylene molecule, ormeloxifene, has shown potent anti-cancer activity, including in PrCa cells. ***The central hypothesis of our proposal is that ormeloxifene inhibits growth of advanced stage PrCa cells by modulating β-catenin-AR/ER signaling pathway. Further, it induces cell death via inducing PARP and/or caspase mediated apoptotic pathways.*** The information gathered from this study will provide insight for developing a novel therapeutic modality for advanced stage PrCa. ***To test this hypothesis, the following specific aims were proposed:***

SPECIFIC AIMS:

AIM 1: To examine the effect of ormeloxifene on β-catenin-AR/ER signaling pathways.

AIM 2: Determine the apoptotic pathways activated by ormeloxifene to induce cell death in PrCa cells.

AIM 3: To evaluate the therapeutic efficacy of ormeloxifene for PrCa treatment in mouse model systems.

Ormeloxifene (ORM): Utilization of clinically approved drugs for other indications as anti-cancer agents (repurposing a drug) appears to be an interesting approach because of their established safety profile in human. The ongoing scenario attracts and welcomes the repositioning and budging of existing established drugs which could complement de novo drug development. Ormeloxifene **(Fig. 1)** is a non-hormonal, non-steroidal synthetic molecule for human use as an oral contraceptive (6,7). Recently, its anti-cancer activity has been reported against advanced breast cancer (8) and head and neck squamous cell carcinoma (HNSCC) (9). Additionally, our recent studies show a potent anti-cancer activity of ormeloxifene (ORM) in various cancer cell lines including AR sensitive and AR refractory metastatic PrCa cells. ***Moreover, ORM is reported to have an excellent therapeutic index and is safe for chronic administration*** (10). Therefore, we believe that ORM has a great repurposing potential for PrCa chemoprevention/treatment. Successful examples of drugs repurposing are anti-diabetic drug metformin and the birth control hormone medroxyprogesterone acetate. In this study we proposed to investigate effects of ORM on β-catenin-AR/ER signaling pathways **(AIM 1)**, apoptosis **(AIM 2)** and evaluate its anticancer potential in clinically relevant PrCa cell lines and animal models **(AIM 3)**.

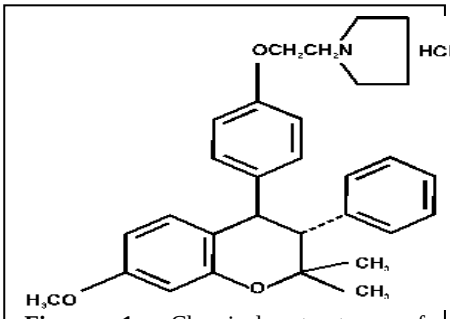


Figure 1: Chemical structure of ormeloxifene (MW 490.50).

Research work performed during the previous funding cycle (2014-15): we hired the post-doc fellow/Scientist for this project and performed some experiments under specific aims # 1. We performed functional assays to determine the effects of ORM on cell invasion, and cell migration, and colony formation

using androgen-independent C4-2 PrCa cell lines. Additionally, we performed some docking studies of ORM with some potential molecular targets of PrCa. We observed that ORM has some potential binding sites with metastasis associated protein 1 (MTA1). We further investigated the effects of ORM on MTA1 protein levels by Western blot analysis. Our results indicate that ORM treatment of C4-2 cells inhibits the protein levels of MTA1. Our lab investigated that MTA1 protein can be inhibited by *via* ectopic overexpression of protein kinase D1. We observed that ORM induces the expression of PKD1 as determined by Western blot analysis, and qRT-PCR. Key research findings of this year are summarized below.

1. ORM inhibits invasion and motility of C4-2 cells:

2. ORM activates tumor suppressive PKD1 and inhibits oncogenic PKD3 in C4-2 cells:

3. ORM interacts with and inhibits protein levels of Metastasis Associated Protein 1 (MTA1)

4. ORM enhances the sensitivity of chemotherapeutic drug Docetaxel (DTX):

It has been reported that MTA1 is involved in DTX drug resistance (11) and DTX treatment does not inhibit the expression of MTA1 in PrCa cells **(Fig. 2A).** However, ORM treatment effectively inhibited the expression of MTA1 (**Fig. 2A)**. Thus, we hypothesized that ORM treatment may enhance the DTX sensitivity in PrCa cells. To prove our hypothesis, we performed cell proliferation and colony formation assays in C4-2 cells treated alone or in combination with DTX. Results illustrated that ORM treatment potentiates the effects of DTX as determined by MTS **(Fig. 2B)**. Further our colony formation results also depicted significant ($P<0.05$) reduction in colony formation compared with alone ORM and DTX treatment. These results indicate that ORM has potential to induce the DTX effects in PrCa. ORM in combination with Overall, these results suggest that ORM which induces PKD1 expression and inhibit MTA1 can sensitize the DTX resistance in PrCa

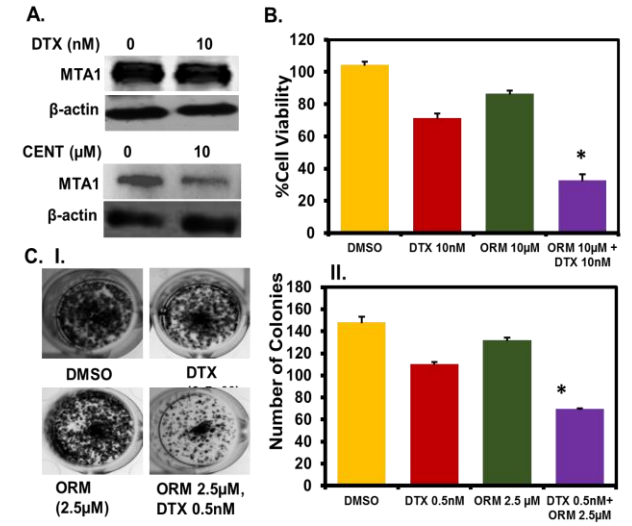


Figure 2: PKD1 overexpression and MTA1 inhibition sensitizes the effects of DTX in C4-2 cells **A.** Effects of DTX or CENT treatment on protein levels of MTA1 in C4-2 cells as determined by W.B analysis. **C**. Effects of CENT alone or in combination with DTX on cell proliferation as determined by MTS assay, and colony formation (CI-II) of C4-2 cells.

<u>Research work performed during the previous funding cycle (2015-16):</u> During this funding cycle, we have completed remaining proposed experiments of **Task 1** and majority of **Task 2**. In **Task 3**, we completed xenograft study in athymic nude mice. We showed functional impact of ORM for inhibiting the growth of hormone refractory prostate cancer cells *in vitro* and in ectopic xenograft mouse model. We determine the effect of ORM on β-catenin signaling pathways, interaction of β-catenin and AR in PrCa cells. Our results demonstrated that ORM inhibits β-catenin signaling network in PrCa cells. ORM treatment inhibited translocation of β-catenin from cytoplasm into nucleus and inhibited its downstream target gene TCF-4. It has been reported that β-catenin interacts and transactivates AR signaling pathways *via* non-androgen dependent mechanism (12). Our results have shown that ORM inhibits physical interaction of β-catenin and AR in C4-2 cells and C4-2 cells derived xenograft tumor tissues as determine by immunoprecipitation/Western blot analysis. Next we performed molecular modeling to determine whether ORM directly interacts with AR, ER, GSK3-β, and β-catenin proteins. We observed strong binding pocket of ORM in ER, AR, β-catenin, and GSK3-β. ORM treatment showed arrest in G0/G1 phase of cell cycle in PC3 cells and inhibited the expression of various cell cycle regulator protein including MCL1, Cyclin D1, CDK2 and enhanced the expression of p21 and p27. ORM treatment inhibited the expression of various EMT markers (N-Cadherin, Slug and Snail) and enhanced the expression of E-Cadherin. To translate our *in vitro* results into *in vivo*, we performed xenograft studies in athymic nude mice using C4-2 and PC3 cells. ORM administration (100 and 500 μg) i.p thrice a week significantly ($P<0.01$) inhibited the growth of PC3 cells derived xenograft tumors as determined by decrease in tumor volume and weight in ORM treated mice compared to control groups. We also observed a significant

(P<0.01) inhibition of PC3 cells derived xenograft tumors in ORM treated (500 µg) group. All of these results suggest that ORM is a potent chemotherapeutic drug and could be used for prostate cancer treatment. Our main findings of this years are summarized below.

1. ORM treatment inhibits the growth of hormone-refractory prostate cancer cells.
2. ORM treatment arrests cell cycle in G0/G1 phase.
3. ORM treatments modulates cell cycle regulatory proteins (p21, p27 cyclin D1 and MCL1) in PrCa cells.
4. ORM treatment inhibits EMT, MMPs, invasion, and migration of PrCa cells.
5. ORM inhibits androgen receptor (AR) signaling and its interaction with β-catenin.
6. ORM docks with β-catenin, GSK3β, and AR/ER.
7. ORM treatment inhibits the growth of PrCa cells derived xenograft tumors in athymic nude mice **(Fig. 3).**
8. ORM treatment inhibited the expression of PCNA, AR and β-catenin in xenograft tumors

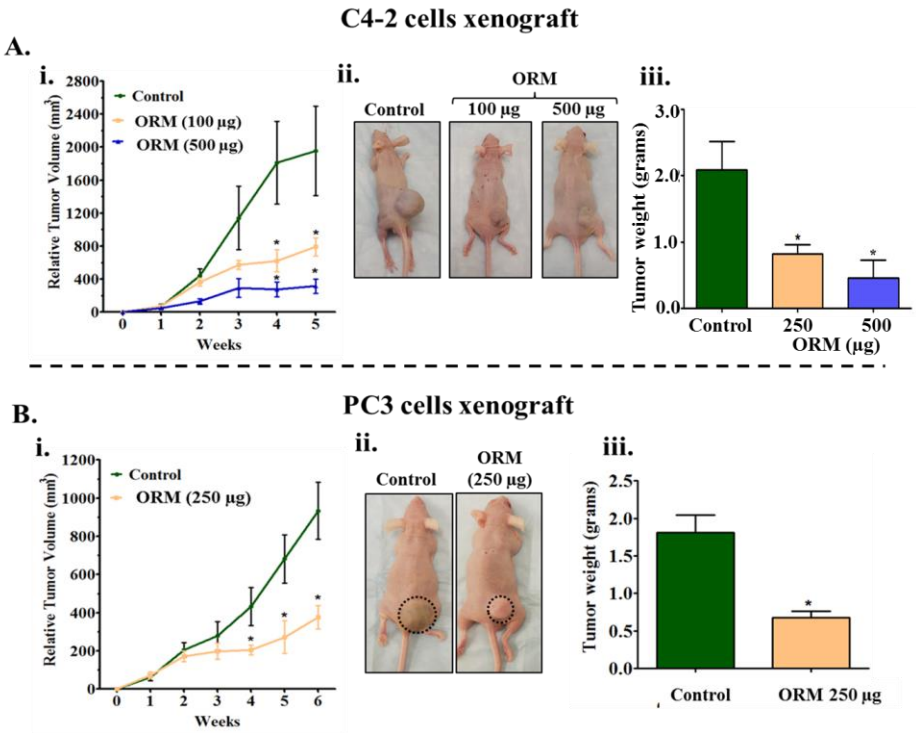


Figure 3: ORM inhibits tumor growth of PrCa cells in athymic nude mice. A. Effect of ORM on C4-2 xenograft tumors in athymic nude mice. Ai Line graph is showing mean tumor volume of 4 mice in each group at 1-5 weeks. **Aii.** Representative C4-2 xenograft tumor bear mouse in each group. **Aiii.** Bar graph representing tumor weight of each group mice. Value in graph represents Mean±SE of 4 mice in each group. **B.** Effect of ORM on PC3 xenograft tumors in athymic nude mice. A total of 10 mice were used in this experiment and were divided into two groups (n=5). $2X10^6$ PC3 cells were injected subcutaneously and ORM treatment (250 µg) was administered 3 times a week till 6 weeks. Mice of both the group were sacrificed when control mice showed a targeted tumor volume 1000 mm^3. **Bi.** Average tumor volume of each group mice at different weeks. **Bii.** Representative mouse picture of control and ORM treated tumor bearing mouse. **Biii.** Bar graph representing tumor weight of each group mice. Value in graph represents Mean±SE of 5 mice in each group.

Research Performed during the 2016-17 Fiscal Period:

We have completed the remaining proposed experiments of Tasks 1, 2, and 3 during this period. Since then, we have observed cell cycle arrest in G0-G1 phase, and we investigated the effect of ORM on apoptotic induction in PrCa cells. We noted that ORM treatment induces apoptosis in both PC3 and DU145 cells as determined by enhanced Annexin V staining in cells and PARP cleavage. We next examined the effect of ORM on mitochondrial membrane potential (Δψm) using TMRE staining, which is a marker of apoptosis. ORM decreased TMRE staining in both DU145 and PC3 cells as determined by fluorescence microscopy and flow cytometry respectively. These results suggest the apoptosis inducing potential of ORM in PrCa cells. We also determined the molecular mechanism of ORM induced inhibition of β-catenin targeting pathways in cancer cells. ORM treatment was found to inhibit nuclear β-catenin in DU145. GSK3β-dependent phosphorylation of β-catenin enhances its proteasomal degradation and inhibits its translocation into the nucleus, thus regulating its various downstream target oncogenes. Our results indicate that ORM activates GSK3β, thereby degrading β-catenin in the cytoplasm and also inhibiting nuclear β-catenin translocation and repressing TCF-4 promoter activity. ORM also inhibited the expression of various motility markers (integrin β5, vinculin, vimentin and phosphorylation of cofflin) in PrCa cells. ORM administration also showed significant (P<0.01) inhibition of prostate tumor growth in TRAMP mice. Histopathological analysis further confirmed significant decrease of poorly differentiated adenocarcinoma in TRAMP mice. ORM treatment inhibited expression of PCNA, vimentin and slug in excised prostate tumor tissues as compared to control group mice. Androgen receptor

signaling plays a key role in the development as well as the progression of PrCa. Accumulating evidence suggests that anti-androgen therapy is effective to reduce the growth of primary as well as castration resistant prostate tumors in humans. However, this therapy still has limitations since after castration, AR signaling is still active in target cells *via* non-androgen activation of AR or the presence of AR splicing variants. ORM has the ability to suppress the function of AR in both LNCaP and 22Rv1 cells. This might be because ORM competes with the LBD of AR. ORM inhibited the expression of the AR splicing variant ARv7 in 22Rv1 cells. More importantly, a known anti-androgen drug (Enzalutamide) for castration resistant prostate cancer does not target AR splicing variants because of a lack of LBD domain of AR. Our preliminary results suggested that ORM targets AR splicing variants and have combinatory effect with Enzalutamide. This novel observation suggests that ORM can be used to improve the therapeutic efficacy of Enzalutamide in castration resistant PrCa. These study are ongoing in our lab and will be performed from no-cost extension funds. We are working to determine the molecular mechanism of this combinatory effect. Moreover, we are also investigating the molecular mechanisms of ORM-induced sensitization of docetaxel therapy of PrCa *in vitro* and *in vivo*.

Task 1: To examine the effect of ormeloxifene on β-catenin/AR/ER signaling

1. ORM inhibits nuclear β-catenin in DU145 cells. We observed that ORM inhibits β-catenin through its sequestration in the cytoplasm. ORM treatment (10 µM) inhibited nuclear β-catenin in DU145 cells **(Fig. 4Ai)** through its sequestration in the cytoplasm as determined by Western blot analysis. This result was further confirmed by confocal microscopy as ORM showed inhibition of β-catenin translocation into the nucleus of PrCa cells as compared to control **(Fig. 4Aii)**. We next evaluated the effect of ORM on lithium chloride (LiCl)-induced β-catenin/TCF promoting activity by transiently co-transfecting the DU145 cells with TCF-firefly luciferase reporter constructs (pTOP-FLASH) and *Renilla* luciferase or (pFOP-FLASH) and *Renilla* luciferase. ORM treatment (10 µM) for 6 hrs, significantly (P<0.01) inhibited lithium chloride (LiCl)-induced TCF-4 promoter activity in DU145 cells **(Fig. 4iii).** We also examined the effect of ORM on activation of GSK3β by Western blot analysis which illustrated a marked increase in phosphorylated GSK3β

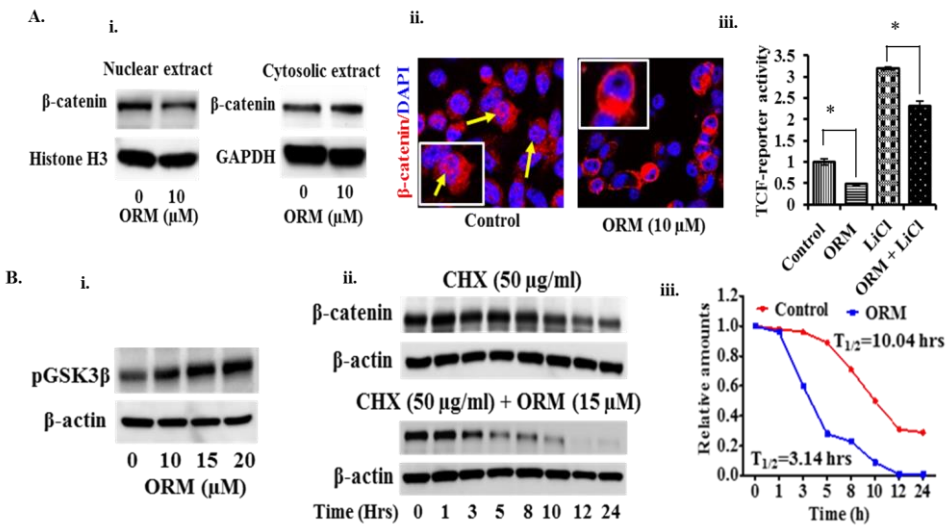


Figure 4: Effect of ORM on β-catenin and GSK3β. A. Effect of ORM on β-catenin distribution in cytoplasm and nucleus of DU145 cells. Briefly, cells were treated with indicated concentrations of ORM for 24 hrs, nuclear extracts were prepared and subjected for Western blot analysis to detect the protein levels of β-catenin. Results demonstrated a decreased expression of nuclear β-catenin and increased expression of β-catenin in the cytoplasm **(Ai)** of DU145 cells. **(Aii)**Effect of ORM on β-catenin localization in PC3 cells as determined by confocal microscopy. Yellow arrows indicate localization of β-catenin in control and ORM treated cells after 24 hrs treatment (Original magnification 40X). **(Aiii)** Effect of ORM on TCF-4 promoter activity. Cells were transiently co-transfected with TCF-firefly luciferase reporter constructs (pTOP-FLASH) (1 µg) and *Renilla* luciferase (200 ng) or (pFOP-FLASH) (1 µg) and *Renilla* luciferase (200 ng). After 24 hrs, cells were treated with LiCl (50 µM) alone or in combination with ORM (10 µM). Cell lysates were prepared 6 hrs post-treatment and firefly and *Renilla* luciferase activity were analyzed via Dual luciferase kit (Promega). The β-catenin/TCF transcription activity was determined by normalizing the firefly luciferase activity to that of *Renilla* luciferase activity and calculating the ratio of TOP-FLASH signal to FOP-FLASH signal. Values in bar graph indicates mean±SE of three wells reading in each group. Asterisk (*) denotes the significant value p<0.01. **B. (Bi)** Effects of ORM on protein levels of phospho GSK3β in DU145 as determined by Western blot analysis. **(Bii)**Effect of ORM on β-catenin degradation as analyzed by pulse chase experiment. Briefly, DU145 cells were treated with CHX (50 µg) alone or in combination with ORM (15 µM) at indicated time points. Protein lysates were prepared and subjected for Western blot analysis to analyze the protein levels of β-catenin. Results indicates protein levels of β-catenin in alone CHX-treated (upper blot) and in CHX and ORM-treated (Lower blot). **(Biii)**Line graph showing quantification of Western blots of Figure Bii. $T_{1/2}$ denotes time point for 50% β-catenin degradation.

protein levels in DU145 cells **(Fig. 4Bi)**. We have since observed activation of GSK3β protein by ORM treatment; thus, we next examined the effect of ORM on β-catenin degradation after using translational inhibitor (cyclohexamide). Results revealed a time-dependent decrease in the protein levels of β-catenin in DU145 cells compared to cyclohexamide treatment alone **(Fig. 4Bii-iii).**

Task 2: To examine the mode of cell death triggered by ORM in PrCa cells

2. ORM treatment induces apoptosis in PrCa cells. Since we observed cell cycle arrest in the G0-G1 phase, we investigated the effect of ORM on apoptotic induction in PrCa cells by flow cytometry analysis. ORM (10-20 µM) dose-dependently increased apoptotic cell populations in both PC3 **(Fig. 5Ai)** and DU145 **(Fig. 5Aii)** cells as determined by enhanced Annexin V positive cells. ORM at 20 µM showed 55.6% and 50% apoptotic PC3 and DU145 cells respectively compared to control group **(Fig. 5Ai-ii)**. We next examined the effect of ORM on mitochondrial membrane potential (Δψm) using TMRE staining **(Fig. 5Bi-ii)**, which is a marker of apoptosis induction through intrinsic pathway. ORM (10-20 µM) dose-dependently decreased TMRE staining in both DU145 and PC3 cells as determined by fluorescence microscopy **(Fig. 5Bi)** and flow cytometry **(Fig. 5Bi-ii),** respectively. We also examined the effect of ORM on PARP cleavage which is a marker for apoptosis. ORM (20 µM) induced PARP cleavage as determined by western blot analysis (**Fig. 5C).** These results suggest the apoptosis-inducing potential of ORM in PrCa cells.

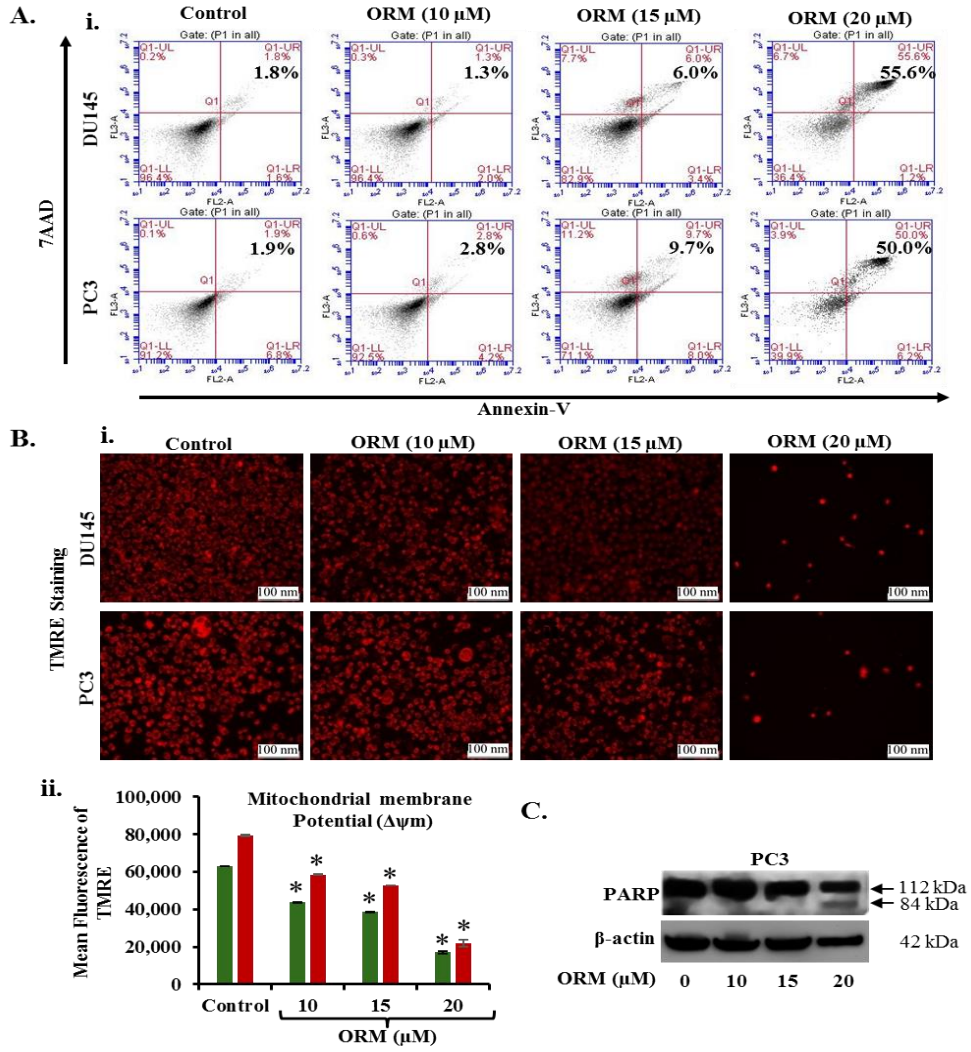


Figure 5: Effect of ORM on apoptosis induction in PrCa cells. PC3 and DU145 cells were treated with indicated concentration of ORM for 24 hrs and processed for apoptosis analysis using Annexin V-7AAD apoptosis kit. **A.** Representative FL3-A and FL2-A plots showing dose-dependent increase of apoptosis in DU145 **(i)** and PC3 cells **(ii). B.** Effect of ORM on mitochondrial membrane potential (Δψm) as determined by TMRE staining. **(i)**Representative Fluorescence images showing dose-dependent effect of ORM on TMRE staining in PC3 and DU145 cells. **(ii)**Bar graph indicating dose-dependent inhibition of mitochondrial membrane potential (Δψm) in ORM-treated PC3 and DU145 cells as determined by flow cytometry. Asterisk (*) denotes the significant value p<0.01**. C.** Effect of ORM on PARP cleavage. Briefly, 70% confluent PrCa cells were treated with ORM (10-20 µM) for 24 hrs. Whole cell lysates was prepared and subjected for Western blot analysis for full and cleaved PARP.

Task 1: To examine the effect of ormeloxifene on β-catenin/AR/ER signaling

3. ORM inhibits motility markers in PrCa cells. Since we observed that ORM inhibits the migratory potential of PrCa cells, we investigated the effect of ORM on various cytoskeletal proteins which help in lamellipodia formation during the migratory process of cancer cells. Results demonstrated that ORM (10 µM) inhibited the expression of integrin β5, vinculin, vimentin and phosphorylation of **cofilin** in PrCa cells **(Fig. 6A).** ORM treatment also inhibited the expression of Integrin β5? (receptor for fibronectin) as determined by confocal microscopy **(Fig. 6B)**. These results suggest that ORM has the ability to suppress cancer cell migratory potential *via* inhibiting these cell motility markers.

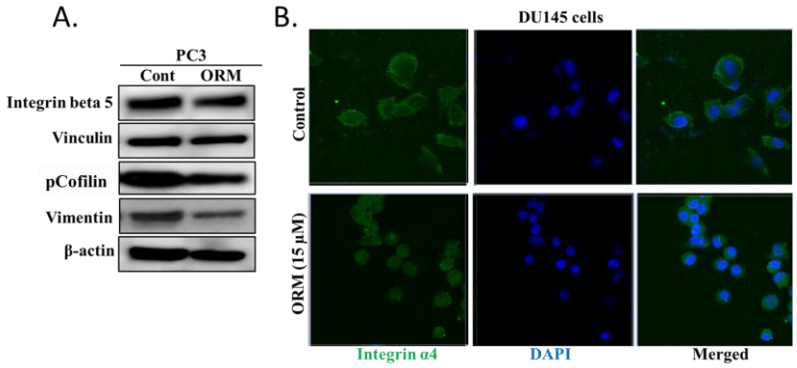


Figure 6: ORM inhibits the expression of motility markers in PrCa cells. A. Effect of ORM on expression of Integrin beta 5, vinculin, phospho Cofilin, and vimentin in PC3 as determined by Western blot analysis. B. Effect of ORM on the expression of Integrin α4 in DU145 cells as determined by confocal microscopy.

Task 3: To evaluate the therapeutic efficacy of ORM for PrCa treatment in TRAMP mouse model

4. ORM inhibits tumor growth in TRAMP mice. Transgenic adenocarcinoma of mouse prostate (TRAMP) mice closely mimics human prostate tumors in many ways. For example, focal adenocarcinoma in TRAMP mice develops rapidly within 10 to 20 weeks. Adenocarcinoma arises in the dorsal lateral lobe, which is considered analogous to the peripheral zone, where the human PrCa originates (13). These mice have been used to identify new molecular targets and to test new therapeutic modalities against PrCa. Moreover, these mice overexpress key oncogenic signaling pathways involved during the development, progression and metastasis of PrCa (14). The main objective of this experiment was to investigate whether ORM treatment inhibits prostate tumor growth and metastasis in an intact mouse model. In this experiment, a total of 22 TRAMP mice (6-7 week old) were purchased from Jackson Laboratory and divided into two groups. ORM treatment (200 µg IP three days a week) was started when mice were 9 weeks old and continued until end points. Vehicle group mice received 0.2 ml vehicle only. None of the mice showed apparent toxicity with ORM administration during entire period of the experiment. Four mice from each group were examined at 18 week of age for prostate tumor development. At this point, none of the mice showed prostate tumor growth in either group (Data not shown). Remaining mice were sacrificed at 35 weeks. As shown in **Figure 7**, ORM administration inhibited the growth of prostate tumor in TRAMP mice as determined by a significant ($P<0.01$) decrease in weight of prostate tumors and genito-urinary tracts (CI)

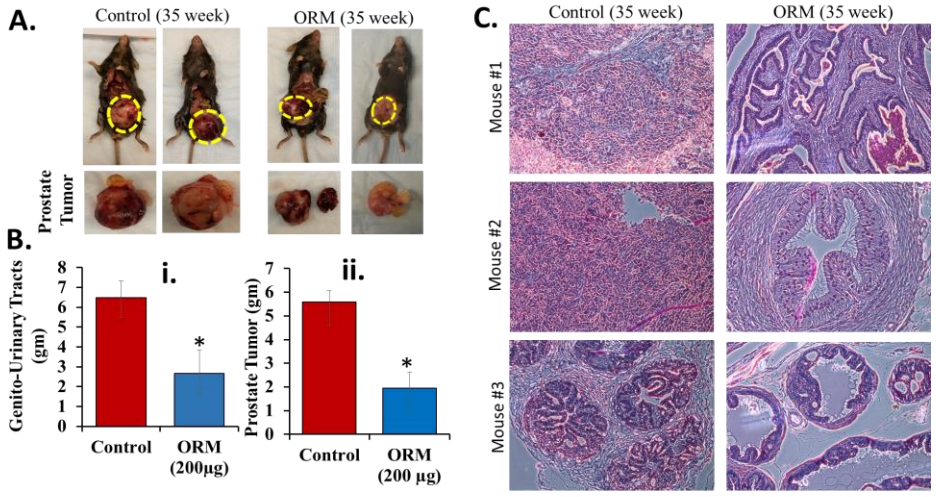


Figure 7: Effect of ORM on prostate carcinogenesis in TRAMP mice. A. Representative pictures of control and ORM treated TRAMP mice and their excised prostate tumors at 35 week. **B.** Bar graph showing mean ±SE of Genito-urinary tracts (i) and prostate tumor (ii) weight of control and ORM treatment group(4 mice each). **C.** Histopathological analysis of prostate tumor of control and ORM treated group. Mouse #1 and 2 in control group are showing poorly differentiated adenocarcinoma while mouse #3 moderately to widely differentiated adenocarcinoma. Representative picture of ORM treatment mice #1, 2, and 3 shows low differentiation. One mouse show moderately differentiated carcinoma. None of the mice in ORM group show poorly differentiated carcinoma.

compared to vehicle-treated mice. Data shown here are mean±SE of prostate tumor of 4 mice in each group. We excluded the remaining three mice of both groups as they did not develop prostate tumors. However, these mice showed presence of SV40-T antigen in tail genotyping by Jackson Laboratory. Histopathological examination of control mice showed mainly poorly differentiated adenocarcinoma (PDAC) at 35 weeks **(Fig. 7 C)**. The PDC exhibited marked polymorphism and high levels of mitosis and apoptosis with neuroendocrine phenotype of large nuclei with fine chromatin and inconspicuous nucleoli and scant cytoplasm within apparent cell membranes **(Fig. 7C)**. ORM treatment inhibited progression of PDAP in TRAMP mice. At 35 week, ORM treatment resulted in focal PIN in 3 mice **(Fig. 7C)**, and diffuse PIN in one mice **(Fig. 7C).** At the same time point (35 weeks), one of the four animals showed a small PDAC in the ORM treated group. These data suggest the potential chemotherapeutic effect of ORM against progression of PCa.

5. ORM inhibits the expression of PCNA, vimentin, CD63, and MMP3 in excised tumor tissues of TRAMP mice. We next evaluated the key oncogenic signaling components involved in aggressive prostate tumor microenvironments. Accumulating evidence suggests that tumor associated macrophages is one of the components of tumor microenvironments (TMEs) involved in development and metastasis of PrCa (15). CD63 is one of the markers for TAMs which was shown to be overexpressed in aggressive prostate tumor types. Thus, we examined the expression of CD63 in excised prostate tumor tissues of TRAMP mice. ORM treatment inhibited the expression of CD63 compared to control as determined by immunohistochemistry **(Fig. 8).** Since we observed that ORM treatment inhibits the metastatic phenotypes of PrCa cells *via* targeting EMT markers and MMPs, thus, we examined the effect of ORM on the expression of vimentin and MMP3 in excised prostate tumor tissues. Our results demonstrated effective inhibition of both vimentin and MMP3 expression with ORM treatment as compared to control. These findings indicate that ORM targets componets of prostate TME.

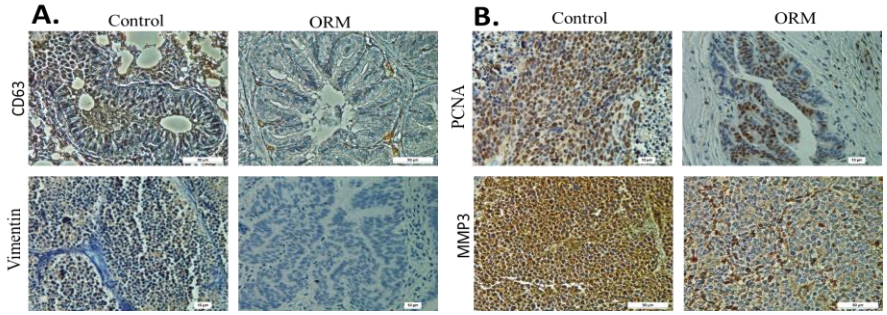
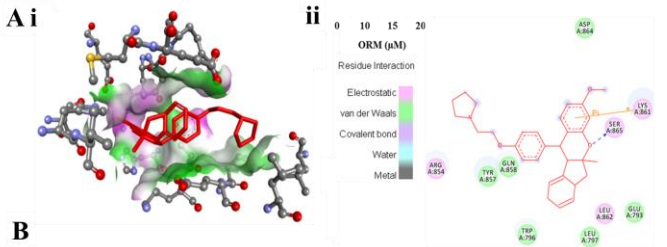


Figure 8: Effect of ORM on the expression of cell proliferative and TME markers in prostate of TRAMP mice. A. Representative Immunohistochemistry images are showing the expression of CD63 and Vimentin in excised prostate tissues of control and ORM treated TRAMP mice at 35 weeks. **B.** ORM inhibits the expression of PCNA and MMP3 in excised prostate tissues of TRAMP mice as determined by IHC analysis. Images were captured at 40 X.

6. ORM inhibits androgen receptor (AR) transactivation and its interaction with β-catenin: It has been shown that androgen receptor (AR) plays an important role in the prostate carcinogenesis (3). Thus, AR is one of the molecular targets for prostate cancer therapy. To investigate whether ORM interacts with AR, we performed molecular docking using ORM as ligand with androgen receptor (PDB ID: 1E3G) (16). using autodock 4.2 suit by employing Lamarckian genetic algorithm (17). The grid map illustrating the active site pocket for ligands were calculated by autogrid and the dimension of the grid for 1E3G, 1SJ0, 2NV7, 4DJS and 4ACH were 52x40x48, 40x44x56, 50x40x60, 56x50x90 and 60x62x70 grid points respectively with a spacing of 0.375 Aº between the grid points and centered on the ligand. Docking was accomplished by each cycle with an initial population of 150 individuals and the remaining parameter set as default. Ten conformational docking poses were created and the



B

S.N.	Protein Name	Binding Energy (Kcal/mol)	Protein Ligand Interaction			
			No of H Bonds	Pi-Interaction	Amino Acid Residue	Distance (A°)
1.	Androgen receptor (1E3G)	-8.0	1	1	SER865, LYS861	3.4

Figure 9: Molecular docking of ORM with AR. A. Docking analysis of ORM with androgen receptor (PDBID:1E3G) **(Ai, ii). B.** Inserted table showing the ORM binding capacity with AR.

best docked conformation was selected based on the autodock binding energy (18).The confirmations with the most favorable free binding energy were selected for analyzing the interactions between the target receptor and ligands by visualization with Discovery Studio software (version 3.5). Auto docking 4.2 was used to determine the orientation of inhibitor bound in the active site of AR and the conformation with the highest binding energy value for AR was chosen for further analysis and results of these studies are given in inserted table of Fig. 9**.** Our docking results revealed that ORM binds into the active site of AR (1E3G), with minimum binding energy (ΔG) -8.0 kcal/mol, -8.3 kcal/mol. ORM strongly binds with amino acid residue of AR at Ser865 and Lys861 aminoacids **(Fig. 9 Ai-ii).** Since, we observed that ORM interacts with LBD of AR, we sought to determine whether ORM inhibits androgen-induced proliferation of PrCa cells. In this experiment, LNCaP cells were grown in charcoal-treated hormone free medium. After 70% confluency, cells were treated with synthetic androgen (R1881,1µM), ORM (10 µM) or a combination of these agents. Twenty four hrs post-treatment, cell proliferation was examined by MTS assay. Results demonstrated that ORM treatment significantly (P<0.01) inhibited androgen-induced growth of LNCaP cells **(Fig. 10A).** ORM treatment (10-20 µM) of C4-2 cells dose-dependently inhibits the expression of AR protein as determined by Western blot analysis **(Fig. 10B).** Next, we were interested if ORM enhances degradation of AR protein in PrCa cells. For this, we performed pulse chase experiment by treated LnCaP cells with translational inhibitor (cycloheximide) followed by ORM treatment. Results clearly indicate that ORM destabilizes AR protein in PrCa cells **(Fig. 10 C-D).** Next we, analyzed the effect of ORM on AR transactivation by performining AR and PSA promoters activity. Results revealed ORM significantly (P<0.01) inhibits luciferase activity of AR **(Fig. 10E)** and its target gene PSA **(Fig 10F)**. These results indicate that ORM treatment inhibits transactivation of AR in C4-2 cells. It has been reported that after castration or androgen-ablation therapy AR still activated either with the low level of androgen or by various non-androgen ligands. β-catenin is one of the non-androgen co-activator of AR (12,19-

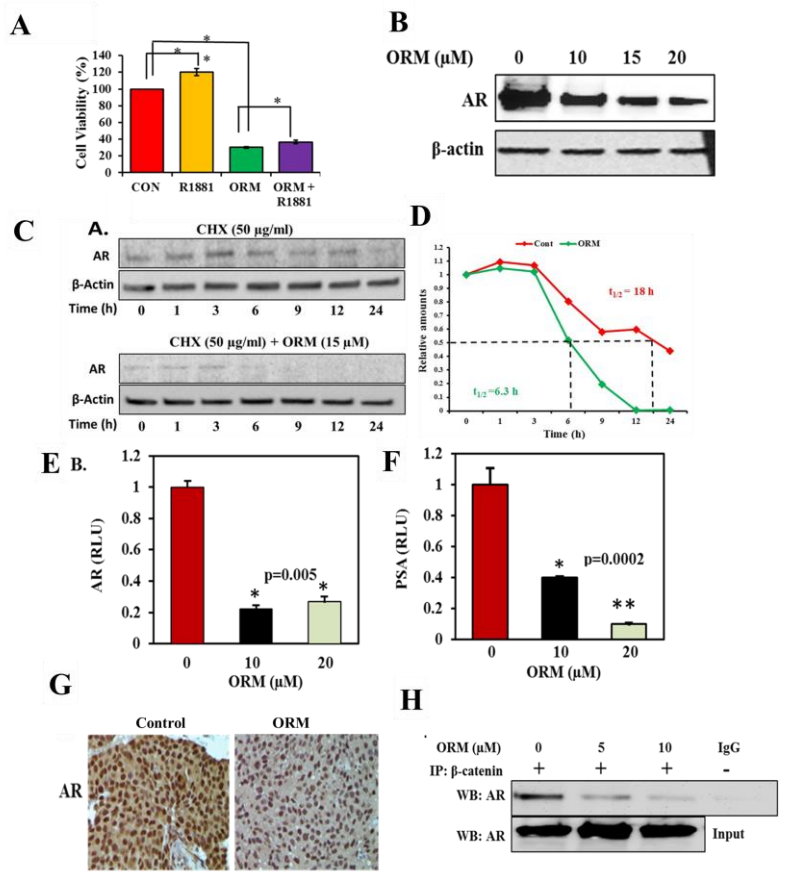


Figure 10: ORM treatment suppresses transactivation of AR and inhibits β-catenin-AR interaction in PrCa cells. A. Effect of ORM on R1881-induced growth of androgen-dependent LNCaP cells. Briefly, cells were treated with R1881 (10 nM) alone or in combination with ORM (10 µM). MTS assay was performed to analyze the cell growth. Data in bar graph represents mean ±SE of 5 wells in each group. Asterics represent significant value P<0.01. **B.** Effect of ORM on AR expression in C4-2 cells as determined by Western blot analysis. **C.** Effect of ORM on AR degradation as analyzed by pulse chase experiment. Briefly, LNCaP cells were treated with CHX (50 µg) alone or in combination with ORM (15 µM) at indicated time points. Protein lysates were prepared and subjected for Western blot analysis to analyze the protein levels of AR. Results indicate protein levels of AR alone CHX-treated (upper blot) and in CHX and ORM-treated (Lower blot). **D.** Line graph showing quantification of Western blots of Figure A. $T_{1/2}$ denotes time point for 50% AR protein degradation. **E.** Effect of ORM on AR promoter activity. Briefly, C4-2 cells were transiently transfected with AR promoter plasmids (1 µg) along with renilla luciferase plasmids (100 ng). After 24 h post-transfection, cells were treated with ORM (10 µM) for 12 h. Promoter activity was measured on illuminometer by using dual luciferase assay kit (Promega). Bar graph indicates significant inhibition of AR promoter activity with the treatment of ORM. **RLA:** Relative luciferase unit. **F.** Effect of ORM on PSA promoter activity in C4-2 cells. Similar experimental method was followed for PSA promoter activity. **G.** Effect of ORM on AR expression in C4-2 cells xenograft tumors as determined by immunohistochemistry. **H.** Effect of ORM on the interaction of β-catenin and AR. Result indicate ORM treatment inhibits interaction of β-catenin and AR as determined by Immunoprecipitation/Western blot analysis. IgG was used as a negative control. Lower blot indicate input for AR with each group.

22), it physically interacts with AR and enhances AR transactivation. We have shown that β-catenin is one of the molecular targets of ORM. Therefore, we were interested to explore effect of ORM on β-catenin-AR in PrCa cells. Our immunoprecipitation and Western blot analysis results revealed that ORM treatment inhibits interaction of β-catenin with AR in C4-2 cells **(Fig. 10D).** Overall these results suggest that ORM has the potential to inhibits both androgen and non-androgen independent growth of PrCa cells.

Research work performed during the funding cycle (2017-18): We have successfully completed the proposed tasks during the 2017-18 academic year.

Task 1:To identify the detailed molecular mechanisms for ORM-specific targeting of ARv7

7. ORM sensitizes the effect of enzalutamide via targeting AR splice variant ARV7. Enzalutamide (ENZ) is a second-generation anti-androgen drug that has shown promising results in improving the survival of mCRPC patients. However, developing ENZ therapy resistance is a major hurdle encountered by Uro-oncologists treating mCRPC patients. (23). ENZ exerts its growth inhibitory activity by competing with and displacing the natural ligands (testosterone and dihydrotestosterone) of androgen receptor (AR) and inhibiting AR translocation into the nucleus to repress its target genes (24). Emerging evidence supports the concept that development of metastatic castration resistant PrCa (mCRPC) is largely due to expression of AR splicing variants (ARVs) which are missing variable portions of the C-terminal domain and ligand binding domain (25-27). Among all splicing variants ARV7 and AR^{v567es} have shown more clinical relevance indeveloping mCRPC and ENZ therapy resistance (28,29). A recent study indicates that higher expression of ARV7 and AR^{v567es} in CRPCs are associated with CRPC developing more rapidly and reduced patient survival (27). ARV7 protein was found to be overexpressed in bone metastatic samples (30), circulating tumor cells (27) of CRPC patients treated with ENZ. These results suggest that AR splicing variants are potential molecular target for the management of mCRPC to overcome enzalutamide resistance. Our results demonstrated that ORM inhibits both AR and AR splice variant in androgen-independent 22Rv1 PrCa cells as determined by Western blot analysis **(Fig. 11A)**. In this experiment we used 22Rv1 cells as these cells express multiple AR splicing variants that remain constitutively active, leading to ligand-independent induction of target gene expression and cell growth (28,29). ORM (2.5-20 µM) treatment of 22Rv1 cells for 12 hrs inhibited the protein levels of both full length AR and ARV7 in 22Rv1 cells **(Fig. 11A).** However, no effect was observed at mRNA expression of ARv7 **(Fig. 11B).** These results prompted us to further investigate its enzalutamide sensitization effect in these cells. ORM treatment showed additive effect with Enzalutamide in 22Rv1 cells **(Fig. 11C).** Various molecular mechanisms contributing to generation of AR spliced variants in CRPC and ENZ resistance have been reported (31,32). However, a more comprehensive approach is needed to further investigate the underlying molecular mechanisms for regulating AR splice variants in ENZ resistance, which will help in developing novel strategies to treat mCRPC. We also investigated the new molecular mechanism of ARv7 regulation in PrCa cells. Stat3 is a protein hub for several oncogenic signaling pathways and regulates the expression of key effector molecules involved in tumor cell survival (33), proliferation (34,35), angiogenesis (36) and metastasis (37). Activation of Stat3 required for indirect phosphorylation at Tyrosine 705 (Y705) residues typically through Janus Associated Kinases (JAK's) in response to cytokines IL-6 and IL-10, or direct phosphorylation by receptor tyrosine kinases (i.e. EGFR, VEGFR, IGFR) and non-receptor tyrosine kinases such as Src family kinases (38) leading to its subsequent dimerization and nuclear localization. Constitutive activation of Stat3 has been reported in PrCa progression and mCRPC (39-41).

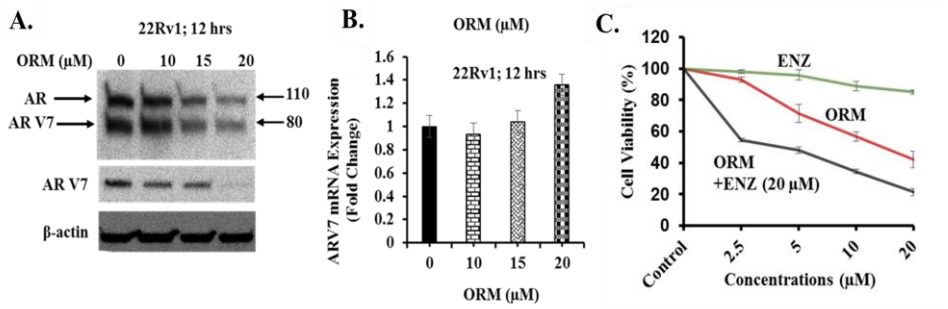


Figure 11: ORM treatment inhibits ARv7 and sensitizes the effect of enzalutamide. **A.** Effect of ORM (12 hrs treatment) on protein levels of full length AR and ARv7 in 22Rv1 cells as determined by Western blot analysis. **B.** Effect of ORM on mRNA expression of AR (i), ARV7 (ii), and PSA (iii) after 12 hrs cells treatment as determined by qPCR analysis. **C.** Effect of ORM alone or in combination with enzalutamide on growth of 22Rv1 cells (24 hrs treatment) as determined by MTT assay.

Stat3 has been linked highlighting the significance of AR/Stat3 co-operation in PCa progression (42). A recent study suggested targeting Stat3-AR signaling suppresses the growth of ENZ resistance PrCa cells (43). To investigate whether Stat3 interacts with ARv7, we performed immunoprecipitation and western blot analysis in 22Rv1 cells. Our results revealed that Stat3 physically interacts with ARv7 as indicated in Figure 12A. We next confirmed these results by double immunofluorescence using primary specific antibodies of ARv7 and Stat3 followed by and human prostate tumor tissues of high grade, we perfWe observed Stat3 interaction with ARv7 in CRPC cells and human prostate tumor tissues. We observed that transcription factor Stat3 physically interacts with ARv7 in 22Rv1 cells **(Fig. 12A)** as well their co-localization in human prostate tumors **(Fig. 12C).** Interestingly, ORM treatment of 22Rv1 cells for 12 hrs inhibited Stat3 and ARv7 interaction. These results suggest that Stat3 might play a role in regulation of ARv7. However, further detail investigation is required to study *in vitro* model system and in enzalutamide sensitive and resistant model system.

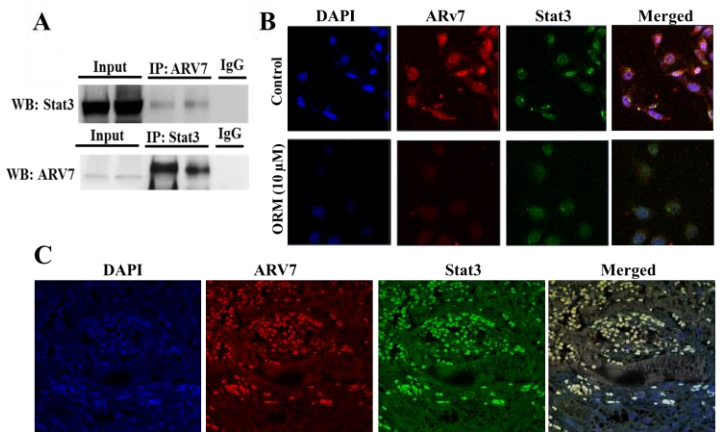


Figure 12: Stat3 physically interacts with ARV7 in PrCa cells and tissues. A. Immunoprecipitation and WB analysis results indicating interaction of Stat3 and ARV7 proteins in 22Rv1 cells. **B.** ORM inhibits Stat3-ARv7 interaction in 22Rv1 cells as determined by confocal microscopy. **C.** Double immune-fluorescence images of Stat3 and ARv7 proteins co-localization in human prostate tumor tissues as determined by double immunofluorescence using specific primary antibodies of Stat3 and ARv7. Images (40X) were captured by confocal microscope.

7. **ORM sensitizes the effect of enzalutamide in xenograft mouse model.** We observed the similar sensitization effect of ORM in xenograft mouse model **(Fig.13).** ORM (10 mg/kg; ip administration, 3 days/week) significantly ($P<0.01$) inhibited growth of 22Rv1 cells derived xenograft tumors in athymic nude mice as determined by a significant ($P<0.01$) decrease of tumor volume **(Fig. 13A)** and tumor weight **(Fig. 13B) .** ENZ (10 mg/kg; ip administration, 3 days/week) alone did not show any significant tumor growth inhibition. However, a combination treatment of ORM (10 mg/kg) and ENZ (10 mg/kg) (ip administration, 3 days/week) showed a more pronounced effect in reducing prostate tumor growth **(Fig. 11B,C).** As expected, no effect was observed on the expression of ARv7 in excised xenograft tumor of ENZ treated mice when compared to control group mice as determined by using ARv7 specific antibody **(Fig. 13C).** Both ORM alone or combination treatment with ENZ showed inhibition in the expression of ARv7 when compared to control **(Fig. 13C).**

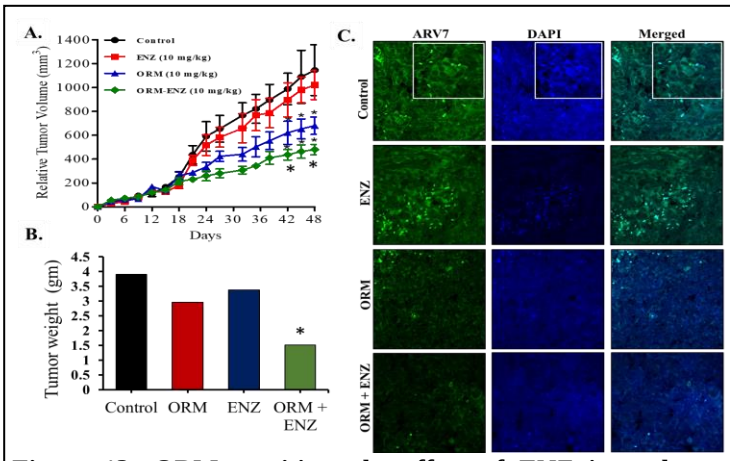


Figure 13: ORM sensitizes the effect of ENZ in androgen-independent 22Rv1 cells *in vitro* and *in vivo*. A. Effect of ORM alone or in combination with ENZ in 22Rv1 cells xenograft tumors. In this experiment, 22Rv1 cells ($1x10^6$) subcutaneously in athymic nude mice and two weeks post-implantation ORM alone or in combination with ENZ at indicated doses was given 3 days a week for 6 consecutive weeks. Line bar graph indicating tumor volume measures by digital caliper. B. Excised prostate tumor weight of indicated groups's xenograft tumors. n=5 in each group. **C.** Immunofluorescence analysis of ARv7 in excised tumor tissues of indicated group mice as determined by confocal microscopy. Asterics represents significant value of $p<0.01$.

Key Research Accomplishments:

Status: All proposed studies were successfully completed.

Task 1: To examine the effect of ormeloxifene on β-catenin-AR/ER signaling pathways.

Status: Completed

Task 2: Determine the apoptotic pathways activated by ormeloxifene to induce cell death in PrCa cells.
Status: Completed
Task 3: To evaluate the therapeutic efficacy of ormeloxifene for PrCa treatment in mouse model systems.
Status: Completed
Task 4: To investigate the therapeutic effects of ORM alone or in combination with Enzalutamide *in vivo*.
Status: Completed

Publications fully and partially pertinent to this Grant:

1: Nagesh PKB, Chowdhury P, Hatami E, Boya VKN, Kashyap VK, Khan S, Hafeez BB, Chauhan SC, Jaggi M, Yallapu MM. miRNA-205 Nanoformulation Sensitizes Prostate Cancer Cells to Chemotherapy. Cancers (Basel). 2018 Aug 25;10(9). pii: E289. doi: 10.3390/cancers10090289. PubMed PMID: 30149628; PubMed Central PMCID: PMC6162422.

2: Almabadi HM, Nagesh PKB, Sahay P, Bhandari S, Eckstein EC, Jaggi M, Chauhan SC, Yallapu MM, Pradhan P. Optical study of chemotherapy efficiency in cancer treatment via intracellular structural disorder analysis using partial wave spectroscopy. J Biophotonics. 2018 Dec;11(12):e201800056. doi: 10.1002/jbio.201800056. Epub 2018 Sep 26. PubMed PMID: 29869394.

3: Nagesh PKB, Hatami E, Chowdhury P, Kashyap VK, Khan S, Hafeez BB, Chauhan SC, Jaggi M, Yallapu MM. Tannic Acid Induces Endoplasmic Reticulum Stress-Mediated Apoptosis in Prostate Cancer. Cancers (Basel). 2018 Mar 7;10(3). pii: E68. doi: 10.3390/cancers10030068. PubMed PMID: 29518944; PubMed Central PMCID: PMC5876643.

4: Ganju A, Chauhan SC, Hafeez BB, Doxtater K, Tripathi MK, Zafar N, Yallapu MM, Kumar R, Jaggi M. Protein kinase D1 regulates subcellular localisation and metastatic function of metastasis-associated protein 1. Br J Cancer. 2018 Feb 20;118(4):587-599. doi: 10.1038/bjc.2017.431. Epub 2018 Feb 20. PubMed PMID: 29465084; PubMed Central PMCID: PMC5830591.

5: Sahay P, Ganju A, Almabadi HM, Ghimire HM, Yallapu MM, Skalli O, Jaggi M, Chauhan SC, Pradhan P. Quantification of photonic localization properties of targeted nuclear mass density variations: Application in cancer-stage detection. J Biophotonics. 2018 May;11(5):e201700257. doi: 10.1002/jbio.201700257. Epub 2018 Jan 17. PubMed PMID: 29222925.

6: Hafeez BB, Ganju A, Sikander M, Kashyap VK, Hafeez ZB, Chauhan N, Malik S, Massey AE, Tripathi MK, Halaweish FT, Zafar N, Singh MM, Yallapu MM, Chauhan SC, Jaggi M. Ormeloxifene Suppresses Prostate Tumor Growth and Metastatic Phenotypes via Inhibition of Oncogenic β-catenin Signaling and EMT Progression. Mol Cancer Ther. 2017 Oct;16(10):2267-2280. doi: 10.1158/1535-7163.MCT-17-0157. Epub 2017 Jun 14. PubMed PMID: 28615299; PubMed Central PMCID: PMC5774234.

7: Chowdhury P, Roberts AM, Khan S, Hafeez BB, Chauhan SC, Jaggi M, Yallapu MM. Magnetic nanoformulations for prostate cancer. Drug Discov Today. 2017 Aug;22(8):1233-1241. doi: 10.1016/j.drudis.2017.04.018. Epub 2017 May 16. Review. PubMed PMID: 28526660; PubMed Central PMCID: PMC5565688.

8: Nagesh PKB, Johnson NR, Boya VKN, Chowdhury P, Othman SF, Khalilzad-Sharghi V, Hafeez BB, Ganju A, Khan S, Behrman SW, Zafar N, Chauhan SC, Jaggi M, Yallapu MM. PSMA targeted docetaxel-loaded superparamagnetic iron oxide nanoparticles for prostate cancer. Colloids Surf B Biointerfaces. 2016 Aug 1;144:8-20. doi: 10.1016/j.colsurfb.2016.03.071. Epub 2016 Mar 26. PubMed PMID: 27058278; PubMed Central PMCID: PMC5100699.

9: Yallapu MM, Khan S, Maher DM, Ebeling MC, Sundram V, Chauhan N, Ganju A, Balakrishna S, Gupta BK, Zafar N, Jaggi M, Chauhan SC. Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer. Biomaterials. 2014 Oct;35(30):8635-48. doi: 10.1016/j.biomaterials.2014.06.040. Epub 2014 Jul 12. PubMed PMID: 25028336; PubMed Central PMCID: PMC4220612.

Publications under review/preparation pertinent to this Grant:

1. Sikander M, Malik S, Hafeez BB, Ganju A, Jaggi M, **Chauhan SC.** Cucurbitacin inhibits the growth of hormone refractory prostate cancer cells *via* modulating glucose metabolism. *Communicated in* **Cancer 2018** (Communicated).
2. Hafeez BB, Sikander, Ganju A, Jaggi M, **Chauhan SC.** ORM suppresses the growth of prostate tumor and metastasis in TRAMP mice. **Carcinogenesis 2017** (Under preparation)
3. Hafeez BB, Sikander, Ganju A, Jaggi M, **Chauhan SC.** ORM inhibits the growth of hormone refractory prostate cancer via inhibition of androgen and no-androgen activation of AR signaling. (**Prostate Under preparation)**.

Grants Submitted on Natural Extension of This Project:

DOD Impact award: 04/01/19 to 03/31/2022

1. PI: Chauhan SC, **Co-PI:** Hafeez BB

Title: Novel Therapeutic Strategy for Metastatic Castration Resistant Prostate Cancer

NIH/RO1 07/01/19 to 06/31/2024

2. PI: Chauhan SC, **Co-PI:** Hafeez BB

Title: Novel Therapeutic Strategy for Metastatic Castration Resistant Prostate Cancer

The major goals of these projects are **1)** to investigate the molecular mechanisms of ORM to suppress metastatic CRPC and overcome enzalutamide resistantm, **2)** to identify molecular factors (Stat3 and lncRNA MALAT1) involved in regulation of AR splice variants (ARv7 and ARv567es), and **3)** to elucidate the correlation of Stat3-ARv7 and MALAT1-ARv7 with enzalutamide resistance and poor prognosis in mCRPC patients.

Other Grants Submitted for Extramural Funding on Prostate Cancer:

1. DOD Idea development award 04/01/2018 to 03/31/2021

PI: Hafeez BB, **Co-Investigator:** Chauhan SC

Title: Role of WAVE2 in Tumor Microenvironment and Metastasis of Prostate Cancer

2. DOD Health disparity award 06/01/2018 to 05/31/2021

PI: Hafeez BB, **Co-Investigator:** Chauhan SC

Title: Role of PKCε in Prostate Cancer Health Disparity

3. DOD Idea development award 04/01/2018 to 03/31/2021

PI: Jaggi M, **Co-Investigator:** Chauhan SC

Title: PKD1 modulated miRNA in Prostate cancer

4. NIH/R01 4/01/2018 to 03/31/2023

PI: Jaggi M, **Co-Investigator:** Chauhan SC

Title: Modulation of Tumor Microenvironment and Metastasis by PKD1

References

1. Damber JE, Aus G. Prostate cancer. Lancet **2008**;371:1710-21
2. Sahoo SK, Ma W, Labhasetwar V. Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. International journal of cancer Journal international du cancer **2004**;112:335-40
3. Kaarbo M, Klokk TI, Saatcioglu F. Androgen signaling and its interactions with other signaling pathways in prostate cancer. BioEssays : news and reviews in molecular, cellular and developmental biology **2007**;29:1227-38
4. Verras M, Sun Z. Roles and regulation of Wnt signaling and beta-catenin in prostate cancer. Cancer letters **2006**;237:22-32
5. Sarkar FH, Li Y, Wang Z, Kong D. Novel targets for prostate cancer chemoprevention. Endocrine-related cancer **2010**;17:R195-212
6. Kamboj VP, Setty BS, Chandra H, Roy SK, Kar AB. Biological profile of Centchroman--a new post-coital contraceptive. Indian journal of experimental biology **1977**;15:1144-50
7. Misra NC, Nigam PK, Gupta R, Agarwal AK, Kamboj VP. Centchroman--a non-steroidal anti-cancer agent for advanced breast cancer: phase-II study. International journal of cancer Journal international du cancer **1989**;43:781-3
8. Nigam M, Ranjan V, Srivastava S, Sharma R, Balapure AK. Centchroman induces G0/G1 arrest and caspase-dependent apoptosis involving mitochondrial membrane depolarization in MCF-7 and MDA MB-231 human breast cancer cells. Life sciences **2008**;82:577-90
9. Srivastava VK, Gara RK, Bhatt ML, Sahu DP, Mishra DP. Centchroman inhibits proliferation of head and neck cancer cells through the modulation of PI3K/mTOR pathway. Biochemical and biophysical research communications **2011**;404:40-5
10. Singh MM. Centchroman, a selective estrogen receptor modulator, as a contraceptive and for the management of hormone-related clinical disorders. Medicinal research reviews **2001**;21:302-47
11. Yu L, Su YS, Zhao J, Wang H, Li W. Repression of NR4A1 by a chromatin modifier promotes docetaxel resistance in PC-3 human prostate cancer cells. FEBS letters **2013**;587:2542-51
12. Yang F, Li X, Sharma M, Sasaki CY, Longo DL, Lim B, *et al.* Linking beta-catenin to androgen-signaling pathway. The Journal of biological chemistry **2002**;277:11336-44
13. Hurwitz AA, Foster BA, Allison JP, Greenberg NM, Kwon ED. The TRAMP mouse as a model for prostate cancer. Curr Protoc Immunol **2001**;Chapter 20:Unit 20.5
14. Hafeez BB, Zhong W, Mustafa A, Fischer JW, Witkowsky O, Verma AK. Plumbagin inhibits prostate cancer development in TRAMP mice via targeting PKCepsilon, Stat3 and neuroendocrine markers. Carcinogenesis **2012**;33:2586-92
15. Maolake A, Izumi K, Shigehara K, Natsagdorj A, Iwamoto H, Kadomoto S, *et al.* Tumor-associated macrophages promote prostate cancer migration through activation of the CCL22-CCR4 axis. Oncotarget **2017**;8:9739-51
16. Sack JS, Kish KF, Wang C, Attar RM, Kiefer SE, An Y, *et al.* Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural agonist dihydrotestosterone. Proceedings of the National Academy of Sciences **2001**;98:4904-9
17. Fuhrmann J, Rurainski A, Lenhof HP, Neumann D. A new Lamarckian genetic algorithm for flexible ligand-receptor docking. Journal of computational chemistry **2010**;31:1911-8
18. Ansari MF, Siddiqui SM, Ahmad K, Avecilla F, Dharavath S, Gourinath S, *et al.* Synthesis, antiamoebic and molecular docking studies of furan-thiazolidinone hybrids. European Journal of Medicinal Chemistry **2016**;124:393-406
19. Chesire DR, Ewing CM, Gage WR, Isaacs WB. In vitro evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis. Oncogene **2002**;21:2679-94
20. Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC. The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. The Journal of biological chemistry **2002**;277:17933-43

21. Pawlowski JE, Ertel JR, Allen MP, Xu M, Butler C, Wilson EM, *et al.* Liganded androgen receptor interaction with beta-catenin: nuclear co-localization and modulation of transcriptional activity in neuronal cells. The Journal of biological chemistry **2002**;277:20702-10
22. Truica CI, Byers S, Gelmann EP. Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. Cancer research **2000**;60:4709-13
23. Chen X, Lu J, Xia L, Li G. Drug resistance of enzalutamide in CRPC. Curr Drug Targets **2017**
24. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science **2009**;324:787-90
25. Wadosky KM, Koochekpour S. Androgen receptor splice variants and prostate cancer: From bench to bedside. Oncotarget **2017**;8:18550-76
26. Cao S, Zhan Y, Dong Y. Emerging data on androgen receptor splice variants in prostate cancer. Endocr Relat Cancer **2016**;23:T199-t210
27. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Zhu Y, *et al.* Clinical Significance of Androgen Receptor Splice Variant-7 mRNA Detection in Circulating Tumor Cells of Men With Metastatic Castration-Resistant Prostate Cancer Treated With First- and Second-Line Abiraterone and Enzalutamide. J Clin Oncol **2017**;35:2149-56
28. Liu X, Ledet E, Li D, Dotiwala A, Steinberger A, Feibus A, *et al.* A Whole Blood Assay for AR-V7 and ARv567es in Patients with Prostate Cancer. J Urol **2016**;196:1758-63
29. Shi XB, Ma AH, Xue L, Li M, Nguyen HG, Yang JC, *et al.* miR-124 and Androgen Receptor Signaling Inhibitors Repress Prostate Cancer Growth by Downregulating Androgen Receptor Splice Variants, EZH2, and Src. Cancer research **2015**;75:5309-17
30. Efstathiou E, Titus M, Wen S, Hoang A, Karlou M, Ashe R, *et al.* Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer. European urology **2015**;67:53-60
31. Zhang Z, Zhou N, Huang J, Ho TT, Zhu Z, Qiu Z, *et al.* Regulation of androgen receptor splice variant AR3 by PCGEM1. Oncotarget **2016**;7:15481-91
32. Thomas JD, Longen CG, Oyer HM, Chen N, Maher CM, Salvino JM, *et al.* Sigma1 Targeting to Suppress Aberrant Androgen Receptor Signaling in Prostate Cancer. Cancer research **2017**;77:2439-52
33. Fukada T, Hibi M, Yamanaka Y, Takahashi-Tezuka M, Fujitani Y, Yamaguchi T, *et al.* Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. Immunity **1996**;5:449-60
34. Leslie K, Lang C, Devgan G, Azare J, Berishaj M, Gerald W, *et al.* Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3. Cancer research **2006**;66:2544-52
35. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, *et al.* Stat3 as an oncogene. Cell **1999**;98:295-303
36. Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, *et al.* Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene **2002**;21:2000-8
37. Huang S. Regulation of metastases by signal transducer and activator of transcription 3 signaling pathway: clinical implications. Clinical cancer research : an official journal of the American Association for Cancer Research **2007**;13:1362-6
38. Bishop JL, Thaper D, Zoubeidi A. The Multifaceted Roles of STAT3 Signaling in the Progression of Prostate Cancer. Cancers **2014**;6:829-59
39. Tam L, McGlynn LM, Traynor P, Mukherjee R, Bartlett JM, Edwards J. Expression levels of the JAK/STAT pathway in the transition from hormone-sensitive to hormone-refractory prostate cancer. British journal of cancer **2007**;97:378-83
40. Don-Doncow N, Marginean F, Coleman I, Nelson PS, Ehrnstrom R, Krzyzanowska A, *et al.* Expression of STAT3 in Prostate Cancer Metastases. European urology **2017**;71:313-6
41. Mohanty SK, Yagiz K, Pradhan D, Luthringer DJ, Amin MB, Alkan S, *et al.* STAT3 and STAT5A are potential therapeutic targets in castration-resistant prostate cancer. Oncotarget **2017**;8:85997-6010

42. Hsu FN, Chen MC, Lin KC, Peng YT, Li PC, Lin E, *et al.* Cyclin-dependent kinase 5 modulates STAT3 and androgen receptor activation through phosphorylation of Ser(7)(2)(7) on STAT3 in prostate cancer cells. American journal of physiology Endocrinology and metabolism **2013**;305:E975-86
43. Thaper D, Vahid S, Kaur R, Kumar S, Nouruzi S, Bishop JL, *et al.* Galiellalactone inhibits the STAT3/AR signaling axis and suppresses Enzalutamide-resistant Prostate Cancer. Scientific reports **2018**;8:17307