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TITLE: A Combination Approach for Treatment of Castration-Resistant Prostate Cancer

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<b>15. SUBJECT TERMS</b> 20(S)-protopanaxadiol-aglycone; prostate cancer; androgen receptor splice variants					
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## **INTRODUCTION:**

Abiraterone, enzalutamide, and taxanes are first-line treatments for metastatic castration-resistant prostate cancer (mCRPC). They prolonged overall survival of the patients. However, both primary and acquired resistance are common. Accumulating evidences point to a role of androgen receptor splice variants (AR-Vs) in mediating resistance to these therapies [1-10]. The purpose of this study is to develop 20(S)-protopanaxadiol-aglycone (PPD), which we showed to downregulate the levels and activities of AR-Vs [11, 12], as an antidote to overcome resistance to these therapies.

## **KEYWORDS:**

20(S)-protopanaxadiol-aglycone; prostate cancer; androgen receptor splice variants

## **ACCOMPLISHMENTS:**

### **What were the major goals of the project?**

**Major Task 1:** To elucidate the involvement of LSD1 in taxane-mediated AR-V upregulation.

Subtask 1: determine the effect of LSD1 inhibitor on AR-FL and AR-V proteins

0% completed

Subtask 2: determine the effect of LSD1 knockdown on AR-FL and AR-V proteins

30% completed

Subtask 3: submit animal protocol for approval

100% completed

Subtask 4: submit ACURO appendix for approval

100% completed

Milestone: *elucidation of the involvement of LSD1 in taxane-mediated AR-V upregulation*

Proposed completion date: 12/31/15

Not achieved

Milestone: ACURO Approval

Proposed completion date: 2/28/16

Actual completion date: 8/19/15.

**Major Task 2:** To determine ARBS2 involvement in taxane-mediated AR-V upregulation

Subtask 1: generate LN-AI and LNCaP95 stable clones with ARBS2 deletion

40% completed

Subtask 2: assess the *in vitro* effect of taxanes on AR-FL and AR-V levels in parental LN-AI and LNCaP95 cells and ARBS2-deleted stable clones

0% completed

Subtask 3: assess the *in vivo* effect of taxanes on AR-FL and AR-V levels in parental LN-AI and LNCaP95 cells and ARBS2-deleted stable clones

0% completed

Milestone(s): generation of LN-AI and LNCaP95 cells with ARBS2 deletion; determination of ARBS2 involvement in taxane-mediated AR-V upregulation

Proposed completion date: 5/31/17

Not achieved

**Major Task 3:** To assess the contribution of the selective stability of AR-V proteins to taxane-mediated AR-V upregulation

Subtask 1: measure the stability of AR-V and AR-FL proteins after taxane treatments  
0% completed

Subtask 2: determine the impact of proteasome inhibitor on taxane modulation of AR-FL and AR-V proteins  
0% completed

Subtask 3: evaluate the rates of AR-FL and AR-V translation after taxane treatments  
0% completed

Milestone(s): determination of the contribution of the selective stability of AR-V proteins to taxane-mediated AR-V upregulation; determination of whether taxanes affect the translation rate of AR-FL and AR-V

Proposed completion date: 12/31/17

Not achieved

**Major Task 4:** To determine the impact of PPD on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization

Subtask 1: determine the impact of PPD on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization  
20% completed

Subtask 2: determine the impact of enzalutamide on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization  
20% completed

Subtask 3: determine the impact of EPI-001 on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization  
0% completed

Milestone(s): determination of the impact of PPD, in comparison with enzalutamide and EPI-001, on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization

Proposed completion date: 3/31/18

Not achieved

**Major Task 5:** To determine the impact of PPD on AR-FL, AR-V7, and AR<sup>v567es</sup> subcellular localization

Subtask 1: determine whether PPD affects AR-V nuclear translocation  
0% completed

Subtask 2: determine whether PPD affects androgen-independent AR-FL nuclear translocation  
0% completed

Subtask 3: determine whether PPD affects androgen-induced AR-FL nuclear translocation  
0% completed

Milestone(s): determination of the impact of PPD on AR-FL, AR-V7, and AR<sup>v567es</sup> subcellular localization

Proposed completion date: 5/31/18

Not achieved

**Major Task 6:** To elucidate the role of AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization in PPD induction of AR-FL and AR-V degradation

Subtask 1: determine whether disruption of AR-V or AR-FL dimerization can attenuate PPD induction of AR-V or AR-FL degradation  
0% completed

Subtask 2: investigate the impact of PPD, in the presence or absence of taxanes, on poly-ubiquitination of dimerization-deficient mutants of AR-V and AR-FL  
0% completed

*Milestone(s): elucidation of the role of AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization in PPD induction of AR-FL and AR-V degradation*

Proposed completion date: 8/31/18  
Not achieved

**Major Task 7:** To determine the potential of using PPD, in comparison with EPI-001, to improve the efficacies of abiraterone, enzalutamide, and cabazitaxel

Subtask 1: determine the potential of using PPD, in comparison with EPI-001, to improve the antitumor efficacies of abiraterone, enzalutamide, and cabazitaxel  
20% completed

Subtask 2: determine PPD concentrations in the tumors  
0% completed

Subtask 3: molecular and histological analyses of the tumors  
0% completed

*Milestone(s) Achieved:* preclinical determination of the potential of using PPD, in comparison with EPI-001, to improve the antitumor efficacies of abiraterone, enzalutamide, and cabazitaxel

Proposed completion date: 5/31/17  
Not achieved

**Major Task 8:** To determine the efficacies of PPD, in comparison with EPI-001, against abiraterone-, enzalutamide-, and taxane-resistant CRPC

Subtask 1: develop abiraterone-resistant LuCaP23CR xenograft line (LuCaP23CR-abiR)  
0% completed

Subtask 2: determine the efficacies of PPD, in comparison with EPI-001, against the growth of abiraterone-, enzalutamide-, and taxane-resistant CRPC  
0% completed

Subtask 3: determine PPD concentrations in the tumors  
0% completed

Subtask 4: molecular and histological analyses of the tumors  
0% completed

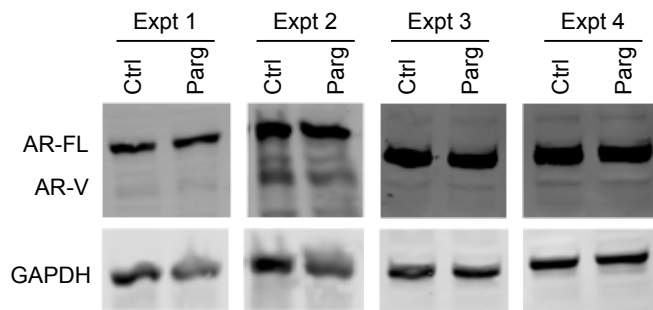
*Milestone(s) Achieved:* preclinical determination of the efficacies of PPD, in comparison with EPI-001, against abiraterone-, enzalutamide-, and taxane-resistant CRPC

Proposed completion date: 8/31/18  
Not achieved

## What was accomplished under these goals?

Task 1-1: To determine the effect of LSD1 inhibitor on AR-FL and AR-V proteins

Androgen represses the transcription of the AR gene via liganded full length AR (AR-FL) binding to an enhancer element in the AR gene (termed ARBS2) and recruiting lysine-specific demethylase 1 (LSD1) to demethylate mono- and di-methyl H3K4 histone (H3K4me1, 2) [13]. We hypothesize that, as a result of inhibition of AR-FL transactivation by taxanes, LSD1 cannot be recruited to the ARBS2 enhancer element in the AR gene to inhibit AR-gene transcription. This leads to enhanced production of AR pre-mRNA and thereby an increase of AR-FL and AR-V mRNAs. Increased mRNA expression together with the selective stability of AR-V proteins after taxane treatment provides a feedforward mechanism leading to accumulation of AR-V proteins. To test this hypothesis, we treated VCaP cells with 2 mM pargyline, an LSD1 inhibitor in the presence of dihydrotestosterone (DHT) and assessed the impact on AR-FL and AR-V protein levels by Western blot analysis. Figure 1 showed the results from 4 independent experiments. No significant change of AR-FL or AR-V proteins was observed after pargyline treatment.

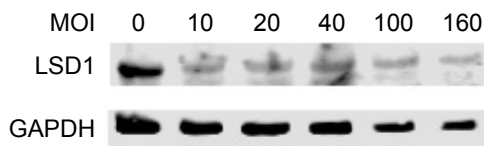


**Figure 1. Pargyline treatment of VCaP cells did not affect the levels of AR-FL or AR-V protein.**

Western blot analysis was performed with the pan-AR antibody (N-20). Data are from 4 independent experiments (Expt).

Task 1-2: determine the effect of LSD1 knockdown on AR-FL and AR-V proteins

We thought that the lack of pargyline effect might be due to potential non-specific activities of pargyline in the cells. We therefore resorted to knocking down LSD1 expression specifically by using an LSD1 shRNA lentiviral construct (Sigma). We used LSD1-shRNA-encoding lentivirus at different multiplicity of infection (MOI) to infect VCaP cells were able to achieve sufficient knockdown of LSD1 protein as assessed by Western blotting (Figure 2). However, we later found out that our cells were contaminated with mycoplasma. We have decontaminated our cell culture facility and obtained new cells and will repeat the above experiments with these new cells.



**Figure 2. LSD1 knockdown.** VCaP cells infected with lentivirus encoding LSD1 shRNA at the indicated multiplicity of infection (MOI), and the cells were subjected to Western blot analysis.

Task 1-3: submit animal protocol for approval

We submitted an amendment of our animal protocol to our institutional IACUC committee to include the work proposed in this funded project, and it was approved on June 23, 2015.

Task 1-4: submit ACURO appendix for approval

We submitted an ACURO appendix to the DOD, and it was approved on August 19, 2015.

Task 2-1: generate LN-AI and LNCaP95 stable clones with ARBS2 deletion

As described above, androgen represses the transcription of the AR gene via liganded AR-FL binding to the ARBS2 enhancer element, which is a 399-bp region in AR intron 2 [13]. To determine the role of ARBS2 in mediating taxane induction of AR mRNA expression, we used the Clustered Regularly Interspaced Short Palindromic (CRISPR) genome-editing technology to delete ARBS2 in the AR gene locus. We designed 5 pairs of gRNAs for deletion of the ARBS2 region and cloned the gRNAs individually into the LentiCRISPRv2 construct, which also encodes cas9. These constructs were then individually cotransfected into 293T with lentiviral packaging plasmids, and the packaged lentivirus was used to infect LNCaP95 cells. Cutting efficiencies of the gRNAs were subsequently assessed by using the Surveyor assay. As shown in Figure 3, gRNA1 and gRNA2 at the 5'-end of ARBS2 and gRNA10 at the 3'-end of ARBS2 have the best cutting efficiency. We are currently cloning the gRNA1 and gRNA10 pair as well as the gRNA2 and gRNA10 pair into the LentiCRISPRv2 construct to produce two dual gRNA and cas9 plasmids. These plasmids will be used to generate ARBS2-deleted cells.

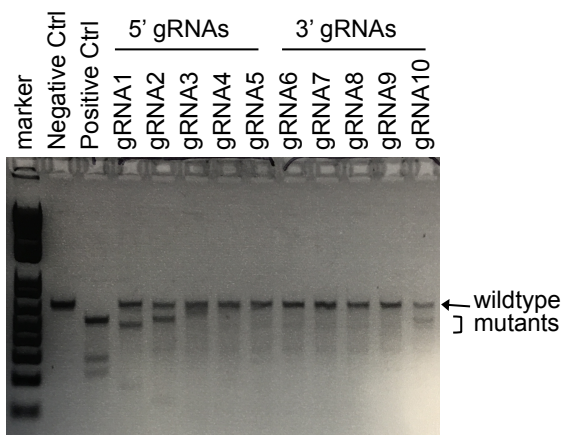


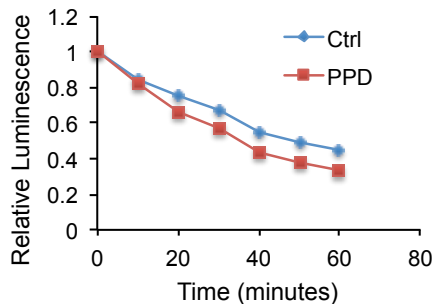
Figure 3. Cutting efficiencies of 10 gRNAs. Surveyor assay was conducted in LNCaP95 cells infected with lentivirus encoding the indicated gRNA.

Task 4-1: determine the impact of PPD on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization

The NanoLuc Binary Technology (NanoBiT) is a platform recently developed by Promega to assess protein-protein interactions [14]. It is a split luciferase system containing two complementary luciferase fragments of a small, engineered luciferase, NanoLuc. These



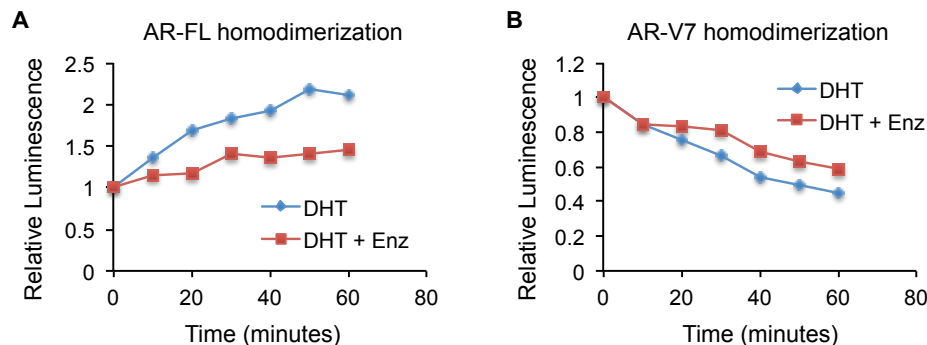
fragments exhibit extremely low luciferase activity separately, but regain activity when brought together through the interaction of their fused protein partners. This complementation response is rapid and reversible, thereby allowing quantitative measurement of protein interaction dynamics. In addition, because of the small size of NanoLuc, it provides minimal steric interference on the fused protein partners. Since NanoBiT depends on the relative orientation of the fusion proteins, we generated all possible combinations of N- and C-terminal fusions through cloning the AR-V7 or -FL cDNA either in front of or after the two complementary luciferase fragments, LgBiT and SmBiT. Different pairs of the fusion protein constructs were transfected into the AR-null M12 cells (to avoid confounding effect of endogenous AR), and the fusion constructs showing the highest NanoBiT signals were chosen for further analysis. Preliminary study showed that PPD might attenuate AR-V7 homodimerization (Figure 4). We are repeating the experiments to confirm the data and will also test the impact of PPD on AR-FL/AR-FL and AR-FL/AR-V dimerization.



**Figure 4. PPD inhibition of AR-V7 homodimerization.** The AR-V7-SmBiT and the AR-V7-LgBiT constructs were co-transfected into M12 cells. At 48 hours after transfection, the cells were trypsinized and seeded in a 96-well plate in equal density for incubation with either vehicle control or PPD, and the luminescence signal was measured continuously for up to 60 minutes.

Task 4-2: determine the impact of enzalutamide on AR-FL/AR-FL, AR-FL/AR-V, and AR-V/AR-V dimerization

Using the NanoBiT system generated above, we showed that enzalutamide inhibited androgen-induced AR-FL homodimerization but did not affect AR-V7 homodimerization (Figure 5). This is consistent of lack of ligand-binding domain of the AR-Vs. We are

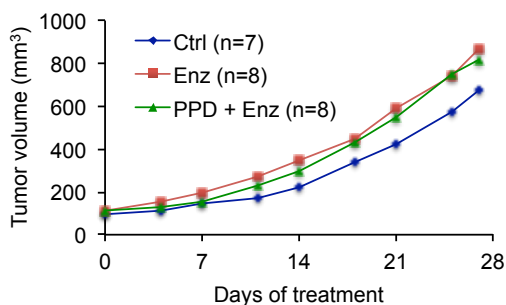


**Figure 5. Enzalutamide inhibition of androgen-induced AR-FL homodimerization but not AR-V7 homodimerization.** The AR-FL-SmBiT and the AR-FL-LgBiT constructs (A) or the AR-V7-SmBiT and the AR-V7-LgBiT constructs (B) were co-transfected into M12 cells. At 48 hours after transfection, the cells were trypsinized and seeded in a 96-well plate in equal density for incubation with either dihydrotestosterone (DHT) or DHT + enzalutamide (Enz), and the luminescence signal was measured continuously for up to 60 minutes.

repeating the experiments to confirm the data and will also test the impact of enzalutamide on AR-FL/AR-V dimerization.

Task 7-1: determine the potential of using PPD, in comparison with EPI-001, to improve the antitumor efficacies of abiraterone, enzalutamide, and cabazitaxel

We assessed whether combined therapy with PPD and enzalutamide is more efficacious than enzalutamide monotherapy in inhibiting tumor growth in 22Rv1 xenograft model. The 22Rv1 cells were inoculated into 6-8-week-old nude mice. When the tumors reach  $\sim 100 \text{ mm}^3$ , the mice were be randomized to receive vehicle, enzalutamide, or PPD + enzalutamide. PPD (40 mg/kg/day) and Enz (10 mg/kg/day) were given by oral gavage. As shown in Figure 6, combined treatment with PPD and enzalutamide was more efficacious than enzalutamide alone in inhibiting the growth of 22Rv1 tumors. We are doing molecular analyses with the tumors and will expand to other models.



**Figure 6. Combined treatment with PPD and enzalutamide (Enz) is more efficacious than enzalutamide monotherapy in inhibiting the growth of 22Rv1 xenograft tumors.**

**What opportunities for training and professional development has the project provided?**

Nothing to Report.

**How were the results disseminated to communities of interest?**

Nothing to Report.

**What do you plan to do during the next reporting period to accomplish the goals?**

During the next reporting period, we will work on completing Major Tasks 1 and 2, continue working on Major Tasks 4 and 7, and start to work on Major Tasks 3 and 8.

## **IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

The research develops a combination therapy regimen for treatment of mCRPC. Disease progression after abiraterone, enzalutamide, and cabazitaxel is all accompanied by persistent

AR signaling, which can be independent of androgen. Using PPD to downregulate the levels and activities of AR-Vs in addition to inhibiting androgen activation of AR by abiraterone, enzalutamide, or cabazitaxel is expected to be more efficacious than the latter alone in treating mCRPC. In addition, developing intervention strategies using an affordable natural product such as PPD will significantly reduce the economic burden associated with prostate cancer treatment.

**What was the impact on other disciplines?**

Nothing to Report.

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**

Nothing to Report.

**CHANGES/PROBLEMS:**

**Changes in approach and reasons for change:**

We used the CRISPR technology instead of the TALEN method to delete ARBS2 in the AR gene locus. This is because the CRISPR technology is much easier than the TALEN method and has become the most commonly used genome-editing tool. We also used the NanoBiT platform instead of the BRET assay to detect AR-FL and AR-V dimerization. This is because the NanoBiT platform has lower background and is more robust than the BRET system.

**Actual or anticipated problems or delays and actions or plans to resolve them:**

Our cells were contaminated with mycoplasma, and this slowed down our progress significantly. We have decontaminated our cell culture facility and obtained new cells and will repeat the experiments with these new cells.

**Changes that had a significant impact on expenditures:**

Nothing to Report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:**

Nothing to Report.

**PRODUCTS:**

**Publications, conference papers, and presentations:**

Yang Zhan, Guanyi Zhang, Xiaojie Wang, Yanfeng Qi, Shanshan Bai, Dongying Li, Tianfang Ma, Oliver Sartor, Erik K. Flemington, Haitao Zhang, Peng Lee, and **Yan Dong**. (2016) Interplay between cytoplasmic and nuclear androgen receptor splice variants mediate castration resistance. *Molecular Cancer Research*, 2016 Sep 26. doi: 10.1158/1541-7786.MCR-16-0236. [Epub ahead of print]. PMID: 27671337.

Subing Cao, Yang Zhan, and **Yan Dong**. (2016) Emerging data on androgen receptor splice variants in prostate cancer. *Endocrine-Related Cancer*, Invited review

**Website(s) or other Internet site(s):**

Nothing to Report.

**Technologies or techniques:**

Nothing to Report.

**Inventions, patent applications, and/or licenses:**

Nothing to Report.

**Other Products:**

Nothing to Report.

**PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

**What individuals have worked on the project?**

Name	Yan Dong	Tianfang Ma	Lianjin Jin
Project Role	PI	Technician	Postdoctoral Fellow
Researcher Identifier	N/A	N/A	N/A
Nearest Person month worked	1.2	0.6	0.6
Contribution to Project	Supervise Dr. Jin and Mr. Ma to conduct the experiments and prepare the results for progress report	Conducted experiments	Performed experiments
Funding Support	N/A		N/A

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

The PI has obtained the following supports:

Department of Defense, W81XWH-16-1-0317 (role: PI) 07/15/16 - 07/14/19

Level (%) of effort: 10%

Title: Contribution of the androgen receptor mRNA per se to castration resistance  
The major goal of this project is to test the hypothesis that the elevated androgen receptor mRNA levels in response to androgen-directed therapies promote castration-resistant progression of prostate cancer through a non-coding function of the androgen receptor mRNA.

Specific Aims:

- Aim 1: confirm the role of the AR 3'-UTR in mediating castration resistance.
- Aim 2: assess miRNA regulation by the AR 3'-UTR.
- Aim 3: elucidate the AR ceRNA regulatory network.
- Aim 4: elucidate the contribution of ceRNA regulation to AR-3'-UTR-mediated castration resistance.

Contracting Officer: Kathy E. Robinson  
US Army Medical Research Acquisition Activity  
Phone: 301-619- 8803  
Email: kathy.robinson@amedd.army.mil  
No overlap

**What other organizations were involved as partners?**

Nothing to Report.

**SPECIAL REPORTING REQUIREMENTS:**

none

**APPENDICES:**

None

**References:**

1. Cao, B., et al., *Androgen receptor splice variants activating the full-length receptor in mediating resistance to androgen-directed therapy*. *Oncotarget.*, 2014. **5**(6): p. 1646-1656.
2. Li, Y., et al., *Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines*. *Cancer Res.*, 2013. **73**(2): p. 483-489.

3. Mostaghel, E.A., et al., *Resistance to CYP17A1 Inhibition with Abiraterone in Castration-Resistant Prostate Cancer: Induction of Steroidogenesis and Androgen Receptor Splice Variants*. *Clinical Cancer Research*, 2011. **17**(18): p. 5913-5925.
4. Nadiminty, N., et al., *NF-kappaB2/p52 Induces Resistance to Enzalutamide in Prostate Cancer: Role of Androgen Receptor and Its Variants*. *Mol.Cancer Ther.*, 2013. **12**(8): p. 1629-1637.
5. Yamamoto, Y., et al., *Generation 2.5 antisense oligonucleotides targeting the androgen receptor and its splice variants suppress enzalutamide-resistant prostate cancer cell growth*. *Clin Cancer Res*, 2015. **21**(7): p. 1675-87.
6. Antonarakis, E.S., et al., *AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer*. *N.Engl.J Med.*, 2014. **371**(11): p. 1028-1038.
7. Efsthathiou, E., et al., *Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer*. *Eur Urol*, 2015. **67**(1): p. 53-60.
8. Martin, S.K., et al., *N-terminal targeting of androgen receptor variant enhances response of castration resistant prostate cancer to taxane chemotherapy*. *Molecular Oncology*, 2015. **9**(3): p. 628-639.
9. Thadani-Mulero, M., et al., *Androgen receptor splice variants determine taxane sensitivity in prostate cancer*. *Cancer Res.*, 2014. **74**(8): p. 2270-2282.
10. Zhang, G., et al., *Androgen receptor splice variants circumvent AR blockade by microtubule-targeting agents*. *Oncotarget*, 2015. **6**(27): p. 23358-71.
11. Cao, B., et al., *20(S)-protopanaxadiol-aglycone downregulation of the full-length and splice variants of androgen receptor*. *International Journal of Cancer*, 2013. **132**: p. 1277-1287.
12. Cao, B., et al., *20(S)-protopanaxadiol inhibition of progression and growth of castration-resistant prostate cancer*. *PLoS One*, 2014. **9**(11): p. e111201.
13. Cai, C., et al., *Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1*. *Cancer Cell*, 2011. **20**(4): p. 457-471.
14. Dixon, A.S., et al., *NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells*. *ACS Chem Biol*, 2016. **11**(2): p. 400-8.