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TITLE: Targeting the Aberrant Androgen Receptor in Advanced Treatment Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR: Stephen R Plymate

RECIPIENT: University of Washington
Seattle, WA 98104-2499

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**Purpose:** We hypothesize that: 

i) therapeutic agents that target AR-Vs as well as AR-FL will effectively disrupt the lethal mitotic phenotype; 

ii) the AR-V-driven transcriptome can provide biomarkers to identify patients at risk for progression and death from CRPC; 

iii) elucidation of mechanism(s) by which AR-Vs activate a lethal phenotype will direct the design of future therapies for CRPC, particularly peptidomimetics that target specific AR-Vs or key AR interacting cofactors.

**Scope:** 

**Aim 1.** Instigate clinical trials to assess whether two novel AR-NTD targeting agents (AT13387 or T6) can reverse resistance to abiraterone and/or MDV3100.

**Aim 2.** Evaluate if expression of specific AR-Vs or transcriptomes can serve as predictive and prognostic biomarkers for disease progression.

**Aim 3.** Identify the key effectors and regulators of signaling by AR-Vs in CRPC and assess their potential as biomarkers and therapeutic targets.

**Progress:** 


**Significance:** Unique signaling components of the AR-Vs have been identified as well as coregulators. Clinical trials with HSP-90 inhibitors are effective against AR-Vs toxicity of these agents needs to be further assessed.

**Subject Terms:** Nothing Listed

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### Targeting the Aberrant Androgen Receptor in Advanced Treatment Resistant Prostate Cancer

**E-Mail:** splymate@u.washington.edu

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**U.S. Army Medical Research and Materiel Command**

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Subject: Metastatic CRPC that arises following therapy with abiraterone or MDV3100 expresses AR-Vs, alone or in combination with AR-FL. We hypothesize that: i) therapeutic agents that target AR-Vs as well as AR-FL will effectively disrupt the lethal mitotic phenotype and thereby improve the prognosis of patients with advanced CRPC; ii) the transcriptome driven by AR-Vs can be employed to provide biomarkers that will identify patients at risk for progression and death from CRPC or serve as a prognostic biomarkers of the utility or response to third and new generation therapeutic agents. **Purpose:** elucidation of the mechanism(s) by which AR-Vs activate a lethal phenotype will direct the design of future therapies for CRPC, particularly development of peptidomimetics that target specific AR-Vs or key AR-V interacting proteins. **Scope:** Aim 1. Instigate clinical trials to assess whether two novel AR-NTD targeting agents (AT13387 and T6) can reverse resistance to the third generation endocrine therapies (abiraterone and MDV3100). **Aim 2.** Evaluate if expression of specific AR-Vs or correlate transcriptomes can serve as predictive and prognostic biomarkers for disease progression through various states of prostate cancer. **Aim 3.** Identify the effectors and regulators of signaling by AR-Vs in CRPC and assess their potential as novel AR-V enrichment biomarkers and therapeutic targets.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

   prostate, cancer, androgen receptor, splice variants, HSP 90 inhibitors, peptidomimetics, enzalutamide, androgen receptor co-regulators, transcriptome, chromatin-looping

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

   **What were the major goals of the project?**
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.
**Aim 1. Evaluate whether novel AR-targeting agents (resorcinol HSP90 inhibitor, AT13387; AR peptidomimetic, T6) that can affect both AR-FL and AR-Vs are active in abiraterone- and MDV3100-resistant CRPC.** We will study 1) archival tissue, 2) fresh tumor biopsies (whenever possible), and 3) circulating tumor cells and plasma from patients enrolled in clinical trials funded by external non-DOD sources to test for effective AR-FL and AR-V protein depletion and transcriptional blockade. A multi-center randomized Phase I/II trial (PI: de Bono) of the next-generation, resorcinol HSP90 inhibitor AT13387 activity in castrate patients with metastatic CRPC progressing on abiraterone acetate, either in combination with abiraterone acetate or as a single agent. The trial was supported by Astex Pharmaceuticals. This proposal independently conducted the planned corroborative biomarker studies in biopsies collected in the Phase I part. To determine effect of galeterone, a novel selective CYP17 lysase inhibitor and AR antagonist, on AR-FL and AR-Vs in chemotherapy-naive CRPC patients within a phase I/II trial. This trial was to be funded by Tokai Pharmaceuticals. This proposal was to independently study the association of response with several corroborative biomarker studies in tissue biopsies, and CTCs. To evaluate the effect of enzalutamide in combination with AZD5363 on AR-FL and AR-Vs in patients with chemotherapy-naive mCRPC within a Phase I/II trial. This is an investigator-initiated trial led by Johann de Bono (PI) of AZD5363, a potent and selective inhibitor of the kinase activity of AKT/PKB in combination with enzalutamide. This proposal was to independently study the association of response with several corroborative biomarker studies in tissue biopsies, and CTCs.

**Phase I/II trial of the T6 peptidomimetic in metastatic MDV3100-resistant or abiraterone-resistant CRPC patients.** Tissue acquisition will be covered by non-DOD funds, all biomarker and tissue analysis including circulating tumor cell analysis will be covered by this DOD proposal.

**Aim 2. Evaluate AR-FL, AR-V and AR-V-driven transcriptomes in various disease states of PCa and their association with treatment outcome and development of resistance.** We will evaluate AR-FL and AR-V expression and the AR-V-driven transcriptome in (1) Hormone-sensitive tissues from neoadjuvant studies of ADT, ADT + ketoconazole and dutasteride; ADT+IGF-IR inhibition; and MDV3100, and the new HSP90 inhibitor AUY922; (2) CRPC tissues from the warm autopsy program of 80 patients who died of metastatic CRPC (3) CRPC tissues from the a phase I/II clinical trial of MDV alone vs MDV3100 + AKT inhibitor; (4) MDV3100-resistant and abiraterone-resistant CRPC tissue from the clinical trials included in this proposal and described in aim 1 (HSP90 inhibitor+abiraterone; T6). This study will delineate both the frequency of expression of AR-FL and AR-Vs and the variant transcriptome in various stages of disease and establish the association of these factors with disease progression and resistance to treatment.

**Aim 3. Determine the effectors and regulators of aberrant AR splice variant signaling in CRPC to identify potential biomarkers and novel therapeutic targets.** We will first integrate expression profiling, ChIP-seq, and ChIA-PET in cell line models of CRPC to identify the effectors and key regulatory elements of AR-Vs and AR-FL. Concomitantly, using the same systems, we will characterize the co-regulator profiles of AR-Vs and AR-FL using a novel proteomics methodology, RIME. The findings will be validated in prostate cancer xenografts and tumor explants representing clinically relevant treatment groups to determine how aberrant AR signaling is altered in response to MDV3100, abiraterone, and novel AR-targeted strategies tested in aim 2. These studies are likely to yield two novel, clinically relevant outcomes: (1) new cellular and molecular indicators of aberrant AR signaling, lethal CRPC, and response to current and novel therapeutics, and (2) new therapeutic targets for CRPC. With respect to this latter outcome, we anticipate identifying new AR co-regulators that would provide the basis for the design and development of additional peptidomimetics, which represent a transformative class of compounds for targeting the AR.
Task 1. Development of organization and administrative structure

- Drop box set-up and scientific and administrative contacts identified
- Private (https://sharepoint.washington.edu/crpcvar-intranet/SitePages/Home.aspx) and Public (http://depts.washington.edu/crpcvar/) TIA websites have been established
- Schedule for monthly teleconferences established and have been conducted regularly since June 2014.
- Met with scientific advisory board members at the Endocrine Society Meeting, Chicago IL, 19 June 2014; will meet again at Prostate Cancer Foundation meeting, Carlsbad CA, 23 October 2014
- Met with members of the lay advisory board July 2014.

Task 2. Multicenter Phase I/II trial of the HSP90 resorcinol inhibitor, AT13387. Tissue, circulating tumour cells, and blood biomarker studies. [N.B. – Study funded by Astex Pharmaceutical for patient and drug costs – Johann de Bono (PI) - Phase 1 (Months 1-6), Phase 2 (Months 3-24)]

- Enrollment in this study is complete, one subject is still ongoing (August 2014). Forty-eight patients were enrolled into two-dose groups for each regimen. This proposal independently conducted the planned corroborative biomarker studies in biopsies collected in the Phase I part.
- Evaluation of AR and variants in tissue biopsies is ongoing

Task 3. Peptidomimetic Phase I/II trial. Acquisition of tissues, CTCs, and blood. The clinical portion of this study will be funded outside of this proposal — R. Montgomery (PI), University of Washington.

- Development of a clinically usable peptidomimetic is still underway.

Task 4. Evaluate AR, AR-Vs and AR-V transcriptome in various disease states of PCa and its association with treatment outcome and development of resistance. (CRPC specimens transcriptome and genomic alterations started, 10% complete as of 9/26/2014)

- Materials have been transferred from clinical studies to the different sites (Luo, Dehm, Plymate) for examination of AR variants

Task 5. To assess AR-FL and AR-Vs chromatin interactions and AR-Vs binding partners in relevant cell line models, both before and after treatment with MDV3100, T6, or AT13387

- Analyzed ChIP-seq data from the engineered CWR-R1 lines. These data have been incorporated into a paper that is currently under review at JCI.
- Have conducted RIME/RIME-SILAC for the engineered CWR-R1 lines. Additionally, we have completed AR RIME analysis of two of Amina Zoubeidi’s enzalutamide-resistant LNCaP lines (using an enz-sensitive LNCaP model as a control).
- Initial ChIP–seq for V7 demonstration run completed 9/26/2014

Task 6. Validate the role of novel genomic mechanisms underlying CRPC-associated AR signaling in prostate cancer xenografts and tissue explants (588 SCID mice)

- Tissue explants have been harvested and processing begun
- All animal protocols have IACUC and ACURO approval
- Analyses on in vivo data from castrate-resistant VCaP studies is nearing completion.
What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. Evaluate whether novel AR-targeting agents (resorcinol HSP90 inhibitor, AT13387; AR peptidomimetic, T6) that can affect both AR-FL and AR-Vs are active in abiraterone- and MDV3100-resistant CRPC.

SOW Task 2. Study 1: A multi-center randomized Phase I/II trial of the next-generation, resorcinol HSP90 inhibitor AT13387 in metastatic CRPC progressing on abiraterone

Enrollment in this study is complete, one subject is still ongoing (August 2014). Forty-eight patients were enrolled into two-dose groups for each regimen. This proposal independently conducted the planned corroborative biomarker studies in biopsies collected in the Phase I part.

Fresh tissue biopsies post-AT13387 treatment were collected from 17 patients. For seven patients matched fresh tumour biopsy collected before starting AT13387 treatment was available. For the remaining ten patients archival FFPE tissue was available for IHC analyses. One of the matched biopsies contained <50 cancer cells resulting in a total of 6 paired pre- and post-dose biopsies available for comparison.

Protein expression levels evaluated the impact of HSP90 inhibition on key client proteins implicated in prostate cancer, specifically AR and GR as well as the induction of HSP72 in paired fresh tumour tissue biopsies obtained before and after AT13387 treatment. Immunostaining was assessed using a quasi-continuous nuclear H score, created by multiplying each intensity level (0 for absent, 1 for weak stain, 2 for moderate, and 3 for intense stain) by the corresponding percentage of positive cancer cells, and then summing the results to obtain a maximum H-score of 300.
AR protein expression was evaluated using a mouse monoclonal antibody against the N-terminal domain (AR-NTD) (AR441, Dako; #M3562) which detected both AR-FL and AR splice variants; a rabbit monoclonal antibody directed against the C-terminus (AR-CTD) (EP670Y; Abcam; #ab52615), specific for AR-FL, was also utilised to specifically study wildtype AR.

AR-NTD was highly expressed in CRPC biopsies taken before starting AT13387 treatment both in the nucleus and in the cytoplasm. Comparison of paired biopsies obtained pre-dose (Screening) and post-dose (Cycle 1 Day 17) demonstrated a reduction in nuclear AR-NTD in 5/6 patients (Figure 1A) (Paired t-test p= 0.01). AR-CTD did not significantly change following AT13387 treatment (data not shown). Representative images are shown in Figure 2.

Changes in tumor cell proliferation following AT13387 treatment was evaluated by measuring the proliferation marker Ki67 in paired tumour tissue biopsies. Overall no significant changes were observed (Figure 1B). Ki67 results were recorded as the percentage of Ki67 positive cells detected in at least 100 cancer cells
Figure 2. Representative images of AR, GR and HSP72 IHC staining in paired biopsies taken before and after AT13387 administration.

Micrographs show AR-NTD, HSP72, Ki67, and GR expression by DAB immunohistochemistry method in (A) lymph node biopsy and (B) bone marrow trephine collected at screening and Cycle 1 Day 17.

This study was the first clinical trial of an HSP90 inhibitor in combination with abiraterone and prednisone. The MTDs reached for once-weekly (220 mg/m²) and twice-weekly (120 mg/m²) regimens. Fatigue and diarrhea were the commonest toxicities. Combination treatment resulted in increased toxicity compared to previous studies of AT13387 monotherapy; this was not predicted based on the toxicity profile of each agent alone; this is possibly explained by the different study patient populations evaluated. Seven (30.4%) and 4 (15.4%) patients discontinued treatment as a result of toxicity in Regimen 1 and Regimen 2, respectively. PK studies demonstrated AT13387 exposures increased in a dose-proportional manner and were similar to those observed in previous Phase 1 dose escalation study, suggesting no effect from abiraterone on AT13387 PK. Abiraterone exposures appeared to be lower when co-administered with AT13387 (Cycle 1-Day1/Day2) versus screening. No direct metabolic drug-drug interactions had been expected. The reason for this effect is unknown but could be due to reduced gastrointestinal transit time as a result of the diarrhea observed on days of dosing with AT13387.

PD studies in cancer tissue were limited by the number of patients who had paired pre- and post-dose biopsies. However, in the tumor tissue biopsies collected 24-48 hours post-dose a significant induction of HSP72 was noted; this was accompanied by only a modest depletion of AR and no change in tumor proliferation or apoptosis. In our study, we observed some depletion of AR protein as assessed by an antibody directed against the AR-NTD post-AT3387 treatment, however the presence of residual nuclear AR NTD coupled with lack of changes in PSA, ERG
expression and Ki67 suggested that the degree and/or duration of depletion was not enough to translate into effective blockade of AR signaling and reduction of proliferation. The lack of activity and modest pharmacodynamic changes did not justify the further evaluation of AT13387 in combination with abiraterone at the doses and schedules evaluated in this study. AR-V7 detection by IHC was not possible due to the poor performance of the Arv7 specific antibody available in IHC assay. Evaluation of AR-FL and AR-V7 mRNA levels by ddPCR is ongoing. Novel antibodies to AR-V7 and AR-V567 are now being generated to further test these tumor samples.

Results of the Phase I part of the trial have been presented at ESMO 2014 Congress, Madrid

Study 2: A randomized phase I/II trial of Enzalutamide in combination with AZD5363 (RE-AKT trial) in patients with mCRPC.

The study has received full Ethics approval (August 2014) and will open to recruitment at the Royal Marsden hospital in October 2014. The clinical portion of this study and biopsies will be funded outside of this proposal. Grant funding will support part of, but not all, the corroborative biomarker analyses including

2.a. Protein expression analysis (PTEN, AR, AR-Vs, PSA, UBE2C, ERG,UGTB17) on archival and fresh tumor biopsy tissue (baseline and on-treatment, circulating tumor cells (CTCs), and plasma DNA) (months 12-30)

2.b Evaluation of PTEN status on the impact of combined AR and AKT blockade on AR and AR-Vs protein expression in tumour biopsies and association with clinical response (months 24-30)

2.c. Analysis and publication of results. We expect that the phase II part of the trial may extend beyond the three-year limit of this proposal but funding for the trial is outside of the scope of this proposal.

The phase I run-in stage will enroll up to 18 patients and will evaluate the safety and tolerability of oral AZD5363 administered in combination with oral enzalutamide and characterize the pharmacokinetics and pharmacodynamics of AZD5363 and enzalutamide when administered in combination. The randomized phase II part will evaluate the anti-tumor activity of the combination versus enzalutamide and matching placebo. The target accrual for the randomized phase II is 100 patients. In addition the study include a single stage expansion cohort (n=20) of enzalutamide alone followed by the addition of AZD5363 upon progression to explore whether the addition of AZD5363 to enzalutamide can reverse resistance.

In collaboration with Dr Jun Luo from Hopkins we are now discussing the possibility of evaluating CTC AR splice variant mRNA expression in blood samples acquired from these patients prior to and after treatment (Antonorakis et al, 2014). These costly but critically important analyses are not covered by this grant but will be the subject of a separate grant call that is focused on the development of this research assay to a fully analytically validated assay as well as the interrogation of the 1000 blood samples taken from these patients.
SOW Task 3: Peptidomimetic Phase I/II trial. We established protocols for rapid cGMP production of our peptidomimetics. In collaboration with Janssen pharmaceuticals, we obtained several grams of cGMP grade T6. Our extensive studies with the cGMP grade T6 peptidomimetic revealed that while the T6 peptidomimetic had significant activity in vitro against prostate cancer cell lines and in vivo against prostate cancer xenografts, its pharmacological properties had to be improved prior to use in clinical trials. Specifically, T6 had poor solubility despite multiple formulations and was potent at a high concentration (micromolar concentrations). In partnership with Janssen pharmaceuticals, over 900 versions of T6 using a systematic medicinal chemistry approach were created to improve solubility, oral bioavailability and potency. We have tested these compounds and have found more potent versions with improved pharmacologic parameters. We are currently further refining these compounds to improve potency to nanomolar efficacy, while retaining solubility and minimizing off target activity. In addition, using biotinylated compounds, we were able to identify potential interacting partners of the WHTLF motif. These binding partners will be validated with knockdown experiments and will enable, with crystallization studies, the elucidation of the AR protein interface. With both the medicinal chemistry approach and the rational characterization of the AR interface we are targeting, we are confident that we will generate a new lead peptidomimetic derived from T6 using this approach and ready this lead for IND-enabling studies by year 3. These studies also enabled the generation of appropriate AR variant constructs with point mutations in specific regions to enable more rapid validation of additional compounds.

Aim 2. Evaluate AR-FL, AR-V and AR-V-driven transcriptomes in various disease states of PCa and their association with treatment outcome and development of resistance.

SOW Task 4. Dr. Jun Luo’s laboratory at the Johns Hopkins University initiated projects relevant to the collaborative studies proposed in this Transformative Impact Award project. On January 24th, 2014, we completed inter-institutional transfer of 50 metastatic prostate tumor samples
under a material transfer agreement between Johns Hopkins University and the University of Washington. The materials were fresh-frozen metastatic prostate tumor samples prepared by Dr. Colm Morrissey (co-investigator at the University of Washington). Dr. Luo has purified total RNA and DNA from all these samples. In addition, Dr. Luo acquired 15 tumor specimens from the laboratory of Dr. Johann de Bono, co-Investigator at Institute of Cancer Research UK. Using these samples, a project characterizing a novel androgen receptor mutation was carried out in a combined set of 100 metastatic prostate tumor samples. This novel AR mutation was named A236D and recently reported in a published study (Antonarakis et al, 2014). However, it was determined that this novel AR mutation was only detected in one patient sample collected at the Johns Hopkins University, and none of the 50 metastatic samples from UW and the 15 tumor specimens from ICR UK was positive for the mutation. Thus, A236D is characterized as rare AR mutation. Other studies investigating the full-length and variant AR expression levels are still ongoing.

Dr. Dehm has received tissues and genomic DNA from the Prime site, and has begun to conduct AR gene re-sequencing analysis, analyze data, and report findings to the study PI Dr. Plymate. The subcontract to the U of M site was not established until February 2014 (half-way through budget period 1), so we were unable to align our studies on this project with the parent-grant start date (Sept 2014). This resulted in a delay in hiring staff for the project.

We obtained an IRB for evaluation of clinical samples (STU 032014-034: Deciphering androgen receptor signaling in primary prostate tissue) for studies in primary prostate explants. We have tested these samples for AR-Vs expression and have ongoing studies with the Tilley and Dehm labs.

Dr. Plymate's lab has now surveyed TMAs of all LuCaP xenografts with AR-V7 monoclonal antibody; these xenografts demonstrated >90% positive staining with the AR-V7 monoclonal antibody Figure 3. Those that were not positive were confirmed AR-negative neuroendocrine tumors. QT-rtPCR of these same xenograft demonstrated that they all expressed AR-V7 message except for the neuroendocrine lines, Figure 4. In addition we have stained this TMA with our new ARv567es polyclonal antibody and compared the results to RNA-seq and qt-rtPCR of these lines. So far at least 5 different xenografts have shown expression of ARv567es and were also positive with the ARv567es antibody. DNA has been isolated from these xenografts to be sent to the Dehm lab for further examination of the AR

Figure 5. LuCaP xenografts in castrate mice that express ARv567es (∗)
gene. IHC has also been used to stain the TMAs of metastases from our rapid autopsy program. We are preparing DNA from the AR-V positive and negative metastases for PCR and genomicDNA to send to Dr. Dehm for ARgene evaluation. We have sent DNA from those that are positive as well as representative negative DNA samples to Dr. Dehm for interrogation of the AR gene structure.

**Aim 3. Determine the effectors and regulators of aberrant AR splice variant signaling in CRPC to identify potential biomarkers and novel therapeutic targets.**

**SOW Task 5.**

5.a. ChIP-seq and RNA-seq on VCaP and LNCaP APIP cells stably transfected with inducible lenti-virus expressing AR-Vs vector with triple flag tag (Months 1-18) (Plymate, Carroll and Tilley laboratories)
- We assisted Dr. Dehm in analyzing his ChIP-seq data from the engineered CWR-R1 lines. These data have been incorporated into a paper that is currently under review at JCI.

5.d. RIME-SILAC performed on cell lines with and without treatment to determine AR and AR-Vs binding partners (Months 1-15) (Carroll, Raj, Tilley and Plymate laboratories)
- We have conducted RIME/RIME-SILAC for the engineered CWR-R1 lines. Additionally, we have completed AR RIME analysis of two of Amina Zoubeidi’s enzalutamide-resistant LNCaP lines (using an enz-sensitive LNCaP model as a control).

1) Major activities: The primary activities of the Adelaide group in this reporting period have been conducting RIME experiments to identify AR and AR-V interacting proteins, and ChIP-seq to characterize AR/AR-V cistromes and signaling pathways.

2) Specific objectives: 1) To identify the key co-regulators and DNA regulatory elements of AR and ARVs in CRPC; 2) To identify the key effectors of AR/AR-V signaling in CRPC.

3) Significant results/outcomes: 1) RIME and RIME-SILAC have been used to identify the AR and ARV interactomes in the CWR-R1 model of CRPC. We are currently characterizing the function of novel interacting proteins in terms of their role in AR/AR-V signaling in CRPC. One such novel factor, GRHL2, appears to be a key co-regulator of AR/AR-V signaling in prostate cancer, and its role in disease progression is being assessed using a range of model systems. 2) ChIP-seq was used to identify the DNA regulatory elements involved in AR/AR-V signaling in the CWR-R1 model of CRPC. We are currently comparing and contrasting the AR/AR-V “cistromes” to identify DNA binding sites that play an important role in CRPC. 3) We have optimized conditions for the RNA-seq experiments, and plan to commence these in the next 4-6 weeks.

We have shown that one of the major genes that is up-regulated by the AR-Vs, UGT2B17, also serves as a co-activator of the AR in the absence of ligand and is also a co-activator of the AR-Vs. Further, although AR is required for UGT2B17 expression addition of ligand decreases UGT2B17. As shown in Figure 6, UGT2B17 enhances AR-V7 and AR\(^{v567es}\) activity using a probasin-luciferase ARE reporter construct in cells were no AR ligand is added. The cells shown are AR-negative M12 cells that have been stably transfected with either AR-V7 or AR\(^{v567es}\) (A.
and B.). In Figure C, we have used an siRNA against UGT2B17 to examine its effects on AR-V7 mRNA levels and see when UGT2B17 is decreased in LNCaP 95 cells AR variant is increased suggesting that in the absence of an AR enhancer additional AR-V7 in generated by the cell to maintain viability.

B UGT2B17 enhances AR-V7 and ARv567es activity using a probasin-luciferase ARE reporter construct in cells were no AR ligand is added. The cells shown are AR-negative M12 cells that have been stably transfected with either AR-V7 or ARv567es (A. and B.). In Figure C, we have used an siRNA against UGT2B17 to examine its effects on AR-V7 mRNA levels and see when UGT2B17 is decreased in LNCaP 95 cells AR variant is increased.
Aim 3b.2 Acquired AR-FL or AR-Vs after LBD Inhibition (VCaP) and MDV resistant VCaP cells.

SOW Task 6. We have initiated studies with the VCaPCR line. Our primary objective is to evaluate how differences in AR axis components may influence response to agents targeting these pathways, specifically AR variants and intratumoral androgens. The VCaPCR work has been compared to LuCaP35CR tumors, with both models treated with enzalutamide (MDV, C terminal inhibitor) or abiraterone (ligand synthesis inhibitor). Parallel studies with the N terminal inhibitor T6 and the combination of T6 and MDV will be initiated shortly. As shown in Figure 7 we observe several distinct patterns of response to AR signaling directed therapies, including primary refractory tumors, slow drifters with an intermediate response, and sensitive tumors with more prolonged response.

Figure 7. Tumor growth curves for VCaPCR (A-C) and LuCaP35CR (D-F) xenografts treated with vehicle (A,D), MDV3100 (B, E) or abiraterone (C,F). Curves have been color coded to highlight the distinct tumor growth patterns including no response (black curves in B, C, red curves in E, F), intermediate response (black curves in E,F) and robust tumor growth inhibition (blue curves).

Figure 8. Androgen levels in VCaPCR (A,T, B,DHT) and 35CR (C,T, D,DHT) tumors. Levels are color coded according to timepoint (pink d0, d21), or growth curve (red rapid, black intermediate, blue slow).
Analysis of tumor androgen levels from tumors taken up to day21 shows that levels of T and DHT were not as well suppressed in VCaPCR (Figure 8 A,B pink dots) vs LuCaP35 (Fig 8 C,D pink dots). Surprisingly, in both cases treatment with MDV also decreased tumor androgen levels compared to d0 values, although generally not as effectively as abiraterone. Androgen levels were generally suppressed in the recurrent tumors (although still remaining detectable) and did not correlate with the tumor growth patterns. The more robust suppression of tumor androgens at day 21 by MDV and abiraterone in LuCaP35 may account for the more pronounced therapeutic response in this tumor line.

To begin dissecting whether anticipated alterations in genes such as AR and constitutively active AR variants would be associated with responses to therapy, we profiled the full length AR, the common AR splice variant ARV7, as well as the glucocorticoid receptor (GR, NR3C1), which has recently been demonstrated to mediate resistant to enzalutamide. Figure 9 shows that VCAPCR demonstrates transient increases in AR-FL at d21 (Fig 9A), and increases in ARV7 and GR at d21 that are sustained at end of study (EOS) (Fig 9 C, E), suggesting each of these may be playing a role in resistance to MDV and ABI in this model.

LuCaP35CR shows a (slight) transient rise in AR-FL at d21, no increase in ARV7 at d21 or EOS, and a transient
increase in GR at d21. The transient increase in GR suggests this may be a bridging mechanism of resistance, whereas in VCaP where GR levels are even higher at EOS than d21 suggesting a persistent role. The AR results in 35CR contrast with a previous study of Abi in this model which did show an increase in AR and ARV7 in Abi treated tumors; however, comparison of cycle thresholds suggests basal levels of these transcripts were higher to begin with in these higher passage tumors (not shown). There was no association between these transcripts and tumor growth patterns.

These data demonstrate that growth patterns in response to Abi and MDV in our xenograft models (primary refractory, induced refractory and sensitive) parallel responses observed in human studies, suggesting mechanisms of resistance in pre-clinical studies will be relevant to human disease. However, we do not find obvious associations between growth patterns and either androgen levels or AR or GR expression. RNA seq analysis of the recurrent tumors is currently ongoing to more comprehensively identify potential drivers/predictors of resistance and sensitivity. Second generation MDV resistant versions of 35CR and VCaPCr have been generated and treatment studies in these lines will be initiated in the coming year.

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

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”*

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

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

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The Dame Roma Mitchell Cancer Research Laboratories at University of Adelaide has a Consumer Advisory Group (CAG), members of which possess diverse skills and backgrounds and have a strong interest in prostate cancer and other men’s health issues. Additionally, a number of the members are prostate cancer survivors. All members of the CAG have been kept abreast of the project activities by way of face to face meetings with the research team, regular
email updates and, in some cases, individual meetings / teleconferences. We encourage our CAG to provide input on research direction and how it could best achieve outcomes that would impact on stake-holders.

Dr. Selth has also presented results/outcomes from this project at public events (e.g. men’s health meeting at Millicent, South Australia; Adelaide Prostate Cancer Support Group).

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

(Aim 1, Task 3) We will conduct a randomized phase I/II trial of enzalutamide in combination with AZD5363 (RE-AKT trial) in patients with mCRPC.
The study has received full Ethics approval (August 2014) and will open to recruitment at the Royal Marsden hospital in October 2014. The clinical portion of this study and biopsies will be funded outside of this proposal. Grant funding will support part, but not all, of the corroborative biomarker analyses including

3.a. Protein expression analysis (PTEN, AR, AR-Vs, PSA, UBE2C, ERG, UGTB17) on archival and fresh tumor biopsy tissue (baseline and on-treatment, circulating tumor cells (CTCs), and plasma DNA) (months 12-30)
3.b Evaluation of PTEN status on the impact of combined AR and AKT blockade on AR and AR-Vs protein expression in tumour biopsies and association with clinical response (months 24-30)
3.c. Analysis and publication of results. We expect that the phase II part of the trial may extend beyond the three-year limit of this proposal but funding for the trial is outside of the scope of this proposal.

The phase I run-in stage will enroll up to 18 patients and will evaluate the safety and tolerability of oral AZD5363 administered in combination with oral enzalutamide and characterize the pharmacokinetics and pharmacodynamics of AZD5363 and enzalutamide when administered in combination. The randomized phase II part will evaluate the anti-tumor activity of the combination versus enzalutamide and matching placebo. The target accrual for the randomized phase II is 100 patients. In addition the study include a single stage expansion cohort (n=20) of enzalutamide alone followed by the addition of AZD5363 upon progression to explore whether the addition of AZD5363 to enzalutamide can reverse resistance.

(Aim 2, Task 4)
In collaboration with Dr. Jun Luo from Hopkins we are now discussing the possibility of evaluating CTC AR splice variant mRNA expression in blood samples acquired from these patients prior to and after treatment (Antonorakis et al, 2014). These costly but critically important analyses are not covered by this grant but will be the subject of a separate grant that is
focused on the development of this research assay to a fully analytically validated assay as well as the interrogation of the 1000 blood samples taken from these patients.

We have obtained the tissue microarrays from TAPs, and MDV-neoadjuvant studies, these will be stained with the antibodies to AR-V7 and AR\textsuperscript{v567es}. Samples that are positive for either of these variants will be confirmed by RT-PCR and genomic DNA sent to Dr. Dehm for analysis of the AR gene.

(Aim 3, Task 5) During the next reporting period we will continue to evolve the structure of the T6 peptidomimetic to increase solubility and should have it in the mouse studies described in Aim 3.b2.

In the next reporting period, we plan to continue analysis and validation of the RIME and ChIP-seq data to identify the co-regulators and DNA regulatory elements that are crucial for AR and AR-Vs signaling in CRPC. We also plan to commence and complete RNA-seq analysis of select AR and AR-Vs driven cell line models of CRPC to identify the key effectors of aberrant AR signaling in lethal forms of prostate cancer. Collectively, these experiments will also allow further assessment of the similarities and differences between AR and AR-Vs signaling. Another study that we will initiate in the next reporting period is to validate some of these key co-regulators, effectors and DNA regulatory elements in more clinically-relevant model systems, such as xenografts, explants, and patient samples.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We have shown that the narrow therapeutic index of the HSP90 inhibitor AT13387 prevents the administration of a high enough dose to sufficiently impact AR-Vs expression. These studies, which comprise part of the Pharmacological Audit Trail for this trial, indicate that targeting HSP90 is unlikely to generate therapeutic benefit for this disease.

We have demonstrated that the measurement of AR-V7 in circulating tumor cells may indicate whether or not a patient will respond to abiraterone or enzalutamide (MDV). These data have been published (Antonorakis et al, 2014). The impact of these data on the patient are that choices of therapy may be made that will significantly change patient cost and avoid time spent on treatments that have no effect and would delay progression to effective therapy.
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

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

1. The HSP90 trial was discontinued due to poor tolerability of the combination and lack of anti-tumor activity. We plan to evaluate an alternative therapeutic strategy combining enzalutamide plus AKT inhibition. Preclinical studies suggest that AKT inhibition inhibits key spliceosome factors including SRPK1, decreasing splicing. We will now evaluate this oral potent AKT1/2/3 to evaluate whether this impacts AR splice variant generation.

2. Our initial plan was to develop the T6 peptidomimetic for clinical trials. While the T6 peptidomimetic was biologically active, it lacked the ideal pharmacologic characteristics for translation to clinical trials. This conclusion was based on extensive testing of T6 in combination with Janssen Pharmaceuticals. We have since generated more than 900 versions of T6 and have improved its pharmacologic properties: however, at the time of this writing, we do not yet have a lead compound. We are applying further medicinal chemistry approaches to improving T6 and have made significant progress towards a more potent, soluble and non-toxic drug. We are pursuing multiple strategies to inform the further development and will continue to do so in the year ahead. We anticipate the generation of a lead in year 2. Thus, while we initially proposed a timeline of IND application in year 2, this will likely be postponed to year 3 of the study.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.
Initial delays in obtaining IACUC approvals at the different sites have been resolved. These approvals as well as ACURO approval have now been resolved.
Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

A novel, costly assay on CTC mRNA expression of AR-V7 has been developed. Due to the significant costs associated with this novel assay we are unable to cover these costs with this grant and alternative funding is being sought for those studies.

Initial delays in subaward inception led to delays in hiring and expenditures during the first year. In addition, there were delays in invoicing the University of Washington from some sites that gave the appearance that work was not being done at an appropriate pace. As is clear from this report, this was not the case and those delays were administrative and not functionally related to work accomplished. We have addressed the causes for these delays in expenditures and do not expect them to occur in the future. However, it should be recognized that the PI and co-PIs do not have direct control over each institution’s administrative components and thus some of these problems cannot be discovered until after they occur, but at this time we have so far been able to identify and rectify the problem.

Dr. Plymate’s UW salary total was $27,000 with 30% on this grant; his University salary this next year will be $100,000 and thus necessitates a minor change in overall budgeting.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects - none

Significant changes in use or care of vertebrate animals- none

Significant changes in use of biohazards and/or select agents - none

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

• Publications, conference papers, and presentations
  Report only the major publication(s) resulting from the work under this award.
**Journal publications.** List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

deBono, J. 776P - A Phase 1/2 study of AT13387, a heat shock protein 90 (Hsp90) inhibitor in combination with abiraterone acetate (AA) and prednisone (P) in patients (pts) with castration-resistant prostate cancer (mCRPC) no longer responding to AA. ESMO 2014 Congress. Conference presentation.


**Books or other non-periodical, one-time publications.** None

**Other publications, conference papers and presentations.** None
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Name: Roberta Ferraldeschi, MD
Project Role: Clinical Research Fellow
Nearest person month worked: 12

Contribution to Project: Clinical trials procedures, samples acquisition, CTC and tissue analyses overview, data analysis and interpretation

Name: Jonathan Welti
Project Role: HSO
Nearest person month worked: 12

Contribution to Project: Tissue and circulating biomarkers analyses for clinical studies, DNA and RNA extraction, IHC analyses, ddPCR analyses
Name: Daniel Nava Rodriguez  
Project Role: Clinical Research Fellow  
Nearest person month worked: 8  
Contribution to Project: Tissue and sections review, IHC scoring, tissue micro and macro dissection, DNA extraction  
Funding Support: Stand Up to Cancer grant

Name: Wayne Tilley, PhD  
Project Role: Site leader for Adelaide, co-PI  
Nearest person month worked: 6  
Contribution to Project: Prof Tilley oversees all research being conducted in the Dame Roma Mitchell Cancer Research Laboratories at the University of Adelaide, communicates research findings to the PI and other co-PIs and co-investigators, presents research findings to scientific and lay audiences, prepares research publications.  
Funding Support: In addition to funding from the DOD, Prof Tilley’s salary is provided by the University of Adelaide

Name: Luke Selth, PhD  
Project Role: Post-doctoral fellow  
Nearest person month worked: 6  
Contribution to Project: Dr. Selth oversees all research being conducted in the Dame Roma Mitchell Cancer Research Laboratories at the University of Adelaide, presents research findings to scientific and lay audiences and prepares research publications.  
Funding Support: In addition to funding from the DOD, Dr. Selth’s salary is partially supported by Cancer Australia/Prostate Cancer Foundation of Australia.

Name: Scott Townley  
Project Role: Research assistant  
Nearest person month worked: 6  
Contribution to Project: Mr. Townley is conducting ChIP-seq experiments to characterize the AR and ARV cistromes in CRPC. He also provides general research support to Drs Paltoglou and Coutinho.
<table>
<thead>
<tr>
<th>Name:</th>
<th>Steve Paltoglou, PhD</th>
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<tbody>
<tr>
<td>Project Role:</td>
<td>Post-doctoral scientist</td>
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<td>Nearest person month worked:</td>
<td>6</td>
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<tr>
<td>Contribution to Project:</td>
<td>Dr. Paltoglou is carrying out RIME experiments to identify new interacting partners of the AR and AR\textsuperscript{V567es}. He is also conducting functional analysis of candidate interactors to assess their importance in terms of regulating AR/ARV action in CRPC.</td>
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<tr>
<th>Name:</th>
<th>Isabel Coutinho, PhD</th>
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<tr>
<td>Project Role:</td>
<td>Post-doctoral scientist</td>
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<td>Nearest person month worked:</td>
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<tr>
<td>Contribution to Project:</td>
<td>Dr. Coutinho is analyzing and comparing the AR and AR-Vs transcriptomes using RNA-seq; assisting Dr. Paltoglou in analyzing the function of candidate AR/AR-Vs co-regulators; and developing a novel proteomic technique (multiple reaction monitoring) to measure AR and AR-Vs expression in prostate cancer models and clinical samples.</td>
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**Funding Support:**

Dr. Coutinho’s salary is supported by: 1) Cancer Australia/Prostate Cancer Foundation of Australia; and 2) Ray and Shirl Norman Cancer Research Trust

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<thead>
<tr>
<th>Name:</th>
<th>Scott M. Dehm, PhD</th>
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<tr>
<td>Project Role:</td>
<td>U of Minnesota Site project leader (Co-PI)</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>1</td>
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<tr>
<td>Contribution to Project:</td>
<td>Co-investigator, leader of studies conducted at U of Minnesota site.</td>
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<tr>
<th>Name:</th>
<th>Yingming Li, MD</th>
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<tr>
<td>Project Role:</td>
<td>Research Associate, Dehm Lab</td>
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<tr>
<td>Nearest person month worked:</td>
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<tr>
<td>Contribution to Project:</td>
<td>Conducts DNA studies</td>
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<thead>
<tr>
<th>Name:</th>
<th>Stephen Plymate, MD</th>
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<tr>
<td>Project Role:</td>
<td>PI</td>
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<tr>
<td>Nearest person month worked:</td>
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</tr>
<tr>
<td>Contribution to Project:</td>
<td>Leader of entire TIA</td>
</tr>
</tbody>
</table>
Name: Cynthia Sprenger, PhD  
Project Role: Project Scientific Coordinator  
Nearest person month worked: 9  
Contribution to Project: Oversees projects in Plymate Lab; coordinates exchange of information/results between labs

Name: Gang Liu, PhD  
Project Role: Research Scientist  
Nearest person month worked: 6  
Contribution to Project: IHC, ChIP, and PCR on variants (Plymate Lab)

Name: Kathryn Soriano-Epilepsia  
Project Role: Technician  
Nearest person month worked: 6  
Contribution to Project: Performs animal studies and assists with PCR (Plymate Lab)

Name: Colm Morrissey, PhD  
Project Role: Research Asst. Professor  
Nearest person month worked: 1  
Contribution to Project: Oversees TMAs

Name: Xiaotun Zhang, Ph.D  
Project Role: Acting Asst. Professor  
Nearest person month worked: 2  
Contribution to Project: IHC of TMAs

Name: Jun Luo, PhD  
Project Role: Site leader for Johns Hopkins, Co-PI  
Nearest person month worked: 2.4 calendar months  
Contribution to Project: Oversees projects at JHU

Name: Yan Chen, BS  
Project Role: Technician  
Nearest person month worked: 12 calendar months  
Contribution to Project: Performs experiments related to JHU project
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:
Location of Organization: (if foreign location list country)
Partner’s contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner’s facilities for project activities);
- Collaboration (e.g., partner’s staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and
- Other.

Organization Name: Janssen Pharmaceuticals
Location of Organization: Beerse, Belgium
Contribution to project: * Generation of GMP quality T6
* Generation of additional peptidomimetics
* Testing of additional peptidomimetics

8. SPECIAL REPORTING REQUIREMENTS.
nothing to report

9. Appendices
n/a