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TITLE: Biomarkers for Taxane Sensitivity and Hormonal Resistance in Patients with
Castration-Resistant Prostate Cancer

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14. ABSTRACT This is an annual report for a one year Hypothesis Development award initially accepted for funding 1/4/2013. Because of sequestration and delays in approvals between UW and DOD, the award was initiated 2/1/2014. The objective of this project was to utilize the presence of splice variant androgen receptor (AR) in circulating tumor cells (CTC) or disseminated tumor cells (DTC) to predict sensitivity to chemotherapy (docetaxel). Progress: Aims 1 and 2 are nearly complete. Aim 1, which was to confirm that prostate cancer cells spiked with AR splice variant expressing cells and transcriptome could be detected has been completed with the Adnagen CTC assay after initial failure of the Rarecyte assay to reliably isolated CTC. Aim 2 was to detect CTC and DTC from men with advanced prostate cancer. We have collected matched CTC, DTC and metastasis biopsies on 21 patients with metastatic prostate cancer. Please see the detailed report for information regarding the presence of new types of AR splice variants detected in metastasis biopsies. The final analysis of the types of splice variants in the biopsies and correlation with presence of the same splice variants in CTC and DTC is pending and is anticipated within six months.					
15. SUBJECT TERMS Androgen receptor, splice variant, circulating tumor cells, castration resistant prostate cancer					
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1. **INTRODUCTION:** The ability to define mechanisms of resistance to the new generation of hormonal therapies such as abiraterone and enzalutamide is critical to determining appropriate therapy for men with prostate cancer. One proposed mechanism is the development of androgen receptor splice variants (ARsv) which are autonomously active and predicted to make tumors resistant to hormonal therapy and sensitive to chemotherapy. This project proposes to isolate ARsv from circulating tumor cells (CTC) and disseminated tumor cells (DTC) from bone marrow and determine if presence or absence of ARsv predicted sensitivity to therapy.
2. **KEYWORDS:** Prostate cancer, androgen receptor, circulating tumor cells, disseminated tumor cells, enzalutamide, abiraterone, metastasis

3. **OVERALL PROJECT SUMMARY:**

Progress;

Aim 1 Determine whether AR variants and the associated mitotic transcriptome can be isolated from blood spiked with AR variant transfected LNCaP and blood samples from men with CRPC after treatment with abiraterone.

1a. Determine if splice variant AR and its transcriptome can be detected using CTC isolation. Peripheral blood is spiked with 0, 1, 5, 50, 100, 500 and 1000 LNCaP transfected with ARV567ES, or ARV7 per 3.5 ml of blood. Controls are untransfected LNCaP. Methods used are processing of samples by Rarecyte, collection of purified LNCaP and extraction for RNA to perform qRT-PCR for splice variants and variant transcriptome.

Results:

The Rarecyte assay originally proposed for this project did not reproducibly isolate CTC below 1000 cells/3.5 ml of blood. Transcripts isolated from successful isolations did not match between duplicates and this assay for isolation of CTC was abandoned. As an alternative approach, we utilized the AdnaTest (Adnagen) assay as modified by Antonarakis et al (1). This assay reliably isolated splice variant transcript as detected by QT-PCR from as low as 5 spiked LNCaP 95 cells overexpressing AR V7 splice variants in 5 ml of blood (spiking experiment utilized 0, 5, 10 and 50 cells in female whole blood). Control experiments utilizing LNCaP alone vs. ARsv expressing LNCaP 95 were used and the assay demonstrated the ability to detect overexpression of UBE2C in the variant transfectant cells compared to controls (data not shown).

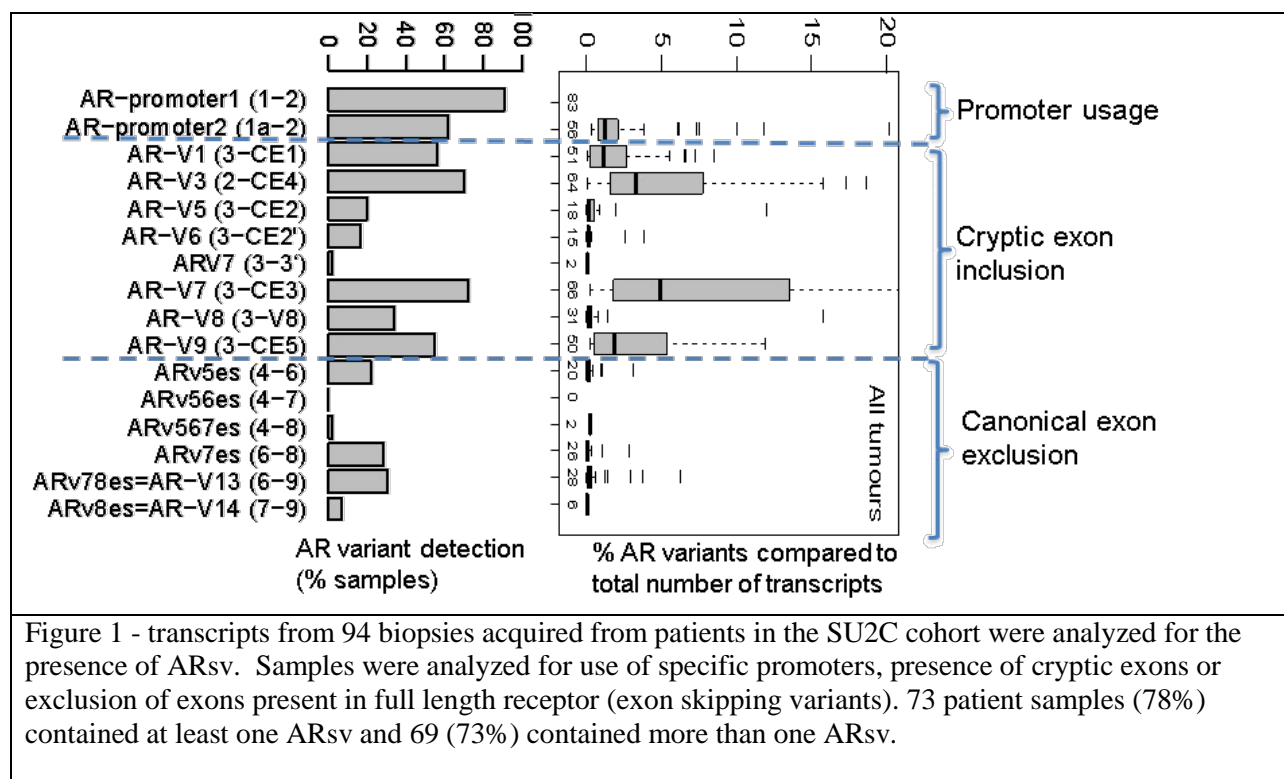
1b. Determine if CTC from abiraterone resistant prostate cancers contain splice variant AR and mitotic transcriptome.

Aim 2. Determine whether AR variants and the associated mitotic transcriptome can be isolated from DTC acquired from men with metastatic CRPC after abiraterone therapy.

Results: We have been a leading site in the SU2C/AACR/PCF “Dream team” CRPC biopsy effort

(https://www.standup2cancer.org/dream_teams/view/precision_therapy_for_advanced_prostate_cancer)

As a component of this project, the investigators on the current DOD proposal have interrogated the metastasis biopsy RNA seq data and have identified multiple AR splice variants which have previously not been detected in clinical biopsy specimens (see Figure 1). The manuscript including this data is in review at *Cell*.



As a component of another DOD proposal, Stephen Plymate has generated constructs of multiple previously uncharacterized ARsv from the above analysis and several are constitutively active. Those that are active are the previously described ARV7, AR V567es and two novel splice variants ARV5es and ARV56es, both of which are exon skipping variants. These constructs are being further interrogated for dimerization with each other and with wild type AR. It appears that there are multiple clinically relevant ARsv which have not been interrogated as to their significance in clinical specimens. As a result of this work we have collected CTC and DTC from twenty one patients undergoing SU2C metastasis biopsy at our site. All patients are beginning or finishing

therapy with abiraterone or enzalutamide. We are awaiting the biopsy RNA seq data for identifying the ARsv from individual metastasis. The CTC/DTC from these patients have been isolated by Adnatest with PCR of the isolated RNA pending. These samples will then be analyzed by primer sets already developed to identify the known ARsv (ARV7 and ARV567es) as well as the novel, constitutively active ARsv referenced above.

The DTC from marrow aspirate at the time of the biopsies are currently being analyzed by the Agilent assay for transcript expression. These results are anticipated in 3 months.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Modification of Adnatest to isolate AR splice variants, including multiple new ARsv from CTC
- Acquisition of paired metastasis biopsies, CTC and DTC from men with metastatic, resistant prostate cancer (CRPC)
- Identification of other potentially clinically relevant AR splice variants

5. CONCLUSION: The importance of the proposed research (when complete) will be to provide critical analysis of the presence of AR splice variants which have been shown to associate and potentially mediate resistance to AR targeted therapies. The ability to identify the known ARsv (ARV7 and ARV567es) and new novel ARsv (ARV5es and ARV56es) from both biopsy (which establishes expression in tumor) and a circulating biomarker (CTC or DTC) will be required before doing the analysis of the significance of the novel ARsv on sensitivity and resistance to AR targeting agents. The plan in the next six months is to acquire the RNA seq data (biopsies sent for sequencing) from the SU2C biopsies and associate this with ARsv transcripts from concurrently isolated CTC.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: Nothing to report

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES: Nothing to report

9. OTHER ACHIEVEMENTS: Nothing to report

10. REFERENCES:

Antonarakis ES, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med*. 2014 Sep 11;371(11):1028-38. doi: 10.1056/NEJMoa1315815. Epub 2014 Sep 3.

11. APPENDICES: None