

AWARD NUMBER: W81XWH-15-2-0050

TITLE: Noninvasive Detection of AR-FL/AR-V7 as a Predictive Biomarker for
Therapeutic Resistance in Men with Metastatic Castration-Resistant Prostate Cancer

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14. ABSTRACT Understanding primary and acquired resistance to abiraterone and enzalutamide, and developing analytically validated and clinically qualified predictive biomarkers, remains a critically important unmet medical need. We propose non-invasive detection of full-length androgen receptor (AR-FL) and the androgen receptor splice variant 7 (AR-V7) (AR-FL/AR-V7) as a predictive biomarker for therapeutic resistance in men with metastatic castration-resistant prostate cancer. Using a laboratory-developed, RNA-based assay modified from a commercially available circulating tumor cell (CTC) detection platform, we have developed standard operating procedures and performed extensive internal validation and quality control studies to determine its feasibility for detection of AR-FL/AR-V7 in blood samples. Although our recent studies show data supporting this predictive biomarker, analytical validation is required prior to clinical use, and a large-scale, multi-institutional study is needed to further establish clinical utility. The overall objective of the project is to enable precision therapy of metastatic castration-resistant prostate cancer by developing non-invasive tests for the AR-FL/AR-V7.					
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1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Understanding primary and acquired resistance to abiraterone and enzalutamide, and developing analytically validated and clinically qualified predictive biomarkers, remains a critically important unmet medical need. We propose non-invasive detection of full-length androgen receptor (AR-FL) and the androgen receptor splice variant 7 (AR-V7) (AR-FL/AR-V7) as a predictive biomarker for therapeutic resistance in men with metastatic castration-resistant prostate cancer. Using a laboratory-developed, RNA-based assay modified from a commercially available circulating tumor cell (CTC) detection platform, we have developed standard operating procedures and performed extensive internal validation and quality control studies to determine its feasibility for detection of AR-FL/AR-V7 in blood samples. Although our recent studies show data supporting this predictive biomarker, analytical validation is required prior to clinical use, and a large-scale, multi-institutional study is needed to further establish clinical utility. The overall objective of the project is to enable precision therapy of metastatic castration-resistant prostate cancer by developing non-invasive tests for the AR-FL/AR-V7.

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

Prostate cancer, CRPC, AR-V7, liquid biopsy, resistance, abiraterone, enzalutamide

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

Major Task 1: Development of robust and standardized SOPs pertaining to the accurate and reliable detection of AR-FL/AR-V7.

Subtask 1: To conduct essential study planning activities including IRB and HRPO approval, ordering of a common set of reagents, equipment readiness, protocol review, distribution of SOPs, personnel assignment, and review of documentation requirements (Months 1-6).

Subtask 2: Testing SOPs pertaining to the accurate and reliable detection of AR-FL/AR-V7.

ĩ Determine the lower and upper detection limit and detection dynamic range (Months 4-12).

ĩ Determine assay sensitivity, primer specificity.

ĩ Evaluate reproducibility among the test sites.

ĩ Standardize analytical SOPs.

Subtask 3: Development of robust SOPs for sample collection, processing, and transfer (Months 7-12).

ĩ Distribution of existing preanalytical SOPs developed by Dr. Luo.

ĩ Further optimization and standardization following comparison of two different SOPs.

Major Task 2: Correlation between CTC AR expression with contemporaneously acquired fresh CRPC biopsy expression, and with expression detected in cell-free exosome RNA.

Subtask 1: Correlation between CTC AR expression with contemporaneously acquired fresh CRPC biopsy expression. (Months 7-24).

Subtask 2: Correlation between CTC AR expression with expression detected in cell-free exosome RNA. (Months 7-24).

Major Task 3: Development of new CTC selection and molecular detection platforms

Subtask 1: Evaluation of new CTC selection platform for the purpose of detection of AR-FL/AR-V7 (Months 12-24).

Subtask 2: Evaluation of new molecular detection platforms (Months 12-24).

Major Task 4: Clinical validation of the AR-FL/AR-V7 test

Subtask 1: Prospective recruitment of 300 patients with mCRPC initiating standard-of-care treatment with abiraterone, enzalutamide, or chemotherapy consenting for blood draw (baseline, 2nd at the time of response if any, and 3rd time at the time of progression), and optional biopsy (~n=50) (Months 12-30)

Subtask 2: Biomarker implementation in certified labs (Months 12-30).

Subtask 3: Data analysis (Months 30-36).

Major Task 5: Biomarker-embedded trial of enzalutamide and AKT inhibitor

Subtask 1:

ı Recruit, consent, and enroll 140 patients/human subjects to Phase I/II trial.

ı Evaluation of the association between CTC counts, ARFL/ AR-V7 expression, and PTEN status, and all these parameters to response to treatment (Months 6-30).

Subtask 2: Collection and documentation of 20 pre and post-treatment biopsies from men enrolled in the trial for collaborative studies with Dr. Luo (Months 6-12).

Major Task 6: Alternative approaches

Subtask 1: Formulation of additional biomarker-driven clinical trials (Months 24-36).

Subtask 2: Additional studies according to FDA/EMA guidance (Months 24-36).

What was accomplished under these goals?

Task 1: We have completed this task. All regulatory documents are in place and all required collaborative agreements have been signed. We have distributed SOPs and compared the data across different institutions. The test has been analytically validated at Johns Hopkins University. We have recruited a total of 40 patients into the study. All relevant biomarker and baseline clinical data have been collected.

Task 2: On-going

Task 3: On-going

Task 4: On-going

Task 5: Samples are being obtained routinely from patients and are being processed to the cDNA stage. Once the SOP is optimized the samples will be analyzed for AR/ AR-V7 expression.

Task 6: Future work

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

The laboratory of Dr. Luo hosted a Scientific Officer from the Prof. de Bono group to train in the Adnatest to ensure good technical practice.

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?

We will continue to recruit patients into this study by coordinating with collaborating sites. In addition, we are currently evaluating novel detection methods using biopsies. An abstract has been submitted to GU ASCO 2017. We expect to complete Task 2 and 3 during year 2 of the project period, and we expect to recruit ~200 patients into this study by the end of year 2.

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?

Following analytical validation, we have realized patient benefit by making a clinical grade test available to patients at the Johns Hopkins University. A manuscript describing our experience in analytical validation of the test is currently in press.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

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

Nothing to Report.

What was the impact on society beyond science and technology?

We believe men with metastatic CRPC will benefit from the availability of the test. A separate study is ongoing to evaluate how the test results are utilized by providers and patients and whether the availability of the test resulted in better patient outcome.

- 5. CHANGES/PROBLEMS:** The PD/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. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to Report.

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.

Nothing to Report.

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.

Nothing to Report.

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

Nothing to Report.

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

Nothing to Report.

- 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.”

i 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).*

1. Lokhandwala P, Riel S, Haley L, Lu C, Chen Y, Silberstein J, Zhu Y, Zheng G, Lin M, Gocke C, Partin A, Antonarakis E, **Luo J**, and Eshleman J. Analytical Validation of AR-V7 Detection in a CLIA Laboratory Setting. The Journal of Molecular Diagnostics. 2016. In Press. (*Corresponding author).

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

i Website(s) or other Internet site(s)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

i Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

i Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

i Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Role	Percent Effort
Sokoll , Lori	Logistical and regulatory consult, Co-Investigator	5.00
Luo , Jun	Principle Investigator, overall management	30.00
Demarzo , Angelo	Tissue-based studies, Co-Investigator	3.99
Eshleman , James	CLIA lab activities, Co-Investigator	4.02
Paller , Channing	Oncology planning, Co-Investigator	3.67
Isaacs , William	Scientific guidance, Co-Investigator	7.83
Antonarakis , Emmanuel	Oncology lead, Co-Investigator	8.40
Wang , Hao	Statistician, Co-Investigator	15.00
Lu , Changxue	quality control, protocol development	50.00
Zhu, Yezi	technological development	60
Taylor, Maritza	protocol coordination, lab management	100

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

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

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

- 9. APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.