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TITLE: “Inhibition of the Androgen Receptor Amino-Terminal Domain by a Small Molecule as Treatment for Castrate-Resistant Prostate Cancer”

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Inhibition of the Androgen Receptor Amino-Terminal Domain by a Small Molecule as Treatment for Castrate-Resistant Prostate Cancer

Purpose: The hypothesis of this study is that EPI-001 that targets the AR NTD will inhibit AR-driven recurrence of prostate cancer resistant to current methods of androgen deprivation or blockade.

Scope: Aim 1 will determine the impact of EPI-001 on castration sensitive tumor regression and re-growth in LuCaP xenografts and on growth of their castration resistant forms. Aim 2 will examine the impact of EPI-001 on castration sensitive and castration resistant growth of tumors with differing tumor androgen levels and differing ratios of ARv567es to full-length AR. Aim 3 will elucidate the specific molecular mechanisms by which EPI-001 inhibits the activity of full-length AR and truncated ARv567es variants using in vitro models.

Progress: This is the final report on this award. The SOW has been completed and the EPI compound had transitioned to Phase 1 clinical trial.

Findings: We have clearly shown that EPI-001 and -002 can suppress the growth of AR-variant driven prostate cancers. We have also shown that Intratumoral androgens play a major role in determining response to N-terminal inhibition. We have shown in this reporting year that EPI combined with MDV is additive on suppressing the growth of castrate-resistant xenografts. Further, we show that the expression of stimulatory co-regulators of the AR do not blunt the activity of EPI on suppression of AR activity.

Significance: Based on these studies to this point EPI has entered Phase 1 clinical trials.
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1. **INTRODUCTION:**

Data has been published from our group as well as others that the AR- splice variants are at least markers of resistance to enzalutamide and abiraterone when found in circulating tumor cells and are harbingers of a more lethal disease. Thus the need to develop and understand the mechanisms of action of EPI compoundson the constitutively active AR splice variants is urgent. Furthermore, in this years report we will show new information on the mechanism of action of EPI compounds and how they effect AR-V interaction with chromatin. Finally, we will show the significance of newly developed specific AR-V antibodies and how they will be used to further dissect the mechanisms of action of the EPI compounds.

2. **KEYWORDS:**

Prostate Cancer, Castration Resistant, Androgen Receptor, EPI-001, N-terminus, therapy

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?**

**Objective/Hypothesis:** The hypothesis of this study is that EPI-001 that targets the AR NTD will inhibit AR-driven recurrence of prostate cancer resistant to current methods of androgen deprivation or blockade.

**Specific Aims**

**Aim 1** will determine the impact of EPI-001 on castration sensitive tumor regression and re-growth in LuCap xenografts and on growth of their castration resistant forms. **Aim 2** will examine the impact of EPI-001 on castration sensitive and castration resistant growth of tumors with differing tumor androgen levels and differing ratios of AR\(^{v567es}\) to full-length AR. **Aim 3** will elucidate the specific molecular mechanisms by which EPI-001 and its derivatives inhibits the activity of full-length AR and truncated AR\(^{v567es}\) variants using in vitro models.
During the previous reporting period, we addressed aims 1 and 2. Aim 2 was completed in the previous reporting period. In this reporting period we have completed aim 1. Specifically, we have shown in human xenografts that are resistant to abiraterone and enzalutamide, that the addition of the EPI-derivative, EPI-002 (which is the active isomer of EPI-001), that the addition of EPI-002 markedly enhanced or reversed the resistance seen with abiraterone and/or enzalutamide, figure. Note that both N and C-terminus blocking agent was required to inhibit the tumor growth. Although EPI-002 alone was effective in some cases tumors it was not effective in all; however, the addition of enzalutamide markedly enhanced its effectiveness. Western blots of representative tumors from each cohort demonstrated that AR-V7 protein expression was elevated over controls with the addition of enzalutamide and markedly elevated with the addition of EPI-002 and enzalutamide. Thus providing proof that EPI-002 is effective at inhibiting the resistant effects of constitutively active AR-variants. Thus completing the goal of aim 2. In this last year of the study we have completed aim 3. In a paper which has been submitted to Clinical Cancer Research we have shown that the over-expression of SRC-1,-2, or-3, or p300, known co-regulators of AR that are increased in CRPC, do not effect the binding of EPI-002 to the AF1 activating domain of the androgen receptor nor its ability to suppress AR reporter activity. Additionally, we have shown that the length of the CAG repeat in exon 1 does not effect EPI-002 binding or activity. Finally, we have shown that that several gain of function mutations in the AR do not overcome the ability of EPI-002 to suppress AR activity.

This is the report of the final year’s work under this proposal and accomplishes the goals set out at the beginning of this project. Importantly, we have shown that the EPI-based compounds are effective in vitro and in vivo in preclinical studies. Based on this data, a version of EPI-002 has been approved for phase 1 clinical trials and the first castrate resistant patient was entered on trial in December 2015.
What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Results of this year’s work were presented at our Pacific Northwest Prostate SPORE symposium. This talk is presented in Seattle and telecast to Oregon Health Sciences University and University of British Columbia. These meetings are attended by scientists as well as patient advocates.

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

Nothing to report

4. IMPACT:

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

The impact of this study set standards for development on N-terminal androgen receptor inhibitors. Furthermore, it demonstrated the pathway to development of drugs targeting the androgen receptor in castration resistant prostate cancer.
What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

The results of this DOD grant led to the generation of ESSA Pharma in order to take the EPI class of compounds forward into clinical trials
What was the impact on society beyond science and technology?

Nothing to report

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

Final report – nothing to report
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

N/A

Significant changes in use or care of human subjects

Significant changes in use of biohazards and/or select agents

Nothing to report
6. PRODUCTS:

• Publications, conference papers, and presentations
  Report only the major publication(s) resulting from the work under this award.

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report
Other publications, conference papers and presentations.

Nothing to report

• **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
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”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567

Nothing to report
<table>
<thead>
<tr>
<th>Name:</th>
<th>Lisha Brown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>Research Technician</td>
</tr>
<tr>
<td>Researcher Identifier (e.g. ORCID ID):</td>
<td>1</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
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</tr>
<tr>
<td>Contribution to Project:</td>
<td>Ms. Brown performed laser-capture microdissection and RNA preparation from prostate tumors.</td>
</tr>
<tr>
<td>Funding Support:</td>
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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? 
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.
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](https://ers.amedd.army.mil) for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on [https://www.usamraa.army.mil](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.