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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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14. ABSTRACT 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: Tasks 1 and 3: We have completed the EPI-001 treatment in 5 xenograft lines in the first year of this study. These were done following castration and in castrate resistant growth states. Tasks 4 and 5: We have begun to measure intratumoral androgens and begin IHC analysis of these tumors. A distinct AR variant transcriptome has been identified and reported. Findings: We have clearly shown that EPI-001 can suppress the growth of AR-variant driven prostate cancers. We have also shown that tumors with both AR-full length and variant receptors may respond to both N- and C-terminal agents. Significance: EPI-001 is effective at inhibiting castration resistant prostate cancer driven by AR-variants in vivo. This class of compounds should be considered for clinical trial.					
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Introduction: We and others have shown that the emergence of resistance to current methods of androgen receptor blockade including MDV-3100 (enzalutamide) and abiraterone is associated with an increase in androgen receptor (AR) splice variants that are constitutively active and cannot respond to these agents. The **purpose** of this proposal was to determine if an agent that acts on the N-terminus of the AR which would include the splice variants can suppress the growth of these resistant tumors. Currently, the only N-terminal agent that has been reported is EPI-001. In this study we will evaluate the effects of EPI-001 on a series of human prostate cancer xenografts with variable levels of intracrine androgens and AR splice variants.

Body: Task 1. (Aim 1.A) Determine effects of EPI-001 on human prostate xenografts in intact animals compared to castration. Year 1-2

Task 3. Determine the impact of EPI-001 on castration resistant growth of tumors that have differing intratumoral levels of androgens and differing ratios of AR^{v567es} to full-length AR. Year 1-2

Task 4. Histology of xenografts (Years 1-3)

Task 5. Intratumoral androgens (Years 1-3)

Work accomplished in year one on Tasks 1, 3, 4, and 5

We have completed the studies on LuCaP 86.2 and 136 xenografts. LuCap 35v, 96ai, and 23.1 xenografts have also been completed and growth curves and molecular analysis are in process. The results of these studies are presented in graphic form in figure 1. As can be seen EPI-001 performed significantly better than castration alone or MDV3100 in this LuCap 86.2, a variant driven xenograft, $p < 0.05$ EPI vs castration or MDV3100.

Intratumoral steroid levels remained low in this xenograft even with EPI-001 treatment. Since the original publication of this xenograft it has been shown that there is an intragenic rearrangement of the AR gene with a decrease in exons 5, 6, and 7 and potentially the xenograft generates AR^{v567es} via a genomic mechanism rather than non-genomic. The results of LuCaP 136 are interesting as shown in figure 1 in that the xenograft responded to both EPI-001 and MDV3100, $p < 0.05$ EPI and MDV3100 vs castration. At first glance this appeared to be a dichotomous response but as we have shown (1). LuCaP 136 can express both AR-FL and AR^{v567es} depending on the intracrine androgen status of the tumor. Furthermore, we have shown that LuCaP 136 can alter its androgen profile following castration to either produce testosterone (T) as its intracrine androgen or dihydrotestosterone (DHT). When DHT is produced the tumor responds to MDV3100 but when DHT is shut down we see that it responds to EPI-001. We are currently examining the gene expression responses to therapy in this line.

In figure 2, we see that treatment with EPI-001 in LuCaP 86.2 tumors decreases the nuclear expression of AR and as shown in figure 1 decreases tumor growth over a 5 week period. This finding was somewhat unexpected because we had not seen abnormalities in nuclear AR localization in our in vitro studies. However, these findings are similar to other N-terminal AR inhibitors that we have studied.

In figure 3 we demonstrated the staining for canonical AR and neuroendocrine proteins in LuCaP 86.2 xenograft tumors.

Additional data that has been published supported in part by this proposal includes a survey of primary prostate cancer and metastases from UW tissue microarrays (2). Because at least 25 c-terminal AR splice variants have been described but antibodies are available for only one of these, we used C- and N-terminal specific AR antibodies to define the incidence of AR variants in primary and metastatic disease. We used the concept that nuclear N-terminal AR antibody staining in the absence of C-terminal would be indicative of a variant. This was born out in the ratio of N-C

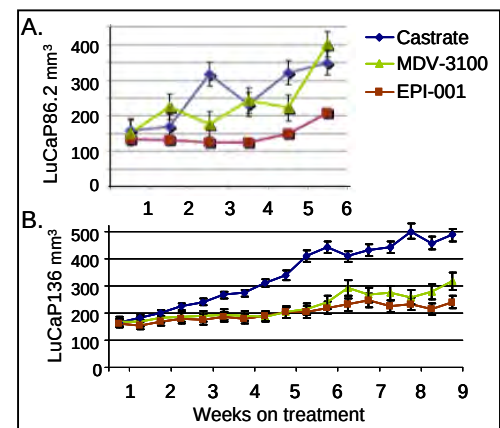


Figure 1. Response of LuCaP 86.2 and LuCaP 136 xenografts in SCID mice treated with either EPI-001 or MDV-3100 per gavage 5 out of 7 days.

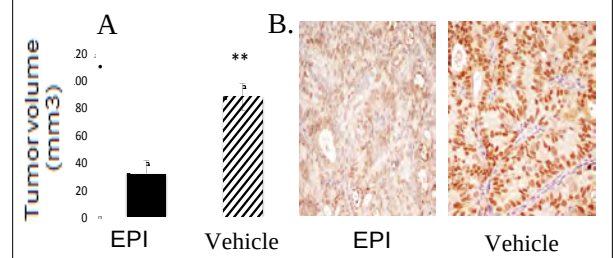


Figure 2. (A) Mean tumor volume of LuCaP86.2 tumors treated with EPI or DMSO daily for 6 weeks. (B) N' AR staining in EPI-treated tumors compared to vehicle.

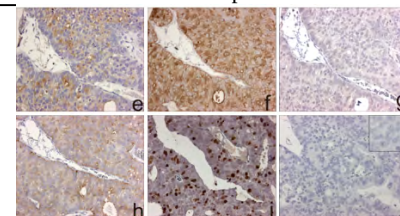


Figure 3. Immunohistochemical staining for (e) PSA (f) PSMA (g) chromogranin a (h) synaptophysin (i) Ki67 (j) negative control.

terminal staining of 1:1 in primary tumors but dropped significantly in metastatic lesion indicating that 35-40 per cent of the metastatic lesions. We further confirmed that the AR variant was active in these lesions by showing an elevation of the unique AR variant expression profile. Finally, during year one of this proposal we were also able to show that the AR variants have a gene expression profile that is unique and distinct from the canonical AR gene expression profile (3).

Key Research Accomplishments:

- **Tasks 1 and 3.** We have completed the EPI-001 treatment in 5 xenograft lines in the first year of this study. These were done following castration and in castrate resistant growth states.
- **Tasks 4 and 5.** We have begun to measure intratumoral androgens and begin IHC analysis of these tumors. A distinct AR variant transcriptome has been identified and reported.

Reportable Outcomes:

Sun, S Mosteghal, E Sprenger, C Nelson, P Bluemn, E Luo, J and Plymate S. Loss of the androgen receptor(AR) ligand-binding domain transforms the AR transcriptome in prostate cancer. AACR Advances in Prostate Cancer Research abst B11. Feb 2012

Sadar, M Myung, J Anderson, R McEwan, I Plymate, S et al . Developing small-molecule inhibitors to the androgen receptor N-terminus domain for the treatment of advanced prostate cancer. AACR Advances in Prostate Cancer Research abst B14. Feb 2012

Based on these outcomes so far we have applied for two additional sources of funding:

1. DOD – Transformative award
2. Pacific Northwest NIH Prostate SPORE Renewal – Project 5

Conclusions: After the first year of funding we have clearly shown that EPI-001 can suppress the growth of AR-variant driven prostate cancers. We have also shown that tumors with both AR-full length and variant receptors may respond to both N- and C-terminal agents. The role that intracrine androgens may play in this response needs to be determined. In order to accomplish this we will add LnCaP and VCaP prostate cancer cell lines with an inducible AR^{v567es} construct to our next years experiments starting with in vitro studies. These results so far have been sufficient to lead to endeavors to initiate a phase 1 clinical trial of EPI-based compounds in castrate resistant prostate cancer

References:

1. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *The Journal of Clinical Investigation*. 2010;120(8):2715-30. Epub 2010/07/21.
2. Zhang X, Morrissey C, Sun S, Ketchandji M, Nelson PS, True LD, et al. Androgen receptor variants occur frequently in castration resistant prostate cancer metastases. *PloS one*. 2011;6(11):e27970. Epub 2011/11/25.
3. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer research*. 2012;72(14):3457-62. Epub 2012/06/20.