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TITLE: Targeting the HSP40/HSP70 Molecular Chaperone Axis as a Novel Treatment Strategy for Castrate-Resistant Prostate Cancer

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CONTRACTING ORGANIZATION: The Geneva Foundation, Tacoma, WA

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14. ABSTRACT

Technical Abstract: Heat shock proteins Hsp40, Hsp70 and Hsp90 are molecular chaperones required for stabilization/activation of nuclear receptors, including full-length androgen receptor (AR) and glucocorticoid receptor (GR). Although ligandbinding domain (LBD) targeted (LBDT) therapy initially inhibits AR function and improves patient survival, this treatment almost invariably leads to emergence of castration-resistant prostate cancer (CRPC). CRPC is frequently characterized by elevated expression of alternative nuclear receptors able to at least partially maintain the AR transcriptional program. These alternative receptors include GR, which is expressed in approximately 30% of LBDT therapy-sensitive prostate cancer, but is expressed at a much higher frequency in CRPC and in those patients with a poor response to LBDT therapy. Additionally, elevated expression of a number of constitutively active AR splice variants lacking the LBD (ARv, particularly ARv7, which correlates with poor prognosis, reduced survival, and resistance to LBDT therapy, and ARv567es) is a frequent occurrence in CRPC. While full length GR and AR depend on the Hsp40/Hsp70/Hsp90 chaperone axis for activity, the chaperone requirements of ARv are not known. Because Hsp90 interacts with the LBD, ARv are insensitive to Hsp90 inhibitors. However, based on strong preliminary data, we believe that ARv, like GR and AR, retain dependence on Hsp40/Hsp70 and we will test this hypothesis using combined biophysical, genetic, biochemical and pharmacological approaches, including novel small molecules able to bind and inhibit both Hsp40 and Hsp70. We envision a synergistic group of studies that will result in a detailed and comprehensive picture of the specific chaperone dependence of these individual nuclear receptors. Together with in vivo xenograft data and with ex vivo evaluation of patient tumor biopsy tissue, we expect to obtain proof-of-principle confirmation that inhibition of Hsp40 and Hsp70 represents a novel strategy to target the continued nuclear receptor dependence of CRPC. Using in vitro and in vivo models, we will test whether this targeting strategy also can abrogate or delay onset of resistance in LBDT therapy-naïve patients.

Impact: The proposed research program is responsive to the goals and mission of the Depart of Defense Prostate Cancer Research Program (PCRP). Our proposal addresses the PCRP Overarching Challenge of developing effective treatments and mechanisms of resistance for men with high-risk or metastatic prostate cancer. Our proposal addresses the FY15 PCRP Focus Areas of (1) Mechanisms of Resistance and Response: Understanding primary and acquired resistance as well as exceptional response to therapy, and (2) Therapy: Identification of targets and pathways (Hsp40/Hsp70) and optimization (including sequencing and combination therapies of chaperone inhibitors) of therapeutic modalities, including metastatic prostate cancer.

15. SUBJECT TERMS
Cancer, Prostate Cancer, Oncology

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1. INTRODUCTION: Heat shock proteins Hsp40, Hsp70 and Hsp90 are molecular chaperones required for stabilization/activation of nuclear receptors, including full-length androgen receptor (AR) and glucocorticoid receptor (GR). Although ligand-binding domain (LBD) targeted (LBDT) therapy initially inhibits AR function and improves patient survival, this treatment almost invariably leads to emergence of castration-resistant prostate cancer (CRPC). CRPC is frequently characterized by elevated expression of alternative nuclear receptors able to at least partially maintain the AR transcriptional program. These alternative receptors include GR, which is expressed in approximately 30% of LBDT therapy-sensitive prostate cancer, but is expressed at a much higher frequency in CRPC and in those patients with a poor response to LBDT therapy. Additionally, elevated expression of a number of constitutively active AR splice variants lacking the LBD (ARv, particularly ARv7, which correlates with poor prognosis, reduced survival, and resistance to LBDT therapy, and ARv567es) is a frequent occurrence in CRPC. While full length GR and AR depend on the Hsp40/Hsp70/Hsp90 chaperone axis for activity, the chaperone requirements of ARv are not known. Because Hsp90 interacts with the LBD, ARv are insensitive to Hsp90 inhibitors. However, based on strong preliminary data, we believe that ARv, like GR and AR, retain dependence on Hsp40/Hsp70 and we will test this hypothesis using combined biophysical, genetic, biochemical and pharmacological approaches, including novel small molecules able to bind and inhibit both Hsp40 and Hsp70.

2. KEYWORDS: heat shock proteins, chaperones, protein-protein interactions, androgen receptor, androgen receptor splice variant, glucocorticoid receptor, proteostasis, protein, chaperone inhibitors, folding, protein turnover, degradation.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Understand how Hsp40 and Hsp70 interact with GR, AR and ARv. We will perform nuclear receptor binding assays in CRPC cell lysates using tagged nuclear receptors and tagged chaperones to identify which partners interact in cells. These studies will be coupled with a suite of biophysical, biochemical and genetic approaches, which we will use to map the individual receptor-chaperone protein-protein interactions and to quantify their affinities. Finally, we will determine which of the closely related Hsp40 and Hsp70 paralogs are most important for nuclear receptor stability/function using newly described and well-validated shRNA libraries.

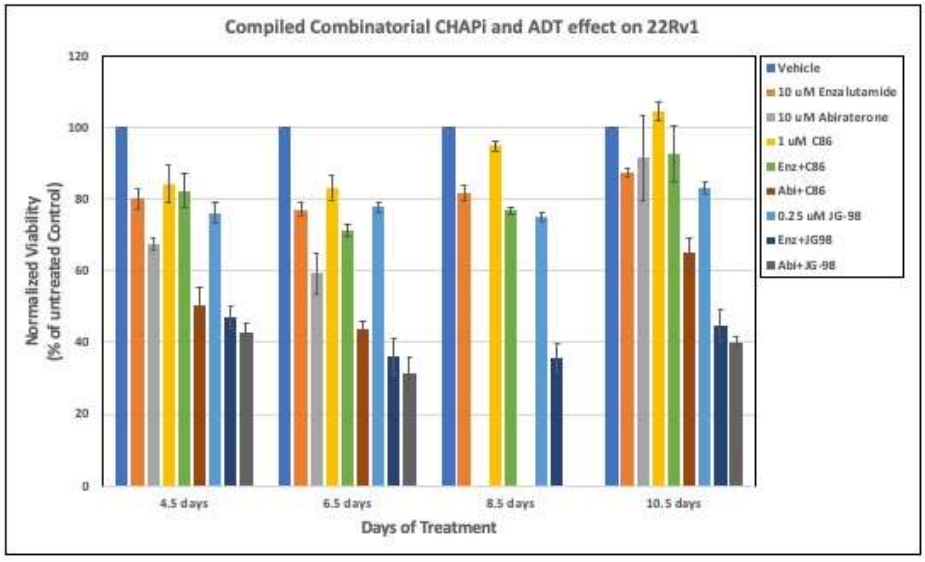
Specific Aim 2: Examine the sensitivity of ARv, GR and AR to pharmacologic inhibition of Hsp40 and/or Hsp70. We will assess the impact of chaperone inhibitors on AR, ARv and GR expression and activity in CRPC cells *in vitro* and on CRPC xenograft growth *in vivo*. We will also determine whether these inhibitors can prevent emergence of resistance to LBD-targeted therapy *in vitro* and *in vivo*. These pharmacologic studies will complement and extend the biochemical and biophysical studies of the first Aim. CRPC xenograft growth *in vivo* with select compounds was to be completed in months 1-12. We have validated the in vivo single agent activity of both JG231 and C86, as well as their combinatorial activity.

Specific Aim 3: Establish proof-of-principle efficacy data using short-term *ex vivo* cultures of freshly obtained PCa tissue to monitor Hsp40 and Hsp70 inhibitor effects on nuclear receptor expression and activity.

- This aim proved not to be feasible due to the poor structural consistency of fresh prostate cancer tissue obtained from prostatectomies. Because of this, long-term (e.g., several days) ex vivo cultures were not feasible.

What was accomplished under these goals? The goals of SA1 were accomplished and data are included in a *Cancer Research* publication (Moses et al, 2018), provided and discussed in the 2018—2019 Annual Report.

The goals of SA2 were partially accomplished during the 2018-2019 year. We validated the in vitro and in vivo dependence of CRPC cells and xenografts on Hsp40 and Hsp70 interaction with full-length GR, AR and AR splice variants, as discussed in the 2018-2019 Annual Report and as published in *Cancer Research* (Moses et al., 2018). However, due to technical difficulties that we had not fully appreciated, we were not able to complete the goal of determining whether Hsp40 and/or Hsp70 inhibitors could prevent or delay emergence of resistance to LBD-targeting therapy (including androgen synthesis inhibitors and androgen binding inhibitors). Instead, we re-focused this goal to determine whether inhibitors of one or both of these chaperones may be synergistic with standard of care inhibitors of androgen synthesis and/or inhibitors of androgen binding to AR. We have obtained preliminary in vitro data (see below) that demonstrate the possibility that synergy, or at least combinatorial activity, can be obtained in a CRPC cell model by varying the concentrations of enzalutamide (androgen binding antagonist), abiraterone (androgen synthesis inhibitor), JG-98 (Hsp70 inhibitor) and C86 (Hsp40 inhibitor).



Likewise, the main goal of SA3, to establish ex vivo cultures of fresh prostate cancer tissue to evaluate drug effects, was not accomplished due to the unexpectedly poor structural consistency of fresh prostate cancer tissue obtained from prostatectomies, which severely impacted the viability of long-term ex vivo cultures.

The current plan is to further explore the possibilities raised by our preliminary findings for SA2, and to expand these studies both in vitro and in vivo. The emergence of the Covid-19 pandemic caused the closure of NIH labs in March, 2020. Labs only partially re-opened in July, 2020 (with less than full staff). Animal facilities did not reopen until September, 2020. Thus, the remaining in vitro and in vivo experiments planned will take place between February, 2021 and the end of September, 2021 thanks to the No Cost Extension Status of this Award, which was aapproved several weeks ago.

What opportunities for training and professional development has the project provided?
Frank Echtenkamp joined the team as a senior postdoctoral fellow and received additional training in translational research. Genesis Rivera-Marquez is currently receiving similar training, as well as intense molecular biology training.

How were the results disseminated to communities of interest? Via invited virtual lectures.

What do you plan to do during the next reporting period to accomplish the goals?
We will determine whether inhibitors of Hsp40 and/or Hsp70 can work synergistically with standard of care inhibitors of androgen synthesis and androgen binding to AR. These experiments will be performed in vitro and in vivo using CRPC cell lines and xenografts as described in SA2. When warranted, additional findings of interest will be communicated to the scientific community in lectures and/or publications. Hopefully, the continued presence of Covid-19 will not interfere with these experiments.

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?
The molecular chaperones Hsp40 and Hsp70 were shown to interact with the NTD (N-terminal domain) of glucocorticoid receptors (GR) and androgen receptors (AR) and thus were shown to interact with both full-length GR and AR, and with AR splice variants lacking the ligand binding domain. Likewise, both full-length GR, AR and AR splice variants were shown to be destabilized at the protein level by inhibitors of Hsp40 and Hsp70. In vitro transcriptional activity of full-length GR, AR and AR splice variants was shown to be inhibited. Inhibitors of these chaperones, both singly and combined, displayed in vivo anti-tumor activity in prostate cancer xenografts resistant to standard of care therapy targeting both androgen synthesis and androgen binding to AR.

What was the impact on other disciplines? Since the NTD of nuclear receptors is disordered to varying degrees, it is likely that not just GR and AR, but other nuclear receptors also interact via their NTD with Hsp40 and Hsp70 to prevent aggregation and improve stability. It is likely that other nuclear receptors in addition to GR and AR depend on these interactions for function. It is likely that other nuclear receptor-driven cancers in addition to prostate cancer, (e.g., estrogen receptor-driven breast cancer) may also be impacted by inhibitors of Hsp40 and Hsp70.

What was the impact on technology transfer? A collaborator on this project, Jane Trepel, was granted a patent on the Hsp40 inhibitor she identified and which is used in this project, for treating prostate cancer (US Patent 10,071,945). The partnering PI, Jason Gestwicki, was granted a patent on Hsp70 inhibitors used in this project (US Patent 9,808,448).

What was the impact on society beyond science and technology? Nothing to report

5. CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:*

Changes in approach and reasons for change
As stated above, due to technical difficulties that we had not fully appreciated, we were not able to complete the goal of determining whether Hsp40 and/or Hsp70 inhibitors could prevent or delay emergence of resistance to LBD-targeting therapy (including androgen synthesis inhibitors and androgen binding inhibitors). Instead, we have re-focused this goal to determine whether inhibitors of one or both of these chaperones may be synergistic (in vitro and/or in vivo) with standard of care inhibitors of androgen synthesis and/or inhibitors of androgen binding to AR. Unfortunately, these experiments have been delayed due to emergence of the Covid-19 pandemic. We will make every reasonable effort to complete them during the remainder of FY21 (with remaining funds).

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report other than what is described above.

Changes that had a significant impact on expenditures
NIH labs were shut down in March 2020 due to emergence of the Covid-19 pandemic. Labs were only partially reopened in July 2020, but animal facilities were not fully reopened until September 2020. Due to these changes, a significant amount of planned expenditures to purchase animals, lab supplies, etc. were not made. We plan to make these expenditures during the remainder of FY21.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. Examples of products include:

Publications, conference papers, and presentations
Nothing to report
Journal publications.
Nothing to report

Other publications, conference papers, and presentations. Nothing to report

Inventions, patent applications, and/or licenses: See page 7.

Other Products: Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Provide the following information on participants:
What individuals have worked on the project?

1) PI
Name: Len Neckers, PhD
Project Role: PI
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 1
Contribution to Project: no change
Funding Support: N/A

2) Key personnel
Name: Frank Echtenkamp PhD
Project Role: Postdoctoral fellow
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 5
Contribution to Project: all cell-based *in vitro* work
Funding Support: N/A

Name: Genesis Rivera-Marquez, MS
Project Role: Postbac student

Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 5
Contribution to Project: cell-based *in vitro* work
Funding Support: N/A

Name: Jane Trepel
Project Role: Collaborator
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 1
Contribution to Project: C86 identification, planned qPCR assays to detect AR and ARv in prostate cancer tissues
Funding Support: N/A

Name: Peter Pinto, MD
Project Role: Collaborator/Prostate surgeon
Researcher Identifier (e.g., ORCID ID):
Nearest person month worked: 0.5
Contribution to Project: provide human prostate tissue
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
There has been no change

What other organizations were involved as partners?
UCSF – Jason Gestwicki, partnering PI

8. SPECIAL REPORTING REQUIREMENTS:
This is a collaborative grant. The collaborative PI is Dr. Jason Gestwicki (UCSF).

9. APPENDICES:
Nothing to report