AWARD NUMBER:	W81XWH-16-1-0562
TITLE:	"Targeting the HSP40/HSP70 Molecular Chaperone Axis as a Novel Treatment Strategy for a Castrate-Resistant Prostate Cancer"
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CONTRACTING ORGANIZATION:	The Geneva Foundation, Tacoma, WA
REPORT DATE:	October 2021
TYPE OF REPORT:	Annual Report
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual Report	30Sep2020-29Sep2021

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

5a. CONTRACT NUMBER
W81XWH-16-1-0562

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*

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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 Aims:

1. Using a combination of purified proteins, CRPC cells and cell lysates, we will identify the unique Hsp40/Hsp70 interactions with GR, AR and Arv and we will explore their functions(s).
2. Using CRPC cells, we will examine sensitivity of GR, AR & Arv to pharmacologic inhibition of the Hsp40/Hsp70 axis in vitro and resultant effects on xenograft growth in vivo.
3. We will test short-term ex vivo culture of freshly obtained prostate cancer tissue provides confirmatory evidence that inhibiting Hsp40/Hsp70 modulates nuclear receptor expression/activity.

What was accomplished under these goals? Most of 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. In the upcoming cycle (NCE), we will use a validated chaperone shRNA library, obtained from our collaborator Jason Gestwicki, to determine which of the closely related Hsp40 and Hsp70 paralogs are most important for nuclear receptor stability/function.

The goals of SA2 were partially accomplished during the 2018-2019 cycle. 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 previous 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. In the previous cycle, we obtained preliminary in vitro data 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). We have confirmed and validated those preliminary data in the current report.

As previously reported, he 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. In the current cycle (a final NCE was granted several weeks ago) we plan to perform a series of in vivo xenograft studies to confirm the in vitro findings described in SA2. The continuing disruption in performing animal studies, and difficulty in recruiting necessary personnel, as a consequence of the continuing impact of COVID-19, did not allow us to generate the in vivo data that we had planned to accomplish in the previous cycle. Within the last 6 weeks, necessary personnel have joined the lab, in vivo animal studies are able to be performed at nearly normal capacity, and we hope to generate animal data during the just approved final NCE cycle.

What opportunities for training and professional development did the project provide?
Frank Echtenkamp joined the team as a senior postdoctoral fellow and received additional training in translational research. Dr. Echtenkamp is now the lead postdoctoral fellow on this study, and he has recently been approved for promotion to Staff Scientist based on his accomplishments. Genesis Rivera-Marquez is currently a Geneva Foundation collaborator who continues work on the project while developing further expertise in molecular and cellular biology.

How were the results disseminated to communities of interest? Via invited virtual seminars (e.g., lecture as part of the Proteostasis Consortium Virtual Seminar Series in July, 2021; lecture to be delivered at the CSSI XIth International Online Symposium on Heat Shock Proteins in Biology and Medicine, October 27-29, 2021).

What do you plan to do during the next reporting period to accomplish the goals and objectives?
As discussed above, during this NCE we plan to complete the in vivo CRPC xenograft studies described in SA2. Also, using a validated shRNA chaperone library, recently made available to us, we will determine which of the closely related Hsp40 and Hsp70 paralogs are most important for nuclear receptor stability/function.

4. IMPACT:

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 constitutively active AR splice variants retaining the NTD but 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. Thus, we demonstrated that chaperone inhibitor targeting of nuclear receptor NTD may represent a novel treatment strategy for CRPC.

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 demonstrate functional dependence on these interactions. 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?

Nothing to report during the current reporting period.

What was the impact on society beyond science and technology?

Nothing to report during the current reporting period.

5. CHANGES/PROBLEMS:

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. Although in vitro experiments are consistent with this hypothesis, as discussed above in vivo experiments have been further delayed due to continued impact of the Covid-19 pandemic at NIH. We will make every reasonable effort to complete these in vivo studies during the remainder of FY21 and FY22 (based on a recently approved NCE).

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

Due to the ongoing impact of COVID-19 on NIH activities and on disruption of the reagent and equipment supply chain, a significant amount of planned expenditures to purchase animals, lab

supplies, etc. were not made during the previous cycle. We plan to make these expenditures during the remainder of FY21-FY22 cycle.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects: Nothing to report.

Significant changes in use or care of vertebrate animals: Nothing to report (other than a delay in using vertebrate animals, as discussed above).

Significant changes in use of biohazards and/or select agents: Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations:
Lecture presented as part of the Proteostasis Consortium Virtual Seminar Series in July, 2021; lecture to be delivered at the CSSI XIth International Online Symposium on Heat Shock Proteins in Biology and Medicine, October 27-29, 2021.

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.

Other Products:
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Len Neckers, PhD
Project Role:	No change
Researcher Identifier (e.g. ORCID ID):	0000-0001-9639-7249
Nearest person month worked:	.1 months
Contribution to Project:	No change
Funding Support:	No change

Name:	Jane Trepel
Project Role:	No change
Researcher Identifier (e.g. ORCID ID):	0000-0002-7681-6554
Nearest person month worked:	.05 months
Contribution to Project:	No change
Funding Support:	No change

Name:	Peter Pinto
Project Role:	No change
Researcher Identifier (e.g. ORCID ID):	0000-0002-0190-5931
Nearest person month worked:	.05 months
Contribution to Project:	No change
Funding Support:	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
Nothing to report (however, UCSF – Jason Gestwicki, partnering PI)

8. SPECIAL REPORTING REQUIREMENTS:

This is a collaborative grant. The partnering PI is Dr. Jason Gestwicki (UCSF). However, Dr. Gestwicki has completed his work on this award and has not requested an NCE.

9. APPENDICES:

Nothing to report.