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TITLE: Mechanisms of Resistance to Androgen Deprivation Therapy in Advanced Castration-Resistant Prostate Cancer (CRPC)

PRINCIPAL INVESTIGATOR: Joshua W. Russo

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall hypothesis is that expression of the dipeptidase DPP4 is downregulated in prostate cancer (PCa) as a mechanism of resistance to androgen deprivation therapy (ADT). My overall objective is to demonstrate that DPP4 downregulation is a mechanism of ADT-resistance and PCa progression and to identify the specific pro-survival growth factor/cytokine targeted by DPP4 for degradation and its associated signaling cascade. Aim 1 will assess the effect of DPP4 downregulation and overexpression on the sensitivity of PCa xenografts to castration. Aim 2 will identify the pro-survival growth factor/cytokine targeted by DPP4 for degradation and the downstream signaling cascades effected. Aim 3 will extend the significance of DPP4 downregulation into primary PCa and CRPC clinical specimens and assess the interaction of DPP4 inhibition with ADT.

15. SUBJECT TERMS

DPP4, CD26, prostate cancer, castration-resistance, androgen deprivation therapy, growth factor, cytokine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

When men develop prostate cancer (PCa) that spreads or metastasizes to other parts of their body, the first and second line treatments used by doctors attempt to block the effects of the androgen hormones testosterone and dihydrotestosterone (DHT) on prostate cancer cells. This type of therapy is called androgen deprivation therapy or ADT because it deprives the prostate cancer cells of these important androgens. Testosterone and DHT bind to the androgen receptor (AR) within cancer cells and stimulate the growth and progression of prostate cancer. Gonadotropin-releasing hormone agonists (GnRH-agonists), abiraterone, and enzalutamide are three drugs commonly used to block the pro-cancer hormone signaling that occurs through testosterone and DHT. GnRH agonists inhibit the testicular production of androgen hormones. Abiraterone inhibits an enzyme called CYP17A1, which decreases the levels of testosterone and DHT. Enzalutamide blocks the ability of testosterone and DHT to bind to androgen receptor (AR) and stimulate prostate cancer cells. These drugs are initially effective at stopping prostate cancer progression, but in nearly all men the cancer eventually becomes resistant. The subject of this research proposal is determining how downregulation of the gene *DPP4* and its protein product mediates prostate cancer resistance to ADT and how DPP4 inhibitors used to treat Type II diabetes influence prostate cancer progression. Over the past 2 years I have shown that DPP4 downregulation is tightly associated with PCa progression in preclinical models and in clinical biopsy materials. In the VCaP xenograft model and in the majority of clinical cases, as PCa becomes resistant to ADT, AR signaling is restored. DPP4 is similar to PSA in that it is an AR-stimulated gene. However, in the resistant setting, while PSA expression is restored, DPP4 expression is not. This suggests that the continued downregulation of DPP4 might have functional significance in PCa survival, especially since DPP4 is known to degrade various pro-survival growth factors and cytokines. Of greater significance, I have also shown that inhibitors of DPP4 enzyme activity decrease the effectiveness of ADT. My overall hypothesis is that DPP4 expression is downregulated in PCa progression in order to increase local concentrations of prosurvival growth factors/cytokines to overcome androgen deprivation. As it will be difficult to identify therapies capable of increasing DPP4 protein expression within PCa cells, over the past year of pandemic-abbreviated work, I have developed cell lines that have doxycycline-inducible overexpression and knockdown of DPP4 protein. The next important step in this work will be to use these cell lines in xenograft models to identify the pro-survival growth factor that is degraded by DPP4 and the kinase signaling cascade the growth factor activates to promote ADT resistance. This will allow us to target the growth factor and its associated receptor/kinase cascade directly to block ADT resistance.

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

DPP4, CD26, prostate cancer, castration-resistance, androgen deprivation therapy, growth factor, cytokine

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?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Goal 1: Assess the functional significance of DPP4 downregulation in PCa xenograft setting.
Milestone: Production of stable cell lines capable of inducible knockdown and overexpression of DPP4 (Month 4) – 100% Complete
Milestone: Establishment of VCaP xenografts, PC-BID-1 and LuCaP-35 PDXs, and VCaP xenografts with stable inducible nonsense and DPP4 shRNA and stable inducible overexpression of DDP4 (Month 10-11) – 90% Complete
Milestone: Assess the functional significance of DPP4 downregulation in the PCa xenograft setting (Month 15) – 50% Complete

Goal 2: Determine the signaling cascades effected by DPP4 downregulation/inhibition and the corresponding growth factors/cytokines targeted by DPP4 that are responsible for ADT resistance.
Milestone: Identification of signaling cascades effected by DPP4 downregulation/inhibition (Month 20) – 25% Complete

Goal 3: Determine the clinical significance of DPP4 expression and concurrent ADT/DPP4 inhibitor treatment on PCa progression. (Month 20) – 50% Complete

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. **Major Activities:** The major focus of the past year has been the establishment of VCaP xenograft tumors that have doxycycline-inducible overexpression of either an shRNA against DPP4 or a cDNA for DPP4. Once established these xenografts will be used to conduct experiments to assess the effects of DPP4 knockdown and overexpression on the efficacy of castration. In addition they will also be used to determine which kinase signaling cascades are most clearly effected by the alteration of DPP4 expression in the xenograft setting using mass-spec phosphoproteome analysis of VCaP xenografts following overexpression of DPP4 in the castrate setting and knockdown of DPP4 in the intact setting. The dox-inducible VCaP cell lines stably expressing the shRNA and cDNA against DPP4 have been successfully established. The dox-inducible VCaP-shDPP4 cells have been tested in xenografts for their response to doxycycline and the construct has been found to effectively knockdown DPP4 protein expression. Twenty of these VCaP-shDPP4 xenografts were started in order to test the effect of DPP4 knockdown on castration-sensitivity. However, the xenografts matured in early April 2020 during the initial stages of the coronavirus shutdown at our hospital and all the mice had to be euthanized by the beginning of May due to tumor size. The coronavirus shutdown has also delayed the use of the dox-inducible VCaP-DPP4 overexpressing cell line in the xenograft setting.

2. **Specific Objectives:** My overall objective is to demonstrate the functional significance of DPP4 downregulation in mediating resistance to ADT in CRPC and identifying the mechanism by which this resistance occurs. My specific objectives include demonstrating the functional significance of DPP4 downregulation in preclinical models, identifying the specific growth factor/cytokine and associated signaling cascade that is upregulated in response to DPP4 downregulation, and correlating DPP4 expression/inhibition with PCa progression.

3. Significant Results and Key Outcomes:

VCaP cell lines with doxycycline-inducible DPP4 shRNA and cDNA have been established and placed into the xenograft setting. A major goal of the first year of this award was the establishment of VCaP cell lines with doxycycline-inducible knockdown (shRNA) or overexpression (cDNA) of DPP4. Given my hypothesis that DPP4 is targeting specific prosurvival cytokines/growth factors for degradation, I reasoned that the best place to see alterations in the levels of these cytokines/growth factors and their signaling cascades was in the VCaP xenograft setting where I had already demonstrated that DPP4 activity levels have a significant effect. I have now established these VCaP cell lines and shown when they are induced, they specifically decrease or cause overexpression of DPP4 protein levels **(Figure 1)**.

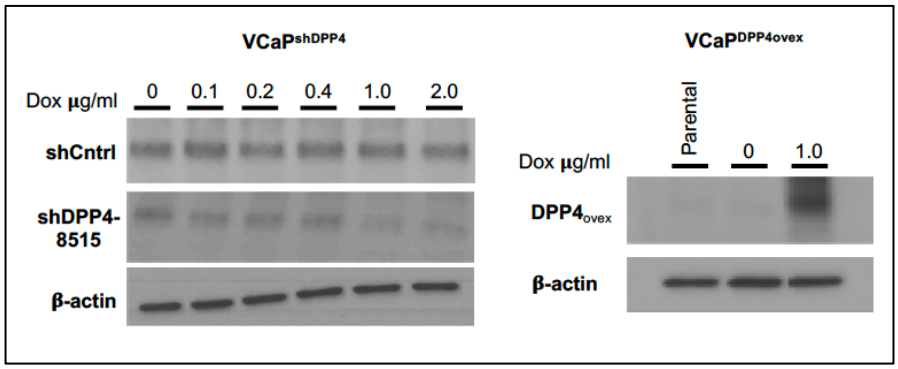

Figure 1. Dox-Inducible VCaP cell lines stably expressing DPP4 shRNA and cDNA. Immunoblot for DDP4 and β-actin protein levels in whole cell lysates taken from VCaP^{shCntrl}, VCaP^{shDPP4}, and VCaP^{DPP4ovex} cell lines. VCaP^{shDPP4} cells show significant knockdown of DPP4 protein compared to VCaP^{shCntrl} cells at 1.0-2.0 µg/ml doxycycline. VCaP^{DPP4ovex} cells show significantly increased DPP4 expression compared to uninduced and parental VCaP cells. Dox – doxycycline.

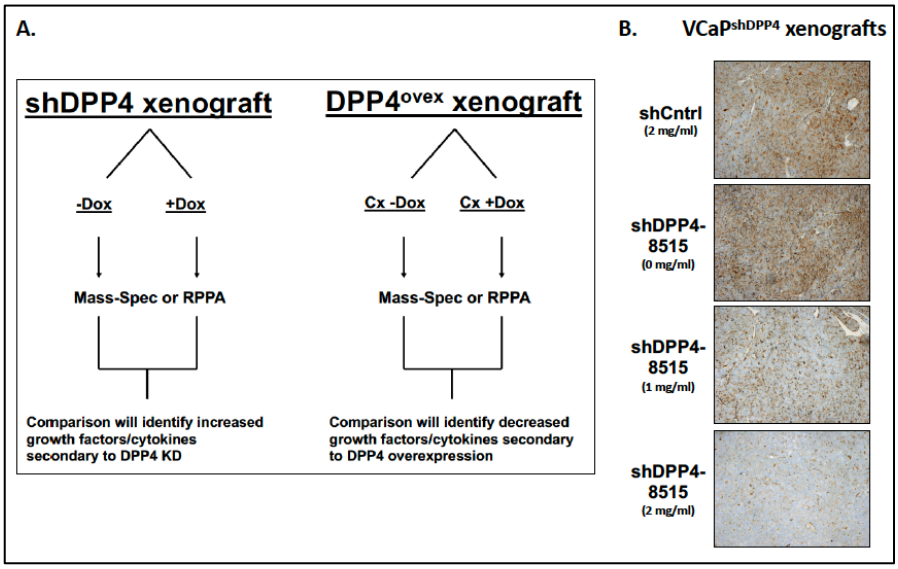

Figure 2. Dox-Inducible VCaP xenografts. A) Schematic representation of experiments planned for the VCaP^{shDPP4} and VCaP^{DPP4ovex} xenografts. B) IHC for DPP4 protein in VCaP^{shCntr} and VCaP^{shDPP4} xenograft tumors following 1-2mg/ml doxycycline induction for 7 days. shDPP4-8515 is the same cell line labeled VCaP^{shDPP4} in Figure 1.

These cells lines will be used to establish dox-inducible xenografts for use in experiments to determine the kinase signaling cascades effected by alterations in DPP4 expression and use this information to backtrack to possible cytokine/growth factors that DPP4 targets for degradation. Briefly, VCaP-shDPP4 and VCaP-DPP4 xenografts will be established in intact immunocompromised mice. VCaP-shDPP4 will be doxycycline-induced for 5 days to knockdown DPP4 protein expression and thereby upregulate the prosurvival cytokine/growth factors targeted for degradation by DPP4 in this setting as well as the kinase signaling cascade the cytokine/growth factor activate **(Figure 2A)**. VCaP-DPP4 xenografts will be castrated, then doxycycline induced to overexpress DPP4 and thereby cause increased degradation of the specific prosurvival cytokines/growth factors as well as the activity of the kinase signaling cascades they regulate. The specific kinase signaling cascades (and possibly the cytokines growth factors) will be identified by mass-spec and phosphoproteome mass-spec or alternatively reverse phase protein array (RPPA). I have established VCaP-shDPP4 xenografts and have successfully tested the ability of the construct to knockdown DPP4 protein levels, getting significant DPP4 protein knockdown with 7 days of doxycycline induction **(Figure 2B)**. A cohort of 20 mice (10 VCaP control-shRNA, 10 VCaP-shDPP4) were started in Feb. 2020 and matured in April 2020 during our hospital's coronavirus shutdown. Unfortunately, due to the moratorium on animal studies forced by the shutdown these mice had to be euthanized in early May due to tumor size restrictions,

so the experiment was wasted. The same restrictions have thus far delayed the demonstration of DPP4 overexpression in the xenograft setting for the VCaP-DPP4 cell line.

4. **Other Achievements:** Another group within our lab has significant interest in determining the characteristics of early prostate cancer that would make these tumors vulnerable to newly developed immune checkpoint blockade therapies, including anti-PDL1/PD1 therapies. They are working to characterize the "hot immunophenotype" of prostate cancer that will respond to anti-PDL1/PD1 therapies. It is well known that DPP4, also known as CD26, is expressed on a number of immune cells and can have immunomodulatory effects on T-cells and antigen presenting cells. During my own studies to determine the clinical significance of DPP4 expression and concurrent ADT/DPP4 inhibitor treatment on PCa progression, I found several primary prostate cancers where there was significant foci of tumor infiltrating lymphocytes (TILs) in areas where there was little tumor DPP4 expression by immunohistochemistry (IHC), while adjacent areas of DPP4 positive tumor had no TILs **(Figure 3)**. While not part of this award, the immunoncology group in our lab is now including DPP4 in their panel of IHC markers they are using to characterize the "hot immunophenotype" of clinical prostate cancer specimens. In conjunction with this, we have also performed IHC for DPP4 on several prostate cancer GEMM models including PTEN$^{-/-}$, high-Myc, and P53$^{-/-}$ **(Figure 4)**. Of interest is the finding that DPP4 is expressed at very high levels in mouse prostate stromal cells, while its expression decreases in the developing prostate cancer, similar to that seen in humans. Also of interest is the finding that unlike the PTEN$^{-/-}$ and high-Myc models, in p53$^{-/-}$ mouse prostate cancer, DPP4 appears to be ubiquitously expressed within the tumor. These studies are not a focus of the research conducted in this award and none of these studies were or will be conducted using funds from this award.

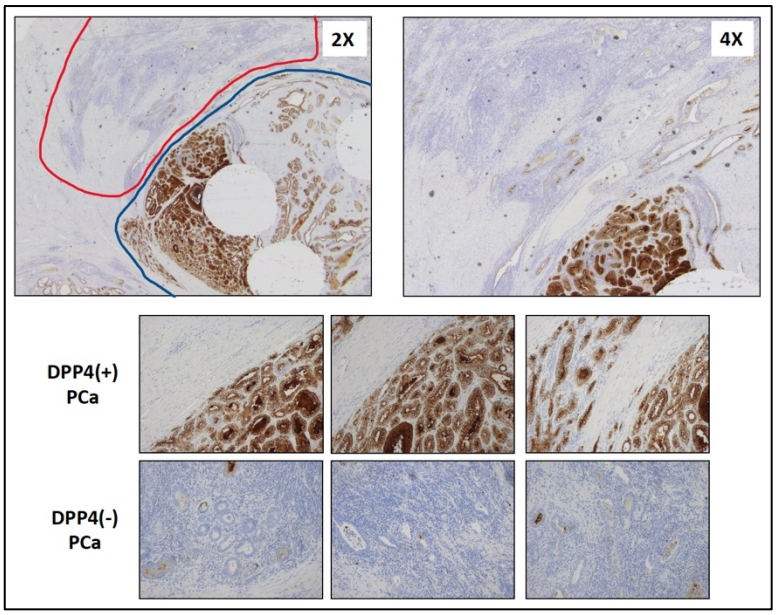

Figure 3. DPP4 downregulation in tumors associates with TILs. IHC for DPP4 protein expression in adjacent foci of primary prostate cancer. (Upper) Low power images. Red area – foci of primary prostate cancer with no DPP4 staining and significant lymphocyte infiltration. Blue area – adjacent foci of primary prostate cancer with high DPP4 expression and low/absent lymphocyte infiltration. (Lower) Higher power images of DPP4 positive (+) and negative (-) foci.

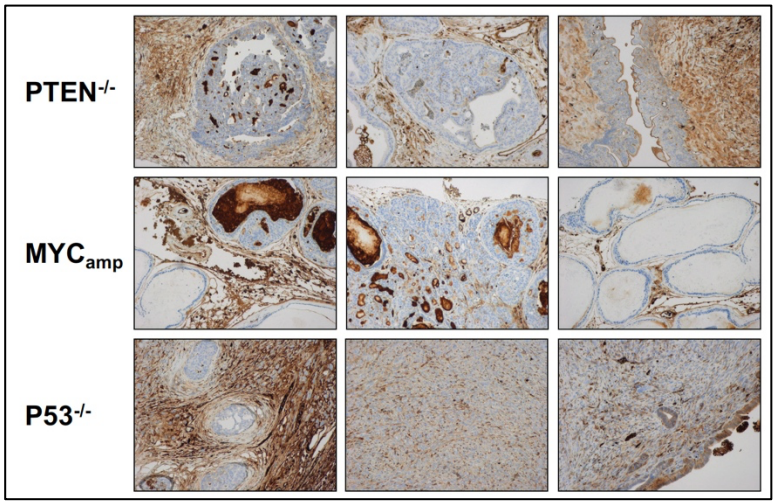

Figure 4. DPP4 expression in GEMMs. IHC for DPP4 protein expression in genetically modified mouse models (GEMMs). Images of 3 separate tumors each for the PTEN$^{-/-}$, MYC$_{amp}$ (high-Myc), and p53$^{-/-}$ models.

5. **Stated Goals Not Met**: All milestones in Goal #1 will be completed within the next year as dox-inducible VCaP^{shDPP4} and VCaP^{DPP4ovex} xenografts are grown up and the effects of DPP4 knockdown and overexpression on the efficacy of castration in VCaP tumors are tested. Goal #2 to determine the signaling cascades effected by DPP4 downregulation/inhibition and the corresponding growth factors/cytokines targeted by DPP4 that are responsible for ADT resistance has not yet been met. The dox-inducible VCaP^{shDPP4}

and VCaP^{DPP4ovex} xenografts are the major means by which the studies in this goal will be achieved. Unfortunately, animal experiments have been one of the areas of research most effected by the coronavirus pandemic lock-downs and subsequent investigator restrictions at my institution. Using these xenografts in the experiments outlined above in combination with mass-spec phosphoproteome methods to identify signaling cascades effected by DPP4 downregulation/inhibition will the primary focus in the remaining year of funding. Goal #3 to determine the clinical significance of DPP4 expression and concurrent ADT/DPP4 inhibitor treatment on PCa progression is continuing to proceed. I expect this goal to be reached by the end of the funding period.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Through this project I have had extensive one-on-one work with my mentor, Dr. Steven Balk. This includes a weekly progress report style meeting. I also present my work every 6 weeks to the lab group as a whole and one a year to the BIDMC Cancer Center as a whole.

I had an abstract accepted for poster presentation on this research at the American Association for Cancer Research (AACR) Special Conference on Advances in Prostate Cancer Research that was to be held in March 12-15, 2020, but this conference was cancelled due to the coronavirus pandemic.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results from these studies were to have been presented in poster presentation format at the American Association for Cancer Research (AACR) Special Conference on Advances in Prostate Cancer Research that was to be held in March 12-15, 2020, but this conference was cancelled due to the coronavirus pandemic.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The primary focus of the next reporting period will be to perform the experiments outlined in Aim 2 of the award. These studies will use the dox-inducible VCaP^{shDPP4} and VCaP^{DPP4ovex} cell lines already developed to establish xenografts in immunocompromised mice. These xenografts will then undergo combinations DPP4 knockdown and overexpression +/- castration to demonstrate the effects of altered DPP4 expression on castration efficacy, while simultaneously providing tumor tissues for mass-spec phosphoproteome analysis to identify the signaling cascades and growth factors/cytokines most effected by DPP4 alteration.

4. **IMPACT:** D*escribe 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an 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."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

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:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency

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.

The coronavirus pandemic and the resulting restrictions put in place to halt the spread of the virus have negatively impacted the progress of my research program. Massachusetts was one of the early coronavirus epicenters in the US and due to climbing rates of infection and deaths the state government decided to close down all non-essential businesses, including biomedical research.

Beth Israel Deaconess Medical Center (BIDMC) announced it would shut down all research operations starting on March 19th. The two weeks leading up to this date saw my efforts refocused on freezing down and storing precious cell lines and patient-derived xenograft models, in order to ensure they were not lost during the shutdown. After March 19th only 2 members of our 12 member lab were allowed to enter the lab space for brief periods of time to perform essential maintenance tasks, such as liquid nitrogen storage replenishment and mouse euthanasia. From March 19th-July 6st (~3.5 months) no new xenograft/PDX studies could be initiated, no new mice for tumor transplant could be ordered, and no cell culture experiments could be performed. During this time period a cohort of 20 $VCaP^{shDPP4}$ and $VCaP^{shCntrl}$ matured around late-APRIL. These mice were to be used to assay for kinase signaling cascade alterations and growth factor/cytokine expression following DPP4 knockdown. These experiments could not be performed and all mice had to be euthanized when the tumors reached $2mm^3$.

On July 6th BIDMC entered Phase II of reopening. This resulted in renewed lab access for all lab members, but on a shift schedule with the lab divided into two shifts to allow social distancing. Shifts last 6hrs and have one hour buffers in between. No one is allowed to come early for or overstay their shift unless the extra time is before 8AM or after 9PM and then only if social distancing can be maintained. Our lab has two adjacent cell culture hoods shared by 5 people per shift. Only one person can be in the cell culture space at a time. This causes significant bottlenecks in workflow as most of the lab works only with cell culture methods.

Due to our lab's size and the spacing requirements of social distancing, I have been effectively locked out of the lab from March 19th-July 6th. Over the past 2.5 months we have slowly started up lab operations again. Animal studies continue to be difficult due to restrictions on investigator numbers within specific animal housing rooms and procedure areas. I am hopeful that I will begin animal experiments again within the month of October. I expect the current guidelines on number of investigators in the animal facility will remain in place and will not become more restrictive over the remaining fall and winter months. This expectation is based on the previous actions of the hospital administration and city/state government. It is unlikely that coronavirus infection rates in Massachusetts will be allowed to rise again to the levels seen in April and May without the state and city enacting new restrictions. It is unlikely that these new restrictions will affect the research in our lab.

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.

Restrictions on work and animal facility access by our institution during the ongoing coronavirus pandemic have slowed expenditure of those funds to be used for animal xenograft studies.

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

Nothing to report.

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."*

- **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).*

Nothing to report.

  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.

- **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.

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

Nothing to report.

- **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.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

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*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Joshua W. Russo
Project Role: Principal Investigator
Research Identifier: ORCID ID – 0000-0001-5481-0090
Nearest person month worked: 10
Contribution to Project: Dr. Russo performed all the animal studies and in vitro work.
Funding Support: CDMRP PCRP Early Investigator Award, PCF Young Investigator Award

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.*

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.*