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TITLE: **The RET Kinase Signaling Axis in Neuroendocrine Prostate Cancer Plasticity**

PRINCIPAL INVESTIGATOR: **Dr. Yuzhen Zhou**

CONTRACTING ORGANIZATION: **Dana-Farber Cancer Institute**

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The RET Kinase Signaling Axis in Neuroendocrine Prostate Cancer Plasticity

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Aggressive variant prostate cancer (AVPC) is associated with loss of expression/function of RB1 and/or Tp53. AVPC represents lethal a subgroup prostate cancer with approximately 1-2 years survival rates in patients. Lineage plasticity driven by epigenetic regulators including EZH2 is implemented as a major mechanism driving AVPC. Currently there are no therapeutic approaches to provide durable response in patients who have progressed to AVPC. The receptor tyrosine kinase RET is found to be highly expressed in AVPC patients comparing with CRPC. We generated 1) Pten(floxed/floxed):Rb1(floxed/floxed):RET(+/+); 2) Pten(floxed/floxed):Rb1(floxed/floxed):RET(floxed/+) and 3) Pten(floxed/floxed):Rb1(floxed/floxed):RET(floxed/floxed) mice to determine the dependence of RET kinase as driver and therapeutic target in AVPC. All floxed alleles were targeted by Cre-recombinase expression driven by a probasin promoter. This allows to targeted excision exlcusive to the mouse prostatatic epithelium. While some complications were encountered, we successfully generated a small number of mice representing all three genotypes. Aging studies are ongoing and will conclude in December 2020 – January 2021. We also collected prostate tissues from 10 weeks-old mice which either Cre+ or Cre- to generate 3D organoids from these mice. 3D organoids are also being generated into 2D cell lines.

Due to the relocation of my mentor – Dr. Leigh Ellis to Cedars-Sinai Medical Center in October 2020, I am unable to join him in Los Angeles and continue this fellowship.

15. SUBJECT TERMS Cancer Biology, Epigenetics, Lineage Plasticity, Chromatin Remodeling					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Blocking androgen synthesis or signaling through the androgen receptor (AR) is the first line treatment. However, prostate cancer inevitably develops castration resistant prostate cancer (CRPC) and an emerging subset (35%) of CRPC patients develops a highly aggressive tumor phenotype designated aggressive variant prostate cancer (AVPC) and treatment provides modest 1-2 years survival rates. AVPC is characterized by low to absent AR levels and expression of neuronal, reprogramming and stem related gene signatures. Previously, we observed tyrosine phosphorylation of RET, suggesting activity of this kinase in a patient with prostate AVPC. Our objectives are to 1.) Functionally assess the role of RET kinase in the transition to AVPC, 2.) Determine if RET kinase define the EZH2 interactome and drive chromatin landscape alteration and promote the transition of AVPC, 3.) Evaluate the effect of co-inhibition of RET kinase and EZH2 on the treatment of AVPC, and re-sensitizing AVPC to enzalutamide. These all are important and unanswered questions involving the most lethal PCa phenotype observed clinically. Our success will drive the PCa field forward, and significantly alter clinical management of patients with AVPC.

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

Prostate cancer; Castration Resistance; ADT; Enzalutamide; Lineage Plasticity, AVPC; NEPC; RET; Tyrosine Kinase, EZH2; Methylation; Epigenetic.

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.

1 Major goal 1#: Maintain RET kinase knockout in AVPC mouse models
1). Obtain IACUC approval for animal protocol and submitted for ACURO approval.
2). Train Dr. Zhou to competence in animal husbandry for generation of knockout mice.
2 Major goal 2#: Assess the chromatin landscape and gene expression in RET kinase knockout AVPC mouse models
1). Train Dr. Zhou to proficiency in RNA-seq and ChIP-seq data acquisition and analysis and also learn to analyze data from RIME experiment.
3 Major goal 3#: In vitro combination treatment of RET and EZH2 inhibitors with enzalutamide in pre-clinical AVPC organoid models
4 Major goal 4#: In vivo combination treatment of RET and EZH2 inhibitors with enzalutamide in pre-clinical human xenograft and murine transplant models
5 Major goal 5#: Conduct general activities relative to presenting and publishing research

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 Accomplishment for major goal 1#: Maintain RET kinase knockout in AVPC mouse models

1). We have submitted ACURO on Sep, 2019 based on the animal protocol 17-003 approved by IACUC on Mar, 2017. The renewal of animal protocol was completed on Feb, 2020 and new ACURO was also submitted on Mar, 2020

2). Dr. Zhou was firstly trained by Animal Research Facility for the general concept of mouse research. After that, Dr. Ellis and his colleagues trained Dr. Zhou for the specific skills in generating knock-out mice model for prostate cancer, including husbandry, set up mating pairs and genotyping the litters. In addition to that Dr. Zhou was also showed the castration surgery. After almost one-year work on the mice model, Dr. Zhou have got competence in mice and successfully maintained RET floxed alleles into AVPC mice to generate AVPC-RET +/lox and AVPC-RET lox/lox mice.

By the end of May 2020, we had already had 9 mice for PbCre: Ptenlox/lox; Rb lox/lox; RET +/+, 13 mice for PbCre: Ptenlox/lox; Rb lox/lox; RET +/lox and 6 mice for PbCre: Ptenlox/lox; Rb lox/lox; RET lox/lox. These mice will gradually reach the endpoint 30-40 weeks. We will collect the prostate tissue (lobes separately) and analyze the histology and molecular alteration based on the different RET genotype.

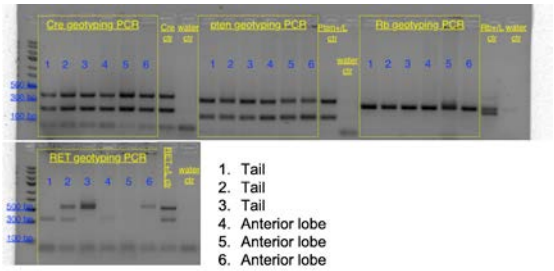

Figure 1. genotype result of experiment mice. sample 1 and 4 were from mice with genotype of PbCre: Ptenlox/lox; Rb lox/lox; RET +/+; sample 2 and 5 were from mice with genotype of PbCre: Ptenlox/lox; Rb lox/lox; RET +/lox, sample 3 and 6 were from mice with genotype of PbCre: Ptenlox/lox; Rb lox/lox; RET lox/lox.

2 Accomplishment for major goal 2#: Assess the chromatin landscape and gene expression in RET kinase knockout AVPC mouse models

1). For the increasingly strict screening for the bioinformatic bootcamp Dr. Zhou failed to attend training from this camp in 2019.

2). Prostate tissue collecting haven't finished as the endpoint of experiment mice was 30-40 weeks and we didn't have enough number of mice at that age. it'll be at the end of Sep, 2020 when some mice reached endpoint. Therefore, we don't have adequate numbers of tissue to execute RNA-seq or ChIP-seq.

3). RIME: we had just finish testing the EZH2 antibody that would be used for RIME on Mar, 2020 and we were also planning for the pre-testing ChIP assay to make sure RIME works in best condition. However, this experiment was stuck by the pandemic.

3 Accomplishment for major goal 3#: In vitro combination treatment of RET and EZH2 inhibitors with enzalutamide in pre-clinical AVPC organoid models

1). AVPC organoid had already been generated and the RET inhibitor AD80 alone and with enzalutamide was also tested in this organoid model by my colleagues Dr. Morel. But Ezh2 inhibitors haven't been tested yet.

3). We have collected adequate amount of prostate tissue from PbCre-; Pten(lox/lox);RB(lox/lox);RET(+/+) and PbCre-; Pten(lox/lox);RB(lox/lox);RET(+/lox) and PbCre-; Pten(lox/lox);RB(lox/lox);RET(lox/lox) mice. These tissues could be generated oganoid. RET could be deleted by infection Cre adenovirus to organoid. Once I was learning to generate organoid from tissue of the three RET genotypes but failed at the first time. Organoid generating experiences were also got from this failure and lesson.

4 Accomplishment for major goal 4#: In vivo combination treatment of RET and EZH2 inhibitors with enzalutamide in pre-clinical human xenograft and murine transplant models

N/A

5 Major goal 5#: Conduct general activities relative to presenting and publishing research

N/A

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.

1). Animal husbandry for generation of knockout mice were trained by my mentor Dr. Ellis and Dr. Burkhart. The skills including calculating frequency of specific genotype of litters from their parents; setting up mating pairs; weaning the litters; getting biopsy and genotyping.

2). One-on-one talk with my mentor Dr. Ellis about the project every week helps a lot in broadening scientific horizons.

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.

Nothing to report.

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.

Nothing to report.

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:*

The mentor of this fellowship Dr. Ellis will leave Dana Farber Cancer Institute and Dr. Yuzhen Zhou decided to stay here for family consideration. Dr. Ellis decided to stop the fellowship because the remote supervising will be inappropriate.

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.

Nothing to Report

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.

We have to stop this fellowship.
The mentor of this fellowship Dr. Ellis will leave Dana Farber Cancer Institute and Dr. Yuzhen Zhou decided to stay here for family consideration. Dr. Ellis decided to stop the fellowship because the remote supervising will be inappropriate.

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).*

No. we had just finished the mice modeling and still haven't done detailed analysis based on this model. And we also haven't got all the data from organoid study. We didn't get complete data to write an article.

- **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).

No. We did plan to finish the experiment in 2020 and attend some conference and communicate out data. The pandemic slowed down everything. We didn't finish as planned and it's impossible to have high quality scientific communication if we didn't have much accomplishment to share.

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.*

No. We did plan to finish the experiment in 2020 and attend some conference and communicate out data. The pandemic slowed down everything. We didn't finish as planned and it's impossible to have high quality scientific communication if we didn't have much accomplishment to share.

- **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: Dr. Yuzhen Zhou
Project Role: Principal investigator
Researcher Identifier: *ORCID* ID: 0000-0003-0461-2963
Nearest person month worked: 12
Contribution to Project: Dr. Yuzhen Zhou had performed mice model establishment and maintaining, including the trouble shooting during this period.

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.