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TITLE: Determining the Role of CDK12 in Driving Prostate Cancer Initiation, Progression, and Responses to Therapeutics

PRINCIPAL INVESTIGATOR: Valeri Vasioukhin, Ph.D.

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
Valeri Vasioukhin, Ph.D.

E-Mail: vvasiouk@fredhutch.org

5d. PROJECT NUMBER

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5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
1100 Fairview Ave N, J6-300
Seattle, WA 98109-4433

8. PERFORMING ORGANIZATION REPORT NUMBER

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
CDK12 is inactivated in 6.9% of metastatic castration-resistant prostate cancer; however, the functional and clinical significance of this event is not clear. In this grant we are investigating the role and mechanisms of Cdk12 in prostate cancer using tissue specific gene targeting in mice. During the reporting period we performed all the studies that were originally planned for the first year. We generated mice with prostate-specific inactivation of Cdk12 with and without overexpression of MYC and presently expanding and analyzing these mutant mice.

15. SUBJECT TERMS
Prostate cancer, CDK12, mouse models of cancer

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	8	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction

CDK12 is inactivated in 6.9% of metastatic castration-resistant prostate cancer (PC). Loss of *CDK12* in PC significantly co-occurs with amplification of *MYC* (TCGA cohort, q=0.024). Genomes of human *CDK12*-null PCs display focal tandem duplications which makes them distinct from all other PC types. This genomic signature indicates a specific function of CDK12 in the maintenance of genome integrity which is presently not well understood. In this study, we will develop and analyze mouse models with prostate-specific deletion of *Cdk12* alone (*Cdk12* cKO), or in combination with overexpression of *Myc*. We will determine the role and mechanisms of *Cdk12* in prostate epithelium and prostate cancer, and analyze potential specific drug and immunotherapy vulnerabilities of *Cdk12*-negative prostate cancer.

2. Keywords

Prostate cancer, CDK12, mouse models of cancer

3. Accomplishments

What were the major goals of the project?

Major Task 1: DoD ACURO and HRPO protocols review and approval. Months 1-3. Completed 100%.
Major Task 2: Generate and analyze PB-Cre/Cdk12fl/fl/TdTomato and PB-Cre/Cdk12fl/fl/PB-Myc/TdTomato mice. Months 1-24. Completed 50%.
Major Task 3: Maintenance of original and newly generated mouse lines. (note this task will be repeated each year). Months 3-36. Completed 30%.
Major Task 4: Treatment of prostate organoids and mice with prostate tumors from aim 1 and human LuCaP xenografts with PARPi, ATMi, ATRi or Carboplatin. Months 12-36. Completed 0%.
Major Task 5: Treatment of prostate organoids, mice with ERG+ prostate tumors and human LuCaP xenografts with CDK12 inhibitor. Months 1-36. Completed 30%.
Major Task 6: Treatment of mice with prostate tumors from aim 1 with anti-PD1 and anti-CTLA4. . Months 24-36. Completed 0%.

What was accomplished under these goals?

Major Task 2: We are generating mice with PB-Cre/Cdk12fl/fl and PB-Cre/Cdk12fl/fl/PB-Myc genotypes with and without TdTomato allele and working on the analysis of the prostate gland phenotypes. We are performing breeding, pups genotyping, weaning, aging. Also performing histological and immunohistochemical analyses of the prostate glands and distant organs from aged mice. Generated many mice with intermediate genotypes that are being used in breedings for obtaining the mice with final genotypes. Presently generated n=25 PB-Cre/Cdk12fl/fl and n=11 PB-Cre/Cdk12fl/fl/PB-Myc mice.
Major Task 3: We maintain original PB-Cre, Cdk12fl/fl, PB-Myc, and TdTomato mouse lines, and their various combinations used to produce PB-Cre/Cdk12fl/fl/TdTomato and PB-Cre/Cdk12fl/fl/PB-Myc/TdTomato genotypes. We are performing breeding, pups genotyping, weaning,
Major Task 5: We are generating and aging PBCre/PTENf/f/TdTomato and PBCre/PTENf/f/PB-ERG/TdTomato mice. We are performing breeding, pups genotyping, weaning, aging. Generated many mice with intermediate genotypes that are being used in breedings for obtaining the mice with final genotypes. Presently generated n=14 PBCre/PTENf/f/TdTomato, n=32 PBCre/PTENf/f/PB-ERG/TdTomato mice.

What opportunities for training and professional development has the project provided?

Nothing to Report during this period.

How were the results disseminated to communities of interest?

Nothing to Report during this period.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue working on the grant along the lines described in our original proposal.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report during this period.

What was the impact on other disciplines?

Nothing to Report during this period.

What was the impact on society beyond science and technology?

Nothing to Report during this period.

5. Changes/Problems

No changes or problems occurred or are anticipated to occur.

6. Products

Generated novel mouse model with prostate epithelium-specific deletion of Cdk12 with and without overexpression of Myc.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project for one month or more?

Name:	**Valeri I. Vasioukhin**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6730-0281
Nearest person month worked:	3
Contribution to Project:	Dr. Vasioukhin has generated mouse models, performed data analysis, and overseen the conduct of the study.
Funding Support:	N/A
Name:	**Sheng-You Liao, PhD**
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-2352-9666
Nearest person month worked:	3
Contribution to Project:	Dr. Sheng-You Liao performed experiments outlined in the year one of the proposal.
Funding Support:	N/A
Name:	**Luan Phan**
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	5
Contribution to Project:	Luan worked with Dr. Liao and assisted him in the performance of the experiments outlined in the year one of the proposal. In addition, he took care of mouse genotyping.
Funding Support:	N/A

Name:	Dmytro Rudoy
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9
Contribution to Project:	Dmytro helped Dr. Liao with performing the experiments in this proposal and also performed animal work; breeding, clipping, weaning, dissecting, euthanizing of mice in this project.
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?

Changes in support for Valeri I. Vasioukhin, PhD, PI:

- Alpha-Catenin in Regulation of Tissue Homeostasis and Cancer, NCI, R01CA179914 ended 5/31/2019.
- Regulation of Hippo Signaling by Src-Family Kinases, NCI, R01CA188452 ended 8/31/2019.
- Pilot Project. Identifying and Exploiting Therapeutic Vulnerabilities in ERG Positive Prostate Cancer, NCI,5P50CA097186-17, started 3/1/2019 until 2/28/20.
- Basal Cell Polarity Proteins in Normal Tissue Homeostasis and Cancer, NCI, 1R01CA234050-02, started 6/7/2019 until 5/31/2024.

Changes in support for Peter Nelson MD, Co-I:

- Selective Androgen Receptor Modulators for the Treatment of Prostate cancer, NCI, R21CA230138, ended 6/30/2020.
- Therapeutic Targeting of Neuroendocrine Prostate Cancer, DoD/CDMRP, PC170350P1, started 9/1/2019 until 8/31/2021.

What other organizations were involved as partners?

Nothing to Report during this period.

8. Special Reporting Requirement

Please see award quad chart on next page per award terms.

Determining the Role of CDK12 in Driving Prostate Cancer Initiation, Progression, and Responses to Therapeutics

Grant Log: PC180295 / Award: W81XWH-19-1-0211

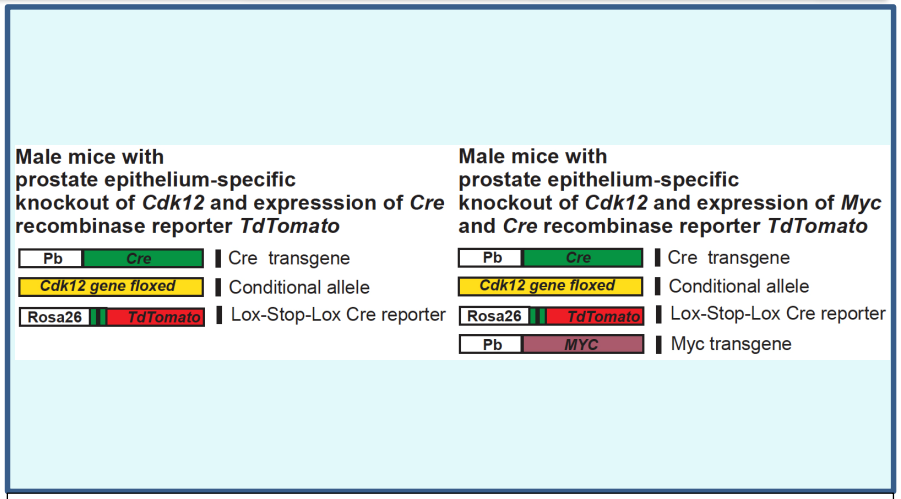
PI: Valeri I. Vasioukhin, PhD **Org:** Fred Hutchinson Cancer Research Center **Award Amount:** $1,056,000

Study/Product Aim(s)

- Aim 1: Characterize the molecular and physiological consequences of prostate-specific Cdk12 loss alone, and together with Myc gain, including assessments of histological (including immune cell repertoire) and molecular features (e.g. genomic copy gains, gene rearrangements, neoantigen load) that accompany the development of neoplasia.
- Aim 2: Determine if Cdk12 loss tumors (with or without Myc gain) respond to inhibitors of genome maintenance: PARPi, ATMi, ATRi or DNA damaging agents: carboplatin, and if ERG+ PC is hypersensitive to inhibition of Cdk12.
- Aim 3: Determine if Cdk12 loss tumors exhibit responses to immune-based therapeutics including PD1/PDL1 inhibitors. This aim will also assess mechanisms of resistance.

Approach

This study will test the hypothesis that mouse prostate-specific inactivation of Cdk12 alone or in combination with Myc overexpression will result in PC, which will show genomic alterations that are concordant with CDK12 null human PC. We also hypothesize that PC in Cdk12 cKO mice will show specific vulnerabilities to a subset of genome maintenance inhibitors and/or immune-based therapeutics. In addition, we hypothesize that ERG-positive PC is hypersensitive to drugs inhibiting CDK12.



Generated and started the analysis of mutant mice with prostate epithelium-specific inactivation of *Cdk12* with and without overexpression of *Myc*.

Timeline and Cost

Activities CY	19	20	21
Characterize the molecular and physiological consequences			
Determine if Cdk12 loss tumors respond to inhibitors of genome maintenance			
Determine if Cdk12 loss tumors exhibit responses to immune-based therapeutics			
Estimated budget ($K)	**$352**	**$352**	**$352**

Updated: 7/22/2019

Goals/ Milestones

CY19 Goal: ACURO and HRPO protocols review and approval *(completed 100%)*
☑ ACURO and HRPO protocols approved.

CY19-21: Maintenance of mouse lines *(completed 30%)*
☐ Maintain the original and newly generated lines, ongoing

CY20 Goal: Generation of mice *(completed 50%)*
☐ Generate and analyze PBCre/Cdk12fl/fl/TdTomato mice and PBCre/Cdk12fl/fl/PBMyc/TdTomato mice

CY21 Goal: Treatment of prostate organoids and mice (*completed 0%)*
☐ Characterization of the effects of PARPi, ATMi, ATRi or Carboplatin
☐ Characterization of effects of CDK12i on ERG+ prostate tumors and human LuCaP xenografts with CDK12 inhibitor
☐ Characterization of the effects of anti-PDL1 and anti-CTLA-4

Comments/Challenges/Issues/Concerns: None to report; spending is on track.

Budget Expenditure to Date (through 6/30/2020)
Projected Expenditure: $369,389 ($1,056,000 total project cost projected)
Actual Expenditure: $368,944

9. References

Nothing to Report during this period.

10. Appendices

Nothing to Report during this period.