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TITLE: Prospective-Retrospective Analysis of PTEN Immunohistochemistry Assay for Prediction of Outcomes in Recurrent and Metastatic Prostate Cancer

PRINCIPAL INVESTIGATOR: Tamara L. Lotan

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21218

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Tamara L. Lotan

E-Mail:tlotan1@jhmi.edu

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14. ABSTRACT

As part of the CDMRP-funded Precision Medicine Biomarker Validating Center, we have developed a robust, highly analytically validated and cost-effective immunohistochemistry (IHC)-based assay to interrogate PTEN loss in prostate cancer. Our PTEN assay is prognostic in multiple cohorts of surgically-treated prostate cancer patients; now, we propose to leverage this body of previous validation studies to test the hypothesis that PTEN loss in primary prostate cancer predicts for a less robust response to hormonal therapies, in the context of two recent, practice-changing Phase III clinical trials for which we have CTEP approval to access specimens. Here, we report on progress to date. We have obtained and immunostained slides from ECOG3805 (CHAARTED) for PTEN. In analyses, we have examined the association of PTEN loss with outcomes in the androgen deprivation therapy (ADT) arm (n=119) and the ADT+Docetaxel arm (n=108). We find that PTEN loss is not a significant predictor overall survival in the trial as a whole and is actually protective in the setting of high volume metastatic disease.

15. SUBJECT TERMS
Prostate cancer, PTEN, ERG, immunohistochemistry, survival

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1. INTRODUCTION:

With the completion of The Cancer Genome Atlas (TCGA) and Stand Up to Cancer (SU2C) sequencing projects for primary and metastatic prostate tumors, the genomic landscape of prostate cancer has largely been elucidated. Yet currently, none of the findings in these studies have improved patient outcomes in the disease, in large part because of the challenges associated with validating prognostic and predictive genomic biomarkers. Among the genomic changes cataloged, PTEN is the earliest and most commonly lost tumor suppressor in primary prostate cancers and its loss portends a poor prognosis and is associated with the development of castrate resistant disease in pre-clinical models. As part of the CDMRP-funded Precision Medicine Biomarker Validating Center, we have developed a robust, highly analytically validated and cost-effective immunohistochemistry (IHC)-based assay to interrogate PTEN loss in prostate cancer. Based on this work, these assays are currently performed in the Johns Hopkins CLIA/CAP-accredited Immunopathology Laboratory.

Hypothesis/Objective: Our PTEN assay is prognostic in multiple cohorts of surgically-treated prostate cancer patients; here, we will leverage this body of previous validation studies to test the hypothesis that **PTEN loss in primary prostate cancer predicts for a less robust response to hormonal therapies**, in the context of two recent Phase III clinical trials for which we have CTEP approval to access specimens.

Here, **we will test the hypothesis generated by preclinical models that PTEN loss predicts for a less robust response to AR-targeted and/or and androgen deprivation therapies**. More specifically, in the context of ECOG 3805, we will examine whether patients with PTEN-deficient metastatic prostate tumors derive additional benefit from docetaxel chemotherapy deployed with androgen deprivation therapy. In the context of RTOG 96-01, we will test whether the addition of AR-targeted therapy to radiation therapy is less beneficial for patients with patients with PTEN-deficient recurrent non-metastatic prostate tumors. In each study, we will further assess whether ERG status modulates the relationship of PTEN to clinical outcomes.

2. KEYWORDS: Prostate cancer, PTEN, ERG, survival

3. ACCOMPLISHMENTS:

What were the major goals of the project?

a. **Specific Aim 1: Test whether PTEN status modifies benefit associated with treatment in ECOG 3805, a phase III trial that demonstrated a benefit for docetaxel chemotherapy at the time of starting androgen deprivation therapy (ADT) for men with high volume metastatic disease.**
    1. **Determine PTEN/ERG status of ~300 tumors from trial (~150 in each arm)**
        a. Obtain HRPO Approval for study (Hopkins IRB approval is in place already)
        b. Obtain tissue slides from ECOG in batches of 30

c. Immunostain and blindly score for PTEN/ERG status

2. **Integrate PTEN/ERG status with de-identified clinical-pathologic data for study patients received from ECOG.** The primary objective will be to assess whether the relative benefit of docetaxel+ADT (androgen deprivation therapy) differs in patients with PTEN loss compared to PTEN intact. Secondary objectives will be to examine the association of PTEN status with outcome, stratified by treatment arm, ERG status, and/or low/high tumor volume.

b. **Specific Aim 2: Test whether PTEN status modifies the benefit associated with treatment in RTOG 96-01, a phase III trial that demonstrated a benefit for AR-targeted therapy with bicalutamide at the time of radiation therapy for non-metastatic PSA recurrence after radical prostatectomy.**

1. Construct Tissue microarray and determine PTEN/ERG status of ~550 tumors from trial

a. Obtain HRPO Approval for study (Hopkins IRB approval is in place already)
b. Generate tissue microarrays (TMAs) from 335 radical prostatectomy specimens in the study; receive ~212 slides of cases with slides available from NRG
c. Immunostain TMAs and tissue slides and blindly score for PTEN/ERG status

2. Integrate PTEN/ERG status with de-identified clinical-pathologic data for study patients received from NRG. The primary objective will be to assess PTEN status by immunohistochemistry (IHC) and assess whether PTEN status modifies the association of treatment (radiation therapy vs. radiation therapy+anti-androgen therapy) with metastasis free survival in patients treated with salvage radiation after biochemical recurrence. Secondary objectives will include similar evaluations of combined PTEN-ERG status, and whether PTEN or PTEN-ERG status are prognostic in these patients, independent of treatment. PTEN/ERG status may also be correlated with next generation sequencing data generated by the Maher-Feng-Tomlins study of the same specimens which is already approved

What was accomplished under these goals?

1) Major activities during this reporting period include HRPO approval of the JHU IRB determination that these studies involved fully deidentified specimens and are not considered human subjects research (A-20180.1 and A-20180.2). Slides for all subjects with available tissue (n=267) were shipped from the ECOG tissue bank and immunostained for PTEN in the Johns Hopkins CLIA-accredited immunopathology laboratory using a highly genetically validated protocol. Dr. Lotan examined all stained slides and scored them for PTEN status. The clinical-pathologic data for these cases were obtained from ECOG and association of PTEN with outcome measures was performed by statistical co-investigator Bruce Trock.

For RTOG96-01, tissue microarrays containing 320 radical prostatectomies from patients in the trial were created by the RTOG tissue biorepository team. Unstained slides from these tissues were sent to Johns Hopkins and have been stained for PTEN and ERG and scanned images segmented and made available on our internal TMAJ viewer for scoring. Scoring will be completed in the next reporting period, as will statistical analysis. An additional 194 cases with unstained slides available were also shipped to Johns Hopkins. These slides are

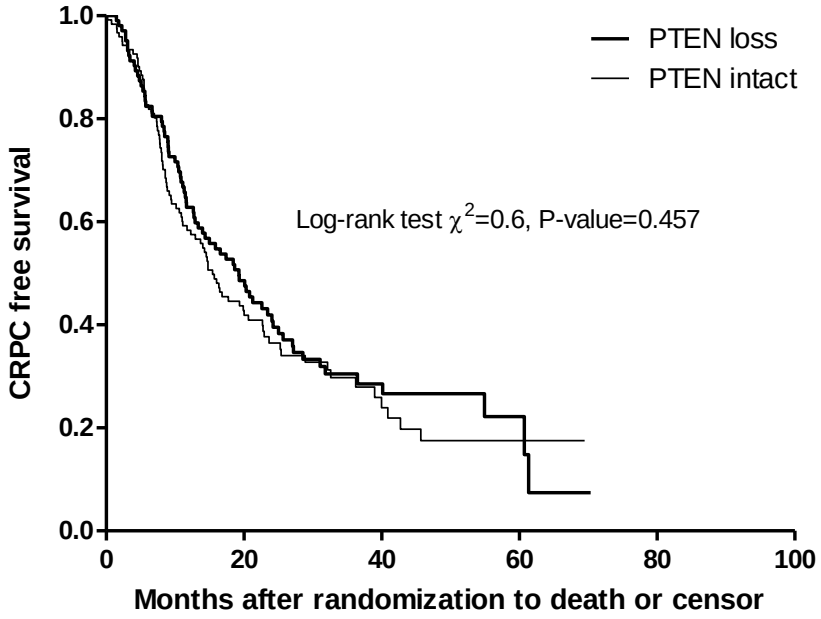
currently stored at -20 degrees Celsius awaiting immunostaining which will be performed for PTEN and ERG in the next reporting period.

2) Specific objectives during this reporting period were to obtain, immunostain and score and analyze PTEN status of tumors with available tissue from the ECOG3805 trial. In addition, construction of the tissue microarrays from the tissues in the RTOG 96-01 trial and staining of these tissue microarrays was another objective.

3) Significant results or key outcomes included HRPO approval and the analysis of PTEN status and its association with outcome in the ECOG3805 trial. Overall, 85% (227/267) of cases had interpretable staining. Of the cases without interpretable staining results, 65% (26/40) had ambiguous PTEN staining results and 35% (14/40) had no tumor sampled on unstained slides. Of the interpretable cases, 46% (105/227) of cases had PTEN protein loss, consistent with PTEN gene deletion based on our prior validation studies and the remainder had intact PTEN protein. When we compared clinical-pathologic variables stratified by PTEN status, cases with PTEN loss tended to have a lower ECOG performance status ($p=0.01$) but age, disease volume, visceral metastases, Gleason score, baseline PSA, assigned treatment arm and percentage developing CRPC and death from prostate cancer were not statistically different between PTEN intact and PTEN lost cases.

In outcomes analyses, time to CRPC was not significantly different between the PTEN loss and PTEN intact groups in the trial as a whole (not stratified by arm, see **Figure 1**) or in each arm considered separately (data not shown). Surprisingly, PTEN loss appeared significantly protective for CPRC survival among patients with high volume metastatic disease in the trial (the patients who benefitted the most from the combined therapies) (**Figure 2,** $p=0.04$ by long rank test). The opposite trend was apparent among patients with low volume disease. We are currently trying to better understand the significance of this finding in additional subset analyses. In addition, stratification by ERG status may provide some insights.

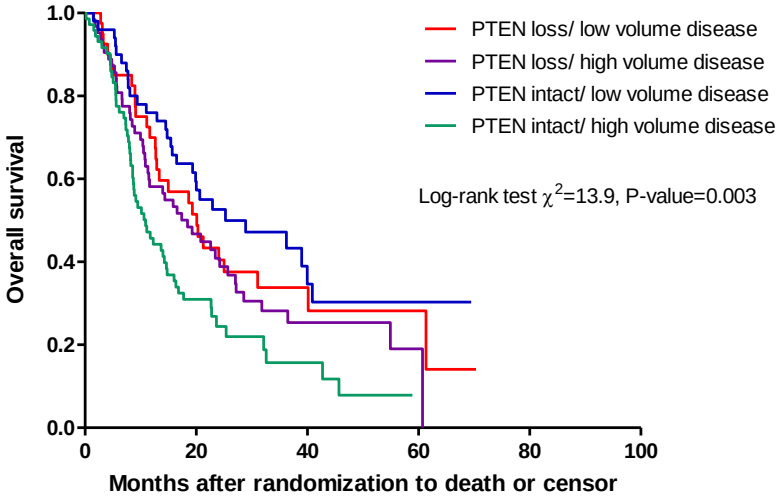
Figure 1: K-M curve for <u>CRPC survival</u> stratified by PTEN (all patients)



At risk	PTEN loss	105	47	16	4
	PTEN intact	122	48	13	2

Median survival time: PTEN loss=19.3 months, PTEN intact=15.4 months

Figure 2: K-M curve for CRPC survival stratified by PTEN and volume of metastatic disease



At risk					
	PTEN loss/ low volume disease	40	20	7	3
	PTEN loss/ high volume disease	65	28	10	2
	PTEN intact/ low volume disease	50	29	9	2
	PTEN intact/ high volume disease	72	20	5	

Median survival time: PTEN loss/ low volume disease =20.1 months, PTEN loss/ high volume disease=18.4months
PTEN intact/ low volume disease=25.3 months, PTEN intact/ high volume disease =10.9 Months

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

Nothing to report

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

In the next reporting period, we will focus on finishing the ERG immunohistochemistry and scoring for ECOG3805. In addition, we will score PTEN and ERG in the RTOG96-01 tissues and perform PTEN and ERG immunohistochemistry in the unstained slides from this cohort. Analyses of the RTOG9601 data will be performed in the third year of the project.

IMPACT

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

We have successfully determined PTEN status on 227 cases from both arms of the landmark ECOG3805 trial. We see a paradoxical effect of PTEN loss in patients with high volume metastatic disease, which may be due to the additional benefit seen in these patients with the dual chemohormonal therapy compared to the hormonal therapy alone. Additional analyses will examine ERG status in this cohort and perform further stratification to parse out whether PTEN and/or ERG may be useful predictive biomarkers of additional benefit from combined chemohormonal therapy.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

4. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

5. **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

Nothing to report

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

Database of PTEN/ERG status in ECOG3805 and RTOG 96-01 trial patients. We will make this available to other researcher upon publication via ECOG and NRG.

6. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	*Tamara Lotan*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*Tlotan1*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Lotan supervised IHC data collection and interpretation.*
Funding Support:	*NCI/NIH, Prostate Cancer Foundation, CDMRP-PCRP*

Name:	*Sanjana Murali*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Murali performed IHC data collection and interpretation.*
Funding Support:	*CDMRP-PCRP*

Name:	*Bruce Trock*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	*Btrock1*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Trock performed statistical data analysis*
Funding Support:	*NCI/NIH, CDMRP-PCRP*

Name:	*Angelo De Marzo*
Project Role:	*Co-investigator*

Researcher Identifier (e.g. ORCID ID):	*Ademarz1*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. De marzo assisted with IHC interpretation and data analysis interpretation*
Funding Support:	*NCI/NIH, CDMRP-PCRP*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

NA—this is the first reporting period for the award

What other organizations were involved as partners?

Organization Name: University of California San Francisco

Location of Organization: San Francisco, CA

Partner's contribution to the project

Collaboration : Dr. Felix Feng is a radiation oncologist who will contribute to interpretation of the RTOG9601 data as it is ascertained. He is in charge of the GU Translational Research Program at NRG.

Organization Name: Dana Farber Cancer Institute

Location of Organization: Boston, MA

Partner's contribution to the project

Collaboration : Dr. Chris Sweeney is an oncologist who was PI of the ECOG3805 trial. He is assisting with data interpretation and analysis.

7. SPECIAL REPORTING REQUIREMENTS

Nothing to report

8. APPENDICES: None