Award Number:
W81XWH-14-1-0475

TITLE: The Thoc1 Ribonucleoprotein as a Novel Biomarker for Prostate Cancer Treatment Assignment

PRINCIPAL INVESTIGATOR: David W. Goodrich, Ph.D.

CONTRACTING ORGANIZATION:
Health Research, Inc., Roswell Park Division
Buffalo, NY 14263

REPORT DATE: DEC 2018

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DEC 2018	Final	09/15/14-09/14/2018

4. TITLE AND SUBTITLE
The Thoc1 Ribonucleoprotein as a Novel Biomarker for Prostate Cancer Treatment Assignment

5a. CONTRACT NUMBER
W81XWH-14-1-0475

5b. GRANT NUMBER
PC130746

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David W. Goodrich, Ph.D.

E-Mail: david.goodrich@roswellpark.org

5d. PROJECT NUMBER
0010512649

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Health Research Inc. and
Roswell Park Cancer Institute
Elm & Carlton Streets
Buffalo, NY 14263-0001

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 NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Active surveillance (AS) is an option for men with low risk prostate cancer in order to reduce over treatment, but few men choose it because current prognostic indicators are imperfect. The objectives of this research are to test whether pThoc1 can improve the assignment of prostate cancer patients to therapy. We have completed the goals articulated in the Statement of Work. For specific aim 1, new prostate cancer TMAs have been constructed using specimens from patients treated at Roswell Park. TMAs have been obtained from PCaP. These TMAs have been immunostained for pThoc1, and the immunostaining scored. pThoc1 levels did correlate with some clinical variables, but not others. Immunostaining did not exhibit racial disparities when controlled for disease aggressiveness. For aim 2, prostate cancer specimens have been obtained from patients enrolled on active surveillance, and retrospective specimens have been obtained from PCaP for patients who whould have been eligible for active surveillance. These specimens have been immunostained for pThoc1 and pThoc1 levels analyzed. pThoc1 levels did correlate with some clinical variables, but not others. For aim 3, ELISA assays for pThoc1 protein and pThoc1 autoantibodies have been developed and used to assay serum samples from prostate cancer patients. pThoc1 autoantibody levels are elevated in the serum of prostate cancer patients relative to the serum of healthy control donors. pThoc1 autoantibody levels correlate with some clinical variables but not others. Overall findings suggest pThoc1 levels tend to correlate with prostate cancer aggressiveness, but these correlations do not reach statistical significance in some cases.

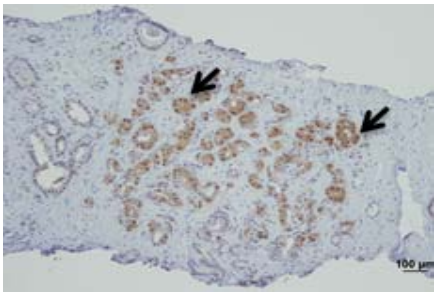
15. SUBJECT TERMS
Prostate cancer, biomarker, active surveillance, prognostic indicator, tissue microarray, immunostaining, ribonucleoprotein

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	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. Introduction



Active surveillance (AS) has been proposed as an option for men with low risk prostate cancer in order to reduce over treatment. Only a fraction of eligible men choose AS, however, because current prognostic indicators are imperfect. Biomarkers that improve upon PSA levels, clinical stage and Gleason score to distinguish between prostate cancers that can be observed safely from those that require immediate treatment could help "right size" recommended treatment. The objectives of this proposal are to test whether pThoc1 can improve the assignment of prostate cancer patients to therapy, to test whether pThoc1 correlates with observed racial disparities in prostate cancer mortality, to determine whether pThoc1 can identify active surveillance patients whose prostate cancer will progress, and to develop methods to quantitate pThoc1 or pThoc1 autoantibody in serum. The general study design is to assay pThoc1 in independent cohorts of clinically annotated prostate cancer biospecimens for which clinical and follow up data is available using previously developed antibody reagents and immunostaining methods. Over treatment is a critical issue complicating the clinical management of prostate cancer. Improving the ability to distinguish aggressive from indolent disease in men newly diagnosed with prostate cancer is recognized as an unmet need by the PCRP Overarching Challenges. Identifying pThoc1 as a biomarker that can help meet this need will have significant impact.

2. Keywords

Prostate cancer, biomarker, active surveillance, prognostic indicator, tissue microarray, immunostaining, ribonucleoprotein

3. Accomplishments

There are three major goals for the proposed work: 1) Characterize pThoc1 levels in independent cohorts of human prostate cancer radical prostatectomy specimens. 2) Characterize pThoc1 levels in a cohort of human prostate cancer patients on active surveillance. 3) Test whether pThoc1 or autoantibodies against pThoc1 can be detected in the serum of prostate cancer patients. These goals have been completed.

1) Characterize pThoc1 levels in independent cohorts of human prostate cancer radical prostatectomy specimens. New prostate cancer TMAs have been constructed from over 1000 patients treated at Roswell Park, and existing TMAs have been obtained from PCaP (PI Mohler). These TMAs have been immunostained for pThoc1 (PI Goodrich)(Figures 1). Increased expression of pThoc1 is observed in cancer lesions compared to adjacent benign glands, consistent with the hypothesis under investigation.

Pathological scoring of most TMAs is complete, but scoring of the large Roswell Park TMA (>1000 specimens) is ongoing (PI Goodrich, Mohler). Interim analysis of pThoc1 immunostaining and clinical data is presented in the appendix (Interim TMA Data Analysis). In summary, pThoc1 immunostaining levels showed significant differences with respect to Gleason grade, stage, NCCN risk level, and prevalence of persistent disease, consistent with the proposed hypothesis. Unexpectedly, significant differences were also observed with respect to age and smoking status. Other significant differences may be detected once analysis of the complete data set is completed. A manuscript describing the results of this work is being prepared for publication.

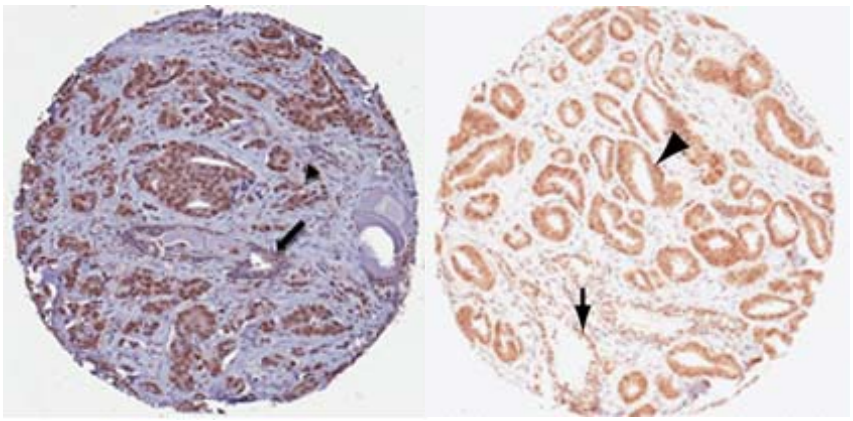


Fig 1. Thoc1 immunostaining of prostate cancer TMAs. Newly constructed prostate cancer TMAs from Roswell Park (left) or TMAs from PCaP (right) were immunostained for Thoc1 protein using our optimized protocol. Increased Thoc1 expression is observed in cancer lesions (arrowhead) compared to cells in an adjacent benign gland (arrow).

A TMA designed to compare Caucasian and African American patients was analyzed to assess racial disparities (PI Goodrich, Mohler). Results are presented in the appendix (Racial Disparities TMA Analysis). In summary, Caucasians prostate cancer patients tended to have higher pThoc1 levels than African American patients. However, this is likely confounded by the older median age of the

Caucasian cohort and the previously detected correlation between pThoc1 level and age. Thus the interim analysis has not detected any clinically relevant racial disparity in pThoc1.

2) Characterize pThoc1 levels in a cohort of human prostate cancer patients on active surveillance. Biopsy specimens from prostate cancer patients qualified for active surveillance have been obtained and are continuing to be enrolled (PI Mohler). Available biopsy specimens have been immunostained for pThoc1 (PI Goodrich)(Figure 2). Pathological scoring of the immunostained biopsy specimen is complete (PI Goodrich)(Figures 3). Analysis of the data is ongoing.

A three gene panel constituting PMP22, CDKN1A and FGFR1 has been shown to stratify low gleason score prostate cancers into indolent and aggressive cancer (Irshad et al., 2013). This panel of genes has been shown to have increased expression in low Gleason score prostate cancers that are likely to remain indolent compared to those which will progress and become agggressive prostate cancer. Thoc1 protein on the other hand is highly expressed in those low Gleason score prostate cancers that are likely to progress to aggressive prostate cancer (Chinnam et al., 2014). Hence, Thoc1 expression in low Gleason score prostate cancer is complimentary to that of the panel of PMP22, CDKN1A and FGFR1. Addition of this complimentary panel of genes to our analysis will help us robustly determine if Thoc1 can stratify low Gleason score prostate cancers into indolent and aggressive prostate cancer. Immunostaining for PMP22, one of the complimentary antigen to Thoc1, has been optimized (PI Goodrich). A set of TMAs made from 700 patients available at RPCI has been immunostained for PMP22 (figure 4). Pathological scoring of this immunostaining and analysis of this data is ongoing.

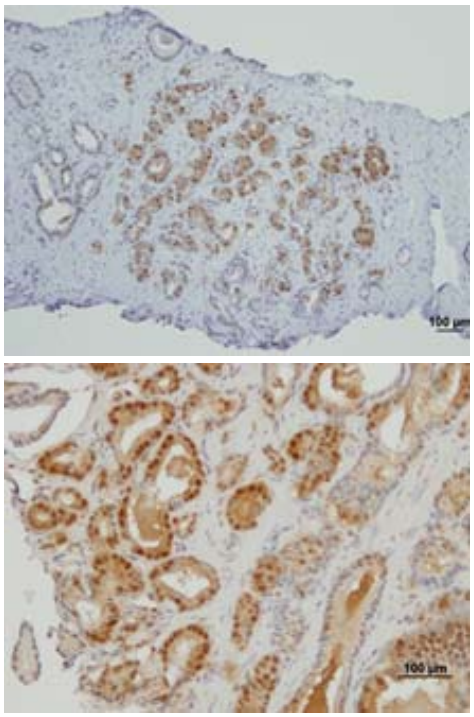


Fig 2: Thoc1 immunostaining of prostate cancer biopsy tissue. Biopsy specimens who qualified for active surveillance from the Roswell Park (top) or PCaP cohorts (bottom) were immunostained for Thoc1 protein. Increased Thoc1 expression is observed in atypical cells of cancerous glands (arrows) compared to adjacent benign gland. Also, the staining pattern is heterogenous.

3) Test whether pThoc1 or autoantibodies against pThoc1 can be detected in the serum of prostate cancer patients. A competitive ELISA assay has been developed and validated for detecting Thoc1 autoantibodies (PI Goodrich) (figure 5). Thoc1 autoantibody levels were measured in serum samples of prostate cancer patients from both PCaP and Roswell Park cohorts using this assay (figure 6). Serum samples from healthy male donors were also analyzed as controls. For the larger PCaP sample set (n=325), the mean serum Thoc1 autoantibody concentration was significantly higher in prostate cancer patients compared to healthy controls (student's t test, $p<0.0001$). There was a statistically significant association of Thoc1 serum autoantibody levels with active surveillance (AS) eligibility, where patients eligible for AS tended to have lower Thoc1 autoantibody levels ($p=0.029$). There was also a trend of higher average Thoc1 autoantibody levels with higher Gleason score, however the association was not statistically significant. Statistically significant associations were not detected in the Roswell Park cohort, likely because of 10-fold smaller sample size (n=32). We not detect racial differences in Thoc1 autoantibody serum concentration levels.

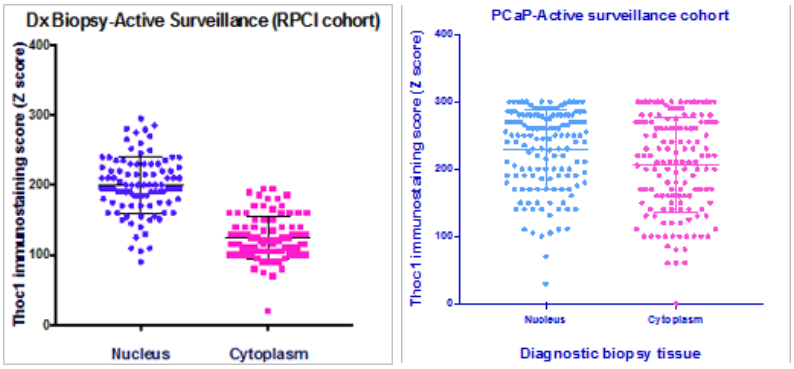


Fig 3. Thoc1 immunostaining scores for prostate biopsy tissue. Graphs show the distribution of nuclear and cytoplasmic Thoc1 immunostaining scores for biopsy specimens from the Roswell Park (left) and PCaP (right) patient cohorts who qualified for active surveillance. Further statistical analysis is underway.

The grant does not support training and professional development, so there is nothing to report.

Given the large data set collected, data analysis is still ongoing. Thus results have yet to be published or presented at public research conferences. Manuscripts are in preparation with anticipated publication in 2019.

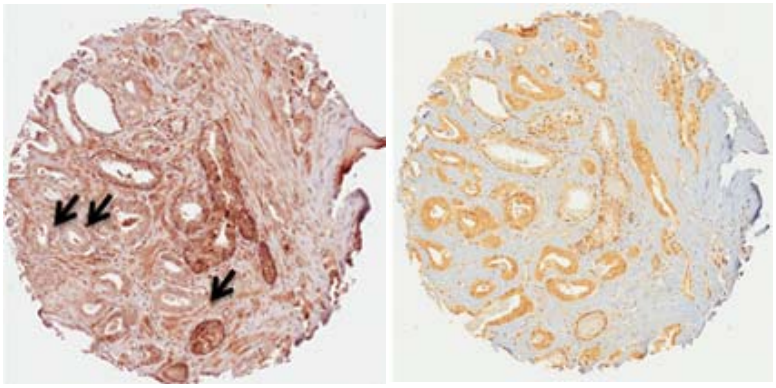


Fig 4: PMP22 immunostaining of Roswell Park prostate cancer TMA. Prostate cancer TMAs from the Roswell cohort were immunostained for PMP22 (left) or Thoc1 (right). Note decreased PMP22 staining in cancer ducts (arrows) whereas the same cancer ducts on adjacent TMA show increased Thoc1 expression.

4. Impact

One major finding from interim data analysis is the confirmation in independent patient cohorts that pThoc1 levels in prostate cancer tissue, as measured by IHC, correlate with clinical measures of disease aggressiveness. Relevant to milestone 1, pThoc1 levels can improve identification of aggressive prostate cancers. We unexpectedly discovered increasing pThoc1 levels with age and smoking. The biological significance of this finding is unknown. We also detected racial differences in pThoc1 levels in prostate cancer patients, with African American patients having lower pThoc1 levels than Caucasian Americans. However this was confounded by the older age of the Caucasian cohort and the correlation with age detected above. Thus we do not detect significant racial disparities in prostate cancer pThoc1 levels (milestone 2).

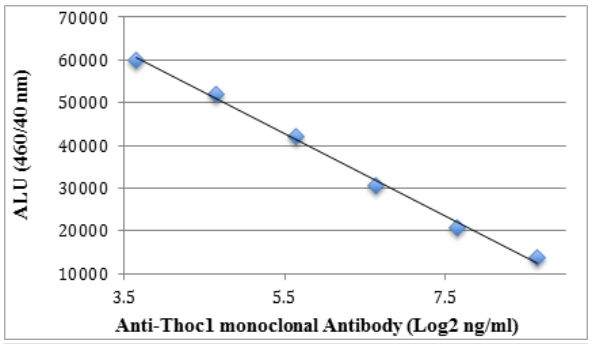


Fig 5: A competitive Elisa assay for measuring anti-Thoc1 autoantibody in patient serum. A competition standard curve was generated from a dilution series (12.5-400 ng/ml) of purified anti-Thoc1 control antibody in competition with anti-Thoc1 biotinylated antibody to demonstrate sensitivity and specificity of the assay.

These findings are potentially significant as pThoc1 levels could potentially be used to more accurately assign patients to active surveillance, reducing prostate cancer over treatment. However, to be clinically feasible, associations between pThoc1 levels and prostate cancer aggressiveness would have to be confirmed in the biopsy specimens used for diagnosis and treatment assignment. Testing this is the goal of specific aim 2. While all the data has been collected, data analysis is not complete. Achieving milestone 3 awaits completion of this data analysis which is expected by early 2019.

Another approach for measuring pThoc1 in a clinically feasible fashion for treatment assignment, is measurement of autoantibodies in serum of prostate cancer patients. Autoantibody generation is hypothesized to increase in prostate cancer patients due to increased extracellular exposure to pThoc1 containing ribonucleoproteins shed by cancer cells. The goals of specific aim 3 were to develop an ELISA assay to measure Thoc1 autoantibodies and test the hypothesis by analysis of prostate cancer patient specimens. Interim data analysis is consistent with the hypothesis. Importantly, Thoc1 autoantibody levels were significantly lower in prostate cancer patients with less aggressive disease who were eligible for active surveillance based on current prognostic indicators, suggesting Thoc1 autoantibody levels may improve identification of patients whose disease is likely to progress (milestone 4). Since this is a serum biomarker, Thoc1 autoantibody levels could also be used to monitor patients on active surveillance to identify those patients whose disease may be progressing. We did not detect racial disparities in Thoc1 autoantibody levels (milestone 5).

What was the impact on the development of the principal discipline(s) of the project? This project has identified biomarkers that correlate with prostate cancer aggressiveness that can potentially be used to help distinguish patients whose disease is likely to progress from those that can safely be monitored by active surveillance. One of these biomarkers is a serum biomarker, potentially permitting non-invasive, continuous monitoring in patients. We have developed an ELISA assay for measuring this serum biomarker, an assay that is not currently available. Further research is warranted to determine if pThoc1 or Thoc1 autoantibodies can be developed into a robust, useful clinical test. It is less likely that a single biomarker like Thoc1 will have sufficient specificity. To increase specificity, we have explored the use of a complementary biomarker whose expression is expected to decrease in aggressive prostate cancers (PMP22). Use of Thoc1 with PMP22 could be used to increase biomarker specificity.

What was the impact on other disciplines? Since elevated Thoc1 expression is observed in other cancers, pThoc1 immunostaining levels or serum Thoc1 autoantibody levels could potentially be used as biomarkers of aggressiveness for other cancers. The presence of the THO ribonucleoprotin complex, or antibodies directed against it, have not been measured in serum or other bodily fluids, either in humans or animal models. These measurements may impact understanding of ribonucleoproteins in general and their ability to elicit autoantibody responses.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology? Nothing to report.

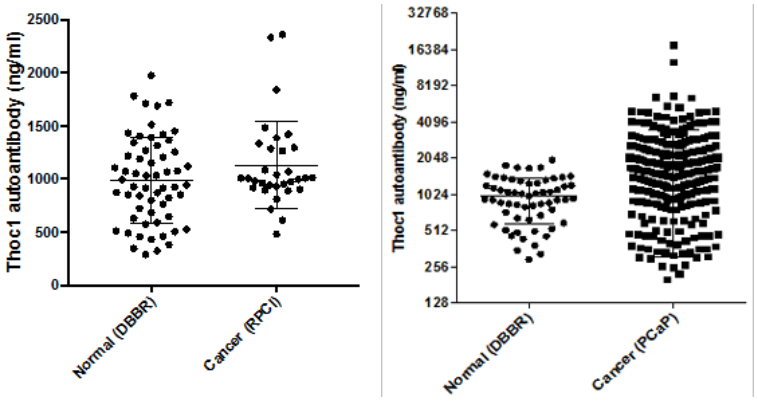


Fig 6: Thoc1 autoantibody levels in the serum of prostate cancer patients. Serum Thoc1 autoantibody levels were measured in prostate cancer patients from the Roswell Park (left) or PCaP cohorts (right). Serum collected from age and race matched healthy volunteers at Roswell Park were also measured.

5. Changes/Problems

Task 1 in specific aim 3, analysis of Thoc1 autoantibodies in mouse models of prostate cancer, was not completed. The ELISA assay was optimized for detection of human autoantibodies, and it was found to be unsuitable for measuring autoantibodies in mouse serum. We proceeded with analysis of human serum samples in the absence of data from mice.

6. Products

As analysis of the large dataset is ongoing, results have not yet been disseminated through publications, conference papers, website(s), or presentations. No inventions, patent applications, and/or licenses have been produced.

ELISA assays were developed for measuring pThoc1 or pThoc1 autoantibodies in serum. If utility of these assays as serum biomarkers of prostate cancer aggressiveness is established, results will be shared through journal publications and meeting presentations.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

David W. Goodrich, Ph.D. (Initiating PI): No change in effort or funding support since the last annual report.

Meenalakshmi Chinnam, Ph.D. (postdoctoral fellow who performed the work for Initiating PI): No change in effort or funding support since last annual report.

James L. Mohler, M.D. (Partnering PI): No change in effort or funding support since the last annual report.

Gissou Azabdaftari, M.D. (Co-Investigator working with the PIs to score the TMAs): No change in effort or funding support since the last annual report.

Kristopher Attwood, Ph.D. (Biostatistician working with the PIs to analyze the data): No change in effort or funding support since the last annual report.

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? No other organizations are involved in the research.

8. Special Reporting Requirements

This grant funds a Synergistic Idea Development Award in collaboration with Dr. James Mohler (Partnering PI, Roswell Park Cancer Institute). Dr. Mohler will be submitting an identical final report to satisfy the special reporting requirements.

9. References:

Irshad S, Bansal M, Castillo-Martin M, Zheng T, Aytes A, Wenske S, Le Magnen C, Guarnieri P, Sumazin P, Benson MC, Shen MM, Califano A, Abate-Shen C. A molecular signature predictive of indolent prostate cancer. Sci Transl Med. 2013 Sep 11;5(202):202ra122.

Chinnam M, Wang Y, Zhang X, Gold DL, Khoury T, Nikitin AY, Foster BA, Li Y, Bshara W, Morrison CD, Payne Ondracek RD, Mohler JL, Goodrich DW.
The Thoc1 ribonucleoprotein and prostate cancer progression. J Natl Cancer Inst. 2014 Oct 8;106(11).

10. Appendices

Interim TMA Data Analysis

Statistical Methods

1. Patient demographic and clinical characteristics are summarized by cohort using the appropriate descriptive statistics. Thoc1 immunostaining scores (nuclear and cyto) for both PCa and benign tissue are summarized by cohort using the mean, median, and standard deviation; and presented graphically using dot-plots. Comparisons were made using the Mann-Whitney U and Fisher's exact test, as appropriate.

2. The Thoc1 immunostaining scores are summarized by patient characteristic using the mean, median, standard deviation, and inter-quartile range (IQR). Associations were evaluated using the two-sided Jonckheere's trend test.

3. The relationship between the different Thoc1 immunostaining scores were presented graphically using a scatter plot matrix; and evaluated using the Spearman correlation coefficient.

4. The association between Thoc1 immunostaining scores (dichotomized at the median) and time-to-event outcomes are summarized using standard Kaplan-Meier methods and evaluated using the stratified log-rank test.
    a. Overall Survival (OS) = Time from RP until death or last vital follow-up.
    b. PCa Specific Survival (PCaS) = Time from RP until death due to PCa or last vital follow-up.
    c. Progression-free Survival (PFS) = Time from RP until progression (AUA, Phoenix, or Tx), death due to PCa, or last recurrence follow-up.
        i. The analysis of PFS excludes patients that had persistent disease.
    d. RP Failure-free Survival (RPFS) = Time from RP until persistent disease, progression (AUA, Phoenix, or Tx), death due to PCa, or last recurrence follow-up.

5. All analyses were conducted in SAS v9.4 (Cary, NC) at a significance level of 0.05.

Results

1. There were n=152 patients from PCaP and n=184 from Roswell PArk that had at least one Thoc1 immunostaining score. Summary of patient characteristics and Thoc1 scores:
    a. There were significant differences in age, smoking status, Gleason grade, stage, NCCN risk level, and prevalence of persistent disease.
    b. The Thoc1 PCa nuclear and benign cyto intensities were also significantly different; where the Roswell cohort tended to have higher PCa and lower benign values.

2. Summary of associations between Thoc1 scores and patient characteristics.
    a. For PCa nuclear intensity, there were significant associations with age ($p=0.016$) and race ($p<0.001$), Gleason grade ($p=0.006$), stage ($p=0.006$), and NCCN risk ($p=0.045$); where intensity increased with age, grade, stage, and risk; and tended to be higher in whites.
    b. For PCa cyto intensity and benign nuclear intensity, there was a significant association with race ($p=0.021$ and $p=0.025$); where whites tended to have higher intensity.
    c. For benign cyto intensity, there was a significant association with stage ($p=0.021$); where intensity decreased with stage.
    d. For the PCa nuclear and cyto groups, there was a significant association with race ($p=0.043$).

3. Summary of associations between Thoc1 scores and the time-to-event outcomes.
    a. For PCa nuclear intensity, there was a significant association with PFS ($p=0.022$); where the subjects with high intensity had slightly poorer outcomes.

b. For benign nuclear and cyto intensity, there was a significant association with OS (p=0.036 and p=0.026); where the subjects with high intensity had slightly poorer outcomes.
c. For the PCa nuclear+cyto groups, there were significant associations with PFS (p=0.036) and RPF (p=0.049); where patients with correlated nuclear and cyto intensity (either both high or both low) tended to have poorer outcomes.
d. No significant associations were observed with respect to PCa cyto intensity or the benign nuclear+cyto groups.

Demographic and Clinical Characteristics

		PCaP	**Roswell Park**	**Overall**	**P-value**
Overall	N	152 (45.2)	184 (54.8)	336 (100%)	
Age	Mean/Std/N	60.4/7.1/152	57.8/7.3/184	59.0/7.3/336	0.002
	Median/Min/Max	61.0/41.0/76.0	57.0/41.0/78.0	59.0/41.0/78.0	
Age*	<= 55	42 (27.6%)	70 (38.0%)	112 (33.3%)	0.030
	55-60	33 (21.7%)	46 (25.0%)	79 (23.5%)	
	60-65	38 (25.0%)	42 (22.8%)	80 (23.8%)	
	> 65	39 (25.7%)	26 (14.1%)	65 (19.3%)	
Race	White	80 (52.6%)	92 (50.0%)	172 (51.2%)	0.63
	Black	72 (47.4%)	92 (50.0%)	164 (48.8%)	
Smoker	Never	41 (27.0%)	64 (35.2%)	105 (31.4%)	0.023
	Former	83 (54.6%)	72 (39.6%)	155 (46.4%)	
	Current	28 (18.4%)	46 (25.3%)	74 (22.2%)	
Other Ca Hx	No	138 (90.8%)		138 (41.1%)	-
	Yes	14 (9.2%)		14 (4.2%)	
	Unknown		184 (100.0%)	184 (54.8%)	
PSA	Mean/Std/N	20.9/163.5/150	8.1/8.7/182	13.9/110.1/332	0.29
	Median/Min/Max	5.4/1.1/2008.0	6.1/0.0/82.1	5.9/0.0/2008.0	
PSA*	<= 10	122 (80.3%)	153 (84.1%)	275 (82.3%)	0.19
	10-20	22 (14.5%)	18 (9.9%)	40 (12.0%)	
	> 20	6 (3.9%)	11 (6.0%)	17 (5.1%)	
	Unknown	2 (1.3%)		2 (0.6%)	
Charlson	0	91 (59.9%)		91 (27.1%)	-
	1	36 (23.7%)		36 (10.7%)	
	2	15 (9.9%)		15 (4.5%)	
	3+	10 (6.6%)		10 (3.0%)	
	Unknown		184 (100.0%)	184 (54.8%)	
Path Gleason Grade	< 7	90 (59.2%)	32 (17.5%)	122 (36.4%)	<.001
	= 7	56 (36.8%)	131 (71.6%)	187 (55.8%)	
	> 7	6 (3.9%)	20 (10.9%)	26 (7.8%)	
Stage	T1	95 (62.5%)		95 (28.3%)	<.001
	T2	52 (34.2%)	119 (64.7%)	171 (50.9%)	
	T3-4	2 (1.3%)	51 (27.7%)	53 (15.8%)	

		PCaP	Roswell Park	Overall	P-value
	Unknown	3 (2.0%)	14 (7.6%)	17 (5.1%)	
NCCN Risk Level	Low	77 (50.7%)	59 (32.1%)	136 (40.5%)	<.001
	Intermediate	61 (40.1%)	78 (42.4%)	139 (41.4%)	
	High	12 (7.9%)	33 (17.9%)	45 (13.4%)	
	Unknown	2 (1.3%)	14 (7.6%)	16 (4.8%)	
Persistent Disease	No	77 (50.7%)	148 (80.4%)	225 (67.0%)	<.001
	Yes	9 (5.9%)	31 (16.8%)	40 (11.9%)	
	Unknown	66 (43.4%)	5 (2.7%)	71 (21.1%)	
Eligible for AS	No	122 (80.3%)		122 (36.3%)	-
	Yes	30 (19.7%)		30 (8.9%)	
	Unknown		184 (100.0%)	184 (54.8%)	

Associations with Thoc1 Immunostaining Scores

		n	Mean (SD)	Median (IQR)	P-value
Age	<=55	78	200.1 (58.8)	201.6 (165.0-240.0)	0.016
	55-60	62	212.2 (58.7)	215.0 (190.0-260.0)	
	60-65	58	220.5 (57.0)	225.0 (178.3-275.0)	
	>65	57	223.5 (49.3)	220.0 (192.8-265.0)	
Race	White	126	226.0 (55.1)	225.0 (192.0-276.5)	<.001
	Black	129	200.2 (55.7)	205.0 (175.3-240.0)	
Smoker	Never	80	210.5 (59.2)	210.0 (182.5-265.0)	0.87
	Former	118	217.2 (52.7)	218.8 (190.0-260.0)	
	Current	56	206.4 (61.9)	209.2 (178.8-263.3)	
Other Ca Hx	No	101	205.2 (40.8)	204.3 (186.0-223.3)	0.11
	Yes	12	223.0 (47.0)	241.3 (190.4-250.8)	
PSA	<=10	206	212.9 (59.2)	215.0 (180.7-265.0)	0.55
	10-20	30	214.1 (46.2)	208.2 (180.0-251.0)	
	>20	15	205.8 (48.3)	210.0 (190.0-225.0)	
Charlson	0	67	203.1 (42.4)	203.5 (186.0-221.7)	0.32
	1	26	213.2 (37.8)	210.0 (196.7-230.3)	
	2	13	213.4 (39.6)	200.0 (178.3-246.0)	
	3+	7	210.5 (55.2)	220.0 (179.2-253.3)	
Gleason Grade	<7	88	204.4 (54.0)	205.9 (178.3-238.8)	0.006
	=7	146	213.4 (56.9)	210.0 (186.0-265.0)	
	>7	20	243.7 (59.3)	252.9 (217.5-290.0)	
Stage	T1	68	202.7 (42.4)	203.9 (181.6-225.3)	0.006
	T2	131	213.1 (59.2)	210.0 (180.0-265.0)	
	T3-4	45	225.7 (62.8)	240.0 (190.0-275.0)	
NCCN Risk Level	Low	98	203.7 (58.3)	203.9 (168.0-250.0)	0.045

		n	Mean (SD)	Median (IQR)	P-value
	Intermediate	109	213.1 (58.1)	215.0 (180.0-265.0)	
	High	36	225.9 (46.5)	217.5 (200.3-261.3)	
Persistent Disease	No	174	212.6 (57.3)	210.0 (180.0-261.7)	0.59
	Yes	32	214.1 (72.3)	243.0 (170.7-272.5)	
Eligible for AS	No	91	206.8 (43.0)	205.0 (186.0-230.3)	0.98
	Yes	22	208.2 (36.6)	205.3 (182.5-225.5)	

Racial Disparities TMA Data Analysis

Statistical Methods

1. Patient characteristics were reported by race using the mean, median, and standard deviation for continuous variables; and using frequencies and relative frequencies for categorical variables. Comparisons were made using eh Mann-Whitney U and Fisher's exact tests, respectively.

   For each tissue type (cancer and benign) and score type (nuclear and cyto), the Thoc1 intensity scores were summarized by race using the mean, median, standard deviation, and inter-quartile range (IQR); and graphically using dot plots. Comparisons were made using the Mann-Whitney U (NP test) and the independent sample T-test (Parametric test).

   The association between race and time-to-event outcomes was evaluated using standard Kaplan-Meier methods, with comparisons made using the log-rank test.
   - Overall Survival (OS) = time from RP until death or last follow-up.
   - Disease Specific Survival (DS) = time from RP until death due to disease or last-follow-up.
   - Freedom from Biochemical Recurrence (BCR) = time from RP until recurrence (NCCN criteria) or last follow-up. BCR is not calculated for patients with persistent disease (per NCCN definition).
   - Freedom from Biochemical Failure (BFR) = time from RP until persistent disease, NCCN recurrence, or last follow-up.
   - Freedom from RP Failure (RPF) = time from RP until persistent disease, NCCN recurrence, post-RP treatment, or last follow-up.
   - Freedom from Metastatic Disease (METS) = time from RP until development of metastatic disease or last follow-up.

2. The Thoc1 intensity scores are summarized by patient characteristic using the mean, median, standard deviation, and IQR. Associations are evaluated using the Mann-Whitney U and T-tests or the Kruskal-Wallis and one-way ANOVA tests, as appropriate.

3. The time-to-event outcomes are summarized by Thoc1 intensity using standard Kaplan-Meier methods, with associations evaluated using the log-rank test.
   a. The Thoc1 intensity scores are categorized into low (less than the 25^{th} percentile), high (greater than the 75^{th} percentile), and intermediate (between the 25^{th} and 75^{th} percentiles).

4. The Thoc1 intensity scores are compared between tissue types (cancer and benign) and score types (nuclear and cyto) using the signed rank and paired T-tests. The correlation between the Thoc1 intensity scores is evaluated using scatter plots and the corresponding Spearman correlation coefficients.

5. All analyses were completed in SAS v9.4 (Cary, NC) ate a significance level of 0.05.

Results

There were a total of n=184 subjects with demographic/clinical data and at least one Thoc1 intensity score.

1. Analysis of racial differences.
    a. *Demographic and Clinical Characteristics*: There were significant differences in age (p<0.001) and tobacco use (p=0.005); where the Caucasian subjects tended to be older and less likely to be active smokers.

    b. *Thoc1 Intensity*: There is a high degree of variability in the Thoc1 intensity scores for both racial cohorts, and significant differences were observed in the cancer tissue for both nuclear (p<0.001) and cyto (p=0.006) intensity. In general, the Caucasians had higher Thoc1 intensity.

    c. *Time-to-Event Outcomes*: There were no significant associations (all p > 0.05).
        i. There were a total of n=183 patients with survival (OS and DS) and metastatic (METS) data.
        ii. There were n=179 patients with adequate follow-up for treatment failure (BFR and RPF).
        iii. There were a total of 31 subjects with persistent disease, so only n=148 have adequate follow-up and data for NCCN biochemical recurrence (BCR).

2. Analysis of Thoc1 intensity and time-to-event outcomes.
    a. *Cancer Tissue – Nuclear*: No significant associations were observed.
    b. *Cancer Tissue – Cyto*: No significant associations were observed.
    c. *Benign Tissue – Nuclear*: No significant associations were observed.
    d. *Benign Tissue – Cyto*: A significant association was observed with OS (p<0.001); where subjects with high Thoc1 intensity (>Q3) had poorer outcomes.

Analysis of Race

		White	**Black**	**Overall**	**P-value**
Overall	N	92 (50.0)	92 (50.0)	184 (100%)	
Age (at Dx)	Mean/Std/N	59.79/6.84/92	55.84/7.17/92	57.82/7.26/184	<.001
	Median/Min/Max	59.00/41.00/78.00	55.00/41.00/69.00	57.00/41.00/78.00	
Age*	<= 55	23 (25.0%)	47 (51.1%)	70 (38.0%)	<.001
	> 55	69 (75.0%)	45 (48.9%)	114 (62.0%)	
Alcohol Use	Never	11 (23.4%)	27 (40.3%)	38 (33.3%)	0.12
	Past/Former	7 (14.9%)	5 (7.5%)	12 (10.5%)	
	Current	29 (61.7%)	35 (52.2%)	64 (56.1%)	
Tobacco Use	Never	32 (35.6%)	32 (34.8%)	64 (35.2%)	0.005
	Past/Former	44 (48.9%)	28 (30.4%)	72 (39.6%)	
	Current	14 (15.6%)	32 (34.8%)	46 (25.3%)	
Clinical Gleason	< 7	46 (51.1%)	40 (43.5%)	86 (47.3%)	0.49
	= 7	36 (40.0%)	40 (43.5%)	76 (41.8%)	
	> 7	8 (8.9%)	12 (13.0%)	20 (11.0%)	
Clinical AJCC Stage	I	2 (2.9%)	6 (9.7%)	8 (6.2%)	0.16
	II	64 (94.1%)	52 (83.9%)	116 (89.2%)	
	III	2 (2.9%)	4 (6.5%)	6 (4.6%)	
Grade	II	28 (30.4%)	26 (28.3%)	54 (29.3%)	0.87
	III	64 (69.6%)	66 (71.7%)	130 (70.7%)	
Pre-RP ADT	No	82 (90.1%)	85 (92.4%)	167 (91.3%)	0.61
	Yes	9 (9.9%)	7 (7.6%)	16 (8.7%)	
Pre-RP XRT	No	35 (100.0%)	46 (100.0%)	81 (100.0%)	-

		White	Black	Overall	P-value
Mets at RP	No	89 (97.8%)	89 (96.7%)	178 (97.3%)	1.00
	Yes	2 (2.2%)	3 (3.3%)	5 (2.7%)	
Persistent Disease	No	77 (86.5%)	71 (78.9%)	148 (82.7%)	0.236
	Yes	12 (13.5%)	19 (21.1%)	31 (17.3%)	
Path Gleason	< 7	17 (18.7%)	15 (16.3%)	32 (17.5%)	0.94
	= 7	64 (70.3%)	67 (72.8%)	131 (71.6%)	
	> 7	10 (11.0%)	10 (10.9%)	20 (10.9%)	
Path AJCC Stage	I/II	57 (71.3%)	42 (61.8%)	99 (66.9%)	0.23
	III	20 (25.0%)	19 (27.9%)	39 (26.4%)	
	IV	3 (3.8%)	7 (10.3%)	10 (6.8%)	
Post=RP ADT	No	77 (84.6%)	79 (86.8%)	156 (85.7%)	0.83
	Yes	14 (15.4%)	12 (13.2%)	26 (14.3%)	
Post-RP XRT	No	72 (79.1%)	67 (73.6%)	139 (76.4%)	0.48
	Yes	19 (20.9%)	24 (26.4%)	43 (23.6%)	
Nodes Examined	Mean/Std/N	2.70/3.84/89	3.00/3.78/92	2.85/3.80/181	0.48
	Median/Min/Max	1.00/0.00/22.00	2.00/0.00/18.00	2.00/0.00/22.00	
Nodes Examined*	0	37 (41.6%)	36 (39.1%)	73 (40.3%)	0.91
	< 15	50 (56.2%)	54 (58.7%)	104 (57.5%)	
	15 +	2 (2.2%)	2 (2.2%)	4 (2.2%)	
Nodes Positive	Negative	52 (100.0%)	53 (94.6%)	105 (97.2%)	0.24
	Positive		3 (5.4%)	3 (2.8%)	
Margin Status	Negative	67 (73.6%)	62 (67.4%)	129 (70.5%)	0.42
	Positive	24 (26.4%)	30 (32.6%)	54 (29.5%)	