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TITLE: PREDICTING PROSTATE CANCER PROGRESSION AT TIME OF DIAGNOSIS

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14. ABSTRACT (200 words)
We have made further progress toward the stated goals of the project over the past year. We have completed accession and processing of all 397 blood specimens from UCSF (Aim 1), as well as blood and urine specimens for 450 PASS participants (Aim 2). We have accessioned >180 of the tissue specimens as well. The remaining blood specimens from University of Washington (Aim 1) are expected within the next several weeks, and we are in the process of collecting the remaining tissue specimens. Preliminary serum studies suggest an association of IL-6 levels, but not TGF-β levels, with upgrading / upstaging between biopsy and surgery. Urinary PCA3 and TMPRSS2:ERG levels are associated with higher baseline risk characteristics, and combining them with PSA improves risk prediction compared to PSA alone. We have begun work on our DNA extraction and aCGH analyses as well, and have collected over 1500 quality of life questionnaires on >500 PASS participants. Two projects focusing on PSA data in PASS have suggested ways of optimizing use of such data in active surveillance protocols. We look forward to completing our analyses and reporting results by the end of the final year.

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Introduction

Identification of new biomarkers that more accurately distinguish indolent from aggressive low-risk prostate cancers would have a major impact on prostate cancer management. Patients with occult aggressive disease could be counseled appropriately for immediate treatment, while those with confirmed indolent disease could select and remain on surveillance with more confidence, and likely with a lesser burden of follow-up testing. Our aims are to validate, in both a pair of radical prostatectomy cohorts and in a multicenter active surveillance cohort, a set of urine, blood, and tissue-based biomarkers with respect to their prognostic utility.

Body

Task 1: Blood and tissue organization for Aim 1
We have completed accession and processing of all serum/plasma specimens from UCSF. As noted last time, the marginal cost for additional ELISA wells is negligible, so we began with N=397 available specimens, i.e., 97 additional specimens beyond the original specified case-control study. We have also pulled >180 tissue specimens from the UCSF pathology archives, and have begun re-reading and punching the cases. For this work we planned to rely on core pathology services at UCSF not explicitly budgeted in the grant. A similar situation applies at UW re: both tissue and blood specimen. Given recent budget constraints and staff cutbacks at both institutions, these tasks have been delayed are behind schedule. However, we have negotiated some additional modest support from Myriad Genetics, our industry partner on this project; this is in the final stages of contracting, and we anticipate we will be able to make up rapidly for lost time in the next few months.

Task 2: Blood, urine, and tissue organization for Aim 2
The total enrollment to the Prostate Active Surveillance Study (PASS) is now over 930. All of these men have contributed baseline urine and serum specimens. Mean follow-up at this point is approximately 3.4 years from diagnosis, and 2.0 years from study enrollment (2.8 years from enrollment for the first 450 enrollees who are the focus of the Aim 2). At study entry, mean age is 63, mean PSA is 5.0, 92% of participants have Gleason score 6, and 96% have < 33% of biopsy cores positive for cancer. Over 220 men have progressed by study criteria. The specified analyses in this grant will focus on the first 450 enrollees. Nearly 150 of these 450 have progressed by study criteria, which is consistent with baseline expectations when the statistical plan was generated.

Task 3: Serum analyses (Aims 1 and 2)
We have completed all TGFβ1 and IL6SR analyses on the N=397 UCSF Aim 1 specimens (Table 1) and the N=505 PASS Aim 2 specimens. All patients were diagnosed in 2000 or

later with low risk disease (diagnosis PSA < 10 ng/ml, clinical stage T1-2, biopsy Gleason grade 2-6) and underwent radical prostatectomy monotherapy within 6 months.

Table 1. UCSF Aim 1 Cohort characteristics at diagnosis among 397 men with low-risk prostate cancer

CHARACTERISTICS AT DIAGNOSIS	
AGE (YEARS), Mean (SD)	58.6 (6.85)
PSA (NG/ML), Median (IQR)	5.2 (4.2-6.5)
BIOPSY CORES % POSITIVE, Median (IQR)	23 (13-41)
CAPRA CLNICAL RISK, Mean (Range)	2 (0-3)
RACE, N (%)	
Asian/Pacific Islander	14 (4)
African American	10 (2)
Caucasian	345 (87)
Mixed	17 (4)
Other	11 (3)

TGFβ1 levels can be affected by platelet integrity in stored specimens, so we also calculated a ratio of PF4 to TGFβ1 to account for potential platelet degranulation in the samples. Serum concentration levels of IL6-SR, TGF-*β*1, and PF4-normalized TGF-*β*1 were reported (Table 2). We found wide ranges in both IL-6 (mean 42.2, SD 12.7, range 13.3-93.7) and TGFβ1 (mean 11.2, SD 7.5, range 0.4-41.3).

Table 2. Plasma concentration levels of biomarker assays among 397 men with low-risk prostate cancer

CONCENTRATION	N	Mean (SD)	Range	Median (IQR)
IL6-SR (NG/ML)	397	42.2 (12.7)	13.3, 93.7	41.7 (32.6-50.2)
TGF-*β*1 (NG/ML)	396	11.2 (7.48)	0.4, 41.3	9.1 (5.6-14.8)
PF4 (NG/ML)	377	1795.4 (1651.66)	0, 16274.7	1449.1 (862.7-2169.2)
RATIO PF4/TGF-*β*1	372	170.4 (104.8)	6, 1492	158.5 (122.5-194.2)

Primary outcomes at RP were rates of upgrade (UG) to Gleason 3+4 or higher and upstage (US) to pT3/4 (Table 3). All patients had at least 6 biopsy cores taken at diagnosis.

Table 3. Rates of upgrading and upstaging at radical prostatectomy among 397 men with low-risk prostate cancer

OUTCOMES		N (%)
UPGRADE	No	226 (57)
	Yes	170 (43)
	Missing	1
UPSTAGE	No	336 (85)
	Yes	61 (15)
ANY CHANGE	No upstage or upgrade	209 (53)
	Upstage only	17 (4)
	Upgrade only	126 (32)
	Both upstage and upgrade	44 (11)
	Missing	1

Associations between levels of serum concentration and UG/US outcome groups were evaluated using logistic regression models that included serum levels as logarithmic values. Models were adjusted for age at diagnosis, Caucasian race, percentage of positive biopsy cores, and diagnostic PSA. A p-value <0.5 was considered significant. Very preliminary analyses suggest that among the UCSF Aim 1 specimens, IL-6 is independently associated with upstaging but not upgrading. Adjusted TGFβ1 shows similar trends, though they do not reach statistical significance among these specimens. (Table 4-5) These analyses will be finalized once the UW specimens have been processed. Aim 2 results on PASS patients have been transferred to the VSIMS database for integration with the rest of the PASS data.

Table 4. TGFβ1 and association with upgrading and upstaging among 397 men with low-risk prostate cancer

	UPGRADE			**UPSTAGE**		
EFFECT	OR	95%CI	P	OR	95%CI	P
TGFβ1 (LOG)	1.08	0.81, 1.40	0.61	1.42	0.98, 2.04	**0.06**
AGE	0.95	0.92, 0.98	<.01	0.94	0.90, 0.98	<.01
CAUCASIAN	0.53	0.28, 1.02	0.06	0.32	0.09, 1.07	0.06
% POS CORES	0.99	0.98, 0.99	0.02	0.98	0.97, 0.99	<.01
PSA	0.88	0.78, 0.98	0.02	0.95	0.82, 1.11	0.52

Table 5. IL-6 SR and association with upgrading or upstaging among 397 men with low-risk prostate cancer

	UPGRADE			UPSTAGE		
EFFECT	OR	95%CI	P	OR	95%CI	P
IL6-SR (LOG)	1.66	0.83, 3.32	0.15	2.84	1.11, 7.29	**0.03**
AGE	0.95	0.92, 0.98	<.01	0.95	0.91, 0.99	0.01
CAUCASIAN	0.51	0.26, 0.97	0.04	0.28	0.08, 0.94	0.04
% POS CORES	0.99	0.98, 1.00	0.01	0.98	0.97, 0.99	<.01
PSA	0.88	0.79, 0.98	0.02	0.96	0.83, 1.12	0.63

Task 4

As noted last year, N=588 PASS participants have had post-DRE urine specimens transferred to GenProbe for analysis of urinary PCA3 and TMPRSS2:ERG levels, all of which have now been processed. Preliminary analyses suggest positive associations between the urinary markers and baseline tumor characteristics. A paper describing associations between these urine markers and baseline risk characteristics on the first N=387 men in PASS has been published (Lin et al, Clin Cancer Res 2013; please see full manuscript attached at end). Analysis on the full cohort and analysis of the markers as predictors of progression on surveillance will be completed this year.

Task 5

As noted previously, the first batch of de-identified tissue specimens from UCSF (N=82) has been transferred to Myriad Genetics for analysis. These results remain blinded to all investigators, but preliminary communications suggest that only 5 cases were non-informative, and Prolaris scores have been successfully computed for the remainder. With respect to the GEMCaP analyses, we have extracted DNA from a first batch of 13 punched samples. The average DNA yield was sufficient at 3 ug per sample, and the DNA quality was good. We explored quantitating the samples by uv-vis spectrophotometry and fluorometry. The latter better predicted array comparative genomic hybridization (aCGH) results as indicated by QC parameters. Time has also been devoted to optimizing the aCGH protocol to improve aCGH QC metrics. This has now been completed. Using the optimized protocol, 8 samples currently have acceptable aCGH results. Once 25 samples have been completed (anticipated within the next few weeks), we can monitor whether the GEMCaP biomarker loci are aberrant and detectable in this patient set.

Task 6

The VSIMS database has been updated to accommodate new tissue-based data fields, and biomarker data are being entered as they become available. We are awaiting maximal follow-up in the PASS cohort before finalizing any biomarker analyses in this

cohort. Likewise, while we have performed preliminary serum studies among the UCSF Aim 1 specimens, we await results from the UW specimens before finalizing these or preparing publications. In the meantime, we have continued analyses of PSA data from PASS participants with the intent of better understanding of PSA kinetics in the active surveillance setting. Two abstracts have been presented as posters and manuscripts are close to completion for both (see below). Finally, we have collected roughly 1500 baseline and follow-up quality of life questionnaires on over 500 PASS participants.

Key Research Accomplishments

- Analysis of 387 baseline urine specimens in PASS (Aim 2) for PCA3 and TMPRSS2:ERG indicates that both markers are associated with higher-volume prostate cancer and with the presence of high Gleason grade tumors at baseline. Both markers combined with PSA yielded better ROC curve results for prediction of high grade disease (AUC 0.70) than any of the markers alone.

- We have found that a declining PSA after initial diagnosis is a strong predictor of subsequent non-progression on active surveillance. This is an important finding given the common scenario of a man who is over-diagnosed with a clinically insignificant prostate cancer when his PSA bumps up transiently for some non-cancer-related cause and then normalizes after a biopsy has been triggered by the rise. Aside from the potential usefulness of declining PSA as a marker in its own right, these findings emphasize the necessity of verifying an unexpected PSA elevation with a repeat assessment.

- We hypothesized that for some men active surveillance might be safely performed with a less intense schedule of observation. We calculated PSA doubling times (PSADT) using the standard q3 month interval of measurement, then re-calculated these values using only every even-numbered measurement or every odd-numbered measurement, finding that in most cases PSADT measured on a q6 month interval is very similar to that measured on a q3 month interval.

Reportable Outcomes

1. A manuscript, “Urinary TMPRSS2:ERG and PCA3 in an Active Surveillance Cohort: Results from a Baseline Analysis in the Canary Prostate Active Surveillance Study” (Lin DW et al, Clin Cancer Res 2013; 19:2442) has been published. A reprint of this report has been included at the end.

2. Two posters were developed based on the PSA analyses described above: "The impact of reducing the frequency of prostate specific antigen testing among men on active surveillance for prostate cancer" (Cooperberg et al) was presented at the 2013 American Urological Association annual meeting; and "Declining PSA values are associated with a lower risk of progression in the Canary/EDRN Prostate Active Surveillance Study (PASS)" was presented at the 2013 American Society of Clinical Oncology Genitourinary Cancers Symposium. (Copies of these posters/abstracts have also been attached.)

3. We have continued to develop the bioinformatics infrastructure and FFPE tissue repository resources at UCSF described last year.

4. Partly drawing on and building from our biomarker validation experience accumulating under this grant and elsewhere, we competed successfully for a 2012 DOD Transformative Impact Award PC121236 "Development, validation, and dissemination of an integrated risk prediction model and decision aid to discern aggressive versus indolent prostate cancer," which is in late stages of budget negotiation.

Conclusion

We have made additional progress during the 2nd year of this project. Plasma collection and analysis are complete for the UCSF Aim 1 specimens and all Aim 2 specimens, and are anticipated to be completed soon for the UW Aim 2 specimens. One of the key deliverables from this effort to date is the annotate plasma repository itself, and we are currently also exploring other biomarker validation opportunities using this same set of specimens. Urine collection and processing likewise are complete for the Aim 2 specimens.

Tissue accession continued to lag in the face of external budget and staffing pressures. However, we have finally resolved these, and expect to catch up to schedule quickly. Once the tissues are collected and punched, the analyses at Myriad and the Paris lab will be able to proceed more quickly than originally anticipated given improvements in lab technologies and processes.

The prostate cancer prognostic biomarker space is in the midst of rapid expansion. Tissue-based assays from Myriad (Prolaris), Genomic Health (OncoType GPS), and GenomeDx (Decipher) have all been released within the past 12 months, and markers such as PCA3 which have been established in the pre-diagnostic space are rapidly being assessed as prognostic candidates. Appropriately validating biomarkers, assessing their independent contribution to prognostic assessment, and determining their optimal clinical use and cost-effectiveness all require carefully designed analyses using well-

described tissue repositories—exactly the sort of work in progress under this grant. We look forward to completing our analyses and reporting results by the end of the final year.

The Impact of Reducing the Frequency of Prostate Specific Antigen Testing Among Men on Active Surveillance for Prostate Cancer

Matthew R. Cooperberg, Sanoj P. Punnen, K. Cary Kelly, Elissa C. Brown, Lisa F. Newcomb, Shanshan Zhao, Ziding Feng, James D. Brooks, Peter R. Carroll, Daniel W. Lin, and the Canary PASS Investigators

University of California, San Francisco; Fred Hutchinson Cancer Center; Stanford University; University of Washington

INTRODUCTION

- Although variation in follow up exists among different surveillance regimens, most protocols require serial PSA assessment every 3 months and many include a definition of progression based on PSA kinetics
- It is unclear that PSA kinetics will be any different if based on semi-annual (every 6 months) rather than quarterly (every 3 months) PSA assessment
- Our objective was to assess the agreement in defining progression based on PSA doubling time (PSAdt) using 3- versus 6-month PSA measurements in a group of men participating in a prospective active surveillance cohort

METHODS

- Men were drawn from the Canary Prostate Active Surveillance Study (PASS), a prospective, multi-institutional cohort on men on active surveillance
- Included men had serial PSA measurements every 3 months up to 42 months following diagnosis
- PSA doubling time (PSAdt) was calculated using every 3 month and every 6 month PSA (using both even and odd numbered assessments for 6 month calculation)
- PSAdt = ln 2/ (ln PSA slope) using minimum of 5 PSA measurements
- PSAdt < 3 years represents progression, while PSAdt > 3 years represents no progression
- Agreement in PSAdt based progression using 3 versus 6 month PSA measurements assessed by Cohen's Kappa coefficient
- Similar analysis of agreement was performed on a subset of low-risk and very low-risk participants
- Kernel density plot used to display difference in PSA slope based on 3-monthly versus 6-monthly PSA measurements
- Bandiwala observer agreement chart used to provide a visual representation of agreement

RESULTS

- Among 831 men in the PASS cohort, 175 had PSA measurements every 3 and 6 months up to 42 months
- When using alternate 6 months measurements, 166 men had PSA measurements available
- Cohort demographics and characteristics are displayed in Table 1
- Table 2 shows agreement in PSAdt based progression using 3 and 6 month PSA measurements, and suggests there was no difference between the two
- Table 3 shows agreement in PSAdt based progression using 3 and 6 month PSA measurements in subset of low and very low risk patients, again with no difference in progression
- A kernel density plot (figure 1) showed no difference in slope of PSA between 3 and 6 month PSA measurements
- Bangdiwala observer agreement chart (figure 2) showed no systematic difference in PSAdt progression when using either 3 or 6-month PSA measurements

Patient Characteristic	PASS cohort (n = 831)	Included Pts (n = 205)	Excluded Pts (n = 626)
Race (N, %)			
Caucasian	747 (90)	189 (92)	558 (89)
African American	49 (6)	5 (3)	43 (7)
Asian	23 (3)	6 (3)	17 (3)
Other or Unknown	12 (1)	4 (2)	8 (1)
Ethnicity (Latino/Hispanic) (N, %)			
Yes	28 (3)	6 (3)	22 (4)
No	794 (96)	192 (94)	602 (96)
Unknown	9 (1)	7 (3)	2
Age at Study Entry (N, %)			
<50	30 (4)	4 (2)	26 (4)
50-60	232 (28)	63 (31)	169 (27)
61-70	446 (53)	99 (48)	341 (55)
>70	129 (15)	39 (19)	90 (14)
Median (IQR)	64 (6)	64 (10)	64 (6)
Mean (SD)	63 (7.2)	64 (7.0)	63 (7.3)
Serum PSA (N, %)			
0-3.99	338 (41)	95 (46)	243 (39)
4.0-10.0	433 (52)	97 (47)	336 (54)
>10	60 (7)	13 (6)	47 (7)
Median (IQR)	4.5 (3.5)	4.2 (3.7)	4.5 (3.4)
Mean (SD)	5.0 (3.2)	4.6 (3.0)	5.1 (3.2)
Clincal T-stage (N, %)			
T1a/b	7 (1)	2 (1)	5 (1)
T1c	726 (87)	176 (86)	550 (88)
T2a	92 (11)	25 (12)	67 (11)
T2b	5 (1)	1 (1)	3 (<1)
T2c	1	0	1
Gleason Score (N, %)			
≤6	762 (92)	189 (92)	573 (92)
7	69 (8)	16 (8)	53 (8)
Volume, % positive cores (N, %)	n = 708	n = 156	n = 552
1-10	379 (54)	81 (52)	298 (54)
11-33	298 (42)	69 (44)	230 (42)
≥34	31 (4)	6 (4)	24 (4)

Table 1: Patient Demographics and Clinical Characteristics

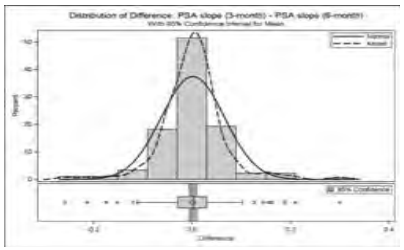


Figure 1: Kernel plot of difference in PSA slope using 3 and 6-month PSA measurements

Table 2: Concordance and discordance of PSAdt Progression categories using 3 and 6-month PSA assessments, b) and 3 and alternate 6-month PSA assessments

PSAdt (3 months)	PSAdt (6 months) No Progression	PSAdt (6 months) Progression	Total
No Progression	153	8	161
Progression	5	9	14
Total	158	17	175

A) Kappa: 0.54, McNemar's test p-value=0.41.

PSAdt (3 months)	PSAdt (6 months) (alternate months) No Progression	PSAdt (6 months) (alternate months) Progression	Total
No Progression	144	7	151
Progression	2	13	15
Total	146	20	166

B) Kappa: 0.71, McNemar's test p-value=0.18.

Table 3: Concordance and Summary of Kappa Coefficient for all patients, low-risk patients, and very low-risk patients

Cohort subset	2 category concordance: 3-6 month Kappa	3-6 month McNemar p-value	3-6 month (alt) Kappa	3-6 month (alt) McNemar p-value
All Participants (175, 166 ppts)	0.54	0.58	0.71	0.18
Low Risk (156, 144 ppts)	0.58	1.0	0.70	0.16
Very Low Risk (77, 74 ppts)	0.36	0.69	0.63	0.06

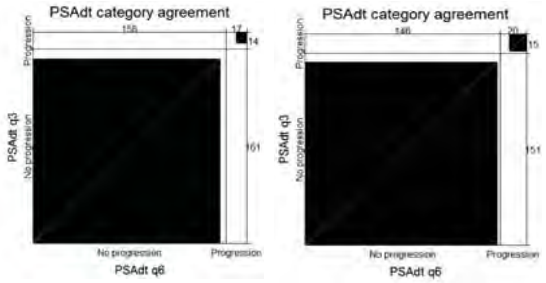


Figure 2: Bangdiwala observer agreement chart: depicts a large square whose dimensions represent total sample size. Within this large square there are two smaller boxes representing no progression and progression. Within each smaller box the darker shaded box represents observed agreement, while the outer lighter shaded box represents the maximum possible agreement given the marginal totals

CONCLUSIONS

- Measuring PSA values every 6 months, as opposed to every 3 months, does not cause substantial differences in the calculation of PSAdt and assessment of progression in active surveillance
- This finding provides evidence that semi-annual PSA testing may be safe for some men on active surveillance

Declining PSA values are associated with a lower risk of progression in the Canary/EDRN Prostate Active Surveillance Study (PASS)

Brooks JD, Brown EC, Cooperberg M, Newcomb LF, Carroll PR, Feng Z, Gleave ME, Lance RS, Sanda MG, Thompson IM, Wei JT, Nelson PS, Lin DW for the Canary Prostate Active Surveillance Study Investigators.

Stanford University, Stanford, CA; University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA; University of California, San Francisco, San Francisco, CA; Vancouver Prostate Centre, Vancouver, BC; Eastern Virginia Medical School, Norfolk, VA; Beth Israel Deaconess Medical Center, Boston, MA; University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Michigan School of Medicine, Ann Arbor, MI

Abstract

Purpose: Active Surveillance of men with low risk prostate cancer entails uncertainty for the patient and physician in determining risk of progression. While PSA determinations are frequently measured in men on active surveillance, no study thus far has found PSA velocity (PSAV) or PSA doubling times that identify patients at risk for clinical progression. However, based on observations in PASS, we hypothesized that men with negative PSAV might be at decreased risk for progression.

Materials and Methods: From 831 PASS participants, we identified 151 who had a serum PSA within 3 months of their diagnosis and at least 5 PSA values over 12-24 months after their diagnosis and prior to progression or last follow-up. Of the 151 patients, 35 progressed as defined by increase in Gleason score or increase in tumor involvement of the core biopsies to ≥ 34%, or increase in clinical stage while 116 men had no progression. PSAV is the slope of the log(PSA) values over time. ROC analysis was used with PSAV as the predictor of biopsy/clinical progression.

Results: PSAV was associated with progression in patients on active surveillance with mean PSAV of 0.12 ng/mL/yr in patients who progressed and -0.05 ng/mL/yr in those that did not progress (p=0.03). While the ROC curve showed a modest improvement in prediction of progression while on surveillance (AUC: 0.62), at the extremes, PSAV was helpful at predicting subsequent progression. In particular, a negative PSAV was associated with a decreased risk of progression. When PSAV was analyzed including only PSA values subsequent to diagnosis in 286 PASS patients, the relationship held, suggesting that the observation was not driven by a spuriously high PSA value immediately prior to diagnosis.

Conclusions: Declining PSAV in men on active surveillance for clinically localized prostate cancer is associated with a lower risk of clinical progression.

Background

- Changes in PSA increases over time (PSA velocity or PSAV) has been associated with increased risk of prostate cancer in men prior to diagnosis.
- High PSAV has been associated with more aggressive prostate cancer and with an increased risk of prostate cancer specific mortality.
- PSAV has shown limited utility in predicting progression in active surveillance (AS) cohorts previously
- We tested whether PSAV, calculated using the prediagnostic PSA was associated with progression in the PASS cohort in which patients were systematically followed.

Prostate Active Surveillance Study (PASS)

- Accrues men with clinically localized prostate cancer who have chosen active surveillance as an initial management plan.
- Serum PSA levels are tested every 3 months; Patients are examined at least every 6 months.
- Clinical data and biospecimens (urine, serum, plasma) are collected serially at 6 month intervals. Germline DNA collected at enrollment; tissue (fresh and frozen collected at biopsy.
- Biopsies done by 1 year after diagnostic biopsy, then at least every other year.
- Biospecimens are sent to a central repository with procedures for specimen allocation and use.
- If there is progression as defined by histologic or clinical criteria a participant is offered radical treatment. He may opt to continue active surveillance.
- Data is managed using a central data system with rigid quality control and reporting.
- Since August 2008 over 830 participants have enrolled in PASS.

Demographics of the Patient Cohort

Age at study entry	n (%)
<50	4 (2)
50-60	54 (36)
61-70	75 (50)
>70	18 (12)
Median (range)	62 (47-79)
Mean	63

Serum PSA	n (%)
0 – 3.99	54 (36)
4.0 – 10.0	91 (60)
> 10	6 (4)
Mean	4.7
Median (range)	4.5 (0.3-14)

Clinical Stage	n (%)
T1a	1 (1)
T1c	126 (83)
T2a	23 (14)
T2b/c	1 (1)

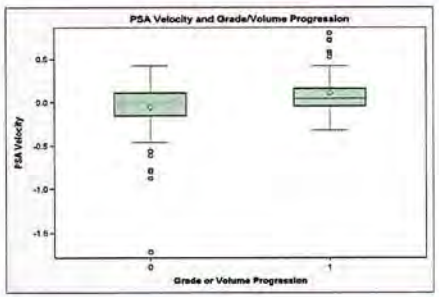
Gleason score at Study entry	n (%)
4-5	0 (0)
6	149 (95)
7 (3+4)	8 (5)
7 (4+3)	0 (0)
8-10	0 (0)

Race	n (%)
Asian	7 (5)
White	135 (89)
Black	6 (4)
Other	3 (2)

Methods

- PSAV = slope of the log(PSA) values over time
- Progression is defined as grade or volume progression during study biopsies. In participants who were on active surveillance prior to enrollment, progressions at baseline are not considered as progression events.
- Starting PSA value (PSA_1) occurs within 3 months prior to the date of initial prostate cancer diagnosis.
- All subsequent PSA values must occur within two years of the starting PSA value.
- The PSA values must span at least a year.
- All participants have had a follow-up biopsy.
- Both progressors and non-progressors have an average of seven PSA values in their PSAv calculation (p=0.49, Student's t-test).

PSAV and Progression



Grade/Volume Progression	N	PSA Velocity (ng/mL/yr) Mean (SD)	Median (IQR)	p*
Yes	35	0.12 (0.27)	0.08 (0.24)	0.03
No	116	-0.05 (0.28)	0.01 (0.27)	

*Two-sided Wilcoxon

Thresholding PSAV Improves PPV and NPV for Progression

Values of PSAV thresholds, Sensitivity, Specificity, NPV, and PPV at varying fixed values of sensitivity and specificity

PSAV threshold (ng/mL/yr)	Sensitivity	Specificity	NPV	PPV
0.11	0.37 (0.20-0.57)	0.75	0.80	0.31
0.13	0.34 (0.14-0.51)	0.80	0.80	0.34
0.19	0.23 (0.09-0.43)	0.85	0.79	0.31
0.24	0.17 (0.06-0.31)	0.90	0.78	0.34
0.26	0.17 (0.06-0.29)	0.95	0.79	0.51
0.41	0.17 (0.06-0.29)	0.99	0.79	0.84
-0.07	0.75	0.34 (0.22-0.59)	0.82	0.26
-0.11	0.80	0.32 (0.21-0.57)	0.84	0.26
-0.12	0.85	0.28 (0.17-0.43)	0.86	0.26
-0.17	0.90	0.23 (0.16-0.37)	0.89	0.26
-0.20	0.95	0.21 (0.12-0.31)	0.93	0.27
-0.25	0.99	0.16 (0.10-0.28)	0.98	0.26

Underlying NPV: 77% (116/151)

- Rising PPV associated with higher PSAV indicates a higher risk of progression on subsequent biopsy. For example PSAV of 0.41 ng/mL/yr is associated with an 84% risk of progression
- Negative predictive value indicates the chance that there will be no progression on subsequent biopsy. For patients with a PSAV of -0.25 ng/mL/yr there is a 98% chance of non-progression
- Redoing the analysis using only PSAs subsequent to diagnosis confirms that positive PSAV is associated with progression, while negative PSAV is associated with non-progression (p=0.001 in a set of 286 patients with availalbe PSA data meeting the criteria outlined under methods).

Conclusions

- Many patients on active surveillance show negative PSAV
- In general, negative PSAV is associated with a lower risk of progression on surveillance
- Larger negative PSAV is associated with a higher negative predictive value; that is, a much lower risk of progression and this could potentially inform clinical decisions
- While negtive PSAV is sometimes a product of biopsies for spurious elevations od screening PSAs, negative PSAV continues to be observed in men on AS after diangosis and continues to be associated with a lower risk of progression.

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Clinical Cancer Research

Urinary TMPRSS2:ERG and PCA3 in an Active Surveillance Cohort: Results from a Baseline Analysis in the Canary Prostate Active Surveillance Study

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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Urinary TMPRSS2:ERG and PCA3 in an Active Surveillance Cohort: Results from a Baseline Analysis in the Canary Prostate Active Surveillance Study

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Abstract

Purpose: Active surveillance is used to manage low-risk prostate cancer. Both PCA3 and TMPRSS2:ERG are promising biomarkers that may be associated with aggressive disease. This study examines the correlation of these biomarkers with higher cancer volume and grade determined at the time of biopsy in an active surveillance cohort.

Experimental Design: Urine was collected after digital rectal examination prospectively as part of the multi-institutional Canary Prostate Active Surveillance Study (PASS). PCA3 and TMPRSS2:ERG levels were analyzed in urine collected at study entry. Biomarker scores were correlated to clinical and pathologic variables.

Results: In 387 men, both PCA3 and TMPRSS2:ERG scores were significantly associated with higher volume disease. For a negative repeat biopsy, and 1% to 10%, 11% to 33%, 34% or more positive cores, median PCA3, and TMPRSS2:ERG scores increased incrementally ($P < 0.005$). Both PCA3 and TMPRSS2:ERG scores were also significantly associated with the presence of high-grade disease. For a negative repeat biopsy, Gleason 6 and Gleason $\geq$7 cancers, the median PCA3, and TMPRSS2:ERG scores also increased incrementally ($P = 0.02$ and $P = 0.001$, respectively). Using the marker scores as continuous variables, the ORs for a biopsy in which cancer was detected versus a negative repeat biopsy (ref) on modeling was 1.41 (95% CI: 1.07–1.85), $P = 0.01$ for PCA3 and 1.28 (95% CI: 1.10–1.49), $P = 0.001$ for TMPRSS2:ERG.

Conclusions: For men on active surveillance, both PCA3 and TMPRSS2:ERG seem to stratify the risk of having aggressive cancer as defined by tumor volume or Gleason score. *Clin Cancer Res; 19(9); 2442–50. ©2013 AACR.*

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Introduction

The prostate-specific antigen (PSA) screening era has been associated with a well-established stage migration of prostate cancer, such that a high proportion of newly diagnosed prostate cancers exhibit features that associate with a very low risk of invasion, metastasis, and consequent morbidity and mortality (1). Multiple studies have examined the natural history of these low-risk neoplasms, showing that the vast majority of men with this diagnosis die of causes other than prostate cancer, even if they are managed without primary curative treatment (2–4). Nevertheless, as a designation of low-risk cancer does not equate to complete absence of risk, the majority of contemporary patients with low-risk prostate cancer choose to pursue immediate curative therapy such as surgery or radiotherapy with the attendant costs and side effects (1, 5–7). These practice patterns have spawned substantial debate regarding overdiagnosis, overtreatment, and the use of PSA-based prostate cancer screening (8–10).

To address the problem of overtreatment, a deferred treatment strategy termed active surveillance has been used by clinicians as an approach to manage low-risk

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Translational Relevance

The identification of biomarkers that, at the time of diagnosis, associate with the presence of, or progression to, aggressive prostate cancer will transform the clinical management of this malignancy. If patients and their physicians have reliable and valid tools for estimating the risk of disease-specific morbidity, then more patients might opt for and adhere to active surveillance regimens, and consequently reduce overtreatment and the attendant substantial costs and harms. Also, a marker or marker panel with high accuracy for progression on active surveillance will identify those men who could be placed on less intensive surveillance protocols with fewer repeated prostate biopsies, reducing the risks and costs of invasive procedures. The study presented here is a step toward validating such biomarkers.

prostate cancer. Active surveillance incorporates serial PSA measurements, physical examinations, and repeat prostate biopsies to monitor for either the presence of occult aggressive disease or progression to a phenotype more commonly associated with metastasis and mortality. Acceptance of active surveillance has been limited for several reasons including the lack of consensus on optimal selection criteria and triggers for intervention, lack of long-term outcomes data, inconsistent study designs in the current active surveillance series, and fear among both patients and providers of losing the window of curability. Of importance, prostate cancer is well described to exhibit a pattern of multifocality that can manifest as independent lesions with different pathologic grades and distinct molecular features (11). Undersampling of the prostate by standard biopsy techniques, the lack of knowledge regarding the rates of cancer progression and a lack of diagnostic imaging modalities capable of accurately assessing tumor volume and histology have prompted the incorporation of repeat tissue assessments by biopsy into active surveillance protocols (12–16). Though morbidity is low (17, 18), the discomfort, cost, and continued undersampling problem inherent in the prostate biopsy procedure advocate for the development of noninvasive biomarkers capable of reflecting events throughout the prostate gland and suitable for repeat measurements over time.

PCA3 and the TMPRSS2:ERG fusion are 2 prostate cancer-specific biomarkers that hold promise for stratifying risk in an active surveillance setting. PCA3 is a prostate-specific noncoding mRNA that is significantly overexpressed in prostate carcinoma compared with benign prostatic tissue (19, 20). Urinary PCA3 levels have been investigated for prostate cancer early detection (21, 22) and importantly are correlated with histologic grade and tumor volume in prostatectomy specimens (23–26). Of the genomic alterations involving ETS oncogene family members, a rearrangement involving the androgen-regulated *TMPRSS2* gene with the ERG transcription factor (TMPRSS2:ERG) is the most prevalent (27), occurring in approximately half of the prostate cancers diagnosed in Caucasians (28), and have been correlated in some reports with aggressive disease (29, 30). A clinical grade, quantitative TMPRSS2:ERG urine assay has been developed and measurements of TMPRSS2:ERG transcript levels associate with cancer volume and grade at prostatectomy, and upgrading from biopsy histologic assessments (31). The combination of both TMPRSS2:ERG and PCA3 improved the performance of PSA for detection of prostate cancer and predicting clinically significant cancer (31). The goal of the present study was to determine whether urinary PCA3 and TMPRSS2:ERG mRNA levels are associated with higher volume or grade prostate cancer in a multi-institutional active surveillance cohort.

Materials and Methods

Canary prostate active surveillance study cohort

The Canary Prostate Active Surveillance Study (PASS) clinical protocol (clinicaltrials.gov NCT00756665) was approved by the Institutional Review Boards at Stanford University (Stanford, CA), University of British Columbia (British Columbia, Canada), University of California at San Francisco (San Francisco, CA), University of Texas Health Sciences Center at San Antonio (San Antonio, TX), University of Washington (Seattle, WA), Veterans Affairs Puget Sound Health Care System (Seattle, WA), and Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA; Coordinating Center), and the study opened for enrollment in late 2008; subsequently the protocol was approved and enrollment was opened at Beth Israel Deaconess Medical Center (Boston, MA), Eastern Virginia Medical School (Norflok, VA), and University of Michigan (Ann Arbor, MI; ref. 32). At the time of the present analysis, November 10, 2010, 413 men provided written informed consent for entry into this prospective, observational, active surveillance study. The enrollment criteria for PASS include: histologically confirmed adenocarcinoma of the prostate, ECOG performance status of 0 or 1, clinical T1 and T2 disease, no previous treatment for prostate cancer including hormonal therapy, radiotherapy surgery, or chemotherapy, and the willingness to undergo serial prostate biopsies. Participants enrolled in Canary PASS are followed with serum PSA measurements every 3 months, clinical examination and digital rectal examination (DRE) every 6 months, and serial repeat prostate biopsy 6 to 12 months after the initial diagnosis, 24 months after the initial diagnosis, and every other year thereafter. In an attempt to make this multicenter study reflect community practice, standard biopsy templates were not mandated, however, at least 10 core biopsy regimens are required and 97% of repeat biopsy regimens were 12 core regimens or more. At study entry and each follow-up visit, blood (plasma and serum) and post-DRE urine are collected, and DNA is collected from peripheral blood at study entry. Deidentified demographic, clinical, and pathologic data

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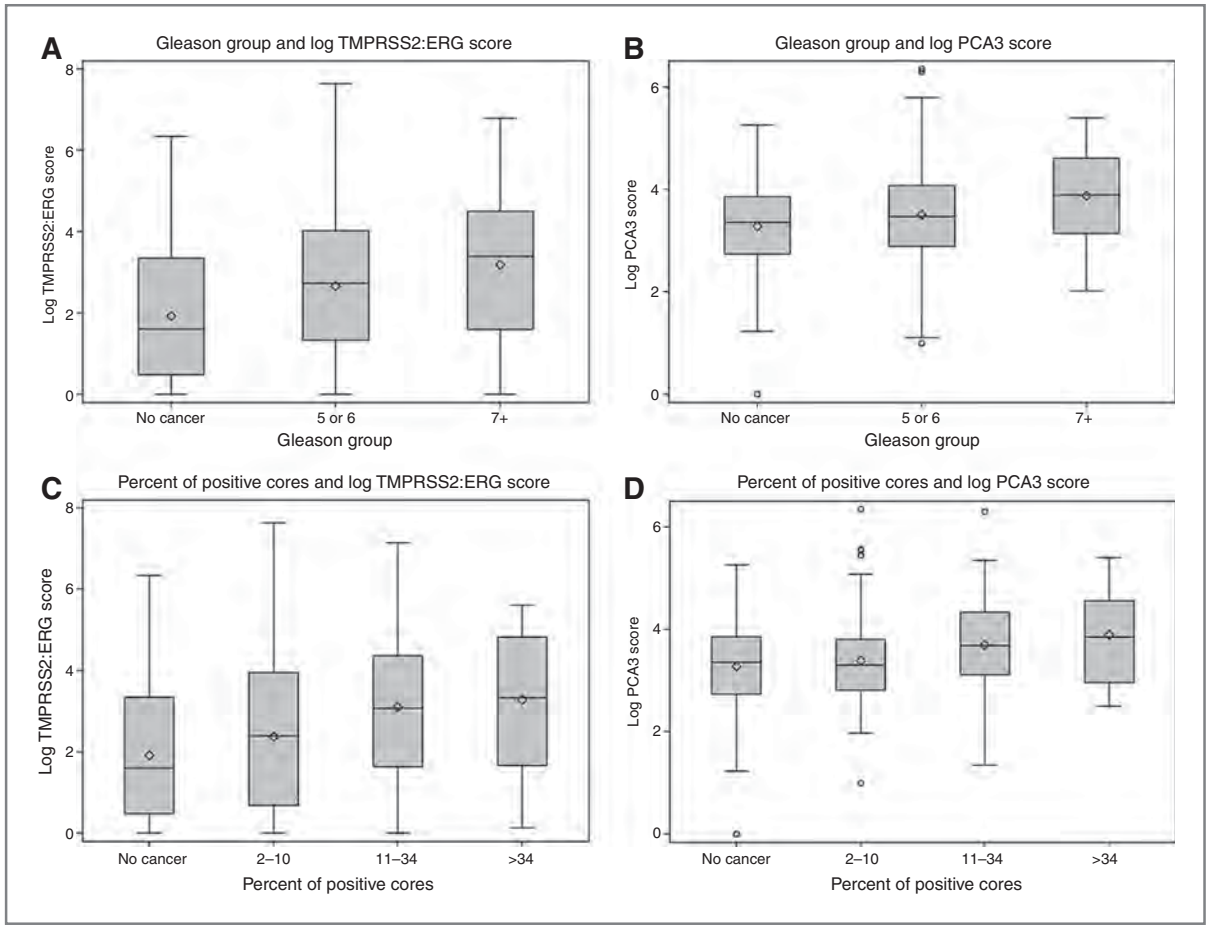

Figure 1. Box and whisker plots of Kruskal–Wallis correlations between (A) TMPRSS2:ERG and (B) PCA3 scores and Gleason score associated with specimen collection; (C) TMPRSS2:ERG and (D) PCA3 scores and tumor volume, defined by the percent of biopsy cores with tumor involvement, associated with specimen collection.

are stored in a central data repository at the FHCRC managed by the National Cancer Institute's (NCI) Early Detection Research Network Data Management and Coordination Center (EDRN DMCC), and specimens are housed in a central biospecimen repository at FHCRC. A collaboration agreement that governs study conduct and specimen and data use has been executed at all participating institutions. Specimens are available to the research community upon approval of the PASS Biomarker Review Committee.

The initial 413 consecutive men enrolled in PASS were included in this study. Of these, 2 were excluded due to problems with sample preservation, 10 participants did not provide a urine specimen, and 14 were excluded because their specimens yielded uninformative results, leaving 387 with evaluable specimens. At study entry, the median time since diagnosis was 10.4 months (range of 6 days to 18 years); 284 (54%) participants were within 1 year of their diagnosis. One hundred and ninety six men (51%) had undergone a single prostate biopsy (i.e., diagnostic biopsy) and 49% of men had previously been using active surveillance to manage their prostate cancer and had repeat surveillance biopsies conducted since their diagnosis—106 men (27%) had undergone 2 biopsies on or after diagnosis, 55 (14%) had undergone 3 prior biopsies, and 29 (8%) had undergone 4 or more biopsies. Although all subjects enrolled had at least 1 biopsy with carcinoma, 20% of participants had a subsequent prostate biopsy session that did not identify cancer. In 302 participants (78%), the biopsy that was associated with study entry was conducted at a mean of 6.5 months (range of 0.2–46.2 months, $s = 5.5$) before study entry. In the remaining 85 participants (22%), the biopsy associated with study entry was a surveillance biopsy conducted on the day of study entry and specimen collection was conducted immediately before the biopsy. Importantly, 91% of urine samples were obtained within 12 months of the biopsy. In this study, biopsies were evaluated for Gleason score by a local genitourinary-trained study pathologist using the 2005 WHO/ISUP modified Gleason system (33). Tumor volume

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was defined as the percentage of biopsy cores with cancer involvement.

PCA3 and TMPRSS2:ERG urine assay

Urine specimens were collected at each clinical site at the time of study entry. Specimens were collected after attentive DRE involving 3 sweeps of each lateral prostate lobe, put on ice, and processed within 4 hours by mixing with an equal volume of urine transport medium (detergent-based stabilization buffer; PROGENSA PCA3 Urine Specimen Kit, Hologic Gen-Probe Inc.). Specimens were stored at −70°C until analysis with grouped shipments on dry ice to the Central Repository and to Hologic Gen-Probe. Assays were conducted by Hologic Gen-Probe to determine amounts of PCA3, TMPRSS2:ERG, and PSA mRNAs in each specimen using the PROGENSA PCA3 assay or the second-generation developmental TMPRSS2:ERG assay as described previously (22, 31). Operators were blinded with respect to subject clinical information at the time of testing and did not participate in data analysis. PCA3 and PSA RNA measurements were conducted in duplicate, and TMPRSS2:ERG RNA levels were measured in triplicate. Samples with an average PSA transcript level of more than 7,500 copies/mL were considered informative. PCA3 scores were calculated as 1,000 × (average urine PCA3 copies/mL)/(average PSA copies/mL). TMPRSS2:ERG scores were calculated as 100,000 × (average urine TMPRSS2:ERG copies/mL)/(average PSA copies/mL).

Statistical analysis

Statistical analyses were conducted at the EDRN DMCC using SAS version 9.2. Descriptive statistics summarized clinical factors. Spearman rank correlation coefficients were calculated between PCA3 and TMPRSS2:ERG scores and continuous clinicopathologic variables. Disease volume and grade were divided into clinically meaningful categories, and nonparametric Mann–Whitney and Kruskal–Wallis tests were conducted to compare PCA3 and TMPRSS2:ERG among the groups. Univariate logistic regression models with log-transformed PCA3 and log-transformed TMPRSS2:ERG were fit separately to provide ORs for prediction of positive disease and high-grade disease, respectively. Receiver operating characteristic (ROC) curves were plotted for serum PSA, PCA3, and TMPRSS2:ERG and the area under the curves (AUC) were analyzed using the DeLong method for comparing correlated ROC curves (34). Multivariable logistic regression models included PCA3, TMPRSS2:ERG, PSA, and other study covariates commonly associated with prostate cancer including DRE results, family history of prostate cancer, race, and age. The linear scores from these multivariable models were used to plot ROC curves.

Results

Characteristics of participants at the time of initial urine specimen collection are given in Table 1. The majority of participants were Caucasian (91%), 4% were African American, 3% were Asian, and 2% have other or unknown racial backgrounds. The Gleason score of the biopsy associated with urine specimen collection was 6 in 72% of the participants, with one participant having a Gleason score reported as 5, and the Gleason sum was ≥7 in 8% of participants; 20% of the participants had a negative repeat biopsy associated with specimen collection. Ninety-three percent of participants had PSAs of less than 10, 84% were with clinical stage T1c disease, and 94% of participants with a known number of positive cores had less than 34% of cores involved with cancer.

In this active surveillance cohort, the mean urine PCA3 score was 49 with a median of 31 (IQR 42). The mean urine

Table 1. Participant characteristics at urine specimen collection

Race	*n* (%)
Caucasian	351 (91)
African American	15 (4)
Asian	13 (3)
American Indian/Alaska Native	2 (1)
Other or unknown	6 (1)
Ethnicity (Latino/Hispanic)	
Yes	13 (3)
No	366 (95)
Unknown	8
Age at study entry	
<50	13 (3)
50–60	105 (27)
61–70	201 (52)
>70	68 (18)
Median (range)	64 (38–84)
Mean	63.8
Serum PSA	
0–3.99	170 (44)
4.0–10.0	190 (49)
>10	27 (7)
Mean	4.8
Median (range)	4.4 (0.25–28.8)
Clinical stage	
T1a	5 (1)
T1c	322 (83)
T2a	55 (14)
T2b	4 (1)
T2c	1
Gleason score	
No cancer detected	79 (20)
5–6	278 (72)
7	27 (7)
8–9	3 (1)
Volume; % positive cores	
No cancer detected	79 (20)
2–10	112 (29)
11–33	108 (28)
≥34	19 (5)
Unknown	69 (18)

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Table 2. Spearman rank correlation of clinicopathologic variables with PCA3 and TMPRSS2:ERG scores

	Variable	*N*	r_s	*P*-value
Serum PSA	PCA3 score	387	0.09	0.07
	T2:ERG score	387	0.03	0.5
Age	PCA3 score	387	0.25	<0.0001
	T2:ERG score	387	0.04	0.47
Prostate volume	PCA3 score	302	0.007	0.9
	T2:ERG score	302	0.03	0.56
Body mass index	PCA3 score	387	−0.03	0.61
	T2:ERG score	387	−0.08	0.13
Number of prior biopsies	PCA3 score	387	0.07	0.16
	T2:ERG score	387	0.09	0.08
Time from biopsy to urine collection	PCA3 score	387	0.009	0.9
	T2:ERG score	387	0.05	0.3
Time from diagnosis to urine collection	PCA3 score	387	0.09	0.07
	T2:ERG score	387	0.07	0.17
Gleason score at study entry	PCA3 score	387	0.13	0.01
	T2:ERG score	387	0.2	0.0001
Tumor volume at study entry (% positive cores)	PCA3 score	294	0.18	0.002
	T2:ERG score	294	0.3	<0.001

TMPRSS2:ERG score was 55 with a median of 12 (IQR 60). We examined the correlations of both markers to clinicopathologic variables of disease (Tables 2 and 3). Both PCA3 and TMPRSS2:ERG scores were significantly correlated to biopsy Gleason score and tumor volume, assessed by percentage of biopsy cores with cancer ($P < 0.01$ for all comparisons). Although others have looked at linear lengths, biopsy Gleason score and percentage of cores with cancer have been shown to independently predict outcome in men who undergo surgery (35–37). There was no significant correlation of the urine markers to serum PSA, prostate volume, body mass index, number of prior biopsies, time from biopsy to urine collection, time from initial prostate cancer diagnosis (Table 2), family history, or clinical stage (Table 3). We also found no significant correlations between urine PCA3 or TMPSS2:ERG scores with IPSS score, PSA doubling time, or the use of statins, diabetes medications, 5 α-reductase inhibitors, or NSAIDs (data not shown). TMPRSS2:ERG score was not correlated with age, but PCA3 levels were positively correlated with advancing age ($P < 0.0001$), as has been observed by others (38).

We further evaluated the associations between PCA3 and TMPRSS2:ERG and tumor histology (Fig. 1 and Table 3). We found a significant sequential increase in both PCA3 and

Table 3. Correlation of clinicopathologic variables with PCA3 and TMPRSS2:ERG scores

			PCA3 Score		TMPRSS2:ERG Score	
	Parameter	*N*	Median (95% CI)	*P*	Median (95% CI)	*P*
Family	Yes	99	34 (26–43)	0.28[a]	16 (8–25)	0.37[a]
History	No	265	30 (27–35)		11 (7–15)	
Clinical	T1	327	30 (27–34)	0.18[a]	12 (8–15)	0.32[a]
T-stage	T2	60	38 (28–49)		22 (3–48)	
Gleason score	No cancer detected	79	27 (24–31)	0.02[b]	5 (2–8)	0.001[b]
	5 or 6	278	31 (27–35)		14 (9–18)	
	≥7	30	48 (31–92)		29 (13–78)	
	No cancer detected	79	27 (24–31)	0.004[b]	3 (2–8)	<0.0001[b]
Tumor volume	1–10	112	28 (22–35)		10 (4–14)	
	11–33	108	40 (31–51)		20 (14–31)	
	≥34	19	46 (18–90)		27 (4–115)	

[a]Mann–Whitney test.
[b]Kruskal–Wallis test.

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TMPRSS2:ERG as Gleason grade increased. For negative repeat biopsy, Gleason 5 to 6, and Gleason ≥7, the median PCA3 scores were 27 (95% CI: 24–31), 31 (95% CI: 27–35), 48 (95% CI: 31–92), $P = 0.02$, and median TMPRSS2:ERG scores were 5 (95% CI: 2–8), 14 (95% CI: 9–18), 29 (95% CI: 13–78), $P = 0.001$, respectively (Table 3). Using log-transformed biomarker scores as continuous predictors, both PCA3 and TMPRSS2:ERG urine measurements associated with a positive biopsy versus a negative biopsy (reference) with ORs for PCA3 of 1.41 (95% CI: 1.07–1.85; $P = 0.01$) and for TMPRSS2:ERG of 1.28 (95% CI: 1.10–1.49; $P = 0.001$). The ORs for a Gleason score of 7 or above versus less than 7 for PCA3 and TMPRSS2:ERG are 1.67 (95% CI: 1.10–2.52; $P = 0.02$) and 1.24 (95% CI: 1.01–1.53; $P = 0.05$), respectively. We also observed a sequential increase in the marker scores as volume increased. For a negative repeat biopsy, and 1% to 10%, 11% to 33%, ≥34% positive cores, median PCA3 scores were 27 (95% CI: 24–31), 28 (95% CI: 22–35), 40 (95% CI: 31–51), 46 (95% CI: 18–90), $P = 0.004$, and median TMPRSS2:ERG scores were 3 (95% CI: 2–8), 10 (95% CI: 4–14,) 20 (95% CI: 14–31), 27 (95% CI: 4–115), $P < 0.0001$, respectively. The ORs for a biopsy with ≥34% positive cores versus <34% (reference) are 1.64 (95% CI: 0.97–2.74; $P = 0.06$) for PCA3 and 1.16 (95% CI: 0.98–1.63; $P = 0.08$) for TMPRSS2:ERG.

In ROC analysis (Fig. 2), we compared the area of the curve (AUC) for the prediction of Gleason ≥7 disease at study entry of serum PSA alone or with the urine biomarkers. The AUC for PSA alone was 0.68, the AUC for the 2 markers alone 0.66, and the AUC for the combination of both markers and PSA was 0.70. The addition of the markers was not significantly different from the AUC for PSA alone ($P = 0.08$), although there was a trend toward significance. Similar results were found in ROC analysis for the prediction of more than 34% positive cores (see Supplementary material). Results from multivariable logistic regression models were not significant after adjusting for covariates (see Supplementary Material).

Discussion

We report the correlation of urinary levels of PCA3 and TMPRSS2:ERG transcripts with clinical characteristics at the time of study entry in a multiinstitutional, prospective active surveillance cohort. We find that in univariate analyses, both markers seem to stratify for baseline risk of disease aggressiveness as defined by biopsy Gleason score or volume of tumor (% of positive cores). However, although there is a trend toward these biomarkers improving the power of PSA to predict high grade or volume disease (Fig. 2), the increase of the markers is not significant.

Men diagnosed with clinically localized prostate cancer are offered a variety of treatment strategies including active surveillance or primary therapies with curative intent. However, decision making for these men is currently impacted by the lack of high specificity for detection of occult aggressive disease or identification of a disease that is likely to progress to an aggressive phenotype, and the majority of men with newly diagnosed low-risk prostate cancer opt for primary curative treatment (1, 6, 7), despite a growing body of evidence that treatment may often be safely delayed (13–15, 39) or avoided all together (2–4). Greater acceptance of active surveillance is limited by several factors. For example, entry into active surveillance programs and triggers for intervention are currently based on a number of clinical parameters including PSA (value, density, kinetics), clinical stage, and biopsy results (Gleason score, core involvement; refs. 13–16, 32), however, there is no consensus as to the optimal criteria for safely or effectively using active surveillance (40). Furthermore, prostate biopsies, which are an integral part of active surveillance regimens, are invasive and frequently underestimate the grade and extent of disease (41, 42).

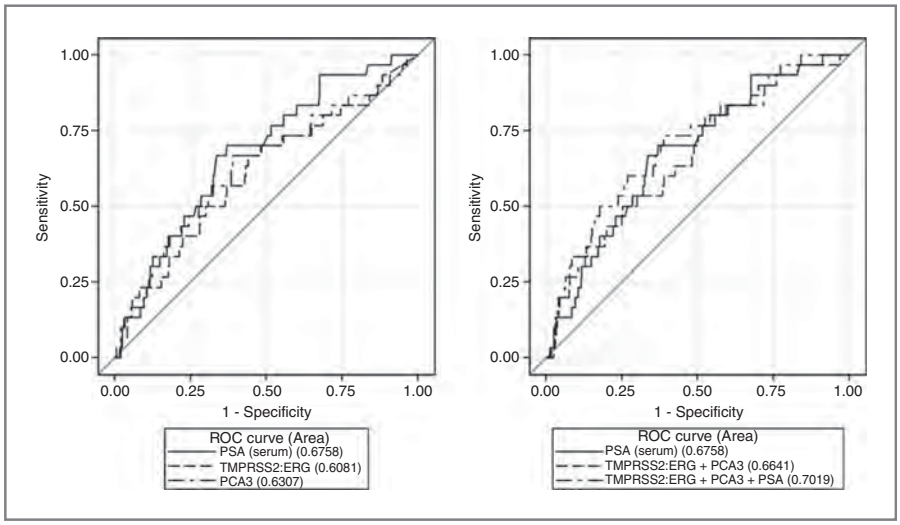

Figure 2. ROC analysis of serum PSA, TMPRSS2:ERG, and PCA3, alone and in combination, for prediction of high Gleason grade (≥7) at time of specimen collection. AUC(PSA) does not differ significantly from AUC(TMPRSS2:ERG; $P = 0.38$), AUC(PCA3; $P = 0.51$), AUC(TMPRSS2:ERG +PCA3; $P = 0.86$), or AUC(TMPRSS2:ERG +PCA3+PSA; $P = 0.08$).

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The present study begins to address an unmet need for a noninvasive biomarker test that can provide a higher degree of specificity for detecting aggressive disease than currently available clinical metrics. This study is based on the PASS cohort, which is a contemporary, multiinstitutional active surveillance cohort with prospective collection and centralized data and specimen storage. In PASS, high-quality specimens and data are maintained by on-site training for standardized specimen collection and processing procedures along with regular site visits and data audits. The clinical study is designed to meet the primary objective of confirming biomarkers that predict the presence of or progression to aggressive disease (32).

Broad eligibility criteria were used in PASS to allow most men who choose to manage their prostate cancer using active surveillance to enroll in the study, including men with primary disease features that are not currently considered low risk. This broad scope of disease characteristics allows for biomarker studies, such as the one presented here, that should provide greater insight into the natural history of prostate cancer and be more informative than studies conducted using strict entry criteria. Another aspect of the PASS design is that it allows participants who were diagnosed with low-grade/stage disease to enroll in the study on the day of a serial repeated biopsy, with specimen collection immediately before the biopsy. In this situation, the repeat biopsy may show evidence of disease progression (e.g., higher grade or volume of disease), yet the participant samples are still included in this present study, and the Gleason score from the biopsy at the baseline visit is used in the association analyses. This study includes 85 such participants, accounting for 15 of the 30 participants with a Gleason score $\geq$7 associated with specimen collection.

A limitation of this study is the inherent and well-recognized undersampling of the prostate by current biopsy procedures. There are several studies that report lack of correlation of PCA3 score with initial biopsy Gleason grade or progression (31, 43), despite strong correlations with prostatectomy Gleason grade (23–26). However, in this study, nearly half of the participants had at least one repeat biopsy, suggesting more adequate sampling in our cohort when compared with previous studies. As many of the participants in this study had undergone multiple prostate biopsy sessions at study entry, when we evaluated our data for the highest Gleason score at any timepoint (versus the single biopsy closest to study entry), the TMPRSS2:ERG score was not found to be statistically significant (P = 0.40), although PCA3 remained so ($P = 0.0019$). Similarly, using the highest Gleason score, the OR for a Gleason score of 7 or above versus less than 7 for TMPRSS2:ERG was not significant (1.08, 0.91–1.30, P = 0.39) and for PCA3 remained significant (1.63, 1.14–2.34, P = 0.0007), suggesting that PCA3 may perform better in predicting aggressive disease than TMPRSS2:ERG. A further limitation involves the interobserver variability in Gleason scoring, especially for a relevant subset of cancers in which it is difficult to distinguish tangentially sectioned pattern 3 versus poorly formed pattern 4 glands (44). However, in PASS, most biopsies are read by a study pathologist at each site, and the study pathologists have routine consensus meetings in which questionable cases are reviewed. Finally, the power of this study is limited by a relatively uniform cohort and a small number of Gleason grades $\geq$7. As such, the ROC analysis in Fig. 2 revealed a trend toward statistical significance, but was likely underpowered because of the lack of high-grade disease at study entry.

In conclusion, both PCA3 and TMPRSS2:ERG seem to stratify risk at time of enrollment, for men on active surveillance, of having aggressive cancer as defined by tumor volume or Gleason score. While there is a statistically valid trend toward these markers, especially PCA3, predicting higher grade and volume cancer, further work is needed to determine their clinical use for men on active surveillance. The results presented here are encouraging, but the clinically relevant question is how these biomarkers aid in the prediction of the presence of occult aggressive disease or progression to an aggressive phenotype over time. To address these important questions, we are continuing to expand our cohort, collect and analyze longitudinal clinical data and specimens, and follow participants to collect long-term disease status.

Disclosure of Potential Conflicts of Interest

Z. Feng is a consultant/advisory board member of Gen-Probe. I.M. Thompson, Jr. has honoraria from speakers' bureau from ASCO and AUA. P.S. Nelson is a consultant/advisory board member of GenProbe. No potential conflicts of interest were disclosed by the other authors.

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