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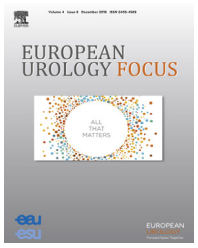

Prostate Cancer

PTEN Loss but Not ERG Expression in Diagnostic Biopsies Is Associated with Increased Risk of Progression and Adverse Surgical Findings in Men with Prostate Cancer on Active Surveillance

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Abstract

Background: Active surveillance (AS) is an option for men with low-risk prostate cancer (PCa). PTEN and ERG have been considered as potential biomarkers of PCa progression and survival.
Objective: To study the role of ERG and PTEN status in the Prostate Cancer Research International: Active Surveillance (PRIAS) trial diagnostic biopsies (DBxs) in predicting surveillance discontinuation and adverse surgical findings in subsequent radical prostatectomy (RP).
Design, setting, and participants: A total of 231 patients were recruited to the PRIAS between 2007 and 2013 in Helsinki. DBx tissue for immunohistochemistry (IHC) was available from 190 patients. Tissue microarrays (TMAs) were constructed from 57 specimens of subsequent RPs. DBxs containing grade group (GG) 1 PCa and RP TMA sections were stained with ERG and PTEN antibodies, and scored as either negative or positive.
Outcome measurements and statistical analyses: Outcomes were followed up by biopsy GG upgrade (GG $\geq$ 2) and protocol-based treatment change, as well as adverse findings in RP (GG $\geq$ 3 or pathological stage $\geq$ 3). Clinical variables and biomarker status in DBx were correlated in Cox regression analysis and cumulative survival in Kaplan–Meier analysis, and finally, Gray's competing risk analysis was performed and nonprotocol-based discontinuation was considered as a competing event.
Results and limitations: In both uni- and multivariate Cox regression analyses, only the number of positive cores in the DBx, the number of rebiopsy sessions, and PTEN status at diagnosis were significantly associated with rebiopsy GG upgrade, treatment change, and adverse histopathology in RP. In Kaplan–Meier analysis, PTEN loss was associated with a shorter time to GG upgrade and treatment change. Patients with PTEN loss had a higher probability for protocol-based discontinuation but not for competing risk factors compared with patients with intact PTEN. Biopsy ERG status was concordant with RP TMA ERG status, while PTEN was not. Limitations include a retrospective analysis of prospective cohort data.
Conclusions: PTEN status at diagnosis is a potential biomarker for identifying patients with PCa on AS with a high risk for progression or adverse findings on subsequent RP.
Patient summary: A simple diagnostic biopsy-based analysis of PTEN status may help identify patients with high risk for prostate cancer progression.

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1. Introduction

Active surveillance (AS) has emerged as an option to avoid or postpone the adverse effects of curative treatments in men with low-risk prostate cancer (PCa). After 5-yr follow-up, half of the patients are directed to other treatments, still a substantial number of them showing favorable findings in subsequent radical prostatectomy (RP) [1]. Only a few factors available at diagnosis, namely, prostate-specific antigen (PSA) density (PSAD) and the number of positive cores in diagnostic biopsies (DBxs), are known to be associated with progression and treatment change during surveillance [2]. There is an unmet need for better prognostic and predictive tools for patients considered for AS. Biomarkers, tissue genomics, and magnetic resonance imaging (MRI)-based tools have been proposed, but none have yet been widely accepted for clinical practice.

ERG oncogene is commonly fused with an androgen-regulated transcription factor, *TMPRSS2,* in PCa. *ERG: TMPRSS2* fusions are present in 36–78% of primary PCas [3–5] and have been suggested to occur early in carcinogenesis. Recently, it was postulated that ERG protein expression in DBxs would predict progression during AS [6].

PTEN is a tumor-suppressor gene that is inactivated by genetic alterations in 18–42% of PCa [7–10]. Loss of PTEN activates the phosphoinositide 3-kinase pathway and causes the activation of mammalian target of rapamycin pathway, which leads to increased cell growth and proliferation. PTEN loss has also been shown to be associated with poor prognostic parameters, such as adverse pathological features(high Gleason score [GS] and advanced pathological stage [pT]) [11,12], recurrence, shortened disease-specific survival (DSS) after surgery [11,13], shortened metastasis-free survival (MFS) after RP [12], and DSS in castration-resistant PCa [7,14]. A recent study of matched archival biopsy and surgical specimens showed that PTEN loss in GS 3 + 4 biopsies predicted locally advanced disease in RP [15].

IHC has been shown to be an accurate and cost-effective method to detect both ERG fusion protein and PTEN expression, and PTEN IHC may detect nondeletion inactivation of the gene, especially in the second allele, and thus may be superior to fluorescence in situ hybridization [12,16,17].

In this study, we evaluated both ERG and PTEN IHC status in DBx of patients with low-risk PCa on AS according to the Prostate Cancer Research International: Active Surveillance (PRIAS) protocol, to test whether these biomarkers would predict later progression at the time of diagnosis and possible adverse findings in subsequent RP.

2. Patients and methods

Between October 2006 and March 2013, 231 patients were enrolled in the Helsinki arm of the PRIAS trial. Paraffin blocks of the DBxs were not available for 28 patients, and 203 patients were included in the final biomarker analysis. Of the study patients, 53 (26.1%) had two positive cores and the remainder (73.9%) had just one positive core at diagnosis. Subsequently, 59 patients (29.1%) underwent RP, of whom we had the complete tissue material available for tissue microarray (TMA) construction. C of data and tissue material was completed in November 2015. The study was approved in 2006 by the Institutional Ethics Committee (HUS 276/E6/06).

2.1. RP TMA construction

All the separate cancer foci in the RP specimen were marked on the diagnostic hematoxylin and eosin glass slides and subsequently punched into recipient TMA blocks with a 1.0-mmdiameter core device. Altogether, four TMA blocks were constructed, constituting of a minimum of three cores per cancer focus and one adjacent benign core per each RP specimen.

2.2. Immunohistochemistry

Altogether, two 4 μm consecutive sections were cut from FFPE (formalin-fixed paraffin-embedded) biopsies, as well as three sections from each RP TMA (one each for ERG and PTEN, and one for negative control, omitting the primary antibody), and were mounted on electrically charged glass slides. IHC staining was performed using an autostainer (Dako A/S, Glostrup, Denmark). Briefly, IHC was performed after heat-induced epitope retrieval on consecutive slides with 1:100 dilution for PTEN antibody (D4.3 XP; Cell Signaling Technology, Danvers, MA, USA) and 1:300 dilution for ERG antibody (EPR 3864; Abcam PLC, Cambridge, UK). For each slide, digital whole-slide images were acquired at 0.33 μm/pixel resolution using a Pannoramic P250 Flash II whole slide scanner (3DHistech, Budapest, Hungary) equipped with a 20× objective. The images were uploaded to the WebMicroscope virtual microscope platform (Fimmic, Helsinki, Finland).

2.3. Scoring of protein expression

Two independent investigators (U.L. and T.M.) evaluated the expression of ERG and PTEN in biopsy sections and RP TMA cores. Internal staining controls were endothelium for ERG and benign epithelium for PTEN. Nuclear ERG expression was considered negative or positive (>90% of nuclei stained positive for ERG antibody) and cytoplasmic PTEN expression as either negative or positive, similarly to previous studies [11–13]. PTEN was considered lost in cancer if the staining intensity was markedly reduced compared with benign glands (internal positive control).

2.4. Statistical analysis

Progression was defined as discontinuation due to changes in protocol follow-up parameters (grade group [GG] >1, >2 positive biopsies, PSA doubling time (PSA-DT) <3 yr, stage >cT2) or GG upgrade alone. Outcome events were defined as GG upgrade (increase in biopsy to GG2 or more during follow-up), PRIAS protocol-based treatment change (GG > 1,> 2 positive biopsies, PSA-DT <3 yr, stage >cT2) and adverse RP findings (GG $\geq$ 3, pT $\geq$ 3). Age, PSA, biomarker status, extent of PCa in DBx (one core vs two cores), and the number of rebiopsy sessions were correlated to outcomes in uni- and multivariable Cox regression analysis, as well as Kaplan–Meier cumulative survival analysis with Mantel–Haenszel log rank statistics. Gray's competitive risk analysis was performed between the protocol- and nonprotocol-based reasons for discontinuation. By competitive risk analysis, we assessed whether the biomarker expression would explain PCa-related discontinuation rather than other causes of dropout. Fisher's χ^2 analysis was applied to the cross correlations of biopsy and RP IHC scores. All the statistical analyses were conducted with IBM SPSS v.23 (SPSS Inc., Armonk, NY, USA) and R Statistical Software v.3.3.2 (Foundation for Statistical Computing, Vienna, Austria).

3. Results

Demographics of the entire AS patient cohort with detailed information on clinical variables and distribution of PTEN and ERG expression in DBx are shown in Table 1 and Supplementary Table S1 in online version at DOI: 10.1016/j.euf.2017.03.004, respectively. The median follow-up time for the entire cohort was 46.2 mo (0.7–107.5 mo). During surveillance, 106 (52.2%) patients enrolled to AS discontinued, 72 (67.9%) due to PRIAS protocol-based reasons and 34 (32.1%) for other reasons. Nonprotocol-based reasons for discontinuation were RP/radiotherapy without progression (four patients), MRI findings solely (four patients), death by other causes (five patients), and watchful waiting (21 patients).

Of the patients who underwent protocol-based discontinuation, 34 (47.2%) discontinued due to having three or more positive cores, 35 (48.6%) due to GG upgrade, and 20 (27.8%) due to PSA-based reasons (PSA-DT <3 yr). Sixteen (22.2%) patients had more than one protocol-based reason for discontinuation. All the patients who discontinued the PRIAS due to protocol-based reasons underwent treatment with curative intention, 59 (81.9%) underwent RP, and the remaining 13 (18.1%) underwent radiotherapy.

Table 2 – Concordance of ERG and PTEN IHC status in diagnostic biopsies and RP specimens.

	RP ERG negative (n = 23)	RP ERG positive (n = 21)	p value
Biopsy ERG negative	19	6	<0.001
Biopsy ERG positive	4	15	
	RP PTEN negative (n = 5)	RP PTEN positive (n = 41)	p value
Biopsy PTEN negative	2	7	0.248
Biopsy PTEN positive	3	34	

IHC = immunohistochemistry; RP = radical prostatectomy.

The median percentage of cancer in DBx was 1 (0.06–14) and not significantly different between the study groups. The median diagnostic PSA was not significantly different between the patients with PTEN loss and those with intact PTEN (Table 1), neither was there a difference between the ERG-positive and ERG-negative patients (Supplementary Table S1 in online version at DOI: 10.1016/j.euf.2017.03.004). We had complete matching of ERG DBx data for 44 patients and PTEN DBx data for 46 patients undergoing subsequent RP. ERG expression was concordant in DBx and RP, while PTEN expression was not (Table 2).

Table 1 – Patient characteristics and distribution of PTEN expression status.

	Study population (n = 203)	PTEN positive (n = 161)	PTEN negative (n = 29)
Age at diagnosis (yr), median (IQR)	63.4 (59.4–68.0)	63.4 (59.4–67.9)	63.4 (59.4–69.3)
PSA (ng/ml), median (IQR)	5.6 (4.4–6.9)	5.6 (4.35–6.7)	5.9 (4.2–7.8)
PSA density, median (IQR)	0.14 (0.11–0.16)	0.14 (0.11–0.16)	0.13 (0.11–0.16)
Prostate volume, median (IQR)	40.4 (33.0–50.3)	40.4 (33.5–50.5)	43.4 (35.3–53.0)
Number of benign biopsies prior to diagnosis, n (%)			
0	147 (72.4)	114 (70.8)	24 (82.8)
1	42 (20.7)	35 (21.7)	4 (13.8)
2	10 (4.9)	8 (5.0)	1 (3.4)
3	3 (1.5)	3 (1.9)	0 (0)
4	1 (0.5)	1 (0.6)	0 (0)
Total number of PRIAS biopsy sessions [a] (n = 193), n (%)			
1	14 (7.3)	12 (7.8)	1 (3.7)
2	69 (35.8)	53 (34.6)	13 (48.1)
3	57 (29.5)	47 (30.7)	5 (18.5)
4	35 (18.1)	30 (19.6)	3 (11.1)
5	13 (6.7)	6 (3.9)	5 (18.5)
6	5 (2.6)	5 (3.3)	0 (0)
Number of positive cores at diagnosis (n = 203), n (%)			
1	150 (73.9)	119 (73.9)	19 (65.5)
2	53 (26.1)	42 (26.1)	10 (34.5)
Cancer location (n = 194), n (%)			
Unilateral	175 (90.2)	139 (89.7)	25 (89.3)
Bilateral	19 (9.8)	16 (10.3)	3 (10.7)
Biopsy ERG staining (n = 190)			
Positive	74 (38.9)	56 (35.2)	17 (58.6)
Negative	116 (61.1)	103 (64.8)	12 (41.4)
PSA-DT ≤3 yr at 1-yr follow-up (n = 203)	155 (76.4)	122 (75.8)	21 (72.4)
Age at discontinuation, median (IQR)	66.3 (61.8–70.5)	64.82 (61.1–67.3)	66.7 (61.9–71.0)
PRIAS status (n = 203)			
Nonprotocol-based discontinuation [b]	34 (16.7)	27 (16.8)	5 (17.2)
Protocol-based discontinuation	72 (35.5)	53 (32.9)	15 (51.7)
Continuing on PRIAS	97 (47.8)	81 (50.3)	9 (31.0)

IQR = interquartile range; PSA = prostate-specific antigen; PRIAS = Prostate Cancer Research International: Active Surveillance; PSA-DT = PSA doubling time.
[a] One diagnostic biopsy, two to six rebiopsies.
[b] Four discontinued due to anxiety, 21 discontinued due to watchful waiting, five died for other reasons, and four discontinued due to MRI findings.

In Kaplan–Meier survival analyses, PTEN loss indicated significantly shorter time for all study outcome events during follow-up as compared with intact PTEN (Fig. 1). ERG protein expression status did not delineate differences in the event-free survival during the follow-up, although there was a trend that positive ERG expression in DBx leads to worse cumulative survival after several years of surveillance (Fig. 1). When the PTEN-negative biopsies were stratified according to ERG status, the ERG-positive patients with PTEN loss had the shortest event-free survival compared with the ERG-negative patients with PTEN loss or patients with intact PTEN (Supplementary Fig. S1 in online version at DOI: 10.1016/j.euf.2017.03.004). In competing risk analysis, PTEN status was significantly associated with an

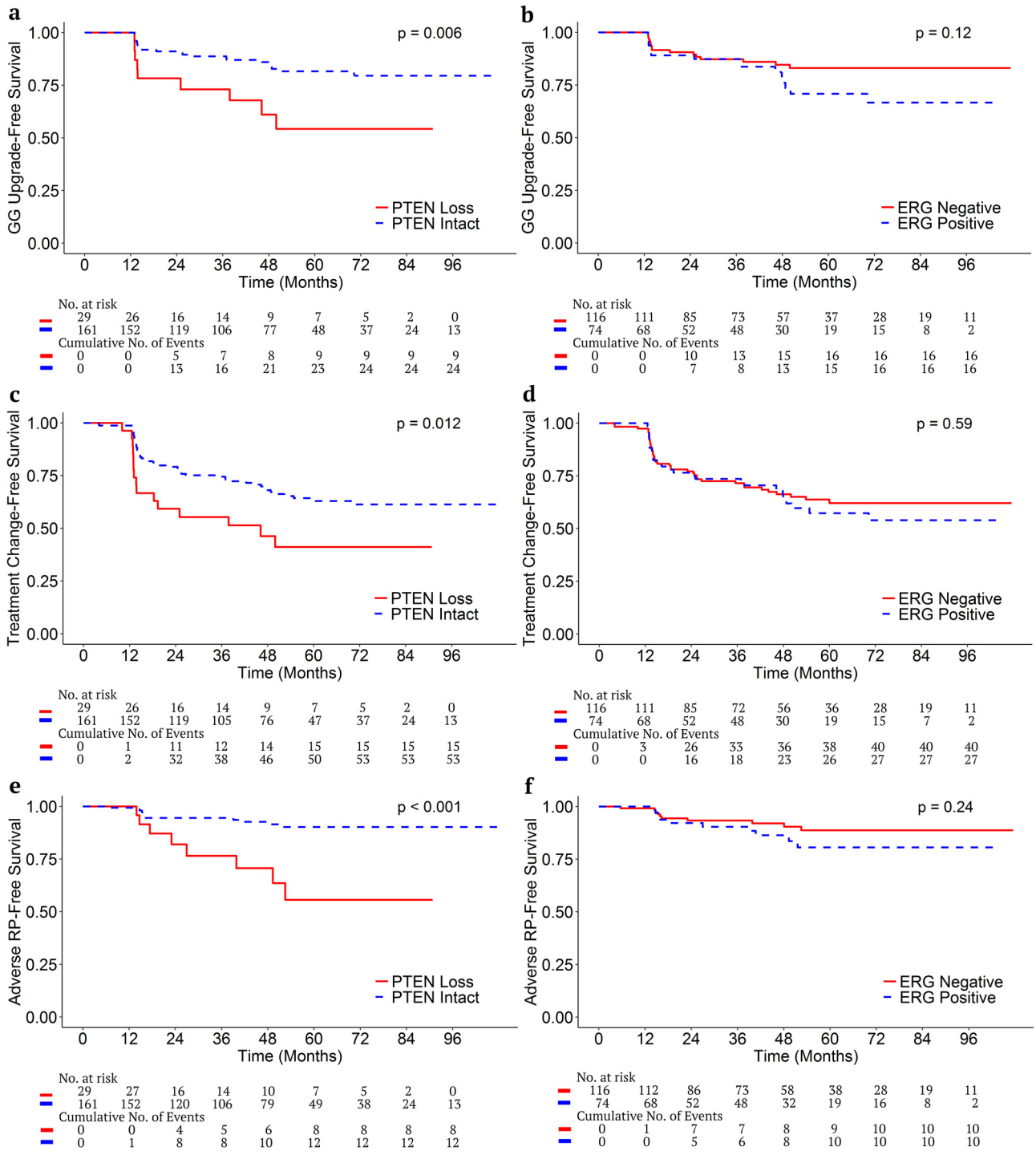

Fig. 1 – Kaplan–Meier survival curves for (A) biopsy PTEN expression versus GG upgrade, (B) ERG expression versus GG upgrade, (C) PTEN expression versus protocol-based treatment change, (D) ERG expression versus protocol-based treatment change, (E) PTEN expression versus adverse RP findings, and (F) ERG expression versus adverse RP findings. GG = grade group; RP = radical prostatectomy.

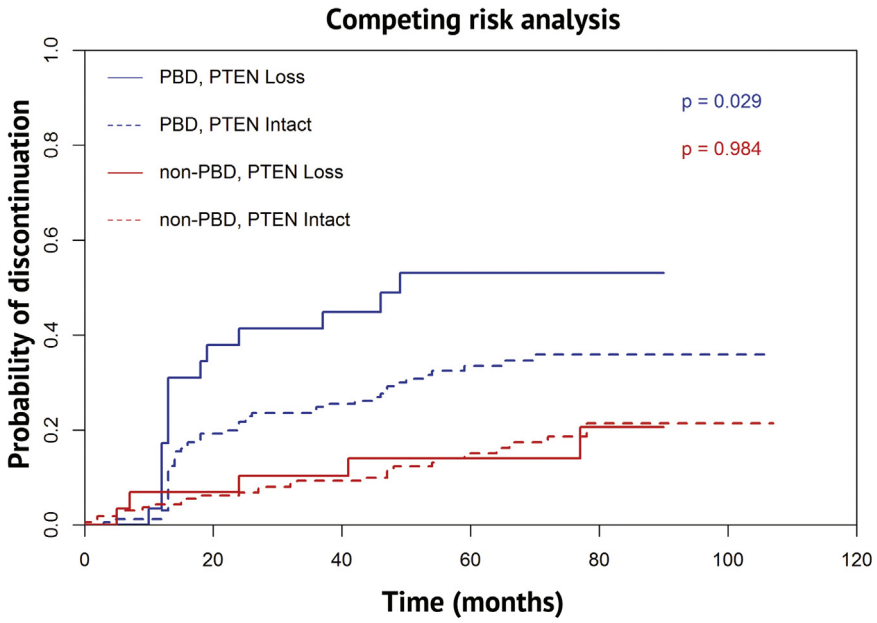

Fig. 2 – Gray's competing risk analysis of PTEN status and protocol-based discontinuation (PBD) versus nonprotocol-based discontinuation (non-PBD).

increased probability of protocol-based treatment change ($p = 0.029$) but was not associated with nonprotocol-based treatment change (Fig. 2).

In Cox regression analyses, the number of pre-DBx sessions was not associated with any of the outcomes in uni- or multivariable analysis (data not shown). PSAD was significantly associated only with treatment change in univariate analysis. The number of positive biopsy cores in DBx was significantly associated with all the outcomes. Hazard ratio (HR) for two positive cores versus one positive core only ranged from 1.83 to 3.02 in multivariable regression analyses (Table 3). If the patient underwent more biopsy sessions during the follow-up, it is less likely that he/she would harbor adverse findings in RP, have higher-grade cancer in

Table 3 – Uni- and multivariate Cox regression analysis.

Outcome and variable	Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Rebiopsy grade group upgrade						
Age at diagnosis	1.02	0.96–1.07	0.46	1.04	0.98–1.10	0.244
Diagnostic PSA	1.12	0.93–1.34	0.22	1.07	0.86–1.33	0.54
PSA density (10×)	1.72	0.70–4.24	0.24	1.27	0.36–4.42	0.71
Number of positive cores at diagnosis	2.52	1.26–5.03	**0.009**	2.26	1.07–4.78	**0.032**
Number of rebiopsy sessions (1 vs 2–5)	0.17	0.04–0.72	**0.016**	0.24	0.11–0.55	**0.001**
PTEN loss in diagnostic biopsies	2.8	1.30–6.03	**0.008**	2.57	1.16–5.70	**0.02**
ERG positivity in diagnostic biopsies	1.59	0.81–3.16	0.18	1.19	0.58–2.47	0.63
Treatment change						
Age at diagnosis	0.991	0.958–1.026	0.615	1.02	0.98–1.06	0.34
Diagnostic PSA	1.018	0.898–1.153	0.782	0.963	0.824–1.126	0.637
PSA density (10×)	2.248	1.206–4.192	**0.011**	1.666	0.733–3.789	0.223
Number of positive cores at diagnosis	1.9	1.174–3.074	**0.009**	1.725	0.999–2.976	**0.05**
Number of rebiopsy sessions (1 vs 2–5)	0.184	0.108–0.315	<**0.001**	0.185	0.104–0.329	<**0.001**
PTEN loss in diagnostic biopsies	2.049	1.154–3.367	**0.014**	2.31	1.264–4.189	**0.006**
ERG positivity in diagnostic biopsies	1.142	0.701–1.862	0.593	1.037	0.610–1.763	0.894
Adverse RP findings						
Age at diagnosis	0.991	0.928–1.059	0.794	1.002	0.923–1.086	0.971
Diagnostic PSA	1.076	0.848–1.364	0.548	0.978	0.716–1.335	0.888
PSA density (10×)	2.195	0.666–7.227	0.196	1.445	0.284–7.36	0.657
Number of positive cores at diagnosis	3.246	1.351–7.802	**0.009**	3.23	1.223–8.532	**0.018**
Number of rebiopsy sessions (1 vs 2–5)	0.194	0.070–0.535	**0.002**	0.187	0.061–0.57	**0.003**
PTEN loss in diagnostic biopsies	4.706	1.921–11.531	**0.001**	4.745	1.84–12.232	**0.001**
ERG positivity in diagnostic biopsies	1.682	0.700–4.042	0.245	1.518	0.588–3.92	0.388

CI = confidence interval; HR = hazard ratio; PSA = prostate-specific antigen; RP = radical prostatectomy. Significant double sided p-values in **bold**.

subsequent biopsies, or undergo treatment change based on any protocol-based reasons. If the patient was not found with significant PCa at the first rebiopsy, he/she had considerably decreased the risk for treatment change, GG upgrade, or adverse RP findings in the following biopsies (HR 0.07–0.18, Table 3). The strongest predictor of the study outcomes was, however, PTEN protein expression. If a patient would have PTEN loss, he/she would harbor 2.31–5.29 times higher risk for disease progression or adverse RP findings (Table 3).

4. Discussion

GG1 PCa is generally considered clinically insignificant and may not need radical treatment with a risk for side effects without any shown benefit in reducing mortality [18,19]. After RP, the oncological outcome is excellent, but men on AS may have better quality of life and AS seems to be more cost effective [20–22]. Biopsies hold uncertainties such as sampling error and subjectivity of grading, and thus, biomarkers of aggressive or nonindolent behavior regardless of GG are needed. Here, we showed that PTEN loss in DBxs in men with PCa on AS predicts worse outcome, that is, shorter duration of AS and worse findings in subsequent RP. One earlier study has shown the association of biopsy PTEN protein loss to upgrading in RP specimen [23]. A recent study in GG2 biopsies showed that PTEN loss correlates with cancer extent in biopsies as well as GG upgrade, stage increase, and nonorgan-confined disease in RP [15]. These results together with our results support the use of PTEN IHC in delineating elevated risk in clinically and histologically low-risk PCa.

The distribution of PTEN and ERG expression was concordant with our previous findings in large RP cohorts [13], and PTEN loss occurs in 29/190 (15.3%) patients in the DBx cohort, whereas ERG was positive in 74 (38.9%) of the DBxs. It is important to notice that PTEN expression in DBx was prognostic despite PTEN expression not being concordant between DBx and RP lesions. This not only reflects the focal heterogeneity of PCa, but also underlines the strength of DBx PTEN IHC as an indicator of disease progression and adverse features. In our study, PTEN-negative biopsies were unilateral in 89.3% of cases, and 73.9% of patients had only one positive biopsy, suggesting a good correlation with sampling the relevant focus related to cancer progression. Our results strongly suggest that PTEN status needs to be considered as an additional tool for patient selection into AS protocol or tailored follow-up, in order to avoid the risk of undue treatment delays and negative consequences of unnecessary repeat biopsies: septic infections and patient discomfort. Contrary to PTEN, ERG expression was concordant in DBx and RP specimen. This confers that *ERG:TMPRSS2* fusion is an early phenomenon during PCa development and is a more common event than PTEN loss in low-risk PCa. A considerable number of ERG-positive biopsies were PTEN negative (76.7%), which suggests that PTEN loss is a subsequent event to ERG fusion already in low-grade PCa.

PTEN deletions have been shown to correlate with early PSA recurrence in both ERG-positive and ERG-negative PCa [8]. In two large patient cohorts, PTEN loss combined with negative ERG IHC was significantly associated with shorter DSS as compared with PTEN loss in ERG-positive PCa after RP [11,13]. We can postulate that the current finding that DBx ERG+/PTEN– patients have shorter survival than ERG−/PTEN– patients most probably reflects the early progression propensities in the androgen-sensitive, low-grade/low-risk PCa. The opposite is most probably true for higher-grade and higher-stage tumors, especially after androgen deprivation therapies.

We found no differences in risk or time to outcome events in Cox regression and Kaplan–Meier analyses when stratifying patients by DBx ERG status, which is in contrast with the results of Berg et al [6]. We were also able to analyze ERG status in matching RP specimens for patients who underwent RP, and found that ERG status in DBx and RP was significantly concordant, in line with the follow-up study by Berg et al [24]. However, the cohort utilized by Berg et al [6] featured a subset (20%) of patients whose clinicopathological features exceeded the protocol limits of the PRIAS (ie, DBx GS = 3 + 4, PSA >10 ng/ml, and/or >3 positive cores), whereas our cohort was enrolled strictly according to the PRIAS criteria. Definition of progression also varied. Besides histological upgrade, Berg et al [6] used more than three positive cores or bilateral positive cores, and PSA-DT <3 yr as criteria for progression. It is generally agreed that Gleason pattern 3 is a very low-risk finding, whereas Gleason pattern 4 poses a clearly increased risk for aggressive biological behavior. Therefore, we also had Gleason pattern 4 or 5 in the repeat biopsies (GG upgrade) as an independent criterion for progression. Nevertheless, these conflicting results indicate that the use of ERG status in the stratification of low-risk AS candidate PCa patients is unclear, and further studies in other larger prospective AS cohorts are needed. Our results do not support the independent role of ERG protein expression in predicting disease progression in low-risk PCa.

In contrast to ERG, the prognostic difference of PTEN expression in early PCa shown here also seems to translate to clinically relevant end points in the later stages of the disease, as emphasized by shortened DSS and MFS after surgery [11,13], and shorter DSS in castration-resistant PCa [7,14]. This further supports the validity of our findings and the role of PTEN in prostate carcinogenesis.

Our study is not without limitations, the most important ones being its retrospective nature and limited sample size. We also did not have a comparative analysis of biomarker expression in DBx and consecutive biopsies, especially those with GG upgrade. Further, a spatial and temporal analysis of ERG and PTEN expression, compared with MRI-guided biopsies, would yield even more relevant information on the heterogeneity of these markers.

5. Conclusions

In conclusion, PTEN loss in biopsies at the time of diagnosis is a strong indicator of disease progression during AS of low-risk PCa. PTEN IHC status analysis together with the number of positive biopsies for cancer is recommended. The

cumulative evidence of PTEN loss being a strong predictor of impaired survival and therapy resistance throughout the PCa continuum warrants careful consideration for earlier radical treatment, and perhaps consideration for tailored follow-up or therapies for PTEN-negative patients.

Author contributions: Thomas Mirtti had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Rannikko, Mirtti.
Acquisition of data: Lokman, Vasarainen, Erickson.
Analysis and interpretation of data: Erickson, Rannikko, Lokman, Mirtti.
Drafting of the manuscript: Erickson, Rannikko, Lokman, Mirtti.
Critical revision of the manuscript for important intellectual content: Rannikko, Mirtti.
Statistical analysis: Erickson, Mirtti, Rannikko.
Obtaining funding: Mirtti, Rannikko.
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References

[1] Bokhorst LP, Valdagni R, Rannikko A, et al. A decade of active surveillance in the PRIAS study: an update and evaluation of the criteria used to recommend a switch to active treatment. Eur Urol 2016;70:954–60.
[2] Iremashvili V, Burdick-Will J, Soloway MS. Improving risk stratification in patients with prostate cancer managed by active surveillance: a nomogram predicting the risk of biopsy progression. BJU Int 2013;112:39–44.
[3] Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
[4] Shah RB, Bentley J, Jeffery Z, DeMarzo AM. Heterogeneity of PTEN and ERG expression in prostate cancer on core needle biopsies: implications for cancer risk stratification and biomarker sampling. Hum Pathol 2015;46:698–706.
[5] Bostrom PJ, Bjartell AS, Catto JW, et al. Genomic predictors of outcome in prostate cancer. Eur Urol 2015;68:1033–44.
[6] Berg KD, Vainer B, Thomsen FB, et al. ERG protein expression in diagnostic specimens is associated with increased risk of progression during active surveillance for prostate cancer. Eur Urol 2014;66:851–60.
[7] Mithal P, Allott E, Gerber L, et al. PTEN loss in biopsy tissue predicts poor clinical outcomes in prostate cancer. Int J Urol 2014;21:1209–14.
[8] Krohn A, Diedler T, Burkhardt L, et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. Am J Pathol 2012;181:401–12.
[9] Cuzick J, Yang ZH, Fisher G, et al. Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer. Br J Cancer 2013;108:2582–9.
[10] Krohn A, Freudenthaler F, Harasimowicz S, et al. Heterogeneity and chronology of PTEN deletion and ERG fusion in prostate cancer. Mod Pathol 2014;27:1612–20.
[11] Ahearn TU, Pettersson A, Ebot EM, et al. A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. J Natl Cancer Inst 2015;108, pii: djv346.
[12] Lotan TL, Gurel B, Sutcliffe S, et al. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. Clin Cancer Res 2011;17:6563–73.
[13] Lahdensuo K, Erickson A, Saarinen I, et al. Loss of PTEN expression in ERG-negative prostate cancer predicts secondary therapies and leads to shorter disease-specific survival time after radical prostatectomy. Mod Pathol 2016;29:1565–74.
[14] Ferraldeschi R, Nava Rodrigues D, Riisnaes R, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. Eur Urol 2015;67:795–802.
[15] Guedes LB, Tosoian JJ, Hicks J, Ross AE, Lotan TL. PTEN loss in Gleason score 3 + 4 = 7 prostate biopsies is associated with non-organ confined disease at radical prostatectomy. J Urol. In press. doi:10.1016/j.juro.2016.09.084.
[16] Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol 2011;35:1014–20.
[17] Lotan TL, Wei W, Ludkovski O, et al. Analytic validation of a clinical-grade PTEN immunohistochemistry assay in prostate cancer by comparison with PTEN FISH. Mod Pathol 2016;29:904–14.
[18] Wilt TJ, Brawer MK, Jones KM, et al. Radical prostatectomy versus observation for localized prostate cancer. N Engl J Med 2012;367:203–213.
[19] Hamdy FC, Donovan JL, Lane JA, et al. 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med 2016;375:1415–24.
[20] Eggener SE, Badani K, Barocas DA, et al. Gleason 6 prostate cancer: translating biology into population health. J Urol 2015;194:626–34.
[21] Vasarainen H, Lokman U, Ruutu M, Taari K, Rannikko A. Prostate cancer active surveillance and health-related quality of life: results of the Finnish arm of the prospective trial. BJU Int 2012;109:1614–9.
[22] Koerber F, Waidelich R, Stollenwerk B, Rogowski W. The cost-utility of open prostatectomy compared with active surveillance in early localised prostate cancer. BMC Health Serv Res 2014;14:163.
[23] Lotan TL, Carvalho FL, Peskoe SB, et al. PTEN loss is associated with upgrading of prostate cancer from biopsy to radical prostatectomy. Mod Pathol 2015;28:128–37.
[24] Berg KD, Brasso K, Thomsen FB, et al. ERG protein expression over time: from diagnostic biopsies to radical prostatectomy specimens in clinically localised prostate cancer. J Clin Pathol 2015;68:788–94.