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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Recurrent Prostate Cancer Genomic Alterations Predict Response to Brachytherapy Treatment

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Abstract

Background: This study aimed to evaluate the association of recurrent molecular alterations in prostate cancer, such as *ERG* rearrangements and phosphatase and tensin homolog gene (*PTEN*) deletions, with oncologic outcomes in patients with prostate cancer treated with brachytherapy.

Methods: Ninety-two men underwent I-125 brachytherapy with a 145 Gy delivered dose between 2000 and 2008. Pretreatment prostate biopsies were analyzed by immunohistochemistry (IHC) and FISH for *ERG* rearrangement and overexpression, *PTEN* deletion, and expression loss. Univariable and multivariable Cox-regression analyses evaluated association of *ERG* and *PTEN* status with biochemical recurrence (BCR).

Results: Within a median follow-up of 73 months, 11% of patients experienced BCR. Of 80 samples with both IHC and FISH performed for *ERG*, 46 (57.8%) demonstrated rearrangement by FISH and 45 (56.3%) by IHC. Of 77 samples with both IHC and FISH for *PTEN*, 14 (18.2%) had *PTEN* deletion by FISH and 22 (28.6%) by IHC. No significant associations were found between *ERG*, *PTEN* status, and clinicopathologic features. Patients with concurrent *ERG* rearrangement and *PTEN* deletion demonstrated significantly worse relapse-free survival rates compared with those with *ERG* or *PTEN* wild type ($P < 0.01$). In multivariable Cox regression analysis adjusted for the effects of standard clinicopathologic features, combined *ERG* rearranged and *PTEN* deletion was independently associated with BCR (HR = 2.6; $P = 0.02$).

Conclusions: Concurrent *ERG* rearrangement and *PTEN* loss was independently associated with time to BCR in patients undergoing brachytherapy. Future studies are needed to validate prostate cancer molecular subtyping for risk stratification.

Impact: Identifying patients in the *ERG*-rearranged/*PTEN*-deleted molecular subclass may improve treatment personalization. *Cancer Epidemiol Biomarkers Prev; 23(4); 594–600. ©2014 AACR.*

Introduction

Prostate cancer is a clinically heterogeneous disease; in Europe, 92,000 men were estimated to have died of advanced prostate cancer in 2012 (1), whereas a significant proportion of men had indolent disease that would not have affected their lifespan.

Brachytherapy can provide local radiation delivery in or near tumors while potentially minimizing the adverse effects and toxicities (2–4). The response to brachytherapy is quite variable, with 5-year biochemical recurrence-free survival rates ranging from 71% to 96% (3, 4). Preoperative nomograms from large studies (5–8) have helped improve risk stratification significantly. Nevertheless, 20% to 40% of patients with intermediate risk prostate cancer will fail primary treatment (9). Recent studies suggest that the variability in clinical outcomes may reflect molecular and genetic heterogeneity, which has led to the search for prognosis-specific genetic alterations (10).

Furthermore, the discovery of different molecular subclasses of prostate cancer (11–14) may help transition to more precise treatment regimens and modalities as demonstrated in other cancers. In breast cancer, the identification of clinically relevant molecular subtypes has led to the development of targeted management strategies, such as trastuzumab (15) for those expressing human epidermal growth factor receptor 2, and the use of PARP inhibitors for the treatment of triple-negative breast cancer who demonstrate *BRCA1* mutations (16). Recent studies have also identified differential responses to radiation therapy according to molecular subtypes in breast cancer (17, 18).

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A major advance has been the discovery of recurrent fusions between androgen-regulated genes and ETS family transcription factors in a majority of prostate cancers, most commonly as a fusion of *TMPRSS2* gene and transcription factor *ERG* (19, 20). The *TMPRSS2:ERG* fusion has been associated with deletions in several tumor suppressor genes including the phosphatase and tensin homolog gene (*PTEN*; refs. 21 and 22), which normally acts to deactivate phosphoinositide 3-kinase–dependent signaling.

ETS gene rearrangements and *PTEN* deletions have now been found to be common molecular events and may be important in prostate carcinogenesis. *PTEN* deletions are found in approximately 40% of prostate cancer specimens, and have been associated with advanced disease and poorer prognosis (21, 22–26). The relationship between ETS rearrangements and clinical outcomes has been inconsistent. In general, population-based studies of watchful-waiting cohorts have found ETS rearrangements to be associated with poorer prognosis (27), whereas retrospective radical prostatectomy cohorts have found conflicting associations (10, 28–30). In a recent study of a watchful-waiting cohort, *PTEN* loss and ETS gene rearrangements were found to be associated with poorer cancer-specific survival (31). Several studies indicate that *PTEN* status may influence response to radiation therapy (32, 33), whereas *ERG* status may not be associated. Although suggested to provide a response advantage (34), the association between *PTEN* loss and ETS gene rearrangements has not been formally studied in patients undergoing radiation therapy previously.

The major objective of this study was to characterize the association of *PTEN* deletions and *ERG* fusions with oncologic outcomes in patients with prostate cancer treated with brachytherapy.

Materials and Methods

Patient population and specimen collection

This institutional review board–approved study included 92 men with a positive biopsy for prostate cancer treated with interstitial brachytherapy (I-125 permanent implant with a delivered dose of 145 Gy) between 2000 and 2008 from Santa Chiara Hospital in Trento, Italy, Santa Maria del Carmine Hospital in Rovereto, Italy, and Bolzano Hospital in Bolzano, Italy. One third of the patients received short-term neoadjuvant androgen deprivation therapy (ADT), either bicalutamide or flutamide, for 4 to 6 months pre-brachytherapy. Patients were assigned to risk groups (low, intermediate, and high) based upon clinical stage, initial biopsy Gleason Score, and prostate-specific antigen (PSA) levels as per the National Comprehensive Cancer Network (35). Biochemical relapse (BCR) was defined according to the Phoenix criteria (PSA nadir + 2 ng/mL; refs. 36). International Prostate Symptom Scores (IPSS) were collected before initiating brachytherapy (Table 1).

Table 1. Study cohort demographics

Number of patients		92
Age (y) (mean ± SD)		65.8 ± 5
cT, *N* (%)	cT1	50 (54)
	cT2/3	42 (46)
PSAi (ng/mL), *N* (%)	<4	7 (8)
	4 ≤ *x*< 10	59 (64)
	>10	26 (28)
Risk group, *N* (%)	L	55 (60)
	I	32 (35)
	H	5 (5)
Gleason score, *N* (%)	6	68 (74)
	7	16 (17)
	8	8 (9)
International prostate symptom score, *N* (%)	<8	80 (87)
	≥ 8	12 (13)
Volume transrectal ultrasound (cc) (mean ± SD)		34 ± 9
BCR, *N* (%)	No event	82 (89)
	Event	10 (11)
RFS (mo) (mean ± SD)	No event	72 ± 28
	Event	50 ± 33
OS (mo) (mean ± SD) (median)	No event	72 ± 28 (70)
	Event	90 ± 30 (96)
Hormonal therapy pre-implant	No	58 (63)
	Yes	34 (37)

For this study, all hematoxylin and eosin (H&E)–stained sections (12 for each patient) from formalin-fixed paraffin-embedded pretreatment biopsies were centrally reviewed by 2 study pathologists (P. Dalla Palma and M. Barbareschi) who were blinded to clinicopathologic parameters and patient outcomes. For each patient, a paraffin block, which was representative of the highest Gleason score, was selected for IHC and FISH evaluations.

Immunohistochemistry analysis

Two 4 μm sections were prepared from each block for immunostaining for ERG and PTEN. Rabbit monoclonal antibodies were utilized (ERG: EPR3864, Ventana, at 1:100 dilution; PTEN: 138G6, Cell Signaling Technology, at 1:25) with an automatic immunostainer (Leica Bond MAX, Leica Biosystem), with antigen retrieval (Bond Polymer Refine Detection, Leica Biosystem). Two pathologists performed a semiquantitative evaluation of nuclear ERG expression using a Fourtier grading system: negative (0), weakly (1+), moderately (2+), and strongly (3+) positive. Any positive staining with >5% of total tumor cells was considered positive for ERG expression (*ERG*+). ERG expression of endothelial cells was utilized as the positive internal control of the immunohistochemical reaction (37). Cytoplasmic and nuclear PTEN expression was scored with the same

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grading system as ERG. Each tumor focus was scored as negative or positive for PTEN protein by comparing staining in malignant glands and adjacent benign glands and/or stroma, which provided an internal positive control. Cases lacking PTEN expression in all or some tumor cells in presence of positive internal controls in the surrounding benign glands and/or stroma were classified as *PTEN*del. Tissue quality was adequate for ERG and for PTEN immunohistochemistry (IHC) assessments for 86 patients (93.5%). IHC scoring was blinded with respect to FISH results.

FISH analysis

Two 4-μm-thick tissue sections from each block were cut for FISH analysis. *ERG* rearrangement status was determined by 2 observers using a dual-color break-apart interphase FISH assay as previously described (19, 38). Briefly, *ERG* rearrangement status was determined using differentially labeled probes spanning the centromeric (BAC clone RP11-24A11, labeled red) and telomeric (BAC clone RP11-372O17, labeled green) regions of *ERG*. *PTEN* deletion was detected using a gene-specific probe (BAC clone CTD-2047N14) and a reference probe, located at 10q25.2 (RP11-431P18). Deletion was defined as fewer than 2 copies of the gene specific probe per nucleus in the presence of 2 reference signals. All clones were tested on metaphase spreads. At least 100 nuclei were evaluated per tissue biopsy using a fluorescence microscope (Olympus BX51; Olympus Optical). Tissue quality was adequate for *ERG* rearrangement and *PTEN* loss status evaluation in 82 cases (89.1%).

Statistical analysis

TMPRSS2:ERG gene rearrangement leading to the overexpression of ERG protein expression as determined by IHC or FISH will be referred to as *ERG*+, *PTEN* deletions will be referred to as *PTEN*del, and the respective molecular subclasses are referred to as *ERG*+/*PTEN*del, *ERG*wt/*PTEN*del, *ERG*+/*PTEN*wt, and *ERG*wt/*PTEN*wt. Differences in variables with a continuous distribution across categories were assessed using the Mann–Whitney *U* test (2 categories). The Fisher exact test and the χ^2 test were used to evaluate the association between categorical variables. Univariable recurrence-free and cancer-specific survival probabilities were estimated using the Kaplan–Meier method. Differences were assessed using the log-rank test. Uni- and multivariable Cox regression analyses addressed factors associated with disease recurrence, cancer-specific, and all-cause mortality. Multivariable analysis was done using forward step-wise logistic regression. Multivariable analyses were performed using ERG and PTEN status by IHC, as status by FISH was not significantly associated with time to relapse-free survival on univariable analysis ($P = 0.09$). All tests were 2-sided, with a P value of <0.05 considered to be statistically significant. All analyses were performed with SPSS 20 (SPSS Inc., IBM Corp.).

Results

Clinical characteristics

The patient characteristics are listed in Table 1. Of the 92 men, 5% (5/92) and 35% (32/92) had high-risk and intermediate-risk disease. Overall, 11% (10/92) of the patients developed BCR with a median overall follow-up of 73 months (range 1–138 months). In total, 37% (34/92) underwent neoadjuvant ADT.

Comparison of IHC and FISH for PTEN and ERG

Of the 92 patients, 80 men (87%) had both IHC and FISH performed for *ERG* rearrangement and 77 (83.7%) for *PTEN* deletion status. *ERG*+ frequency was 57.8% (46/80) when evaluated by FISH and 56.3% (45/80) by IHC, with a concordance of 97.8% ($P < 0.01$). ERG IHC staining was generally either diffusely positive (2+ or 3+ intensity) or completely negative. For the 77 men that had IHC and FISH for *PTEN*, 22 (28.6%) had *PTEN*del by IHC and an additional 14 (18.2%) had hemizygous loss of *PTEN* by FISH, with 4 exhibiting *PTEN*del by FISH and IHC ($P = 1.0$).

In comparing the 86 men that had IHC performed for both ERG and PTEN, 18 (20.9%) were *ERG*+/*PTEN*del, 5 (5.8%) were *ERG*wt/*PTEN*del, and 30 (34.9%) were *ERG*+/*PTEN*wt ($P = 0.01$). A representative *ERG*+/*PTEN*del prostate biopsy is shown in Fig. 1. For the 82 men that had FISH performed for *PTEN* and *ERG*, 7 (8.5%) were *ERG*+/*PTEN*del, 7 (8.5%) were *ERG*wt/*PTEN*del, and 39 (47.6%) were *ERG*+/*PTEN*wt.

Association of PTEN and ERG with clinicopathologic features

Rearrangement of *ERG* by FISH or expression of ERG protein by IHC did not differ according to patient age, PSA, clinical stage, risk-factor grouping, use of neoadjuvant ADT, biopsy Gleason score, or pre-brachytherapy IPSS scores (all $P > 0.05$). The deletion of *PTEN* by FISH or IHC was also not associated with any of the previously mentioned clinicopathologic features (all $P > 0.05$; Table 2).

Association of PTEN and ERG with oncologic outcomes

The median follow-up time was 73 months. Within the follow-up, 10 (11%) developed BCR, and 2 (2.2%) died of disease. From Kaplan–Meier analysis, the actuarial recurrence-free survival was significantly lower for those with moderate and high-risk disease compared with low-risk (log rank P-value < 0.01) diseases. Those who were *PTEN*-del by IHC displayed significantly shorter times to recurrence ($P < 0.01$; Fig. 2A), as did *ERG*+ patients by FISH and IHC ($P = 0.02$; Fig. 2B). Estimated times to recurrence-free survival were not significantly associated with *PTEN*del by FISH.

We hypothesized that *ERG* and *PTEN* status could be used as classifiers to define molecular subgroups. *ERG*+/*PTEN*del patients identified by IHC had significantly

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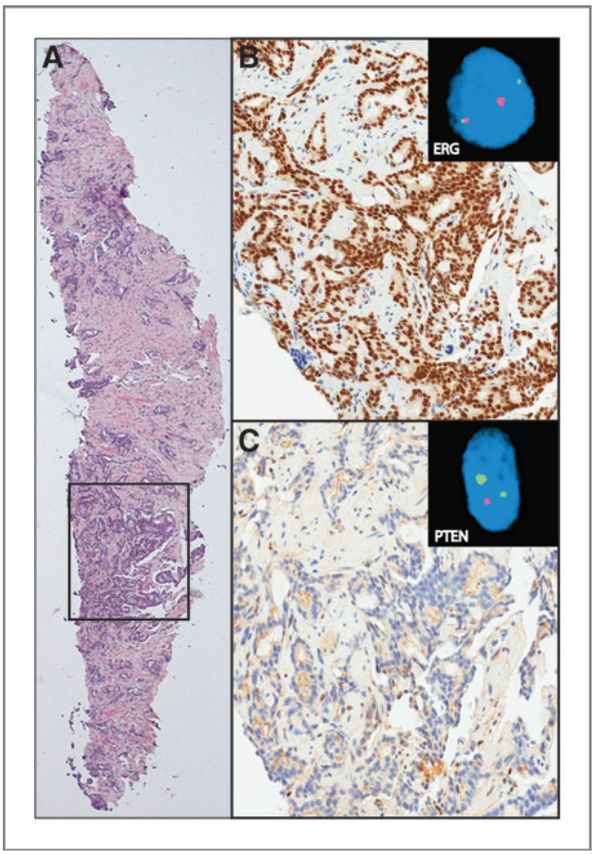

Figure 1. Detection of ERG and PTEN status by IHC and FISH. An ERG+/PTENdel biopsy is represented. A, H&E of needle biopsy showing prostatic adenocarcinoma, Gleason score 7. B, strong, diffuse nuclear ERG IHC staining of tumor glands. *ERG* break-apart FISH assay showing ERG translocation (inset). C, absence of PTEN IHC staining in tumor glands. FISH assay showing hemizygous *PTEN* loss (inset).

lower rates of recurrence-free survival than *ERG*wt/*PTEN*wt patients (log-rank *P*-value <0.01; Fig. 2C), whereas *ERG*+/*PTEN*del by FISH was not significantly associated with time to relapse-free survival ($P = 0.09$). Use of ADT was not associated with BCR or survival in this cohort. In a subset analysis of only those with Gleason 6 on prostate biopsy, *ERG*+/*PTEN*del patients exhibited significantly shorter times to BCR than *ERG*wt/*PTEN*del or *ERG*wt/*PTEN*wt patients ($P = 0.03$). The 2 patients who died of disease were *ERG*+/*PTEN*del.

In multivariable Cox regression analysis, adjusting for age and biopsy Gleason score, *PTEN*del by IHC remained independently associated with BCR [HR = 1.80; *P*-value = 0.01; 95% confidence interval (CI), 1.51–24.2]. The *ERG*+/*PTEN*del subtype was also independently associated with BCR (HR = 2.60, *P*-value = 0.02; 95% CI, 1.62–111.9).

Discussion

Recent discoveries in the genomic landscape and molecular pathways of prostate cancer (10, 12–14) have helped spur the search for molecularly distinct subclasses of prostate cancer that may have differential responses to various therapies. This represents the first known study to investigate the association between the *ERG*+/*PTEN*del subtype and biochemical recurrence in patients with prostate cancer treated with brachytherapy. *ERG*+/*PTEN*-del patients exhibited shorter times to BCR compared with *ERG*wt/*PTEN*del or *ERG*+/*PTEN*wt. After adjusting for disease characteristics, *ERG*+/*PTEN*del subtype was independently associated with BCR in patients that underwent brachytherapy.

Prior studies have reported that the lack of ETS gene fusions and lack of *PTEN* loss (*ERG*wt/*PTEN*wt) were associated with good prognosis in patients undergoing radical prostatectomy or in a conservatively treated watchful waiting cohort (39–41). However those who were *ERG*+/*PTEN*del had faster BCR rates in the prostatectomy cohort (41), whereas the *ERG*wt/*PTEN*del patients had significantly lower survival rates than *ERG*+/*PTEN*del patients in the watchful waiting cohort (31). This discrepancy may reflect differences in the outcomes measured or study sampling methods, but may also reflect true differences in the response to different treatment modalities among distinct molecular subtypes. Larger sample sizes across different treatment modalities will help to further characterize the importance of molecular subtypes in prostate cancer.

To our knowledge, few studies have interrogated the influence of molecular subclasses of prostate cancer on brachytherapy treatment response, specifically. In a recent publication, Dal Pra and colleagues (42) looked at *ERG* status alone in pretreatment biopsies in patients with prostate cancer treated by image-guided radiotherapy (IGRT), and identified no association between *ERG* status and biochemical-free relapse rate. Another study reported that tumors with *c-MYC* amplification alone, or combined with *PTEN* loss, were prognostic for BCR after IGRT (32).

ETS gene fusions and *PTEN* deletions do not exist in isolation but have been found to have complex interactions altering androgen receptor signaling. Chen and colleagues (22) reported that ETS positive cancers that lose *PTEN* exhibit partial restoration of androgen receptor transcription resulting in early-onset invasive prostate cancer, in contrast to the suppression of androgen receptor when there is loss of *PTEN* in ETS negative samples. Several other studies have demonstrated the subclonal loss of *PTEN* in prostate cancers (13, 40, 43), whereas ETS rearrangements tend to occur homogenously in both metastatic and primary prostate cancer samples, indicating that often *PTEN* deletion occurs as a relatively late event compared with ETS fusions in prostate carcinogenesis. These data indicate that patients with ETS gene rearrangements that develop loss of *PTEN* exhibit a distinct molecular environment, with potentially differing responses to treatments (44). In support of this observation, a recent study found that PARP inhibition using rucaparib was able to sensitize cells that exhibited *PTEN* loss and ETS rearrangements to low-dose radiation (34).

Several groups have explored the biologic mechanisms by which *ERG* rearrangement and *PTEN* deletion

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Table 2. Association of ERG and PTEN IHC status with clinicopathologic features

		ERG IHC			PTEN IHC			ERG/PTEN IHC				
		Neg	Pos	*P*-value	Neg	Pos	*P*-value	Neg/no loss	Pos/no loss	Neg/loss	Pos/loss	*P*-value
Age	≤ Median	23	26	0.58	35	13	0.94	20	15	3	10	0.86
	> Median	16	23		28	10		13	15	2	8	
PSAi (ng/mL)	< 4	3	4	0.97	4	2	0.93	1	3	1	1	0.46
	$4 \leq x < 10$	24	31		40	14		20	20	4	10	
	> 10	12	14		19	7		12	7	0	7	
cT	1	24	22	0.21	35	10	0.32	19	16	4	6	0.21
	2/3	15	27		28	13		14	14	1	12	
Risk group	H	3	2	0.62	2	3	0.18	2	0	1	2	0.44
	I	12	19		22	9		11	11	1	8	
	L	24	28		39	11		20	19	3	8	
Gleason score	6	29	36	0.68	49	14	0.13	26	23	2	12	0.18
	7	6	10		11	5		5	6	1	4	
	8	4	3		3	4		2	1	2	2	

may confer radiation resistance. A recent study showed that ERG confers radioresistance through increased DNA damage response efficiency, by interacting with PARP1 and increasing its activity (45). Similarly, it has been suggested that loss of PTEN function delays the repair of radiation-induced double-stranded breaks (46).

In addition, in our study, we confirm that there is a high concordance between IHC and FISH for the detection of *ERG* rearrangements, as previously reported (37, 47). However, *PTEN* assessment is less concordant between PTEN protein loss by IHC and *PTEN* genomic loss by FISH. This is because of the fact that loss of PTEN protein expression may be caused by variable genomic and epigenomic mechanisms, such as inversions and mutations of *PTEN*, recently described rearrangements disrupting PTEN-interacting proteins such as MAGI2 (14) or post-translational inactivation, all of which were not detectable by FISH (48, 49).

There are several limitations to consider in our study. The study was retrospective in design with the inherent biases and confounders of all retrospective studies. Inherent in prostate cancer studies is inter- and intratumoral heterogeneity, which can confound the association of outcomes with molecular subclasses. This study has a relatively small sample size, and the current findings should be substantiated in independent studies on larger cohorts. In addition, there is significant heterogeneity in management strategies with neoadjuvant ADT, and may influence the times to BCR.

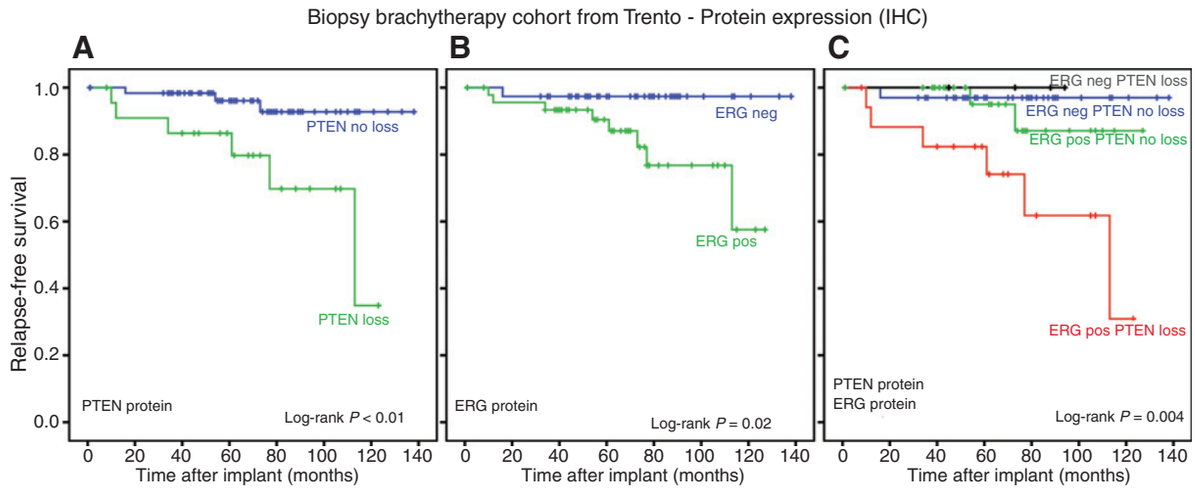

Figure 2. Prostate cancer relapse-free survival according to PTEN and ERG IHC status. Kaplan–Meier curves are reported with respect to recurrence-free survival for PTEN loss identified by IHC (A), for *ERG*+ prostate cancer identified by IHC (B), and for their combination (C).

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Conclusions

Concurrent *ERG* rearrangement and loss of *PTEN*, which seems to represent a biologically relevant molecular subclass, was independently associated with time to BCR and worse prognosis in patients undergoing brachytherapy. Identifying patients in this subclass may predict failure to radiotherapy and may therefore improve treatment personalization by suggesting alternative management strategies. Larger prospective studies are needed to validate the molecular subtyping of prostate cancer for risk stratification.

Disclosure of Potential Conflicts of Interest

J.M. Mosquera has a commercial research grant from Ventana Medical Systems, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Study supervision: P. Dalla Palma, J.M. Mosquera, M. Barbareschi, F. Demichelis

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