Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180

Cancer Epidemiology, Biomarkers & Prevention

*Research Article*

# Recurrent Prostate Cancer Genomic Alterations Predict Response to Brachytherapy Treatment

Jacqueline Fontugne[1], Daniel Lee[1,2], Chiara Cantaloni[5], Christopher E. Barbieri[1,2], Orazio Caffo[6], Esther Hanspeter[9], Guido Mazzoleni[9], Paolo Dalla Palma[7], Mark A. Rubin[1,2,3], Giovanni Fellin[8], Juan Miguel Mosquera[1,3], Mattia Barbareschi[7], and Francesca Demichelis[4,5]

## 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).

**Authors' Affiliations:** Departments of [1]Pathology and Laboratory Medicine and [2]Urology; [3]Institute for Precision Medicine; [4]Institute for Computational Biomedicine, Weill Medical College of Cornell University, New York, New York; [5]Centre for Integrative Biology, University of Trento; Departments of [6]Medical Oncology, [7]Pathology, and [8]Radiotherapy and Medical Physics, Ospedale Santa Chiara, Trento; and [9]Department of Surgical Pathology, Central Hospital, Bolzano, Italy

**Corresponding Authors:** Francesca Demichelis, Centre for Integrative Biology, University of Trento, Via Sommarive, 14, 38123 Povo, Trento, Italy. Phone: 39-0461-285305; Fax: 39-0461-283937; E-mail: demichelis@science.unitn.it; and Juan Miguel Mosquera, Weill Medical College of Cornell University, NewYork-Presbyterian Hospital, 525 East 68th Street, ST-1015B, New York, NY 10065. Phone: 212-746-2700; Fax: 212-746-8816; E-mail: jmm9018@med.cornell.edu

**doi:** 10.1158/1055-9965.EPI-13-1180

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180



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

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180



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 $\chi^2$ 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

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180



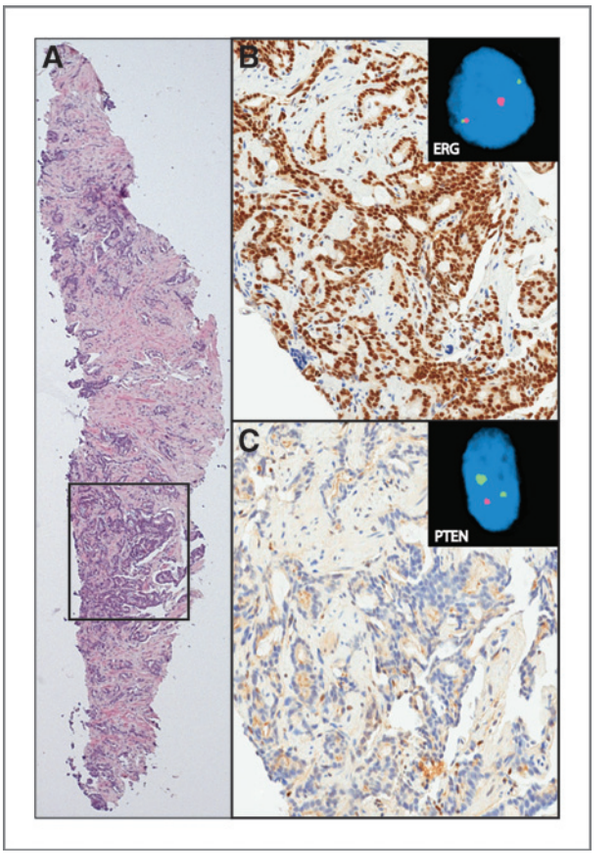


Figure 1. Detection of ERG and PTEN status by IHC and FISH. An ERG+/PTENdel biopsy is represented. A, H&E of needle biopsy showing prostatic adenocarcinoma, Gleason score 7. B, strong, diffuse nuclear ERG IHC staining of tumor glands. *ERG* break-apart FISH assay showing ERG translocation (inset). C,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

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180



**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.

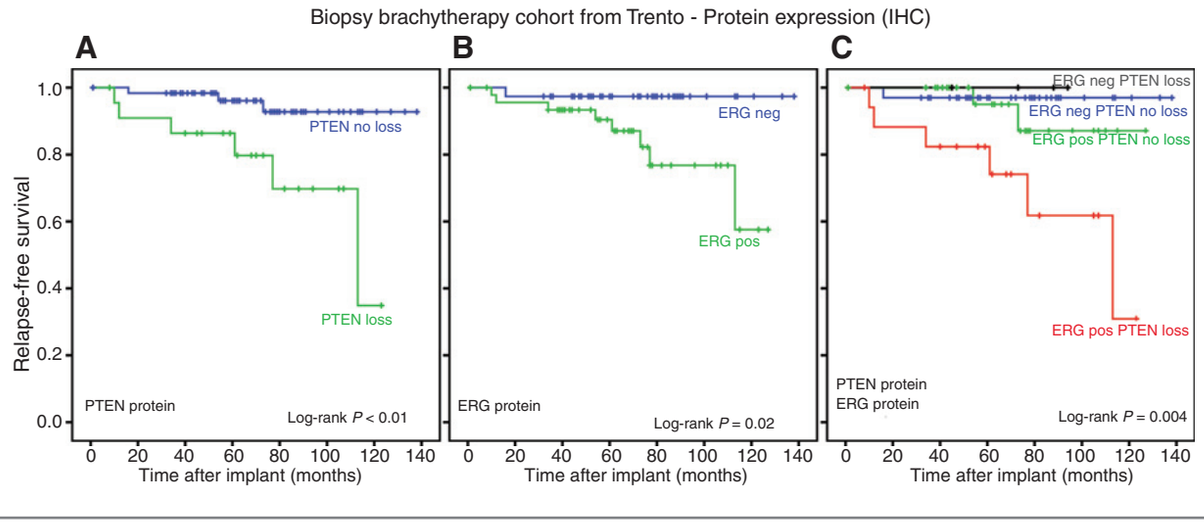


**Figure 2.** Prostate cancer relapse-free survival according to PTEN and ERG IHC status. Kaplan–Meier curves are reported with respect to recurrence-free survival for PTEN loss identified by IHC (A), for *ERG*+ prostate cancer identified by IHC (B), and for their combination (C).

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180



## 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

**Conception and design:** O. Caffo, M.A. Rubin, G. Fellin, J.M. Mosquera, F. Demichelis
**Development of methodology:** P. Dalla Palma, F. Demichelis
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** J. Fontugne, C. Cantaloni, O. Caffo, E. Hanspeter, G. Mazzoleni, G. Fellin, J.M. Mosquera, M. Barbareschi, F. Demichelis
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** J. Fontugne, D. Lee, C.E. Barbieri, P. Dalla Palma, J.M. Mosquera, M. Barbareschi, F. Demichelis
**Writing, review, and/or revision of the manuscript:** J. Fontugne, D. Lee, C.E. Barbieri, O. Caffo, M.A. Rubin, G. Fellin, J.M. Mosquera, M. Barbareschi, F. Demichelis
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** D. Lee, E. Hanspeter, G. Mazzoleni, F. Demichelis
**Study supervision:** P. Dalla Palma, J.M. Mosquera, M. Barbareschi, F. Demichelis

## Acknowledgments

The authors thank T.Y. MacDonald for her assistance in performing FISH assays.

## Grant Support

This study was supported by the Fondazione Trentina per la Ricerca sui Tumori (F. Demichelis and C. Cantaloni), by the Associazione Italiana per la Ricerca sul Cancro (AIRC, IG 13562), and by the Early Detection Research Network (5U01 CA11275-07 for M.A. Rubin, J. Fontugne, and J.M. Mosquera).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 10, 2013; revised December 31, 2013; accepted January 15, 2014; published OnlineFirst February 10, 2014.

## References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374–403.
2. Ferrer M, Guedea F, Suarez JF, De Paula B, Macias V, Marino A , et al. Quality of life impact of treatments for localized prostate cancer: Cohort study with a 5-year follow-up. Radiother Oncol 2013;108:306–13.
3. Peinemann F, Grouven U, Bartel C, Sauerland S, Borchers H, Pinkawa M, et al. Permanent interstitial low-dose-rate brachytherapy for patients with localised prostate cancer: a systematic review of randomised and nonrandomised controlled clinical trials. Eur Urol 2011;60:881–93.
4. Peinemann F, Grouven U, Hemkens LG, Bartel C, Borchers H, Pinkawa M, et al. Low-dose rate brachytherapy for men with localized prostate cancer. Cochrane Database Syst Rev 2011:CD008871.
5. Stephenson AJ, Scardino PT, Eastham JA, Bianco FJ Jr, Dotan ZA, Fearn PA, et al. Preoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. J Natl Cancer Inst 2006;98:715–7.
6. Stephenson AJ, Kattan MW, Eastham JA, Bianco FJ Jr, Yossepowitch O, Vickers AJ, et al. Prostate cancer-specific mortality after radical prostatectomy for patients treated in the prostate-specific antigen era. J Clin Oncol 2009;27:4300–5.
7. Briganti A, Joniau S, Gontero P, Abdollah F, Passoni NM, Tombal B, et al. Identifying the best candidate for radical prostatectomy among patients with high-risk prostate cancer. Eur Urol 2012;61:584–92.
8. Eisenberg MS, Karnes RJ, Kaushik D, Rangel L, Bergstralh EJ, Boorjian SA. Risk stratification of patients with extraprostatic extension and negative lymph nodes at radical prostatectomy: identifying optimal candidates for adjuvant therapy. J Urol 2013;190:1735–41.
9. Nichol AM, Warde P, Bristow RG. Optimal treatment of intermediate-risk prostate carcinoma with radiotherapy: clinical and translational issues. Cancer 2005;104:891–905.
10. Barbieri CE, Bangma CH, Bjartell A, Catto JW, Culig Z, Gronberg H, et al. The mutational landscape of prostate cancer. Eur Urol 2013;64:567–76.
11. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
12. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9.
13. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y, et al. Punctuated evolution of prostate cancer genomes. Cell 2013;153:666–77.
14. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214–20.
15. Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005;23:3676–85.
16. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, et al. Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 2011;364:205–14.
17. Nguyen PL, Taghian AG, Katz MS, Niemierko A, Abi Raad RF, Boon WL, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol 2008;26:2373–8.
18. Kyndi M, Sorensen FB, Knudsen H, Overgaard M, Nielsen HM, Overgaard J. Estrogen receptor, progesterone receptor, HER-2, and response to postmastectomy radiotherapy in high-risk breast cancer: the Danish Breast Cancer Cooperative Group. J Clin Oncol 2008;26:1419–26.
19. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
20. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007;448:595–9.
21. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet 2009;41:619–24.

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180




22. Chen Y, Chi P, Rockowitz S, Laquinta PJ, Shamu T, Shukla S, et al. ETS factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to PTEN loss. Nat Med 2013; 19:1023–9.
23. Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci U S A 2011;108:17087–92.
24. Weischenfeldt J, Simon R, Feuerbach L, Schlangen K, Weichenhan D, Minner S, et al. Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. Cancer Cell 2013;23:159–70.
25. Krohn A, Diedler T, Burkhardt L, Mayer PS, De Silva C, Meyer-Kornblum M, 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.
26. Liu S, Yoshimoto M, Trpkov K, Duan Q, Firszt M, Corcos J, et al. Detection of ERG gene rearrangements and PTEN deletions in unsuspected prostate cancer of the transition zone. Cancer Biol Ther 2011; 11:562–6.
27. Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007;26:4596–9.
28. Darnel AD, Lafargue CJ, Vollmer RT, Corcos J, Bismar TA. TMPRSS2-ERG fusion is frequently observed in Gleason pattern 3 prostate cancer in a Canadian cohort. Cancer Biol Ther 2009;8:125–30.
29. Petrovics G, Liu A, Shaheduzzaman S, Furusato B, Sun C, Chen Y, et al. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene 2005;24:3847–52.
30. Saramaki OR, Harjula AE, Martikainen PM, Vessella RL, Tammela TL, Visakorpi T. TMPRSS2:ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. Clin Cancer Res 2008;14: 3395–400.
31. Reid AH, Attard G, Ambroisine L, Fisher G, Kovacs G, Brewer D, et al. Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. Br J Cancer 2010;102:678–84.
32. Zafarana G, Ishkanian AS, Malloff CA, Locke JA, Sykes J, Thoms J, et al. Copy number alterations of c-MYC and PTEN are prognostic factors for relapse after prostate cancer radiotherapy. Cancer 2012; 118:4053–62.
33. Rosser CJ, Tanaka M, Pisters LL, Tanaka N, Levy LB, Hoover DC, et al. Adenoviral-mediated PTEN transgene expression sensitizes Bcl-2-expressing prostate cancer cells to radiation. Cancer Gene Ther 2004; 11:273–9.
34. Chatterjee P, Choudhary GS, Sharma A, Singh K, Heston WD, Cieski J, et al. PARP inhibition sensitizes to low dose-rate radiation TMPRSS2-ERG fusion gene-expressing and PTEN-deficient prostate cancer cells. PLoS ONE 2013;8:e60408.
35. Mohler J, Bahnson RR, Boston B, Busby JE, D'Amico A, Eastham JA, et al. NCCN clinical practice guidelines in oncology: prostate cancer. J Natl Compr Canc Netw 2010;8:162–200.
36. Roach M 3rd, Hanks G, Thames H Jr., Schellhammer P, Shipley WU, Sokol GH, et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. Int J Radiat Oncol Biol Phys 2006;65: 965–74.
37. Park K, Tomlins SA, Mudaliar KM, Chiu YL, Esgueva R, Mehra R, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia 2010;12:590–8.
38. Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, et al. TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res 2006; 66:8337–41.
39. Nagle RB, Algotar AM, Cortez CC, Smith K, Jones C, Sathyanarayana UG, et al. ERG overexpression and PTEN status predict capsular penetration in prostate carcinoma. Prostate 2013;73: 1233–40.
40. Yoshimoto M, Ding K, Sweet JM, Ludkovski O, Trottier G, Song KS, et al. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. Mod Pathol 2013;26:435–47.
41. Yoshimoto M, Joshua AM, Cunha IW, Coundry RA, Fonseca FP, Ludkovski O, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 2008;21:1451–60.
42. Dal Pra A, Lalonde E, Sykes J, Warde F, Ishkanian A, Meng A, et al. TMPRSS2-ERG status is not prognostic following prostate cancer radiotherapy: implications for fusion status and DSB repair. Clin Cancer Res 2013;19:5202–9.
43. Gumuskaya B, Gurel B, Fedor H, Tan HL, Weier CA, Hicks JL, et al. Assessing the order of critical alterations in prostate cancer development and progression by IHC: further evidence that PTEN loss occurs subsequent to ERG gene fusion. Prostate Cancer Prostatic Dis 2013; 16:209–15.
44. Demichelis F, Attard G. A step toward functionally characterized prostate cancer molecular subtypes. Nat Med 2013;19:966–7.
45. Hans S, Brenner JC, Salboch A, Jackson W, Speers C, Wilder-Romans K, et al. Targeted radiosensitization of ETS fusion-positive prostate cancer through PARP1 inhibition. Neoplasia 2013;15: 1207–17
46. Pappas G, Zumstein LA, Munshi A, Hobbs M, Meyn RE. Adenoviral-mediated PTEN expression radiosensitizes non-small cell lung cancer cells by suppressing DNA repair capacity. Cancer Gene Ther 2007; 14:543–9.
47. Chaux A, Albadine R, Toubaji A, Hicks J, Meeker A, Platz EA, 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.
48. Bhalla R, Kunju LP, Tomlins SA, Christopherson K, Cortez C, Carskadon S, et al. Novel dual-color immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma. Mod Pathol 2013;26:835–48.
49. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, 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.

Published OnlineFirst February 10, 2014; DOI: 10.1158/1055-9965.EPI-13-1180

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

# Recurrent Prostate Cancer Genomic Alterations Predict Response to Brachytherapy Treatment

Jacqueline Fontugne, Daniel Lee, Chiara Cantaloni, et al.

*Cancer Epidemiol Biomarkers Prev* 2014;23:594-600. Published OnlineFirst February 10, 2014.