Prostate Cancer

PTEN Protein Loss and Clinical Outcome from Castration-resistant Prostate Cancer Treated with Abiraterone Acetate

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Article info

Abstract

Background: Loss of the tumor suppressor phosphatase and tensin homolog (PTEN) occurs frequently in prostate cancers. Preclinical evidence suggests that activation of PI3K/AKT signaling through loss of PTEN can result in resistance to hormonal treatment in prostate cancer.

Objective: To explore the antitumor activity of abiraterone acetate (abiraterone) in castration-resistant prostate cancer (CRPC) patients with and without loss of PTEN protein expression.

Design, setting, and participants: We retrospectively identified patients who had received abiraterone and had hormone-sensitive prostate cancer (HSPC) and/or CRPC tissue available for PTEN immunohistochemical analysis.

Outcome measurements and statistical analysis: The primary end point was overall survival from initiation of abiraterone treatment. Relationship with outcome was analyzed using multivariate Cox regression and log-rank analyses.

Results and limitations: A total of 144 patients were identified who had received abiraterone post-docetaxel and had available tumor tissue. Overall, loss of PTEN expression was observed in 40% of patients. Matched HSPC and CRPC tumor biopsies were available for 41 patients. PTEN status in CRPC correlated with HSPC in 86% of cases. Loss of PTEN expression was associated with shorter median overall survival (14 vs 21 mo; hazard ratio [HR]: 1.75; 95% confidence interval [CI], 1.19–2.55; $p = 0.004$) and shorter median duration of abiraterone treatment (24 vs 28 wk; HR: 1.6; 95% CI, 1.12–2.28; $p = 0.009$). PTEN protein loss, high lactate dehydrogenase, and the presence of visceral metastases were identified as independent prognostic factors in multivariate analysis.

Conclusions: Our results indicate that loss of PTEN expression was associated with worse survival and shorter time on abiraterone treatment. Further studies in larger and prospective cohorts are warranted.

Patient summary: PTEN is a protein often lost in prostate cancer cells. In this study we evaluated if prostate cancers that lack this protein respond differently to treatment with abiraterone acetate. We demonstrated that the survival of patients with loss of PTEN is shorter than patients with normal PTEN expression.

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1. Introduction

Prostate cancer (PCa) is the most common malignant tumor in men and carries significant morbidity and mortality [1]. Substantial improvements have been made in the molecular characterization of this disease, but these have not yet been translated into relevant stratification in clinical practice [2].

Loss of the tumor suppressor phosphate and tensin homolog (PTEN) is one of the most common molecular aberrations in PCa and has been correlated with a poor prognosis [3–10]. As a lipid phosphatase and negative regulator of the PI3K/AKT/mTOR pathway, PTEN controls a number of cellular processes including survival, proliferation, metabolism, migration, and cellular architecture [11]. Preclinical data have proposed a role for PTEN loss and activation of the PI3K/AKT pathway in regulating androgen receptor (AR) transcriptional output and in driving resistance [5,12–14].

PTEN has attracted great interest as a biomarker in PCa. Initial assessments of PTEN loss mostly focused on genomic deletions of the PTEN locus identified by fluorescence in situ hybridization (FISH) [7,15]. However, multiple mechanisms account for loss of PTEN protein expression including genomic deletion, mutation, microRNA, and promoter methylation [16], and FISH may therefore be systematically underestimating the frequency of loss of PTEN in PCa [8,15–18].

Reliable analysis of PTEN status by immunohistochemistry (IHC) in routinely processed clinical formalin-fixed and paraffin-embedded (FFPE) pathology specimens has been established [19], and good concordance has been demonstrated between FISH detection of PTEN deletions and the overall cellular PTEN protein expression by IHC [8,15, 18,20]. Importantly, assessment of PTEN protein expression by IHC offers the advantage of detecting loss of PTEN by mechanisms other than genomic deletion [8,15].

In this retrospective study we investigated PTEN protein expression in hormone-naive prostate cancer and castration-resistant prostate cancer (CRPC) tissue and its association with clinical outcome in metastatic CRPC patients treated with the CYP17 inhibitor abiraterone acetate (abiraterone).

2. Patients and methods

2.1. Patient cohort

Potentially eligible cases were identified from a population of men with CRPC treated at the Royal Marsden NHS Foundation Trust between January 2006 and December 2013. Patients were included in this study if they had received abiraterone plus prednisolone treatment following docetaxel for metastatic CRPC and had available paraffin tissue blocks from metastatic sites or primary tumors for PTEN IHC. Exclusion criteria were previous treatment with a PI3K/AKT inhibitor or enzalutamide. Patients with histologic features supporting a diagnosis of pure neuroendocrine or small cell cancer were not included. All patients gave their written informed consent and were enrolled in institutional protocols approved by the Royal Marsden NHS Foundation Trust Hospital (London, UK) ethics review committee (reference no. 04/Q0801/60).

Demographics and clinical data were retrospectively collected from the hospital electronic patient record system.

2.2. Tissue samples

PCa tissue was obtained from prostate needle biopsies, transurethral resections of the prostate, prostatectomies or PCa metastases within bone (bone marrow trephine), lymph node, or viscera (needle biopsies) (Supplementary Table 1). A subset of patients had matched therapy-naive hormone-sensitive primary tissue and CRPC fresh tumor samples available. All tissue blocks were resectioned and reviewed by a pathologist (D.N.R. or K.T.) for confirmation of the adequacy of the material (≥50 viable cells).

2.3. PTEN immunohistochemistry

PTEN protein expression was determined by IHC on 4-μM-thick FFPE sections as previously described [15,21]. Briefly, PTEN immunoreactivity was investigated using a rabbit monoclonal anti-PTEN antibody 138G6 (catalog no. 9559; Cell Signaling Technology, Inc, Danvers, MA, USA) [19] detected using the Vectastain Elite ABC kit (catalog no. PK-6101; Vector Laboratories, Burlingame, CA, USA). Nuclear and cytoplasmic staining intensity were semiquantitatively assessed using the H-score formula: 3 times percentage of strongly staining cells and 2 times percentage of moderately staining cells and percentage of weakly staining cells, giving a range of 0–300 [22]. PTEN-positive controls included normal prostate tissue and 22RV1 xenograft tissue, and PTEN-loss controls included PC3 (PCa cell line-PTEN null) xenografts. Endothelial cells and stroma were used as internal positive controls for PTEN. Because there is no validated standard definition for PTEN positivity or loss on the basis of our extensive literature review and personal discussions, we devised a binary classification system in which cases were considered PTEN negative if they either showed a complete absence of PTEN staining or weak intensity staining compared with internal control in no more than 10% of cancer cells (H-score ≤10). The evaluation of all IHC sections was done by a pathologist (D.N.R.) blinded to the patients' clinical characteristics and outcome data. The PTEN IHC assay and binary classification system was validated in a series of PCa specimens for which we had available PTEN genomic status by FISH (n = 103).

A fraction of tumors showed prominent intratumor heterogeneity for PTEN expression with clearly distinct PTEN-positive and PTEN-negative areas, suggesting two clear populations of tumor cells in which one population had PTEN loss and the other did not. For the purpose of data analysis, a case was considered PTEN negative if any tumor area showed a complete absence of PTEN staining. For the purpose of survival analysis when a change in PTEN status was observed between patient-matched hormone-sensitive prostate cancer (HSPC) and CRPC samples, cases were classified accordingly to the PTEN status in the CRPC sample.

2.4. Fluorescence in situ hybridization

PTEN FISH was performed as described previously [7,21,23] on 4-μM FFPE tissue slices adjacent to hematoxylin and eosin sections that were confirmed to contain a minimum of 50 intact cells. All the tissues were then scanned using an Ariol SL-50 scanner (Applied Imaging); all areas of tumor were scored for PTEN loss by FISH status by an operator blinded to the IHC results. Heterozygous deletions were recorded with >30% of cells containing one signal for the locus probe and two or more signals for the chromosome 10 control probe. Homozygous deletions were recorded by the loss of both copies of PTEN locus probe and the presence of two or more signals for chromosome 10 control probe in >30% of cells as cut-off.

2.5. Statistical analyses

Biochemical response to abiraterone was defined per the Prostate Cancer Working Group Criteria 2 as a ≥50% decline in prostate-specific antigen

(PSA) from baseline, confirmed at least 3 wk later [24]. Survival was measured from the first date of abiraterone treatment to the date of last contact or to the date of death from any cause. The Kaplan-Meier product-limit method was used to estimate the duration of abiraterone treatment and overall survival. Independent sample *t* tests and Pearson chi-square were used to study the association of PTEN loss with continuous and categorical variables, respectively. All tests were two sided, and a *p* value ≤0.05 was considered statistically significant. Univariate and multivariate analyses of the independent factors for overall survival were performed using the Cox regression model with a 95% confidence interval (CI). High/low values for accepted normal ranges were used for laboratory parameters [25]. PSA was highly skewed, and the logarithm function was used to transform this variable. Twenty-seven patients had at least one missing baseline variable. Descriptive statistics and survival analyses were performed using IBM SPSS Statistics v.22.

3. Results

3.1. Validation of immunohistochemical cut-off

Because no validated standard definition for PTEN positivity or loss by IHC exists, we first determined if our PTEN IHC assay and binary classification system was sensitive for detecting PTEN loss in clinical PCa specimens. We evaluated PTEN protein by IHC in a series of PCa specimens for which we had available PTEN genomic status by FISH (n = 103). In patients with no deletion of PTEN, a variable degree of cytoplasmic and nuclear PTEN protein immunostaining was evident (Fig. 1), whereas patients with homozygous loss of both PTEN alleles had loss of PTEN protein by IHC with a median cytoplasmic H-score of 0 (range: 0–10) (Fig. 1). According to our classification system, 100% of patients with homozygous loss were classified as PTEN negative. PTEN expression in patients with heterozygous loss was low/absent with a median cytoplasmic PTEN H-score of 0 (range: 0–80) (Fig. 1), confirming previous observations that patients with heterozygous loss commonly have loss of PTEN expression by IHC [8,15]. Taken together, these results confirmed our PTEN IHC was sensitive for the detection of genomic PTEN loss and the validity of our cut-off to classify PTEN status.

3.2. Metastatic prostate cancer patients commonly have loss of PTEN protein expression

A total of 144 patients were identified who had received treatment with abiraterone in the post-docetaxel setting and had tissue available for PTEN analysis. One single tissue sample was available for 95 patients; 42 patients had two tissue samples collected at different time points, and 7 had three tissue samples available for analysis. PTEN protein was scored by IHC in 200 tissue samples from 144 patients (Supplementary Table 1). PTEN loss occurred in 38% (54 of 140) of the primary tumor samples and 50% (30 of 60) of the metastatic CRPC samples. There was no significant association between PTEN loss and specimen type (primary vs metastatic; p = 0.1 by Pearson chi-square test). Of note, however, was that the rate of PTEN loss in liver metastases was higher than that in other sites (70% [7 of 10]). Nine of the 140 primary tumor samples (6%) (eight prostate needle biopsies, one radical prostatectomy) showed prominent intratumor heterogeneity for PTEN expression, with distinct areas positive for PTEN, whereas other areas showed absence of PTEN staining (Fig. 2).

Overall, PTEN loss was demonstrated in 40% of patients (57 of 144). Forty-nine patients had two samples collected at different times evaluated for PTEN expression. The median interval between the first and second sample collection was 4.7 yr (interquartile range [IQR]: 2.2–8.9). Intrapatient concordance was demonstrated in 90% of the cases (44 of 49). According to our classification, a change in PTEN status was only observed in 5 of 49 patients. The PTEN status by IHC in primary prostate tissue and distant metastases is presented in Supplementary Table 2.

3.3. PTEN status does not usually change with the development of castration-resistant prostate cancer

We next examined PTEN status in matched same-patient HSPC and CRPC tissue to evaluate if the frequency of PTEN loss changed with disease progression. Matched HSPC and CRPC tissue samples were available for 41 patients. Staining
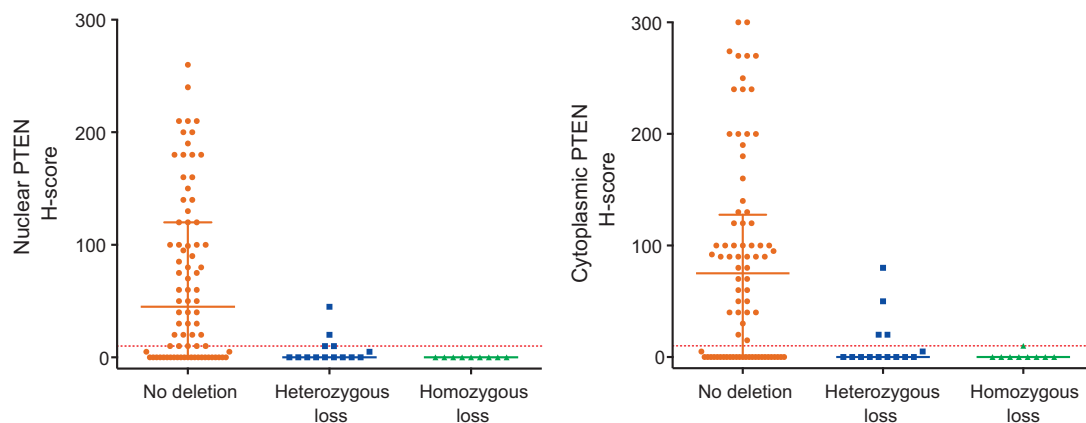

Fig. 1 – Cytoplasmic (right) and nuclear (left) PTEN H-score in specimens with no somatic cell deletion of PTEN by fluorescent in situ hybridization (FISH) (no deletion); heterozygous somatic PTEN loss by FISH (heterozygous loss); and homozygous (biallelic) somatic PTEN loss by FISH (homozygous loss). Staining intensity in prostate cancer cells was scored 0–3 (negative; weak; moderate; intense), and this value was multiplied by the percentage of cancer cells staining positively to generate an H-score (0–300). PTEN protein loss was defined as an H-score ≤10 (red line). FISH = fluorescent in situ hybridization.
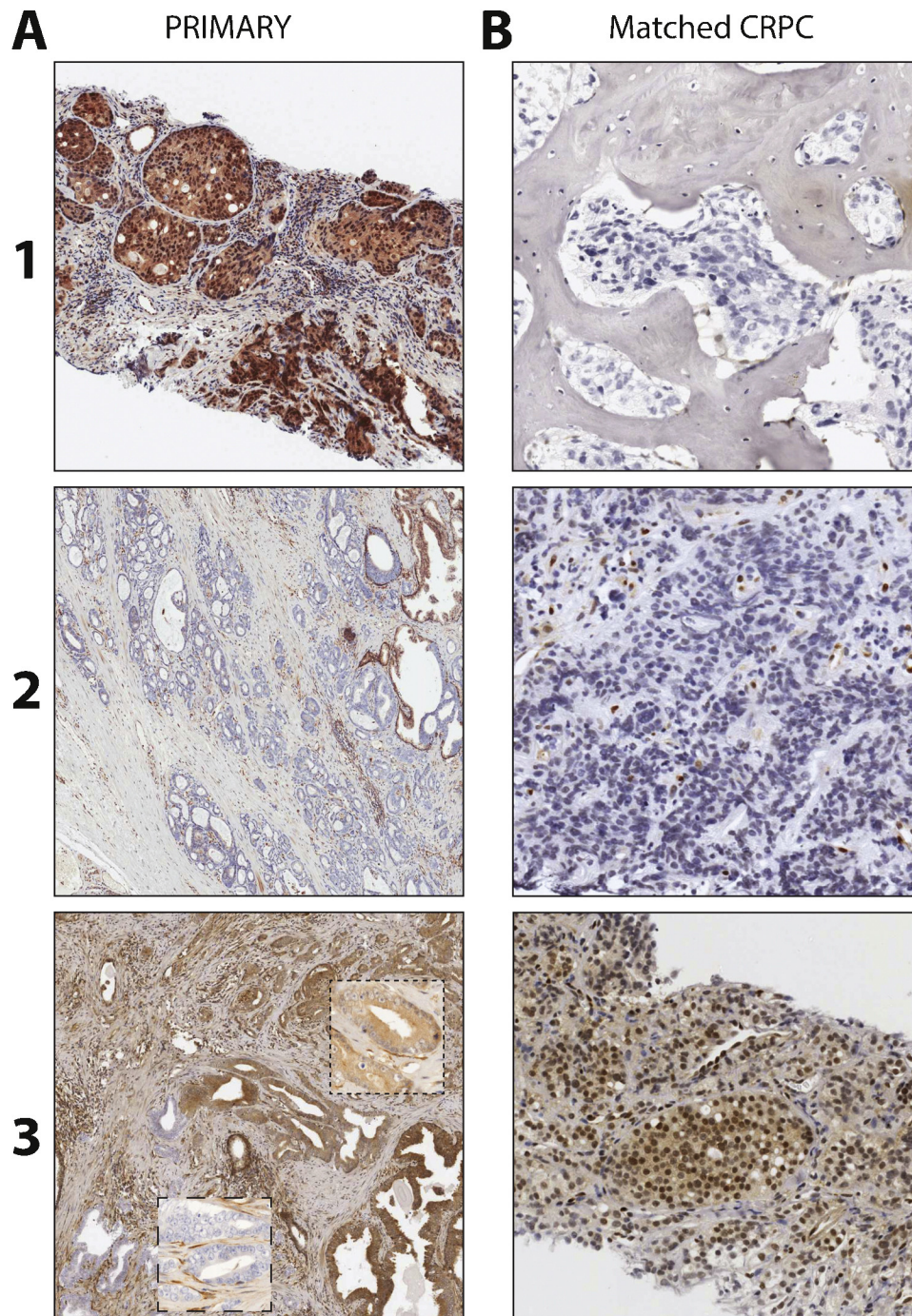
Fig. 2 – Micrographs show PTEN expression by diaminobenzidine immunohistochemistry method in six samples. (A) Column A displays three micrographs of primary prostate adenocarcinoma. (A1) Needle biopsy with moderate nuclear and cytoplasmic PTEN protein expression (×100 magnification). (A2) Prostatectomy specimen with extensive PTEN protein negative adenocarcinoma infiltration. In the upper right-hand corner, cytoplasmic PTEN protein–positive benign glands are adjacent to invasive carcinoma (×50 magnification). (A3) Prostatectomy specimen showing heterogeneous PTEN protein expression (×50 magnification). Two inserted photographs at ×200 magnification demonstrate cytoplasmic positivity (upper right: short dashes square) and negativity (bottom left: long dashes square). Nuclear staining was predominantly negative in both areas. (B) Column B shows three matched metastatic castration-resistant prostate cancer samples acquired at a later time point from the same patients as the respective diagnostic samples in column A. (B1, B2) PTEN negativity has been verified in bone marrow and liver metastases, respectively (×200 magnification). (B3) PTEN nuclear and cytoplasmic positivity is demonstrated in tumor nests of a lymph node biopsy (×200 magnification). CRPC = castration-resistant prostate cancer.

consistent with loss of PTEN expression was found in 44% (18 of 41) and 57% (22 of 41) of the HSPC and CRPC tumor specimens, respectively. Overall concordant PTEN status was evident between matched HSPC and CRPC tissue

samples in 86% of cases (32 of 37 patients); a change in classification from PTEN positive to PTEN negative was reported in three patients (7%), and conversely from PTEN negative to PTEN positive in two patients (5%). Intratumor

heterogeneity in PTEN expression was observed in 5 of the 41 HSPC and in 1 CRPC; 3 of the 5 heterogeneous HSPC samples demonstrated eventual complete loss of PTEN expression in the CRPC sample; 1 retained PTEN expression; and 1 showed heterogeneity also in the CRPC sample.

3.4. Loss of PTEN expression associates with worse clinical outcome

At the commencement of abiraterone treatment, the median age was 68 yr (IQR: 63–73), and all patients had

Table 1 – Demographic and clinical characteristics of patients at the time of abiraterone initiation

	Overall $n = 144$	PTEN negative $n = 57$	PTEN positive $n = 87$	p value
Age, yr				
Median	68	66	69	>0.9[a]
IQR	63–73	62–72	65–73	
Gleason score at diagnosis, n (%)				
≤6	15 (10)	7 (12)	8 (10)	0.06[b]
7	33 (23)	19 (33)	14 (16)	
8–10	71 (49)	23 (41)	48 (55)	
NA	25 (17)	8 (14)	17 (19)	
Sites of metastases, n (%)				
Bone	128 (88)	52 (91)	76 (87)	0.7[c]
Nodal	75 (51)	28 (48)	47 (54)	0.7[c]
Visceral	25 (17)	14 (24)	11 (12)	0.03[c]
ECOG PS, n (%)				
0	35 (24)	13 (23)	22 (25)	
1	86 (60)	35 (61)	51 (59)	0.4[b]
2	7 (5)	4 (7)	3 (3)	
NA	16 (11)	5 (9)	11 (13)	
PSA, μg/l				
Median	213	155	237	0.5[a]
IQR	60–681	56–660	67–762	
Hemoglobin, g/dl				
Median	11.6	11.8	11.5	0.9[a]
IQR	10.5–12.7	10.4–12.6	10.5–12.8	
NA	17	4	13	
Alkaline phosphatase, IU/l				
Median	131	155	124	0.2[a]
IQR	69–253	77–251	69–272	
NA	13	4	9	
Lactate dehydrogenase, IU/l				
Median	188	216	181	>0.9[a]
IQR	154–246	154–343	155–226	
NA	22	7	15	
Albumin, g/l				
Median	35	35	36	0.4[a]
IQR	33–38	32–38	33–38	
NA	14	4	10	
Previous treatments for CRPC, n (%)				
Docetaxel	144 (100)	57 (100)	87 (100)	
Cabazitaxel	11 (8)	4 (7)	7 (8)	0.8[c]
Other agents	19 (21)	8 (14)	11 (12)	0.8[c]
Systemic therapy after abiraterone, n (%)				
Cabazitaxel	43 (30)	17 (30)	26 (30)	>0.9[c]
Other agents	42 (29)	24 (42)	31 (36)	0.3[c]

CRPC = castration-resistant prostate cancer; ECOG PS = Eastern Cooperative Oncology Group performance status; IQR = interquartile range; NA = not available; PSA = prostate-specific antigen.
The p values refer to significance of difference compared with PTEN positive.
[a] Mann-Whitney test.
[b] Chi-square test for trend.
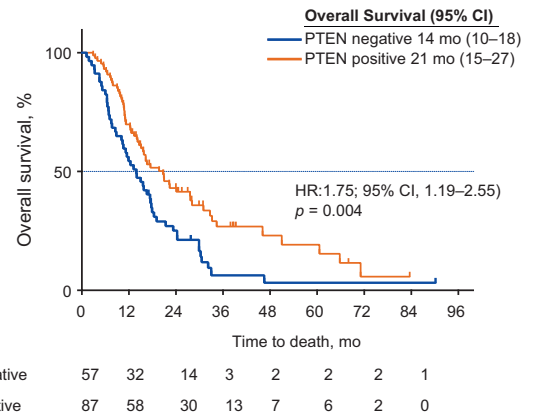[c] Pearson chi-square test.

Fig. 3 – Kaplan-Meier survival curves from initiation of abiraterone treatment according to PTEN expression status demonstrating a significantly shorter overall survival for patients with PTEN protein loss. CI = confidence interval; HR = hazard ratio.

radiologically detectable metastatic disease. The most common sites of metastases were bone (88%), lymph nodes (51%), and visceral (17%). Eastern Cooperative Oncology Group performance status was 0 or 1 in 121 patients (84%). Fifty-four patients (38%) received abiraterone within a clinical trial. The median follow-up was 16 mo (range: 1–90 mo). Table 1 details the demographic and clinical characteristics at the time of abiraterone initiation. Of note, a higher percentage of patients in the PTEN-negative group had visceral metastases at the time of abiraterone initiation (24% vs 12%; $p = 0.03$) (Table 1). There were no other significant differences in baseline characteristics between the PTEN-positive and PTEN-negative groups. After progression on abiraterone, 30% of patients in each group received cabazitaxel treatment.

In univariate analyses, loss of PTEN expression was significantly associated with a shorter median overall survival (14 vs 21 mo; hazard ratio [HR]: 1.75; 95% CI, 1.19–2.55; $p = 0.004$; Fig. 3). Loss of PTEN protein expression,

Table 2 – Multivariate Cox regression analysis for overall survival

	HR	95% CI	p value
PTEN status, negative vs positive	1.56	1.02–2.40	**0.04**
Low albumin, yes vs no	0.96	0.43–2.11	>0.9
High ALP, yes vs no	1.39	0.83–2.30	0.2
Low hemoglobin, yes vs no	1.81	0.94–3.47	0.07
High LDH, yes vs no	1.59	1.00–2.52	**0.048**
Visceral metastases, yes vs no	1.97	1.09–3.55	**0.02**
logPSA[*]	1.09	0.79–1.50	0.6
Age[*]	1.02	0.98–1.06	0.3
ECOG PS ≥2, yes vs no	0.97	0.33–2.85	>0.9
Previous cabazitaxel, yes vs no	1.96	0.72–5.30	0.2

ALP = alkaline phosphatase; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; LDH = lactate dehydrogenase; PSA = prostate-specific antigen.
Values in bold are statistically significant at $\alpha = 0.05$. Hospital high/low values for accepted normal ranges were used for laboratory parameters.
[*] Continuous variables.

Table 3 – Abiraterone activity according to PTEN expression

	PTEN negative n = 57	PTEN positive n = 87	p value
PSA decline			
≥50%, n (%)	18/56[*] (32)	38/87 (43)	0.2
≥30%, n (%)	24/56[*] (43)	48/87 (55)	0.2
Duration of abiraterone treatment, wk	24	28	**0.009**

PSA = prostate-specific antigen; PTEN = phosphatase and tensin homolog.
Values in bold are statistically significant at α = 0.05.
[*] Data for one patient are missing.

high lactate dehydrogenase levels, and the presence of visceral metastases were identified as independent factors for overall survival in multivariate Cox regression analysis (Table 2).

Confirmed PSA declines of at least 50% in the absence of radiographic progression were observed in 18 of the 56 patients with loss of PTEN (32%) and in 38 of the 87 patients (43%) (p = 0.2). The median duration of abiraterone treatment for patients in the PTEN-negative group was shorter than that for the PTEN-positive group (24 vs 28 wk; HR: 1.6; 95% CI, 1.12–2.28; p = 0.009) (Table 3).

4.   Discussion

Loss of PTEN is a common molecular aberration in PCa and believed to be critically important in regulating AR signaling output [5,13,14]. Preclinical studies have demonstrated the existence of reciprocal feedback regulation between the AR and PI3K/AKT pathway in PTEN-deficient models that confers survival advantage and resistance to single pathway inhibition [5,26].

In this retrospective study we investigated PTEN expression in metastatic CRPC patients who had received treatment with abiraterone post-docetaxel and its association with clinical outcome. We used a specific antibody directed against the extreme carboxy-terminal sequence of human PTEN protein [3,8,15,19] and used a stringent cut-off to define PTEN negativity that was validated in cases with biallelic genomic losses by FISH. Loss of PTEN expression occurred in 40% of patients and was associated with a shorter duration of abiraterone treatment and poorer overall survival. The study was retrospective in design with the inherent biases and confounders of all retrospective studies including the lack of data on comorbidities and comprehensive data on tumor volume and radiologic responses. Nonetheless, our data indicate that patients with loss of PTEN may have a worse clinical outcome when treated with abiraterone. These findings require replication in an independent data set but support the evaluation of PTEN as a biomarker in trials with combinations of novel AR-targeting drugs (abiraterone or enzalutamide) and PI3K/AKT inhibitors for patients with CRPC.

Inherent in PCa studies is intratumoral heterogeneity that can lead to misclassification and confound the association with outcomes. In keeping with previous studies, heterogeneity of PTEN expression was observed in 6% of primary tumors, mostly obtained in our cohort by needle biopsies [3,8]. Nevertheless, with our binary classification system, we observed good concordance (90%) between same-patient samples collected at multiple time points and in matched HSPC and CRPC tissue (86%). These findings require further validation in large independent cohorts, with possible interrogation of heterogeneity in primary and especially in metastatic disease.

5.   Conclusions

To the best of our knowledge, this is the first report of an association between PTEN protein expression status and clinical outcome in metastatic CRPC patients. PTEN loss has been shown to be frequently, but not always, associated with the presence of transmembrane protease, serine 2-v-ets avian erythroblastosis virus E26 oncogene homolog (*TMPRSS2-ERG*) rearrangements [9,27,28]. Discordant results have been published concerning the prognostic effect of loss of PTEN in the contest of ERG fusion [7,9,29,30]. PTEN loss is also accompanied by frequent alterations in the PI3K/AKT signaling pathway network involving inositol polyphophate-4 phosphatase, type II, 105kDa (*INPP4B*); PH domain and leucine rich repeat protein phosphatase 1 (*PHLPP*); and phosphoinositide-3-kinase, regulatory sub-unit 1 (alpha) (*PIK3R1*) [27]. Characterization of PTEN, along with ERG and possibly other key proteins implicated in this pathway, as part of larger studies with well-powered analyses are now warranted to better define the impact of PTEN loss on response to novel AR-targeting agents including studies of their interaction with ETS gene rearrangements.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2014.10.027.

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