



Docetaxel Treatment in PTEN- and ERG-aberrant Metastatic Prostate Cancers

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Abstract

Background: Loss of PTEN is a common genomic aberration in castration-resistant prostate cancer (CRPC) and is frequently concurrent with ERG rearrangements, causing resistance to next-generation hormonal treatment (NGHT) including abiraterone. The relationship between PTEN loss and docetaxel sensitivity remains uncertain.

Objective: To study the antitumor activity of docetaxel in metastatic CRPC in relation to PTEN and ERG aberrations.

Design, setting, and participants: Single-centre, retrospective analysis of PTEN loss and ERG expression using a previously described immunohistochemistry (IHC) binary classification system. Patients received docetaxel between January 1, 2006 and July 31, 2016.

Outcome measurements and statistical analysis: Response correlations were analyzed using Pearson's χ^2 tests and independent-sample *t* tests. Overall (OS) and progression-free survival (PFS) were analyzed using univariate and multivariate (MVA) Cox regression and Kaplan-Meier methods.

Results and limitations: Overall, 215 patients were eligible. Established metastatic CRPC prognostic factors were well balanced between PTEN loss (39%) and normal patients (61%). PTEN loss was associated with shorter median OS (25.4 vs 34.7 mo; hazard ratio [HR] 1.66, 95% confidence interval [CI] 1.18–2.13; *p* = 0.001). There were no differences in median PFS (8.0 vs 9.1 mo; univariate HR 1.20, 95% CI 0.86–1.68; *p* = 0.28) and PSA response (53.4% vs 50.6%; *p* = 0.74). PTEN loss was an independent prognostic factor in MVA. ERG status was available for 100 patients. ERG positivity was not associated with OS or PFS. Limitations include the retrospective nature and the single-centre analysis.

Conclusions: Our findings suggest that metastatic CRPC with PTEN loss might benefit more from docetaxel than from NGHT.

Patient summary: In this study we found that metastatic prostate cancer with loss of the PTEN switch may benefit more from docetaxel than from abiraterone.

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1. Introduction

Prostate cancer (PC) is the most common malignancy in men and a common cause of cancer-related death in Western countries [1]. Molecular characterization of metastatic castration-resistant PC (mCRPC) through whole-exome and transcriptome sequencing has offered an insightful understanding of its biology, identifying aberrations of the androgen receptor (AR); gene fusions including those involving *TMPRSS2* and *ERG*; and *PTEN* loss, commonly via deletion [2].

PTEN acts as a phosphatase regulator of the *PI3K/AKT* pathway, which is also involved in regulating AR signaling and in hormonal resistance in preclinical models [3]. *PTEN* loss is an early and stable event in the carcinogenesis process and is associated with poor prognosis [4–7] and short response to next-generation hormonal treatment (NGHT) such as abiraterone acetate (AA) [8]. This has prompted investigators to design studies evaluating the efficacy of the combination of NGHT and *PI3K/AKT* inhibitors [9,10].

The impact of *PTEN* loss, which commonly co-occurs with *ERG* genomic rearrangements, on the taxane sensitivity of mCRPC has not yet been clearly elucidated. Therefore, in this retrospective study we investigated *PTEN* protein expression in both hormone-naïve PC and mCRPC samples from patients with advanced disease and evaluated clinical outcomes and the association of docetaxel response with *PTEN* status. We then analyzed the association of *PTEN* loss and *ERG* expression and retrospectively evaluated the impact of high *ERG* expression on outcome from docetaxel in this cohort of patients.

2. Patients and methods

2.1. Patient cohort

Potentially eligible cases were identified from a population of men with mCRPC treated at the Royal Marsden NHS Foundation Trust between January 2006 and July 2016. Patients were included in the study if they had received docetaxel treatment for mCRPC (either as first-line treatment or after NGHT) and had paraffin tissue blocks from metastatic sites or diagnostic samples for *PTEN* immunohistochemistry (IHC) available. Exclusion criteria were previous treatment with a *PI3K/AKT* inhibitor and histologic features of neuroendocrine or small cell cancer. All patients gave their written informed consent and were enrolled in institutional protocols approved by the Royal Marsden NHS Foundation Trust Hospital ethics review committee (reference no. 04/Q0801/60). Demographic and clinical data were retrospectively collected using the hospital electronic patient record system.

2.2. Tissue samples

PC tissue was obtained from prostate needle biopsies, transurethral resections of the prostate, prostatectomies, or PC metastases at the time of castration resistance within bone (bone marrow trephine), lymph nodes, or viscera (needle biopsies). All tissue blocks were sectioned and reviewed by a pathologist (D.N.R.) for confirmation of the adequacy of the material (>50 viable cells).

2.3. *PTEN* IHC

PTEN protein expression was determined via IHC on 4-mm-thick formalin-fixed, paraffin-embedded sections as previously described [11,12]. In brief, *PTEN* immunoreactivity was investigated using rabbit monoclonal anti-*PTEN* antibody 138G6 (Cell Signaling Technology, Danvers, MA, USA) [13] and detected using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). The intensity of nuclear and cytoplasmic staining was semiquantitatively assessed using the H-score formula as previously defined [8]. *PTEN*-positive controls included normal prostate tissue and 22RV1 xenograft tissue, and *PTEN*-loss controls included PC3 (*PTEN*-null PC cell line) xenografts. Endothelial cells and stroma were used as internal positive controls for *PTEN*. A binary classification was used for IHC *PTEN* positivity or loss according to validation studies previously published by our group [8]. Cases were considered *PTEN*-negative if they either showed a complete absence of *PTEN* staining or weak-intensity staining compared to the internal control in no more than 10% of cancer cells (H-score >10). All IHC sections were evaluated by a pathologist (D.N.R.) blinded to the patients' clinical characteristics and outcome data.

A small 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 this data analysis, a case was considered *PTEN*-negative if any tumor area showed a complete absence of *PTEN* staining. For the survival analyses, when a change in *PTEN* status was observed between patient-matched hormone-naïve PC and CRPC samples, cases were classified according to the *PTEN* status in the CRPC sample.

2.4. *ERG* IHC

Antigen retrieval was conducted by heating slides in Tris-EDTA buffer (pH 8.1) using a microwave. Protein blocking was performed to eliminate nonspecific background staining using serum-free protein block #X0909 (Dako, Glostrup, Denmark). The primary antibody was #ab92513 from Abcam (Cambridge, UK) diluted 1:200 in Dako antibody diluent. The detection kit was a REAL EnVision detection system and DAB reagent (Dako). A negative control serum (rabbit IgG control antibody I-1000; Vector Laboratories) was used instead of the primary antibody for the negative controls. Control sections included a VCaP xenograft, a PC3 xenograft, and normal prostate tissue. Cases were scored by a pathologist (D.N.R.) blinded to clinical data using a modified H-score (HS) method, which is a semiquantitative assessment of staining intensity that reflects antigen concentration. HS was determined according to the formula $[(\% \text{ of weak staining}) \times 1] + [(\% \text{ of moderate staining}) \times 2] + [(\% \text{ of strong staining}) \times 3]$, yielding a range from 0 to 300.

2.5. Statistical analysis

Biochemical response to docetaxel was defined according to Prostate Cancer Working Group Criteria 3 as a 30% decline in prostate-specific antigen (PSA) from baseline, confirmed at least 3 wk later [14]. Survival was measured from the first date of docetaxel treatment to the date of last contact or the date of death from any cause. Progression-free survival (PFS) was defined as the time from docetaxel initiation to the time of progression during or beyond the discontinuation of docetaxel because of radiological and/or biochemical progression or death. In patients with measurable disease on computed tomography imaging, the radiographic response was also assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 [15]. The Kaplan-Meier product limit method was used to estimate the duration of docetaxel treatment, PFS, and overall survival (OS) by *PTEN* status. Independent-sample *t* tests and Pearson's χ^2 tests were used to investigate the association of *PTEN* loss

with continuous and categorical variables, respectively. All tests were two-sided, and $p \leq 0.05$ was considered statistically significant.

Approximately 50% of patients were missing one or more independent factors at baseline, 30% of patients were missing values for all laboratory measurements. These values were considered to be missing at random from clinical notes and it was thought to be unlikely that there were systematic differences between the missing and observed values. To avoid a loss in precision, multiple imputation by chained equations was conducted using baseline patient and tumor characteristics. PTEN status and visceral disease were completely observed and were included in the imputation model with the Nelson-Aalen estimate and censoring indicators for mortality or progression depending upon the analysis. ECOG performance status ≥ 1 , Gleason score ≥ 8 and previous experience of AA were imputed using logistic regression models; albumin, \log_{10} alkaline phosphatase, hemoglobin, \log_{10} neutrophil to lymphocyte ratio and \log_{10} lactate dehydrogenase were imputed using linear regression models which assumed normality. In total, after a 100 imputation burn-in, 50 imputations were used and results were combined using Rubin's rules. Univariate and multivariate analyses of PTEN status, ERG status and other potential independent factors for OS, duration of docetaxel treatment and PFS were performed using the Cox regression model with a 95% confidence interval (CI). Descriptive statistics and survival analyses were performed using Stata v13.1 (Stata Corp., College Station, TX, USA).

3. Results

3.1. Tissue samples and patient characteristics

We identified 215 patients who received treatment with docetaxel and had tissue available for PTEN analysis. A single tissue sample was available for 160 patients, while 55 patients had matched samples collected at the time of diagnosis and in the castration-resistant phase. A total of 270 samples were scored for PTEN by IHC. Inpatient concordance was present in 87% of the matched samples (48 of 55) with a change in PTEN status observed in only seven of 55 patients (13%). Overall, PTEN loss was demonstrated in 83 of the 215 patients (39%).

Key baseline patient characteristics are listed in Table 1. Patients received a median of eight cycles of docetaxel, with median treatment duration of 5.1 mo. There were no significant differences in hemoglobin, albumin, lactate dehydrogenase, neutrophil/lymphocyte ratio, or performance status between PTEN-loss and PTEN-positive patients before docetaxel initiation; only alkaline phosphatase levels were higher in PTEN-loss patients ($p = 0.02$). Globally, 33 patients (15.4%) had visceral metastases at docetaxel initiation, with no significant difference between the groups (14.5% vs 15.9%; $p = 0.77$).

3.2. Outcomes

Median OS from the start of docetaxel treatment for the whole cohort was 29.3 mo (95% confidence interval [CI] 26.6–35.1); 180 patients (83.7%) had died by the time of data cutoff. Median PFS was 8.9 mo (95% CI 8.1–10.3). Patients with PTEN loss had worse OS than patients with normal PTEN expression (25.4 vs 34.7 mo; univariate hazard ratio [HR] 1.66, 95% CI 1.23–2.24; $p = 0.001$; Fig. 1) in both univariable and multivariable (MVA) Cox regression

analyses (Table 2). PTEN loss, higher lactate dehydrogenase levels, and lower albumin remained strongly associated with worse OS in MVA ($p < 0.05$).

There was no difference in PFS observed between patients whose tumors had PTEN loss and those with PTEN-positive disease (median 8.0 vs 9.1 mo; HR 1.20, 95% CI 0.86–1.68; $p = 0.28$; Fig. 1B, Supplementary Table 1), with a similar median number of docetaxel cycles (7.5 vs 8.0; $p = 0.29$) and median time on docetaxel (5.0 mo [95% CI 4.2–5.5] vs 5.2 mo [95% CI 4.7–6.0]; $p = 0.23$). Overall, 86 patients (40.1%) received further treatment with cabazitaxel; of these, 56 (65.1%) had tumors with PTEN loss.

Data on PSA response were available for 143 patients. The overall median PSA decline was 53.3% (95% CI 61.7% to –42.9%); 74 of the 143 patients (51.8%) experienced a PSA response. Patients receiving docetaxel as first-line therapy for mCRPC were more likely to experience a PSA response than those receiving second-line docetaxel (58.4% vs 38.5%; $p = 0.03$). There was no difference in PSA response rate between patients with and without PTEN loss (53.5% vs 50.6%; $p = 0.795$; Fig. 2). Furthermore, 128 patients (59.5%) had scans available for assessment of radiological response. Of these 128, 55 patients (43.0%) had bone-only disease and 73 (57.0%) had measurable disease by RECIST. Among the latter 73 evaluable patients, 23 (31.5%) had a partial response during docetaxel treatment or at treatment completion. Response rates were not different between PTEN-loss and PTEN-positive mCRPC (28.6% vs 33.3%; $p = 0.67$; Table 3).

3.3. ERG status and correlation with outcome

To further characterize this mCRPC population, we evaluated ERG status in 100 tumors. IHC revealed 58 tumors (58%) with ERG-negative status and 42 (42%) with ERG positivity. ERG status was consistent between matched hormone-naïve and CRPC samples from the same patient, with only one patient having discordant hormone-naïve and CRPC ERG staining. There was a significant association between ERG-positive staining and PTEN loss (Fisher's exact test, $p = 0.02$; Supplementary Table 2). Despite this, no difference was observed in terms of OS (univariate HR 0.94, 95% CI 0.60–1.47; $p = 0.79$), PFS (HR 1.08, 95% CI 0.65–1.77; $p = 0.77$), and time on docetaxel (HR 1.06, 95% CI 0.70–1.58; $p = 0.79$) when patients were dichotomized according to ERG tumor status (Supplementary Figs. 1–3). In the subgroup with known ERG status, PTEN loss remained associated with worse survival (univariate HR 1.62, 95% CI 1.20–2.18; $p = 0.002$).

4. Discussion

Hyperactivation of the PI3K/AKT/mTOR pathway, generally through loss of PTEN function, is one of the most common aberrations driving progression in mCRPC [2]. PTEN loss of function can be due to different genomic (deletion, microdeletions, and rearrangements, including intronic rearrangements) and nongenomic mechanisms (methylation, miRNA, pseudo-gene expression) [2]. At the post-

Table 1 – Patient characteristics at baseline.

	Overall	PTEN-positive	PTEN loss	p value
Patients (n)	215	132	83	
Median age, yr (IQR)	70 (66–75)	68 (63–73)	66 (61–72)	0.23
Gleason score at diagnosis, n (%)				0.66
≤6	17 (7.9)	10 (7.6)	7 (8.4)	
7	51 (23.7)	28 (21.2)	23 (27.7)	
8–10	113 (52.6)	71 (53.8)	42 (50.6)	
Missing	34 (15.8)	23 (17.4)	11 (13.3)	
Sites of metastases at start of DTX, n (%)				0.78
Bone only	84 (39.1)	48 (36.4)	36 (43.4)	
Nodal	63 (29.3)	40 (30.3)	23 (27.7)	
Visceral	33 (15.4)	21 (15.9)	12 (14.5)	
Missing	35 (16.3)	23 (17.4)	12 (14.5)	
ECOG performance status, n (%)				0.46
0	78 (36.3)	30 (36.1)	48 (36.4)	
1	78 (36.3)	33 (39.8)	45 (34.1)	
2	5 (2.3)	3 (3.6)	2 (1.5)	
Missing	54 (25.1)	17 (20.5)	37 (28.0)	
Prostate-specific antigen				0.15
Median, ng/ml (IQR)	116 (47–404)	139 (58–569)	109 (32–369)	
Missing, n (%)	59 (27.4)	39 (29.6)	20 (24.1)	
Hemoglobin				0.81
Median, g/dl (IQR)	12 (11–13)	12 (11–13)	12 (11–13)	
Missing, n (%)	80 (37.2)	53 (40.2)	27 (32.5)	
Alkaline phosphatase				0.02
Median, IU/l (IQR)	127 (76–259)	116 (72–203)	211 (81–435)	
Missing, n (%)	79 (36.7)	52 (39.4)	27 (32.5)	
Lactate dehydrogenase				0.35
Median, IU/l (IQR)	192 (149–239)	188 (146–239)	197 (156–245)	
Missing, n (%)	84 (39.1)	56 (42.4)	28 (33.7)	
Albumin				0.19
Median, g/l (IQR)	36 (32–38)	36 (33–39)	35 (32–38)	
Missing, n (%)	80 (37.2)	53 (40.2)	27 (32.5)	
Neutrophils				0.99
Median (IQR)	4.6 (3.5–6.8)	4.6 (3.6–6.9)	4.5 (3.3–6.9)	
Missing, n (%)	81 (37.7)	54 (40.9)	27 (32.5)	
Lymphocytes				0.72
Median (IQR)	1.2 (0.8–1.6)	1.2 (0.8–1.6)	1.1 (0.8–1.7)	
Missing, n (%)	81 (37.7)	54 (40.9)	27 (32.5)	
Neutrophil/lymphocyte ratio				0.65
Median (IQR)	4.0 (2.5–8.8)	4.0 (2.4–9.0)	4.0 (2.4–8.6)	
Missing, n (%)	81 (37.7)	54 (40.9)	27 (32.5)	
Previous abiraterone, n (%)				0.69
Yes	51 (23.7)	31 (23.5)	20 (24.1)	
No	159 (74.0)	97 (73.5)	62 (74.7)	
Missing	5 (2.3)	4 (3.0)	1 (1.2)	

IQR = interquartile range; DTX = docetaxel; ECOG = Eastern Cooperative Oncology Group.

translational level, PTEN function is regulated by various modifications, including phosphorylation, oxidation, and ubiquitination, with intrapatient heterogeneity in approximately 10% of cases [16,17].

PTEN loss results in hyperactivation of the PI3K/AKT/mTOR pathway, which in turn is highly related to the activity of the AR pathway [3]. While PI3K/AKT/mTOR activation can suppress AR transcriptional output and stability [18], PI3K/AKT/mTOR signaling is activated following androgen deprivation, especially in patients with PTEN loss [19].

In the present analysis for patients treated with docetaxel, we confirmed the prognostic importance of PTEN loss in mCRPC. However, we found no evidence that docetaxel antitumor activity is impaired in PTEN-loss mCRPC, with no difference in the number of cycles administered, the duration of docetaxel treatment, or the

PSA or RECIST response between PTEN-loss and PTEN-positive tumors. However, this may not be the case in earlier stages of the disease, as PTEN loss was associated with shorter PFS among 57 patients treated on a trial of adjuvant docetaxel after radical prostatectomy [20].

In this study, in the PTEN-positive group, 31 patients (23.5%) received AA before chemotherapy and 97 patients (73.5%) received AA after chemotherapy. In the PTEN-loss group, 20 patients (24.1%) received AA before docetaxel and 62 patients (74.7%) received AA after docetaxel (Table 1). Therefore, the two groups were well balanced in term of anticancer treatments. We previously showed that AA has lower antitumor activity against PTEN-loss tumors [8], which might explain why patients with PTEN-loss tumors experience shorter OS despite no difference in term of PFS on docetaxel.

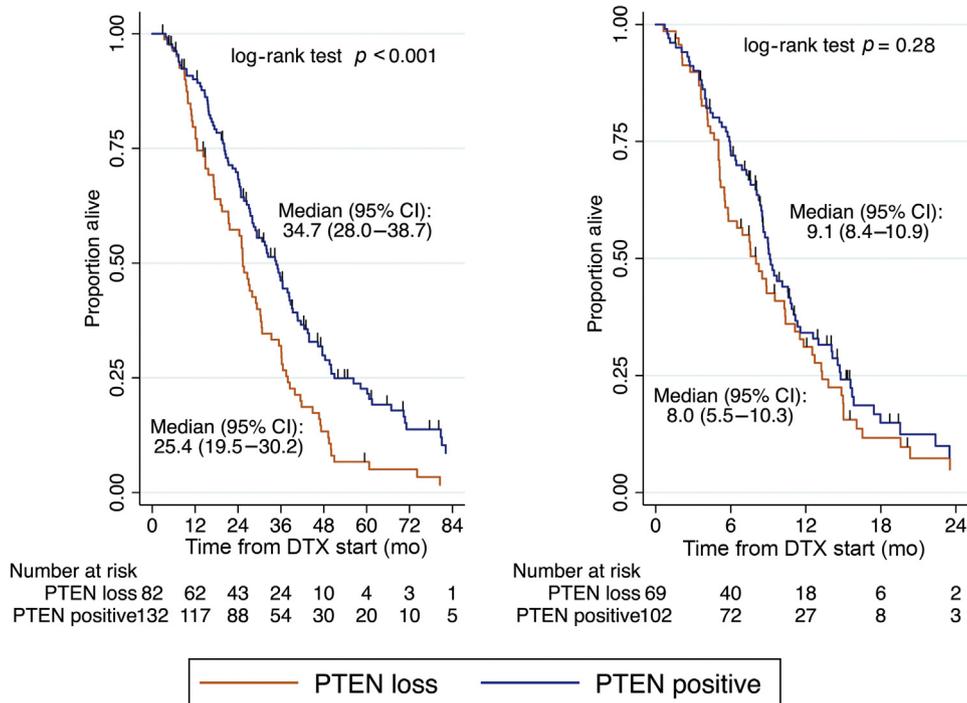


Fig. 1 – Kaplan-Meier curves for (A) median overall survival (OS) and (B) median progression-free survival (PFS) from the start of docetaxel chemotherapy for patients with PTEN loss and those with PTEN-positive tumors. CI = confidence interval; DTX = docetaxel.

These data were recently confirmed by a phase 2 trial of AA + ipatasertib/placebo in which patients with PTEN-loss tumors in the AA + placebo arm had significantly shorter radiographic PFS when compared to the PTEN-positive group. Conversely, co-targeting of AR and AKT using AA + ipatasertib in combination improved outcomes compared to AA alone in PTEN-loss cancers [9]. Taken together, these data suggest that docetaxel might be a preferable option for this patient population.

As mCRPCs with PTEN deletion are enriched in ERG genomic rearrangements [21], with PTEN loss postulated as being a later event to ERG rearrangements [22], we

analyzed ERG status in the tumors from 100 patients in this cohort. Gene fusions involving TMPRSS2 and ERG can be detected by IHC and/or fluorescent in situ hybridization (FISH), and are common in PC (30–50%) [23], being highly associated with ERG protein overexpression [24]. The role of these ERG rearrangements in prognosis and survival remains controversial, although a recent meta-analysis of 5074 men treated with radical prostatectomy revealed no association between ERG rearrangements and clinical outcome [25–28]. A recent study evaluating ERG rearrangements in peripheral blood mononuclear cells using quantitative reverse transcription polymerase chain

Table 2 – Univariable and multivariable Cox regression analyses for overall survival.

	Univariable		Multivariable	
	HR (95% CI)	p value	HR (95% CI)	p value
PTEN status (loss)	1.66 (1.23–2.34)	0.001	1.73 (1.21–2.46)	0.003
Previous abiraterone	1.52 (1.06–2.17)	0.02	1.40 (0.90–2.18)	0.13
Hemoglobin (g/dl)	1.00 (0.97–1.03)	0.94	–	–
Albumin (g/l)	0.92 (0.87–0.97)	0.002	0.94 (0.88–1.00)	0.05
ALP (log ₁₀ IU/l)	2.02 (1.14–3.58)	0.02	1.11 (0.59–2.11)	0.73
LDH (log ₁₀ IU/l)	5.33 (1.39–20.49)	0.02	4.78 (1.33–17.22)	0.02
NLR (log ₁₀)	1.09 (0.78–1.52)	0.62	–	–
ECOG PS ≥1	1.74 (1.23–2.46)	0.001	1.45 (0.94–2.24)	0.09
Gleason score ≥8	1.43 (1.02–2.00)	0.04	1.37 (0.93–2.02)	0.11
Visceral disease	1.65 (1.10–2.46)	0.01	1.57 (0.97–2.53)	0.07

HR = hazard ratio; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; PSA = prostate-specific antigen; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; NLR = neutrophil/lymphocyte ratio.

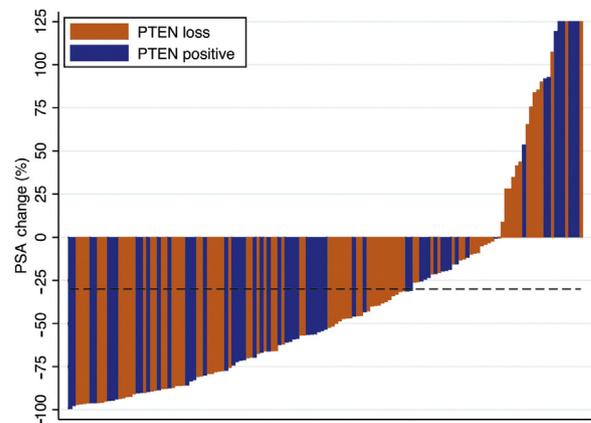


Fig. 2 – Waterfall plot of prostate-specific antigen (PSA) change for patients with PTEN loss and those still PTEN-positive. The bar indicates a 30% decline in PSA from baseline.

Table 3 – PSA and RECIST responses to treatment.

	Patients, n (%)			p value
	Total	PTEN-positive	PTEN loss	
PSA response ^a	74 (51.8)	43 (50.6)	31 (53.5)	0.74
No PSA response	69 (48.3)	42 (49.4)	27 (46.6)	
RECIST response (PR)	23 (31.5)	15 (33.3)	8 (28.6)	0.67
No RECIST response (SD or PD)	50 (68.5)	30 (66.7)	20 (71.4)	

PSA = prostate-specific antigen; RECIST = Response Evaluation Criteria in Solid Tumors; PR = partial response; SD = stable disease; PD = progressive disease.

^a A PSA response was defined as a 30% PSA decline from baseline.

reaction demonstrated that TMPRSS2-ERG was associated with taxane resistance in mCRPC. However, the incidence of ERG rearrangements detected with this method appeared to be particularly low (16%) compared to IHC and FISH tumor tissue-based testing [29]. Our analyses confirm that ERG positivity is a common event in PC and correlates with PTEN loss; however, we found no association between ERG status and clinical outcome from or response to docetaxel in mCRPC. Moreover, ERG status was not prognostic in our population.

4.1. Limitations

Patients in this study came from a single centre, so these findings may not be generalizable to patients treated at other institutions and require prospective confirmation through a multicenter study. Furthermore, the patient cohort was retrospectively collected and so could suffer from selection bias.

5. Conclusions

We have shown for the first time and in the largest series on PTEN loss reported to date that despite being a prognostic factor, independent of ERG status, PTEN loss does not alter response to taxane-based chemotherapy. We envision that these findings may be relevant to treatment selection. Prospective trials are warranted to determine whether mCRPC patients with PTEN loss might be better served by docetaxel treatment rather than NGHT.

Author contributions: Johann S. de Bono 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.

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Drafting of the manuscript: Rescigno, Lorente.

Critical revision of the manuscript for important intellectual content: de Bono.

Statistical analysis: Dolling, Lorente.

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Supervision: Tunariu, de Bono.

Other: None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.euo.2018.02.006>.

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