ACTA ONCOLOGICA
2023, VOL. 62, NO. 12, 1698–1706
https://doi.org/10.1080/0284186X.2023.2254480

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Ki67 and prostate specific antigen are prognostic in metastatic hormone naïve prostate cancer

Vasiliki Spyratou[a], Eva Freyhult[b], Anders Bergh[c], Camilla Thellenberg-Karlsson[d], Pernilla Wikström[c], Karin Welén[a,e] and Andreas Josefsson[a,f,g]

[a]Department of Urology, Institute of Clinical Sciences, University of Gothenburg, Gothenburg, Sweden; [b]Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala, Sweden; [c]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden; [d]Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden; [e]Sahlgrenska Center for Cancer Research, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [f]Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden; [g]Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden

ABSTRACT

Background: For metastatic hormone naïve prostate cancer patients, androgen deprivation therapy (ADT) with escalation therapy including docetaxel and/or androgen targeting drugs is the standard therapy. However, de-escalation is preferable to avoid unnecessary side effects, especially from docetaxel, but markers to identify these patients are lacking. The purpose of the present study was to investigate the potential of PSA and Ki67 immunoreactive scores as prognostic and treatment-predictive markers.

Material and methods: Prostate biopsies from 92 patients with metastatic hormone naïve PC (PSA > 80 ng/mL or clinical metastases) were immunohistochemically evaluated for PSA and Ki67. Gene expression analysis was performed with Clariom D microarrays to identify the phenotypic profile associated with the immunohistochemistry scores of biopsies. Cox regression analysis for progression free survival after ADT adjustment for age, ISUP, and serum PSA and Kaplan-Meier analyses were performed to assess prognostic values of Ki67, PSA, and the Ki67/PSA ratio.

Results: The immunohistochemical score for PSA was the strongest prognostic factor for progression-free and overall survival after ADT. Consequently, the ratio between Ki67 and PSA displayed a stronger prognostic value than Ki67 itself. Further, mRNA expression data analysis showed an association between high Ki67/PSA ratio, cell-cycle regulation, and DNA damage repair. In an exploratory sub-analysis of 12 patients treated with early docetaxel as addition to ADT and matched controls, a high Ki67/PSA ratio showed potential to identify those who benefit from docetaxel.

Conclusion: PSA and Ki67 immunoreactive scores are prognostic in the metastatic hormone-sensitive setting, with PSA being superior. The combination of Ki67 and PSA did not give additional prognostic value. The results suggest immunohistochemical scoring of PSA to have potential to improve identification of patients responding well to ADT alone.

ARTICLE HISTORY
Received 22 March 2023
Accepted 28 August 2023

KEYWORDS
Metastatic prostate cancer; biomarker; androgen deprivation therapy; docetaxel; prostate specific antigen

Introduction

The development of new treatments strategies for patients with advanced prostate cancer have resulted in significant improvement in overall survival and the addition of docetaxel to androgen deprivation therapy (ADT) has been the standard of care for 5–7 years. Phenotypically and genotypically different subtypes have been demonstrated [1–5], and there is increasing evidence that a subtype of prostate cancer with low androgen receptor (AR) signaling responds poorly to androgen-targeted therapies [2,4,5], and may be in most need of docetaxel in addition to ADT. In contrast, patients with intact AR signaling and lower proliferation could have a good response to ADT alone. Clearly, a one-size fits all approach is not appropriate and strategies for patient selection are needed. Despite this pressing unmet clinical need, no validated biomarkers exist for guiding the selection of patients with advanced prostate cancer for early add-on therapy, although there are some possible clinical parameters, such as tumor burden, with potential for patient selection [6,7].

Although serum PSA is used for diagnosis and monitoring of prostate cancer, it cannot be used for treatment selection in advanced stages, since the levels mostly reflect tumor burden and not tumor biology. In the tumor cells, a low PSA expression is linked to dedifferentiation and limited AR signaling [8], and tissue PSA staining has previously been shown to be a strong prognostic biomarker for PSA progression-free survival (PFS) in a cohort of 12,000 prostate cancer cases [9]. The proliferation marker Ki67 is the most studied

CONTACT Andreas Josefsson andreas.josefsson@umu.se Umeå University, Försörjningsvägen 1, Trc By 6m vån 3, 901 85 Umeå, Sweden
Supplemental data for this article can be accessed online at https://doi.org/10.1080/0284186X.2023.2254480.

prognostic tissue biomarker in prostate cancer [10], and its expression in cancer tissue in the prostate is associated with higher Gleason scores and the development of metastatic disease [11,12].

We have previously shown that immunoreactivity of Ki67 and PSA in combination could distinguish three groups of patients with poor, intermediate, and good prognosis, respectively, in a cohort of men initially managed with watchful waiting [13]. Also in bone metastases, the combinatory PSA and Ki67 score provided prognostic information about different subgroups [2]. Further, we recently showed that AR, PSA, and Ki67 in the primary tumor to some extent could predict the phenotype of the metastases, and also were prognostic for survival after ADT [14].

The aim of the present study was to evaluate the potential of PSA and Ki67 staining of prostate biopsies, individually and combined, to predict treatment response to ADT in patients with hormone naïve metastatic disease from two prospective biomarker studies. Further, an exploratory substudy aims to evaluate if these markers could help in identifying patients with the most benefit of docetaxel therapy or suitable for de-escalation from the standard of care. Further, we aimed to define the gene expression patterns associated with the expression of these markers to enable identification of targetable pathways to improve therapy for patients with metastatic prostate cancer.

Materials and methods

Patient population

The present study includes systematic prostate biopsies from 92 patients with PSA > 80 ng/mL and/or clinical manifestations of metastatic PC at the time of diagnosis from two prospective biomarker studies at Sahlgrenska University Hospital, Gothenburg ($N = 63$) and at Norrland's University Hospital, Umeå, Sweden ($N = 29$) between 2012 and 2018 (Table 1). Thirteen patients were excluded after the biopsy procedure; three were treated with radiotherapy, three received other hormonal treatment, three were lost for follow up, two were treated for other cancer forms, and two died of other cause shortly after inclusion. The remaining 79 patients were evaluable for response to ADT, see Figure 1. Twelve of these patients were treated with early docetaxel and two with abiraterone in combination with ADT. Serum PSA was followed approximately every third month after initiation of ADT. Castration-resistant prostate cancer (CRPC) was defined as either (1) biochemical progression with at least two consecutive rises in serum PSA after nadir, a total increase of > 1 ng/ml and reaching an absolute value > 2 ng/ml, (2) radiological progression with the appearance of two or more new lesions on bone scan, according to the Swedish guidelines, or (3) prostate cancer death.

Immunohistochemistry and evaluation

Standard immunohistochemistry methods were used on new sections of 4 μm formalin-fixed paraffin embedded (FFPE) prostate needle biopsies for staining with A0562(DAKO) and 30-9 (790.4286, Ventana) for PSA and Ki-67, respectively, using Benchmark Ultra (see supplementary material for details). Sections from the Gothenburg cohort were scanned with the Aperio image scope-pathology slide viewing software, and digital evaluation was performed using the image viewing software NDP.view2 (Hamamatsu). Evaluation of the sections from

Table 1. Baseline characteristics.

Clinical variables	All ($N = 92$)	Survival analysis ($N = 65$)a	ClariomD ($N = 56$)
Age at start of ADT (years)	70.0 (64.0–78.2)	71.0 (65.0–81.0)	71.0 (64.0–77.2)
Serum PSA at start of ADT (ng/mL)	270.0 (124.5–780.0)	330.0 (121.5–927.5)	320.0 (157.5–927.5)
Tumor stage			
T1–2	13	11	4
T3–4	37	31	17
TX	42	23	35
ISUP grade /Gleason score (GS)			
ISUP 2–3/GS 7	17	10	11
ISUP 4–5/GS 8–10	75	55	45
PSA score IHC	9.0 (5.4–10.9)	9.0 (5.5–10.9)	8.1 (5.2–10.1)
Ki67 score IHC	18.6 (11.2–31.2)	18.8 (11.2–32.1)	17.9 (11.2–29.4)
Type of treatment			
Medical castration[b]	83	59	52
Surgical castration	6	6	1
Radiation therapy	3	0	3
Adjuvant therapy			
Abiraterone acetate	2	0	1
Docetaxel	12	0	5
None	78	65	50
Time from biopsy to relapse or last follow up (days)		380.0 (175.0–658.0)	291.5 (154.0–458.2)c
Cause of death			
Prostate cancer	47	47	31
Other death	1	1	1
Alive	17	17	6
Excluded from survival analysis	27		18
Time from biopsy to prostate cancer death (days)		1202.0 (567.0–1780.2)	956.5c (440.8–1476.0)

Ranges are presented as interquartile range (IQR). N; number, x; unknown.
[a]For primary ADT without added docetaxel
[b]Medical castration; GnRH agonist/antagonist.
[c]Only calculated for patients included in survival analysis.

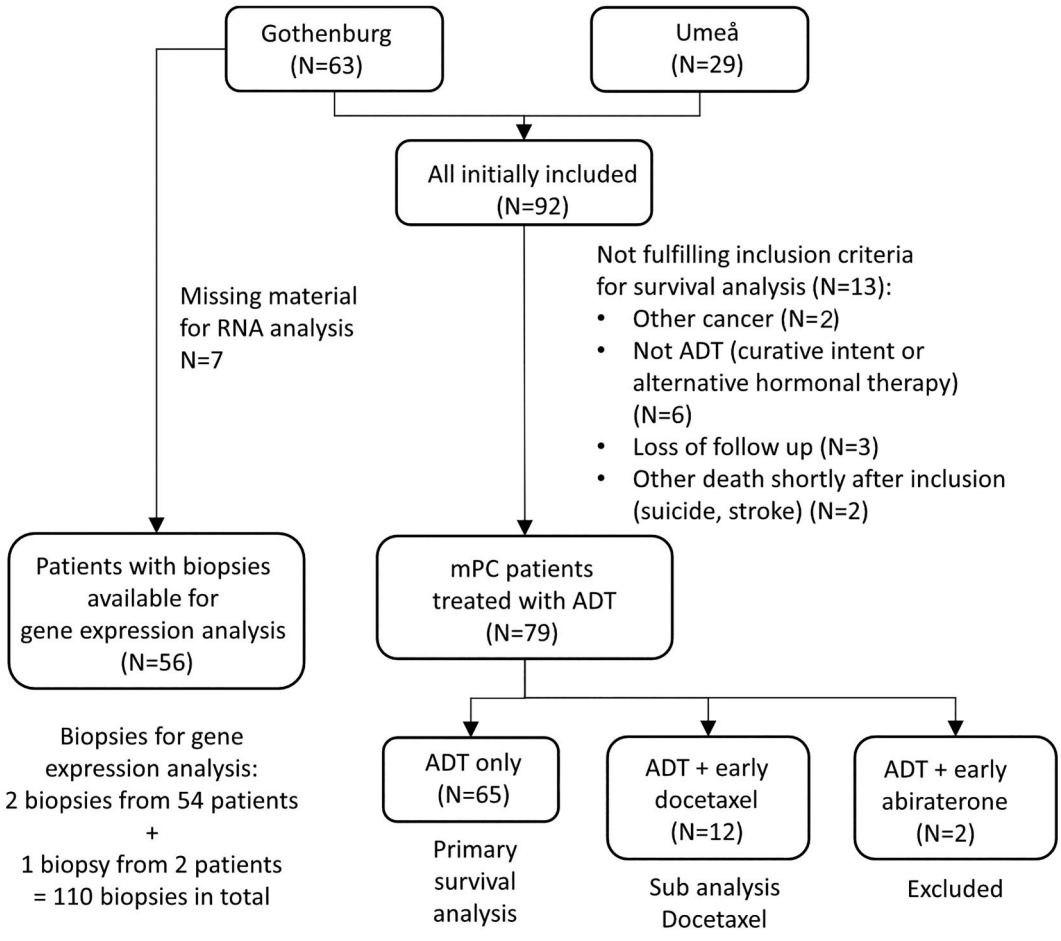

Figure 1. Flow chart of the patient selection for the different analyses.

Umeå was performed in SlideViewer (3DHistech Ltd.). The cancer tissue for each biopsy was divided in quartiles, resulting in 8 or 4 different scores for each patient, of which the mean was used as the score for each patient.

Hematoxylin and eosin-stained biopsies were Gleason graded according to the 2014 ISUP Consensus Conference. PSA immunoreactivity score was calculated using a percentage scale for distribution (0: no staining, 1: 1–25%, 2: 26–50%, 3: 51–75%, 4: 76–100%) multiplied with the score for intensity (0: negative, 1: weak, 2: moderate, 3: strong) resulting in a score of 0–12. Ki67 score was calculated as a mean percentage of strongly positive Ki67 epithelial nuclei at least 500 randomly selected malignant cells at 400× magnification. The distribution of the PSA and Ki67 scores in the two cohorts correlated well (Supplementary Figure S1).

RNA isolation and gene expression analysis

RNA was extracted from 4 × 10 μm FFPE sections using the Invitrogen PureLink™ FFPE total RNA Isolation Kit (K156002, ThermoFisher, USA) as per the manufacturer's protocol. RNA was quantified, and quality assessed on a DeNovix DS −11 FX + Spectrophotometer (DeNovix Inc, Wilmington, USA). The RNA was amplified and hybridized using the GeneChip™ WT Pico Reagent Kit before the expression was analyzed using the Clariom™ D Human Transcriptome Array (both Applied Biosystems, Thermofisher) at the Uppsala Array and Analysis Facility. Raw data was normalized in Transcriptome Analysis Console software (Thermofisher) and analysis carried out in R (http://www.r-project.org).

Ethical approval

The study is conducted with respect to the Global data protective regulation (GDPR) and is ethically approved by the Local Ethical committees with the numbers 2013-57-31 M, 974-16 (T1138-18) and 936-12 (T113-18).

Statistics

All statistical analyses were performed using R v4.2.1. PSA and Ki67 score values, as well as serum PSA and Ki67/PSA ratio, were log2 transformed before statistical analyses, due to the observed distribution of values. The Ki67/PSA ratio was computed as Ki67 score/(PSA score +1), to avoid dividing with zero. Cox regression was used to estimate the hazard ratio, both in univariate and multivariate models of survival. Kaplan–Meier, restricted mean survival time (RMST) and the log rank test were used to analyze association between survival outcome and categorized variables. Genes associated with Ki67/PSA ratio were identified using the limma R-package [15]. In the linear model, run (batch) was included as covariates, as was age (at biopsy), ISUP and

log2(PSA) (at biopsy). Many patients were represented by two samples and in the calculations, these paired samples were considered through duplicate correlations (function duplicateCorrelation). Correlation was performed by Spearman's rank test for continues values and Cohan's kappa for the quartiles of the Ki67 and PSA IHC.

For the exploratory analysis regarding possible potentials of the biomarkers PSA, Ki67 and their ratio for the effect of early docetaxel, the ccoptimalmatch R-package was used to identify three matched controls per docetaxel-treated case, matched on $\log_2$ ("biomarker") (maximum distance 1.3) and ISUP (maximum distance 1). Docetaxel treated cases were split in two groups (high and low) based on the biomarker value. Kaplan-Meier analysis was used to compare the progression-free survival between docetaxel-treated cases and their matched "ADT alone" controls in these two groups (high and low) separately.

All statistical analyses were based on two-sided tests, and $p < .05$ was considered statistically significant, except in the gene association analysis where Benjamini-Hochberg's FDR method for multiple testing correction was adopted and the significance threshold 5% FDR was used.

Pathway analysis

Genes showing significant correlations (adjusted *p*-value <.05) between transcript levels and log2Ki67/PSA were analyzed by the MetaCore analysis tool for pathway enrichment analysis (Clarivate analytics, UK). The sets of genes being positively or negatively associated with Ki67/PSA were analyzed separately for the probability of being significantly enriched (false discovery rate (FDR) <.05) in pre-defined pathway maps, considering the numbers of enriched gene products in the data vs. the total number of genes per pathway. *p* values were adjusted by considering the rank of the pathway, given the total number of pathways in the MetaCore ontology.

Results

Patient characteristics

Of the 92 patients, 79 were available for analysis of PFS and overall survival (OS) for ADT. Fourteen of these patients received early docetaxel ($n = 12$) or abiraterone acetate ($n = 2$) as addition to ADT and were excluded from the primary analysis. All patients stayed on their ADT or additional therapy until relapse. For baseline characteristics see Table 1. For the 65 patients in the primary survival analysis, ADT was performed by GnRH agonists with or without 30 d of bicalutamide as flair protection ($n = 48$), GnRH agonist after one month of GnRH antagonists ($n = 11$), surgical castration ($n = 6$). At last follow-up, castration resistant relapse had occurred in 60 of the 65 patients, and 17 of the patients were still alive. Of the baseline clinical characteristics (age, ISUP, and serum PSA) only ISUP was significantly associated with PFS and OS in multivariate analysis (Supplementary Table S1).

PSA immunoreactivity score outperforms Ki67 score as prognostic marker for PFS and OS

After adjustment for age, ISUP and baseline serum PSA, Cox regression analysis showed that an increased Ki67 score increases the risk for progression and death (HR = 1.30; $p = .047$ and HR = 1.41; $p = .022$, respectively) (Table 2). The PSA score had an even better prognostic potential, with the risk of progression and death decreasing with increasing PSA scores (HR = 0.53 and 0.37, respectively and $p < .0001$ for both) (Table 2). The patients in the highest Ki67 quartile or lowest PSA quartile had the worst prognosis, but these cut-offs did not always identify the same patients, illustrated by the tabulation of the quartiles (Supplementary Table S2). We therefor hypothesized that a ratio between Ki67 and PSA IHC score would further discriminate patients with different outcomes. This ratio was prognostic for both progression-free and overall survival ($p < .0001$ for both) (Table 2) but did not confer stronger prognostic potential compared to PSA score alone. In line with this, analyzing PSA and Ki67 scores in a multivariable cox regression model together with age, ISUP, and serum PSA, showed that only PSA score was independently prognostic for relapse and overall survival (Table 3). Dividing the immunoreactivity scores in quartiles, Kaplan–Meier analysis showed that all three parameters (Ki67, PSA and Ki67/PSA) could identify the groups with best and worst prognosis, confirming the strongest prognostic potential for

Table 3. Multivariable survival analysis with Cox regression for the immunoreactive score of PSA and Ki67.

Outcome	Variable	*N* (events)	HR	*p*
Relapse	Ki67 score	64 (59)	1.07 (0.81, 1.42)	.62
	PSA score		0.55 (0.39, 0.78)	6.6E-04
	Age		0.99 (0.96, 1.02)	.59
	ISUP		1.35 (0.94, 1.94)	.1
	serum PSA		1.07 (0.95, 1.21)	.28
Death	Ki67 score	64 (47)	1.08 (0.78, 1.48)	.65
	PSA score		0.38 (0.25, 0.59)	1.1E-05
	Age		1.02 (0.99, 1.06)	.26
	ISUP		1.21 (0.79, 1.83)	.38
	serum PSA		1.06 (0.93, 1.19)	.39

Table 2. Survival analysis with Cox regression for the immunoreactive score of PSA and Ki67.

Outcome	Variable	*N* crude (events)	HR crude	*P* crude	*N* adj. (events)	HR adjusteda	*P* adjusted[a]
Relapse	Ki67 score	65 (60)	1.39 (1.11, 1.75)	0.0049	64 (59)	1.30 (1.00, 1.70)	0.047
	PSA score		0.51 (0.38, 0.68)	6.5E-06		0.53 (0.39, 0.71)	3.5E-05
	Ki67/PSA		1.38 (1.18, 1.60)	4.8E-05		1.34 (1.13, 1.59)	6.3E-04
Death	Ki67 score	65 (48)	1.52 (1.16, 1.99)	0.0025	64 (47)	1.41 (1.05, 1.91)	0.022
	PSA score		0.38 (0.27, 0.54)	6.2E-08		0.37 (0.25, 0.54)	3.3E-07
	Ki67/PSA		1.54 (1.28, 1.85)	4.8E-06		1.54 (1.25, 1.90)	5.4E-05

[a]Adjusted for serum PSA, ISUP, and age.

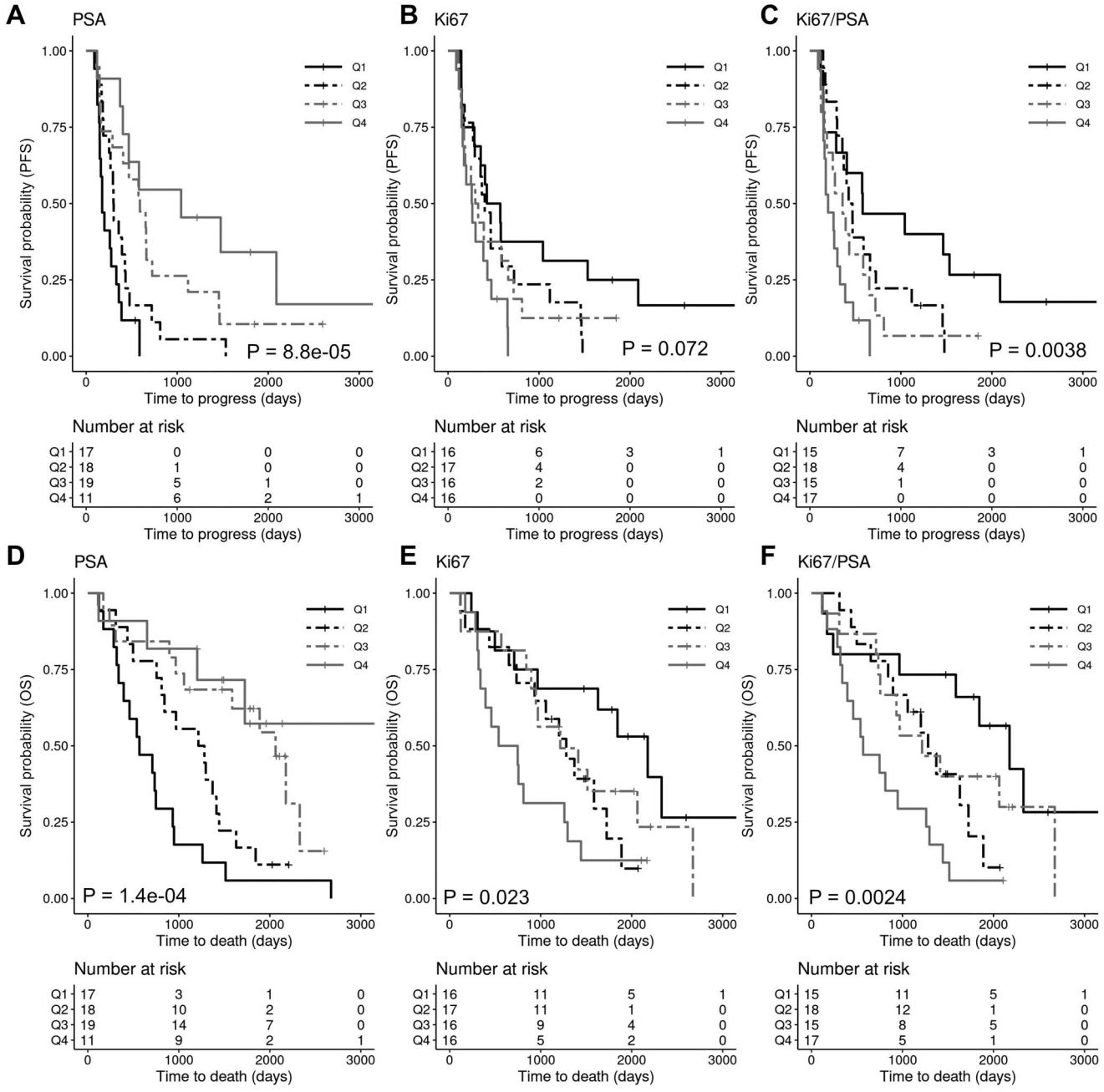

Figure 2. Kaplan–Meier analysis of survival in relation to Ki67 and PSA immunoreactivity scores. Progression-free survival (upper panels) and overall survival (lower panels) in patients grouped in quartiles based on PSA score (A,D), Ki67 score (B,E), and Ki67/PSA ratio (C,F). *p* values are indicated in the graphs.

PSA (Figure 2). Images of PSA and Ki67 stainings are shown in Supplementary Figure S2.

Gene expression of MKI67 and KLK3: correlations and prognostic value

110 biopsies from the Gothenburg cohort (2 biopsies/patient ($N = 54$), except for two patients from which only 1 biopsy was available) were used for comprehensive whole genome expression profiling. IHC scores for Ki67 and PSA correlated well with the mRNA expression of their coding genes MKI67 and KLK3, respectively (Supplementary Figure 3(B,C)). High mRNA levels of KLK3 were, after adjustment for ISUP, age, and serum PSA, associated with a decreased risk of progression and death, in line with the results from the immunoreactivity analysis, while MKI67 levels displayed no significant associations with prognosis after adjustments (Table 4).

The Ki67/PSA ratio is associated with cell cycle regulation and DNA damage repair

The mRNA expression data was analyzed for the correlation with the immunoreactivity scores for Ki67 and PSA. In total, 238 genes (40 down and 198 up) were significantly correlated to the Ki67 score, 436 (280 down and 156 up) genes were correlated to the PSA score, and 472 genes (116 down and 356 up) correlated to the Ki67/PSA ratio. The overlap

Table 4. Survival analysis with Cox regression for the gene expression levels of KLK3 and MKI67.

Outcome	Variable	*N* crude (events)	HR crude	*P* crude	*N* adj. (events)	HR adjusted[a]	*P* adjusted[a]
Relapse	MKI67	38 (36)	1.53 (0.90, 2.60)	0.11	38 (36)	1.19 (0.62, 2.29)	0.61
	KLK3		0.56 (0.35, 0.91)	0.018		0.46 (0.26, 0.80)	0.0061
Death	MKI67	38 (32)	2.01 (1.12, 3.63)	0.021	38 (32)	1.72 (0.86, 3.43)	0.12
	KLK3		0.62 (0.41, 0.93)	0.021		0.50 (0.30, 0.83)	0.0076

[a]Adjusted for serum PSA, ISUP, and age.

Table 5. Gene enrichment analysis for genes associated with Ki67/PSA ratio.

Top 10 pathways significantly associated with high Ki67/PSA ratio			Pathways significantly associated with low Ki67/PSA ratio		
Enriched Pathway Maps	FDR	*P* value	Enriched Pathway Maps	FDR	*p* value
1. Cell cycle: Chromosome condensation in prometaphase	5.52E-14	1.35E-16	1. Androgen receptor activation and downstream signaling in prostate cancer	1.66E-03	5.07E-05
2. Cell cycle: Spindle assembly and chromosome separation	6.73E-12	3.31E-14	2. Recurrent gene fusions in prostate cancer	1.66E-03	5.70E-05
3. Cell cycle: Initiation of mitosis	9.62E-12	7.09E-14	3. Transcription targets of androgen receptor involved in prostate cancer	1.66E-03	6.60E-05
4. DNA damage: ATM/ATR regulation of G2/M checkpoint: nuclear signaling	8.42E-11	1.06E-12	4. Cell adhesion: ECM remodeling	3.12E-03	1.48E-04
5. DNA damage: Double-strand break repair via homologous recombination	8.42E-11	1.38E-12	5. Protein folding and maturation: Bradykinin/Kallidin maturation	2.99E-02	1.66E-03
6. Cell cycle: Role of APC in cell cycle regulation	8.42E-11	1.45E-12	6. Pro-oncogenic action of androgen receptor in breast cancer	3.12E-02	1.98E-03
7. DNA damage: ATM/ATR regulation of G2/M checkpoint: cytoplasmic signaling	2.11E-07	4.15E-09	7. Neutrophil chemotaxis in asthma	3.27E-02	2.34E-03
8. Cell cycle: The metaphase checkpoint	3.55E-07	4.48E-09	8. Mechanisms of deltaF508 CFTR activation by S-nitrosoglutathione	3.79E-02	8.39E-03
9. Cell cycle: Transition and termination of DNA replication	1.39E-06	3.42E-08			
10. Abnormalities in cell cycle in SCLC	6.93E-08	2.57E-06			

Displayed pathways are selected based on a p value $<.05$ and a FDR $<.05$.

between these groups of significantly associated genes is shown in Supplementary Figure S4 and Supplementary Tables S3–S5 and illustrates that there are about 30% of genes that are unique for their association to the Ki67/PSA ratio and not to the PSA and Ki67 scores separately. Gene ontology and pathway analysis of the genes significantly associated to the Ki67/PSA ratio demonstrated that a high ratio (high Ki67 and low PSA) was associated with increased expression of genes involved in regulation of the cell cycle and DNA damage repair. Genes associated with a low ratio (low Ki67 and high PSA) were involved in androgen receptor activation, cell adhesion, and neutrophil chemotaxis (Table 5).

Potential for Ki67 and PSA as markers for outcome of early docetaxel as addition to ADT

Docetaxel is microtubule-stabilizing and anti-mitotic [16], and since high Ki67/PSA scores were associated with cell cycle regulation and DNA damage repair, we hypothesize that patients with high Ki67/PSA ratio may have the most benefit of adding docetaxel to ADT. To assess this, we selected the 12 patients receiving early docetaxel and for each of them three control patients receiving only ADT, matched on Ki67/PSA ratio and ISUP, resulting in 48 individuals in total. The Ki67, PSA, and Ki67/PSA values for this selected cohort are shown in Figure 3(A). This cohort was divided in two by the median Ki67/PSA value among the 12 treated patients. PFS was analyzed for the low ($n = 20$) and high ($n = 28$) Ki67/PSA groups separately, comparing patients with and without early docetaxel therapy. The same was also performed for PSA and Ki67 separately.

The results displayed a beneficial effect of docetaxel on progression free survival in patients with a high Ki67/PSA ratio ($p = .041$), while the effect seemed negligible in the low Ki67/PSA group (Figure 3(B)). When dividing the patients according to PSA score neither of the groups displayed any effect of docetaxel addition (Figure 3(C)). However, the Ki67 high group, like the high Ki67/PSA group, showed a possible benefit in progression free survival of docetaxel (not significant: $p = .075$), while the low Ki67 low group did not (Figure 3(D)). Overall survival was not assessed due to the

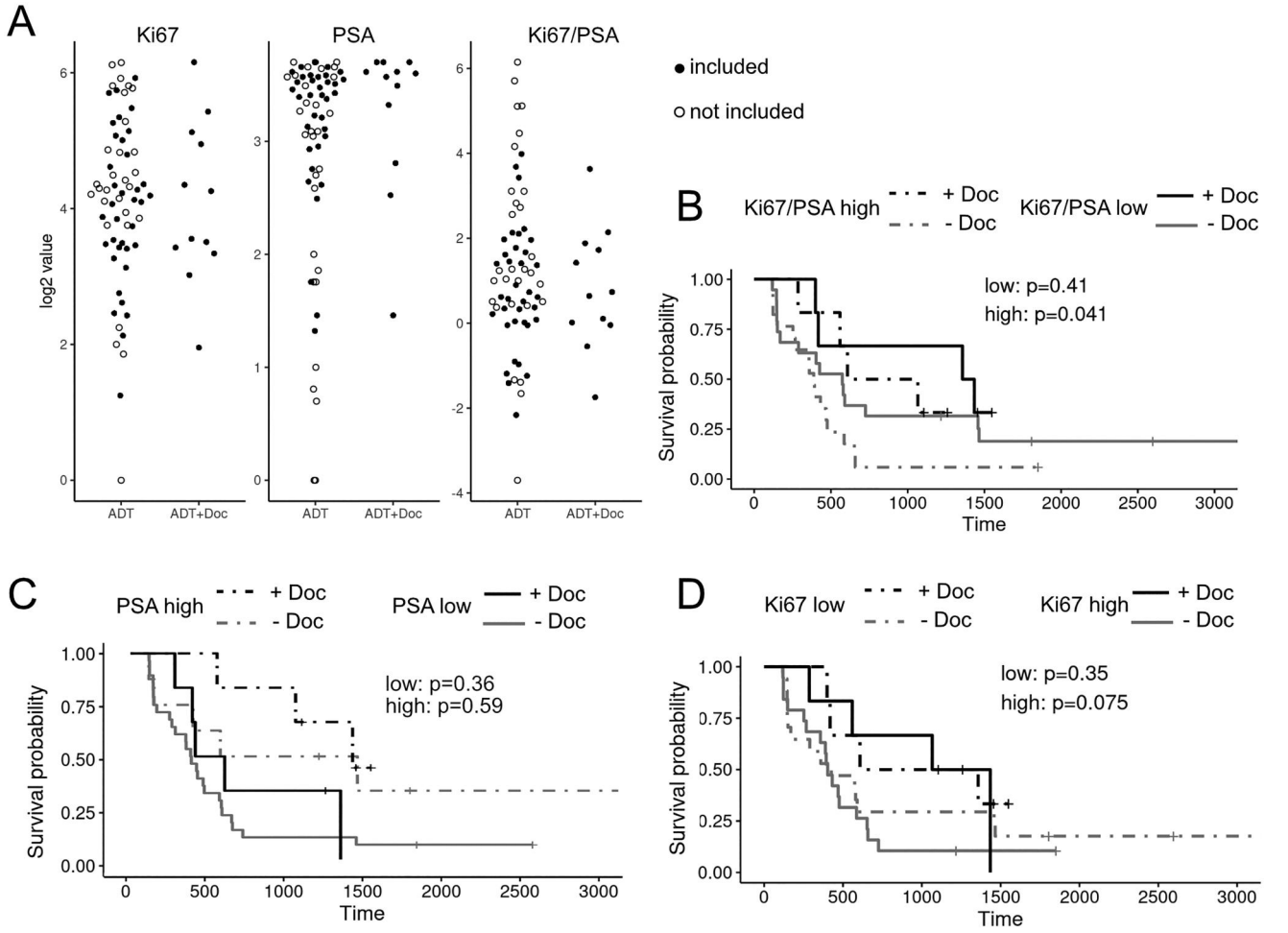

Figure 3. Sub-analysis of PFS among patients who received docetaxel in addition to ADT. (A) Illustration of the docetaxel-treated patients and their matched controls regarding PSA, Ki67, and Ki67/PSA. Filled circles indicate matched cases included in the sub analysis, open circles represent the patients not included in the sub analysis. (B–D) Kaplan–Meier analysis of progression-free survival after ADT with or without additional docetaxel (±doc) in relation to the (B) Ki67/PSA ratio (high or low), (C) PSA score, and (D) Ki67 score. *p* values are shown in the graphs.

difference in use of CRPC treatments (docetaxel, abiraterone, and enzalutamide) between the groups, which was too large to allow comparison.

Discussion

Markers to identify patients who are in need of the growing repertoire of treatments for metastatic prostate cancer are urgently needed. This paper illustrates the potential of immunoreactivity for PSA, and of its combination with Ki67, to distinguish between good and poor responders to ADT given as mono therapy. In addition, it suggests that readily available immunohistochemical scoring may be used to identify patients with the highest potential to benefit from adding docetaxel to ADT.

Today, guidelines recommend adding docetaxel or androgen signaling inhibition (ASI) to ADT for metastatic prostate cancer, with equal beneficial effects reported for docetaxel and abiraterone acetate [6,17,18]. Further, triple treatment with ADT, docetaxel, and ASI are discussed to be standard of care despite increased side effects [15]. However, the quality-of-life (QoL) adjusted survival indicates that the reduced QoL for the combination therapy may not balance the increased survival for all patients [19]. Thus, there is a need for treatment predictive biomarkers to select de-escalation from triple therapy to avoid or postpone docetaxel side effects.

The present study demonstrates the strong potential of immunoreactivity of PSA to identify good or poor responders to ADT alone, which is in line with previous evidence that tissue expression of PSA has prognostic value [9,20–22]. Compared to the proliferative index measured by Ki67 staining, which, although not clinically used, is broadly accepted to be associated with prognosis, PSA is more powerful. This is also in line with previous head-to-head comparisons of PSA and Ki67 as prognostic tissue markers in other clinical stages of prostate cancer [2,14]. However, although these earlier studies identified a combination of Ki67 and PSA as even more efficient, in the situation of primary metastatic prostate cancer, this seems not to be true for the prognostic potential for response to ADT. This may be explained by the relatively high proliferative index in the majority of these tumors (median 18.8, interquartile range 11.2–31.2), making the tumors with lowest Ki67 scores still highly proliferative and limiting the impact of this parameter for prognostic purposes for the response to ADT.

Although a true comparison of efficacy of the addition of early docetaxel is not possible in the present set-up, we used our patient material, including the 12 patients who received addition of docetaxel to ADT, as a pilot study evaluating if docetaxel addition gives more benefit to cases with a high Ki67/PSA ratio. The results suggest that patients bearing tumors with a low Ki67/PSA ratio, i.e., the combination of high AR activity and low proliferation, may not need the addition of docetaxel to ADT. In contrast, the patients with high Ki67/PSA ratio showed the worst response to ADT alone and clearly benefitted from the added docetaxel. In this situation, the proliferative index using Ki67 was almost as good to prognosticate benefit of docetaxel as the Ki67/PSA ratio, while PSA on its own was not powerful at all, illustrating the different potential for these markers depending on the nature of the intended therapy.

Recently, it was shown that a high Decipher GC risk score based on whole transcriptomic profiling was associated with most benefit from addition of docetaxel to ADT [23]. Further, the luminal B subtype from the PAM50 gene expression identifier associated with both high proliferation and high androgen activity, was found to both have the best response to ADT [5] and being the only subgroup to benefit with increased OS from additional docetaxel to ADT [23], possibly indicating the Luminal B patient group in need of multimodal treatment, regardless of choice of treatment. In the present sub study, OS could not be properly analyzed due to the large difference in CRPC treatments received between the patients who got addition of docetaxel to ADT, recruited to the study recently, and those recruited earlier of whom many only received total androgen blockade with bicalutamide. However, this study points out the possibility that classification using basic tissue detection of Ki67 and PSA could be equally efficient in identifying patients benefitting from addition of docetaxel to ADT as elaborate transcription profiling algorithms.

In addition to docetaxel, ASIs are used as early treatment in combination with ADT. Since a low Ki67/PSA ratio is dependent on a high PSA score, indicating a high AR activity, it could suggest a better response to ADT. In line with that, patients with CRPC tumors classified as PAM50 luminal B, having a higher AR activity, achieved significantly better survival after treatment with ASI drugs compared to patients with basal tumors [24]. Thus, the Ki67/PSA ratio may also be useful in selecting patients for the addition of ASI to ADT in the hormone sensitive phase, possibly together with information of presence of intraductal carcinoma of the prostate, which recently was shown to be predictive for ASI in this setting [25]. In the present study, however, only two patients were treated with abiraterone acetate, and no such analyses could be performed.

The gene expression profile associated with the Ki67 and PSA immunoreactive scores largely reflect the expected pathways, including cell cycle regulation in one end and AR signaling in the other. The appearance of DNA damage repair pathways together with the proliferative profile in the more aggressive part of the spectrum may indicate that Ki67/PSA reflects properties important for PARP-inhibitors. Further, AR inhibitors exert a synergistic effect with PARP inhibitors in impairing DNA repair and downregulating DNA repair gene expression [26,27], properties which may also be connected to the low AR activity in the high Ki67/PSA patients. Thus, it may be speculated that the Ki67/PSA ratio may have a potential to be a biopsy-based tool for patient selection also for PARP inhibitors. However, efficacy of PARP inhibitors in the hormone naïve setting is yet to be defined, and phase III trials are ongoing for genetically selected patient cohorts (AMPLITUDE (NCT04497844) and TALAPRO-3 (NCT04821622))

Limitations of the present study include the retrospective design and as an explorative hypothesis-generating study, results need to be validated in prospective clinical trials. Also, inclusion of more clinical variables such as tumor burden, lactate dehydrogenase, hemoglobin, and others would have strengthened the evaluation regarding independency of the investigated markers. Further, the small study size and the long inclusion time (during which the treatment for mCRPC have changed) compromise the possibility to draw conclusions of overall survival.

In conclusion, we show evidence that PSA immunoreactivity in primary metastatic hormone sensitive prostate cancer is a strong prognostic marker for identifying patients with best response to ADT. Further, the ratio of Ki67 and PSA scores correlates with a gene expression profile associated with cell cycle regulation and DNA damage repair. Accordingly, a small exploratory sub study suggests Ki67/PSA as a possible treatment predictive marker for identification of patients benefitting from the addition of docetaxel to ADT, an interesting option for future studies in larger patient cohorts.

Acknowledgments

We thank Robert Buckland, Karin Larsson, Pernilla Andersson, and Susanne Gidlund for excellent technical assistance. Thanks also to Marie Lundholm, Kerstin Almroth and Lars Rönnlund help with follow up data.

Disclosure statement

A.B. and P.W. have a pending patent application ('Methods for diagnosis and prognosis of prostate cancer', EP2020/054681). Other authors declare no conflicts of interest

Funding

This work was supported by grants from Swedish Research Council (2022-00946) and the Swedish Cancer Society (Grant numbers 21-1856, 20-1055, 19-0053, 19-0054), Knut and Alice Wallenberg foundation (KAW 2020.0235), the research foundation of Ulla och Karl-Erik Winberg, and the Swedish Prostate Cancer Federation.

ORCID

Karin Welén http://orcid.org/0000-0001-6480-636X

Data availability statement

The data generated in this study are not publicly available due to information that could compromise patient privacy or consent but are available upon reasonable request from the corresponding author.

References

[1] Quigley DA, Dang HX, Zhao SG, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. Cell. 2018; 175(3):889. doi:10.1016/j.cell.2018.10.019.

[2] Thysell E, Vidman L, Ylitalo EB, et al. Gene expression profiles define molecular subtypes of prostate cancer bone metastases with different outcomes and morphology traceable back to the primary tumor. Mol Oncol. 2019;13(8):1763–1777. doi:10.1002/1878-0261.12526.

[3] Ylitalo EB, Thysell E, Jernberg E, et al. Subgroups of castration-resistant prostate cancer bone metastases defined through an inverse relationship Between androgen receptor activity and immune response. Eur Urol. 2017;71(5):776–787. doi:10.1016/j.eururo.2016.07.033.

[4] You S, Knudsen BS, Erho N, et al. Integrated classification of prostate cancer reveals a novel luminal subtype with poor outcome. Cancer Res. 2016;76(17):4948–4958. doi:10.1158/0008-5472.CAN-16-0902.

[5] Zhao SG, Chang SL, Erho N, et al. Associations of luminal and basal subtyping of prostate cancer With prognosis and response to androgen deprivation therapy. JAMA Oncol. 2017;3(12):1663–1672. doi:10.1001/jamaoncol.2017.0751.

[6] Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. N Engl J Med. 2015;373(8):737–746. doi:10.1056/NEJMoa1503747.

[7] Yanagisawa T, Rajwa P, Thibault C, et al. Androgen receptor signaling inhibitors in addition to docetaxel with androgen deprivation therapy for metastatic hormone-sensitive prostate cancer: a systematic review and meta-analysis. Eur Urol. 2022;82(6):584–598. doi:10.1016/j.eururo.2022.08.002.

[8] Goldstein NS. Immunophenotypic characterization of 225 prostate adenocarcinomas with intermediate or high gleason scores. Am J Clin Pathol. 2002;117(3):471–477. doi:10.1309/G6PR-Y774-X738-FG2K.

[9] Bonk S, Kluth M, Hube-Magg C, et al. Prognostic and diagnostic role of PSA immunohistochemistry: a tissue microarray study on 21,000 normal and cancerous tissues. Oncotarget. 2019;10(52): 5439–5453. doi:10.18632/oncotarget.27145.

[10] Zhao L, Yu N, Guo T, et al. Tissue biomarkers for prognosis of prostate cancer: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2014;23(6):1047–1054. doi:10.1158/1055-9965.EPI-13-0696.

[11] Richardsen E, Andersen S, Al-Saad S, et al. Evaluation of the proliferation marker ki-67 in a large prostatectomy cohort. PLoS One. 2017;12(11):e0186852. doi:10.1371/journal.pone.0186852.

[12] Richardsen E, Uglehus RD, Due J, et al. COX-2 is overexpressed in primary prostate cancer with metastatic potential and may predict survival. A comparison study between COX-2, TGF-beta, IL-10 and Ki67. Cancer Epidemiol. 2010;34(3):316–322. doi:10.1016/j.canep.2010.03.019.

[13] Hammarsten P, Josefsson A, Thysell E, et al. Immunoreactivity for prostate specific antigen and Ki67 differentiates subgroups of prostate cancer related to outcome. Mod Pathol. 2019;32(9): 1310–1319. doi:10.1038/s41379-019-0260-6.

[14] Wikstrom P, Bergstrom SH, Josefsson A, et al. Epithelial and stromal characteristics of primary tumors predict the bone metastatic subtype of prostate cancer and patient survival after androgen-deprivation therapy. Cancers. 2022;14(21):5195. doi:10.3390/cancers14215195.

[15] Thomas C, Baunacke M, Erb HHH, et al. Systemic triple therapy in metastatic hormone-sensitive prostate cancer (mHSPC): ready for prime time or still to be explored? Cancers. 2021;14(1):8. doi:10.3390/cancers14010008.

[16] Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov. 2010;9(10):790–803. doi:10.1038/nrd3253.

[17] James ND, de Bono JS, Spears MR, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med. 2017;377(4):338–351. doi:10.1056/NEJMoa1702900.

[18] Sydes MR, Spears MR, Mason MD, et al. Adding abiraterone or docetaxel to long-term hormone therapy for prostate cancer: directly randomised data from the STAMPEDE multi-arm, multi-stage platform protocol. Ann Oncol. 2018;29(5):1235–1248. doi:10.1093/annonc/mdy072.

[19] Menges D, Yebyo HG, Sivec-Muniz S, et al. Treatments for metastatic hormone-sensitive prostate cancer: systematic review, network meta-analysis, and benefit-harm assessment. Eur Urol Oncol. 2022;5(6):605–616. doi:10.1016/j.euo.2022.04.007.

[20] Abrahamsson PA, Lilja H, Falkmer S, et al. Immunohistochemical distribution of the three predominant secretory proteins in the parenchyma of hyperplastic and neoplastic prostate glands. Prostate. 1988;12(1):39–46. doi:10.1002/pros.2990120106.

[21] Erbersdobler A, Isbarn H, Steiner I, et al. Predictive value of prostate-specific antigen expression in prostate cancer: a tissue microarray study. Urology. 2009;74(5):1169–1173. doi:10.1016/j.urology.2009.02.061.

[22] Schlomm T, Iwers L, Kirstein P, et al. Clinical significance of p53 alterations in surgically treated prostate cancers. Mod Pathol. 2008;21(11):1371–1378. doi:10.1038/modpathol.2008.104.

[23] Hamid AA, Huang HC, Wang V, et al. Transcriptional profiling of primary prostate tumor in metastatic hormone-sensitive prostate cancer and association with clinical outcomes: correlative analysis of the E3805 CHAARTED trial. Ann Oncol. 2021;32(9):1157–1166. doi:10.1016/j.annonc.2021.06.003.

[24] Aggarwal R, Rydzewski NR, Zhang L, et al. Prognosis associated with luminal and basal subtypes of metastatic prostate cancer. JAMA Oncol. 2021;7(11):1644–1652. doi:10.1001/jamaoncol.2021.3987.

[25] Naito Y, Kato M, Kawanishi H, et al. Clinical utility of intraductal carcinoma of the prostate in treatment selection for metastatic hormone-sensitive prostate cancer. Prostate. 2023;83(4):307–315. doi:10.1002/pros.24462.

[26] Asim M, Tarish F, Zecchini HI, et al. Synthetic lethality between androgen receptor signalling and the PARP pathway in prostate cancer. Nat Commun. 2017;8(1):374. doi:10.1038/s41467-017-00393-y.

[27] Li L, Karanika S, Yang G, et al. Androgen receptor inhibitor-induced "BRCAness" and PARP inhibition are synthetically lethal for castration-resistant prostate cancer. Sci Signal. 2017;10(480): eaam7479. doi:10.1126/scisignal.aam7479.