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An Immunohistochemical Signature Comprising PTEN, MYC, and Ki67 Predicts Progression in Prostate Cancer Patients Receiving Adjuvant Docetaxel After Prostatectomy

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Abstract

BACKGROUND—Loss of the tumor suppressor PTEN is common in prostate cancer and may have prognostic significance. The authors examined PTEN and additional protein markers in primary tumors from patients with high-risk, localized prostate cancer who received adjuvant docetaxel in a prospective multicenter trial (TAX2501).

METHODS—Fifty-six of 77 patients enrolled in TAX2501 had primary prostatectomy specimens available for immunohistochemical analysis of PTEN, MYC, ERG, tumor protein p53 (p53), antigen KI-67 (Ki67), and phosphorylated forms of Akt, mammalian target of rapamycin (mTOR), and S6 ribosomal protein. Protocol-defined progression included a prostate-specific antigen (PSA) level ≥0.4 ng/mL, radiologic/clinical recurrence, or death. Univariate and multivariable proportional hazards regression analyses were used to investigate the influence of PTEN status (and other protein markers) on progression-free survival (PFS).

RESULTS—In this exploratory, post hoc analysis, PTEN protein loss (vs presence) was observed in 61% of patients and was associated with lower preoperative PSA levels, higher clinical stage, lower Ki67 expression, the presence of p53, and the presence of ERG. In univariate analysis, the factors associated with PFS included Gleason sum, seminal vesicle invasion, PTEN status, MYC expression, and Ki67 expression. In multivariable analysis, only 3 variables emerged as independent prognostic factors for PFS: PTEN status ($P = .035$), MYC expression ($P = .001$), and Ki67 expression ($P < .001$). A prognostic model was constructed that incorporated clinical covariates as well as information on PTEN, MYC, and Ki67.

CONCLUSIONS—The current results indicated that PTEN status, MYC expression, and Ki67 expression in primary tumor samples may predict PFS more accurately than clinical factors alone in men with high-risk prostate cancer who receive adjuvant docetaxel after prostatectomy. If

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validated, these hypothesis-generating findings may have prognostic and therapeutic implications and may aid clinical trial design.

Keywords

PTEN; MYC; Ki67; high-risk prostate cancer; adjuvant docetaxel; progression-free survival; prognostic model

INTRODUCTION

Prostate cancer is the most common noncutaneous cancer in American men, and over 240,000 new cases are diagnosed annually in the United States.[1] Most patients present with early stage disease and undergo definitive local treatment with prostatectomy and/or radiation therapy; however, 20% to 40% of men develop evidence of biochemical relapse,[2,3] and a significant subset of these men will develop lethal metastatic disease. Pathologic risk factors for disease recurrence and cancer-specific mortality after radical prostatectomy include positive surgical margins, extracapsular extension, high Gleason score, positive lymph nodes, and seminal vesicle invasion.[4] The natural history of patients who have unfavorable risk factors suggests the presence of micrometastatic disease at diagnosis in a significant fraction of these men.

The advent of effective systemic chemotherapy for patients with metastatic, castration-resistant prostate cancer has provided the impetus for evaluating the role of this modality in patients with high-risk disease at earlier stages in an attempt to eradicate potential micrometastases. The receipt of docetaxel in the adjuvant/neoadjuvant setting by men with high-risk prostate cancer is currently under evaluation in several ongoing randomized trials.[5-9] It is clear from several phase 2 studies of neoadjuvant docetaxel in this patient population that, although there appears to be activity in a subset of patients, treatment failures occur frequently; thus, prognostic markers in the adjuvant/neoadjuvant setting could have clinical utility for patient selection and trial design.

The phosphatase and tensin homolog (*PTEN*) gene encodes a widely expressed tumor suppressor protein that possesses lipid phosphatase and protein phosphatase activity. Its most well studied catalytic activity results in removal of the 3'-phosphate from phosphoinositide-binding protein 3 (PIP3), converting it to phosphatidylinositol-diphosphate (PIP2) and, thus, countering the activity of the phosphatidylinositol-3 kinase (PI3K) signaling pathway.[10] This results in inhibition of downstream targets, such as Akt, mammalian target of rapamycin (mTOR), and S6 kinase, which have several functions, including prevention of apoptosis and stimulation of cell proliferation and migration.[11,12] Studies using radical prostatectomy samples have suggested that approximately 40% to 80% of locally confined prostate cancers have loss of at least 1 allele of *PTEN* as determined by fluorescence in situ hybridization (FISH) analysis.[13-15] Preclinical and clinical studies have demonstrated that PTEN loss is associated with prostate cancer recurrence after surgery[16] and resistance to chemo-therapy[17] as well as the time to prostate cancer metastasis after radical prostatectomy.[15]

TAX2501 was a prospective, multi-institutional, phase 2 study[18] of adjuvant, weekly docetaxel (6 cycles of docetaxel at 35 mg/m^2 on days 1, 8, and 15 of a 28-day cycle) for patients who underwent radical prostatectomy and were considered at high risk for progression, defined as a ≥50% chance of recurrence at 3 years. We previously demonstrated the feasibility and utility of interrogating PTEN protein levels in archival formalin-fixed, paraffin-embedded tissue samples using immunohistochemistry (IHC). In those studies, loss of PTEN protein correlated well, albeit not perfectly, with loss of 1 or

both alleles of *PTEN* as determined by either FISH or high-density single-nucleotide polymorphism microarrays.[15] In the current study, we generated tissue microarrays using prostatectomy samples from patients in the TAX2501 study and used this IHC assay to examine whether PTEN protein status in primary tumors was associated with progression-free survival. In addition, we sought to analyze other biologically relevant, cancer-related proteins, including downstream targets of PI3K signaling, such as phosphorylated Akt (p-Akt), mTOR (p-mTOR), and S6 ribosomal protein (p-S6). Furthermore, to determine their influence on progression, we also evaluated the expression levels of MYC,[19,20] a well known onco-gene linked to prostate cancer, as well as the tumor suppressor protein TP53 and the proliferation marker antigen KI-67 (Ki67). Finally, because several recent studies have suggested synergy between loss of PTEN and the presence of a transmembrane protease, serine 2-E26 oncogene homolog (*TMPRSS2-ERG*) gene fusion,[21] we also examined whether these tumors expressed the ERG protein, which is an excellent surrogate of *TMPRSS2-ERG* fusion status.[22-24]

MATERIALS AND METHODS

Study Population

The patient population consisted of men who participated in TAX2501, a prospective study of adjuvant docetaxel in patients with high-risk prostate cancer. Details of that trial design are provided in the original report.[18] Briefly, all patients underwent radical prostatectomy for presumed localized prostate carcinoma. Pathologic findings were reviewed centrally for confirmation of the original diagnosis, including disease stage and tumor grade. The risk of recurrence was calculated using a validated prognostic tool based on a multivariate Cox proportional hazards model that included information on Gleason score, seminal vesicle invasion, lymph node involvement, and surgical margin status.[25] Patients were required to have a recurrence risk score ≥2.84, which translated into a risk of progression ≥50% at 3 years. Treatment consisted of 6 cycles of intravenous docetaxel at a dose of 35 mg/m^2 on days 1, 8, and 15 of a 28-day cycle. Patients were evaluated weekly during chemotherapy treatments and every 3 months thereafter with physical examinations (including digital rectal examinations), prostate-specific antigen (PSA) measurements, as well as computed tomography and bone scans. Progression-free survival (PFS) was calculated from the date of prostatectomy to the date of progression. Protocol-defined progression included a PSA level ≥0.4 mg/mL, radiologic/clinical evidence of disease recurrence, or death from any cause (whichever occurred first). This research was carried out in accordance with the prior approval of the Institutional Review Board Committee of our institution.

Protein Marker Analysis

From the patients enrolled in the TAX2501 trial,[18] we prepared tissue microarrays (TMAs) by selecting the highest grade/largest tumor per patient from radical prostatectomy specimens, which we sampled with 4-fold redundancy. The TMAs were constructed as previously described.[26]

IHC assays for MYC (1472-1; 1:300 dilution; rabbit monoclonal; Epitomics, Burlingame, Calif) and PTEN (clone D4.3, 9188; 1:50 dilution; rabbit monoclonal; Cell Signaling Technology, Danvers, Mass) were performed as previously described.[15,19] IHC for p-S6 (serine 240/244; rabbit polyclonal; Cell Signaling Technology) and p-mTOR (serine 2448; rabbit monoclonal; 2976; Cell Signaling Technology) were performed as previously described.[27] IHC for p53 (clone D07; mouse monoclonal; 1:800 dilution; Dako, Carpenteria, Calif) was performed using the Catalyzed Signal Amplification Kit (Dako). IHC for Ki67 (mouse monoclonal; clone 7B11; Zymed Laboratories, South San Francisco, Calif) was performed using the mouse PowerVision+ kit (Leica Microsystems, Buffalo Grove, Ill). IHC

for ERG was performed as previously described.[23] All IHC stains were developed using 3-3'-diaminobenzidine chromogen and counter-stained with hematoxylin. TMA spots with artifactual folds or those lacking tissue target representation were omitted from further analysis.

TMA slides were scanned using the Aperio Scan-Scope CS virtual slide scanner (Aperio, Vista, Calif), and composite TMA core images were viewed using the TMAJ software package developed by the Johns Hopkins TMA Core Facility (available at http://tmaj.pathology.jhmi.edu; [accessed May 21, 2012]). For PTEN, IHC scoring was performed visually as a dichotomous variable in which the carcinoma cells within the TMA spot were scored as either positive or negative/markedly decreased.[15] ERG protein also was scored dichotomously as either present or absent within tumor cells in a given TMA spot. For TP53, we visually estimated the percentage of tumor cells that were stained positive, and a given case was scored as positive if >10% of tumor cells were stained positive, as previously reported.[28] All other makers were subjected to semiautomated image analysis using the FriDA open source software package, as previously described,[20] in which normal prostatic glands were excluded by manual circling. For nuclear markers, such as MYC, we obtained the area score as the number of positively stained pixels within nuclei (brown mask) divided by the total nuclear area (brown plus blue mask). We also obtained a composite "intensity-weighted area ratio" score by combining the area score with the median intensity of positive pixels using the following equation: log(area score × median intensity) (available at: http://jco.ascopubs.org/content/28/25/3958.full; [accessed May 21, 2012]). For markers with predominantly cytoplasmic (or cytoplasmic and nuclear) staining, such as p-Akt, pmTOR, and p-S6, we obtained an area score as follows: (positive pixels)/(total nuclear area). For these markers, the combined score was obtained with the following equation: log(area score × median intensity). For Ki67, we used FrIDA image analysis tools to obtain the number of positively staining tumor nuclei and divided this number by the total nuclear area (brown area plus blue area) to obtain the Ki67 score. For all markers with more than 1 TMA core, the average score of all cores was used as the final score for that patient (except as noted above for PTEN loss).

Statistical Analysis

Of the 8 protein markers we investigated, 3 were treated as categorical/dichotomous variables (PTEN, p53, and ERG), and 5 were treated as continuous variables (S6, mTOR, Akt, MYC, and Ki67). For the continuous markers, the intensity-weighted area ratios were log transformed. Spearman rank correlation coefficients were calculated to examine the correlations of the continuous markers with each other. Univariate and multivariable Cox regression analyses were performed to evaluate the association between each protein markers and the risk of disease progression, with the multivariable model adjusted for the following covariates: pathologic Gleason sum, seminal vesicle invasion, surgical margin status, and lymph node involvement. Proteins in the same pathway (eg, Akt, mTOR, S6) tended to have a strong correlation with each other. Thus, only 1 was picked from this cluster (Akt) to be included in the multivariable model. A global test was performed for the entire marker panel as a whole using the likelihood-ratio test, and subsequent analyses were conducted only if the global test was significant. A final set of informative markers was selected using a backward elimination procedure, and those with a P value < .05 were chosen for retention in the model. Furthermore, the likelihood-ratio test for goodness of fit was used to assess whether the addition of the immunohistochemical markers to the known clinical risk factors improved risk prediction. Each marker was added sequentially to a basic multivariable model that contained clinical covariates only to determine whether the added variable significantly improved risk prediction beyond the preceding model.

For the purposes of the current analysis, progression was defines as a PSA level ≥0.4 mg/mL, radiologic/clinical recurrence, or death (whichever occurred first). If patients were removed from the study before documented progression or if they were lost to follow-up, then they were censored at the last date that they were known to be progression-free. The PFS distribution was estimated using the Kaplan-Meier method, and differences in PFS across different patient strata were compared using the log-rank test. The median PFS was reported along with 95% confidence intervals (CIs) using the method of Brookmeyer and Crowley. Hazard ratios were estimated with 95% CIs. All tests were 2-sided and were considered statistically significant at $P < .05$. All analyses were conducted using the SAS statistical software package (version 9.2; SAS Institute, Inc., Cary, NC).

RESULTS

Patient and Tumor Characteristics

From 77 patients enrolled in the TAX2501 trial, we were able to obtain 57 primary tumor specimens that were suitable for IHC analysis of the candidate proteins (PTEN and p53 analyses were only possible in 56 patients). Baseline patient and tumor characteristics of the 57 evaluable primary tumor specimens are listed in Table 1. Consistent with the high-risk disease status of the enrolled patients, Gleason scores were 8 to 10 in 58% of patients, 62% of patients had seminal vesicles invasion, 39% of patients had lymph node involvement, and 65% of patients had positive surgical margins. After a median follow-up of 37.4 months (range, 10.4-44.5 months), 74% of patients (n = 42) had disease progression. The median PFS was 13.0 months (95% CI, 9.8-16.2 months) for the overall cohort.

Protein Marker Analysis

Loss of PTEN established by IHC analysis was observed in 61% of evaluable patients (a patient's tumor was considered to have lost PTEN if any of the TMA cores had absent or markedly decreased staining). There was a trend toward an association between PTEN loss and lower pre-operative PSA levels ($P = .015$), higher clinical stage ($P = .094$), lower Ki67 expression ($P = .064$), the presence of p53 ($P = .082$), and the presence of ERG ($P = .099$). PTEN loss was not associated with Gleason score, lymph node involvement, seminal vesicle invasion, positive surgical margins, or with the expression of p-S6, p-mTOR, p-Akt, or MYC (Table 2).

Analysis of the 7 other markers of interest revealed that p-Akt expression was strongly correlated with levels of p-S6 ($r^2 = 0.40$; $P = .002$) and p-mTOR ($r^2 = 0.51$; $P < .001$) and that p-S6 and p-mTOR levels also were strongly correlated ($r^2 = 0.54$ and $P < 0.001$), all of these proteins are members of the same signaling pathway. For this reason, only p-Akt expression was included in subsequent multivariable analyses, whereas p-S6 and p-mTOR were removed from the model to avoid multicollinearity. There were no strong correlations between any of the other protein markers that we analyzed.

Prognostic Factors for Progression-Free Survival

In univariate Cox regression analysis (Table 3), the following factors were associated with a greater risk of disease progression after adjuvant docetaxel chemotherapy: seminal vesicle invasion ($P = .024$), a Gleason score of 9 or 10 ($P = .023$), loss of PTEN ($P = .031$), increasing Ki67 expression ($P < .001$), and decreasing MYC expression ($P = .036$). Considering PTEN status, the PFS rate at 18 months in patients with intact PTEN was 45.5% (95% CI, 25.7%-65.3%) compared with 25.7% (95% CI, 12%-39.4%) in patients with PTEN loss ($P = .026$). In multivariable analysis, only 3 variables remained independently prognostic for progression: PTEN loss (hazard ratio [HR], 2.93; 95% CI, 1.08-7.92; $P = .035$), Ki67 expression (HR, 1.43; 95% CI, 1.21-1.68; $P < .001$), and MYC

expression (HR, 0.28; 95% CI, 0.13-0.61; $P = .001$) (Table 3). In the final multivariable model (Table 4), the risk of progression was greater for patients with PTEN deficiency (HR, 3.26; 95% CI, 1.42-7.47), for men with increasing Ki67 expression (HR, 1.34; 95% CI, 1.20-1.50), and for those with decreasing MYC expression (HR, 0.52; 95% CI, 0.29-0.93). When each of these 3 IHC markers was incorporated, in turn, into the basic prognostic model using clinical variables only, this resulted in an improved fit of the model in each case, suggesting that the prognostic ability of the model was enhanced.

Finally, we tested the fit of the prognostic model after sequentially adding multiple protein markers (1 at a time) to the basic model comprising clinical covariates only. In this regard, adding PTEN and/or Ki67 significantly improved risk prediction over the basic model that used clinical factors alone. The prognostic ability of the model reached a plateau when information about PTEN, Ki67, and MYC was added to the clinical factors (Table 5). It is noteworthy that the fit of the prognostic model did not improve further after the addition of data on p53, p-Akt, or ERG, suggesting that these additional proteins do not add to the predictive accuracy of the model.

DISCUSSION

Approximately 30% to 60% of men who receive local therapy for prostate cancer have evidence of subsequent disease progression, and this rate is increased in patients who have high-risk features.[4] In an attempt to improve outcomes, ongoing randomized clinical trials are investigating the role of docetaxel chemotherapy in the adjuvant and neoadjuvant settings in men with high-risk prostate cancer. However, predictive markers of response to therapy (that would be of clinical utility for patient selection and clinical trial design) remain poorly defined. The current results suggest that loss of the PTEN tumor suppressor protein in primary tumor tissue may be an independent, negative prognostic factor for PFS in men with high-risk prostate cancer who receive adjuvant docetaxel after undergoing radical prostatectomy, although these data must be viewed as exploratory and hypothesis-generating. In addition, increased expression of Ki67 and decreased expression of MYC were associated independently with higher rates of progression in this patient population. Furthermore, the prognostic accuracy of the progression model was optimized after incorporating combined information about PTEN status, Ki67 expression, and MYC expression.

Sixty-one percent of patients in the current study demonstrated PTEN loss. Although this is similar to the incidence of PTEN loss reported in prior studies of patients undergoing radical prostatectomy for localized prostate cancer,[13,14] it is at the higher end of the range. This high rate of loss likely relates to the very-high-risk nature of our study cohort, because other populations had PTEN loss in as few as 20% of men.[29] In the current analysis, PTEN loss did not correlate with Gleason sum. Although this may seem surprising, our study was highly enriched for prostate cancers with a Gleason sum >6; therefore, the correlation between elevated Gleason sum and PTEN loss may not be as apparent in this group of patients. In addition, PTEN status in this patient population outperformed all other clinical variables (including Gleason sum, seminal vesicle invasion, lymph node involvement, and surgical margin status) in its ability to predict progression after adjuvant docetaxel chemotherapy. This is consistent with other studies, indicating that functional loss of PTEN is associated with cancer recurrence in patients postprostatectomy[16] and resistance to chemotherapy in preclinical experiments.[17]

It is noteworthy that, although inactivation of PTEN leads to activation of the mTOR signaling pathway, increased phosphorylation of downstream targets in this pathway (Akt, mTOR, S6) was not associated with PTEN loss in this group of patients nor with an

augmented risk of disease progression. However, it is possible that the lack of association of PTEN loss and downstream target activation was the result of delays in tissue fixation and/or processing, consistent with recent studies indicating that several phosphorylated proteins examined in breast cancer specimens demonstrated marked discordance between levels observed in core-needle biopsies and levels observed in resection specimens.[30] This was attributed to delays in tissue fixation (increased total cold ischemic time) in resection specimens compared with needle biopsies. Alternatively, it is possible that the effects on the PI3K/mTOR pathway mediated by PTEN loss are not reflected in steady-state levels of these various phosphorylated proteins or that PTEN loss results in effects on prostate cancer in addition to the PI3K/mTOR pathway (eg, c-Jun N-terminal kinase [JNK] signaling and/or enhancer of zeste homolog 2 [EZH2] overexpression).[31,32]

The observation of improved PFS with increasing MYC protein expression levels is intriguing. *MYC* is a proto-oncogene implicated in the control of cellular growth, proliferation, cell survival, differentiation and apoptosis. *MYC* is 1 of the most frequently activated onco-genes in human cancer, and its amplification and/or overexpression is commonly observed in many cancer types including aggressive prostate cancer.[33] Interestingly, in a neoadjuvant study of docetaxel chemotherapy in patients with high-risk localized breast cancer, women with *MYC* amplification (detected from presurgical tumor biopsy specimens using FISH) demonstrated higher rates of pathological complete responses (30%) than women without *MYC* amplification (11%).[34] In a separate neoadjuvant breast cancer study examining mRNA expression signatures, tumors with coactivation of both the MYC and E2F pathways demonstrated the highest sensitivity to docetaxel chemotherapy.[35] Finally, it has recently been shown in prostate cancer cell lines that over-expression of DNA-binding protein inhibitor ID-1 (ID1) (a protein known to induce docetaxel sensitivity) is mediated by binding of MYC to the ID1 promoter, thereby enhancing ID1 expression.[36] These studies, together with our own observations, begin to suggest a potential role for MYC expression in predicting docetaxel response in patients with prostate (and other) cancers. Alternatively, we have previously demonstrated that steady-state levels of the MYC protein, although they are elevated in most prostate cancers, do not correlate with *MYC* locus amplification as evaluated by FISH.[19] Thus, it is plausible that elevated MYC protein levels are related to improved outcomes in patients with prostate cancer, regardless of adjuvant treatment with docetaxel.

Our study has several limitations. First, this retrospective analysis was not part of the original study design of the TAX2501 trial and represents a post hoc analysis. Therefore, our data are preliminary and were limited by the historic nature of this comparison and by the finding that primary tumor specimens for IHC analysis were not available from all patients. Furthermore, the sample size was relatively small, which limited our ability to exclude the possibility that unequal distribution of clinical-pathologic parameters in this patient cohort may have biased the observed results. Because the TAX2501 study enriched for patients with multiple high-risk features, baseline clinical characteristics may not be reflective of the general prostatectomy population, possibly explaining why clinical covariates did not predict PFS in multivariable analysis (as indicated in other studies). In addition, in the current study, we used a weekly docetaxel schedule that differed from the conventional 3-weekly schedule that is standard for metastatic disease, possibly limiting the utility of our findings. Finally, the median follow-up in this study was relatively short; therefore, we did not have the ability to examine clinical and IHC variables that influenced overall survival.

One additional significant limitation of our current analysis was the lack of requirement of the TAX2501 study to collect postprostatectomy prechemotherapy PSA information. For this reason, we were unable to include postoperative prechemotherapy PSA data in the univariate and multivariable regression models. Therefore, it remains possible that some of

the molecular markers that were significant in our analyses may be acting as surrogates of other progression parameters, such as postoperative prechemotherapy PSA.

Despite these limitations, the association of PTEN loss, high Ki67 expression, and low MYC expression with diminished PFS after adjuvant docetaxel in men with high-risk prostate cancer merits further evaluation and should now be confirmed in a larger, independent cohort. If validated as a predictive marker of response to treatment, then this IHC signature potentially may contribute to treatment decisions, patient selection, and clinical trial design by distinguishing high-risk patients who are more likely to benefit from adjuvant chemotherapy from those who may be candidates for other approaches.

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Table 1

Baseline Patient and Tumor Characteristics (n = 57)

Characteristic	No. of Patients (%)
Age, y	
Mean±SD	57.8±7.4
Median [range]	58 [42-75]
Preoperative PSA, ng/mL	
Mean±SD	14.3±14.1
Median [range]	10.1 [1.8-83.1]
Recurrence risk score	
Mean±SD	4.1±0.6
Median [range]	4.1 [2.9-5.4]
Ethnicity	
Caucasian	52 (91.2)
Black	3 (5.3)
Other	2 (3.5)
Clinical tumor classification	
T1	22 (38.6)
T2	6 (10.5)
T3	6 (10.5)
Missing	2 (3.5)
Seminal vesicle invasion	
Positive	35 (61.4)
Negative	22 (38.6)
Surgical margin status	
Positive	37 (64.9)
Negative	20 (35.1)
Lymph node involvement	
Positive	22 (38.6)
Negative	34 (59.6)
Missing	1 (1.8)
Pathologic Gleason sum	
7	24 (42.1)
8	7 (12.3)
9	24 (42.1)
10	2 (3.5)
PTEN status	
Loss	35 (61.4)
Presence	21 (36.8)
Missing	1 (1.8)

Abbreviations: PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; SD, standard deviation.

Table 2

Correlation Between PTEN and Other Clinical and Immunohistochemical Variables

	No. of Patients (%)		
Variable	**Loss of PTEN, n = 35**	**Presence of PTEN, n = 21**	***P***
Preoperative PSA, ng/mL			
Mean±SD	10.4±7.4	20.7±19.6	.015[a]
Median [range]	7.6 [2.2-32.2]	13.9 [1.8-83.1]	
Recurrence risk score			
Mean±SD	4.1±0.6	4.2±0.7	.837[a]
Median [range]	4.1 [2.9-5.4]	4.1(3.3-5.4)	
Lymph node involvement			
Positive	14 (40)	8 (38.1)	.999[b]
Negative	21 (60)	13 (61.9)	
Clinical tumor classification			
T1	11 (31.5)	13 (61.9)	.094[b]
T2	20 (57.1)	6 (28.6)	
T3	4 (11.4)	2 (9.5)	
Seminal vesicle invasion			
Positive	23 (65.7)	11 (52.4)	.401[b]
Negative	12 (34.3)	10 (47.6)	
Surgical margin status			
Positive	20 (57.1)	16 (76.2)	.249[b]
Negative	15 (42.9)	5 (23.8)	
Pathologic Gleason sum			
7	15 (42.9)	9 (42.9)	.999[b]
8-10	20 (57.1)	12 (57.1)	
S6			
Median [range][c]	0.99 [–0.79, 1.83]	0.86 [–0.99, 1.68]	.407[d]
mTOR			
Median [range][c]	1.08 [–1.40, 1.83]	0.98 [–0.67, 1.68]	.397[d]
Akt			
Median [range][c]	1.01 [–0.97, 1.79]	0.79 [0.07-1.80]	.987[d]
MYC			
Median [range][c]	3.57 [2.17-4.07]	3.62 [–0.50, 4.11]	.906[d]
Ki67			
Median [range][c]	2.09 [0.64-7.52]	2.62 [0.33-18.51]	.064[d]
p53			
Presence	26 (74.3)	10 (47.6)	.082[d]

	No. of Patients (%)		
Variable	**Loss of PTEN, n = 35**	**Presence of PTEN, n = 21**	***P***
Absence	9 (25.7)	11 (52.4)	
ERG			
Presence	22 (62.9)	8 (38.1)	.099[d]
Absence	13 (37.1)	13 (61.9)	

Abbreviations: ERG, v-ets erythroblastosis virus E26 oncogene homolog; Ki67, antigen KI-67; mTOR, mammalian target of rapamycin; p53, tumor protein p53; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; S6, S6 ribosomal protein; SD, standard deviation.

[a] This *P* value was obtained using a nonparametric Wilcoxon rank-sum test.

[b] This *P* value was obtained using the Fisher exact test.

[c] For this marker, the area score was calculated as the number of positively stained pixels within nuclei (brown mask) divided by the total nuclear area (brown plus blue mask). A composite “intensity-weighted area ratio” score also was obtained by combining the area score with the median intensity of positive pixels (for details, see Protein Marker Analysis).

[d] This *P* value was obtained using univariate logistic regression.

Table 3

Association of Clinical and Immunohistochemical Variables With Progression-Free Survival Based on Cox Proportional Hazards Regression Models

	Univariate Analysis		Multivariable Analysis[a]	
Variable	**HR (95% CI)**	***P***	**Adjusted HR (95% CI)**	**Adjusted *P***
Preoperative PSA[b]				
Continuous variable	1.01 (0.99-1.04)	.213	0.98 (0.95-1.01)	.115
Seminal vesicle invasion				
Positive vs negative[c]	2.18 (1.11-4.29)	.024	1.43 (0.65-3.14)	.378
Surgical margin status				
Positive vs negative[c]	1.03 (0.55-1.94)	.931	1.18 (0.34-4.12)	.799
Lymph node involvement				
Positive vs negative[c]	1.19 (0.64-2.22)	.585	2.45 (0.77-7.83)	.130
Pathologic Gleason sum[c]				
9-10 vs 7	2.18 (1.11-4.29)	.023	1.74 (0.64-4.75)	.277
8 vs 7	1.65 (0.63-4.31)	.305	1.61 (0.46-5.72)	.460
9-10 vs 8	1.32 (0.53-3.28)	.547	1.04 (0.30-3.60)	.946
ERG				
Presence vs absence[c]	0.86 (0.47-1.58)	.631	1.52 (0.66-3.51)	.330
Akt[b]				
Continuous variable	1.16 (0.78-1.74)	.460	1.22 (0.75-2.01)	.426
S6[b]				
Continuous variable	1.12 (0.76-1.65)	.574	–	–
mTOR[b]				
Continuous variable	1.14 (0.76-1.70)	.519	–	–
MYC[b]				
Continuous variable	0.63 (0.41-0.97)	.036	0.28 (0.13-0.61)	.001
Ki67[b]				
Continuous variable	1.26 (1.14-1.40)	< .0001	1.43 (1.21-1.68)	< .0001
p53				
Presence vs absence[c]	1.21 (0.62-2.33)	.578	0.61 (0.27-1.40)	.244
PTEN				
Loss vs presence[c]	1.82 (1.08-3.66)	.031	2.93 (1.08-7.92)	.035

Abbreviations: CI, confidence interval; ERG, v-ets erythroblastosis virus E26 oncogene homolog; HR, hazard ratio; Ki67, antigen KI-67; mTOR, mammalian target of rapamycin; p53, tumor protein p53; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; S6, S6 ribosomal protein.

[a] The multivariable analysis excluded S6 and mTOR to avoid multicollinearity.

[b] Continuous variable.

[c] Categorical variable.

Table 4

Variables Retained in the Final Multivariable Cox Regression Model

Marker	Adjusted HR (95% CI)[a]	Chi-Square Statistic	*P*[b]
Ki67	1.34 (1.20-1.50)	26.4	< .0001
PTEN loss vs presence	3.26 (1.42-7.47)	7.79	.005
MYC	0.52 (0.29-0.93)	4.90	.027
Global test of the full marker panel[c]	–	31.1	< .0001

Abbreviations: CI, confidence interval; HR, hazard ratio; Ki67, antigen KI-67; PTEN, phosphatase and tensin homolog.

[a] HRs were adjusted for the clinical covariates (seminal vesicle invasion, surgical margin status, lymph node involvement, and pathologic Gleason sum).

[b] A significant *P* value suggests an improvement in the model fit over the model that contained clinical covariates only.

[c] Based on the likelihood-ratio test for the model that included clinical covariates only compared with the model that included clinical covariates plus the immunohistochemical marker panel (comprising Ki67, PTEN, and MYC).

Table 5

Nested Prognostic Model for Progression-Free Survival Examining the Effect of Multiple Immunohistochemical Covariates Added to Clinical Covariates

Model[a]	Chi-Square Statistic	*P*[b]
Clinical covariates only (reference)[c]	–	–
Clinical covariates+PTEN	10.23	.001
Clinical covariates+Ki67	10.47	.001
Clinical covariates+PTEN+Ki67	13.31	.0003
Clinical covariates+PTEN+Ki67+MYC	4.09	.043
Clinical covariates+PTEN+Ki67+MYC+p53	2.07	.150
Clinical covariates+PTEN+Ki67+MYC+p53+Akt	0.77	.379
Clinical covariates+PTEN+Ki67+MYC+p53+Akt+ERG	0.63	.428

Abbreviations: ERG, v-ets erythroblastosis virus E26 oncogene homolog; Ki67, antigen KI-67; p53, tumor protein p53; PTEN, phosphatase and tensin homolog.

[a] Each model was compared with the preceding model to determine whether there was evidence of improvement in the fit of the model based on the likelihood-ratio test that had a chi-square distribution with 1 degree of freedom.

[b] A significant *P* value suggests an improvement in the fit of the model compared with the preceding model.

[c] Clinical covariates included seminal vesicle invasion, surgical margin status, lymph node involvement, and pathologic Gleason sum.