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ERG Status at the Margin is Associated with Biochemical Recurrence after Radical Prostatectomy with Positive Surgical Margins

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Abstract

Positive surgical margins at radical prostatectomy are associated with an increased risk of biochemical recurrence (BCR). However, there is considerable variability in outcomes, suggesting that molecular biomarkers -- when assessed specifically at the margin tumor tissue -- may be useful to stratify prognosis in this group. We used a case-cohort design for the outcome of BCR, selecting 215 patients from a cohort of 813 prostatectomy patients treated at Johns Hopkins from 2008–2017 with positive margins and available clinical data. Tissue microarrays (TMA) were created from the tumor adjacent to the positive margin and stained for PTEN, ERG, and Ki-67. Cases were scored dichotomously (PTEN, ERG) or by Ki-67 staining index, using previously validated protocols. The analysis employed Cox proportional hazards models weighted for case-cohort design. Overall, 20% (37/185) of evaluable cases had PTEN loss, 38% (71/185) had ERG expression, and median Ki-67 expression was 0.42%. In multivariable analysis adjusting for CAPRA-S score, adjuvant radiation, as well as Grade Group at the positive margin, ERG-positive tumors were associated with a higher risk of BCR compared to those that were ERG-negative (HR=2.4; 95% CI: 1.2–4.9; p=0.012), regardless of PTEN status at the margin, and adding ERG to clinical-pathologic variables increased the concordance index from 0.827 to 0.847. PTEN loss was associated with increased risk of BCR on univariate analysis (HR=3.19; 95% CI: 1.72–5.92; p=0.0002), but this association did not remain after adjusting for clinical-pathologic variables (HR=1.06; 95% CI: 0.49–2.29; p=0.890). Thus, in the setting of prostate tumors with positive surgical margins after prostatectomy, ERG-positive tumors with or without PTEN loss at the

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Disclosures

Ethics and Concent to Participate: This study was approved by the Johns Hopkins IRB under a waiver of consent.

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All other authors report no conflict.

positive margin are associated with a significantly higher risk of BCR after adjusting for clinical-pathologic variables. If validated, ERG status may be helpful in decision-making surrounding adjuvant therapy after prostatectomy.

Keywords

Prostate cancer; radical prostatectomy; positive surgical margins; PTEN; ERG; Ki-67

Introduction:

A positive surgical margin at radical prostatectomy (RP) indicates incomplete excision of the prostate tumor and is a widely accepted marker of unfavorable prognosis [1–3]. Despite an established increased risk of disease progression in patients with a positive surgical margin, many are cured with surgery alone. Given these heterogeneous outcomes, the significance of a positive surgical margin for triggering treatment with adjuvant radiation and hormonal therapies remains controversial. Clinicians typically evaluate a constellation of clinical pathologic parameters, such as tumor grade and presence or absence of extraprostatic extension, before recommending adjuvant therapy. However, treatment decision-making paradigms remain variable between different providers.

Part of the observed heterogeneity in outcomes following a radical prostatectomy with positive surgical margins is likely due to the fact that not all margins are created equal. Since prostate cancer is frequently multiclonal and multifocal, the tumor at the positive surgical margin may be representative of the dominant nodule or it may represent a different clone. Previous studies have shown that the Grade Group at the margin, as well as the length and location of the margin are associated with risk of BCR after RP [4–11]. Accordingly, cases where the Grade Group at the margin is lower than the dominant nodule may have less impact on outcomes [4–11]. Due to the multiclonality of primary prostate cancer, molecular biomarkers may help to further stratify risk associated with a positive surgical margin. Though RNA-based molecular biomarkers - such as the Decipher assay - have been developed to help predict which surgical patients may benefit from adjuvant radiation [12], due to their high tissue requirement for adequate RNA isolation, to our knowledge these assays have not been tested specifically on the tumor at the positive surgical margin. In this context, an *in situ* molecular biomarker test might be expected to be most useful as it can be performed and interpreted in a small area of tissue directly adjacent to the surgical margin.

Our group and others have previously developed and validated *in situ* surrogate biomarkers for underlying molecular changes common in prostate cancer, including *ERG* gene rearrangement [13], *PTEN* deletion [14], and Ki-67 labeling index [15]. When performed on the dominant tumor nodule, many of these markers are associated with adverse outcomes in surgical cohorts. Here, we tested whether one or several of these markers, when performed on tumor tissue directly adjacent to the positive margin, might predict for risk of biochemical recurrence (BCR) among patients with positive surgical margin.

Materials and Methods:

Patients and samples:

After institutional review approval, the Johns Hopkins Pathology database was queried for cases with positive margins at RP. The study used a case-cohort design, nested in the cohort of prostatectomy (RP) patients treated at Johns Hopkins Hospital from 2008–2017, with positive surgical margins based on tumor cells in contact with the inked surface, known status with regard to biochemical recurrence (BCR), and with complete clinical-pathologic variables. At our institution, men were followed up with PSA assays every 3 months after surgery for the first year, semiannually for the second year, and annually thereafter. A detectable serum PSA level of at least 0.2 ng/mL was evidence of biochemical recurrence. There were 813 patients in this cohort, including 224 BCR cases. A random sample was drawn from this cohort without regard to BCR status; this comprised the *subcohort*, representative of a control group. A random sample was also drawn from the remaining BCR cases that were not included in the subcohort; combined with the BCR patients in the subcohort this comprised the case group.

Different surgeons performed the RP using standardized procedures. The prostatic tissue for all RPs was fixed with formalin overnight, and the margins were entirely inked at grossing. The entire prostate was submitted for pathologic evaluation with rare exceptions. Two pathologists confirmed the surgical margin was positive (DCS, TLL) in all cases and one pathologist (DCS) annotated tumor areas adjacent to the positive margin. The annotated sites were used to construct tissue microarrays (TMA) with quadruplicate 0.6 mm diameter core sampling from tissue within 3 mm of the positive surgical margin using a manual tissue arrayer (Figure 1).

Immunohistochemistry (IHC):

IHC was performed at Johns Hopkins CLIA-accredited laboratory using genetically validated and previously described protocols on the Ventana Benchmark immunostaining system [14]. The following markers were utilized: PTEN (Cell Signaling Technology, Clone D4.3 XP), ERG-9FY BIOCARE (BioCare, Catalog # CM421C), and KI67 ULTRA (Ventana, Catalog # 790–4286).

Image analyses:

Three trained urological pathologists (TL, DCS, and AM) blindly scored the ERG and PTEN assays using a validated and previously described scoring system. In brief, ERG was scored as positive if any sampled tumor gland showed nuclear ERG expression and negative if no sampled tumor gland showed ERG expression [13]. PTEN was scored as lost if some or all tumor sampled in the cores showed loss [14].

The Ki-67-stained TMAs were scanned at 40X magnification on Nano Zoomer Digital Pathology scanner (Hamamatsu), and the images were imported to QuPath software. A trained pathologist (AM) identified and annotated areas of interest (tumor), excluding staining artifacts and benign prostate glands from the annotations. The software identified the Ki-67-positive and –negative nuclei within the region of interest and the Ki-67 labeling

index was calculated as the ratio of Ki-67 positive cells divided by the total number of tumor cells in the area of interest.

Statistical analysis:

Wilcoxon rank sum test was used to compare continuous variables, and chi-square test or Fisher's exact test used for categorical variables. The case-cohort design permits an unbiased assessment of failure time outcomes with efficiency approaching that of the full cohort [16]. BCR risk associated with PTEN and ERG, adjusted for CAPRA-S score and other potential confounding factors, was evaluated in multivariable Cox proportional hazards models, with Lin-Ying weighting and robust sandwich variance estimator for the case-cohort design [17,18]. Improvement in multivariable model fit for addition of PTEN, ERG, and Ki-67 was assessed with the change in robust score statistic [19,20], and concordance index weighted for case-cohort design [21]. Statistical significance for all analyses was set at $p<0.05$. All analyses were performed with SAS v9.4 (SAS Institute, Cary, NC) and R v4.0.5 (http://cran.r-project.org/).

Results:

Out of 215 patients originally included in the tissue microarray, 89% (192/215) had evaluable tumor tissue present on the tissue microarray for PTEN status determination and 92% (197/215) had evaluable ERG status. The vast majority of those that were inevaluable had no tumor tissue sampled on the tissue microarray (91% or 21/23 for PTEN and 89% or 16/18 for ERG), while the remainder had insufficient internal control immunostaining for interpretation. Of the evaluable cases, 185 had complete ERG, PTEN and clinical-pathologic information available. Of these, there were 154 patients selected into the subcohort, including 29 BCR cases, and there were 31 BCR cases sampled outside of the subcohort, for a total of 60 BCR cases, and effective sample size of 214 (subcohort cases are counted once as controls prior to their recurrence time, and once as cases). The median follow-up in the subcohort was 2 years (IQR: 1–3).

Clinical-Pathologic and Molecular Features of the Cohort:

The clinical-pathologic characteristics of the positive surgical margin case-cohort are presented in Table 1, stratified by BCR status. Patients with BCR had significantly higher pre-operative PSA, Gleason Grade Group, pathologic stage at RP and CAPRA-S scores (which includes preoperative prostate-specific antigen levels, RP Grade Group, surgical margins status, extraprostatic extension, seminal vesicle invasion, and lymph node invasion) compared to those without ($p<0.0001$). Median age and year of surgery were similar between those with and without BCR, though median length of follow-up was significantly higher for those with BCR than for those without ($p=0.003$). Pathologic characteristics of the tumor present at the surgical margin were also associated with BCR status. Higher Grade Group tumor present at the positive surgical margin ($p<0.0001$) and extraprostatic extension at the surgical margin ($p=0.001$) were both more common in patients with BCR; positive margin length did not differ significantly ($p=0.159$). Grade Group at the positive margin was the same as the primary tumor grade group for 45% of cases; in the majority of cases the Grade Group at the margin was less than in the primary tumor (Supplemental Table 1). Only 17 patients in the case-cohort received adjuvant radiation therapy, and adjuvant radiation was

more frequent among cases without BCR, although this difference did not reach statistical significance. PTEN loss was significantly associated with BCR, with 38% of cases with BCR showing PTEN loss compared to 11% of cases without BCR (p<0.0001). Similarly, ERG expression was more common among patients with BCR, with 55% of cases with BCR expressing ERG compared to 30% without BCR. Ki-67 labeling index was also higher in the BCR group compared to the cases without BCR (p=0.04).

Univariable and multivariable proportional hazards models:

In univariable analyses, preoperative PSA, Grade Group at RP, pathologic stage, and CAPRA-S score were all significantly associated with BCR in men with a positive surgical margin (p<0.0001, Table 2). A positive margin in an area of extraprostatic extension (p=0.0007), and Grade Group at the margin (p<0.0001) were significantly associated with BCR, the latter showing a nearly monotonic increase with grade. Among molecular parameters, ERG positivity (HR: 1.86; 95% CI: 1.03–3.34; p=0.038), PTEN loss (HR: 3.19; 95% CI: 1.72–5.92; p=0.0002), and Ki-67 (HR: 1.59; 95% CI: 1.23–2.06; p=0.0004) at the positive margin were each significantly associated with increased risk of BCR. Examining the four-category combined PTEN/ERG status at the positive margin, tumors with PTEN loss and ERG positivity at the margin, were associated with significantly higher BCR risk compared to the reference PTEN intact/ERG-negative cases (HR: 3.86; 95% CI: 1.86–8.01; p=0.002), while risk was not significantly increased among those with PTEN loss/ERG negative, and PTEN intact/ERG positive (Table 2).

In multivariable analysis adjusting for the clinical model consisting of CAPRA-S score, Grade Group at the positive margin, and receipt of adjuvant radiation, ERG positivity, but not PTEN loss was significantly associated with BCR, HR: 2.44 (95% CI: 1.22–4.88; p=0.012), and HR: 1.06 (95% CI: 0.49, 2.29; p=0.890), respectively. The concordance index for the model was 0.847 (Table 3A), compared to 0.827 for the clinical model (CAPRA-S, Grade Group at margin and adjuvant radiation therapy) alone. The model was not improved when combined PTEN/ERG status replaced the two biomarkers individually. Similar hazard ratios were observed for ERG positivity combined with PTEN intact, HR: 2.57 (95% CI: 1.20–5.54), and combined with PTEN loss, HR: 2.54 (95% CI: 1.22–5.27) (p=0.028). The concordance index remained unchanged at 0.847 (Table 3B), and the robust score statistic nonsignificantly increased from 40.23 to 40.26 (p=0.862), indicating no improvement over the model with ERG and PTEN individually. Ki-67 at the positive margin was no longer significant after adjusting for the clinical factors in a multivariable model (data not shown).

Discussion:

A positive surgical margin creates anxiety for patients and surgeons alike, yet the clinical significance of this finding remains controversial. In this context, biomarkers to further stratify risk may be useful. Positive margins are more common in patients with higher grade and higher stage disease [22] and though associated with risk of BCR [1,23], positive margins are inconsistently associated with prostate cancer mortality in adjusted analyses [1–3]. Given the multifocality and multiclonality of many primary prostate tumors, more recent studies have probed whether some of this variability in outcomes may be due to tumor-specific

pathologic features at the surgical margin. In support of this hypothesis, positive margin length [4,24–26] and the Grade Group of the tumor at the positive margin [4–8] have both been shown to be associated with risk of BCR, though association of these features with prostate cancer specific mortality has only been assessed in a few studies [7].

Better characterization of the tumor left behind in the patient after surgery has potential utility in determining whether the patient could benefit from adjuvant therapy. Three randomized clinical trials have supported the utility of adjuvant radiation therapy (versus observation) for patients with high risk clinical features, which were defined to include extraprostatic extension, seminal vesicle invasion or positive surgical margins[27-29]. However, some trials analyzed cases with positive margins or extraprostatic extension as a single group and none of these trials examined the utility of further pathologic classification of the positive margin itself (margin length or Grade Group of margin) with respect to the magnitude of benefit from radiation therapy. In our own case-cohort of patients with positive surgical margins, only a small minority of patients received adjuvant therapy, however the protective benefit with respect to risk of BCR was significant in multivariable analyses, confirming previous studies.

Nonetheless, there is no question that adjuvant radiotherapy leads to over-treatment of some patients and significant morbidity, thus more refined selection of patients for adjuvant radiation therapy is critical. To avoid overtreatment, many clinicians have largely avoided recommending adjuvant therapy, favoring salvage radiotherapy at the time of BCR instead. This is evident in our own cohort, where only 9% of men with positive surgical margins – a group at high risk for an early pelvic recurrence -- received adjuvant radiotherapy. Because the adjuvant trials were conducted prior to the current era of routine post-operative PSA testing and salvage radiation therapy, contemporary trials have compared the efficacy of salvage versus adjuvant radiation therapy for progression-free survival, event-free survival or metastasis-free survival [30–32]. Strikingly, all three trials indicated no evidence of improvement in 5-year event-free survival for adjuvant therapy compared to early salvage radiotherapy, with significantly higher toxicity among those patients receiving adjuvant radiotherapy. However, because these trials enrolled patients with a variety of high risk features and were not limited to those with positive surgical margins, they do not allow for a more nuanced analysis of which subsets of men might potentially benefit more from earlier therapy with adjuvant radiation. Indeed, those at the highest risk for pelvic recurrence due to positive surgical margins, particularly in an area of an aggressive tumor, might derive additional benefit from early radiation therapy prior to evidence of BCR. Conversely, it is conceivable that those with a baseline low risk for pelvic recurrence might not benefit from early salvage radiation at the time of BCR.

Ultimately, molecular parameters may further refine which patients with positive surgical margins are at the highest risk of local recurrence. While some work has been done using RNA-based biomarkers in radical prostatectomy tissue to stratify patients who may benefit from adjuvant radiation [12], these studies have been performed on the dominant tumor nodule which may not be the tumor clone left behind when surgery is not curative. To our knowledge, the current study is one of the first to examine the molecular phenotype of tumor tissue specifically at the surgical margin. Using a case-cohort design, we demonstrate

that tumors with *ERG* gene rearrangements at the positive margin are at 2.4 times the risk of BCR compared to those lacking *ERG* rearrangement, independent of whether PTEN is intact or lost, and adjusted for other clinical-pathologic parameters including CAPRA-S score, Grade group at the positive margin, and use of adjuvant radiation. Although PTEN at the margin was significantly associated with BCR in univariate analyses, it was no longer significant once ERG and clinical variables were included in the model. Similarly, Ki-67 labeling index, assessed as a continuous variable, was significantly associated with BCR in univariate, but not multivariable analyses.

Though PTEN has been associated with adverse outcomes in large number of prior studies of surgically-treated prostate cancer patients [33], ERG has not been identified as a consistent risk factor for BCR in meta-analyses of similar cohorts [34]. Notably, however, none of these previous studies focused exclusively on patients with positive surgical margins. In the current study, it is notable that in the univariable analysis, cases with PTEN loss showed significantly increased hazard ratios for BCR, as expected, but this association did not remain after adjusting for clinical-pathologic variables. PTEN loss is enriched among prostate tumors with *ERG* rearrangements and our group and others have previously found that the relatively rare subset with PTEN loss in an ERG-negative background is at highest risk for metastasis, though this has not been consistent for the outcome of BCR [35,36]. In the current study, the hazard ratio for this group in the multivariable model for risk of BCR was a modest 1.2, with a confidence interval spanning 1.0. ERG-negative cases with PTEN loss (only 10 cases total in the cohort) exhibited the highest CAPRA-S scores, and were most strongly confounded by CAPRA-S in the multivariable model. This suggests that there is no increased risk of BCR for ERG-negative cases with PTEN loss among patients with positive surgical margins, after adjusting for other clinical-pathologic factors which likely confounded the univariable analysis. Given that ERG has not been reliably associated with adverse outcomes in other cohorts, it is surprising that only ERG (and not PTEN) status remained associated with risk of BCR in multivariable analyses in our current study. This may be due to the unusual study design, where all patients had positive surgical margins. Perhaps in the setting of local recurrence, ERG positivity (or some phenotype related to ERG positivity, such as androgen signaling) may predispose to earlier or more common PSA recurrence. Future studies examining other endpoints such as metastasis or death will be critical to better understand this finding.

Taken together, our data support the feasibility and potential utility of molecular testing of tumor tissue specifically at the positive surgical margin for further risk stratification. ERG positivity, but not PTEN loss, was associated with a significantly increased risk of BCR, even after adjusting for other known clinical-pathologic risk factors in patients with positive surgical margins. While Ki-67 has been associated with adverse outcomes in large studies of prostate cancer [15], technical differences between laboratories and difficulties establishing a reproducible cut-point for low and high risk tumors have prevented its widespread clinical use [33], and may be reflected in our current findings where Ki-67 was not independently prognostic. Given the ease and low expense of performing and interpreting dichotomously scored immunostains, ERG could potentially be employed clinically if validation studies support these results. There are some limitations of the current study, including its retrospective nature, the use of BCR rather than metastasis-free survival as an

outcome measure and the utilization of tissue microarrays which may not sample the full heterogeneity of marginal tumor tissue.Tumor tissue was sampled within 3 millimeters of the inked positive margin, though not directly including the ink due to difficulty of punching these areas of the block and surrounding cautery artifacts. However, to our knowledge, this is the first study to examine the utility of molecular biomarkers in a cohort of patients, all of whom had positive surgical margins, and the first to examine the molecular status at the margin tumor tissue specifically. Future work will examine whether other molecular assays, such as RNA-based tests [37], may add additional information beyond that gleaned from ERG. Ultimately, it will be essential to examine promising markers in prospective clinical trials to determine whether patients with positive surgical margins for molecularly aggressive tumors might benefit more from adjuvant versus salvage radiotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability:

The tissue microarray images, as well as clinical-pathologic and immunohistochemistry datasets analyzed for the current study are available from the corresponding author on reasonable request.

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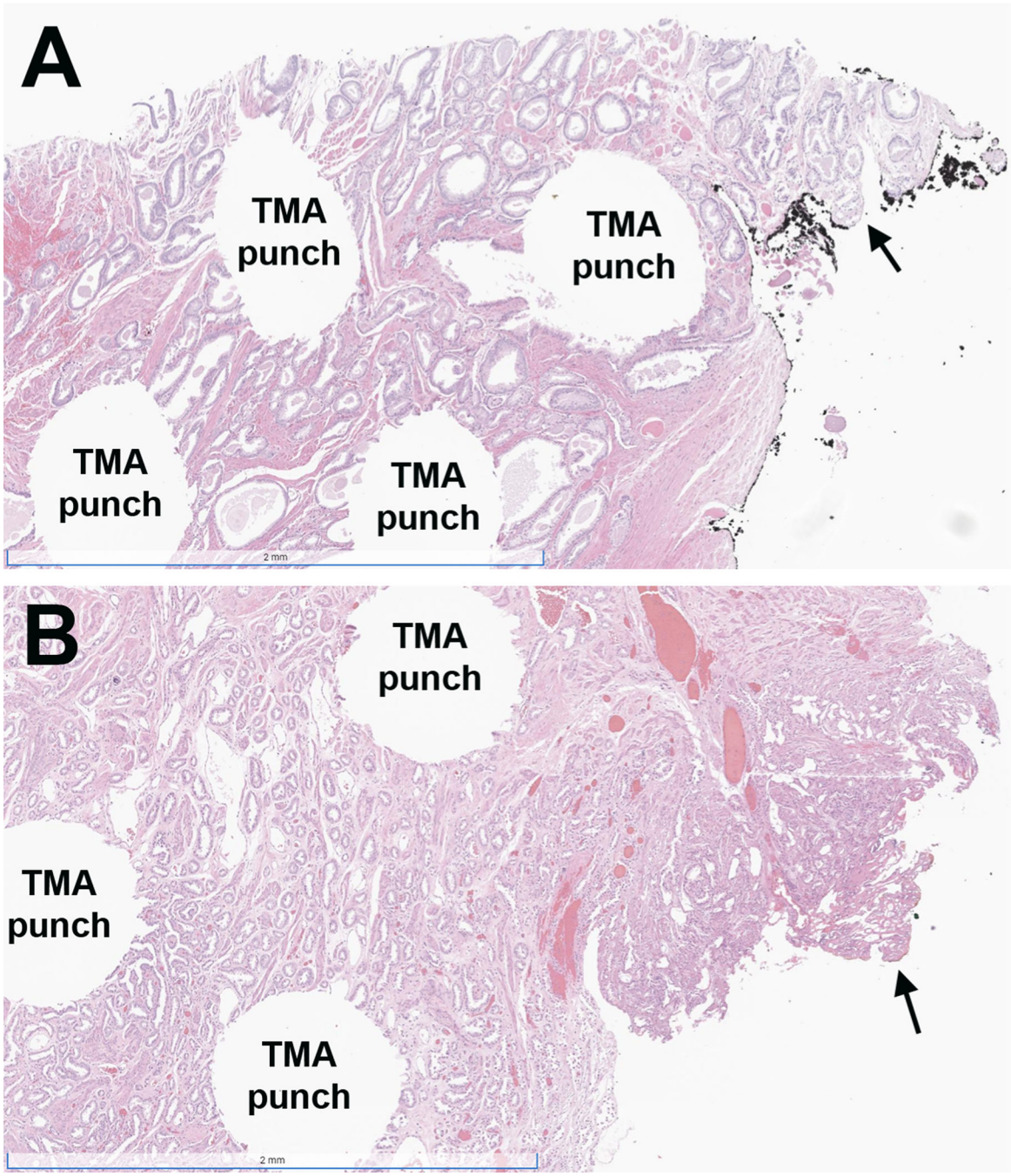

Figure 1: Hematoxylin and eosin (H&E) stained tissue sections of tumor blocks used for tissue microarray (TMA) construction after punching.

(A) TMA punches are indicated by circular holes and are within 3 millimeters of the inked margin which is positive for tumor (arrow). **(B)** TMA punches are indicated by circular holes and are within 3 millimeters of the cauterized and faintly inked margin which is positive for tumor (arrow). Scale = 2 mm.

Table 1.

Clinical-pathologic and molecular variables stratified by biochemical recurrence (BCR) status (n=185).

IQR, interquartile range; RP, prostatectomy; GS, Gleason score; IPI, intraprostatic incision; EPE, extraprostatic extension.

Variable	No BCR (N=125)	BCR (N=60)	p-value*
Median age, yrs, (IQR)	60 (54–65)	59 (54–63)	0.347
Year of RP, median (IQR)	2012 (2010–2014)	2011 (2009–2014)	0.366
Follow-up years, median (IQR)	2.0 (1.0–4.0)	3.0 (2.0–5.0)	0.003
Race, n (%) White African American Other	 100 (80.0) 17 (13.6) 8 (6.4)	 51 (85.0) 8 (13.3) 1 (1.7)	0.369
PSA ng/ml, median (IQR)	5.7 (4.7–8.0)	8.0 (5.1–13.0)	0.005
RP Grade Group, n (%) 1 (GS 6) 2 (GS 3+4) 3 (GS 4+3) 4/5 (GS 8–10)	 32 (25.6) 58 (46.4) 15 (12.0) 20 (16.0)	 2 (3.3) 20 (33.3) 20 (33.3) 18 (30.0)	<0.0001
Pathologic stage, n (%) T2N0 T3aN0 T3bN0 N1	 60 (48.0) 57 (45.6) 6 (4.8) 2 (1.6)	 9 (15.0) 30 (50.0) 17 (28.3) 4 (6.7)	<0.0001
CAPRA-S, median (IQR)	4.0 (3.0–5.0)	6.5 (5.0–8.5)	<0.0001
Grade Group at positive margin, n (%) 1 (GS 6) 2 (GS 3+4) 3 (GS 4+3) 4 (GS 8) 5 (GS 9–10)	 83 (66.4) 25 (20.0) 8 (6.4) 3 (2.4) 6 (4.8)	 18 (30.0) 16 (26.7) 14 (23.3) 7 (11.7) 5 (8.3)	<0.0001
Length of positive margin, mm (IQR)) (n=180)	2 (1–4)	3 (1–6)	0.159
Margin type, n (%) IPI EPE	 84 (67.2) 41 (32.8)	 25 (41.7) 35 (58.3)	0.001
Adjuvant radiation, n (%)	15 (12.0)	2 (3.3)	0.056
PTEN at positive margin, n (%) Intact Loss	 111 (88.8) 14 (11.2)	 37 (61.7) 23 (38.3)	<0.0001
ERG at positive margin, n (%) Negative Positive	 87 (69.6) 38 (30.4)	 27 (45.0) 33 (55.0)	0.001
Ki-67 %, median (IQR)	0.39 (0.14–0.69)	0.58 (0.26–1.12)	0.039

*Wilcoxon rank sum test used for continuous variables, chi-square test or Fisher's exact test used for categorical variables.

Table 2:

Univariable analyses of association of BCR with clinical-pathologic and molecular variables.

HR, hazard ratio; CI, confidence interval; RP, prostatectomy; GS, Gleason score; IPI, intraprostatic incision; EPE, extraprostatic extension

Variable	HR (95% CI	p-value
Age (per year)	1.00 (0.95, 1.04)	0.880
Year of prostatectomy	1.15 (1.00, 1.33)	0.054
PSA (per 1 ng/ml)	1.07 (1.04, 1.10)	<0.0001
RP Grade Group, n (%)		<0.0001
1 (GS 6)	1.0 (reference)	
2 (GS 3+4)	4.82 (1.09, 21.20)	
3 (GS 4+3)	18.95 (4.17, 86.18)	
4 (GS 8)	13.04 (2.15, 78.98)	
5 (GS 9–10)	15.59 (3.14, 77.41)	
Pathologic T stage, n (%)		<0.0001
T2N0	1.0 (reference)	
T3aN0	2.95 (1.32, 6.62)	
T3bN0	22.50 (9.26, 54.64)	
N1	13.09 (2.82, 60.90)	
CAPRA-S (per unit)	1.4 (1.24, 1.67)	<0.0001
Grade Group at positive margin		
1 (GS 6)	1.0 (reference)	<0.0001
2 (GS 3+4)	2.99 (1.37, 6.52)	
3 (GS 4+3)	6.17 (2.55, 14.93)	
4 (GS 8)	11.52 (4.01, 33.05)	
5 (GS 9–10)	4.09 (1.34, 12.53)	
Length of positive margin (per mm)	1.09 (0.99, 1.20)	0.072
Positive margin type, EPE vs. IPI	2.82 (1.55, 5.12)	0.0007
Adjuvant radiation (yes vs. no)	0.20 (0.05, 0.82)	0.026
PTEN at margin, loss vs. intact	3.19 (1.72, 5.92)	0.0002
ERG at margin, positive vs. negative	1.86 (1.03, 3.34)	0.038
PTEN/ERG status at positive margin		0.002
intact/negative	1.0 (reference)	
intact/positive	1.36 (0.64, 2.89)	
loss/negative	2.81 (0.92, 8.60)	

Variable	HR (95% CI	p-value
loss/positive	3.86 (1.86, 8.01)	
Ki67, % (per 1%)	1.59 (1.23, 2.06)	0.0004

Table 3:

Multivariable analysis of association of biochemical recurrence with clinical pathologic and molecular variables (n=185).

Variable	HR (95% CI)	p-value
A. Model with PTEN and ERG individually		
CAPRA-S (per unit)	1.40 (1.22, 1.60)	<0.0001
Grade Group at positive margin		
1 (GS 6)	1.0 (reference)	0.007
2 (GS 3+4)	2.52 (1.04, 6.10)	
3 (GS 4+3)	4.51 (1.81, 11.24)	
4 (GS 8)	3.38 (0.93, 12.23)	
5 (GS 9–10)	1.03 (0.22, 4.89)	
Adjuvant radiation (yes vs. no)	0.14 (0.04, 0.49)	0.002
PTEN loss vs. intact	1.06 (0.49, 2.29)	0.890
ERG positive vs. negative	2.44 (1.22, 4.88)	0.012
Concordance index	0.847	
B. Model with combined PTEN/ERG status		
CAPRA-S (per unit)	1.39 (1.21, 1.60)	<0.0001
Grade Group at positive margin		
1 (GS 6)	1.0 (reference)	0.016
2 (GS 3+4)	2.56 (1.06, 6.19)	
3 (GS 4+3)	4.72 (1.82, 12.26)	
4 (GS 8)	3.51 (0.94, 13.17)	
5 (GS 9–10)	1.00 (0.19, 5.16)	
Adjuvant radiation (yes vs. no)	0.14 (0.04, 0.49)	0.002
PTEN/ERG status at positive margin		0.028
intact/negative	1.0 (reference)	
intact/positive	2.57 (1.20, 5.54)	
loss/negative	1.22 (0.24, 6.16)	
loss/positive	2.54 (1.22, 5.27)	
Concordance index	0.847	

Abbreviations: HR, hazard ratio; CI, confidence interval; GS, Gleason score