



Prostate Cancer Gene Expression Adds Prognostic Information beyond Criteria such as Stage, Gleason Score, PSA, AUA Criteria, and CAPRA Score

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Disclosures

- SM Falzarano - research funding, Genomic Health, Inc.
- C Magi-Galluzzi - research funding, Genomic Health, Inc.
- T Maddala - employee, Genomic Health, Inc.
- D Cherbavaz - employee, Genomic Health, Inc.
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- C Millward - employee, Genomic Health, Inc.
- EA Klein - research funding, Genomic Health, Inc.

Rationale for Developing a Genomic Assay for Prostate Cancer

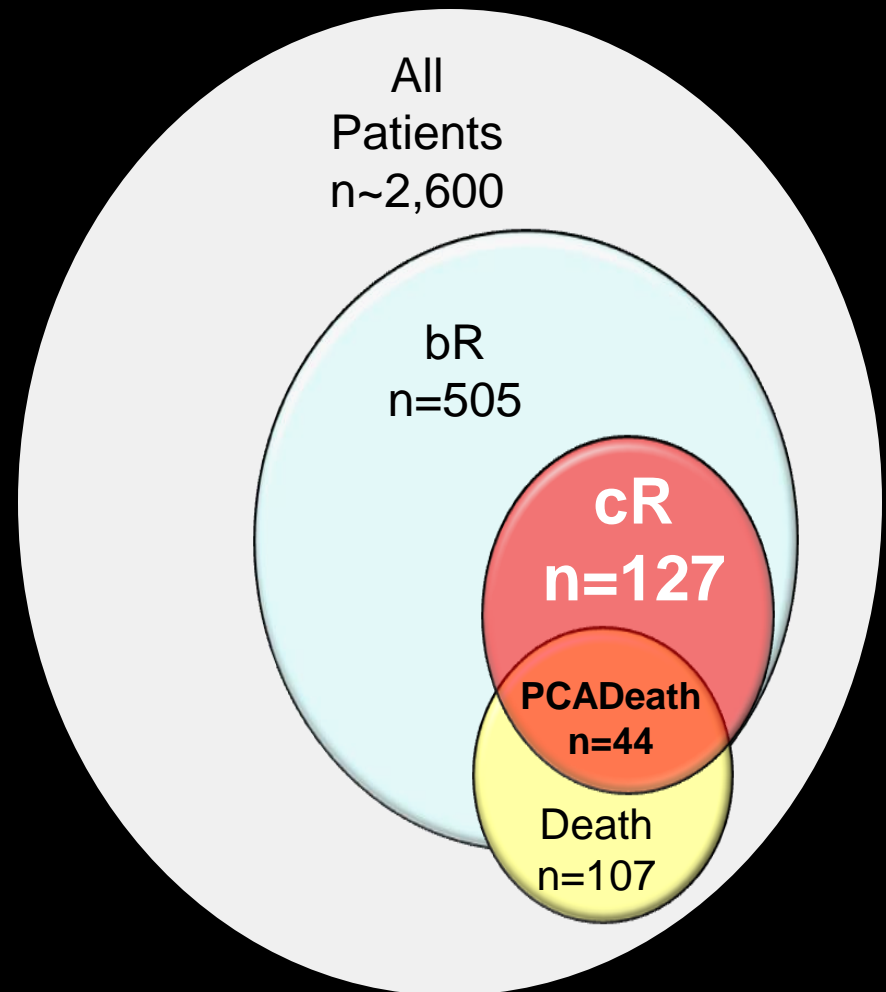
- Prognosis of localized prostate cancer (PCA) is currently based on clinical and pathologic features
- Current schema do not fully account for individual tumor biology and provide only a general estimate of aggressiveness
- A genomic test that distinguishes between clinically indolent and aggressive disease could help decide between active surveillance and immediate treatment

Objectives

- As a first step in the development of a biopsy-based genomic assay for localized PCA, we conducted a large study in radical prostatectomy (RP) tissue, with the following objectives:
 - Identify genes whose quantitative expression predicts clinical recurrence (cR) after RP
 - Determine if genes provide additional prognostic information beyond clinical and pathological covariates
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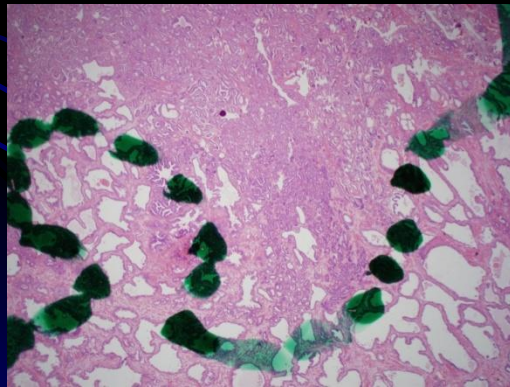
Study Design

- Patients:
 - Men with clinically localized PCA treated with RP at Cleveland Clinic between 1987-2004
 - 5.8 years median follow up
- Design:
 - Stratified cohort sampling method
 - Total sample size=501
 - 1:3 ratio cR to non-cR
- Stratification:
 - Clinical stage (T1 vs. T2)
 - Year of surgery (<1993 vs. ≥1993)
 - Gleason score (≤7 vs. >7)
- Primary endpoint:
 - Clinical Recurrence (biopsy proven local recurrence, or distant metastasis)



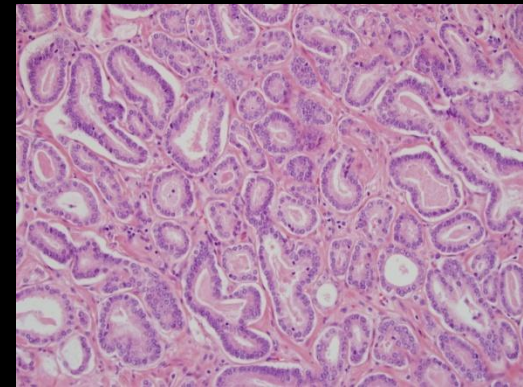
Laboratory Methodology

- Surgical pathology central review was conducted at Cleveland Clinic
- Six 10 μm unstained sections, as well as a beginning and ending 5 μm H&E slide, were obtained from selected paraffin-embedded tissue blocks
- Manually-dissected tissue enriched for tumor from the primary (PGP) and highest (HGP) Gleason patterns was submitted for qRT-PCR gene expression analysis (data presented for PGP only)
- Gleason score (GS) was assessed according to the 2005 International Society of Urological Pathology Consensus Conference criteria*



PCA specimen marked for manual microdissection, low magnification (20X)

Manual macrodissection



PCA sample enriched for tumor assayed, high magnification (200X), GS 3+3=6

Analysis Methodology (primary Gleason pattern only)

- Identify genes associated with outcome (cR)
 - Cox Proportional Hazards regression evaluating the association between gene expression and outcome
 - Unadjusted univariate analyses
 - Adjusted for clinical and pathological covariates (pathology T stage, GS, PSA, AUA risk group, CAPRA risk group)
 - Controlling for a False Discovery Rate of 10%*
- Identify co-expressed gene modules/groups
 - Cluster Analysis

Evaluatable Patients and Genes

501 sampled patients

Excluded 61 with
insufficient tumor

440 patients with RT-PCR
gene expression data

Excluded 9 with gene outlier
profile or clinically ineligible

431 patients evaluatable for
clinical/pathology/lab

732 test genes
normalized RT-PCR data

Excluded 5 due to poor
performance

**727 evaluatable test
genes**

Patient Characteristics (N=431)

Characteristic	Values	%*
Age (mean=61, range: 42-77)	≤70	93
	>70	7
Race	Caucasian	83
	Black/Afro-Caribbean	12
	Other	5
Baseline PSA	≤4	14
	>4-10	68
	>10-20	13
	>20	5
Clinical T Stage	cT1	66
	cT2	34
AUA risk group	Low	56
	Intermediate	32
	High	12
CAPRA risk group**	0-1	39
	2	32
	3	15
	4	6
	5	6
	6+	2
Pathology T Stage	pT2	50
	pT3	50
Surgical Gleason score	≤6	25
	7	62
	≥8	13

* Weighted percent functionally represents the entire cohort of 2,600 patients

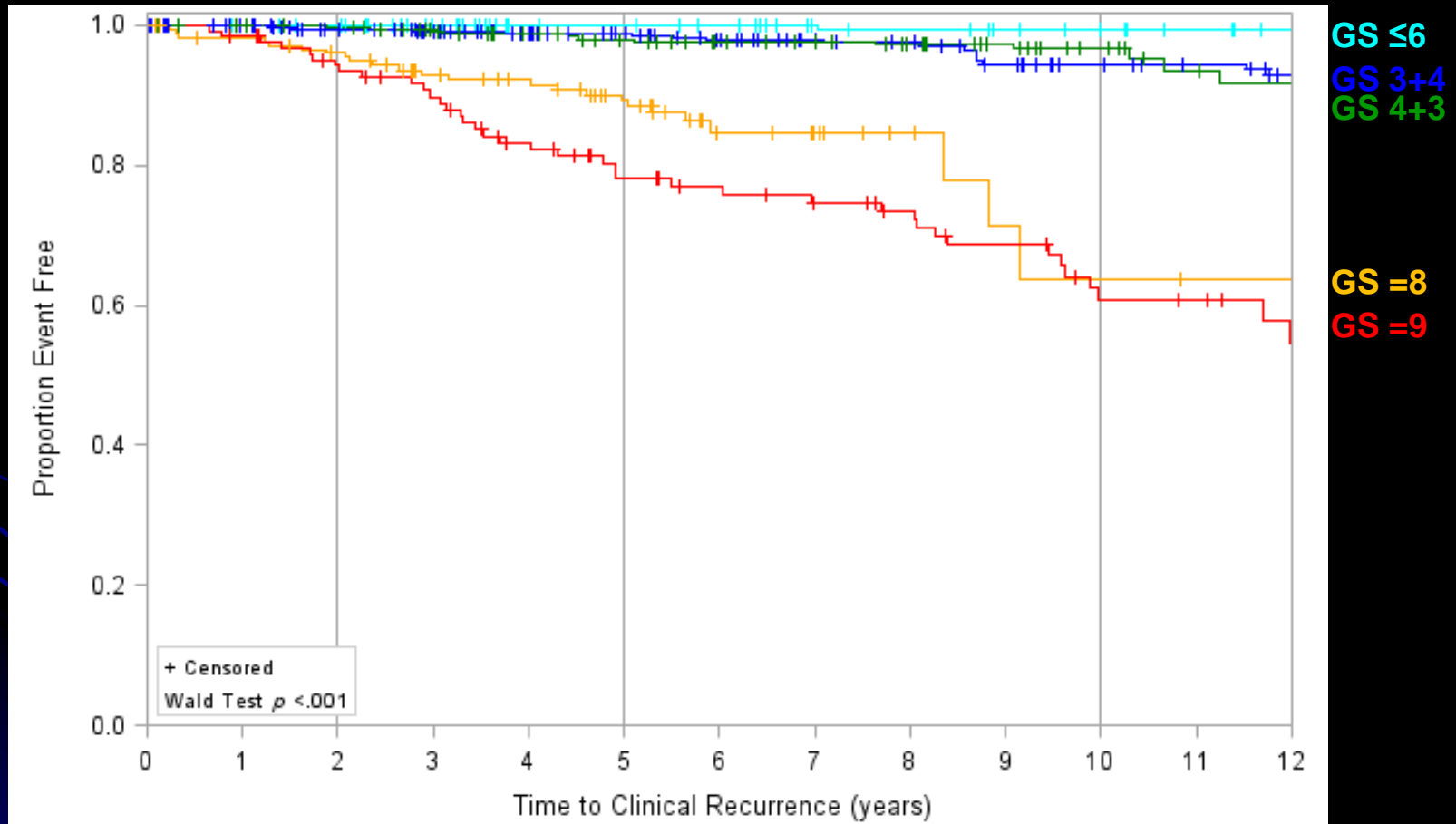
** CAPRA risk score calculated using the Johns Hopkins modification (Zhao KH, Hernandez DJ, Han M, *et al.* Urology. 2008;72:396-400)

Clinical and Pathological Covariates are Significantly Associated with Clinical Recurrence-Free Interval (cRFI)

Variable	HR	95% CI	P-value
Baseline PSA			0.020
≤4 vs. >20	0.25	(0.09,0.74)	0.012
(>4 and ≤10) vs. >20	0.41	(0.20,0.81)	0.010
(>10 and ≤20) vs. >20	0.63	(0.28,1.43)	0.272
Surgical Gleason score			<0.001
≤6 vs. ≥8	0.08	(0.04,0.15)	<0.001
7 vs. ≥8	0.16	(0.09,0.26)	<0.001
Pathology T Stage (pT3 vs. pT2)	2.10	(1.08,4.08)	0.028
Surgery year (>1993 vs. ≤1993)	0.27	(0.17,0.44)	<0.001


In univariate analysis, age at surgery and clinical stage were significantly associated with clinical recurrence-free interval (cRFI), but those variables were not significant in multivariate analyses

Surgical Gleason Score (GS) is Strongly Associated with cRFI



Number of Genes Significantly Associated with cRFI after Clinical and Pathological Covariate Adjustment

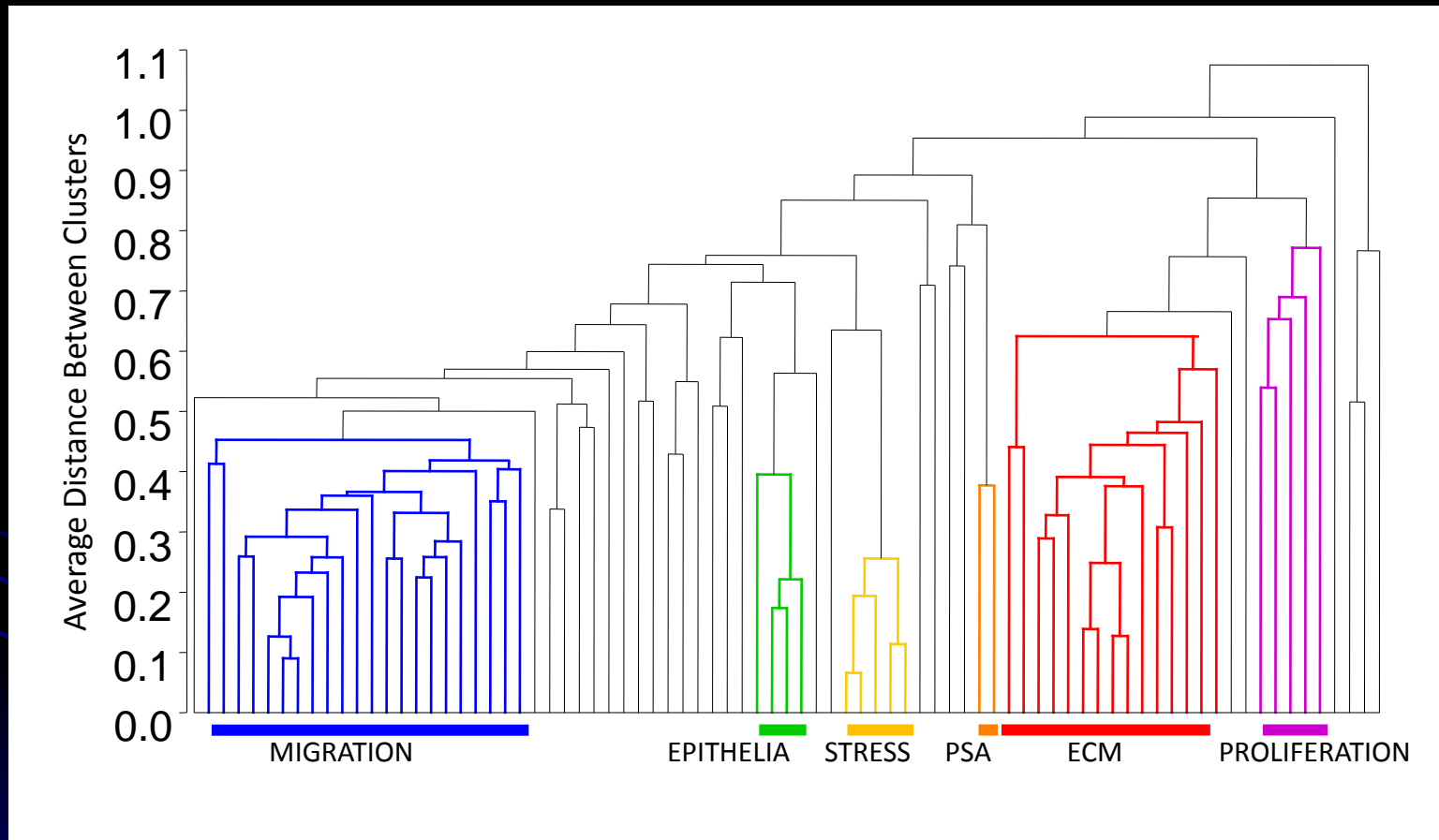
295 (41%) genes (unadjusted for covariates) are significantly ($p < 0.05$) associated with cRFI



Clinical and Pathological covariate used for adjustment	N (%) of genes significantly associated after adjustment
Pathology T Stage (pT)	221 (75%)
Surgical Gleason score (GS)	99 (34%)
GS, pT and baseline PSA	82 (28%)
AUA risk group	244 (83%)
CAPRA risk group	271 (92%)

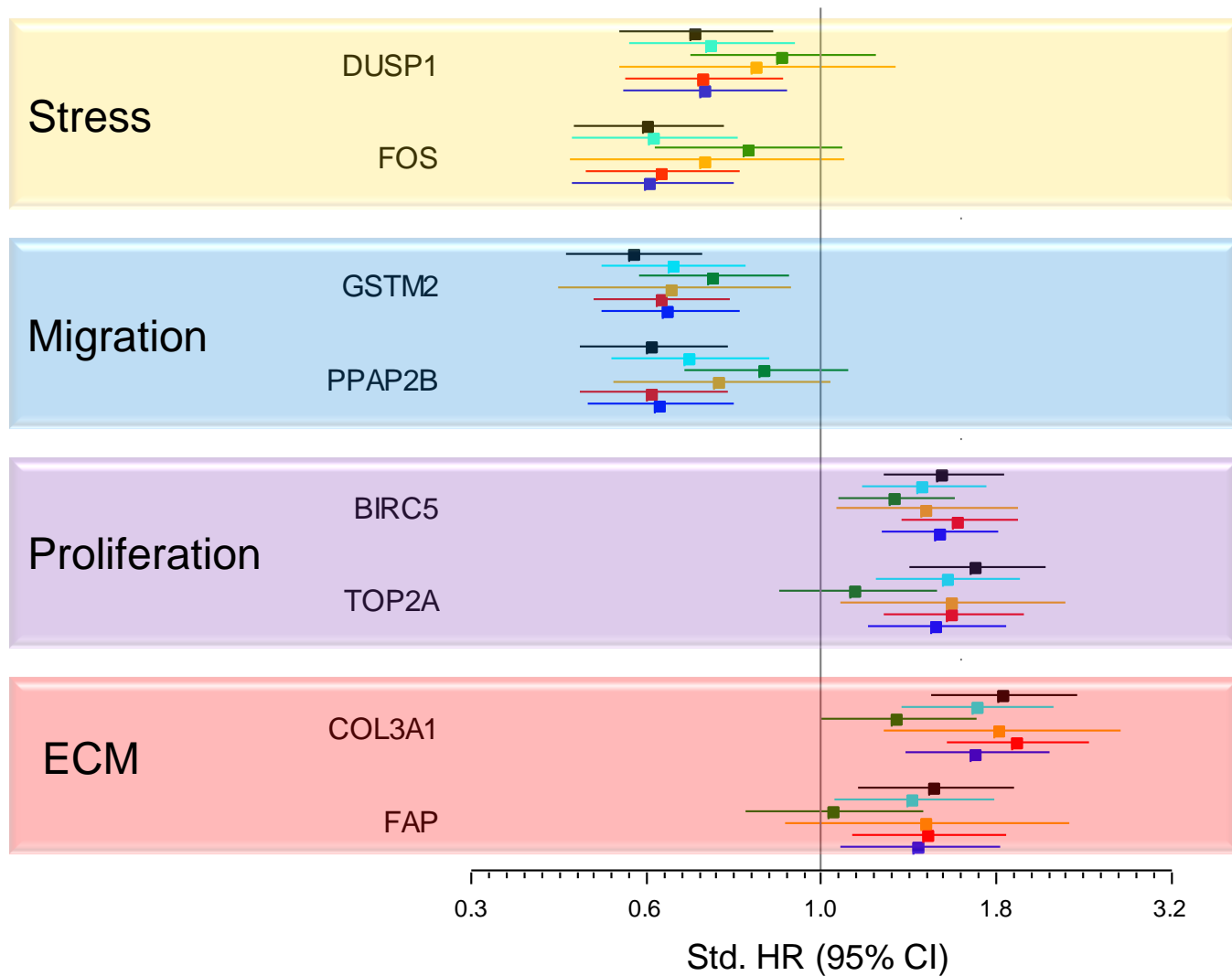
- Controlling for a false discovery rate of 10% all 295 genes are associated with cRFI
- Many genes add prognostic information beyond clinical and pathological covariates

Genes Associated with cRFI Cluster into Several Gene Groups

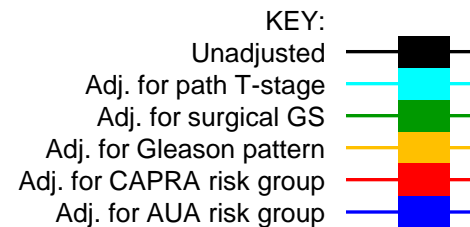


- Many genes cluster into co-expression modules or gene groups
- Gene groups identified are relevant to prostate cancer biology

Multiple Gene Groups Add Prognostic Value beyond Clinical and Pathological Covariates



Standardized hazard ratio is the hazard ratio per 1 standard deviation increase in gene expression



Conclusions

- Prostate cancer has an inherently diverse biology with a strong correlation between gene expression and risk of recurrence
- Many genes were identified as significantly associated with cRFI, ~ 50% with hazard ratios >1.4
- Genes and gene groups add prognostic information beyond traditional clinical and pathological covariates

Perspectives

- These data will be used to develop a prognostic multi-gene algorithm used in the context of other clinical and pathologic features (such as Gleason score) to distinguish clinically indolent PCA from aggressive disease
- They will be the foundation of a biopsy-based genomic test

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