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Prostate cancer: incorporating genomic biomarkers in prostate cancer decisions



Practice points

- PSA testing became the cornerstone of early PCa detection after its approval over 20 years ago. However, due to the low disease mortality rate, controversies have emerged with early detection strategies.
- New biomarker assays have been developed to help reduce the burden of biopsies in men with a low probability of PCa.
- For patients with negative biopsies who are believed to be at high risk for PCa, biomarker tests should be considered to improve specificity of the diagnosis.
- Physicians and patients can consider disease monitoring as an alternative to treatment after careful consideration of the patient's PCa risk, general health, and age. Biomarkers can help with this decision-making.
- Physicians who manage patients with CRPC are faced with complex decisions, given the numerous treatment options available. Beyond PSA, CTC testing offers an opportunity for predictive biomarkers to identify men most likely to benefit from a given therapy.

Prostate cancer is the most common solid tumor malignancy among men in the western world. Even without treatment, PCa-specific mortality rates at 5 and 10 years remain low. Nonetheless, many men with newly diagnosed PCa undergo interventional therapies, underappreciating the biologic aggressiveness and subsequent clinical impact regarding their specific PCa pathology. Improved prognostic biomarkers that can provide individualized patient risk assessment are needed to assist informing treatment decisions for patients and physicians. Currently available biomarkers provide clinical information for disease detection, disease aggressiveness and therapeutic response assessment but are not used routinely. This review will provide an overview of the biomarker landscape, specifically focusing on assays that may stratify patients who might appropriately elect active surveillance versus interventional therapy.

Keywords: active surveillance • genomic biomarkers • prostate cancer

Introduction

Prostate cancer (PCa) is the most commonly diagnosed solid tumor among men in the USA and Europe [1]. Improvements in optimizing screening, diagnosis and treatment have resulted in decreasing PCa mortality [2]. Nevertheless, certain challenges still remain for men facing important disease state decisions, for example:

- Those with an initial decision to biopsy or not:
- Those with an initial negative prostate biopsy, whom might not require a repeat biopsy;
- Those with apparent low risk, localized disease whom might not require intervention.

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Additionally biomarkers, which can improve the precision of risk assessment, are needed to enhance decisionmaking for physicians and patients, especially when the traditional clinical parameters (PSA, DRE, pathology) do not provide an accurate assessment of risk.

Patients with early-stage PCa can benefit from a more precise, personalized assessment of their tumor biology given that current clinical risk assessment tools may be less than adequate.

For those with CRPC, the increasing complexity of treatment decisions has led to research efforts to define predictive biomarkers that identify men most likely to benefit from a given therapy [3].

A new generation of biomarkers (Table 1) has emerged to help improve risk assessment, guide diagnostic strategies and ultimately enhance treatment outcomes through more targeted screening, more accurate diagnosis, improved risk stratification, which should lead to improved treatment recommendations and subsequent selection of therapy [4].

Who should be biopsied? PSA

After its approval by the US FDA in 1986, the availability of PSA dramatically influenced PCa early diagnosis [5,6]. In the USA, approximately 19 million men receive annual PSA testing, which results in more than 1.3 million biopsy procedures and a resultant 240,890 newly diagnosed findings of PCa cases [1].

Nonetheless, reliance on PSA testing alone for the detection of PCa has inherent limitations. First, the test is prostate specific but not PCa specific, and it may give false-positive or false-negative results. Most men with an elevated PSA level (above 4.0 ng/ml) [7] are not found to have PCa; only approximately 25% of men undergoing biopsy for an elevated PSA level actually have PCa. Conversely, a negative result may give false assurances that PCa is not detected, when, in fact, a cancer may still exist. Second, the test does

not always differentiate indolent from aggressive cancer and thus early detection of PCa may not impact eventual mortality from the disease [7] and potentially lead to overtreatment. This limitation of PSA testing was largely responsible for the recent recommendation of the USPTF against continued routine screening [8].

The PLCO Cancer Screening Trial is a large population-based randomized trial designed and sponsored by the National Cancer Institute to determine the effects of screening on cancer-related mortality and secondary end points in men and women aged 55-74 years. Regarding the PCa arm of the trial, after 13 years of follow-up, there was no evidence of a survival benefit for planned annual PCa screening compared with mandated screening. Additionally, there was no clinical impact with benefit for scheduled versus unplanned screening as it related to age, baseline comorbidity or pretrial PSA testing [9]. PLCO had high rate of screening (~50%) in the control arm, thus limiting its conclusions. However, Crawford and colleagues have reported a survival benefit for screening in men without significant comorbidities [10].

Eleven-year follow-up results from the European Randomized Study of Screening for Prostate Cancer study demonstrated that screening does significantly reduce death from PCa [11]. A potential reason for these differing results is that in the US-based PLCO Cancer Screening Trial, at least 44% of participants in the control arm were already PSA-tested prior to being randomized into the study [9], confounding interpretation of the results.

Roobol and colleagues [12] stated that there was "poor compliance with biopsy recommendations" in PLCO, as the trial did not mandate biopsies. Screening test results were sent to the participant and his physician, and together they decided upon subsequent biopsy. Screening test results were sent to the participant and his physician, and they decided upon subsequent biopsy per shared decision-making.

Table 1. Prostate cancer	biomarker overview.		
Biomarkers that assist clin	icians in determining:		
Who should be biopsied?	Who should be re-biopsied?	Who should be treated vs monitored?	Therapeutic response assessment
PSA	PCA3 ConfirmMDx	Onco <i>type</i> DX®	PSA
Phi	PCA3	Prolaris®	СТС
4KScore	PCMT	Decipher™	
	PTEN	ProMark	
	PHI		
CTC: Circulating tumor cell; PCA3: specific antigen.	Prostate cancer antigen 3; PCMT:	Prostate Core Mitomic Test; PHI: Pro	ostate Health Index; PSA: Prostate-

Adapted from [4]

In order to improve the sensitivity and specificity of serum PSA, several PSA derivatives and isoforms (e.g., PSA isoforms and PSA density, among others) have been used The National Comprehensive Cancer Network (NCCN) recommends PSA density when assessing for very low risk PCa patients [13]

Of note, the Goteborg trial, a prospective randomized trial of 20,000 men born between 1930 and 1944, showed that the benefit of PCa screening compared favorably to other cancer screening programs. PCa mortality was reduced by almost half over 14 years of follow-up [14].

Prostate Health Index (Phi)

Efforts have been made to reduce PSA-associated overbiopsying, which may lead to overtreatment in very low and low risk patients. The Prostate Health Index (Phi) was approved by the FDA for use in 2012 in those with serum PSA values between 4 and 10 ng/ml [15] in an effort to reduce the burden of biopsies in men with a low probability of PCa.

The Phi (Phi = [-2] proPSA/fPSA × PSA1/2; proPSA is a PSA subtype and fPSA is free PSA) was initially developed as an additional diagnostic biomarker in men with a serum PSA level of 2–10 ng/ml in European trials; an elevated proPSA/fPSA ratio is associated with PCa [16]. Percent-free PSA, Phi score and PCA3 have been described as markers of specificity within the 2014 NCCN guidelines for early detection of prostate cancer [15].

Phi score has a high diagnostic accuracy rate and can be used in PCa diagnosis. Phi score may be useful as a tumor marker in predicting patients harboring more aggressive disease and guiding biopsy decisions [17].

PHI also predicts the likelihood of progression during active surveillance. Tosoian and colleagues showed that both baseline and longitudinal values of PHI predicted which men would have reclassification to higherrisk disease on repeat biopsy during a median follow up of 4.3 years after diagnosis. Baseline and longitudinal measurements of PHI had C-indices of 0.788 and 0.820 for upgrading on repeat surveillance biopsy, respectively. By contrast, an earlier study in the Johns Hopkins active surveillance, PCA3 did not reliably predict short-term biopsy progression during active surveillance [18].

In patients with persistent suspicion of PCa and a negative biopsy, testing with PCA3 and Phi has been proposed as a way to reduce the number of unnecessary repeat biopsies [19].

4KScore

4KScore is a newly available commercial assay panel that is designed to help predict which men with an elevated PSA will have high-grade disease upon tumor biopsy. By combining measures of total, free, and intact PSA with human kallikrein 2 (hK2) and other clinical parameters, the 4KScore was shown to be better than PCPT at predicting the occurrence of high-grade disease on biopsy [20].

Who should be re-biopsied? PCA3

PCA3 is a noncoding mRNA that has been shown to be elevated in >90% of men with known PCa, but not significantly elevated in normal prostatic glands or in benign prostatic hypertrophy. The PCA3 test is a urine-based assay approved by the FDA as a diagnostic test in the setting of a previous negative prostate biopsy. It may be helpful in deciding when to re-biopsy or avoid doing so, with its attendant morbidity and cost, and adds to the diagnostic information obtained from the PSA test [21]. A high PCA3 score indicates a high probability of PCa, whereas a low score indicates a low likelihood. The mean PCA3 score was statistically significantly higher in men with a positive PCa biopsy, or those with atypical small acinar proliferation and/or high-grade prostatic intraepithelial neoplasia (HGPIN), compared with men who had a negative biopsy [22]. PCA3 testing may fail to identify transition zone cancers because the DRE does not elude cells into the urine.

ConfirmMDx

ConfirmMDx is a tissue-based epigenetic assay to improve patient stratification on the decision for repeat biopsy. It is performed on the archived tissues from the previous negative biopsy and detects an epigenetic field effect resulting from hypermethylation of three genes. This field effect around the cancer lesion can be detected despite the normal histologic appearance of cells, effectively extending the coverage of the biopsy. This test may help in the identification of high-risk men who require repeat biopsies and men without PCa who may avoid unnecessary repeat biopsies (the test has a 90% negative predictive value) [23,24].

PCMT

PCMT is a tissue-based test that identifies a deletion in mitochondrial DNA that indicates cellular change associated with PCa. It detects presence of malignant cells in normal appearing tissue across an extended area [25]. Recent clinical data indicate that this test may be useful for identifying men who do not require a repeat biopsy [26].

PTEN

Dysregulation of PTEN, a tumor suppressor gene, has been associated with poor prognosis in PCa.

Evidence suggests that loss of PTEN is associated with higher Gleason grade, risk of progression and recurrence after therapy [27]. Additionally, it is associated with advanced localized or metastatic disease and death [28]. The PTEN assay is a prognostic fluorescence *in situ* hybridization test typically ordered in conjunction with prostate biopsy tests that will indicate partial or complete deletions of the gene.

Who should be treated versus monitored? Oncotype DX[®]

The Oncotype DX is a multigene RT-PCR expression assay that has been prospectively validated in several contemporary cohorts as an accurate predictor of adverse pathology in men with NCCN very low, low and low-intermediate risk PCa [29]. Using very small biopsy tumor volumes, the assay measures expression of 17 cancer-related genes from four relevant biological pathways and five reference genes. These are combined to calculate a Genomic Prostate Score (GPS), which adds independent predictive information beyond standard clinical and pathologic parameters [30-32]. This enables more confidently selection of active surveillance or immediate therapy as an initial management strategy.

Prolaris®

Prolaris is a tissue-based cell cycle progression signature test that assesses 31 cell cycle progression genes to provide a risk assessment of PCa-specific progression and 10-year disease-specific mortality when combined with standard pathologic parameters [33]. It is designed as a risk stratification tool to help refine treatment/monitoring strategy for patients with PCa. Initially validated to predict prostate cancer specific survival (CSS) following radical prostatectomy, Prolaris has been subsequently validated in the biopsy setting as well.

Decipher[®]

The Decipher RNA assay directly measures the biological risk for metastatic PCa after radical prostatectomy. The test assesses the activity of 22 RNA markers associated with metastatic disease and has been demonstrated to be independently prognostic of PCa death in a high-risk surgical cohort. In a validation study, over 70% of high risk patients had low genomic classifier (GC) scores and good prognosis whereas patients with high GC scores had a cumulative incidence of metastasis over 25% [34]. This assay may better enable application of directed, multimodal or adjuvant therapy for patients with high-risk PCa following radical prostatectomy (RP).

ProMark

ProMark is a prognostic biopsy-based PCa test. It uses immunofluorescent imaging analysis to quantify protein biomarker expression and classify patients' tumors. A clinical validation study demonstrated that ProMark can differentiate indolent from aggressive disease, based on data from standard formalin-fixed, paraffin-embedded tissue. The ability to monitor treatment effects and identify therapeutic targets at the time of treatment consideration are major unmet needs in PCa. PSA and CTCs are two markers designed to address this need [35].

Therapeutic response assessment PSA

In addition to its use in PCa screening and diagnosis, PSA has been used to monitor responses to therapy and has been investigated as a therapeutic target [36]. Newer therapies, such as sipuleucel-T and radium-223 may improve survival without decreasing PSA levels. For cytotoxic chemotherapies or newer oral targeted hormonal therapies, PSA responses may be indicative of clinical response to therapy. Radiographic progression and symptomatology are still key parameters for consideration of changing antineoplastic therapy [37]. Discordance between PSA kinetics and clinical response and progression of disease has been regularly observed. The need for biomarkers - beyond PSA - to predict response to treatment is well recognized. Other serologic tests that are helpful include hemoglobin, alkaline phosphatase, LDH and others.

CTCs

The CTC assay is intended for the enumeration of CTCs (CD45-, EpCAM+, and cytokeratins) in whole blood, which can be a biomarker for therapeutic response to anti-neoplastic regimens. An increased abundance of CTCs in the blood of CRPC patients can predict worse outcomes. Recently, evaluation of individual CTC cells has allowed further prognostication of PCa [38]. CTCs may be useful for predicting treatment response/survival with cytotoxic and hormonal therapies. However, approximately 50% of patients do not have detectable CTC levels by current detection methods. More sensitive CTC detection techniques are under investigation.

Incorporating genomic biomarkers into PCa decision-making: a case report

Despite extensive efforts, few PCa biomarkers have been integrated into clinical practices. The development and validation of biopsy-based genomic assays may provide patients and their clinicians with independent information, and thus the confidence to elect an active surveillance strategy.

The new genomic markers can expand the tools needed to assess tumor aggressiveness. Oncotype DX® helps separate patients with low risk features on biopsy into those with lower and higher risk likelihood of harboring adverse pathology in the prostate at the time of diagnosis. Table 2 describes two such newly diagnosed PCa cases. In the first case (Case 1; courtesy Dr Neal Shore), the patient had Gleason Score 6 disease based on his biopsy. In such a case, a common question for many urologists would be whether to do a confirmatory biopsy or whether the patient would be upgraded after surgical extirpation of the prostate. Having knowledge of adverse pathology (freedom from high-grade disease) allowed the physician and the patient to make a more informed decision regarding active surveillance. In the second case (Case 2: Courtesy Dr Neal Shore), the molecular marker predicted aggressive cancer and thus changed the treatment recommendation from active surveillance to treatment.

Conclusion

PCa biomarkers have the potential in assisting clinicians to improve decisions regarding whom to biopsy, whom to avoid a repeat biopsy, whom to enhance risk assessment, and thereby reduce unnecessary biopsy strategies, as well as overtreatment, and thus achieving more selective therapy for patients with high-risk disease. In effect, clinicians can strive for better outcomes and hopefully remain cost neutral or better yet achieve cost savings to the healthcare system. In the last few years, there has been rapid development of many new and novel biomarkers. These biomarkers should offer and assist clinicians with improved decision-making on when to biopsy, whom to re-biopsy and how to assist patients with treatment decisions. If third-party payers are to appropriately support reimbursement outcomes, prospective data is needed that will demonstrate beneficial and actionable utility metrics, which should positively impact both patient outcomes and cost of care. Table 3 summarizes the characteristics of commercially available biomarkers in PCa.

Future perspective

One reason for the current controversy regarding screening for PCa is the lack of a test or a marker that can differentiate indolent from aggressive disease at the time of initial diagnosis. The ideal screening test should detect not just the presence of PCa but the aggressive PCa that needs to be treated, whereby its finding would impact progression and patient mortality.

Most urologists have experience treating men with apparent low-risk PCa who, at radical prostatectomy, are found to have higher grade and higher stage cancers harboring a significant risk of progression and death. In addition to prognostic markers predicting likelihood of aggressive disease, we will need to develop markers that predict the response to therapies. Molecular diagnostic researchers should ensure

decision whether to treat or pursue a	nolecular diagnostic Onco <i>type</i> D active surveillance.	ox® was used to impact the
Characteristics	Case 1: 60-year-old patient ⁺	Case 2: 67-year-old patient [‡]
PSA	4.0	4.9–10.8 annual visits
Gleason score	3 + 3 = 6	3 + 3 = 6
Number of cores positive/collected	1/12	3/12 (2, 5 and 30% involvement)
>50% tumor involvement in any core	No	No
Stage	T1c	T1c
PSA density	0.10	N/A
Life expectancy	25+ years	15+ years
Initial clinical risk (NCCN)	Very low	Intermediate
Pre-GPS recommendation	Patient undecided	Favored active surveillance
Genomic Prostate Score (GPS)	28	31
Likelihood of favorable pathology	83%	56%
In the expected range of NCCN	Very low	Intermediate
Post-GPS recommendation	Active surveillance	IMRT

¹The molecular marker predicted less aggressive cancer thereby supporting the decision to recommend active surveillance. ⁴The molecular marker predicted aggressive cancer and thus changed the treatment recommendation from active surveillance to treatment. GPS: Genomic prostate score; IMRT: Intensity-modulated radiation therapy; N/A: Not applicable; NCCN: National Comprehensive Cancer Network.

Table 3. Characte	eristics of commonly utilized bic	omarkers in prostate cancer.		
Test	Indication	Science	Results	Cost estimates [†] and other considerations
Oncot <i>ype</i> DX Genomic Health	Biopsy tissue based test NCCN very low, low and intermediate risk provides personalized risk assessment	Assay looks at 17 genes within four pathways (androgen signaling, stromal response, cellular organization, proliferation) to assess tumor aggressiveness	GPS from 0 to 100 Likelihood of freedom from dominant 4 or higher GS and/or non-organ confined disease GPS is reflective of the biology of the tumor at the time of biopsy	US\$3820 Medicare = No ABN required ⁺ Other ins: If estimated out-of-pocket cost > US\$100, company will contact the patient to offer financial assistance program. Tel.: 866 662 6897
Prolaris Myriad Genetics	Biopsy tissue based test for patients who are active surveillance candidates or Post-prostatectomy tissue- based test to determine relative risk of BCR	46-gene expression signature includes cell cycle progression genes selected based upon correlation with prostate tumor cell proliferation	Prolaris score biopsy is < or = or > than AUA risk group and estimated 10-year mortality risk Post-surgical is similar but 10-year risk for BCR	US\$3400 Medicare = no ABN required ⁺ Other ins: If estimated out-of-pocket cost >US\$375, company will contact patient to make arrangements. Financial assistance available. Tel.: 800 469 7423
Decipher GenomeDx Biosciences	Post-prostatectomy tissue- based test used for patients who are candidates for secondary therapy post prostatectomy pT2 with positive margins or pT3 or BCR	Analyzes the activity of 22 genetic markers in multiple pathways across the genome to measure the tumor's biological potential for metastasis after surgery	Decipher reports the probability of metastasis at 5 years after surgery and 3 years after PSA recurrence. AUC: 0.79; HR: 7.3 (decipher high risk); NPV 98.5%	US\$4250 Medicare = no ABN required ⁺ Other ins: Financial assistance program available for out of pocket expenses. 888- 792-1601
ConfirmMDx MDxHealth	Biopsy tissue based test for patients who are repeat biopsy candidates Provides risk stratification on decision for repeat biopsy Eligibility: Prior negative or HGPIN biopsy result in past 24 months	Three-gene methylation assay to detect an epigenetic field effect associated with the cancerization process at the DNA level	Negative ConfirmMDx result: Avoid repeat biopsy and monitor with routine screening. Positive ConfirmMDx result: hypermethylated areas are marked as positive providing repeat biopsy guidance on a prostate map	US\$2473 (\$206 core/block) Medicare = no ABN required [‡] Other ins: Financial assistance program is available for out of pocket expenses. Tel.: 866 259 5644
Know Error Strand Diagnostics	Oral swab and biopsy tissue based test provides DNA tissue matching Confirms pathology and/or confirms Biomarker is performed on correct patient Increases diagnostic accuracy	Buccal swab in the clinic sent for DNA match to pathology specimen; may be used with all tissues. STR profiles assessed from multiplex panel of 16 genetic markers	DNA Match DNA Non-match Contamination	US\$1780 (out-of-network billed charge amount per test) Medicare = US\$293/no ABN required [#] Other ins: Patient is only responsible for in-network' copays/deductibles. As of March 2014, only 2.4% of patients had any out-of-pocket costs and average is US\$65. Tel.: 888 924 6779 ext. 2
Costs listed in this table Without an ABN, the pa ABN: Advanced benefici predictive value; PSA: Pr Adapted from [39].	are estimates and vary by region and institu- trient is not held responsible for any unreimt iary notification; AUA: American Urological ostate-specific antigen; STR: Short tandem	ution. bursed expenses. Association; AUC: Area under the curve; repeat.	; BCR: Biochemical recurrence; GPS: Genomic	Prostate Score; HR: Hazard ratio; NPV: Negative

that the analytic validity of a biomarker test has been established prior to the evaluation of clinical utility. In planning clinical utility studies for biomarkers, protocols should specify the patient population intended to benefit from the decision guided by the test result. For validation studies of all types, prior evidence from early studies must be obtained from cohorts relevant to the intended use population. Another critical aspect is ascertaining that the samples studied are in fact those of the correct patient. To that end, another biomarker, Know Error (Strand Diagnostics) may be utilized to ensure that the specimen chain of custody is indeed accurate.Ideally, clinical validation studies should use metrics that are clinically useful to physicians in order to assess the strength of association between the biomarker assay and PCa. Ideally, such studies should include outcome measures that assess the potential benefits and challenges from the patient perspective, recognizing that these outcomes may occur at different time points and are the result of clinical management decisions guided by test results. The development and use of reimbursement policy approaches to promote clinical utility must be evidence based.

Biomarker platforms that enable healthcare professionals to accurately interpret and communicate the

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results of biomarker diagnostic and predictive testing for patients and their caregivers must be prospectively validated and their contemporaneous use should be promoted. Such strategies should include Continuing Medical Education credits (CME) for biomarkerrelated training, ongoing clinical utility trials, as well as engaging professional societies to develop practice guidelines to assess the incorporation and utilization of biomarker test results when appropriate.

Acknowledgments

The authors acknowledge Clifford Jones for his support in creating Table 3.

Financial & competing interests disclosure

ED Crawford has received honoraria for speaking engagements and advisory boards for MDxHealth and Genomic Health. BS Denes is an employee of Genomic Health. N Shore has received honoraria for speaking engagements and advisory boards for Genomic Health, MDxHealth, and Myriad. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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