

# Current State of Genomic Assays to Improve Risk Stratification in Newly Diagnosed Prostate Cancer: A Multidisciplinary Review and Recommendations

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## Abstract

Although prostate cancer (PCa) has surpassed lung cancer as the most common malignancy in men, many patients have indolent disease that is unlikely to threaten their health during their natural life span. The vast majority of men with PCa are diagnosed at a clinically localized stage and with a lower likelihood of recurrence (metastases/death) however the majority of men are given immediate intervention primarily due to uncertainty of their cancers' malignant potential. Over-treatment of low-risk disease with immediate intervention leads to significant morbidities, quality of life impairment, and health care expenses.

"Pro"active surveillance is a viable treatment alternative for men with low-risk PCa. However, confidently making the decision to choose pro-active surveillance requires effective tools to assess the risk of disease aggressiveness at diagnosis. Clinicopathologic risk stratification guidelines or nomograms are conventional tools used in clinical practice. Although effective, they have inherent challenges, such as their ability to provide personalized risk assessment. Population based tools, while easy to use; lose predictive power when collapsing large variables into one category.

Incorporation of genomic assays with clinicopathologic risk assessment tools affords better risk stratification for patients based on their likelihood of progression and disease-specific mortality. Thus, allowing men with more indolent disease to consider being treated more conservatively, while those with more aggressive disease to be afforded the benefits of immediate intervention.

Oncotype DX<sup>®</sup> Genomic Prostate Score (GPS) and Prolaris<sup>®</sup> are clinically validated genomic assays used in patients with low risk disease who are considering active surveillance. Other pertinent assays to consider in this setting include Decipher<sup>™</sup> and ProMark<sup>™</sup>. Many physicians are unfamiliar with these assays and there is a lack of consensus regarding their role in clinical practice. This manuscript aims to review current evidence and identify recommendations for their clinical utility in the newly diagnosed, positive biopsy, PCa setting.

**Keywords:** Genomic assays; Prostate cancer diagnosis; Malignancy; Genomic alterations.

## Introduction

PCa is one of the most common malignancies in men with approximately 1,80,000 new cases diagnosed in 2016, accounting for 21% of new cancer cases in men. The American Cancer Society now estimates that 1 in every 7 men will be diagnosed with the disease during his lifetime [1]. Although prevalence is high, mortality is low and PCa is one of the few disease settings that offer active surveillance as a viable alternative to treatment. The relative 5 year, 10 year, and 15

year survival rates are approximately 100%, 98%, and 95%, respectively [2]. Furthermore, age adjusted death rates from PCa have declined by approximately 4% each year from 1994 to 2011 [3].

Estimating life expectancy is important for informing decisions around PCa treatment [4]. Over 2.9 million men in the United States who have been diagnosed with PCa are still alive today [5]. PCa is multifocal and heterogeneous [6]. Multiple genomic alterations within the tumor influence a wide range of biologic pathways, which subsequently impact tumor aggressiveness [7]. The presence of frequent genetic differences between regions of individual tumors pose

a challenge to accurate, individualized risk assessment for men with localized PCa [8], thereby impacting treatment decisions.

### Treatment Approach for Localized Prostate Cancer

Despite the low risk of death from PCa, >90% of men diagnosed with low-risk disease receive immediate treatment with surgery or radiation [9]. Such overtreatment of prostate cancers that do not threaten life expectancy results in unnecessary side effects, quality of life impairment and health care expenses [4,10].

Pro-active surveillance is a viable treatment alternative that involves active monitoring with PSA and follow up/confirmatory biopsy (time frame undetermined). This differs from watchful waiting/observation, which involves monitoring the disease with the expectation to deliver definitive therapy upon development of symptoms (disease progression). Several surveillance protocols have been developed, including those from Johns Hopkins [11], University of California San Francisco (UCSF) [12], University of Toronto [13], Memorial Sloan-Kettering Cancer Center [14], and the Royal Marsden Hospital [15]. While there is currently no standard surveillance protocol, it is generally accepted that low-risk PCa should be managed with pro-active surveillance. Some protocols also include Gleason 3+4 patients as candidates for surveillance.

Clinical evidence supports the value of pro-active surveillance [16]. Radical prostatectomy (RP) in patients with low-risk PCa has failed to significantly impact overall and cancer-specific survival in randomized settings. Thus, it seems logical to abstain from aggressive curative treatment in low-risk PCa patients to avoid troublesome side-effects with no gain in life-expectancy [16].

Multiple studies have demonstrated that most patients with low-grade, organ confined disease can be successfully and safely managed with pro-active surveillance. In seven such studies including over 3,700 patients, the combined cancer-specific survival rate was 99.4% with follow-up between 22 months to 82 months [17]. There is evidence supporting the benefits of pro-active surveillance. Although a general agreement on which patients to include and how they should be managed exists, there are large variations in practice. Uncertainties concerning selection and progression criteria, as well as the long-term safety of pro-active surveillance, are still present [16]. Patient preferences should also be incorporated into evidence based decision making.

### Conventional Risk Stratification Tools

To confidently make the decision about pro-active surveillance as a treatment choice, clinicians need effective tools to assess the risk of disease aggressiveness at diagnosis. Several clinical and pathological risk stratification systems have been developed to improve prediction of PCa aggressiveness, including American Urological Association (AUA) [18,19] and NCCN [4] criteria. The widely used Epstein criteria for insignificant disease are similar in definition to NCCN very low risk: Gleason score <6, one-third of positive cores and an involvement of <50% of individual cores [20].

These conventional risk stratification tools are based on PSA levels, Gleason score, clinical stage, and burden of disease (e.g. the number or percent of biopsy cores with cancer) [21,22]. (Table 1)

	<b>Very-low/Low risk</b>	<b>Intermediate risk</b>	<b>High risk</b>
<b>NCCN</b>	Very low	•T2b to T2c or	•T3a or
	•T1c	•Gleason score 7 or	•Gleason score of 8 to 10, or
	•Gleason score <6	•PSA 10 to 20 ng/mL	•PSA >20 ng/ml
	•PSA <10 ng/mL		
	•Fewer than 3 prostate biopsy cores positive, <50% cancer in each core		
	•PSA density <0.15 ng/mL/g		
	Low		
	•T1 to T2a		
<b>AUA</b>	•Gleason score ≤ 6, PSA <10 ng/mL		
	•Stage T1c, T2a and	•Stage T2b or	•Preoperative PSA >20 ng/ml, and/or
	•PSA level ≤ 10 ng/mL and	•Gleason score of 7 or	•Preoperative Gleason score of 8 to 10, and/or
•Gleason score ≤ 6	•PSA level >10 and ≤ 20 ng/mL	•Clinical stage ≥ T2c	

**Table 1:** Common definitions of prostate cancer risk groups

Per the guidelines, the following strategy for personalizing treatment of localized prostate cancer may be considered:

Conventional risk stratification guidelines may be used to begin the discussion of treatment options for localized prostate cancer.

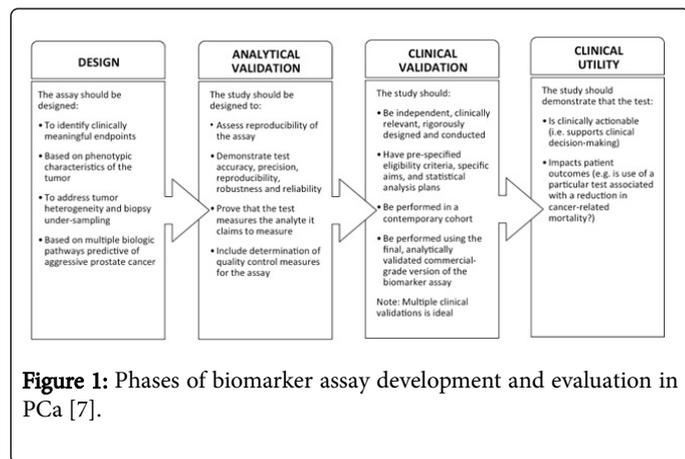
Then, a genomic test may be used to improve risk stratification by incorporating individual underlying tumor biology which provides additional, more personalized information to help further identify potential candidates for pro-active surveillance.

While these tools are effective in identifying patients at risk of aggressive disease, they have inherent challenges, including their ability to predict indolent disease as well as the sensitivity and specificity of biopsies. Genomic assays are being recognized as a complement to conventional clinical and pathologic parameters to personalize the care of cancer patients.

### Genomic Risk Stratification Tools

Given the potential uncertainty around aggressiveness of individual prostate cancers, prognostic assays should be used to supplement standard clinicopathologic parameters and inform treatment decision-making. Molecular diagnostics can provide an objective biologic measure of tumor aggressiveness [7].

Incorporation of genomic assays into standard clinical practice requires a level of validation, which is not often achieved. Some of the key challenges in developing biopsy-based assays for PCa include the heterogeneous and multifocal nature of the disease and the very small amounts of tumor tissue available from diagnostic prostate needle biopsies [23] (Figure 1).

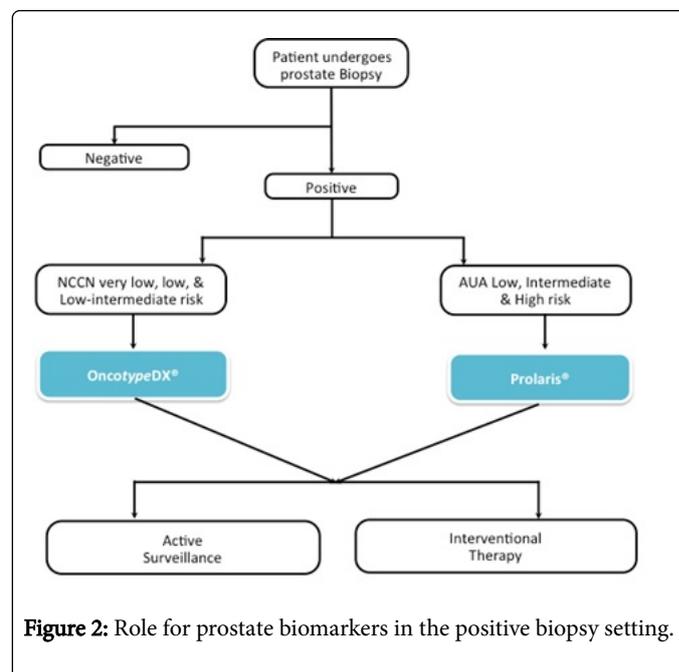


Based on the current evidence the authors believe that the ideal pathway to designing molecular diagnostics should include analytical validation, clinical validation, and clinical utility studies, which demonstrate clinical value [7].

Several genomic assays are commercially available for measuring tumor aggressiveness in the positive prostate biopsy setting: Oncotype DX<sup>®</sup> GPS, Prolaris<sup>®</sup>, Decipher<sup>™</sup>, and ProMark<sup>™</sup>. Each has gone through differing levels of validation for risk assessment. This review focuses on the two assays that appear further along in development and clinical use, as mentioned in the NCCN guidelines [4]: Oncotype DX<sup>®</sup> GPS and Prolaris<sup>®</sup>.

**OncotypeDX<sup>®</sup>:** Oncotype DX<sup>®</sup> is a tissue-based assay which can be utilized along with standard clinical parameters to improve risk stratification of disease aggressiveness for newly diagnosed PCa patients. By assessing diagnostic biopsy samples at the time of diagnosis, it assists with decision-making for active surveillance versus interventional therapy. The assay's genes were selected based on performance in biopsies and prediction of clinically relevant endpoints such as adverse pathology, biochemical recurrence, metastasis, and PCa death. Starting from a list of 732 candidate genes, 17 genes were ultimately selected representing multiple biological pathways that were combined into the Oncotype DX<sup>®</sup> GPS algorithm [24].

The assay measures expression of 12 cancer-related genes: AZGP1, KLK2, SRD5A2, FAM13c (androgen signaling genes), FLNC, GSN, TPM2, GSTM2 (cellular organization genes), TPX2 (cellular proliferation gene), BGN, COL1A1, and SFRP4 (stromal response genes) and five reference genes (ARF1, ATP5E, CLTC, GPS1, and PGK1). The Oncotype DX<sup>®</sup> GPS has been clinically validated in multiple contemporary cohorts as an accurate predictor of adverse pathology in men who have clinical NCCN very low, low, and low intermediate risk disease [24]. It has also been validated as an independent predictor of biochemical recurrence after surgery for localized PCa [25]. Patient selection criteria for this assay include NCCN very low, low, and low intermediate risk patients. These markers assist in the decision making regarding various therapeutic options (Figure 1).



**Prolaris<sup>®</sup>:** Prolaris<sup>®</sup> is a tissue-based test that assesses a cell cycle progression (CCP) signature to provide risk assessment of PCa-specific progression and 10 year disease-specific mortality when combined with standard pathologic parameters. The test provides a quantitative measure of the RNA expression levels of 31 CCP genes, normalized to 15 housekeeper genes [26]. Using either of the diagnostic biopsy samples or post-prostatectomy specimen, the test can assist in further refining treatment and monitoring strategies for both newly diagnosed PCa patients as well as for post-prostatectomy patients. The test has been clinically validated to predict biochemical recurrence after surgery in the post-prostatectomy setting and to predict prostate cancer-specific death when performed on transurethral resection of the prostate [27] or diagnostic needle biopsies [26]. Patient selection criteria for this assay include AUA low, intermediate, and high risk patients.

**Decipher<sup>™</sup>:** Decipher<sup>™</sup> is a genomic assay that assesses post-prostatectomy specimens to measure the biological risk for metastatic PCa. By assessing the activity of 22 genetic markers associated with metastatic disease, the assay has been shown to be independently prognostic of PCa metastases and mortality in a high-risk surgical cohort [28]. This assay may better enable application of directed, multimodal or adjuvant therapy for patients with high-risk PCa

following radical prostatectomy. The test has been clinically validated to predict the likelihood of metastatic disease after radical prostatectomy [29]. Patient selection criteria for this assay include those who are considered as AUA high risk.

**ProMark™:** ProMark™ is a tissue-based PCa test, which became commercially available in 2014. This protein-based test analyzes 8 biomarkers that individually correlate with tumor aggressiveness

(DERL1, CUL2, SMAD4, PDSS2, HSPA9, FUS, YBX1, pS6) [30]. By using immunofluorescent imaging analysis to quantify protein biomarker expression and classify patients' tumors, ProMark™ can differentiate indolent from aggressive disease. Further assay validation trials are planned [31-33]. Patient selection criteria for this assay include those with biopsy/Gleason score 3+3 and 3+4 (Table 2).

	Decipher™	Oncotype DX®	Prolaris®	ProMark™
Indication	Post-prostatectomy tissue based test used for patients who are candidates for secondary therapy post-prostatectomy  pT2 with positive margins or pT3 or BCR	Biopsy tissue based test  predicting the likelihood of favorable pathology at radical prostatectomy	Biopsy tissue based test for determination of prostate cancer-specific mortality risk (used in biopsy setting as well as the post prostatectomy setting)	Differentiating indolent from aggressive disease
Technology	22-biomarker micro array	17-gene assay (5 reference genes; 12 cancer-specific genes in multiple pathways: androgen, stromal, cell organization, proliferation)	45-gene panel (15 reference genes; 31 cancer-specific genes in proliferation pathway/CCP)	Quantitative protein-based immune-fluorescence imaging
Endpoints	5 year metastasis risk following RP	Predicts likelihood of favorable pathology	Predicts 10 year mortality risk in biopsy setting; predicts biochemical recurrence in post-prostatectomy setting)	Identification of indolent vs. aggressive cancer
Appropriate Patients	AUA high risk patients	NCCN very low; low and low intermediate risk patients	AUA low; intermediate and high risk patients	Biopsy/Gleason score 3+3 and 3+4 patients

**Table 2:** Overview of commercially available biomarker assays in the positive biopsy PCa setting.

### Pathologist Considerations

Multiple factors influence the diagnostic yield of prostate biopsies. While many of these factors are fixed, others are controlled either by the urologist (eg. number of cores obtained, method and location of biopsy, amount of tissue obtained) or the pathology lab [34]. It is important to establish effective lines of communication between pathologists and urologists to optimize the yield of prostate biopsies and manage issues that may pose challenges to accurate, individualized risk assessment for treatment selection.

Some of the key issues facing pathologists are specimen preparation, biopsy under-sampling, sampling heterogeneity, staging, and

assessment/observer interpretability. The panel recommends the following best practices for consideration:

**Specimen preparation:** The standard biopsy is 12 cores and it is ideal for each prostate biopsy core to be stored in a separate specimen jar. However, having up to 3 cores from the same location within a container is acceptable and would not preclude accurate histological evaluation of the tissue. Increasing the number of cores in a specimen jar leads to increased tissue fragmentation, tangling of cores, and reduced tissue sampling, which can reduce cancer detection rates and increase the likelihood of equivocal diagnoses (such as atypical small acinar proliferation) [34,35] (Table 3).

	Decipher™	Oncotype DX®	Prolaris®	ProMark™
Specimen tissue requirements	At least 0.5 square centimeters of tumor by area	>/= 1 mm or >/=~5% of a standard TRUS biopsy core; 10 ng RNA; Eight 5 µm slides	2mm (0.5 mm) tumor length 200ng RNA; Seven 3 to 5 µm slides	Four 5 µm slides
Specimen preparation considerations	FFPE block	FPE prostate needle biopsy tissue	FFPE prostate tumor biopsies	FFPE prostate tissue

**Table 3:** Pathologist considerations for commercially available biomarker assays in the positive biopsy PCa Setting.

Both fixation and storage time are considered to be critical steps that determine block quality and its suitability for subsequent genomic and proteomic analysis [36]. It is important that physicians are aware

of key issues related to tissue fixation and tissue conservation. For example, some fixatives may cause significant changes to DNA, RNA transcripts, and proteins thereby invalidating the genomic assay

results. Specimen preparation requirements for select genomic assays are provide in Table 3. For Prolaris<sup>®</sup>, Decipher<sup>™</sup>, and ProMark<sup>™</sup>, formalin-fixed paraffin-embedded (FFPE) block is appropriate, whereas the Oncotype DX<sup>®</sup> assay uses fixed, paraffin-embedded (FPE) prostate needle biopsy tissue.

**Sampling heterogeneity:** Given the heterogeneous and multifocal nature of PCa, biopsy samples may not always represent the pathophysiology of the entire tumor. Evidence indicates that a tumor that is a mix of grade patterns has a genomic pattern of abnormality throughout the tumor that indicates grade irrespective of the region sampled. Exploratory evidence indicates that both Oncotype DX<sup>®</sup> and Prolaris<sup>®</sup> can predict tumor aggressiveness when measured in adjacent tumor tissue [37,38]. This suggests that these assays may be measuring a more generalized field effect and may therefore be less susceptible to tumor heterogeneity and multifocality [7]. Furthermore, given that the phenotype of disease aggressiveness evolves over time [39], genomic markers may help to more accurately assess tumor grade.

**Biopsy under-sampling:** Limited tumor sampling by needle biopsy poses a challenge to the incorporation of genomic-based assays in treatment. For example, under certain circumstances, the OncotypeDX<sup>®</sup> and the Prolaris<sup>®</sup> test results may be returned as “insufficient tissue”. This may be due to tumor exhaustion prior to submission or submission of samples with little or no tumor on the deepest diagnostic sections [40].

Communication between urologists and the pathology laboratories which process their diagnostic samples regarding the desire to pursue genomic testing in biopsy tissue on a recurring basis can be helpful. In certain instances, tissue sectioning practices can be modified during the initial diagnostic workup to ensure that sufficient tumor remains for testing period.

## Discussion

The goal of this paper was to discuss commercially available PCa biomarker tests in use today, specifically focusing on those in the positive biopsy setting. Building on personal clinical experiences, we provide actionable recommendations on how to integrate these assays into clinical practice. Studies have shown that even without treatment, PCa-specific mortality remains relatively low. Nonetheless, many men with PCa undergo interventional therapies, fearing uncertainty with regard to the malignant potential of their specific pathology. Prognostic biomarkers that can provide individualized patient risk assessment are important for informing treatment decisions for patients and physicians. The rationale behind the recommendations in this paper is rooted in the fact that many physicians remain unfamiliar with the available tools for their patients who are considering active surveillance and there remains a lack of consensus regarding the role of these tests in clinical practice. To that end, the authors hope that this manuscript provides timely and practical advice on how to effectively use tools to assess the risk of disease aggressiveness at diagnosis and confidently make decisions about pro-active surveillance as a treatment choice.

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