

# Current & Emerging Role of Tissue-Based Molecular Biomarkers in Prostate Cancer

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**Abstract:** Prostate cancer (Pca) is a common male-specific cancer with diverse genetics and disease phenotypes. Current risk stratification tools for Pca rely on standard clinico-pathological variables like serum Prostate Specific Antigen (PSA) levels, histopathological Gleason scoring, and image-based tumor staging. PSA levels are criticized for reduced specificity, leading to over-diagnosis. Monitoring tumor progression with standard clinico-pathological parameters is challenging. Pca-specific biomarker research has primarily focused on disease diagnosis, but there is a need for novel prognostic and predictive response to treatment biomarkers. Personalized approaches for early detection, risk stratification, and treatment response prediction are essential. Recent advancements in tissue-based molecular biomarker assays through genomics and proteomics technology have created opportunities for improving diagnosis, prognosis, and management of Pca. However, these assays also pose challenges in incorporating them into routine clinical practice. This review discusses the current role of commercially and non-commercially available tissue-based molecular assays approved by FDA or Clinical Laboratory Improvement Amendments (CLIA) in local and advanced diseases and their challenges.

**Keywords:** Prostate cancer; PSA; Gleason score; Risk stratification; Tissue-based biomarkers; Molecular assays; Genomics; Proteomics; Diagnostic biomarkers; Prognostic biomarkers; Predictive biomarkers; CLIA-approved assays; FDA-approved assays; Personalized medicine.

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## Introduction

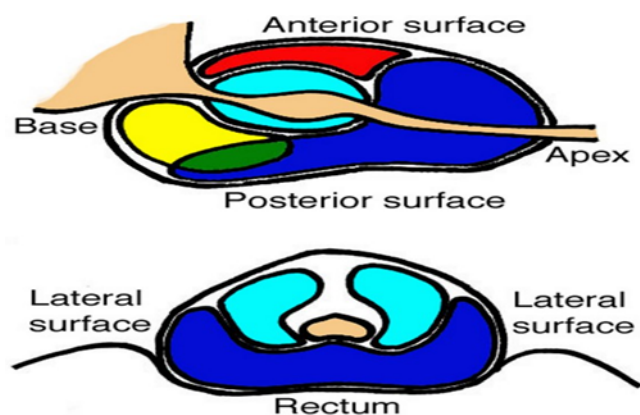
Pca is a major global cause of mortality and morbidity.<sup>1</sup> Biomarkers are essential for early diagnosis, prognosis, treatment response, and staging. They reduce over-diagnosis and differentiate disease subtypes. Biomarkers are classified based on their application, such as screening for asymptomatic or at-risk patients, diagnostic for suspected cancer, prognostic for disease progression with or without treatment, and predictive for disease progression or treatment response. Many Pca tissue-based molecular biomarkers have emerged as clinically useful diagnostic, prognostic, and predictive tools in managing Pca.<sup>2</sup>

### Anatomical location and structure of the human prostate gland

The prostate is a large gland in the male reproductive system that secretes enzymes into semen, aiding in sperm migration during ejaculation.<sup>3</sup> It is located in the pelvic cavity and is accessed during the Digital Rectal Examination (DRE).<sup>4,5</sup> The prostate gland consists of surfaces such as base, apex, anterior, posterior, and infero-lateral<sup>5</sup> (fig 1a & b).<sup>3,6,7</sup> It is divided into three zones: central, transitional, and peripheral. The central zone surrounds ejaculatory ducts, the transitional zone is prone to benign-prostatic-hyperplasia (BPH), and the posterior peripheral zone is

more prone to inflammation and prostate cancer. The DRE is a procedure used to examine the prostate.<sup>8,9</sup>

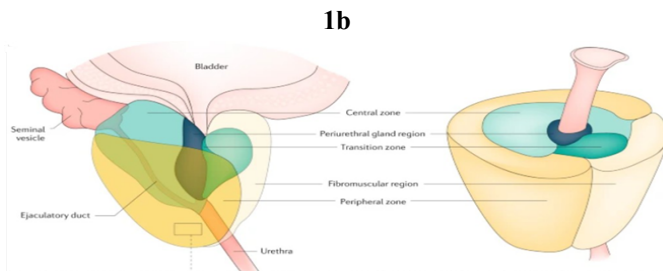
**Fig 1a & 1b<sup>3,6,7</sup>: Prostate gland anatomy**



**Prostate gland anatomical zones Fig 1a:** Anterior fibromuscular zone (red); transition zone (light blue); central zone (yellow); peripheral zone (dark blue) - note that most of the peripheral zone is available for examination through the rectum

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### Sagittal view of prostate zones



**Fig 1b:** Central zone constitutes around 20% of the gland, Transitional zone constitutes around 10% (more prone for (Benign Prostatic Hyperplasia) and peripheral zone constitutes more than 70% of the gland.

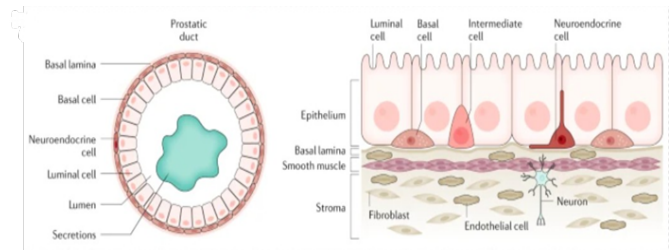
Adapted from *Verze et.al*<sup>3</sup>, *Romero et.al*<sup>6</sup>, *Bott et.al*<sup>7</sup>.

### Histology of prostate gland

The prostate gland, composed of ducts and acini embedded in the stroma, has pro-tumorigenic ability in the tumor micro-environment by stimulating EMT, which causes epithelial cells

to lose adhesion and gain invasive mesenchymal markers. Both normal and cancerous prostate epithelial cells express high levels of Androgen-Receptor (AR), which drives the hormonal dependency of prostate cancer.<sup>10,11,12,13,14,15,16</sup> (Fig 2)

**Figure 2: Prostate gland histology**



### Epidemiology and risk factors

Pca is the second most common cancer globally, with a strong association with age. In the UK, 34% of cases were diagnosed in those over 75.<sup>17,18</sup> The incidence is higher in developed countries compared to developing ones.<sup>1,19,20,21,22</sup>

**Table 1:** Factors influencing prostate cancer risk.<sup>21,22</sup>

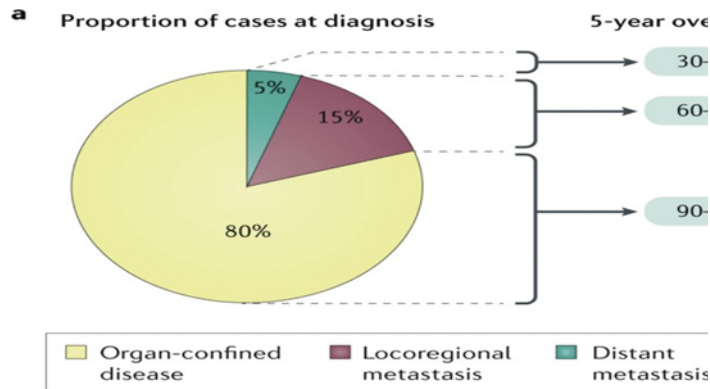
Non-modifiable risk factors	
<b>Advanced age</b>	>66% of Pca in men occur over 65 years.
<b>Family history</b>	2.16 & 1.95-fold of increased risk when father or brother diagnosed with Pca at age <65.
<b>Genetic association</b>	Mutation in BRCA1 & BRCA2 genes (i.e., breast cancer genes) associated with increased risk by 25%.
<b>Ethnicity</b>	African American men, West African ancestry have increased incidence of Pca compared with White and Asian men.
Life-style behaviour	
<b>Smoking</b>	Smoking increases androgen and testosterone level which in turn elevates Pca risk.
<b>Alcohol</b>	Increased risk is associated with increased intake of alcohol per day.
<b>Obesity</b>	Alters metabolism and sex steroid hormone level.
<b>Diet:</b>	Red meat and dairy products increase testosterone level.

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Modified from Shah *et.al*<sup>21</sup>, Bernardo *et.al*<sup>22</sup>

### Prognosis and survival

The prognosis of a tumor depends on its grade and stage (Fig 3) with localized cases having a life expectancy of over 10 years, and distant metastases resulting in a 30% survival rate.<sup>23,24,25,26</sup>



**PCa:** Early detection aims to identify clinically significant diseases in asymptomatic populations, improving outcomes for patients requiring definitive therapy. Screening involves quantifying blood serum PSA levels, a serine protease secreted from prostate epithelial cells.<sup>27,28</sup> PSA was first identified in 1980 in prostate cancer patients' serum. In men, PSA cut-offs for prostate cancer are set at 4 ng/ml, with a level > 4 ng/ml considered appropriate for investigation.<sup>29-31</sup>

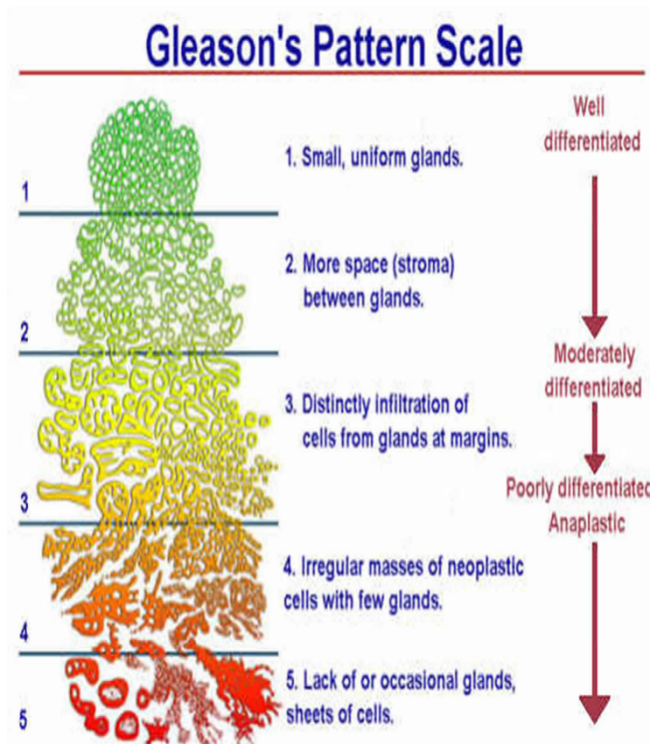
Men with consistently high PSA levels often undergo prostate cancer (Pca) biopsy, although this is considered an imprecise marker due to benign conditions like prostatitis and BPH.<sup>32</sup>

Overdiagnosis and overtreatment of clinically insignificant diseases can lead to complications like erectile dysfunction, urinary incontinence, and psychological effects. Current guidelines recommend informed shared decision-making, information about potential harm and benefits for individual screening, and factors like age and family history considerations.<sup>33,34</sup>

Standard Pca diagnostic criteria include DRE, PSA level, and Magnetic Resonance Imaging (MRI).

Transrectal Ultrasound guided (TRUS) biopsy is mainly used for histopathological diagnosis, but it may miss anteriorly situated tumors.<sup>34</sup> Trans-perineal mapping biopsies (TPMB) are preferred over TRUS for their ability to obtain samples through the perineum, reducing UTI infections.<sup>35</sup> Multiparametric (mp) MRI-guided biopsies are recommended worldwide for increasing diagnostic sensitivity, but they have limitations such as prolonged procedure time, limited availability, and not being cost-effective, making them an economic burden for low-income patients.<sup>34,35,36,37</sup>

Gleason scoring is a prognostic indicator for prostate cancer (Pca), which classifies tumor tissues into well-differentiated glands separated by stroma to poorly differentiated glands.<sup>39,40</sup> Pathologists score the glandular architecture based on histological patterns of each tumor specimen and report it by the summation of two most predominant patterns within the tumor.<sup>38,41,42</sup> (Figure 4)<sup>23</sup>



### Gleason Score and Grade Groups

Gleason Score	Grade Group	Characteristics
6	Grade Group 1	Less aggressive Very slow growing Low risk
3 + 4 = 7	Grade Group 2	Slightly aggressive Slow growing Low to Intermediate risk
4 + 3 = 7	Grade Group 3	Moderately aggressive Fast growing Intermediate to High risk
8	Grade Group 4	Aggressive Rapidly growing High risk
9 - 10	Grade Group 5	Highly aggressive Rapidly growing High risk

The International Society of Urological Pathology (ISUP) introduced grades 1-5 in 2014, which is more effective for differential prognosis of Gleason grading-7 tumors. (Table 2)<sup>23</sup>

**Table 2- Shows Pca risk classification based on ISUP grading<sup>23</sup>.**

Table 2: Pca risk classification based on ISUP grading. <sup>23</sup>				
Type of risk	Measured variables	Low risk	Intermediate risk	High risk
ISUP grade (risk of BCR)	GS	<b>Grade 1:</b> GS 3+3=6	<b>Grade 2 (low-intermediate):</b> GS 3+4=7 (mostly well-formed or fused or cribriform glands)	<b>Grade 4:</b> GS 4+4=8; (only poorly formed or fused or cribriform glands or mostly lacking glandular formation with minimal well-formed glands)

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			<b>Grade 3</b> (high - intermediate): GS 4+3=7 (mostly poorly formed or fused or cribriform glands)	<b>Grade 5:</b> GS 9 or 10 (gland formation is lacking with or without poorly formed or cribriform glands and necrosis)
<b>ISUP:</b> International society of urological pathology, <b>GS:</b> Gleason score, <b>BCR:</b> Biochemical recurrence (i.e., rise in blood PSA level after surgery or radiotherapy)				
Adapted from Rebello <i>et.al</i> <sup>23</sup>				

Gleason grades 1 and 2 are included within Gleason grade 3 and are considered non-aggressive tumors, while Gleason grades 4 and 5 are more aggressive with high metastatic frequency.<sup>23,43,44,45</sup> Histological heterogeneity within multiple foci is the commonest finding, as Pca is a multifocal disease, and each focus may have a different phenotype.<sup>46</sup>

Although Gleason scoring is the strong predictor for Pca, it faces challenges, especially when differentiating grade 3 from ill-formed, small fused, and closely packed glands.<sup>40</sup> Medium

sized well-defined grade-3 glands mixed with smaller-sized glands but still reported as grade-3.

**NICE-2019** classifies localized Pca into three tiers: low, intermediate, and high-risk group. It recommends men with intermediate risk be treated with surgery or radiotherapy. The Cambridge Prognostic Group (CPG) is a five-tiered Pca risk stratification system for non-metastatic cancer, dividing intermediate and high-risk groups into two sections. (Table 3)<sup>43,44</sup>

**Table 3:** Categorisation of risk-tier using NICE & CPG Guidelines.<sup>43,44</sup>

NICE risk group	Criteria	CPG category	Criteria
Low-risk disease	Gleason score ≤ 6 PSA < 10 ng/ml & stage =T1-T2a	CPG-1	Gleason Score 6 (Grade 1) PSA < 10 ng/ml stage =T1-T2
Intermediate risk	Gleason score 7 (or) PSA 10-20 ng/ml (or) Stage =T2b	CPG-2	Gleason score 3 + 4 = 7 (Grade 2) (or) PSA 10-20 ng/ml and stage= T1-T2
		CPG-3	Gleason score 3 + 4 = 7 (Grade 2) PSA 10-20 ng/ml stage =T1-T2. (or) Gleason score 4 + 3 = 7 (Grade 3) & stage= T1- T2
High-risk (or) locally advanced disease	Gleason score 8-10 (or) PSA > 20 ng/ml (or) Stage ≥T2c	CPG-4	One of: Gleason score 8 (Grade 4) (or) PSA > 20 ng/ml (or) Stage =T3
			Any combination of: Gleason score 8 (Grade 4), PSA > 20 ng/ml or Stage =T3 (or) Gleason score 9-10 (Grade Group 5) (or) Stage= T4

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		CPG-5	
<p><b>PSA:</b> Prostate Specific Antigen,  <b>T1</b> Tumor is contained within the prostate and too small on scan / Rectal examination  <b>T2</b> Tumor is still inside the prostate gland but can be felt on rectal examination.  <b>T2a</b> Tumor is only one - half of 1 lobe.  <b>T2b</b> Tumor is &gt; half of one lobe.  <b>T2c</b> Tumor in both lobes.  <b>T3</b> Tumor spread outside prostate, but into close-by tissues  <b>T4</b> Tumor spread into other organs.  <b>NICE-National Institute for health and care in England, CPG-Cambridge Prognostic Group</b>          Modified from <i>NICE Overview</i><sup>43</sup>, Gnanapragasam VJ <i>et.al</i><sup>44</sup>.</p>			

### Localized disease treatment

Primary prostate cancer (Pca) is a hormonally dependent or castration sensitive cancer that relies on androgen receptor activation by ligands like testosterone for growth, making it initially responsive to Androgen Deprivation Therapy (ADT), which blocks testosterone production.<sup>48</sup> (Table 5) shows disease characteristics important for treatment of Pca.<sup>47</sup>

<b>Table 5:</b> Overview of various factors important for Pca treatment. <sup>47</sup>	
S. No	Disease characteristics or factors
1	Tumor growth ( <i>clinical T-Stage</i> ).
2	Tumor behaviour on imaging ( <i>localized, locally advanced, metastatic</i> ).
3	Castration-sensitive
4	Castration-resistant (i.e., Pca stops responding to hormonal treatment such as ADT)
5	Histopathological features ( <i>neuroendocrine/cribriform /intraductal patterns</i> ).
6	BCR ( <i>i.e., Rise in blood PSA level &gt; 0.1 ng/ml</i> ) after surgery or radiotherapy stratifies the patients into low-risk, intermediate and high risk.
7	Gleason scoring
8	ISUP grade
9	Patient characteristics including age, life expectancy, health status
10	Co-morbidities
11	history of germline -mutation
12	Patient's preferences
<b>BCR:</b> Biochemical relapse, <b>ISUP:</b> International society of urological pathologist.	
Modified from Mottet N <i>et.al</i> <sup>34</sup> , D'Amico AV <i>et.al</i> <sup>47</sup>	

The treatment of prostate cancer involves conservative monitoring of symptom-free prostate cancer until disease progression, ideal for low-risk cases. Active surveillance is achieved through scheduled follow-up examinations, including DRE, PSA level, and mpMRI-guided repeat biopsy. Surgical extirpation can be done for intermediate-risk and high-risk localized cases. External beam radiotherapy

(EBRT) can be given for intermediate and high-risk localized disease, and low dose interstitial brachytherapy can be given for intermediate risk and high-risk disease along with ADT. Further imaging is mandatory for high-risk categories due to the risk of metastasis. Treatment options for those with locally advanced diseases at increased risk of relapse include surgery or EBRT along with ADT.<sup>34,50,51,52</sup>

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Most Pca patients receive aggressive therapy regardless of age and disease risk, with low-risk patients having a higher

risk of dying from other causes and less benefiting from active management. Clinical practitioners face challenges in treating low and high-risk localized Pca cases. (Table 6)<sup>49</sup>

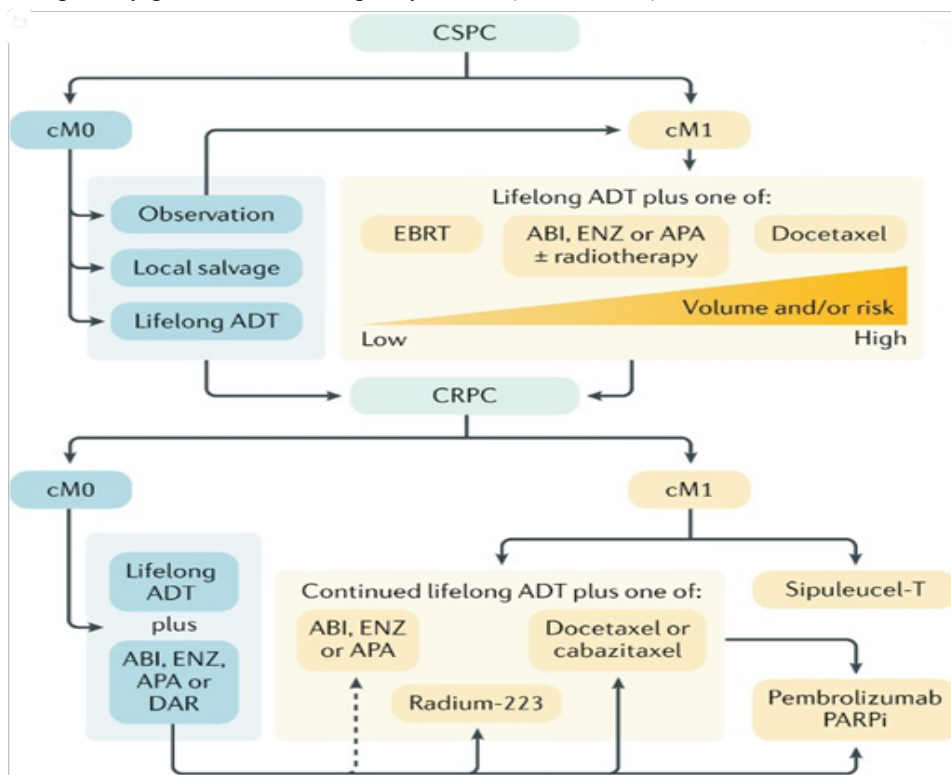
**Table 6:** Challenges met by clinicians in low and high risk localised Pca.<sup>49</sup>

Risk Type	Challenges
Low Risk	Over treatment due to overdiagnosis
	Risk stratification based on imprecise physical examination
	Limited random biopsy sampling
	Variation in PSA level
High Risk	Same challenges as low risk
	Sometimes undertreatment as they receive only palliative therapy with ADT instead of aggressive therapy

Modified from Albertsen PC *et.al*<sup>49</sup>.

### Advanced disease & treatment

Metastatic disease is the advanced stage of prostate cancer, categorized into treatment categories based on presentation stage. Although not curable, the primary goal is to enhance quality of life. (Flow chart 1)<sup>23,54</sup>



### Flowchart 1: Summary of approved therapeutic- strategies in advanced Pca.

BCR=Biochemical Relapse, CSPC=Castration sensitive prostate cancer, CRPC=Castration resistant prostate cancer, cM0=non-metastatic, cM1=metastatic, EBRT=External beam radiotherapy, ARSI=Androgen-receptor signalling inhibitor, ABI=Abiraterone, ENZ=Enzalutamide,

APA=Apalutamide, DAR=Darolutamide, PARPi=Poly (ADP) ribose polymerase inhibitor.

Modified from Rebello RJ *et.al*.<sup>23</sup>, Mateo J *et.al*.<sup>54</sup>

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BCR which is classified as CSPC, is further divided into clinically non-metastatic (cM0) and metastatic disease (cM1). Those with cM0 CSPC receive salvage treatment, life-long ADT until they develop metastasis. Patients with cM1 CSPC receive lifelong ADT along with docetaxel or ARSI with or without radiation. For CRPC, patients receive continuous ADT along with other agents depending on their metastatic behavior. **For cM0 CRPC-** ARSI can be given. **For cM1 CRPC-** ARSI, radium-223 in presence of bone -metastases, or chemotherapy can be given along with continuous ADT. Biomarker driven therapies such as Pembrolizumab and PARPi has shown to be effective in selected patients.<sup>52,53</sup>

**Metastatic castration sensitive prostate cancer (mCSPC)** is a condition where men develop metastatic prostate cancer after local therapy or present with de novo metastatic disease. Systemic ADT with Luteinizing Hormone-Releasing Hormone (LHRH) antagonists or surgical orchiectomy is the standard treatment until radiological/biochemical progression occurs. Combination treatment approaches, including AR-targeting drugs like enzalutamide, apalutamide, abiraterone, darolutamide, or docetaxel, along with continued ADT, are considered standard of care in de novo mCSPC.<sup>55,56</sup>

**Castration-resistant prostate cancer (CRPC)** is the progression of mCSPC either clinically or biochemically. Most CRPC tumors restrain their dependency on AR cistrome through AR gene mutations. The standard treatment for mCRPC is docetaxel/Cabazitaxel with prednisolone along with continued ADT.

Next-generation AR-targeting agents are now effective in treating mCRPC in combination with ADT, blocking extra-gonadal androgen formation or directly inhibiting AR.<sup>76</sup> Radium-223 is effective in improving overall survival and reducing the risk of skeletal events. Sipuleuce-T is an autologous vaccine that activates clearing of tumor cells by T-cell recognition. Immune-checkpoint inhibitors (ICIs) like Pembrolizumab are approved for patients with DNA mismatch-repair deficiency or microsatellite instability.<sup>77</sup>

Despite advances in treatment, the median overall survival for most patients is around 3 years after mCRPC development. Patients with increased PSA level and no signs of metastasis are considered M0-CRPC with occult metastasis. Early treatment with continuous ADT and Androgen Receptor Signaling Inhibitors (ARSI) is proven to be effective.<sup>77,78</sup>

### **Discussion:**

### **Current role of tissue-based molecular biomarkers**

Biomarkers should have high sensitivity and specificity for accurately identifying positive and negative cases of prostate cancer (Pca). Tissue-based molecular biomarkers play a growing role in Pca diagnosis, prognosis, and management. The current and emerging role of these biomarkers is highlighted in the following sections.

#### **As a Diagnostic marker**

Tissue-based molecular biomarkers are becoming increasingly useful in diagnosing prostate cancer (Pca). Hematoxylin & Eosin (H&E)-stained prostate biopsy tissue is typically used for diagnosis, but immunohistochemistry (IHC) staining can be used in specific cases. For instance, ex: an Atypical Small Acinar Proliferation (ASAP) foci in atypical prostate glands, expressing the ERG-Pca-specific protein and Alpha-methyl acyl-CoA racemase, can be detected by IHC for a definitive diagnosis. Histologically, Neuroendocrine Prostate cancer (NEPC) is difficult to distinguish from small cell urinary bladder cancer, in those cases ERG gene alterations detected by Fluorescent In-Situ Hybridization (FISH) technique can confirm tumor origin.<sup>57</sup>

#### **As a Prognostic marker**

Pca prognosis is currently determined through clinical data and histological features, but this method often undertreats low-risk patients and overtreats high-risk ones. To address this, tissue-based molecular biomarkers have been proposed, with multiplex tissue-based biomarker assays like Oncotype Prostate, Prolaris, Promark, and Decipher being developed for clinical use.<sup>57</sup>

#### **As a Predictive biomarker**

Predictive biomarkers help in identifying patients who are likely to benefit from specific clinical treatments, allowing for treatment selection and patient identification. For instance, hormonal therapy for prostate cancer (Pca) may develop resistance through mechanisms like amplification, mutation, or splicing of the AR gene, leading to castration resistant Pca (CRPca). Therefore, molecular analysis of the AR gene may be clinically significant in assessing hormonal therapy resistance.<sup>59</sup>

#### **Technology considerations**

The text discussed the use of IHC & FISH assays in Pca diagnosis, prognosis, and management, while also discussing emerging technologies like micro-array and Next Generation

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Sequencing for detecting sensitive tissue-based molecular biomarkers.

**NGS:** Next Generation Sequencing (NGS) is a DNA sequencing method that accurately detects gene-mutations, copy number variations, and epigenetic alterations. It has been shown to be genetically complex and linked to signaling pathway alterations, providing a clear view for diagnosis, prognosis, and management of prostate cancer. NGS can be done in various ways, including genome-based sequencing, whole genome and exome sequencing, RNA or transcriptome sequencing of mRNA, or non-coding RNA. For instance, genome sequencing of Pca neuroendocrine tissue reveals the deletion of Methyl-thioadenosine Phosphorylase (MTAP), which is more common in CRPC than localized Pca, indicating poor prognosis in Pca patients.<sup>60,61,62</sup>

**Microarray techniques** have enabled the detection of millions of gene expressions in signaling cascades, as well as novel genes and biomarkers crucial for Pca diagnosis and management. These techniques can be divided into cDNA and tissue-microarray, which analyzes numerous tissue samples on a glass slide using IHC or ISH results. The main advantage of tissue-based microarrays is its ability to detect stage-specific biomarkers by simultaneously analyzing tumors from different patients with different aetiologies and

clinical criteria. For example, in formalin fixed paraffin-embedded tissues (FFPE), this technique can detect gene fusions between TMRSS2 and ETS family genes, which correlate with Gleason score.<sup>63,64</sup>

### **New commercially available tissue based molecular biomarkers in localised & advanced Pca**

Risk stratification in men with localized prostate cancer (Pca) depends on PSA level and clinical staging. To address challenges associated with PSA as a biomarker, several tissue-based molecular assays have been developed to provide insights into patient risk stratification. These tests guide urologists in choosing accurate treatment, such as Adjuvant Radiation Therapy (ART) or Salvage Radio Therapy (SRT) for suspected/recurrent malignant disease or HT and predict recurrence and progression risk following localized management.

Most commercially available panels evaluate mRNA signatures in the prostate, but recent technology advancements in cancer epigenetics and protein increase the possibilities of Pca prognosis. These biomarker assays are monitored by The National Comprehensive Cancer Network (NCCN), FDA, and CLIA. Some commercially available and FDA or CLIA approved tissue-based molecular Pca tests and their indications are presented in Table 7.<sup>65,66</sup>

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**Table 7:** Commercially available & FDA /CLIA approved tissue -based molecular test for Pca and their indications.<sup>2,66</sup>

Bio Marker test	Molecular Markers	Test measures	Study outcome	Indications	Clinical use	Organization approval
<b>Repeat Biopsy: Pre-Diagnostic markers</b>						
<u>Confirm MDx</u>	Methylation of 3 TSG	Likelihood of Pca%	Detection of Pca with prior -ve biopsy	Men with prior -ve biopsy and considering the second biopsy	Decision for repeat prostate biopsy in men with prior true -ve biopsy	CLIA - approved

**Confirm-MDx** is a tissue-based epi-genetic assay developed to evaluate the prognostic value of epigenetic alterations in prostate cancer. This test mainly involves quantifying the methylation of three tumor suppressor genes (TSG) in tissues extracted from benign or negative prostate biopsy.<sup>58</sup> The field-cancerization effect was noticed first in the 1950s, when it was found that the tissues surrounding tumor lesions containing the markers are associated with tumor formation of oral squamous-cell carcinoma.<sup>69</sup> The field-effect can translate to changes in cellular morphology, epigenetics, mitochondrial DNA alterations (mtDNA), and alterations in gene expression & protein levels.<sup>67,69,70</sup> The two methylation studies, Methylation Analysis To locate Occult Cancer (MATLOC) and Detection of Cancer Using Methylated

Events in Negative biopsy Tissue (DOCUMENT), validated the utility of confirm-MDx. MATLOC study demonstrated that this test can detect occult Pca with a sensitivity of 68% & specificity of 64%. DOCUMENT study has demonstrated that this test is an independent predictor of Pca with a negative predictive value of around 90%.<sup>64,65</sup>

The role of Confirm-MDx regarding clinical decision making was demonstrated by Wonju et al and found that there was a significant reduction in the rate of repeat biopsy that is only 4.4% of men showing negative Confirm-MDx had a repeat biopsy compared to a repeat biopsy rate of 43% in PLCO clinical trial. This study also showed that all the repeat biopsies with negative confirm -MDx were negative.<sup>71</sup>

**Prognostic biomarkers have been developed to help redefine the clinical prognostics of Pca. Some commonly used and commercially available prognostic biomarker tests include (Table 8)<sup>2,66</sup>**

Bio Marker test	Molecular Markers	Test measures	Study outcome	Indications	Clinical use	Organization approval
<b>After Biopsy: Prognostic markers</b>						
<b>Decipher™</b>	Expression level of 22 RNA markers	GC Score: 0-1.0	Predict the risk of recurrence, metastasis & PCSM after RP	Men with high-risk pathology after RP	Determine if AT Vs ST is to be pursued	CLIA- approved
<b>Decipher™ Biopsy</b>	Expression level of 22 RNA markers	GC Score: 0-1.0	Predict the 5-year risk of metastasis and 10-year risk of PCSM, chance of high-grade Pca on RP	Men with localised Pca	Patients stratification prior to primary management	CLIA approved

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<b>Oncotype DX</b>	Expression levels of 12 cancer genes +5 house-keeping genes (RNA)	GC Score: 0-100	Predict the risk of AP & recurrence after RP from biopsy tissue	Men with very-low & low-risk Pca.	Advising men on AS vs treatment in newly diagnosed low or low-intermediate risk Pca	CLIA - approved
<b>Prolaris</b>	Expression levels of 31 cell-cycle progression genes +15 housekeeping	CCP Score:0-6	Predict the risk of BCR & PCSM after RP. Predict the risk of BCR and PCSM after primary management based on biopsy tissue.	Men with prostate cancer on biopsy	Advising men on AS vs treatment in newly diagnosed low or low-intermediate risk Pca	FDA-approved

*GGG=Gleason grade group, GC=Genomic classifier, AP=Adverse pathology, PCSM=Prostate cancer specific mortality, TSG=Tumour suppressor genes, AT=Adjuvant therapy, ST=Salvage therapy, CLIA=Clinical Laboratory Improvement Amendments, AS= Active surveillance Modified from Kohaar I et.al<sup>2</sup>.*

### **Deciphering the mRNA signatures in the prostate**

Decipher™ is a microarray test that profiles RNA expression levels of 22 genes involved in cell proliferation, differentiation, CCP, and AR signaling (Figure 4).<sup>57,72</sup> The test samples were extracted from FFPE-prostate specimens and the Genomic Classifier (GC) or Decipher score is reported as a number from 0 to 1.0 for both radical prostatectomy (RP) specimens and prostate biopsy specimens. The recent GC score was validated to detect three different score risk groups based on metastasis-free survival. Increased scores suggest aggressive tumors and are useful in guiding the appropriate timing of post-operative ART or SRT. The Decipher™ biopsy test was recently validated, providing risk at RP for pathological upgrading, 5-year development of clinical metastasis, and 10-year PCSM. The GC expression signature for Decipher™ test was developed using retrospective RP specimens from the Mayo-clinic. The study proved that GC was a strong independent transcriptomics predictor of metastasis development (P < 0.001). The AUC for GC was 0.90 in the original cohorts and 0.75 in the second validation cohort.

The Decipher™ test has been shown to significantly change urologists' adjuvant treatment decisions for men at increased

risk of metastasis after prostate cancer (RP). This was demonstrated in a prospective study called PRO-ACT, which evaluated 15 urologist's management decisions before and after the Decipher™ test. The test showed that around 42% of men who were recommended for adjuvant therapy were advised to be under observation after decipher testing, and 60% of men with high-risk were again reclassified into low-risk based on the test results.

The Decipher™ test has also been validated in terms of guiding ADT after ART or SRT post-prostatectomy.<sup>127</sup> Low decipher-score indicates good prognosis, which can change treatment strategies.<sup>73</sup> Recently, a systemic review performed on the Decipher test and its role on PCa treatment by Jairath et al. showed that the Decipher test was an independent prognostic indicator for Adverse Pathology (AP), BCR, metastasis, and cancer-specific survival.<sup>73</sup>

**Oncotype-DX Prostate** is a RT-PCR assay done on FFPE tissues obtained from diagnostic prostate needle biopsies (Figure 4).<sup>57,134</sup> The test measures the expression level of 12 cancer genes responsible for cellular organization, stromal response, androgen signaling and proliferation and measures

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the expression levels of 5 house-keeping genes. These gene combination expressions are used for the calculation of the Genomic-Prostate Score (GPS) ranging from 0-100.<sup>74</sup> The GPS score corresponds to the probability of AP during RP and non-organ confined disease (pT2 or above).

The Oncotype DX test has been validated using three cohorts of patients: RP discovery, prostate biopsy, and a prostate biopsy validation (independent cohort). In multivariate analysis, the odds ratio (OR) for every 20-point rise in GPS was 1.9 when altering for clinical factors such as age, PSA level, clinical stage & biopsy Gleason score. The OR was 2.1 when altering for Cancer of the Prostate Risk Assessment (CAPRA) score.<sup>75</sup>

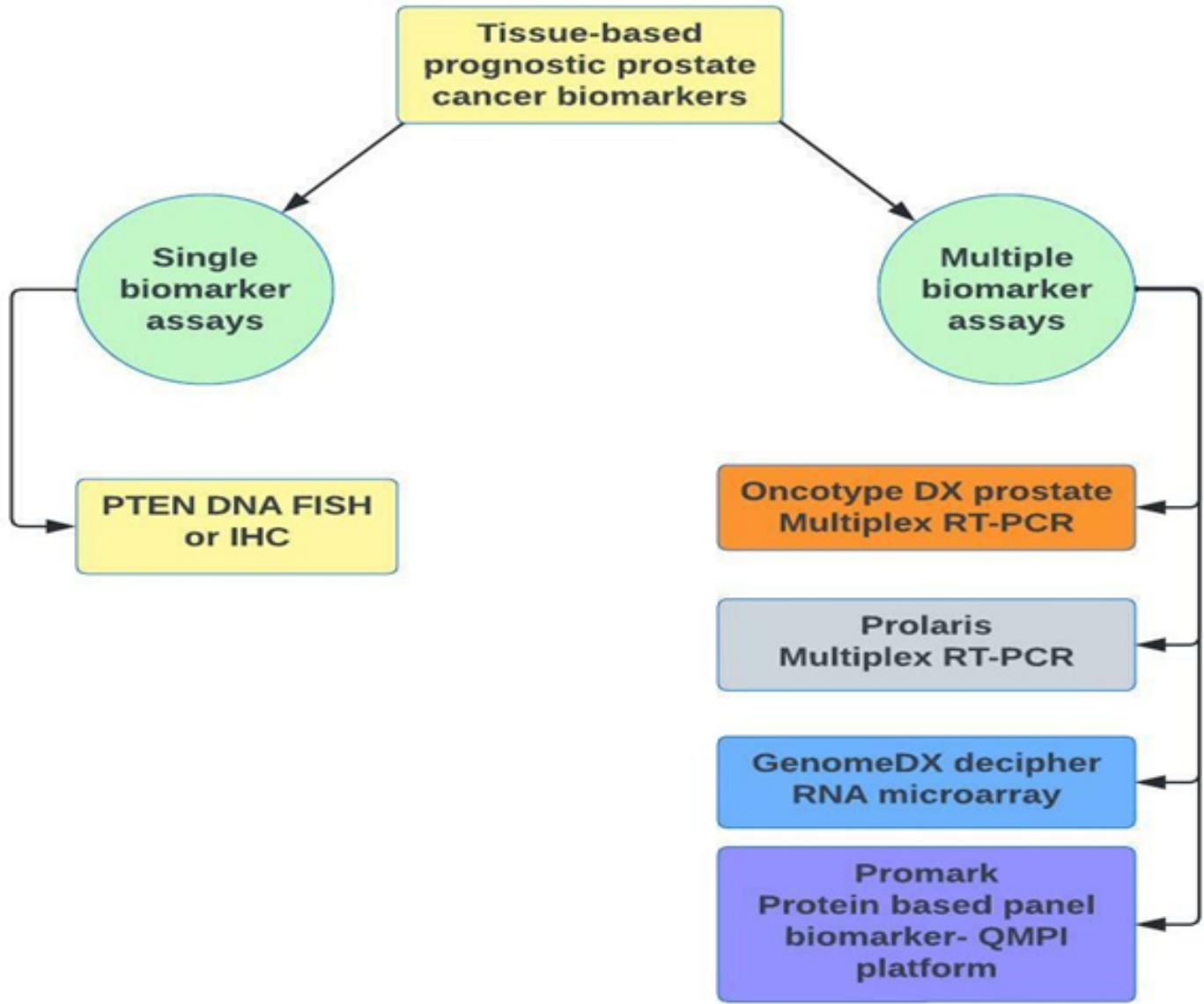
In a recent retrospective study involving low to high-risk patients' cohort with PCSM and metastasis, GPS was found to be the strong independent predictor of adverse oncology-related effects. Since this cohort is not the complete indicative of men undergoing Active Surveillance (AS), it recommends future GPS applications beyond low & intermediate-risk Pca.<sup>74</sup>

### Prolaris:

The test is currently available for both prostate biopsy (Prolaris-biopsy) and RP specimens (Prolaris-RP). This test which runs on total RNA, measures the expression level of 31 Cell Cycle Progression genes (CCP) involved in cancer proliferation and 15 house-keeping genes. The Prolaris-biopsy test, score the risk of 10-year PCSM and 10-year metastasis with definitive management, whereas the Prolaris-RP test score the risk of 10-year BCR. As per NCCN guidelines, prolaris biopsy test may be suggested to men having verylow/low-favourable intermediate-risk disease on prostate biopsy and a life expectancy of minimum 10-years.<sup>130</sup>

In conclusion, the Decipher™ test, Oncotype-DX, Prolaris®, and Prolaris® tests have shown promising results in clinical decision making for prostate cancer patients. These tests can help select patients for active surveillance and guide treatment strategies based on their prognosis and risk factors.

Figure 4: Overview of single & multiple tissue-based biomarker assays for Pca prognostication<sup>57</sup>



Single biomarker assays: PTEN DNA by FISH or IHC.  
 Multiple biomarker assays: RT- PCR assays (Oncotype Dx Prostate and Prolaris), RNA microarray assays (Decipher) and Quantitative multiplex immunofluorescence-QMPI (Promark).

FISH=Fluorescent in situ hybridisation,  
 IHC=Immunohistochemistry, RT-PCR=Reverse transcriptase polymerase chain reaction.

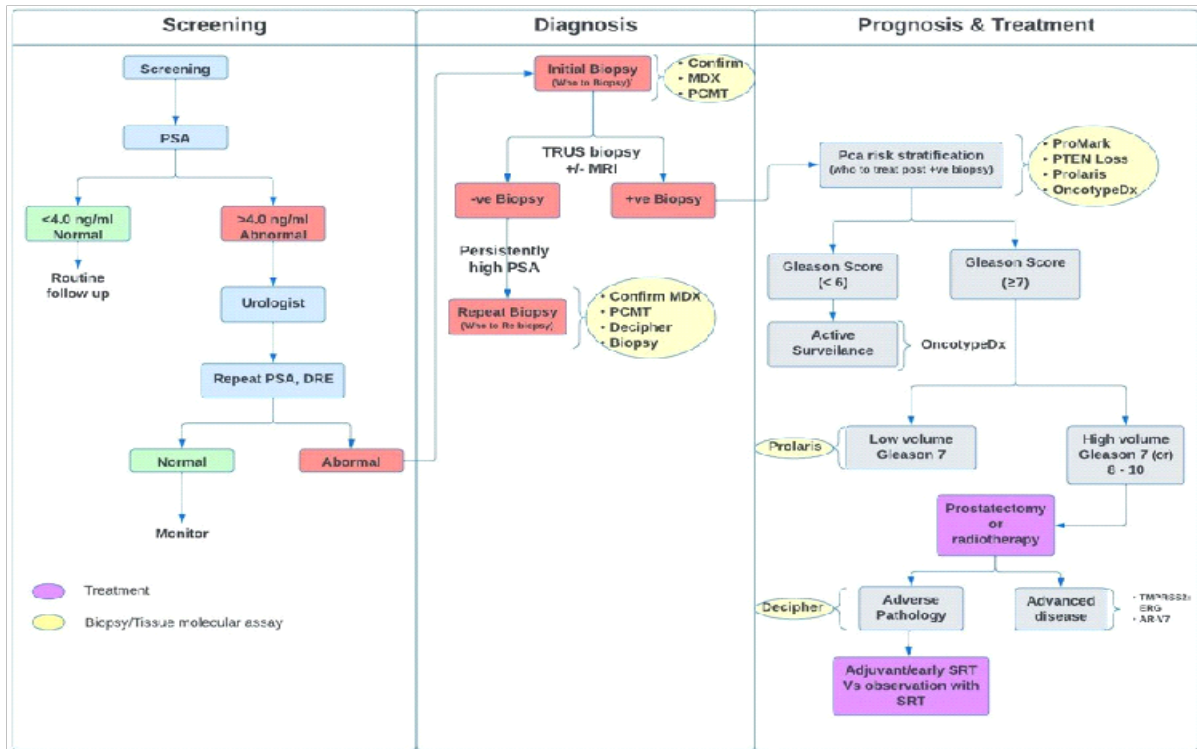
Modified from *Udager AM et.al*<sup>57</sup>

Utility of tissue based molecular biomarkers in diagnosis, prognosis and management of Pca

The emerging Pca tissue-based molecular biomarkers hold huge potential in improving the risk assessment, reducing the over-treatment and offering selective treatment for high-risk disease patients.<sup>66</sup> A schematic flowchart (Figure:5)<sup>68</sup> shows the use and correct timing of some current tissue based molecular biomarkers in the treatment of a Pca patient.

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**Figure 11: Schematic representation of tissue-based molecular biomarker assays for the diagnosis, prognosis and treatment of Pca.**



Patients who are not sure about undergoing biopsy after PSA or those with previous -ve biopsies may benefit from Confirm-MDx, PCMT and Decipher biopsy. Patients with Gleason 6 score on initial biopsy, may benefit from prognostic assays like Oncotype-DX, Prolaris, Promark & PTEN/TMPRSS2: ERG to predict the disease aggressiveness, risk stratification. These assays also provide information on which patients to be treated following +ve biopsy. For those who needs to be treated after RP (Gleason >7) Decipher and Prolaris may be used to detect metastatic risks.

PCMT=Prostate Core Mitotic Test, RP=Radical Prostatectomy, ART=Adjuvant radiotherapy, SRT=Salvage radiotherapy

Modified from *Narayan VM et.al.*<sup>163</sup>

**Limitation of Tissue-based molecular biomarkers in Pca**  
 Tissue based biomarker assays for the Pca should be used within the context of their limitations. **Racial variations:** Majority of the tissue-based biomarkers test have been validated in white Caucasian men cohorts. Though multiple reports suggests that aggressiveness of Pca varies among races, especially higher risk of Pca and death rates in African American men, they are not included in many validation studies till date.<sup>76,77</sup> Hence, further validation is needed while using the genetic risk-classifiers in African American men.

**Cost:** The important issue of these tests is cost, as many tissue-based tests are not under insurance coverage. This financial burden may prevent their usage for certain populations.  
**Tumour-heterogeneity:** The tumour multifocality & heterogeneity of primary Pca should not be excluded. According to Salami & colleagues, gene expression assays on low-grade Pca biopsy tissue sample might not give proper information on the co-existing aggressive disease.<sup>78</sup> In particular, multifocal low & high- grade Pca foci can show different prognostic expressions within the same case.<sup>79</sup> Recently, studies have also demonstrated the potential

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changes to the GC scores in certain patients depending upon the biopsy core or region of prostatectomy samples analysed, indicating the difficulties of genomic risk classification in tumours having genomic and clonal heterogeneity.<sup>79,80</sup> **mp-MRI incorporation:** Most of the tissue-based biomarker studies were conducted in the pre-MRI era. Therefore, it is not clear whether these biomarkers can provide clinically significant information in the treatment of localized Pca better than MRI guided interventions. However, there are few studies that incorporated mp-MRI for characterization of prostate tumours. They found a strong association of radiomic features (i.e., MRI-prostate findings) with known gene signatures that detect aggressive Pca.<sup>81</sup> This demonstrate that the utility of biomarker test along with mp-MRI might provide a new era of clinical utility for Pca.<sup>81</sup> Moreover, due to the insufficient head-to-head comparison trials, there is no evidence to substantiate the superiority of a single tissue biomarker over other. Hence the choice of choosing the biomarker entirely depends on either clinician's decision, patients or financial factors.<sup>130</sup> While some biomarker tests have showed their potential to impact Pca management by providing guidance for AS, ART or SRT, the prospective studies supporting their impact on Pca disease specific outcome is still lacking.<sup>82</sup> Despite of these limitations, NCCN recommends that Prolaris, Decipher, ProMark and Oncotype DX may be used for stratification of risk in patients with low or favourable intermediate-risk Pca.<sup>130</sup>

### Conclusions and Future Directions

In the past 2 decades, the emerging tissue-based molecular assays for Pca are playing significant role in providing diagnostic, prognostic and predictive information beyond

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standard clinicopathological variables. The biomarkers assays which are discussed in this review are available at different stages and contribute effectively for the diagnosis, prognosis and management of Pca by

- (i) reducing unwanted biopsies (i.e., help the doctors in determining the need for biopsy in those with PSA level of 4.0 to 10.0 ng/mL)
- (ii) enhancing risk stratification
- (iii) providing insight into metastasis rate & cancer specific survival
- (iv) and treatment selection which results in a personalised medicine for Pca patients.

The recent advancement in technology have driven the establishment of comprehensive assays which assess thousands of biomarkers simultaneously. NGS technology can detect novel genes and it has higher sensitivity in quantifying rare variants. National guidelines have already begun to represent these tissue-based molecular assays and this will continue as many novel molecular biomarkers are currently discovered. Further, for any biomarker assay to be implemented into clinical practice, clinical trials should demonstrate their specificity, sensitivity and their potential to improve upon current clinical practices. In this context, the novel Pca biomarkers along with other clinical variables including PSA levels, Gleason grading, staging of disease and imaging would definitely improve the personalized risk assessment and disease management. Finally, these tissue-based assays should be cost-effective and well-designed randomized prospective trials should be validated, for implementation of these assays into routine clinical practice, so that the doctors and physicians both can benefit.

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