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Tissue-based biomarkers in prostate cancer

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Abstract

Introduction—Prostate cancer is a heterogeneous disease. Existing risk stratification tools based on standard clinicopathologic variables (prostate specific antigen [PSA], Gleason score, and tumor stage) provide a modest degree of predictive ability. Advances in high-throughput sequencing has led to the development of several novel tissue-based biomarkers that can improve prognostication in prostate cancer management.

Areas Covered—The authors review commercially-available, tissue-based biomarker assays that improve upon existing risk-stratification tools in several areas of prostate cancer management, including the appropriateness of active surveillance and aiding in decision making regarding the use of adjuvant therapy. Additionally, some of the obstacles to the widespread adoption of these biomarkers and discuss several investigational sources of new biomarkers are discussed.

Expert Commentary—Work is ongoing to answer pertinent clinical questions in prostate cancer management including which patients should undergo biopsy, active surveillance, receive adjuvant therapy, and what systemic therapy is best in the first-line. Incorporation into novel biomarkers may allow for the incorporation of a ‘personalized’ approach to management. Further validation will be required and questions of cost must be considered before wide scale adoption of these biomarkers. Tumor heterogeneity may impose a ceiling on the prognostic ability of biomarkers using currently available techniques.

Keywords

active surveillance; adjuvant therapy; biomarker; genomics; prostate cancer

1. Background

Prostate cancer is the most common malignancy in men in the United States, with an estimated 161,360 diagnosed and accounting for 26,730 deaths in 2017.[1] Worldwide, it is the 5th most common cause of cancer death, although the incidence and mortality differ significantly across the globe; reflecting differences in diagnosis, use of prostate specific

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antigen (PSA) testing, genetic susceptibility, environmental factors, and competing causes of mortality.[2, 3]

Prior to advent of PSA testing, most prostate cancer cases were diagnosed by digital rectal exam or after the development of symptoms associated with advanced disease. The introduction of PSA testing in the late 1980s has resulted in an increase in the number of localized and lower-stage prostate cancer diagnoses.[4] This downward stage migration has been dramatic; such that at present the vast majority of prostate cancer diagnoses are made in the localized setting.[5] Increasing understanding of the biology of prostate cancer led to the acknowledgements that prostate cancer is a heterogeneous disease, with high-risk groups best served by aggressive local therapy, and low-risk groups that reasonably undergo active surveillance (AS).[6-8] The backlash against the rapid adoption of aggressive localized therapy for what was now understood to be often low-risk disease led to the United States Preventive Services Task Force (USPTF) “D” recommendation against PSA testing in 2012. [9] However, this blanket approach neglected to acknowledge that while most prostate cancer was, indeed low-risk disease, prostate cancer specific mortality (PCSM) remains one of the leading causes of cancer-related mortality in the US and given a lack screening, models project a potential increase in PCSM of 13-20%.[1, 10] Earlier this year, the USPSTF released draft recommendations that partially reverse course, instead opting for a more nuanced approach – recommending an individualized decision making process between the clinicians and patients between 55-69 years of age.[11] A more rational approach to the problem of overtreatment is to better understand the biology of the cancer. Until recently, simple clinicopathologic variables (e.g. PSA, Gleason grade, tumor stage) were the only available tools to risk-stratify patients.[12-17] While these rudimentary characteristics perform modestly well, technologic advancements in the field of transcriptomics, genomics, metabolomics, proteomics, and epigenomics, stand poised to greatly change our approach to patients with prostate cancer. These advances will go beyond just stratifying patients according to risk of mortality or metastases, but will play key roles in determining the need for adjuvant therapy and directing therapy in the localized and systemic settings.

While this review will focus on specifically tissue-based biomarkers for prostate cancer, there are numerous other commercially available tests. Serum-based tests like 4K score and Prostate Health Index (PHI) score have improved upon PSA. PHI has been shown to increase sensitivity for cancer detection in those with PSA between 2 and 10ng/ml. [18] PHI is also available for those contemplating active surveillance (AS) as it correlates with those with Gleason 7 or greater. [19] 4K score has been shown in a large multi-institutional prospective trial to demonstrate its ability to predict Gleason 7 or greater prostate cancer prior to biopsy. [20] Urine-based tests include PCA3 which has been shown to have similar increased sensitivity in cancer detection of prostate cancer and predict those with Gleason 7 or greater prostate cancer in AS. [18] [19] [21] These are just a few examples of urine and serum-based tests that are commercially available and certainly have a role in the detection and determination of active surveillance versus treatment aspects of prostate cancer management.

In this review, we aim to review commercially available tissue-based biomarkers for prostate cancer (Table 1) and discuss investigational biomarkers that appear most relevant for the near future. All relevant and available data was considered for each biomarker and will highlight its current approved use in the management of prostate cancer (Figure 1).

2. Currently Available Tests

2.1 Decipher™

Decipher™ (GenomeDx Biosciences, Vancouver, BC, Canada) is a transcriptomic microarray that profiles RNA markers from formalin fixed paraffin embedded (FFPE) prostate specimens.[22] This 22-marker signature is known as the Genomic Classifier (GC) and is relayed as a score from 0 to 1.0 available for both radical prostatectomy (RP) and prostate biopsy specimens. The higher the GC score the higher the risk of tumor aggressiveness. At this time, the marketed test provides for the post-RP GC score the prognostic ability for 5-year development of metastasis and 10-year PCSM. Recently a Decipher™ biopsy test has been validated and provides risk at RP for upgrading (Gleason pattern 4 or 5), 5-year development of metastasis and 10-year PCSM.

The GC signature was developed utilizing 545 RP specimens retrospectively from the Mayo Clinic by comparing specimens from a cohort developing metastasis (n=192) against a control cohort (n=353).[22] A training cohort from both groups included 359 samples tested against the expression of over 1.4 million RNA features and through a performance metric analysis identified the 22-marker GC signature, which included markers for cell cycle proliferation, adhesion and motility, immune modulation and androgen signaling. In an analysis between the metastatic cases and the controls, GC was as a strong independent predictor for the development of metastasis. The performance of this test was then tested in the validation cohort of 186 samples with retained prognostic value. The area-under-curve (AUC) for GC was 0.90 for training cohort and 0.75 for validation cohort, which improved upon an AUC of 0.65 for a 'clinical-only' classifier generated from pathologic Gleason score, preoperative PSA, and pathologic characteristics (positive surgical margins, seminal vesicle invasion, extracapsular extension, and lymph node involvement).

After the initial development and validation study, multiple studies have been utilized to validate the GC score (Supplementary Table 1).[22-30] The initial validation study evaluated 219 RP specimens with high risk of metastasis, defined as a PSA ≥ 20 ng/mL, pathologic Gleason score ≥ 8 , pT3b stage or a Mayo Clinic nomogram score ≥ 10 . [22] The GC score was again the strongest predictor of metastasis on multivariate analysis ($p < 0.001$) with an AUC for GC was 0.79 (95% CI 0.68-0.87) which increased to 0.82 (95% CI 0.72-0.88) with all clinical variables incorporated. The GC score was then investigated to identify three distinct score risk groups with significant differences between them regarding metastasis-free survival (GC risk groups: <0.4 , 0.4 to 0.6 and >0.6). The 5-year cumulative incidence of metastasis was 2.4%, 6% and 22.5% respectively for the GC score risk groups ($p < 0.001$). These cutoffs were further calibrated to create the current GC score risk groups for the marketed Decipher™ test of low (<0.45), intermediate (0.45 to 0.60) and high (>0.60). [23]

Multiple studies have externally validated this biomarker signature to predict clinical recurrence in a community setting,[24] metastatic progression,[22, 25-27] and PCSM after radical prostatectomy.[28, 29] These validation studies have shown good discriminatory ability of the test with the AUC ranging between 0.75 and 0.82. Almost all studies compared the discriminatory ability of the GC score to clinical prognostic models such as the Cancer of the Prostate Risk Assessment postsurgical (CAPRA-S),[14] Stephenson nomogram,[15] and Eggener risk model[16] demonstrating improved prognostic discrimination when combined.[24-26, 28, 29]

Given the validity of GC scores in retrospective RP specimens for postoperative risk stratification, a study was then evaluated to demonstrate the ability of GC to predict metastasis in those men who develop biochemical recurrence (BCR) after RP.[30] Of the 85 men examined who had developed BCR after RP, the GC score was an independent predictor of metastatic progression on multivariate analysis and outperformed all clinical variables and models with an AUC of 0.82 (95% CI 0.77-0.86). This suggests a use of this test to identify those men at risk of progression with the development of BCR and potential need for earlier initiation of salvage therapy.

Despite the results of EORTC 22911, SWOG 8794 and ARO906-02/AUO-AP09/95 demonstrating better oncologic outcomes in men with adverse pathology after RP when treated with adjuvant radiation therapy (aRT),[31-33] the routine use of aRT in these patients remains limited in the United States.[34] Reasons for lack of adherence to oncologic guidelines and level 1 evidence may be speculative but patient preference, physician bias, concern for radiation toxicity, and preference for 'early' salvage radiation at the time of BCR are often-cited rationales. Given the ability of GC score to provide postoperative risk stratification after surgery, there is a critical need to identify patients who are most likely to benefit from aRT and biomarkers like Decipher™ may improve patient selection. An initial study evaluated 188 patients who received post-RP radiation therapy retrospectively from two institutions.[35] Utilizing the GC score to create risk strata, the cumulative incidence of metastasis at 5 years for this cohort was 0%, 9% and 29% for low, intermediate and high-risk groups. When comparing those who received aRT versus those who received salvage radiation therapy (sRT) there was no significant difference in the cumulative incidence of metastasis in the low-risk GC group. For all those with GC score ≥ 4 the incidence of metastasis at 5 years was 6% for aRT compared to 23% for sRT ($p < 0.01$). These findings suggest that the GC may be a predictive marker used to determine which patients will benefit from earlier post-operative intervention (i.e. aRT rather than sRT). A recent study reviewed 512 men treated with RP and compared those that received aRT to those managed with initial observation.[36] In this series from four academic institutions, 112 (21.9%) received aRT and 62 (12.1%) developed clinical recurrence, defined as positive prostatic fossa biopsy or radiographic metastatic development. In multivariate analysis pT3b/T4 disease, pathologic GS ≥ 8 , presence of lymph node involvement and a high GC score were independent predictors of clinical recurrence. These predictors were used to develop a novel nomogram stratified by aRT status to predict 5 and 10-year clinical recurrence. The nomogram demonstrates that those with a risk score ≥ 2 have the greatest benefit from aRT. This new model demonstrates that the utility of G score with other pathologic risk factors identifies that men with more aggressive disease (risk score ≥ 2) have the greatest benefit

from aRT. In fact, up to 70% of men eligible for aRT could be spared the radiation given the findings of this model. An accompanying editorial highlighted the ongoing debate regarding the use of genomic data to guide postoperative management of prostate cancer and aptly concluded that currently these strong independent predictive biomarkers are aiding in our existing knowledge but have yet to reliably link to predictive therapeutic interventions and be validated in external cohorts.[37] A prospective analysis of 2,342 RP patients found positive correlation with the GC score and baseline tumor characteristics, but also found a substantial amount of risk reclassification with the aid of the Decipher™ assay.[38] Assuming all men with pT3 or positive margins received adjuvant RT, this study modeled that the use of the Decipher™ test would reduce usage by nearly 36% theoretically. While not as drastic of a reduction as seen in the aforementioned retrospectively created model,[36] this provides evidence from prospectively collected specimens.

Although the data might be compelling, there is still need to evaluate the how the use of Decipher™ affects clinical decision-making. Three studies surveyed prostate cancer practitioners with patient vignettes and suggest that the use of the Decipher™ r test might change treatment recommendations for aRT/sRT in 31-45% of high-risk patients.[39-41] Using the patterns of treatment recommendation change in the DECIDE study,[39] a decision analytic model was designed to provide further evidence that genomic risk classification might have a clinical benefit for prostate cancer patients faced with decisions for second-line therapy after RP.[42] Another prospective study (PRO-ACT) involved the evaluation of 15 community urologists' recommendations in the management of 146 prostate cancer patients with adverse pathologic features. 42.5% of patients who were initially recommended adjuvant therapy were subsequently recommended observation following Decipher™ testing.[43] Similar results were reported in the interim analysis of the PRO-IMPACT study, a prospective study evaluating the impact of the Decipher™ test on decision making for aRT and sRT following RP. In the 150 patients considering aRT, 89% were recommended observation. After Decipher™ testing, 18% had a change in treatment recommendations. In the 115 patients considering sRT, 58% were recommended observation; after Decipher™ testing, 32% had a change in treatment recommendations. In both the aRT and sRT arms, use of the Decipher™ test was significantly associated with decreased decisional conflict overall for providers and patients in the use of Decisional Conflict Scale ($P < 0.001$). In those with low-risk Decipher™ scores, anxiety was significantly decreased in the aRT arm ($P = 0.02$) and marginally significantly decreased in the sRT arm ($P = 0.05$). This prospective interim analysis of the PRO-IMPACT study demonstrates the clinical utility of the GC score in areas of post-prostatectomy prostate cancer with decisional uncertainty and provides guidance to both patients and providers.[44]

Recently the GC score has been extended to include prostate biopsy specimens. Knudsen et al. demonstrated the feasibility of obtaining transcriptome-wide RNA expression of tissue from prostate needle core biopsy specimens. The genome-wide transcriptomic profiles demonstrated that 95% of features detected in RP specimens could be detected in the biopsy tissues with high correlation ($r=0.96$). When the evaluation was limited to the GC score, correlation between biopsy and RP specimens remained relatively high ($r=0.70$) with approximately 25% of matched biopsy and prostatectomy pairs demonstrating discordant

molecular subtyping.[45] Similarly high rates of concordance (86%) are also reported by Lee et al.[46]

Several studies have demonstrated the clinical efficacy of Decipher™ testing on prostate biopsy tissue (Supplementary Table 2). The first report was a study of 57 patients, of which, 8 developed metastasis and 3 died of prostate cancer. On multivariate analysis, GC score from biopsy was the only significant predictor of metastasis within 10 years after RP with an AUC of 0.80 (95% CI 0.58-0.95).[47] A second biopsy study was run on biopsy specimens with National Comprehensive Cancer Network (NCCN) intermediate or high-risk prostate cancer who received definitive radiation with at least 6 months of androgen deprivation therapy. From the 100 patients evaluated, 18 developed metastases and on multivariate analysis each 0.1 unit increase in GC score was significantly associated with time to distant metastasis (HR 1.36, $p = 0.024$). The AUC for 5-year prediction of metastasis was 0.76 (0.57-0.89) for GC score.[48] The largest study evaluating biopsy specimens included 235 patients of which, 130 underwent definitive radiation and 105 underwent RP [46]. During the study follow-up 34 patients developed metastases and 11 patients died of prostate cancer and on multivariate analysis, each 0.1 unit increase in biopsy Decipher™ score was a significant predictor of metastasis (HR 1.37, 95% CI 1.06-1.78, $p=0.018$). The AUC for 5-year prediction of metastasis was 0.74 (95% CI 0.63-0.83). Mortality analysis was limited due to a low rate of PCSM.[49] These retrospective evaluations provide evidence of the utility of Decipher™ testing on prostate biopsy specimens, however there is clearly a need for further prospective evaluation.

2.2 Oncotype DX®

Oncotype DX® (Genomic Health, Redwood City, CA, USA) has a long-standing history in breast cancer,[50, 51] colon cancer,[52] and more recently has become available in prostate cancer.[53] It is a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) expression assay performed on FFPE tissue from diagnostic prostate needle biopsies. The test measures the expression levels of 12 cancer genes related to 4 biological pathways: cellular organization, stromal response, androgen signaling, and proliferation) as well as 5 housekeeping genes.[53] This genomic assay was developed in men with NCCN low or intermediate-risk prostate cancer diagnosed at biopsy. The expression of the panel genes is used to calculate a Genomic Prostate Score (GPS) ranging from 0-100, correlating with the probability of adverse pathology (AP) at the time of radical prostatectomy defined as high-grade disease (primary Gleason pattern 4 or any pattern 5) and/or non-organ-confined disease (pT3 or greater).

The GPS was developed through a RP discovery study, a prostate biopsy study and an independent validation cohort (Supplementary Table 3). Initially 727 genes were evaluated in the RP discovery study to identify those associated with clinical recurrence-free interval. This subset of genes were then evaluated in the prostate biopsy specimen study to identify those associated with adverse pathology at RP, which was narrowed to include the 12-cancer related genes and 5 reference genes. This was then validated in 359 patients with prostate biopsy specimens and RP specimens as an independent predictor of AP in a cohort of men with low- to low-intermediate-risk prostate cancer ($P = 0.002$). In multivariate analysis, the

odds ratio (OR) for each 20-point increase in GPS was OR 1.9 (95% CI 1.2-2.8, $p < 0.005$) when adjusting for clinical variables including age, PSA, clinical stage, and biopsy Gleason score, OR 1.9 (95% CI 1.3-2.8, $p < 0.005$) when adjusting for NCCN risk group and OR 2.1 (95% CI 1.4-3.2, $p < 0.005$) when adjusting for CAPRA score. This demonstrated that GPS adds additional clinically meaningful predictive value to prior validated risk stratification tools.[54] A second validation study confirmed GPS ability to predict AP at the time of radical prostatectomy, and also demonstrated capacity for predicting BCR with a 20 GPS units increase corresponding to a hazard ratio (HR) 2.73; 95%CI 1.84-3.96; $P < 0.001$).[55]

A recently presented study from Kaiser Permanente retrospectively evaluated 259 patients in a cohort that included low to high-risk patients with 64 prostate-cancer specific deaths and 79 metastatic events. GPS was an independent predictor of the adverse oncologic outcomes, with a 20 GPS unit increase corresponding to a HR 2.0 ($P = 0.007$) and HR 2.75 ($P < 0.001$) for metastases and PCSM, respectively.[56] While this cohort is not entirely reflective of those undergoing active surveillance, it suggests potential future applications of GPS beyond low- and intermediate-risk prostate cancer.

The clinical utility of GPS score for prostate cancer was evaluated with a prospective study of 80 patients with low- to low-intermediate-risk NCCN prostate cancer receiving the Oncotype DX[®] test as compared to a baseline group. Compared to the baseline cohort of risk-group-matched men, the GPS-tested cohort saw a 21% increase in AS utilization, a 10% decrease in RP, and 14% decrease in radiation therapy. This was extrapolated into a cost-analysis that determined the use of GPS led to a decreased aggregate health-care cost.[57] This study is a start to confirming the utility of the Oncotype DX[®] test and as prospective studies are developed and published more confidence can be placed in this test.

2.3 Prolaris[®]

Prolaris[®] (Myriad Genetics, Salt Lake City, UT, USA) is a test run on total RNA extracted from FFPE specimens to generate the cell cycle progression (CCP) score which is calculated from an algorithmic analysis of a 46-gene expression panel including 31 cell-cycle progression genes and 15 housekeeping genes.[58] The CCP score was initially reported in 2011[59] and subsequently validated for prognostic value in both newly diagnosed prostate cancer and those who have undergone RP and are at risk of disease recurrence. The score itself is reported as a value from -3 to +3, such that a unit change in the score corresponds to a doubling in expression level indicating a more aggressive tumor profile.[59] This scoring system has recently been updated to now range from 0 to 6 but the concept and unit change remain the same. It is currently available as both a test of prostate biopsy and RP specimens.

The gene-signature of proliferation was initially tested retrospectively in two separate cohorts to identify their prognostic value in prediction of disease outcome. The first cohort included RP specimens and the other composed of men diagnosed with clinically localized prostate cancer on transurethral resection of the prostate (TURP) managed conservatively. On multivariate analysis, 1-unit change in the CCP score was significantly associated with biochemical recurrence in the RP cohort (HR 1.77, 95% CI 1.40-2.22, $P < 0.001$) and PCSM (HR 2.57, 95% CI 1.93-3.43, $P < 0.001$). In both cohorts, CCP score and PSA concentration were the dominant variables in prognostication of clinical outcomes.[59]

A validation study of the CCP score was performed on an independent cohort of RP patients, which demonstrated its ability to be a strong prognostic indicator for increased risk of developing BCR with each unit increase in score when controlling for other clinical characteristics and clinical predictive models (i.e. CAPRA-S).[60] Given this finding CCP score may be helpful in selecting men for adjuvant therapy after RP. Another study also evaluated RP specimens in a smaller cohort and found increased CCP score was associated with increased risk of systemic disease in those with BCR after RP, which may help identify those most likely to benefit from earlier salvage therapy.[61]

Multiple retrospective validation studies of CCP score have demonstrated conserved predictive value in prostate biopsy specimens as well (Supplementary Table 4).[62-66] These validation studies have demonstrated the prognostic ability of CCP scores in biopsy specimens for development of biochemical recurrence after RP[62, 63] or after radiation therapy.[64] Two studies identified the CCP score as a prognostic indicator of PCSM from prostate needle biopsies from men with clinically localized prostate cancer that were initially managed conservatively for at least 6 months.[65, 66] Limitations of these studies include those of retrospective prostate cancer registry in which exact cause of death might not be known[65, 66] and the use of simulated biopsy from RP specimens in certain cohorts.[62, 63] A meta-analysis and systematic review has demonstrated the strength of CCP scores in identifying BCR and PCSM in multiple scenarios and settings.[58]

Two prospective registries have enrolled over 1500 patients collectively with newly diagnosed prostate cancer from prostate biopsy specimens to prospectively demonstrate the clinical utility of the Prolaris® test. Thus far, analysis of these registries is suggesting a change in treatment recommendations in 47-65% of patients based on biopsy derived CCP scores.[67, 68] Ongoing evaluation will be required to demonstrate the accuracy of these recommendations and demonstrate utility in the post-prostatectomy setting.

2.4 ConfirmMDx®

ConfirmMDx® for Prostate Cancer (MDxHealth, Inc., Irvine, CA, USA) is a tissue-based epigenetic test on FFPE prostate tissue from a 12-core biopsy that quantifies the methylation of promoter regions of the *RASSF1*, *GSTPI*, and *APC* genes in benign prostate tissue, relative to the *ACTB* reference gene. [69, 70] This concept is based on the idea of a field-change in histologically normal tissue adjacent to a focus of adenocarcinoma.[71-73] The assay is used to decide on the need for repeat biopsy in a patient with a previously negative biopsy. The European MATLOC and American DOCUMENT validation studies have demonstrated that the epigenetic assay was an independent predictor of prostate cancer detection, and was associated with an 88-90% negative predictive value.[74, 75] A preliminary, retrospective study of 5 urologic practices reported a significant decrease in the rate of repeat biopsies (4.4%) over the course of an 18-month study period with the use of the ConfirmMDx® assay.[76] This study was limited due to its retrospective nature, small patient cohort size (n=138), and industry-sponsored nature. Currently, there is an ongoing prospective randomized trial (PASCUAL) that has already met enrollment criteria which aims to track the clinical utility of the ConfirmMDx® assay by comparison to standard of care.[77]

2.5 PTEN/TMPRSS2:ERG

ERG transcription factor and transmembrane protease, serine 2 (*TMPRSS2*) fusion is a specific and frequently found chromosomal rearrangement in prostate cancer[78] and appears to represent a genetically distinct subset of prostate cancer characterized by *PTEN* mutations.[79] *PTEN* deletions are a frequent finding in malignancy, resulting in deactivation of PI3K signaling which controls cellular proliferation and growth.[80] Loss of *PTEN* has been associated with poorer outcomes in prostate cancer in several studies[81-84] including a report by McCall and colleagues in which PTEN loss was noted in 23% and 52% of hormone sensitive and refractory tumors, respectively. Loss of nuclear PTEN expression was independently associated with lower cancer-specific survival (HR 0.52, P=0.03).[85] The association between *TMPRSS2:ERG* fusion status and clinical outcomes is not clear with multiple retrospective cohort studies reporting contradictory findings. A meta-analysis of over 5,000 men treated with prostatectomy found no significant association with BCR and PCSM.[86] Other studies found no association with *TMPRSS2:ERG* fusion status and response to androgen deprivation therapy. [87, 88] However, not all studies are negative.[89] Ahearn and colleagues followed over 1000 incident prostate cancer from the Health Professional Follow-Up Study and Physician's Health Study with an immunohistochemical assay for PTEN and TMPRSS2:ERG fusion and found that complete PTEN loss was independently associated with risk of lethal progression. When considered in the context of ERG fusion, any PTEN loss was associated with PCSM in fusion negative cases, but PTEN loss in fusion positive cases was not independently predictive of survival. [90] Thus the presence of *TMPRSS2:ERG* fusion may modify the effects of *PTEN* loss on the disease biology. In spite of these complexities, the PTEN/TMPRSS:ERG test (Metamark, Cambridge, MA, USA) is a commercially available assay. It is marketed for patients with atypical pathology, high-grade prostatic *in situ* neoplasia and those with Gleason score 3+3 or 3+4 to provide risk stratification. ProstaVysion™ (Bostwick Laboratories, Glen Allen, VA, USA) offers a complementary assay that also includes a DNA methylation analysis of *HOXD3*. [91]

2.6 ProMark®

ProMark® (Metamark, Cambridge, MA, USA) is a newly developed commercially available protein-based biomarker panel which includes 8 protein markers using a fully automated, quantitative, multiplex immunofluorescence assay on FFPE. Given the pitfalls of intratumoral cellular heterogeneity, a protein-based panel attempts to provide a more accurate classification of the most aggressive tumor areas that may only consist of a few tumor cells from prostate biopsy specimens.[92] The panel provides a score of 0-1 that reflect the probability of AP at time of RP for those who are NCCN very-low or low-risk considering AS. The initial discovery and validation study provided favorable results and refined the 8-biomarker proteomic assay such that a favorable risk score was 0.33 and a nonfavorable risk score was > 0.80. The validation study met its two co-primary endpoints such that it could provide independent prognostic information to separate favorable from non-favorable pathology and Gleason score 6 from non-Gleason score 6 pathology beyond clinical variables.[93] At this time no other studies have been completed and no prospective evaluations of clinical utility have been published.

2.7 Prostate Core Mitomic Test™

The Prostate Core Mitomic Test™ (PCMT; MDNA Life Sciences, West Palm Beach, FL, USA) is a quantitative RT-PCR assay of mitochondrial DNA used to determine the need for repeat prostate biopsy following an initially negative test. The biologic basis of genomic analysis of mitochondrial DNA is that somatic mutations take place at an accelerated rate in this unique circular and small (16,500 bp) genome due to the accumulation of reactive oxygen species resulting from neoplastic transformation. The mutations in mitochondrial DNA of histologically normal tissue may indicate a ‘cancerization’ or field effect similar to that of methylation studies.[94, 95] In the initial reports of this assay in 101 patients with an initially negative biopsy who underwent repeat biopsy within 1 year demonstrated sensitivity, specificity, and negative predictive value of 84%, 54%, and 91%, respectively. [96] This study demonstrated that the assay was able to predict the presence of a missed tumor in 17 out of 20 men a year before diagnosis. Since this original study was published prospective evaluation of clinical utility has not been published.

2. Investigational Tests

An exhaustive review of all potential tissue-based biomarkers in prostate cancer based on preliminarily positive translational research is beyond the scope of this review, and given the rapidly changing landscape of this field, impractical. We point out some particularly intriguing areas that show promise.

An area of biomarker discovery that is currently lacking a commercially available test is in predicting response to radiotherapy in prostate cancer. The Decipher™ GRID is an ongoing database of RNA expression and the first signature developed from this database is a 24-gene Post-Operative Radiation Therapy Outcomes Score (PORTOS). [97] This signature is able to predict those who should undergo postoperative RT (aRT or sRT) after RP to decrease likelihood of developing metastasis. Biomarkers for primary RT have also been recently investigated as well. A recent validation study demonstrated that biomarkers such as Ki-67 and bcl&bax are independent predictors of BCR after RT ± ADT. [98] While these markers are currently investigational there is clearly still a need to identify actionable biomarkers that can identify those patients who may be best suited for RT.

Recent studies on molecular subtype of prostate cancers have shown promise in defining prognosis and response to therapy. Zhao et al. used the PAM50 classifier, the basis of a commercially available Prosigna test (NanoString Technologies, Inc. Seattle, WA, USA) in breast cancer,[99] to sub-classify prostate cancer into luminal A, luminal B, and basal subtypes. After taking into account standard clinicopathologic variables, they found that luminal B was associated with the worst prognosis, followed by basal, and luminal A subtypes. Intriguingly however, luminal B tumors were the most likely to respond to androgen deprivation therapy.[100]

Gain or loss somatic DNA has been implicated in the activation of oncogenes or inactivation of tumor suppressor genes.[101] Prostate cancer is generally characterized by genomic loss[102] and the prognostic value of copy number variation has been explored in prostate cancer.[103-107] While it appears that copy number variation analysis may have future role

in prostate cancer management, these exploratory analyses require validation and development of clinically meaningful assays.

Advances in genomic sequencing has led to identification of single-nucleotide polymorphisms (SNPs) that appear to have wide-ranging predictive implications in prostate cancer, from disease susceptibility, risk of progression, to therapeutic response.[108, 109] Although numbers SNPs have been identified as associated with a particular disease phenotype, further work will be required to determine the clinical relevance of these and to integrate them into meaningful biomarkers.

4. Other Considerations of Tissue-Based Biomarkers

4.1 Racial Variations

One important aspect to consider is the applicability of the genomic tests in racial minority populations. Numerous reports suggest significant differences between prostate cancers in Caucasians and men of other ethnicities.[110-112] With few exceptions,[55] all the commercially available tests were discovered and validated in a fairly homogenous Caucasian patient population, and studies are needed to determine the applicability of these assays in other demographics. This is particularly important in African American (AA) men who experience a higher incidence and mortality due to prostate cancer.[113] While some of these disparities might be partly attributed to socioeconomic factors, genetic differences likely play significant roles.[114, 115] As an example, Yamoah et al compared biomarkers and known signatures in AA men to Caucasian men. They identified 6 of 20 biomarkers with significantly different expression patterns between the two ethnicities. Evaluation of biomarkers in commercially-available assays demonstrated some discrepancy for prediction of 5-year risk of metastases: GC [AA: AUC 0.78 (95% CI 0.59-0.98), European: AUC 0.88 (95% CI 0.66-0.89)], GPS [AA: 0.89 (95% CI 0.81-0.96), European: AUC 0.78 (95% CI 0.66-0.89)], and CCP [AA: AUC 0.60 (0.36-0.83, European AUC 0.70 (95% CI 0.55-0.84)]. [116] A recent report evaluated Decipher™ test results in AA men and found no correlation with pathologic characteristics, thereby questioning its validity outside of the largely Caucasian cohort it has been validated in.[117] Further work is required to ascertain the validity of these and upcoming biomarkers in AA men and other ethnicities.

4.2 Tumor Heterogeneity

The rapid expansion of predictive biomarkers in oncology belies several significant challenges that are still facing this burgeoning field. A major consideration that might place a ceiling on the prognostic ability of tissue-based tests is that of tumor heterogeneity. A recent study by Wei et al. is illustrative of the challenge.[118] The authors analyzed inter and intratumoral heterogeneity in four patients with prostate cancer by performing next-generation sequencing (whole-exome sequencing, copy number alteration, and RNA sequencing) on multiple prostate cancer foci from within the radical prostatectomy specimens of these patients. The clonal evolution of prostate cancer demonstrated significant early divergence and genomic heterogeneity within each specimen. To demonstrate the clinical impact of this heterogeneity, the authors perform a similar genomic analysis used by Oncotype DX®, Decipher™, and Prolaris® and found significant variability in the scores

depending on which prostate cancer foci was used. This will remain an ongoing challenge for tissue-based biomarkers evaluating needle prostate biopsy specimens as we know just from retrospective evaluation of Gleason score that discordance has been reported between 32-73%. [119]

It should be noted that GPS was developed based on prostate needle biopsy specimens accounting for tumor heterogeneity and multifocality. [45] GPS was therefore evaluated for its predictive value in normal-appearing prostate tissue adjacent to prostate cancer in RP specimens. [120] When evaluated for development of clinical recurrence, the normal tissue with GPS score was shockingly significantly associated with a HR/20 units of 1.25 (95% CI 1.01-1.56, $p = 0.024$) as compared to the tumor (HR/20units was 3.42; 95% CI 2.33-5.63; $P < 0.001$). While the significance of this finding is unclear it certainly suggests a possible field effect in prostate cancer. Further knowledge on this field effect will be crucial when evaluating prostate biopsy specimens that are limited by tumor heterogeneity and multifocality.

4.3 Incorporation with Imaging

Almost all studies that demonstrated the added value of genomic biomarker testing have done so by comparison to standard clinicopathologic features. However, the field of prostate cancer management has grown to include the incorporation of multiparametric magnetic resonance imaging (MRI) for tumor characterization. Few studies have attempted to analyze the added benefit of genomic classifiers in this context. One report did retrospectively evaluate those who underwent a multiparametric MRI and Decipher™ testing but found no significant association between MRI categories with either GPS or likelihood of AP in low-risk patients. [121] Another study attempted to characterize the MRI prostate findings as “radiomics” and sequenced the biopsied areas to establish an association with genomics. There was a strong association of the radiomic features identified with known gene signatures that identify aggressive prostate cancer. [122] This provides evidence that the utility of both biomarker testing with advancements in multiparametric MRI may provide a new wealth of clinical utility for prostate cancer.

4.4 Cost

The current costs of the tissue-based tests have been gathered directly from companies and are summarized in Table 2. Given the listed costs, there is a lack of substantial analysis of the cost-effectiveness of additional marker testing, which is understandable given the rapidly shifting landscape of available assays and the incorporation of prostate magnetic resonance imaging (MRI). A recently published analysis of the cost-effectiveness of the Decipher™ assay following prostatectomy suggested this was more effective and less costly than 100% adjuvant radiation therapy or usual care. [42] A report of insurance claims data in the real-world, clinical setting reported an average savings of \$2,286 per patient with the use of Oncotype DX® to promote an increased utilization of active surveillance. [57] At this time most community practitioners are not routinely utilizing these tissue-based biomarker tests, and companies are providing many of the tests at significantly discounted rates, therefore the true cost has not been evaluated outside of theoretical cost-savings scenarios.

4.5 Regulatory Approval

As of the publication of this review, currently Laboratory Developed Tests do not require FDA approval. The abundance of new biomarker-based tests led the Centers for Medicare & Medicaid Services (CMS) to partner with Molecular Diagnostic Services Program (MoIDX™) to establish coverage and reimbursement for molecular diagnostics tests. A summary of MoIDX status is listed in Table 2. Medicare decision makers tend to adhere to the MoIDX recommendations, as do many private insurers. At present, a number of the biomarkers have received MoIDX approval or draft coverage, including Decipher™, Prolaris®, Oncotype DX®, and ConfirmMDx. Full information is available at: [www/palmettogba.com/MoLD](http://www.palmettogba.com/MoLD).

5. Conclusion

A number of tissue-based biomarker assays have been developed in the last half-decade to aid clinical decision making in several scenarios in prostate cancer – including in newly diagnosed men considering active surveillance and in men considering adjuvant therapy. Retrospective data suggests these assays improve upon the prognostic ability of standard clinicopathologic risk grouping, however no prospective randomized trials have confirmed the utility of these tests. Unfortunately, such clinical trials are unlikely to occur. Additionally, there are justified concerns about increasing the burden of healthcare costs and the cost-effectiveness of these assays. Despite these concerns, the decreasing costs of high-throughput testing and multiple tests currently in development suggest that such assays will become a mainstay in the management of prostate cancer for the next generation of urologists and their patients.

6. Expert Commentary

Prostate cancer is an incredibly heterogeneous disease. Although the past two decades has seen ever more nuanced interpretation of clinicopathologic variables, we are still striving to answer pertinent clinical questions including which patients should undergo biopsy, active surveillance, receive adjuvant therapy, and what systemic therapy is best first-line. Advances in new technologies such as next-generation sequencing and their incorporation into novel biomarkers is welcome news in our continuing attempts at developing a ‘personalized’ approach to the management of this disease. In this review we discuss commercially-available tissue-based tests and those ideas that are most promising for future tests. Those tests that have been validated have generally demonstrated improved prognostic capability than use of standard PSA levels, tumor stage, and Gleason scoring. Yet, there is still significant room for improvement. Questions of tumor heterogeneity may impose a ceiling on the prognostic ability of tissue-based biomarkers using currently available techniques. How to integrate this growing body of tests into competing, or as the case may be, complimentary technologies such as multiparametric MRI will be a further challenge. Further validation of new and existing biomarkers will be required in different racial/ethnic groups. Additionally, in a disease with such a high prevalence, we must consider the added costs of these tests. Is it really worth \$3-4,000 to be marginally more accurate in predicting a patient is safe to undergo active surveillance for very-low risk prostate cancer? In many

cases, the biomarker tests will only tell us what we already know. The scale of technological advancement will likely drive the costs of current tests downward, but they will only be replaced by newer and possibly more expensive ones. Despite these questions, it is clear that such tests will be further incorporated into the management of prostate cancer in the near future.

7. Five Year View

In oncology and specifically in prostate cancer there are many clinical dilemmas that cause significant consternation amongst clinicians and their patients. Questions about the need to biopsy, need to re-biopsy, choice of therapy, need for adjuvant or salvage therapy remain unanswered despite highly complex and nuanced interpretation of patient data based on standard clinicopathologic variables. The development of high-throughput measurement of biological molecules, the so-called “-omics” disciplines is poised to change much of how prostate cancer care is delivered. The National Academy of Medicine has put forth recommendations on the development and validation of future candidate omics-based tests[123] and given the prevalence and market share of prostate cancer, it is undoubtable that much work will be devoted to this field. Further discovery and validation of existing genomic biomarkers will likely lead to incorporation of these assays into oncologic guidelines, as they have been for breast cancer. The development of novel therapeutics for prostate cancer will also require the development and validation of corresponding biomarkers. Just as the use of abiraterone and enzalutamide versus taxane-based chemotherapy for metastatic prostate cancer might be guided by the presence of androgen receptor splice variant AR-V7,[124, 125] biomarkers that reflect defects in DNA repair might guide treatment with novel drugs such as the poly(ADP-ribose) polymerase (PARP) inhibitors.[126, 127] In parallel with tissue-based assays, ‘liquid-biopsies’[128] in the form of assays to detect cell-free DNA, circulating tumor cells,[129, 130] RNAs, cell-free proteins, peptides, and exosomes will also be developed. The difficulties facing clinicians will likely be how to incorporate the deluge of new tests in a meaningful and cost-effective way. The difficulties facing future investigators will be to validate their findings and, ideally, compare them to other assays to demonstrate their utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference annotations

* Of interest

** Of considerable interest

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Key Issues

- Advances in high throughput technology is rapidly changing the landscape of modern prostate cancer management with the development of multiple commercially-available biomarkers
- Several tissue-based assays have demonstrated independent prognostic ability in scenarios ranging from the need to re-biopsy a patient, appropriateness of active surveillance, or the possibly utility of adjuvant therapy for advanced disease
- Statistically, many of these assays perform better than standard clinicopathologic variables (PSA, Gleason score, pathologic characteristics of the tumor)
- At present, no tissue-based assay has been shown to have clinical utility in prospective randomized controlled trial for prostate cancer and the cost-effectiveness remains to be determined in most cases
- In the near future, patients will have a wide array of biomarkers available to help guide their decision-making process in prostate cancer. Clinicians should be familiar with the strengths and limitations of these tests.

Figure 1. Utility of Tissue-Based Biomarkers in Prostate Cancer Management

Table 1

Tissue-Based Prostate Cancer Tests

Test	Manufacturer	Type	Test Measure	Study Outcomes	Clinical Use
Decipher™	GenomeDx Biosciences	22 RNA markers	GC Score: 0-1.0	Predicting risk of recurrence, metastasis and prostate cancer specific mortality following RP	Determining if adjuvant therapy versus salvage therapy should be pursued
Decipher™ Biospy	GenomeDx Biosciences	22 RNA markers	GC Score: 0-1.0	Predicting risk of metastasis after primary treatment based on biopsy tissue	Stratification of patients prior to primary treatment
Oncotype DX®	Genomic Health	12 cancer genes + 5 housekeeper genes (RNA)	GPS Score: 0-100	Predicting risk of adverse pathology and recurrence after RP based on biopsy tissue	Counseling men on active surveillance vs. treatment in newly diagnosed low or low-intermediate risk prostate cancer
Polaris®	Myriad Genetics	31 cell-cycle progression genes + 15 housekeeper genes (RNA)	CCP Score: 0-6	Predicting risk of recurrence and prostate cancer specific mortality following RP. Predicting risk of recurrence and prostate cancer specific mortality after primary treatment based on biopsy tissue	Counseling men on active surveillance vs treatment in newly diagnosed low- or low-intermediate risk prostate cancer
ConfirmMDx®	MDxHealth	Methylation of 3 genes	Likelihood of prostate cancer %	Prostate cancer detection with prior negative biopsy	Decision for repeat prostate biopsy with prior negative biopsy
PTEN/TMPRSS2:ERG	Metamark	Mutations	Risk Groupings	Predicting risk of adverse pathology or recurrence after RP based on biopsy tissue	Counseling men on active surveillance vs treatment in newly diagnosed low- or low-intermediate risk prostate cancer Counseling for possible re-biopsy in atypical prostate tissue
ProMark®	Metamark	8 protein markers	ProMark Score Score: 0-100	Predicting risk of adverse pathology or Gleason score >6 on RP based on biopsy tissue	Counseling men on active surveillance vs treatment in newly diagnosed low- or low-intermediate risk prostate cancer
PCMT™	MDNA Life Sciences	Mitochondrial DNA mutations	-	Prostate cancer detection with prior negative biopsy	Decision for repeat prostate biopsy with prior negative biopsy

Abbreviation Key: RP = radical prostatectomy, GC = Genomic Classifier, GPS = Genomic Prostate Score, CCP = Cell Cycle Progression, PCMP = Prostate Core Mitomic Test

Table 2
Coverage and Approval of Most Common Tissue-Based Prostate Cancer Biomarkers

Test	MolDx Status	Cost
Oncotype DX®	Endorsed for clinically very low low risk patients (Favorable Intermediate pending)	\$4,520 Medicare: covered Financial Assistance: available Maximum out-of-pocket: \$100 or discussed with patient
Prolaris®	Endorsed in clinically low and favorable Intermediate risk patients	\$3,400 Medicare: covered Financial Assistance: available Maximum out-of-pocket: \$375 or discussed with patient
Decipher™	Endorsed in the post-prostatectomy setting (Post-biopsy [Decipher™ Biopsy] pending)	\$2,990 Medicare: covered Financial Assistance: available

Abbreviation Key: MolDx = Molecular Diagnostic Services Program