Review Article

Genetic signatures on prostate biopsy: clinical implications

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Abstract: Prostate cancer management remains a topic of intense debate given favorable disease-specific outcomes for the overwhelming majority of patients. Consensus regarding the role of prostate-specific antigen (PSA) screening among the general male population has been elusive, in part due to questions surrounding the reliability of prostate biopsy results and the difficulty identifying patients with localized disease who will one day progress. Until recently, the Gleason score has been the best prognostic indicator available but issues with interobserver reliability and biopsy sampling error fuel therapeutic indecision and likely lead to over- or under-treatment. Prostate cancer genomic testing takes several forms and offers additional information for predicting the clinical behavior of a tumor. Urine based assays take advantage of the prostate's anatomic location in the urinary tract to look for genetic material that is associated with finding prostate cancer on a subsequent biopsy. Prostate biopsy tissue samples give direct access to the cancer genome and provide information beyond what is ascertainable using a microscope. If the Gleason score provides a snapshot of the current state of a given prostate tumor, then these tissues based genomic tests offer a window in its future. Several commercially available tests are currently available but only a small number have been FDA-approved thus far. Even the best products have only provided modest gains in prognostic accuracy over existing clinical risk stratification tools, however, this is a key step in the right direction that is nearly a decade behind other malignancies (i.e., breast). There is published data to suggest that these tests can alter physician practice patterns, but to what degree they will ultimately alter clinically significant outcomes remains to be seen.

Keywords: Prostate cancer; genetic testing; biopsy

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Introduction

Prostate cancer is the most common non-cutaneous malignancy in men with 161,000 new diagnoses in 2017 (1). It is a very heterogeneous disease with variable outcomes, depending on disease stage and grade. Due to serum prostate-specific antigen (PSA) screening of men at risk, most patients are diagnosed with indolent disease that does not impact quality of life or life expectancy and is therefore managed expectantly [active surveillance (AS)]. However, some patients will die of their cancer if left untreated.

Different clinical and pathologic factors, such as PSA level, tumor stage and grade, and presence or absence of lymph node metastasis significantly correlate with the prognosis of the underlying disease. One of the most significant prognostic parameters is the Gleason biopsy grading system (2), comprising five histologic grades of prostate adenocarcinoma: Gleason pattern 1 to 5. Pattern 1 represents the best differentiated, i.e., it is the closest in histologic appearance to benign prostate gland, while pattern 5 is the least differentiated and most aggressive type of prostate adenocarcinoma. The sum of the two

most prevalent Gleason patterns equals the Gleason score (GS). Nowadays, a GS of ≤5 (primary + secondary Gleason pattern) is rarely described, as Gleason patterns 1 and 2 are histomorphologically almost indistinguishable from normal prostate tissue. The most recent modification to this pathological schema is the introduction of grade groups based on the GS, reorienting the numerical system to more accurately reflect the aggressiveness on a scale from 1 to 5 (3). The grade group allows for better discrimination at the lower grade of prostate pathology, specifically for GS 7 which can now be separated into grade group 2 (3+4) or grade group 3 (4+3). This simple modification holds the potential to help improve practices regarding prostate cancer management by highlighting the subtle differences in pathology that might have an impact on prognosis (4).

Microscopic assessment and quantification of Gleason grade is somewhat subjective, and there is evidence that random prostate biopsies might under-sample, and hence under-stage and -grade the existing cancer. Therefore, additional genetic and genomic tools to better characterize the cancer biology are being studied. Genetic testing in prostate cancer (i.e., the study of individual up- or down-regulated genes that have been found to be associated with the grade of the tumor) is important as family predisposition is responsible for about 5–15% of all prostate cancers (5-7). In contrast, genomic studies assess all genes in the genome and their interactions that can directly influence the biology and behavior of the tumor itself; studying these activities can then help to more objectively and independently assess underlying risk of undetected prostate cancer, as well as the tumor grade in patients found to have cancer on prostate biopsy.

The reason why this is so important is because most very low and low risk prostate cancers are, depending on the individual circumstances, age, and life expectancy of the patient, not necessarily being treated nowadays, but closely monitored (i.e., AS). In contrast, localized but more aggressive (intermediate or high-risk) cancers are usually being referred to definitive curative treatment options. Distinguishing the different types of cancers through histological, genetic and genomic analyses therefore helps counseling and reassuring patients, and guiding patients toward the appropriate and necessary management route.

This review is aimed at giving an overview of the different types of genetic and genomic tests that exist to determine the risk of the presence of non-indolent prostate cancer on prostate biopsy, i.e., to determine the necessity for the performance of a prostate biopsy or to repeat a biopsy, and to examine prostate biopsy tissue for further characterization of the cancer biology to aid in the decision-making process with regards to surveillance or treatment in clinical practice.

Prostate cancer genetics

The genetic drivers underlying prostate carcinoma oncogenesis provide information useful for diagnostic, prognostic, and therapeutic purposes. The early efforts within this advanced diagnostic area used immunhistochemical (IHC) staining to determine the level of expression of a specific gene within a pathological specimen. One such study found that expression of p27, a cell cycle regulator, negatively correlated with biochemical recurrence (BCR), with tumors staining at <45% showing a 2.5-fold increase in the risk of BCR (8). Although IHC is still widely used for prostate cancer research and diagnosis [i.e., AMACR, androgen receptor (AR), PSA, etc.], the widespread availability and dwindling costs associated with genetic sequencing techniques offers a more precise assessment of a tumor's mutational profile.

The landmark publication of the Cancer Genome Atlas for prostate carcinoma in 2015 used whole-exome sequencing to develop a molecular taxonomy of 7 distinct subgroups of genetic mutations into which 74% of all tumors examined would be classified (9). For example, overexpression of the E26 transformation-specific (ETS) family of oncogenes, regulated by androgen-regulated stimulation, is present in over 50% of tumors. This overexpression was found to be due to chromosomal rearrangement resulting in fusion of the promoter *TMPRSS2* to an ETS gene, most often *ERG* (10). There are no commercially available therapeutic agents available to date that have been able to take advantage of *TMPRSS2-ERG* rearrangement, however, its high overall prevalence has led to the development of several prognostic tests based on its detection, as will be discussed in subsequent sections.

There are more recent efforts, beyond simply identifying genetic mutations present in prostate cancer, which seek to evaluate clonal evolution within the lifespan of a tumor (11-13). This is particularly important when considering ideal tests for early detection and prognostication, since a mutation present in metastatic disease may not be present in the beginning stages of localized disease. In these circumstances, when such a mutation is detected in a patient otherwise lacking clinical signs or symptoms of distant spread, it may indicate a need for more aggressive treatment,

Table 1 Commercially available non-tissue based genomic tests

Trade name	Genomic marker(s)	Specimen type	Clinical setting	FDA approval
SelectMDx	*HOXC6, DLX1*	Post-DRE urine	Initial or repeat biopsy	No
Progensa PCA3 assay	PCA3 noncoding RNA	Post-DRE urine	After prior negative biopsy	Yes
Mi-Prostate Score (MiPS)	*TMPRSS2:ERG* gene fusion	Post-DRE urine	Initial or repeat biopsy	No
ExoDx Prostate (IntelliScore)	*TMPRSS2:ERG* gene fusion and PCA3 noncoding RNA in exosomes	Voided urine	Initial or repeat biopsy	Yes

DRE, digital rectal exam.

independent of other factors (i.e., PSA, GS, etc.).

Pre-prostate biopsy decision making

The availability of genetic testing comes at the earliest stages of prostate cancer diagnosis when the decision of whether or not to proceed with a prostate biopsy is being contemplated. *Table 1* shows an overview of non-tissue based genomic tests. Given the intense scrutiny surrounding over-diagnosis in prostate cancer, among other malignancies, there is a demand for increased sensitivity and specificity in our screening tests. Like all commercially available genetic testing for prostate cancer, these tests are prognostic, offering insight into which patients might benefit most from a more aggressive therapeutic strategy rather than identifying actionable mutations for targeted treatment. In reviewing the available literature on this topic, it becomes clear that the development of each test has followed a very similar pathway: (I) identifying a cohort of patients with clinically significant prostate cancer or certain adverse clinicopathologic features; (II) genetic sequencing of prostatectomy specimens, prostate biopsies, and/or bodily fluids in these patients to identify mutations, or combinations of mutations, that appear more frequently than in matched benign controls; (III) experimental validation of the newly identified biomarkers to ensure feasibility, and, finally; (IV) clinical validation in a cohort with sufficient follow-up to draw significant conclusions regarding the prognostic ability of the test.

SelectMDx

The SelectMDx test was developed by MDxHealth (Irvine, CA, USA) as a urinary biomarker collected in the first voided urine specimens after digital rectal exam of the prostate (DRE). In the exploratory analysis, the biomarker discovery phase identified 39 potential prostate cancer biomarkers using gene expression profiling data from the transurethral resection of prostate (TURP) or radical prostatectomy (RP) specimens of 133 men (14). The pathology within this cohort included normal prostate, benign prostatic hyperplasia (BPH), low-grade prostate cancer (GS ≤6), high-grade prostate cancer (GS ≥7), castration-resistant prostate cancer (CRPC), and metastatic prostate cancer. Using a separate cohort of men undergoing initial or repeat prostate biopsy for elevated PSA, the researchers identified a panel of 3 genes (*HOXC6*, *TDRD1*, and *DLX1*) that served to increase the AUC for predicting presence of GS ≥7 when compared to PCA3 or PSA alone (AUC 0.77 *vs.* 0.68 *vs.* 0.72, respectively). When clinically validated, the *HOXC6* and *DLX1* expression profiles had the best combination of performance (AUC 0.73) and analytic reproducibility (15). In combination with other clinicopathologic features (PSA, PSA density, age, family history, and any prior prostate biopsy results) the AUC improved to 0.90. It is worth noting that this was only slightly better than the predictive ability of the clinicopathologic features alone which had an AUC of 0.87, though the difference was found to be statistically significant (P=0.018). To date, SelectMDx is still under consideration for FDA approval.

PCA3

Prostate cancer antigen 3 (PCA3) is a noncoding RNA from chromosome 9p21-22 that was found to be overexpressed by 10- to 100-fold in the tissue of men with prostate cancer (16). The Progensa PCA3 score available from Hologic, Inc. (Marlborough, MA, USA) calculated by measuring the level of PCA3 mRNA as a ratio to PSA mRNA within first voided urine sample after brief prostate massage, and has been shown to improve upon the predictive ability of serum PSA for finding any prostate cancer at the time of prostate biopsy (17-22). The critical

validation study was conducted in 1,140 men within the placebo arm of the REDUCE trial who provided post-DRE urine samples prior to their 2- and 4-year per protocol prostate biopsies. Given that all men were required to have a negative prostate biopsy at study entry this represented an entirely repeat biopsy cohort (17). While the median PCA3 score was higher for GS ≥7 (49.5) compared to GS 6 (31.8), there was no statistically significant difference between the AUC for high- and low-grade disease. PCA3 score performed the best when used as part of a model including clinical factors (PSA, percent free PSA, prostate volume, age, and family history) with an AUC of 0.753 compared to 0.612 for PSA alone when the cutoff value was set to >35 when the PSA was between 2.5 and 10 ng/dL. FDA approval is limited to the repeat biopsy setting in large part due to the results of subsequent studies that have shown unacceptably high rates of missed GS ≥7 cancers when applied to biopsy-naïve patients; upwards of 13% on initial biopsy (18,19). Recently, Wei *et al.* (22) argued that PCA3 can still be useful for ruling-in an initial biopsy with a higher cutoff value of >60 when the PSA results are otherwise equivocal.

MiPS

By combining the most commonly identified genetic mutation found in prostate cancer, the *TMPRSS2*:*ERG* gene fusion (10), with PCA3, the University of Michigan MLab (Ann Arbor, MI, USA) introduced the Mi-Prostate Score (MiPS) which measures the expression of each within a urine sample. Unlike PCA3 alone, MiPS has shown utility both in the initial and repeat biopsy setting by outperforming PSA alone, as well as the ERSPC and PCPT risk calculators (23,24). Sensitivity was measured at 91% for detecting GS ≥7 in a cohort that was comprised largely of biopsy-naïve patients (79%), with an AUC 0.842 if combined with the ERSPC risk calculator (23). In an AS cohort, increasing MiPS, measured as a continuous variable, was significantly associated with negative biopsy outcome, GS 6, and GS ≥7 cancer (25). Though it is not yet FDA-approved, this test holds potential for reducing the number of unnecessary biopsies by approximately 50% while maintaining good discrimination for high-grade disease (24,26).

ExoDx Prostate (IntelliScore)

Urinary exosomes are small, bilipid walled vesicles that are shed by cells and can be found in various bodily fluids, including prostatic secretions, while containing highly enriched levels of mRNA compared to total cell RNA (27,28). The Prostate (IntelliScore) from Exome Diagnostics (Waltham, MA, USA) is an FDA-approved test which capitalizes on the fact that both *TMPRSS2*:*ERG* and PCA3 are found at levels nearly 100-fold higher within urinary exosomes than post-DRE urine specimens (28). Clinical validation comes by way of a 2016 study (29) comprised of a training cohort (n=499) and validation cohort (n=1,064) with the specific aim of predicting detection of GS ≥7 with the specific aim of predicting detection of GSn cohort ng/dL. The AUC for exosomes plus clinical parameters (PSA, age, race, and family history) was 0.73 compared to 0.55 for PSA alone. The test showed high sensitivity and negative predictive value (NPV) (92% and 91%, respectively) and led to a 27% reduction in the number of prostate biopsies, however, the tradeoff was that 8% of GS ≥7 cancers would be missed using this method.

It is worth noting that while all of the aforementioned genetic biomarker tests have demonstrated statistical improvement in prostate cancer detection and/or discrimination of higher grade disease, there have been no published studies to date on the cost-effectiveness of these approaches. In real-world scenarios, insurers will demand to see not only clinical efficacy but also a concomitant change in practice patterns that result in significant cost-savings.

Prostate biopsy based genetic testing

There are definite advantages to non-invasive genetic testing with regards to patient comfort and inconvenience, however, tissue samples are still required for more detailed genomic analysis at present time (*Table 2*). The prostate biopsy based tests require a small section of diagnostic material to be sent to a central laboratory for analysis, relying upon the services of local pathologists to select an appropriate sample of tissue. The raw data generated from genetic testing is then interpreted in the context of the validation cohorts for each specific product and then reported to the physician and patient in an easy to read manner that typically provides some type of "score" with a corresponding percentage of patients who developed a specific outcome (i.e., BCR, metastasis, overall survival).

ConfirmMDx

The ConfirmMDx test was also developed by MDxHealth (Irvine, CA, USA). It is a diagnostic tissue-based test to

Table 2 Commercially available prostate biopsy tissue based genomic tests

Trade name	Genomic marker(s)	Specimen type	Outcome of interest	FDA approval
ConfirmMDx	Epigenetic hypermethylation of *GSTP1, APC, RASSF* genes	Non-malignant prostate biopsy tissue	Presence of prostate cancer after negative biopsy	No
OncotypeDx Genomic Prostate Score (GPS)	12 prostate cancer related genes, 5 reference genes	Malignant prostate biopsy tissue	10-year prostate cancer specific mortality, 10-year risk of metastasis	No
Polaris	31 cell-cycle progression related genes; 15 reference genes	Malignant prostate biopsy tissue	10-year prostate cancer specific mortality, 10-year risk of metastasis	No
Decipher	22 genomic markers yielding "genomic classifier" (GC)	Malignant prostate biopsy tissue	10-year prostate cancer specific mortality, 5-year risk of metastasis, presence of GS 4 or 5 on RP specimen	No
Prostate Core Mitomic Test	Subregion of mitochondrial genome	Non-malignant prostate biopsy tissue	Presence of prostate cancer after negative biopsy	No

detect occult prostate cancer in histopathologically negative prostate biopsy tissue, and is mainly based on the "field-effect" (i.e., changes in tissues surrounding cancerous lesions) of epigenetic hypermethylation changes of three genes (*GSTP1*, *APC*, *RASSF*).

Originally described by Lee *et al.* in 1994, epigenetic hypermethylation mutations of regulatory sequences of the pi-class glutathione S-transferase gene (GSTP1) were found to be highly prevalent only in prostate cancer, but not in benign tissue (30). A subsequent cohort study assessing epigenetic changes in prostate cancer tissue confirmed these findings, and was used for the development and optimization of an epigenetic multiplex assay based on the above three genes (31). Two subsequent retrospective clinical validation studies confirmed the predictive accuracy of hypermethylation changes on prostate cancer-negative biopsy tissue for the presence of significant prostate cancer on repeat biopsy: In the MATLOC (Methylation Analysis to Locate Occult Cancer) study, 483 men in the UK underwent ConfirmMDx testing on initial biopsy; this analysis found a sensitivity of 68%, a specificity of 64%, and a NPV of 90% for the absence of cancer on repeat prostate biopsy samples. Interestingly, all cases with a GS of ≥8 tested positive for epigenetic hypermethylation (32). The DOCUMENT (Detection Of Cancer Using Methylated Events in Negative Tissue) trial, a validation study of the MATLOC analysis in a US cohort of men, confirmed these findings, and hypermethylation mutations of regulatory sequences of GSTP1 were again found to be the most accurate predictor for the presence of prostate cancer on repeat biopsy after initial negative biopsy (33).

Detecting areas that test positive for hypermethylation in the identified genes aids in the identification of biopsies with false-negative histopathological results, and might help in the decision-making whether a repeat biopsy is warranted. Consequently, ConfirmMDx test might help decrease the number of unnecessary repeat prostate biopsies. It was incorporated into the NCCN guidelines in 2016 (34).

OncotypeDx Genomic Prostate Score (GPS)

Since 2004, Genomic Health, Inc. (Redwood City, CA, USA) has developed a variety of validated multi-gene real-time polymerase chain reaction (RT-PCR) assays to evaluate the individual underlying cancer biology in patients with different types of cancers, i.e., breast, colon, prostate. The Oncotype DX Prostate Cancer Assay platform is able to examine small amounts of prostate tissue for the expression patterns of 12 cancer-related genes, representing 4 distinct biological pathways in prostate tumorigenesis (stromal response: *BGN*, *COL1A1*, *SFRP4*; cellular organization: *FLNC*, *GSN*, *TPM2*, *GSTM2*; androgen pathway: *FAM13C*, *KLK2*, *AZGP1*, *SRD5A2*; proliferation: *TPX2*). It also includes 5 reference genes (*ARF1*, *ATP5E*, *CLTC*, *GPS1* and *PGK1*). Together, this 17-gene assay has been shown in analytical validation studies to be able to reproducibly calculate the GPS, a score that in numerous subsequent clinical validation studies was confirmed to correlate with adverse pathologic findings on RP specimens and was also able to

predict risk of BCR (35-37). Even in non-cancerous regions in prostatectomy specimens of patients that underwent removal of the prostate for biopsy-proven cancer, the GPS showed similar performance characteristics as in cancerous lesions themselves, suggesting that a "field effect" within the prostate is present as well, as was described earlier (38). A prospective observational study of community-based urology practices in the US examining the use of GPS in the decision-making process of patients with newly-diagnosed prostate cancer is currently being conducted. Preliminary data from this study on the first 258 enrolled patients after one year of follow-up found that men were more likely to choose AS if GPS was used, in comparison to a control cohort in which GPS was not used (62% *vs.* 40%). Overall, 55% of patients who underwent GPS elected AS and were still on AS at one year, compared to 34% of the control group. The rate of patients that continued on AS at the one-year time point was similar between the two groups (89% *vs.* 86%) (39).

In summary, Oncotype DX GPS helps assess underlying tumor biology on prostate biopsy tissue, and has a significant impact in the decision-making between initial treatment or surveillance options for patients with newly-diagnosed prostate cancer. Whether it also has a significant benefit during the course of surveillance remains to be established.

Prolaris

Cancer cells exhibit higher levels of expression from certain cell cycle related genes when compared to their benign counterparts leading to unregulated cellular proliferation, a hallmark of cancer oncogenesis (40). Expression levels from 874 candidate genes were quantified in HeLa cells at various time points as they progressed through the cell cycle in some of the early pioneering work in this field of study. When compared to benign cells, the HeLa cells showed higher levels of those involved in DNA replication and chromosomal segregation. The Prolaris test from Myriad Genetics (Salt Lake City, UT, USA) has taken advantage of these observations to develop a 46-gene cell cycle progression (CCP) score for predicting adverse outcomes from prostate biopsy samples (41).

This test was originally developed and validated for use on RP specimens as a way to improve risk stratification post-operatively and predict BCR (42,43). Of those 874 candidate genes identified by Whitfield *et al.* (40), 31 were selected for use in the CCP score due to their increased level of expression in patients with the adverse outcomes of interest, while an additional 15 genes that exhibited constant expression were used as internal controls (43). In this post-RP setting the 10-year risk of mortality based on AUA risk category alone was 4.8%, but adding the CCP score widened the discriminatory ability of the test from 1.8% to 6.7%. The authors then took this concept a step further and used the CCP score on prostate biopsy specimens where it was expected to make a greater impact in the decision-making process, before a definitive therapy had been carried out. Using core-needle biopsies from 349 "conservatively treated" men with prostate cancer from a UK registry, the CCP score was able to significantly predict prostate cancer mortality with a HR 1.65 per one unit increase (44). Furthermore, the 10-year cancer specific mortality risk could be reported as a percentage based on CCP score, ranging from 19% for score of <0 up to 74.9% for scores of 3 or greater. Subsequent studies have further validated these findings with HR for disease specific mortality on multivariate analysis (controlling for GS, PSA, clinical stage, etc.) ranging from 1.47 to 2.17 per unit increase (41,42). Prolaris offers the potential of identifying men at both increased and decreased risk of prostate cancer death, as shown by Cuzick and colleagues where 14% of the CAPRA low risk (<4% 10-year cancer specific mortality) patients were reclassified into higher-risk groups and 44% of CAPRA intermediate-risk patients were downgraded to low-risk, based on the results of the CCP score (41).

The ability of the Prolaris test to change clinical practice has been published more than any other prostate biopsy based genomic test currently available (45-47). In one such study, physicians were surveyed regarding their pre- and post-test treatment decisions for 331 patients diagnosed with prostate cancer on needle biopsy (45). The Polaris test influenced physicians in at least some way for 97.8% of patients and led to an overall reduction in the therapeutic burden, as measured by change from interventional to non-interventional treatment strategies for this predominantly low-grade (80.1% grade group 1 or 2) cohort. Though the authors found 80.2% concordance with the ultimate treatment delivered, the conclusions drawn from a survey-based study should be considered with a great deal of skepticism. In a larger cohort, Shore *et al.* (47) found that the there was no significant difference in the recommendation for nonintervention between pre- and post-Prolaris test result treatment plans. Rather, what was observed was a "reshuffling" of patients from the intervention and non-intervention groups based on

the Prolaris test, creating no net difference. The rate of RP declined by 34% from pre- to post-test, however, attributing such a change to the test alone is impossible given the numerous other potential factors that could be implicated in the ultimate treatment decision. While such studies may be useful in garnering opinions of physicians using these tests under "real-world" conditions, more objective endpoints (cost-benefit, survival, etc.) are required to truly evaluate clinical impact.

Decipher

The Decipher test (GenomeDx, Vancouver, BC, CA) was originally conceived as a prostatectomy based tissue assay for predicting early metastasis in men with high-risk features (pT3, positive margins, GS ≥8, PSA >20 ng/mL) (48). The initial discovery and validation cohort specifically included men with regional and/or distant metastasis within 5 years of BCR for comparison against those with no evidence of disease recurrence (PSA or other signs) for at least 7 years post-operatively (49). Whole transcriptome RNA microarray and statistical regressions yielded 22 genomic markers from both coding and non-coding regions of the genome to be included in the so-called "genomic classifier" (GC).

Clinical validation in RP specimens showed that the Decipher score outperforms CAPRA-S for the prediction of early metastatic disease, though the absolute difference is small (c-index 0.75 *vs.* 0.72) (50). Using the same cohort of patients, Klein *et al.* (48) applied Decipher to the pre-RP biopsies and compared it to the NCCN risk stratification model (34). The Decipher score alone had a c-index of 0.80 for predicting 10-year post-RP risk of metastasis compared to 0.75 for NCCN risk group category alone; this increased to 0.88 when both Decipher and NCCN were combined. In another biopsy study, each 10% increase in the Decipher score correlated with an increase in risk of metastasis with a HR of 1.53 (51). Prostate cancer specific mortality was not a primary endpoint of this study. However, low- and intermediate-risk Decipher scores correlated with a 0% chance of prostate cancer death within 5-year of receiving definitive therapy.

Prostate Core Mitomic Test

The Prostate Core Mitomic Test (PCMT) from MDNA Life Sciences (West Palm Beach, FL, USA) is based on the "tumor field effect" of prostate cancer and specifically focuses on alterations of the mitochondrial genome (52). Preliminary studies showed that benign tissue obtained from RP specimens containing foci of prostate cancer exhibited equal mutation rate of the mitochondrial genome from all areas sampled, regardless of the pathologic diagnosis (53). This test is commercially available but not FDA-approved as of yet, with minimal published data available. The clinical validation study used men with negative prostate biopsies who underwent repeat biopsy within 1 year, finding a NPV of 91% with AUC 0.75 on the second specimen (54).

Limitations

Definite statistical improvements in prostate cancer prognosis have been reported widely throughout the literature, however, these tests are far from ideal and carry with them a few important limitations to keep in mind when applying them in clinical practice. The biopsy procedure itself is, in fact, a limitation to the application of prostate cancer genomics, much in the same way it limits traditional pathologic diagnosis. When biopsy specimens are compared to the final RP specimen, the tumor grade will be discordant for 25% to 50% of patients, mainly due to biopsy sampling error (55-57). This means that the pathologist will often not be presented with a sample from the most relevant region (i.e., highest GS) based on 12 tissue cores alone. Genomic testing may provide an advantage in this regard since genetic alterations likely precede phenotypic changes detectable on microscopy, but the degree to which all tumor foci are genetically linked is still debatable (polyclonality *vs.* field cancerization) (36,52,58,59). Thus, questions about the role of sampling error persists in prostate-biopsy based genomic tests.

Interobserver variability in Gleason scoring has been well documented but genetic testing offers the theoretical promise of true objectivity because it no longer depends on human interpretation (56,60). However, the tissue-based tests require representative sections to be selected by local pathologists and sent to a central location for expression profiling, introducing some degree of error between individuals. Furthermore, the genetic tests themselves reportedly yielded non-diagnostic results in approximately 10–20% of samples for each of the validation studies previously mentioned (15,17,29,36,48,61).

Finally, perhaps the most significant factor limiting clinical utility of prostate cancer genomics is the lack of available cost-effectiveness data. There have been several

studies examining self-reported changes in physician practice habits (39,45-47,62), but no large-scale analysis of the real-world impact that these tests are having on patient outcomes. There is a hypothesized benefit from gaining additional prognostic information but, on the other hand, without evidence of such benefits (i.e., improved survival, reduced overall cost of treatment, etc.) it is unclear how the currently available products are going to fit into contemporary and future prostate cancer management.

Future directions

Genetic sequencing is no longer restricted to highly specialized laboratories at major universities and the widespread availability of inexpensive next-generation sequencing platforms is sure to generate additional valuable data that will impact the way we care for prostate cancer. The literature is full of publications containing potential future biomarkers touting improvement in risk stratification and higher diagnostic accuracy (63-65). Ultimately, the ideal prostate biopsy based test would not only offer prognostic insights but also provide therapeutic targets to improve disease outcomes. In much the same way as the AR-V7 splice variant identifies men who are unlikely to benefit from enzalutamide therapy (66) or *SPOP* mutation predicts response to PARP-inhibitors (67), genomic information from biopsy specimens could determine suitability for radiotherapy versus RP (68-70).

Conclusions

Prostate cancer management has only begun to integrate genomic biomarkers despite their use for over a decade in other cancers, specifically breast. The tests available at the present time reflect the unmet need for better discrimination for adverse disease outcomes in clinical risk-stratification models and aim to reduce the burden of overtreatment while identifying patients who would benefit most from early, aggressive intervention. The current landscape represents an incremental improvement over the previously available tools based on analysis of retrospective cohorts, but prospectively generated outcomes data will take many more years to mature.

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Footnote

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