

# Genetics and Genomics: Discovery, Validation, and Utility of Novel Tools for management of Prostate Cancer

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## **Abstract**

Genomics is the science of how genes influence human health and disease states. It differs from traditional genetic screening in that the transcriptional activity (or other markers) in full panels of related genes are studied. Compared to simple genetic testing, assessment of expression levels in a panel of genes provides a more nuanced and holistic understanding of genetic modulation of human disease. Genomic testing may be used to great effect in resolving controversial questions on detection and treatment of prostate cancer. Genomic tests are currently in use for numerous facets of prostate cancer care, including screening, biopsy, and treatment planning. The clinical validity (predictive capacity) of these assays has been well established; studies on clinical utility (i.e. usefulness of these tests in guiding patient/provider decisions) have shown promising results. Men's health specialists should be familiar with the role genomic testing will play in contemporary management of prostate cancer.

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## Introduction

Prostate cancer is the most commonly diagnosed solid tumor and the cause of over 27,000 deaths annually in the United States.<sup>1</sup> Globally, there were an estimated 900,000 new cases of prostate cancer in 2008 with over a quarter of a million prostate cancer deaths, making prostate cancer the 6th leading cause of cancer death in men worldwide.<sup>2</sup> The number of men diagnosed and dying of prostate cancer is expected to increase with the ageing of the global population.

While no one can dispute the real world toll of prostate cancer mortality, the practice of screening and subsequently treating prostate cancer has been mired in controversy for decades. There is evidence to suggest that screening for, diagnosing, and treating prostate cancer is associated with an aggregate benefit in terms of mortality.<sup>3</sup> The sheer number of prostate cancer patients drives the high absolute rate of prostate cancer deaths; however, as this tends to be a disease of ageing men, the majority will die of other causes.<sup>4</sup> The diagnosis of prostate cancer requires a tissue diagnosis obtained typically at prostate biopsy; this procedure is generally low risk but carries some potential for complications such as bleeding and infection.<sup>5</sup> If cancer is diagnosed and determined to be localized, curative treatments may include radiation, brachytherapy, and surgery (radical prostatectomy). These all carry substantial potential for morbidity including urinary incontinence, erectile dysfunction, and bowel disturbances.<sup>5,6</sup> Therapy without curative intent (e.g. hormone ablation) has less upfront risk but may lead to substantial declines in quality of life due to loss of testosterone activity.<sup>7,8</sup> The cost of treatment, and management of the inevitable adverse events associated with cancer treatment, also poses a substantial burden on healthcare systems.<sup>9,10</sup>

Due to controversies surrounding the net benefit to risk ratio of prostate cancer screening, the United States Preventative Services Task Force<sup>11</sup> and more recently the Canadian Task Force on Preventive Health Care<sup>12</sup> have recommended against routine screening for prostate cancer with serum PSA testing. These recommendations have raised concerns that as a result, many men with potentially curable prostate cancer may not be screened and later present with lethal and incurable disease.<sup>13</sup>

The traditional prognostic tools used to risk stratify newly diagnosed prostate cancer include biopsy Gleason Score, percent positive tissue on biopsy, clinical stage, serum Prostate Specific Antigen (PSA), and various derivations of PSA (e.g.

free PSA, PSA density, etc).<sup>14</sup> Decisions on treatment are also made based on age and patient comorbidities; given the generally indolent nature of many prostate cancers it is frequently recommended that treatment be reserved for those men who have at least 10 year life expectancy.<sup>14,15</sup>

Existing clinical endpoints can inform decision making, particularly when combined into nomograms and/or summary scores.<sup>16-18</sup> However, there are substantial limitations to existing prognostic tools. Biopsy Gleason score frequently does not correspond to surgical Gleason score.<sup>19</sup> This variability is in some cases attributable to limitations of prostate sampling during biopsy; these limitations may be addressed but never completely eliminated.<sup>20</sup> PSA levels fluctuate for a number of reasons other than malignant potential of the tumor including benign enlargement, inflammation, and infection.<sup>21</sup> Development of additional metrics/tools to more carefully select patients for screening and treatment is a priority.<sup>13</sup> The overarching intent is to help providers identify patients who carry significant risk of clinically relevant prostate cancer. The development of such tools may be targeted at guiding decision making before and/or after prostate cancer diagnosis.

A particularly exciting development in risk stratification for men concerned about, or diagnosed with, prostate cancer is genetic and genomic based testing of cancer tissues. Molecular assays (including genomic tests) have recently been incorporated into the National Comprehensive Cancer Network (NCCN) guidelines for management of low risk prostate cancer as an option to consider for men with clinically localized prostate cancer and at least 5-year life expectancy.<sup>16</sup> It is likely that molecular diagnostics will play an expanding role in management of prostate cancer in the future. In this review we will discuss some of the basic underpinnings of genetic testing for prostate cancer with a focus on assays/protocols that are currently in clinical use. We will also briefly mention non-genomic tests for prostate cancer.

## Methodology

Peer reviewed publications on genomic and other assays for diagnosis and management of prostate cancer were identified from company websites. Reference lists were consulted for additional publications of interest and for additional information on background concepts/principles. (this search was done in June 2015. Manuscripts published after this cut off point were not included).

## Genomic Testing

Most commonly, the genetic material obtained for genomic analysis is Ribonucleic acid (RNA). RNA is transcribed from genes, which are composed of Deoxyribonucleic Acid (DNA). Following transcription RNA is translated by ribosomes into proteins that can have intracellular and extracellular effects. The amount of RNA transcribed from a given gene protein is a proxy measure of the genes effect on cellular metabolic activity.<sup>22</sup> While RNA is commonly used in genomic testing, some non-RNA tests (e.g. epigenetic modifications to DNA structure) may also be considered genomic in nature.<sup>23,24</sup>

There is variability in the quality and quantity of RNA recovered from tissue samples. This heterogeneity complicates genetic analysis of biopsy material when comparing between specimens. Hence, it is standard practice to normalize genetic expression to a series of “housekeeper genes” that are expressed in nearly all cells at similar levels. By indexing the expression of genes of interest to these housekeeper genes a normalized expression pattern is determined, permitting comparison across groups while controlling for variations in expression patterns related to tissue processing.<sup>25</sup>

Aside from relative expression, how reliably given genes can be recovered and their association with known pathways of carcinogenesis must be considered when selecting genes for further development. There are numerous genes that are up- or down-regulated in cancerous tissue compared to controls; selection of genes for further development must therefore focus on those genes which are highly relevant to the particular cancer’s biology AND can be reliably recovered from available tissue.<sup>25,26</sup>

## Development of Genomic Based Tests

The development of genetic testing follows a prescribed series of steps. The first of these is typically described as “discovery” and the second as “validation”. The validation phase may be further subdivided into assessment of analytic validity, clinical validity, and finally clinical utility.<sup>27</sup>

Discovery involves the detection of genes that are expressed variably in tissues of interest, typically cancer tissues versus non-cancer tissues.<sup>22,28</sup> Alternatively, variations in expression

of genes between cancer tissues may be useful in differentiating cancers that will behave more or less aggressively.<sup>26,29-31</sup> Genes with a known relationship to endpoints of interest may be selected for incorporation in an assay; alternatively, gene expression micro-array may be used to efficiently screen a large number of candidate genes as an initial step.<sup>28</sup> Genes that are consistently upregulated or downregulated in comparison to normal tissues are identified as candidates for further study.<sup>22,28</sup>

**Clinical validation** is the confirmatory step in which the results of a genomic test are verified to be associated with a meaningful clinical outcome.<sup>32</sup> It is a critical step that allows clinicians to utilize the novel test with confidence that the assay will provide useful prognostic and/or predictive information for their patient population of interest.<sup>22</sup> Additional important elements of clinical validation include sensitivity and specificity, positive and negative predictive values, genetic penetrance, variations in genetic frequencies between racial or other groups, and confirmation that the test provides information over and above what can be gleaned from existing clinical parameters.<sup>27</sup>

After the assay is optimized an important confirmatory step is **analytic validation**, defined as testing of assay parameters to verify that accurate and precise measurements can be consistently obtained.<sup>27</sup> In the case of genomic testing this typically involves confirming that genes of interest can be reliably identified and their expression quantified from the tissue source of interest despite variations in test conditions.<sup>25,28,32</sup> Specific considerations include accuracy, precision, reproducibility, and robustness (ability to provide meaningful data with variable input).<sup>27</sup>

The final step in development is assessment of **clinical utility**, which is the real world usefulness of the test in influencing patient care. Clinical utility is founded in large part on comparative effectiveness research, in which the assay is studied in the context of real world clinical settings with consideration of cost, actual changes in patient outcome, and risk/benefit ratio.<sup>27,32</sup> This step is critical; regardless of validity only assays that positively influence care decisions for patients are of genuine use in clinical medicine.<sup>27,28,32,33</sup>

TABLE 1, Genomic Tests for Prostate Cancer

Tissue Source	Assay Characteristics	Target Population	Validated Endpoint	Test Report	
<b>Tests Used in Selection for Biopsy</b>					
Confirm <sup>®</sup> MDx	Benign Prostate Biopsy Tissue	Methylation status of Glutathione S-Transferase pi 1 (GSTP1), Adenomatous Polyposis Coli (APC), and Ras association domain family member 1 (RASSF1)	Men with prior negative prostate biopsy	Likelihood of negative result on repeat prostate biopsy	Negative vs Positive Result reported for each biopsy core
Progenesa <sup>®</sup> PCA3	Post-Prostate Massage Urine	mRNA for Prostate Cancer Antigen 3 (PCA3)	Men with prior negative prostate biopsy	Likelihood of negative result on repeat prostate biopsy	PCA 3 score (higher scores imply greater chance of aggressive prostate cancer)
<b>Tests used to determine treatment versus surveillance</b>					
Oncotype Dx <sup>®</sup> Prostate	Highest Gleason Grade from Prostate Biopsy Cores (GS 3+3=6 and 3+4=7)	17 Gene PCR Assay assessing genes related to Proliferation, Androgen Signalling, Stromal Response, and Cellular Organization	Men with NCCN Very Low, Low, and Intermediate (Gleason 3+4) prostate cancer on biopsy	Adverse Pathology (p T3+, Prostatectomy Gleason Grade > 4+3) Biochemical Recurrence	Genomic Prostate Score (range 0-100) with higher scores implying greater risk of adverse pathology at RP
Prolaris <sup>®</sup>	Highest Gleason Grade from Prostate Biopsy Cores	46 Gene PCR assay assessing genes related to Cell Cycle Progression	Men with biopsy proven prostate cancer	Prostate Cancer Specific Mortality at 10 years	Cell Cycle Progression Score (range ~ -3 to 3) with higher scores implying greater risk of mortality at 10 years
<b>Tests to determine risk of disease progression</b>					
Decipher <sup>®</sup>	Highest Gleason Grade from Prostatectomy Specimen	22 Gene Assay assessing genes related to Cell Proliferation, Cell Structure, Differentiation, Motility, and Cell Cycle Progression	Men with pathologically proven high risk prostate cancer	Metastatic Disease after Prostatectomy Biochemical Recurrence and Metastasis after Radiation Therapy	Genomic Classifier Score (0-1) with higher score implying greater risk of metastasis
Prolaris <sup>®</sup>	Highest Gleason Grade from Prostatectomy Specimens	46 Gene PCR assay assessing genes related to Cell Cycle Progression	Men after radical prostatectomy for prostate cancer	Prostate Cancer Specific Mortality at 10 years	Cell Cycle Progression Score (range ~ -3 to 3) with higher scores implying greater risk of mortality at 10 years
<b>Miscellaneous Tests</b>					
Know Error <sup>®</sup>	Buccal Swab	Assessment of Short Tandem Repeats	Men with positive biopsy	Coinfirmation of specimen provenance	DNA Match vs. DNA-Mismatch vs. Contamination with Foreign DNA

## Genomics in Prostate Cancer

Genomics holds tremendous promise to guide decision making in men before and after diagnosis of prostate cancer with the goal of helping patients and providers make more informed decisions about their care. Specifically, genomics may be useful in helping men determine their risk of clinically significant cancer before they undergo biopsy.<sup>23,24,34</sup> Testing may also provide utility for physicians and their patients diagnosed with prostate cancer make a decision about immediate treatment or active surveillance.<sup>26,30,35,36</sup> Tests have also been developed to help men make decisions about the benefit of adjuvant therapies after definitive treatment.<sup>29,31,37</sup> The growing relevance of genomic and other molecular based assays has been highlighted by the National Comprehensive Cancer Network's latest recommendations on the evaluation of low risk prostate cancer.<sup>16</sup> A brief overview of genomic/genetic tests for use in prostate cancer is presented in Table 1.

### Tests Used to Aid in Patient Selection for Repeat Prostate Biopsy After Initial Negative Biopsy

#### *ConfirmMDx*

ConfirmMDx (MDxHealth, Irvine, CA) is a prostate tissue assay that is designed to aid patients at risk for prostate cancer with a prior negative biopsy in decisions about repeat biopsy. The test relies on assessment of methylation (an epigenetic phenomenon) in the promoter region of three genes: glutathione S-transferase pi 1 (GSTP1, plays a role in DNA detoxification), adenomatous polyposis coli (APC, plays a role in apoptosis and cell migration), and Ras association domain family member 1 (RASSF1, plays a role in cell cycle regulation).<sup>24,38</sup> The ratio of methylated genes is normalized to beta-actin copies.<sup>39</sup> The assay is based on the concept of "field cancerization" in which detectable changes (in this case epigenetic) occur in benign tissue adjacent to cancerous tissue.<sup>40</sup> By assessing for epigenetic changes in benign tissue information is provided on the likelihood of malignant disease that was not sampled on the initial biopsy.

Two confirmatory studies of this assay have been performed. The first study (Methylation Analysis to Locate Occult Cancer, MATLOC) was performed in 498 negative prostate biopsy specimens from men who had repeat biopsies within 30 months of the initial biopsy.<sup>24</sup> The second study (Detection Of Cancer Using Methylated Events in Negative Tissue, DOCUMENT) was performed in 350 men negative prostate biopsy specimens

from men who had repeat biopsies within 24 months of the initial biopsy.<sup>23</sup> The negative predictive value of the test in these reports was 88-90%; the assay provided prediction of biopsy outcome independent of classical predictors of prostate cancer including atypical pathology (e.g. high grade prostate intraepithelial neoplasia).<sup>23,24</sup> A clinical utility study in 138 men with prior negative biopsy who subsequently had testing with ConfirmMDx indicated that with median follow up of 9 months just six (4.3%) with negative results had subsequent repeat biopsies, all of which were negative. The principle driver of biopsy in these six patients appeared to be PSA greater than 4 ng/mL.<sup>41</sup> Longer term follow up and assessment of outcomes in men who deferred biopsy will be of interest for future utility studies.

#### *Progenesa® PCA3 Assay*

The Progenesa® PCA3 Assay (Hologic Inc, Bedford, MA) is a urine based test for expression of the Prostate Cancer Antigen 3 (PCA3, which is upregulated in prostate cancer) in post-prostate massage voided urine. The test detects non-coding mRNA for PCA3 in the urine, normalized to the expression of the PSA mRNA and multiplied by 1000 to permit standardization across assays.<sup>42</sup>

The test is utilized to determine the need for repeat biopsy in a patient with prior negative biopsy but persistent concern about occult prostate cancer. The higher the PCA3 score the greater the likelihood of prostate cancer on repeat biopsy. A variety of cut-offs for a positive PCA3 have been proposed, ranging from 10-35.<sup>42</sup> Higher cut-off scores reduce the risk of false positive tests but run the risk of missing potentially relevant cancers.

Numerous studies on PCA3 have produced generally positive results although controversy remains about the usefulness given variability in selected cut off scores. In a meta-analysis of 11 studies, Luo et al reported a range of positive predictive values from 39-86% and negative predictive values ranging from 61-90% in men who had repeat biopsies (i.e. at least two) for prostate cancer.<sup>34</sup> A cut off of 20 for PCA3 produced an AUC of 0.846, which was the optimal result in this meta-analysis. Importantly, the vast majority of evidence indicated that PCA3 was superior to PSA in predicting outcome of prostate biopsy in the setting of prior negative biopsy. Starting with two established nomograms which included total

PSA, estimated prostate volume, prior negative biopsy, normal/abnormal DRE, and/or abnormal transrectal ultrasound, addition of PCA3 slightly improved the performance of an established nomogram for prostate cancer risk in a multivariable analysis of 708 Dutch men referred for repeat screening for prostate cancer after negative biopsy. A more modest but still significant superiority of the model including PCA3 was noted in the sub-set of men who had PSA < 3.0 ng/mL. However, cancer in this series was detected with a sextant biopsy rather than the more standard 10-12 core biopsy now widely in use.<sup>43</sup> Furthermore, the authors used a PCA3 score of > 10 as positive result, a low threshold level for this assay.

The PCA3 has been criticized for relatively low predictive capacity in detection of clinically significant prostate cancer (i.e. Gleason > 7 with extensive malignant tissue on biopsy cores).<sup>14</sup>

## Tests Used to Aid Patient Selection for Definitive Therapy

### *Oncotype*<sup>®</sup> DX

The *Oncotype* DX<sup>®</sup> prostate cancer assay (Genomic Health, Redwood City, CA) is a 17 gene panel designed to aid in risk stratification for patients with NCCN very low, low, and intermediate (Gleason 3+4 only) risk prostate cancer. The assay assesses mRNA expression of 12 cancer related genes from the androgen signaling, stromal response, cellular organization, and proliferation cellular pathways. Expression of these genes is normalized to that of 5 housekeeper genes to calculate a Genomic Prostate Score (GPS).<sup>26</sup> The *Oncotype* DX<sup>®</sup> prostate cancer assay has been optimized so as to yield interpretable results with 1 mm or less of paraffin-embedded fixed prostate cancer tissue. Importantly, a high level of precision was also verified in a series of experiment using varying degrees of RNA input (as low as 5 ng). Further studies were conducted to determine precision and reproducibility of a given specimen using alternative operators, instruments, and times. For a 10 ng standard input the standard deviation for precision on the overall GPS score was 1.86 (on a 100 unit scale); the similar value for reproducibility was 2.11 units on the 100 unit scale.<sup>25</sup>

The *Oncotype* DX<sup>®</sup> prostate cancer assay was initially developed from two subsets of a database of prostate cancer patients from a single institution. The first development study was conducted to assess outcomes in 441 patients who underwent

prostatectomy for prostate cancer in the 1980s to 1990s. Of these men, 111 experienced biochemical recurrence (BCR) and 45 died of prostate cancer. Prostate tumor tissue was obtained from two distinct areas of the prostate tumor, specifically the dominant Gleason pattern and the highest Gleason pattern. In cases where only a single Gleason pattern was present, samples from two distinct areas of tumor were assayed. Two hundred and eighty-eight (288) genes were associated with biochemical recurrence/metastasis and expressed in both tissue segments. To confirm that the genes of interest could be recovered from prostate biopsy tissue, a second development analysis was conducted on biopsy specimens from 167 men with low/intermediate risk prostate cancer who went on to have a prostatectomy at the same institution in the late 1990s to 2000s. The endpoint of interest for this second development study was adverse pathology (AP), present in 58 (35%) of these men and defined as extracapsular extension (pT3+) or high Gleason score (primary pattern 4 or any pattern 5). This definition of adverse pathology corresponds to the updated Epstein criteria which indicate minimal risk of both BCR (within 5 years) and mortality (within 10 years) for men with organ-confined Gleason 3+3=6 or Gleason 3+4=7 disease.<sup>44,45</sup> Fifty-eight genes were associated with likelihood of AP.<sup>26</sup> The twelve genes finally selected for the GPS were determined based on consistency, analytic performance, and association with pathways known to be relevant in prostate cancer prognosis.<sup>26</sup>

The *Oncotype* DX<sup>®</sup> GPS algorithm was then tested in a validation study in a population of 395 men with NCCN low and intermediate risk prostate cancer who underwent radical prostatectomy. The validation endpoint in this study was AP as defined above; 123 men in this study had AP. On multivariable analysis a 20 point change in GPS was associated with an OR for AP between 1.9-2.1 after adjusting for classic clinical parameters, CAPRA score, and NCCN risk classification.<sup>26</sup> The combination of GPS and existing parameters was shown to better predict surgical pathology outcome compared to models using clinical parameters alone based on Receiver Operator Characteristic curves and decision curve analyses.

A second validation study was conducted in 402 men treated with RP at one of two military medical centers (median follow up over 5 years). After adjustment for NCCN risk group, each 20 point increase in GPS was found to correspond to a number of clinically relevant outcomes including AP (HR 2.7

after adjustment for age and NCCN risk group), BCR (HR 2.65, defined as PSA 0.2 ng/mL x 2), and metastasis (HR 3.8, univariable analysis only). Importantly, 20% of this cohort was of African-American race and there was no significant difference in mean GPS between races; it is implied that the genetic signature assessed by *Oncotype Dx*® is applicable to both Caucasian and African-American men.<sup>30</sup> Use of the GPS improved upon the prognostic power of the NCCN risk group for AP; the combination of NCCN and GPS yielded an AUC of 0.69 for AP compared to an AUC of 0.60 with NCCN alone.

The clinical utility of the *Oncotype DX*® Assay was demonstrated in two studies published in 2015. In the first, urologists at three sites provided feedback on pre- and post-GPS treatment recommendations in 158 patients with clinically low risk prostate cancer. In 39% of cases GPS predicted a biological risk different from what was predicted from NCCN alone. This was associated with an 18% change in treatment recommendation with a 24% relative increase in recommendations for active surveillance compared to pre-GPS treatment recommendations. In 85% of cases participant urologists reported increased confidence in their post-GPS recommendation.<sup>46</sup> The second study enrolled patients from 15 urologists and examined actual treatment decisions in 124 clinically low risk prostate cancer patients who utilized GPS compared to 87 similar patients who made treatment decisions in the 1 year period prior to commercial release of the GPS assay. Utilization of AS or watchful waiting (WW) was higher in the GPS group (67%) compared to 43% in the control group (relative difference 56%). As expected, the difference in utilization of AS/WW was greatest in men with post-GPS risk in the very low category. However, the rate of AS/WW utilization was higher for all post-GPS risk categories (very low, low, and intermediate).<sup>47</sup>

### ***Prolaris*®**

*Prolaris*® (Myriad Genetics, Salt Lake City, Utah) is a 31 gene panel designed to aid patients in selecting definitive therapy for prostate cancer and need for adjuvant therapy after radical prostatectomy. The assay assesses the expression of genes related to cell cycle progression (an important component of malignant potential) in malignant cells and normalized to the expression of 15 housekeeping genes; these data are used to calculate a Cell Cycle Progression (CCP) score which typically ranges between -1 to 4 or higher.<sup>36</sup> The genes were selected

from a gene expression database and found to be expressed in anonymous formalin-fixed paraffin embedded prostate cancer specimens.<sup>48</sup> The *Prolaris* test has been utilized in tissue obtained from 2-4 mm segments of formalin fixed prostate biopsies as well as prostate specimens after radical prostatectomy.<sup>29,36</sup>

An early validation study of CCP score studied archived tissues in men who had undergone prostatectomy or transurethral resection of the prostate in the 1980s-1990s. mRNA was recoverable from the prostatectomy specimens in 366 of men who underwent RP. After multivariable analysis a hazard ratio of 1.77 for biochemical recurrence (PSA > 0.3 ng/mL) per unit increase in CCP was reported. In this same study CCP was found to predict time to death from prostate cancer in men with CaP incidentally diagnosed during TURP.<sup>48</sup> A contemporary validation study of CCP in whole prostate specimens utilized a single dominant tumor focus in 413 men who had undergone RP. Per unit increase in CCP score the HR for disease recurrence (PSA > 0.2 ng/mL or salvage treatment) was 1.7 and 2.0 after adjustment for CAPRA-S and a variety of other known prognostic variables, respectively. No men with CCP scores less than -1 (n=44 or 11%) had BCR within 5 years whereas 50% of those with CCP > 1 (n=14 or 3%) had recurrence, irrespective of CAPRA-S scores.<sup>29</sup>

The initial biopsy validation study of *Prolaris*® was conducted in 349 men (90 of whom died of prostate cancer over almost 12 years follow up) with pretreatment PSA and prostate cancer diagnosed by needle biopsy between 1990-1996. In this cohort, CCP was found to be an independent predictor of prostate cancer mortality; a one unit change in CCP was significantly associated with greater HR for prostate cancer specific mortality in men with PSA > 4 ng/mL and Gleason score of 8 or higher; interestingly, a one point change in CCP did not produce a statistically significant change in HR for prostate cancer specific mortality in men with either Gleason 6 or 7 disease AND/OR PSA < 4 ng/mL in this study.<sup>36</sup> A second validation study in biopsy tissue was conducted using the largest tumor focus identified on specimens from 582 patients treated at 3 centers; of note, 283 of these samples were “simulated” biopsies taken as needle cores immediately after RP due to absence of biopsy tissue at one of the centers. The HR for biochemical recurrence in the total cohort was 1.47 per unit increase in CCP score; for metastatic disease the HR was

4.19 after adjustment for known prognostic factors.<sup>35</sup>

In 2015, another validation study was conducted to evaluate the predictive capacity of CCP alone and as part of an integrated prognostic tool with CAPRA score<sup>49</sup> in a pre-specified algorithm to produce a clinical-cell-cycle-risk (CCR) score.<sup>49</sup> CCP scores were generated from prostate needle biopsy tissue from 585 British men diagnosed with prostate cancer between 1990 and 2003. Similar to prior studies, after adjustment for other variables a one-point increase in CCP was associated with a HR of 1.76 for prostate cancer death within 10 years. The integrated CCR showed greater prognostic capacity than either CAPRA or CCP alone; a one-point increase in CCR was associated with a HR of 2.17 for prostate cancer death within 10 years. Similar to prior publications, the 95% confidence interval for predictive capacity of one-point change in CCP included a HR of 1.0 or less for CAPRA of 7 or less, Gleason 3+3, and PSA less than 4 ng/mL.<sup>50</sup> Although the result did not quite attain the standard alpha for significance of 5% the results trended towards significance; a slightly larger sample size may have met criteria for strict statistical significance.

A clinical utility study from an ongoing registry reported on 305 men with prostate cancer (87% of whom had low or intermediate risk disease) who had used a CCP score to make treatment decisions. The number of men who were initially recommended to undergo definitive treatment (as opposed to active surveillance or watchful waiting) declined from 164 to 103. On the other hand, the number of men who were initially recommended to consider active surveillance or watchful waiting declined from 141 to 108. In addition to distinction based on active treatment versus surveillance, the authors reported that for 198 (65%) of these men there was a change in “therapeutic burden” based on a scale in which prostatectomy was considered most burdensome with radiation therapy, brachytherapy, androgen deprivation, active surveillance, and watchful waiting representing progressively less burdensome options. In 93 of 116 (80%) of verified cases the treatment decision was congruent with physician recommendation.<sup>51</sup>

Prolaris has also been investigated for use in prognostication for patients undergoing external beam radiation therapy. Based on biopsy specimens from 141 men with mean follow up of 4.8 years post therapy, the CCP score was independently predictive of BCR (defined as treatment for recurrent prostate

cancer or serum PSA > 2 ng/mL above nadir level) with a hazard ratio of 2.11 per unit increase in CCP score. CCP score was also predictive of prostate cancer specific mortality at the 10 year time point (HR 3.77 per unit increase in CCP for mortality within 10 years). Interestingly, the predictive power of CCP for biochemical recurrence was greater in the first 5 years post-treatment compared to years 5-10.<sup>52</sup>

## Tests Used to Aid Patient Decision Making for Adjunctive Therapy

### *Decipher*<sup>®</sup>

Decipher<sup>®</sup> (GenomeDx Biosciences) is a 22-gene assay that provides information on metastasis risk in patients with high risk pathology who have undergone radical prostatectomy. The assay is performed on RNA, including many non-coding RNAs, extracted from prostate cancer tissue selected from the highest grade present in prostatectomy specimens.<sup>31,37</sup> Specific biological processes mediated by these RNAs include cell proliferation, cell structure, differentiation, motility, and cell cycle progression.<sup>31</sup> From these data a Genomic Classifier (GC) score is generated; the GC ranges from zero to one with higher scores indicative of greater risk of metastasis.<sup>31</sup>

The Decipher assay was designed based on 545 prostate specimens from men who had undergone prostatectomy, including 213 who had metastatic disease. The score remained the most important independent predictor of metastatic disease and prostate cancer specific mortality after multivariable analysis.<sup>31</sup> The assay was then validated in a subset of 219 patients with metastatic disease from a cohort of over 1,000 patients who had high risk features (e.g. PSA > 20 ng/dL, Gleason 8 or higher, seminal vesicle invasion) prior to RP. The assay was the strongest predictor of metastasis with 22% of men with GC scores greater than 0.6 progressing to metastasis within 5 years. No patients with Gleason 6 cancer had scores greater than 0.6. Conversely, in men with Decipher GC score of less than 0.4 metastasis occurred in 14% and 23% of men with Gleason 7 and Gleason 8-10 disease, respectively.<sup>31</sup> Incidentally, almost half of these patients had adjuvant hormonal ablation.

Decipher was used in a subset analysis of 85 men with BCR (PSA > 0.4 ng/mL) after radical prostatectomy. Just 8% of patients with low risk scores (<0.4) developed metastasis within 3 years of BCR versus 40% of men with high risk scores.

On multivariable analysis GC score remained significantly predictive of metastatic disease. Of note, a higher proportion of men in the metastasis group had androgen deprivation therapy within 90 days of surgery (57% versus 24% compared to the no metastasis group) and after 90 days of surgery (59% versus 38%).<sup>53</sup>

Decipher has been of particular interest in prediction of metastasis in high risk patients. To this end Decipher was used in a study of 169 men with high risk (PSA > 20ng/mL, extraprostatic extension, and/or Gleason Score > 8), node-negative prostate cancer who had undergone RP with no adjuvant therapy. Decipher GC score was the strongest predictor of metastasis within 5 years of RP; the odds ratio for metastatic disease increased by 1.48 for each 0.1 unit increase in Decipher GC score.<sup>54</sup> Importantly, amongst the 47 patients with Cancer of the Prostate Risk Assessment Postsurgical (CAPRA-S) scores suggestive of greater than 50% chance of recurrence, 15 (32%) were low risk according to Decipher GC and just one of these patients had metastasis within 5 years.<sup>54</sup> A separate cohort of 185 high risk post-prostatectomy patients identified 82 men with high risk CAPRA-S scores (> 6); of these, 33 had high risk (> 0.6) and 49 had low risk (< 0.6) Decipher GC scores. The number of prostate cancer specific deaths in high and low CAPRA-S risk men was 52% (17/33) and 6% (3/49), respectively. On multivariable analysis, the hazard ratio for prostate cancer specific mortality was 1.81 per 0.1 unit increase in GC.<sup>55</sup>

Decipher was used to predict outcomes in a study of 139 men who had radiation for locally advanced disease after RP (i.e. positive margins or extension beyond the prostatic capsule). The Decipher GC score significantly enhanced the prognostic utility of CAPRA-S<sup>17</sup> and the Stephenson nomogram<sup>18</sup> for the prediction of outcome in this type of patient. The risk for biochemical recurrence and distant metastasis in men with Decipher GC scores indicating high risk (>0.6) was 81% and 17%, respectively, at 8 year follow up. Importantly, in men with intermediate or high risk (>0.4) GC score and detectable PSA prior to radiation treatment the hazard ratio for BCR was 2.2 compared to men with similar Decipher scores and undetectable PSA.<sup>23</sup> Of note, 21% of these men had adjuvant hormone therapy during radiation treatment; this was not shown to impact BCR or metastasis on multivariable adjustment.

## Miscellaneous Genomic Tests

### *Know Error*<sup>®</sup>

There has been concern that the numerous steps in confirming a prostate cancer diagnosis introduce a small but finite risk of error of attribution (e.g. mislabeling of a prostate specimen or contamination of a prostate specimen with DNA from another patient).<sup>56</sup> A study of almost 13,000 prostate biopsies about which there was no baseline concern about possible error indicated that on average 0.22% of biopsies showed transposition (tumor and reference tissue not derived from the same individual) and 1.69% showed evidence of biopsy contamination with presence of DNA not derived from the index patient. Importantly, every clinical and pathology laboratory setting evaluated had at least one case each of transposition and contamination.<sup>57</sup> It is estimated that clinically meaningful errors may occur in more than 4,500 prostate biopsies annually at a cost of \$880 million dollars and over 600 quality adjusted life years (QALY).<sup>58</sup>

The Know Error<sup>®</sup> system (Strand Diagnostics, Indianapolis, IN) is a genetic panel designed to confirm that biopsy tissue is derived from the subject patient. Using cells derived from the inner cheek, Know Error assesses short tandem repeats (STR, a genetic marker with high discriminative value) in the buccal swab section and matches them to STR in the biopsy specimen.<sup>57,59</sup> Cost effectiveness models for use of confirmatory genetic testing have reported generally favorable results, suggesting that as prices for this assay continue to decline it may eventually be useful as a routine part of clinical practice.<sup>60</sup>

### *Non-Genomic Tests for Prostate Cancer*

The following tests are not strictly genomic assays but are relevant in that they are intended to guide decision making in prostate cancer diagnosis/management. They are included in the interest of providing a comprehensive review of this diagnostic space.

### *PHI score*

The Prostate Health Score is a composite measure which incorporates total PSA, free PSA, and the proPSA isoform p2PSA in the formula  $(p2PSA/fPSA)^* \sqrt{tPSA}$ . The PHI is useful for determination of appropriate patients (i.e. at risk of clinically relevant cancer) for prostate biopsy in the setting of negative DRE and PSA between 4 and 10 ng/L.<sup>42,61</sup> The performance of the PHI may be enhanced by accounting for

prostate volume<sup>62</sup> and other biological factors known to be relevant to prostate cancer (e.g. age, DRE, biopsy history).<sup>63</sup> Several studies have suggested that PHI has greater utility than PCA3 in detection of clinically significant prostate cancer.<sup>14</sup>

#### **4Kscore®**

The 4kScore® test (Opko Lab, Nashville, TN) is a serum based panel of kallikreins, serine proteases that cleave proteins. The assay measures human kallikrein 2 (hK2), total PSA, free PSA, and intact PSA. The 4kScore® test is used to stratify risk of aggressive cancer on biopsy and has been shown to enhance prediction of Gleason >7 prostate cancer on biopsy compared to use of existing metrics (i.e. the Prostate Cancer Prevention Trial Risk Calculator, European Randomized Study of Screening for Prostate Cancer multivariable prediction models, free PSA testing, and total PSA testing with or without DRE).<sup>43,64-67</sup> The panel was shown to be predictive of metastasis from prostate cancer in men with baseline PSA > 2 ng/mL.<sup>68</sup> Importantly, no difference in performance was noted between men of African versus European ancestry.<sup>64</sup>

In head to head comparisons the performance of the 4Kscore test was inferior/equivalent to that of the PCA3 test and the PHI in predicting prostate cancer.<sup>43,65</sup> Use of the 4kScore test has been reported to reduce the number of biopsies with minimal increase in risk for delayed detection of higher grade cancers.<sup>64,68,69</sup> It is currently recommended that the 4Kscore test be used in men with modest elevations of PSA.<sup>68</sup>

#### **ProMark®**

Promark® (Metamark Genetics, Cambridge, MA) is a proteomic test which utilizes quantitative immunofluorescent in situ imaging of biopsy tissue to assess the expression of proteins relevant to prostate cancer biology. The initial validation study of the Promark® assay studied tissue sections from prostatectomy specimens. Cancer tissue with both high and low grade characteristics was sampled from each specimen and stained with monoclonal antibodies to proteins known to be relevant to prostate cancer. Western blotting for final candidate markers was performed for confirmatory purposes. From an initial field of 160 potential proteins, twelve were identified that showed predictive capacity in terms of prostate cancer aggressiveness (Gleason score 7 or greater, pathological T3b, and/or node positive/metastatic disease) and lethal outcome. Importantly, these relationships persisted when

tested in both high and low grade tumor tissues.<sup>70</sup> A follow up study using eight of the initial 12 proteins markers in men with biopsy Gleason 3+4 or lower prostate cancer confirmed in development and clinical validation studies that the assay had the capacity to predict Gleason 3+4=7 or lower and/or organ confined prostate cancer. Combination of the assay with NCCN and D'Amico classification further enhanced prognostic utility; using the low end threshold of <0.33 the test had 85% positive predictive value for favorable pathology.<sup>71</sup> Confirmatory validation data and clinical utility data will be of great interest in determining the eventual role of this test in clinical practice.

#### **Conclusions**

Genomic testing holds enormous promise in optimizing the management and care of prostate cancer patients. Integration of genomic tests with classical markers (e.g. PSA, Gleason score, clinical/pathological stage), other genomic assays, and novel non-genetic tools (e.g. proteomic tests) offers the promise of a new era of precision medicine for men with (or at risk of) prostate cancer. Particular challenges for the future include optimizing the decisional impact of these novel tests and ensuring that the information obtained adds meaningful data to clinical decision making in a cost-effective fashion.

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