Molecular Profiles of Prostate Cancer: To Treat or Not to Treat

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Abstract

A major dilemma in the selection of treatment for men with prostate cancer is the difficulty in accurately characterizing the risk posed by the cancer. This uncertainty has led physicians to recommend aggressive therapy for most men diagnosed with prostate cancer and has led to concerns about the benefits of screening and the adverse consequences of excessive treatment. Genomic analyses of prostate cancer reveal distinct patterns of alterations in the genomic landscape of the disease that show promise for improved prediction of prognosis and better medical decision making. Several molecular profiles are now commercially available and are being used to inform medical decisions. This article describes the clinical tests available for distinguishing aggressive from nonaggressive prostate cancer, reviews the new genomic tests, and discusses their advantages and limitations and the evidence for their utility in various clinical settings.

INTRODUCTION

EBRT: external-beam radiation therapy

RP: radical prostatectomy

CNA: copy-number alteration

FFPE: formalin-fixed paraffin-embedded

MP: molecular profile

AS: active surveillance

Prostate cancer (PCa) is the most common lethal malignancy diagnosed in American men, with an estimated 220,800 new cases detected and 27,570 deaths predicted in 2015 (1). Even though many more men with PCa will die with the disease than of it, most men diagnosed—including those with low-risk cancers—have been treated with external-beam radiation therapy (EBRT) or radical prostatectomy (RP), raising concerns about excessive treatment and the consequent adverse effects on quality of life (2–4). A major confounding issue in the selection of treatment for men with PCa is the difficulty in accurately characterizing the nature of the cancer and the risk of subsequent metastases or death from the disease left untreated. The established risk factors at the time of diagnosis are clinical stage, circulating prostate-specific antigen (PSA) levels, and the Gleason grade (Gl) and extent of cancer in a biopsy specimen. These prognostic factors are sufficient for general risk stratification (5) but have limited utility for predicting the long-term risk of metastases, especially among men with low- or intermediate-risk cancers (6).

Genomic analyses of PCa reveal distinct patterns of alterations in individual genes, and, more commonly, in the genomic landscape of the disease, which show promise for improved prediction of prognosis and better medical decision making. Analyses of the extent of copy-number alterations (CNAs) may improve prediction compared with Gl alone or combined with the other standard clinical features (7, 8). Recently, RNA expression profiles, or signatures, of selected gene panels relevant to the biology of PCa have been developed to predict, more accurately than clinicopathologic features alone, the patient's prognosis (risk of metastases and death from cancer), and the risk that a patient harbors a cancer with aggressive pathologic features (9–12). These profiles can be performed on formalin-fixed paraffin-embedded (FFPE) specimens of tissue obtained by 18g thin-needle biopsy cores that contain as little as 0.5 mm of cancer. Several molecular profiles (MPs) are now commercially available and are being used to assist medical decisions, including the critical decision whether a man with a low- or intermediate-risk, clinically localized PCa should be treated immediately with surgery or radiation therapy (with their attendant risks) or whether he can be monitored expectantly in an active surveillance (AS) program, deferring definitive therapy indefinitely unless the cancer becomes more aggressive (13, 14).

We review the clinical tests currently available for distinguishing aggressive from nonaggressive PCa, discuss how these tests are used to assess risk, examine the new genomic tests in detail (including those commercially available and those that are promising but still in development), and assess the advantages and limitations of these tests and the evidence for their utility in various clinical settings.

ASSESSING RISK BASED ON CLINICAL FEATURES OF THE CANCER

Unlike breast cancers, most prostate cancers are not palpable but are discovered because of an elevated PSA level. For PCa screening and for the initial evaluation of a man with an elevated PSA, there is no imaging test comparable to mammography that reliably identifies a target for biopsy [although magnetic resonance imaging (MRI) shows promise] (15), so detection of cancer is almost always by "random" systematic needle biopsies, with the needle guided by transrectal ultrasound into defined segments of the prostate. Once a patient is diagnosed with PCa, his prognosis—in the absence of metastases, which are uncommon—is estimated from the clinical stage or extent of the local tumor (determined by digital rectal examination and imaging), the PSA level, and the needle biopsy findings. The findings of interest are the Gl (the highest grade of cancer in any of the multiple biopsy cores) and the extent of cancer in the biopsy cores (the number or percentage of cores with cancer and the size or amount of cancer in each core, expressed in millimeters or as a percentage). Each of these factors is associated with pathologic stage and

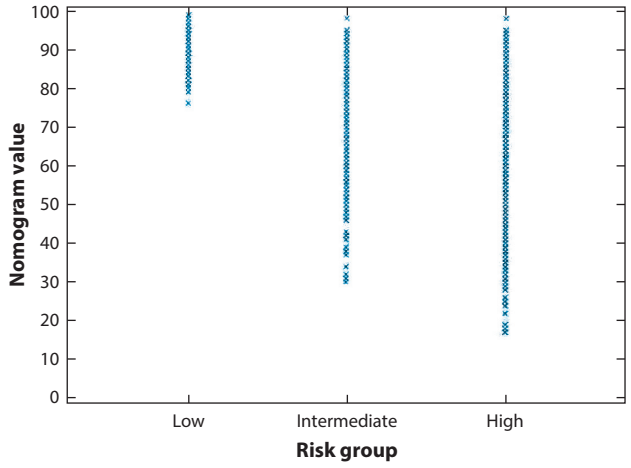

Figure 1

Heterogeneity within American Urological Association risk groups indicated by the wide variation in risk when computed by a validated nomogram (*x*, individual patient nomogram score). From Reference 21 with permission.

prognosis (risk of recurrence after therapy or risk of death from cancer), but when combined in a prognostic algorithm or nomogram, they predict pathologic stage (16) and prognosis (17, 18) more accurately than any of the factors assessed individually.

Despite the greater accuracy of nomograms, in clinical practice most patients are simply assigned to a low, intermediate, or high "risk group" (5, 19) or risk category (20) to facilitate therapeutic recommendations. Risk groups, however, can be misleading (21) (**Figure 1**). Assigning risk using categorical values (e.g., PSA 10–20 ng/ml) and single-factor criteria (the "high-risk" category includes, for example, men with Gl 8–10 cancer regardless of clinical stage or PSA level), with each factor weighted equally, creates heterogeneous groups; men with markedly different outlooks are classified together into a single prognostic group, such as "intermediate risk." Some men in this group have a poor prognosis and a high risk of recurrence, even with early definitive therapy, but others may have a relatively indolent cancer and could safely be observed in an AS program (14).

In addition, sampling error is a concern with random systematic biopsies. Biopsy results underestimate the grade of cancer in 30–40% of patients, as clinicopathologic correlations with surgically removed prostate glands have repeatedly shown (22). The difficulty in accurately assessing the grade and extent of the cancer, as well as the heterogeneity within risk groups, have until recently led many physicians and patients to conclude that PCa, even if low-risk, should be treated immediately with surgery or irradiation in otherwise healthy men who have a life expectancy of more than 10 years.

In contrast, recent clinical trials confirm the slow growth rate and favorable prognosis of most patients with low-risk cancer over a decade or more when simply observed (23, 24), or when managed conservatively in an AS program, delaying definitive therapy until evidence of progression (25, 26). Low-risk cancers are not completely innocuous, however. About one-third of patients with an apparently low-risk cancer develop, over 10 years, a more aggressive cancer that requires definitive therapy, and the success of such delayed therapy, measured by the biochemical recurrence (BCR) rate, is sometimes disappointing (14). A small percentage of patients, whether placed on watchful waiting or enrolled in a formal AS program, develop metastases and die of their

BCR: biochemical recurrence

cancer, either because the initial tumor was underestimated or because a new, more aggressive cancer developed later (14, 23–25).

Consequently, otherwise healthy men who have been diagnosed with an early-stage PCa and have a life expectancy of 10 years or more should be evaluated thoroughly to assess the risk posed by their cancer before being placed on AS. Promising approaches include multiparametric magnetic resonance imaging (mpMRI) (27) and/or a repeat (confirmatory) systematic needle biopsy (28). Multiparametric MRI of the prostate is technically demanding; accurate interpretation requires skill and experience (29). In experienced hands, mpMRI has a reasonably high sensitivity for large or high-grade cancers for which immediate therapy is indicated (30, 31). Suspicious lesions on MRI should be confirmed by targeted biopsy before being considered an indication for therapy because as many as 50% prove to be false positives on biopsy (32). Confirmatory biopsies can also identify most unsuspected large or high-grade cancers in men with apparent low-risk tumors, but biopsy includes some risk of infection and bleeding.

A new, noninvasive approach to risk assessment is molecular characterization of cancer in the biopsy specimen. With the rapid progress in genomic characterization of PCa (7, 33), MPs are now commercially available and are being used clinically to identify men at higher risk for progression and death from PCa who may benefit from treatment, while reducing overtreatment of men with low- or intermediate-risk cancers who are less likely to benefit from therapy that would expose them unnecessarily to treatment-related side effects.

MOLECULAR PROFILES IN PROSTATE CANCER

In PCa, clinical application of genomic testing has been hampered by the difficulty of obtaining adequate frozen samples of cancer by thin-needle (18g) biopsy of the prostate, the small amount of cancer available for analysis, and the known multifocality and heterogeneity of cancer within the prostate. And although the first two problems have technical solutions, the prognostic accuracy of MPs may be limited by the inherent sampling error of prostate biopsies—that is, the cancer in the biopsy specimen may not accurately represent the cancer present in the prostate (34). This drawback is particularly relevant to low-risk, small-volume cancers identified by systematic biopsy, the most frequent presentation of men who are candidates for AS.

Several commercial assays now available perform well on FFPE needle biopsy cores containing as little as 0.5 mm of cancer (9, 35), and more MPs should be available in the near future that assay for DNA CNAs (8) and RNA expression levels of panels of selected genes (11), as well as immunofluorescence assays of protein levels in specific cellular compartments of the tumor (36).

DNA Copy-Number Alterations

PCa is characterized by the accumulation of CNAs, which can result in amplification of oncogenes or deletion of tumor-suppressor genes. Common alterations found in PCa are TMPRSS2-ERG fusion (seen in ~50% of cases), loss of 8p (in 30–50%), and gain of 8q (in 20–40%). Comparative genomic hybridization (CGH) has been used to identify CNAs (37), and array CGH (aCGH) is more accurate than conventional CGH (38). Integrated genomic approaches, such as combining aCGH with single-nucleotide polymorphism arrays, may be even more accurate (39). In an integrated genomic analysis of 181 tumor samples from fresh frozen RP specimens, Taylor et al. (7), in an unsupervised clustering hierarchical analysis, identified five distinct prognostic groups defined by CNAs independent of Gl in the RP specimen. Others have reported similar findings correlating increased percentage of the genome affected by CNA with higher tumor grade, stage, and PSA at the time of diagnosis (40), and with tumor aggressiveness (defined as Gl 8 or higher, stage T3 or

T4, or lymph node or other metastases) (41). Hieronymus et al. (8) expanded the previous analysis of the prognostic significance of CNAs in a contemporary patient cohort. The extent of CNAs, or "CNA burden," defined as the percentage of the autosomal tumor genome bearing CNAs, predicted the risk of both BCR and metastases after RP independent of pretreatment PSA level and biopsy Gl. These investigators were also able to assess CNA burden accurately by whole-genome sequencing in FFPE needle biopsy samples. Thus, CNAs hold promise as a future biomarker that may improve risk stratification of clinically localized PCa.

RNA Expression Profiles

Three commercially available genomic tests use RNA expression profiles for risk stratification in biopsy samples of PCa: the Cell Cycle Progression (CCP) assay (Prolaris®, Myriad Genetics, Inc., Salt Lake City, UT, USA) (42); the Genomic Prostate Score (GPS) assay (Oncotype DX®, Genomic Health, Redwood City, CA, USA) (10); and the Genomic Classifier (GC) profile (Decipher®, GenomeDx Biosciences, San Diego, CA, USA) (11).

Prolaris® Cell Cycle Progression (CCP) assay. The expression levels of genes that regulate the cell cycle are obvious candidate biomarkers of the growth rate of a cancer because they reflect cell proliferation (43). To select a set of genes whose expression could be used to assess prognosis in patients with cancer, investigators at Myriad Genetics evaluated 126 cell cycle–related genes in commercially available FFPE tissue samples of PCa and selected 31 genes whose expression captured a highly reproducible measure of cell cycle activity (**Table 1**). A CCP score was calculated from the average expression of these genes in tissue samples normalized to 15 housekeeping genes whose expression varies little throughout the cell cycle. For the average PCa the CCP score was 0, ranging from −3.0 to 7.0, with a one-unit change representing approximately a doubling of gene expression (9). The commercial assay has a high analytic validity: Reproducible results were obtained from triplicate runs in 97–99% of FFPE tissue samples with at least 0.5 mm of cancer in 18g needle biopsy cores (42, 44).

In initial clinical studies examining the prognostic significance of the CCP score in PCa, high-quality RNA sufficient for analysis was obtained from 77–89% of 5- to 20-year-old archived FFPE tissue samples (9, 42, 45). The clinical validity of the assay was assessed in multiple cohorts, including patients treated conservatively after being diagnosed with PCa by transurethral resection of the prostate or by needle biopsy (9, 42), as well as patients treated with RP (9, 45, 46) or EBRT (47) (**Table 2**). The endpoints were time to death from PCa (in patients managed conservatively) or time to BCR, indicated by a rising serum PSA level, after RP or EBRT. CCP scores in diagnostic biopsy specimens strongly predicted the probability of death from cancer over 10 years in patients managed conservatively (**Figure 2**) (9, 42), as well as time to BCR and to metastases after RP (9, 45, 46). In multivariable analyses, the CCP score added substantial prognostic accuracy to standard clinical features, such as Gl and PSA, as well as to a combination of known clinicopathologic predictive factors (including age, clinical stage, extent of cancer in biopsy, and Ki-67) in predicting time to event, with a hazard ratio (HR) of ~2 (range 1.5–2.6) per unit change in CCP. CCP scores correlated weakly but significantly with Gl ($r = 0.19–0.61$) and PSA ($r = 0.14–0.27$), supporting the premise that the CCP score measures an important biologic feature of the cancer not captured by clinicopathologic characteristics (see **Table 2**) (45).

CCP retained its prognostic value when algorithms combining multiple clinical features, such as Cancer of the Prostate Risk Assessment (CAPRA) score (45) or nomograms, were incorporated into the models (9). The CCP score was especially accurate in predicting early events, such as death from cancer within five years of diagnosis in the conservatively managed cohort (9), and

Table 1 Genes included in molecular profiling tests for prostate cancer

Cell Cycle Progression (9)	Genomic Prostate Score (10)	Genomic Classifier (11)	ProMark (12)
ASF1B	*AZGP1*	*ANO7*	*ACTN1*
ASPM	*BGN*	*CAMK2N1*	*CUL2*
BIRC5	*COL 1A1*	*EPPK1*	*DERLI1*
BUB1B	*DUSP1*	*GLYATL1P4/PCAT-80*	*FUS*
C18orf24	*FAM13C*	*IQGAP3*	*HSPA9*
CDC2	*FLNC*	*LASP1*	*PDSS2*
CDC20	*FOS*	*MYBPC1*	*PLAG1*
CDCA3	*GSN*	*NFIB*	*pS6*
CDCA8	*GSTM2*	*NUSAP1*	*SMAD2*
CDKN3	*KLK2*	*PBX1*	*SMAD4*
CENPF	*LAMB3*	*PCAT-32*	*VDAC1*
CENPM	*SFRP4*	*PCDH7*	*YBX1*
CEP55	*SRD5A2*	*RABGAP1*	
DLGAP5	*TPM2*	*S1PR4*	
DTL	*TPX2*	*THBS2*	
FOXM1		*TNFRSF19*	
KIAA0101		*TSPB*	
KIF11		*UBE2C*	
KIF20A		*ZWILCH*	
MCM10			
NUSAP1			
ORC6			
PBK			
PLK1			
PRC1			
PTTG1			
RAD51C RAD54L			
RRM2			
TOP2A			
TK1			

the effect was strong in men with high-grade cancer and in samples from RP specimens (45), suggesting the CCP score's potential to inform decisions about adjuvant systemic therapy before or after treatment of the primary tumor.

The most immediate application of MPs for PCa, however, is for men with early-stage, low- or intermediate-risk cancer, faced with the difficult choice between AS and immediate definitive therapy. Several studies have addressed the clinical utility of the CCP score in this setting. In a large cohort of men managed conservatively, Cuzick et al. (48) assessed the CCP score in 585 men diagnosed by needle biopsy and followed for a mean of 9.6 years. CCP was independently prognostic of prostate cancer–specific mortality (PCSM) at 10 years (HR 1.8 in multivariable analysis). Of 80 men diagnosed with low-risk cancer by clinical criteria (10 years PCSM <4.3% in men managed conservatively), 19 (24%) were identified as being at higher risk by CCP score, while 31 (6%) of 505 men across a wide range of intermediate- and high-risk cancers were found to be at low risk by CCP score and would therefore be good candidates for AS.

Examining contemporary men whose cancer has clinical features that would make them appropriate candidates for AS (Gl ≤3+4, PSA <10 ng/ml, low tumor stage, no more than 25% of biopsy cores positive), Cuzick et al. (48) reported no cancer deaths within 10 years in those with a low CCP score. When the CCP score was combined with clinical criteria in a large set of men

Table 2 Overview of clinical validation cohorts for the Prolaris Cell Cycle Progression test

Study	N	Endpoint	Hazard ratio	Correlation with clinical variables
TURP (9)	337	Death due to prostate cancer	2.6 [1.9–3.5]	Gleason grade, r = 0.61 PSA, r = 0.27
Needle biopsy (23)	349	Death due to prostate cancer	1.7 [1.3–2.1]	Gleason grade, r = 0.37 PSA, r = 0.14 Extent of cancer, r = 0.28
Radical prostatectomy 1 (9)	366	Biochemical recurrence	1.8 [1.4–2.2]	Gleason grade, r = 0.22 PSA, r = 0.21
Radical prostatectomy 2 (45)	413	Biochemical recurrence	1.7 [1.3–2.3]	CAPRA-S score, r = 0.21
Germany (46)	283	Biochemical recurrence	1.7 [1.2–2.3]	Gleason grade, r = 0.27
North Carolina (46)	176	Biochemical recurrence	1.3 [1.0–1.7]	Gleason score, r = 0.18 Positive cores, r = 0.19
Utah (46)	123	Biochemical recurrence	1.6 [1.0–2.4]	Gleason grade, r = 0.30
EBRT (47)	141	Biochemical recurrence	2.1 [1.1–4.3]	Clinical stage, r = 0.33

Abbreviations: CAPRA-S, Cancer of the Prostate Risk Assessment after Surgery; EBRT, external-beam radiation therapy; PSA, prostate-specific antigen; TURP, transurethral resection of the prostate.

whose samples were submitted for commercial assay (N = 1,718), 29% would qualify for AS by clinical criteria, but 55% would qualify when the CCP score was added to the clinical criteria.

To examine how physicians utilize the Prolaris assay, Myriad sponsored a survey of physicians who reported their recommendations for treatment of 305 patients based on clinical data when they ordered the assay, and again after the CCP scores were available (49). In 94 of the 305 patients (31%), the physicians' recommendation was changed—from no intervention (AS) to intervention (RP, EBRT or systemic therapy) in 33 patients (11%) and the reverse, from intervention to no intervention, in 61 (20%). The greatest change occurred when a high CCP score was reported in

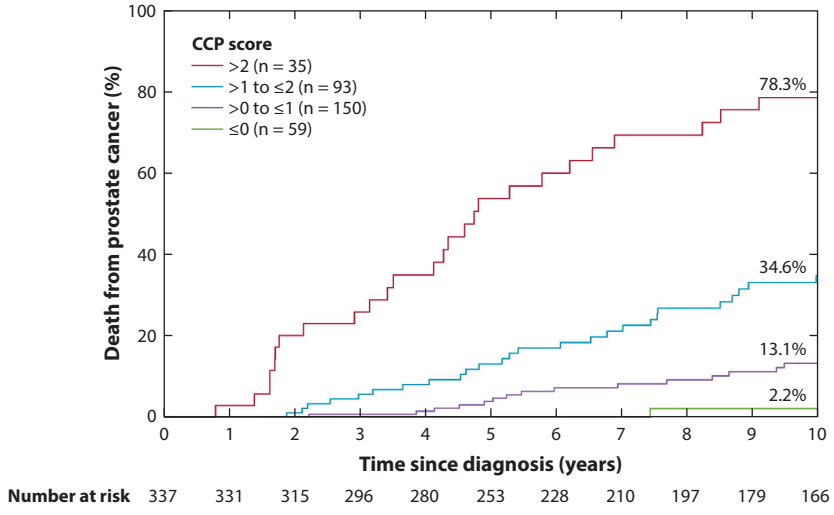

Figure 2

Actuarial time to death from prostate cancer by Cell Cycle Progression (CCP) score in men with prostate cancer managed conservatively. From Reference 9 with permission.

Table 3 Change in treatment category in selected treatment from pre- to post-test across AUA risk categories (from Reference 49 with permission)

AUA risk category	Change in selected treatment from pretest to post-test (N = 205), n (%)			
	Noninterventional to noninterventional	Interventional to noninterventional	Interventional to interventional	Noninterventional to interventional
Low (n = 83)	47 (56)	18 (22)	13 (16)	5 (6)
Intermediate (n = 93)	18 (19)	15 (16)	47 (50)	12 (14)
High (n = 29)	1 (3)	4 (14)	18 (61)	6 (21)

Abbreviation: AUA, American Urological Association.

men with clinically low-risk cancer, or when low scores were reported in men with intermediate-risk cancer (49) (**Table 3**). In another set of 4,261 patients assessed by CCP assay and clinical criteria, the CCP assay identified a more aggressive cancer than clinicopathologic criteria in 37% of patients and a less aggressive cancer in 21%. The majority of cancers, however, were classified as either "less aggressive" or "more aggressive" (one-unit change in CCP score); only 6.5% were considerably less (2.5%) or more (4%) aggressive (a change of two or more units in CCP score) (44).

The major strengths of Myriad's Prolaris assay are its analytic validity and reproducibility, and its clinical validity in predicting important clinical endpoints (e.g., risk of metastases or death from cancer). The CCP score appears to perform equally well in biopsy and in prostatectomy specimens, and it predicts outcomes after conservative management (AS and systemic hormonal therapy), surgery, and radiation therapy.

A concern about the test in biopsy specimens is the sampling error inherent in prostate biopsy: Does the cancer in the biopsy sample accurately represent the biology of all cancer within the prostate?

Extensive analyses of the clinical benefit of the CCP assay on decision making and clinical outcomes have not yet been conducted. A prospective clinical trial in which men are randomly assigned to have the test or not, with the endpoint of higher survival rates in men who had the test, is not feasible, given the long natural history of PCa. However, formal evaluation of clinical utility could be achieved through retrospective analysis of specimens collected prospectively (50) during a randomized controlled trial that compared outcomes after treatment and observation, such as the PIVOT (24) or SPCG-4 trials (51), and then compared outcomes between arms when patients were stratified by CCP score.

Oncotype DX® Genomic Prostate Score (GPS). The Oncotype DX® Genomic Prostate Score (GPS) assay measures the level of expression of 17 genes in FFPE needle biopsy cores, including 12 genes involved in pathways known to play a role in prostate tumorigenesis (cellular proliferation, stromal response, androgen signaling, cellular organization, and stress response) and five housekeeping reference genes (see **Table 1**) (35). The genes selected were developed from an initial group of 732 candidate genes tested in samples from 441 RP specimens for their ability to predict clinical local or distant recurrence after surgery. They were further refined in a preoperative needle biopsy cohort for predicting adverse pathology in the subsequent RP specimen. The level of expression of these 17 genes is used to generate a GPS that ranges from 0 to 100. A 20-point difference in GPS corresponds to the difference between the average score of the highest and lowest quartiles of patients in the test cohort (10). The analytic validity of the test for predicting aggressive PCa has been established using as little as 5 ng of RNA (35). The test produced a reproducible, high-quality result in 96% of needle biopsy specimens when at least 1 mm of cancer was available for analysis (10, 35).

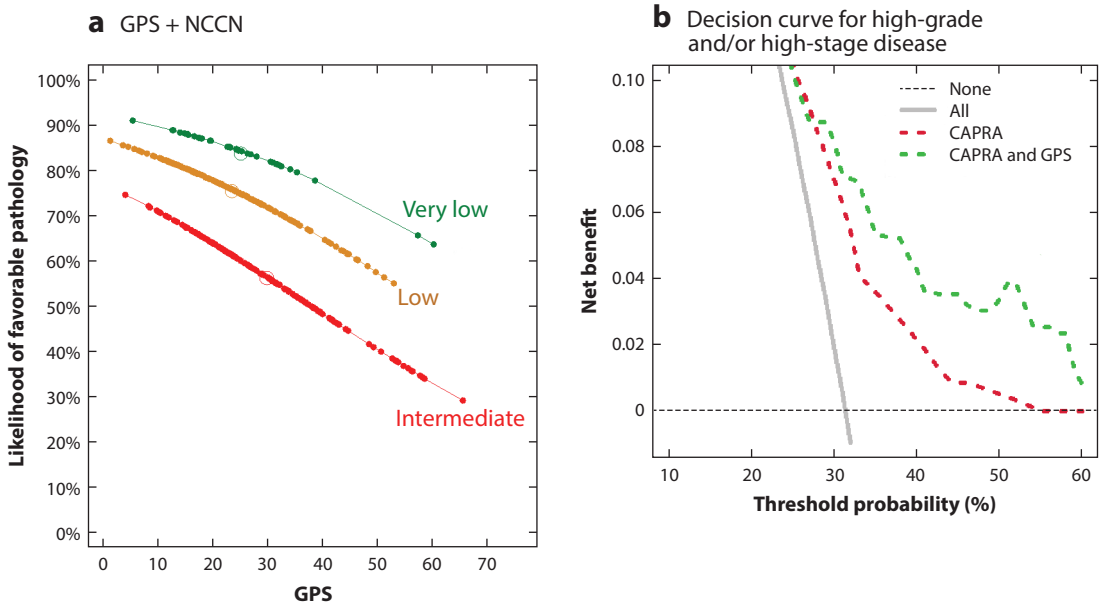

Figure 3

(*a*) GPS measured in a biopsy sample predicts likelihood of favorable pathology in an RP specimen within an NCCN risk group. Open circles correspond to the mean GPS on the *x*-axis and expected likelihood of favorable pathology on the *y*-axis for each level of risk. Curves represent the probability of favorable pathology at each level of clinical risk, incorporating GPS. Distributions of the GPS values by clinical risk group are plotted as solid circles beneath the curves. (*b*) Decision-curve analysis illustrates that net benefit is higher when GPS is combined with a clinicopathologic risk model (CAPRA) than when CAPRA is used alone for predicting the presence of unfavorable RP pathology. From Reference 10 with permission. Abbreviations: CAPRA, Cancer of the Prostate Risk Assessment; GPS, Genomic Prostate Score; NCCN, National Cancer Centers Network; RP, radical prostatectomy.

GPS in specimens from FFPE RP specimens strongly predicted the risk of clinical recurrence (local and/or distant), even when adjusted for the clinicopathologic features of the cancer, including American Urological Association (AUA) risk group (10) (**Figure 3**). The clinical validity of the test was then assessed in needle biopsy specimens from a group of 395 men with low- or low-to-intermediate-risk cancers who were eligible for AS but who chose treatment with RP within six months of diagnosis. The principal endpoint of the study was the discovery of adverse pathology in the RP specimen, which was present in 123 (31%) of the patients and defined as the presence of high-grade cancer (dominant Gl pattern 4 or any Gl pattern 5; i.e., Gl score >3+4) or extension outside the prostate (pathologic stage pT3aN0M0 or higher). In multivariable analyses adjusting for known clinical covariates, GPS in the biopsy specimen significantly predicted adverse pathology [odds ratio (OR) 1.9 for each 20-point increase]. Within any given level of risk assigned by the National Cancer Centers Network (NCCN) criteria (19), GPS identified men with a broad range of risks for adverse pathology (e.g., in the NCCN low-risk category the average probability of favorable pathology was ~75%, but with GPS the probabilities ranged from approximately 55% to 85%) (**Figure 3*a***). GPS was weakly associated with clinical variables, individually or combined (10).

NCCN: National Cancer Centers Network

The clinical utility of the test has not been validated extensively, but the area under the curve (AUC) for predicting unfavorable pathology increased from 0.63 with multiple clinical variables to 0.67 with the addition of GPS; the modest AUC probably reflects the relatively narrow range of cancers included in this select cohort. A more informative method of assessing the effect of

GPS is decision-curve analysis (**Figure 3*b***), measuring the "net clinical benefit" of the test, which confirms that over a range of probabilities, GPS would result in fewer men being treated with surgery for favorable pathology with no change in the number with unfavorable pathology left untreated (10).

The Oncotype DX GPS assay appears to be a reproducible, analytically and clinically valid tool to assess the aggressiveness of PCa in needle biopsy specimens, and it may add important information when patients are making the critical choice between AS and definitive therapy. The published data, however, are limited, and the test's accuracy in predicting metastases or death from cancer remains to be substantiated in larger cohorts. The clinical validation report (10) asserts the ability of the GPS "to discriminate prostate cancer aggressiveness despite tumor heterogeneity, multifocality and limited sampling in biopsy tissue." But the data presented are not convincing, given previous studies documenting the molecular heterogeneity of PCa and the sampling error inherent in prostate biopsy (22, 34).

Decipher® Genomic Classifier (GC). Unlike the MPs described above, which assess the risk of adverse pathology or disease progression by analyzing biopsy specimens, the Decipher Genomic Classifier (GC) is a 22-gene RNA expression profile developed to predict the risk of metastases after RP by analyzing tissue samples from the prostatectomy specimen. The genes included in the final GC model were selected from a high-density transcriptome-wide microarray as those that most closely reflected the development of metastases in a training set of 359 patients. The selected genes include a variety of protein-coding and noncoding RNA loci representing a number of cellular functions: nine code for proliferation or cell cycle progression, five for adhesion and motility, five for cell structure, four for differentiation, and two for immune response; for three the function is unknown (see **Table 1**) (11).

The GC reports a continuous variable score, from 0 to 1, with a higher score reflecting a greater risk of metastases. For RNA extraction, areas of cancer are microdissected from three or four 10-micron sections of the index, or highest-grade, lesion in the FFPE RP specimen. Adequate RNA was obtained from 76% to 92% of clinical samples, and the analytic validity of the GC has been established in >1,500 patient samples (11, 52–54).

The performance of the GC was assessed initially in a study of 192 patients who developed metastases within five years after RP matched with 353 controls who remained free of recurrence for at least seven years, or who developed BCR but no metastases within five years of BCR. In clinical validation studies, the GC proved to be a more accurate predictor of time to metastases (AUC 0.75) than any one or combination of clinicopathologic variables. Comparing GC scores to final Gleason scores in the RP specimens, high GC scores were found in none of the Gl≤6 cancers, 29% of Gl 7 cancers, and the majority of the Gl 8–10 cancers. In multivariable analyses, GC was the only independent predictor of time to metastases (OR 1.4 for each 10% increase in GC). The authors concluded that GC assessed in the primary tumor within the prostate produces a reliable measure of the risk of metastases years after surgery for clinically localized PCa (11).

In a validation study of a separate cohort, Karnes et al. (52) designed a nested case-control study of 256 men (73 with metastases). Adequate RNA was extracted from 92% of the patients, of whom 6% developed metastases within five years. The GC was the most accurate predictor of metastases (AUC 0.79, HR 1.51 for each 10% increase), and it was even more accurate when optimally combined with clinicopathologic variables (0.82). In decision-curve analysis, the combination of GC and clinical features had the highest net benefit across a range of threshold probabilities of metastases. Overall, GC alone or in combination with a nomogram that includes all accepted clinicopathologic variables was able to separate the study group into patients at low risk (2.4%) of metastases at five years (60% of the cohort), those at intermediate risk (6%)—similar to the

group as a whole (20% of the cohort)—and those at high risk (22.5%). Despite the remarkable performance of the GC in these series, the study population was substantially enriched with patients who developed metastases; adjuvant radiation or hormone therapy was administered to 45% and salvage therapy (after BCR) to 70% before the appearance of metastases. Because additional therapy after RP substantially extends the time to appearance of metastases but may not alter long-term survival (55, 56), an MP may prove less accurate when used to predict time to metastases in prospective trials in representative samples of all men treated with surgery.

Klein et al. (54) studied 169 men at high risk for recurrence and metastases after RP. All were free of cancer immediately after the operation (undetectable PSA level) and none received adjuvant therapy. Fifteen developed metastases within five years; of the 154 without metastases at five years, 34 developed metastases later. GC was the most important variable for predicting rapid metastasis and, when combined with widely used clinical risk models such as the Stephenson nomogram (57), yielded the highest net benefit in decision-analysis models. The GC was able to identify a subset of men at high risk for rapid metastasis and death from PCa (50% within seven years) whose cancers were similar in clinicopathologic features to patients at much lower risk of dying of the disease (25% within 15 years). The clinical advantage of the GC risk calculator, compared with other risk models, was its more accurate identification of patients at low risk for metastases, despite high-risk clinicopathologic features, who could be spared additional therapy.

A similar study of the risk of metastases after RP in 185 patients confirmed the accuracy of the GC (**Figure 4**) (53). When integrated with a clinical algorithm incorporating clinicopathologic variables (CAPRA-S) (20), the combined GC and clinical classifier was able to identify patients with a very low risk (0%) of dying of cancer within five years or a much higher risk (53%). Other studies have examined the predictive accuracy of the GC in patients with BCR after RP (58) and after postoperative adjuvant or salvage EBRT (59).

Several studies have evaluated the clinical utility of the GC by assessing its effects on clinical decision making among physicians who were asked to recommend adjuvant therapy for men with no evidence of disease after RP. Representative case histories of men with high-risk PCa were presented, and the physicians were asked whether they would recommend adjuvant therapy (and if so, which). The same physicians were then presented with the GC scores for these patients and asked again for their recommendations. For 31% of the patients, the physicians' recommendation changed, most often from treatment to observation. With knowledge of the GC scores, physicians recommended treatment in only 19% of patients with low-risk scores and in 65% of those with high-risk scores. Treatment tended to be withheld when the GC score predicted $<7\%$ risk of metastases but was recommended when the risk was higher (60).

In summary, the GC is a promising test that can augment clinicopathologic models to predict the risk of metastases after RP. In retrospective studies, the test was informative in $>90\%$ of tissue samples from RP specimens and was able to classify patients as having low (0–2.4%) or high (24–50%) risk of metastases within five years after surgery. Although GC has not been validated across the full spectrum of patients treated with RP, it appears to be an accurate predictor and, given the high level of accuracy of clinical predictors (61), may prove most valuable in patients at intermediate risk by clinicopathologic features.

One impediment to the use of the GC is the current standard of care after surgery for PCa. PSA is such a powerful indicator of disease status after RP that most physicians recommend observing patients who have an undetectable postoperative PSA level, and delaying radiation to the prostatic bed or androgen deprivation hormonal therapy, or both, until the PSA level rises, and, in the absence of detectable metastases, choosing the timing and nature of salvage therapy according to the level and rate of rise of PSA. Neither adjuvant postoperative EBRT nor androgen deprivation therapy is considered the standard of care today, regardless of the risk of recurrence, and clinical

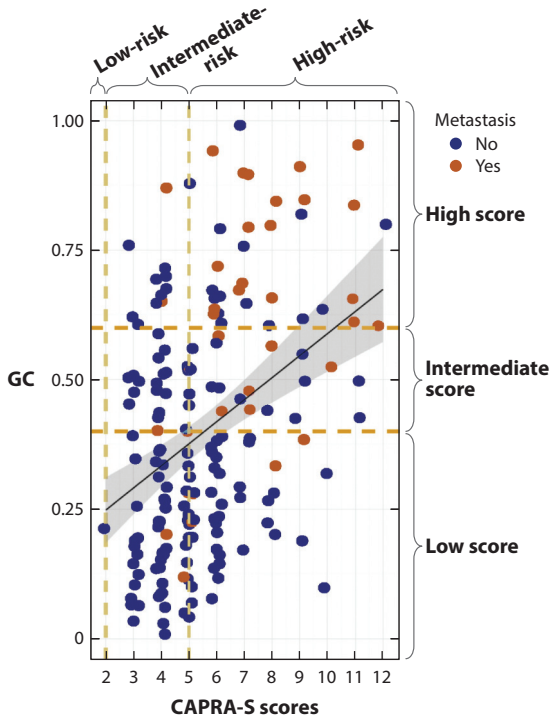

Figure 4

Agreement between GC and CAPRA-S scores predicting the risk of metastases after RP from analyses of tissue specimens from the index cancer in the RP, indicating that the major role for the GC may be more accurate discrimination within the clinical intermediate-risk group. Dashed vertical lines show boundaries for low-risk (≤2), intermediate-risk (3–5), and high-risk (≥6) groups for CAPRA-S. Dashed horizontal lines mark low (<0.4), intermediate (0.4–0.6), and high (≥0.6) GC scores. The regression line is solid black. From Reference 53 with permission. Abbreviations: CAPRA-S, Cancer of the Prostate Risk Assessment Score after Surgery; GC, Decipher® Genomic Classifier; RP, radical prostatectomy.

trials have not clearly established a survival benefit for adjuvant therapy (2, 5, 55, 56). So the clinical utility of the GC may remain limited until the benefits of early postoperative therapy can be shown to justify the risks in a defined subset of patients at substantial risk for metastases.

Proteomic Profile

ProMark™ (Metamark Genetics, Cambridge, MA, USA) is a prognostic test that utilizes immunofluorescence to measure in tumor epithelium the levels of eight protein biomarkers identified in intact tissue sections of FFPE biopsy specimens to predict tumor aggressiveness (see **Table 1**) (12). Described in detail elsewhere (36), the assay was developed to overcome the morphological heterogeneity of PCa by identifying biomarkers predictive of aggressive disease in separate samples of low-grade and high-grade cancer taken within the same RP specimens. The assay produces a continuous risk score from 0 to 1. For the initial clinical study, biopsy samples were scored with ProMark in 276 patients with low- or intermediate-risk PCa. The assay predicted the presence of favorable (GI<4+3 and organ-confined) or unfavorable pathology in the paired RP specimens with moderate accuracy (AUC 0.68). The model was more accurate (AUC 0.75) when combined

with NCCN clinical risk categories (19), suggesting that the eight-biomarker risk score is partly independent of clinicopathologic variables.

The clinical utility of the test stems from its ability to reclassify patients compared with standard risk categories such as NCCNs. Among 96 patients with NCCN intermediate- or high-risk cancer who would not usually be considered candidates for AS, 14 (15%) had a low ProMark score (<0.34), and 71% of these had favorable pathology (positive predictive value 71%), making them candidates for AS. Similarly, of 160 patients with NCCN very low- or low-risk cancer, 12 (8%) had a high ProMark score (>0.80), and of these 58% had unfavorable pathology, more suitable for definitive therapy.

The principal advantage of ProMark is its ability to assess molecular alterations in intact tissue, targeting changes specifically within tumor epithelium. It is uncertain whether such focal changes will better represent molecular abnormalities among all cancers within the prostate than CNAs or RNA profiles on tissue homogenates from microdissected tumor. Although the proteins selected for analysis were deliberately chosen for their ability to predict the risk of metastases in analyses of both low- and high-grade lesions within the same prostate, this test may not fully overcome biopsy sampling error, because low-grade cancers that are remote from a high-grade lesion may have a different MP from cancers immediately adjacent (33, 34, 41). In addition, the clinical comparators used to assess the additional information provided by ProMark did not include some of the more rigorous predictive models such as CAPRA, or nomograms that include all established clinicopathologic parameters. Finally, the endpoint chosen—favorable or unfavorable pathology in the RP specimen—is a proxy for tumor aggressiveness but does not fully predict long-term clinical outcomes.

CONCLUSION

Dozens of MPs of PCa have been reported (33, 34, 41, 62–65), but few MP tests have been sufficiently validated analytically and clinically to be used in practice. The MPs reviewed here are those that are the most promising. Most have progressed to commercial development, but their clinical utility remains to be fully established. Clinical risk assessment for clinically localized PCa, properly conducted, is actually quite accurate. Modern MRI can visualize many nonpalpable lesions that are missed or insufficiently sampled by systematic biopsy. Magnetic resonance images can be fused digitally with real-time ultrasound images to target suspicious lesions within the prostate for biopsy, improving the accuracy of sampling. The challenge for MPs, therefore, is to demonstrate more accurate risk stratification than that achieved with the best clinical tools and predictive models, or similar accuracy without requiring expensive or invasive tests. In reported studies, the proportion of men whose clinical risk category is substantially altered by the MP result is relatively small. Randomized trials (e.g., PIVOT) have shown no advantage to immediate definitive therapy for most men with clinically low-risk cancers; identifying the minority who would benefit will be challenging for MP tests.

Nevertheless, the MPs described in this review all offer promising ways to characterize the biologic aggressiveness of PCa differently—and better than we have in the past. These new tests are likely to become widely used and increasingly valuable in clinical practice, in part because their reproducibility provides assurance relative to the variations in expertise among pathologists in assigning Gleason grade and among radiologists in performing and interpreting MRIs. The decision for observation or definitive therapy is so important, and so laden with potential effects on quality of life, that physicians and patients will continue to seek MPs and genomic classifiers to assist them in making better medical decisions. Better risk stratification will encourage acceptance of AS, help to reduce overtreatment, and alleviate some of the concerns about screening for PCa.

Improved risk stratification may also allow less stringent monitoring of patients on AS, reducing the need for frequent biopsies.

Genomic profiling of PCa is an exciting and rapidly developing field that promises to improve the care and clinical outcomes of patients.

SUMMARY POINTS

1. The lethal potential of prostate cancer (PCa) is difficult to characterize precisely by stage, grade, and PSA level, so most men who are diagnosed with the disease are treated with surgery or radiation, even though many would have lived the remainder of their lives with no symptoms from the disease.
2. Genomic analyses of PCa reveal distinct patterns of alterations in the disease's genomic landscape that may prove useful for improving prediction of prognosis and medical decision making. RNA expression profiles of selected gene panels can be performed on small samples of cancer in biopsy specimens to predict prognosis more accurately.
3. Several molecular profiles (MPs) are commercially available to assist in deciding whether a man with a low- or intermediate-risk PCa should be treated immediately or monitored expectantly in an "active surveillance" program. These assays have sufficient analytic and clinical validity to be used in practice, but their clinical utility remains to be established. One concern about performing these tests in biopsy specimens is the sampling error inherent in prostate biopsy.
4. The challenge for MPs is to demonstrate more accurate risk stratification than can be achieved with the best clinical tools and predictive models, or to demonstrate similar accuracy without requiring expensive or invasive tests.
5. The proportion of men whose clinical risk category is substantially altered by MP results is relatively small, but these new tests are likely to become widely used in clinical practice because their reproducibility provides assurance relative to variations in expertise among pathologists assigning grade and among radiologists performing and interpreting prostate MRIs.

DISCLOSURE STATEMENT

Dr. Scardino declares a financial interest in OPKO Diagnostics, which licensed and is developing for commercial application the "4K score" blood test, used to detect the presence of a high-grade prostate cancer before biopsy.

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7. First large-scale integrated genomic profile of PCa; identified CNAs as an independent prognostic factor.

9. First clinical study of CCP score as independent prognostic feature to predict risk of death from cancer in a conservatively managed cohort.

10. First published study of accuracy of GPS to predict risk of unfavorable pathology in RP specimens from biopsy samples.

11. First study confirming the ability of GC to predict risk of early metastasis after RP.

12. First study of ProMark immunofluoresence assay to predict aggressiveness of prostate cancer from biopsy specimens.

14. Largest and longest prospective study of an AS cohort; includes low- and intermediate-risk patients.

17. Most widely used nomogram; combines pretreatment factors to predict risk of recurrence after RP.

20. First description of CAPRA risk assessment score; uses clinicopathologic features to predict risk of recurrence after RP.

24. Second of two randomized clinical trials (RCTs) comparing radical prostatectomy with observation for clinically localized PCa.

27. Excellent review of accuracy of MRI in detecting "clinically significant" cancers within the prostate.

32. Excellent study of increased accuracy of MRI/ultrasound fusion technology for biopsy detection of high-grade cancer.

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50. Excellent summary of requirements for establishing a new biomarker for use in clinical practice.

51. First RCT to establish survival benefit of RP compared with observation.