Medical Policy

BlueCross BlueShield of Kansas
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Title: Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

See Also: *Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management*

Professional
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Populations	Interventions	Comparators	Outcomes
Individuals: • Who are being considered for initial prostate biopsy	Interventions of interest are: • Testing for genetic and protein biomarkers of prostate cancer	Comparators of interest are: • Standard clinical examination including measurement of percent free prostate-specific antigen	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity • Resource utilization • Quality of life

Populations	Interventions	Comparators	Outcomes
Individuals: • Who are being considered for repeat prostate biopsy	Interventions of interest are: • Testing for genetic and protein biomarkers of prostate cancer	Comparators of interest are: • Standard clinical examination including measurement of percent free prostate-specific antigen	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity • Resource utilization • Quality of life

DESCRIPTION

Various genetic and protein biomarkers associated with prostate cancer. These tests have the potential to improve the accuracy of differentiating which men should undergo prostate biopsy or rebiopsy after a prior negative biopsy. This policy addresses these types of tests, for cancer risk assessment. Testing to determine cancer aggressiveness after a tissue diagnosis of cancer has been made is addressed in Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management.

Objective

The objective of this evidence review is to determine whether testing for genetic and protein prostate biomarkers improves the net health outcome in men for whom an initial prostate biopsy or a repeat prostate biopsy is being considered.

Background

Prostate Cancer

Prostate cancer is the second most common cancer in men, with a predicted 161,360 incidence cases and 26,730 deaths expected in the United States in 2017.[1]

Prostate cancer is a complex, heterogeneous disease, ranging from microscopic tumors unlikely to be life-threatening to aggressive tumors that can metastasize, leading to morbidity or death. Early localized disease can usually be treated with surgery and radiotherapy, although active surveillance may be adopted in men whose cancer is unlikely to cause major health problems during their lifespan or for whom the treatment might be dangerous. In patients with inoperable or metastatic disease, treatment consists of hormonal therapy and possibly chemotherapy. The lifetime risk of being diagnosed with prostate cancer for men in the United States is approximately 16%, while the risk of dying of prostate cancer is 3%.[2] African American men have the highest prostate cancer risk in the United States; the incidence of prostate cancer is about 60% higher and the mortality rate is more than 2 to 3 times greater than that of white men.[3] Autopsy results have suggested that about 30% of men age 55 and 60% of men age 80 who die of other causes have incidental prostate cancer,[4] indicating that many cases of cancer are unlikely to pose a threat during a man's life expectancy.

Grading

The most widely used grading scheme for prostate cancer is the Gleason system.[5] It is an architectural grading system ranging from 1 (well differentiated) to 5 (poorly differentiated); the score is the sum of the primary and secondary patterns. A Gleason score of 6 is low-grade prostate cancer that usually grows slowly; 7 is an intermediate grade; 8 to 10 is high-grade cancer that grows more quickly. Ten-year survival rates stratified by Gleason score have been estimated from the Surveillance, Epidemiology, and End Results registry to be about 98% for scores 2 through 6, 92% for a score of 7 with primary pattern 3 and secondary pattern 4 (3+4), 77% for a score of 7 (4+3), and 70% for scores between 8 and 10.[6]

Numerous genetic alterations associated with development or progression of prostate cancer have been described, with the potential for the use of these molecular markers to improve the selection process of men who should undergo prostate biopsy or rebiopsy after an initial negative biopsy.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed under the Clinical Laboratory Improvement Amendments for high-complexity testing. The following laboratories are certified under the Clinical Laboratory Improvement Amendments: BioReference Laboratories and GenPath Diagnostics (subsidiaries of OPKO Health; 4Kscore®), ARUP Laboratories, Mayo Medical Laboratories, LabCorp, BioVantra, others (PCA3 assay), Clinical Research Laboratory (Prostate Core Mitomic Test™), MDx Health (ConfirMDx), and Innovative Diagnostics (phi™). To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

In February 2012, the Progensa® PCA3 Assay (Gen-Probe; now Hologic, Marlborough, MA) was approved by FDA through the premarket approval process. According to the company's press release, this assay is "indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had 1 or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on the current standard of care, before consideration of Progensa *PCA3* assay results." FDA product code: OYM.

In June 2012, proPSA, a blood test used to calculate the Prostate Health Index (phi; Beckman Coulter, Brea, CA) was approved by FDA through the premarket approval process. The phi test is indicated as an aid to distinguish prostate cancer from a benign prostatic condition in men ages 50 and older with prostate-specific antigen levels of 4 to 10 ng/mL and with digital rectal exam findings that are not suspicious. According to the manufacturer, the test reduces the number of prostate biopsies. FDA product code: OYA.

POLICY

A. The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered **experimental / investigational**:
   1. Kallikrein markers (eg, 4Kscore™ Test)
   2. *PCA3* testing
   3. TMPRSS fusion genes
   4. Candidate gene panels
   5. Mitochondrial DNA mutation testing (eg, Prostate Core Mitomics Test™)
   6. Gene hypermethylation testing (eg, ConfirmMDx®)
   7. Prostate Health Index (phi)

B. Single-nucleotide variant testing for cancer risk assessment of prostate cancer is considered **experimental / investigational**.

Policy Guidelines

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the HUman Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

RATIONALE

This evidence review has been updated with searches of the MEDLINE database. The most recent literature update was performed through July 26, 2017. In 2009, this evidence review was extensively updated and its scope broadened, informed primarily by a 2008 TEC Special Report on prostate cancer genetics and genetic testing.[7] In September 2017, the Blue Cross Blue Shield Association Medical Advisory Panel reviewed this report.

Genetic and protein biomarker tests are best evaluated within the framework of a diagnostic or prognostic test because such frameworks provide diagnostic and prognostic information that assists in clinical management decisions. Assessment of a diagnostic or prognostic tool typically focuses on 3 categories of evidence: (1) analytic validity (ability of the test to accurately and reliably measure the marker of interest); (2) clinical validity (ie, statistically significant association between the test result and health outcomes); and (3) clinical utility (ie, demonstration that use of the diagnostic or prognostic information clinically can improve the net health outcome compared with patient management without use of the tool). Because these tests are used as an adjunct to the usual diagnostic workup, it is important to evaluate whether the tests provide incremental information above the standard workup to determine whether the tests have utility in clinical practice.

This review evaluates evidence for genetic and protein biomarkers for the purpose of guiding decision making about biopsy or rebiopsy (see Appendix Table 1 for genetic testing categories).

Biomarker testing for selection of men for initial prostate biopsy

Clinical Context and Test Purpose

The purpose of genetic and protein biomarker testing for prostate cancer is to inform the selection of men who should undergo initial biopsy. Conventional decision-making tools for

identifying men for prostate biopsy include digital rectal exam (DRE), serum prostate-specific antigen (PSA), and patient risk factors such as age, race, and family history of prostate cancer.

DRE has relatively low interrater agreement among urologists, with estimated sensitivity, specificity, and positive predictive value (PPV) for diagnosis of prostate cancer of 59%, 94%, and 28%, respectively.[8] DRE might have a higher PPV in the setting of elevated PSA.[9]

The risk of prostate cancer increases with increasing PSA levels; an estimated 15% of men with a PSA level of 4 ng/mL or less and normal DRE, 30% to 35% of men with a PSA level between 4 ng/mL and 10 ng/mL, and more than 67% of men with a PSA level greater than 10 ng/mL will have biopsy-detectable prostate cancer.[10,11] Use of PSA levels in screening has improved detection of prostate cancer. The European Randomized Study of Screening for Prostate Cancer (ERSPC) trial and Göteborg Randomised Prostate Cancer Screening Trial trials demonstrated that biennial PSA screening reduces the risk of being diagnosed with metastatic prostate cancer.[12-16]

However, elevated PSA levels are not specific to prostate cancer; levels can be elevated due to infection, inflammation, trauma, or ejaculation. In addition, there are no clear cutoffs for cancer positivity with PSA. Using a common PSA level cutoff of 4.0 ng/mL, the American Cancer Society (2010) systematically reviewed the literature and calculated pooled estimates of elevated PSA sensitivity of 21% for detecting any prostate cancer and 5% for detecting high-grade cancers with an estimated specificity of 91%.[17]

PSA screening in the general population is controversial. The U.S. Preventive Services Task Force recommended against PSA-based screening (D recommendation) in 2012; the recommendations are currently being updated, and the draft released in 2017 advises individualized decision making about screening for prostate cancer after discussion with a clinician for men ages 55 to 69 (C recommendation) and recommends against PSA-based screening in men 70 and older (D recommendation). Guidelines published by the American Cancer Society and the American Urological Association (AUA) have endorsed consideration of PSA screening based on age, other risk factors, and estimated life expectancy.[17-19]

The utility of PSA screening depends on whether screening can lead to management changes that improve the net health outcome. Results from the National Health Service–supported Prostate Testing for Cancer and Treatment trial (2016) demonstrated no difference in 10-year prostate cancer mortality rates between the treatment strategies of active monitoring, radical prostatectomy, and external-beam radiotherapy in clinically localized prostate cancer detected by PSA testing.[20]

Existing screening tools have led to unnecessary prostate biopsies. More than 1 million prostate biopsies are performed annually in the United States, with a resulting cancer diagnosis in 20% to 30% of men. About one-third of men who undergo prostate biopsy experience transient pain, fever, bleeding, and urinary difficulties. Serious biopsy risks (eg, bleeding or infection requiring hospitalization) are rare, with estimated rates ranging from less than 1% to 3%.[21,22]

Given the risk, discomfort, burden of biopsy, and low diagnostic yield, there is a need for noninvasive tests that distinguish potentially aggressive tumors that should be referred for biopsy from clinically insignificant localized tumors or other prostatic conditions that do not need biopsy with the goal of avoiding low-yield biopsy.

The following PICOTS were used to select literature to inform this review.

Patients

The relevant populations are men for whom an initial prostate biopsy is being considered because of clinical symptoms (eg, difficulty with urination, elevated PSA).

The population for which these tests could be most informative is men in the indeterminate or "gray zone" range of PSA on repeat testing with unsuspicious DRE findings. Repeat PSA testing is important because results initially reported to be between 4 and 10 ng/mL frequently revert to normal.[23] The gray zone for PSA levels is usually between 3 or 4 ng/mL and 10 ng/mL, but PSA levels vary with age. Age-adjusted normal PSA ranges have been proposed but not standardized or validated.

Screening of men with a life expectancy of less than 10 years is unlikely to be useful because most prostate cancer progresses slowly. However, the age range for which screening is most useful is controversial. The ERSPC and Rotterdam trials observed benefits of screening only in men up to about 70 years old.

Interventions

For assessing future prostate cancer risk, numerous studies have demonstrated the association between many genetic and protein biomarker tests and prostate cancer. Commercially available tests for selection of men for initial prostate biopsy include those described in Table 1.

Table 1. Commercially Available Tests to Determine Candidate for Prostate Initial Biopsy

Test	Manufacturer	Description
4Kscore	OPKO lab	Blood test that measures 4 prostate-specific kallikreins, which are combined into an algorithm to produce a score
Prostate Health Index (phi)	Beckman Coulter	Blood test that combines several components of PSA with an algorithm to produce a score

PSA: prostate-specific antigen.

In addition to commercially available tests, single-nucleotide variant (SNV) testing as part of genome-scanning tests for prostate cancer risk assessment are offered by a variety of laboratories, such as Navigenics (now Life Technologies), LabCorp (23andme), and ARUP Laboratories (deCODE), as laboratory-developed tests.

Comparators

Standard clinical examination for determining who goes to biopsy might include DRE, review of history of PSA values, along with consideration of risk factors such as age, race, and family history. The ratio of free (or unbound) PSA to total PSA (percent free PSA) is lower in men who have prostate cancer than in those who do not. A percent free PSA cutoff of 25% has been shown to have a sensitivity and specificity of 95% and 20%, respectively, for men with total PSA levels between 4.0 ng/mL and 10.0 ng/mL.[24]

The best way to combine all risk information to determine who should go to biopsy is not standardized. Risk algorithms have been developed that incorporate clinical risk factors into a risk

score or probability. Two examples are the Prostate Cancer Prevention Trial (PCPT) predictive model[25] and the Rotterdam Prostate Cancer risk calculator (also known as the ERSPC-Risk Calculator 4 [ERSPC-RC]).[26] In 2016, the AUA and the Society of Abdominal Radiology recommended that high-quality prostate magnetic resonance imaging (MRI), if available, should be strongly considered in any patient with a prior negative biopsy who has persistent clinical suspicion for prostate cancer and who is under evaluation for a possible repeat biopsy.[27]

Outcomes

The beneficial outcome of the test is to avoid a negative biopsy for prostate cancer. A harmful outcome is failure to undergo a biopsy that would be positive for prostate cancer, especially when disease is advanced or aggressive. Thus the relevant measures of clinical validity are the sensitivity and negative predictive value (NPV). The appropriate reference standard is biopsy, though prostate biopsy is an imperfect diagnostic tool. Biopsies can miss cancers and repeat biopsies are sometimes needed to confirm diagnosis. Detection rates vary by biopsy method and patient characteristics, with published estimates between 14% and 22% for the initial biopsy.[28,29]

Timing

The timeframe of interest for calculating performance characteristics is time to biopsy result. Men who forgo biopsy based on test results could miss or delay diagnosis of cancer. Longer follow-up would be necessary to determine effects on overall survival.

Setting

Initial screening using PSA levels and DRE may be performed in the primary care setting with referral to specialty (urologist) care for suspicious findings and biopsy. Clinical practice on screening methods and frequency vary widely.

Kallikreins Biomarkers and 4Kscore Test

The 4Kscore test (OPKO Lab) uses results from a blood test to generate a risk score estimating the probability of finding high-grade prostate cancer (defined as a Gleason score ≥7) if a prostate biopsy were performed. The intended use of the test is to aid in a decision whether to proceed with a prostate biopsy. A kallikrein is a subgroup of enzymes that cleaves peptide bonds in proteins. The intact prostate-specific antigen and human kallikrein 2 (hK2) tests are immunoassays that employ distinct mouse monoclonal antibodies. The score combines the measurement of 4 prostate-specific kallikreins (total PSA, free PSA, intact PSA, hK2), with an algorithm including patient age, DRE (nodules or no nodules), and a prior negative prostate biopsy.

The manufacturer's website states that the ideal patient for the 4Kscore is one whose other test results are equivocal.[30] The test is not intended for patients with a previous diagnosis of prostate cancer, who have had a DRE in the previous 4 days, who have received 5α reductase inhibitor therapy in the previous 6 months, or who have undergone treatment for symptomatic benign prostatic hypertrophy in the previous 6 months.

Analytic Validity

Measures of analytic validity include sensitivity (detection rate), specificity (1 – false-positive rate); reliability (repeatability of test results), and assay robustness (resistance to small changes in preanalytic or analytic variables). As described above, the 4Kscore combines 4 blood biomarkers. Total and free PSA are measured using Food and Drug Administration

(FDA)–approved kits from Roche Diagnostics. Intact PSA and hK2 are proprietary assays validated by OPKO. Only one published study was found describing any components of analytic validity for a test of kallikrein biomarkers. In 2006, Vaisanen et al reported on results of a new method to reduce false high results by eliminating assay interference in measurement of intact, free PSA and free hK2.[31] Using 1092 female heparin plasma samples as controls and 957 male samples, they optimized the protocol for immunoassays by replacing monoclonal capture or tracer antibodies with F(ab′)2 or recombinant Fab fragments. They tested the new method on another set of 444 samples and found that the optimized assay eliminated 70% to 85% of the falsely elevated results. Other measures of analytic validity were not found in the literature or the 4Kscore website. The laboratories that perform the analyses for 4Kscore are certified under the Clinical Laboratory Improvement Amendments.

Clinical Validity

At least 13 retrospective studies[32-44] and a prospective study[45] have estimated the performance characteristics of a risk score derived from 4 kallikrein (KLK) biomarkers. Many studies appear to be developmental work for the currently marketed version of the test. In general, the comparators used in these studies were other risk calculators or models that included terms for age, total PSA, and occasionally other risk factors. The reference standard was usually biopsy. Some studies performed in Sweden had long-term follow-up from a national registry of prostate cancer. The eligibility criteria included a lower limit of PSA (2 or 3 ng/mL) in most studies with no upper limit, and men with and without positive DRE. The studies included a mix of men who had or did not have previous PSA testing or biopsies. Mathematical methods used to calculate the KLK risk score varied across studies with respect to whether KLK values derived from plasma or serum measurements. Additionally, there was variance across studies in the additional risk factors included in the model (age, DRE, biopsies, other risk factors), and how KLK marker values were entered into the model (linearly, with splines or cubic splines). The area under the receiver operating characteristic (AUROC) curve, or a similar metric, was calculated in all studies. The estimated area under the curve (AUC) for the KLK model ranged from 0.72 to 0.90 and was numerically higher than the comparator in all studies except Carlsson et al (2013), who compared the KLK model with a clinical model including the length of benign tissue.[36] However, the confidence intervals (CIs) for AUC of the KLK model frequently overlapped with those of the comparator. A few studies have provided results for the KLK model calculated with and without each of the 4 KLK. In many cases, the addition of intact PSA and hK2 did not significantly improve the model. In Bryant et al (2015), the CIs of the AUC for 4 KLK model overlapped considerably with a model that included age, total and free PSA for any grade, and high-grade cancer.[44] Nordstrom et al (2015) included a comparison with another biomarker test (phi) and found both tests had very similar AUCs.[34]

Our review of the clinical validity of the 4Kscore only includes studies that stated use of the marketed 4Kscore version of the KLK model. The marketed version of the test appears to have been used in 3 studies.[32,33,45] Cutoffs for categorizing risk into low, medium, or high levels were only given in Konety et al (2015) and therefore sensitivity and NPV have not been calculated.[33] Results of the studies are summarized in Table 2. Two studies were conducted in the United States[33,45] and are discussed in the following paragraphs.

Table 2. Clinical Validity Studies of the 4Kscore for Diagnosing High-Grade Prostate Cancer

Study	Population	Reference Standard	Blinded Comparison With Reference Standard	Performance Characteristics (95% CI)		
				4Kscore	Comparators	
Borque-Fernando et al (2016)[32] (Spain)	51 men scheduled for biopsy for suspicion of prostate cancer	Clinical consensus of 4 uropathologists after review of biopsy of ≥10 cores	NR	AUC=0.79 (0.66 to 0.89)	ERSPC-RC risk calculator AUC=0.81 (0.67 to 0.90)	PCPT risk calculator AUC=0.75 (0.61 to 0.86)
Konety et al (2015)[33] (U.S.)	171 men of high-volume 4Kscore users who had biopsy results	Biopsy	NR	Low vs intermediate/high risk[a]: • Sen=0.97 (0.87 to 0.99) • Spec=0.12 (0.07 to 0.19) • PPV=0.37 (0.29 to 0.45) • NPV=0.87 (0.58 to 0.98)	NA	
Parekh et al (2015)[45] (U.S.)	1012 men scheduled for biopsy regardless of PSA or clinical findings	Biopsy with ≥10 cores	Yes	AUC=0.82 (0.79 to 0.85)	Risk model without intact PSA and hK2 AUC=0.75 (0.71 to 0.79)	PCPT modified risk calculator[b] AUC=0.74 (NR)

AUC: area under the curve; CI: confidence interval; ERSPC-RC: European Randomized Study of Screening for Prostate Cancer Risk Calculator; NA: not available; NPV: negative predictive value; NR: not reported; PCPT: Prostate Cancer Prevention Trial; PPV: positive predictive value; PSA: prostate-specific antigen; Sen: Sensitivity; Spec: Specificity.
[a] Calculated from value provided in article considering low risk to be a negative result and intermediate/high risk to be a positive result.
[b] Excluding the term for family history because it was not known in this cohort.

Performance of the 4Kscore test was validated in 1012 patients enrolled in a blinded, prospective study at 26 urology centers in the United States.[45] Enrollment was open to all men scheduled for a prostate biopsy, regardless of age, PSA level, DRE, or prior prostate biopsy. Each patient underwent a transrectal ultrasound-guided prostate biopsy of at least 10 cores. A blinded blood sample collected before biopsy was sent to OPKO Lab for the 4 KLK markers. Results of the KLK markers, prostate biopsy histopathology, patient age, DRE, and prior biopsy status were unblinded and analyzed.

Most participants (86%) were white; 85 (8%) African American men were included. At baseline, 247 (24%) men had an abnormal DRE, 348 (34%) had a PSA level less than 4 ng/mL, and 104 (10%) had PSA level greater than 10 ng/mL. Approximately 25% of the men were older than 70

years. Biopsies were negative in 54% (n=542) of cases, and showed low-grade (all Gleason grade 6) prostatic cancer in 24% (n=239) and high-grade cancer in 23% (n=231). Statistical analysis of 4Kscore test clinical data had AUROC of 0.82 (95% CI, 0.79 to 0.85) for the detection of high-grade prostate cancer; the AUROC for the PCPT risk calculator model was 0.74, but a precision estimate was not given.

Subsection Summary: Clinical Validity
The intended use population is not well defined. In addition, there is uncertainty regarding clinical performance characteristics such as sensitivity, specificity, and predictive value due to the following factors: a lack of standardization of cutoffs to recommend biopsy, study populations including men with low (<4 ng/mL) and high (>10 ng/mL) baseline PSA levels, positive DRE results likely outside the intended use population, and lack of comparison with models using information from standard clinical examination. African Americans have a high burden of morbidity and mortality but were not well represented in the study populations. The evidence needed to conclude clinical validity is insufficient. Longer term data on the incidence of prostate cancer in men who did not have a biopsy following testing with the marketed version of 4Kscore are not available. However, the Stattin et al (2015) case-control study, which was a nested cohort study of more than 17,000 Swedish men, estimated that, for men ages 60 with PSA levels of 3 or higher and a KLK risk score less than 10%, the risk of metastasis at 20 years was 1.95% (95% CI, 0.64% to 4.66%).[35]

Clinical Utility
No studies reporting direct evidence of utility for clinical outcomes were found. Various cutoffs for the KLK probability score were used in decision curve analyses to estimate the number of biopsies vs cancers missed. Parekh et al (2015) estimated that 307 biopsies could have been avoided and 24 cancer diagnoses would have been delayed with a 9% 4Kscore cutoff for biopsy and 591 biopsies would have been avoided with 48 diagnoses delayed with a 15% cutoff.[45] However, inferences on clinical utility cannot be made due to deficiencies in estimating the clinical validity that are described in the previous section.

Konety et al (2015) reported on results of a survey of 35 U.S. urologists identified through the 4Kscore database at OPKO Lab as belonging to practices that were large users of the test.[33] All 611 patients of participating urologists who were referred for abnormal PSA or DRE and had a 4Kscore test were included. Six percent of the men had an abnormal DRE; the distribution of PSA levels was not reported. Urologists, who received the 4Kscore as a continuous risk percentage, were retrospectively asked about their plans for biopsy before and after receiving the test results and whether the 4Kscore test results influenced their decisions. Scores were grouped into 3 risk categories: less than 7.5%, low risk; 7.5% to 19.9%, intermediate risk; and 20% or more, high risk. The physicians reported that the 4Kscore results influenced decisions in 89% of men and that the test led to a 64.6% reduction in prostate biopsies. The 4Kscore risk categories correlated highly ($p<0.001$) with biopsy outcomes in 171 men with biopsy results. Calculated performance characteristics are shown in Table 2. No other risk calculators were included as comparators.

Absent direct evidence of clinical utility, a chain of evidence might be constructed. The 4Kscore test is associated with a diagnosis of aggressive prostate cancer. The incremental value of the 4Kscore concerning clinical examination and risk calculators in the intended use population is unknown due to deficiencies in estimating clinical validity described in the previous section. There

is no prospective evidence that use of 4Kscore changes management decisions. The chain of evidence is incomplete.

Section Summary: Kallikreins Biomarkers and 4Kscore Test

Published data on most components of the analytic validity of the 4Kscore test are lacking. At least 13 studies have reported on clinical validity of the KLK biomarkers, but only three clearly used the marketed version of the 4Kscore test. The eligibility criteria for these studies had a lower limit for screening PSA but no upper limit. Given that the test manufacturer's website states that the test is for men with inconclusive results, the inclusion of men with PSA levels greater than 10 ng/mL and positive DRE in the validation studies is likely not reflective of the intended use population. Studies that provide data on the incremental value of the components of the test show only small improvements with the intact PSA and hKA components (components specific to the 4Kscore). The 2 studies performed in U.S. men did not provide estimates (with confidence intervals) of validity compared with a standard clinical examination with a ratio of free or unbound PSA to total PSA (percent free PSA). Very few data are available on longer term clinical outcomes of men who were not biopsied based on 4Kscore results. No direct evidence supports the clinical utility of the test, and the chain of evidence is incomplete due to the limitations in estimates of clinical validity and utility.

proPSA and Prostate Health Index

The Prostate Health Index (phi; Beckman Coulter) is an assay combining results of 3 blood serum immunoassays (total PSA, free PSA, [-2]proPSA [p2PSA]) numerically to produce a "phi score." This score is calculated in a routine laboratory using Beckman Coulter equipment and software with the phi algorithm incorporated in the software using the following formula: ([-2]proPSA/free PSA) × √total PSA. It has been suggested that the PSA isoform p2PSA might better distinguish between prostate cancer and benign prostatic conditions.

The phi score has been approved by FDA for distinguishing prostate cancer from benign prostatic conditions in men 50 years and older with above-normal total PSA readings between 4.0 ng/mL and 10 ng/mL who have had a negative DRE. The manufacturer's website states that the test gives men "accurate information on what an elevated PSA level might mean and the probability of finding cancer on biopsy" and when "combined with family and patient history, the phi results can be used to determine the best individualized patient management decisions."[46]

Analytic Validity

The FDA Summary of Safety and Effectiveness Data (SSED) provides data on the analytic validity of the assay.[47] The analytic validity was also reviewed by the National Institute for Health and Care Excellence (NICE) in 2015.[48] The limit of blank of p2PSA was 0.5 pg/mL, the limit of detection was 0.7 pg/mL, and the limit of quantification was 3.23 pg/mL. Accuracy was calculated by the percentage recovery of measured p2PSA pg/mL in 6 male serum samples containing different known amounts of purified p2PSA.

Clinical Validity

Systematic Reviews

Several systematic reviews and meta-analyses have described the clinical validity of p2PSA and phi. The characteristics of the reviews are shown in Table 3. The reviews cover studies reported between 1990 and 2014. All primary studies were observational and most were retrospective. All reviews included studies of men with a positive, negative, or inconclusive DRE; only 2[49,50] of the 5

reviews restricted eligibility to studies including PSA levels between 2 ng/mL and 10 ng/mL. The Wang et al (2014) review included only studies that had sufficient information to distinguish aggressive from indolent prostate cancer.[51] The 2 most recent reviews (Nicholson et al [2015],[48] Pecoraro et al [2016],[49]) included most of the studies covered in the older reviews; we review them in more detail below.

Nicholson et al[48] performed a systematic review and health technology assessment commissioned to support the development of NICE guidance (2015)[52] for diagnosing prostate cancer with the prostate cancer antigen 3 (PCA3) and phi assays. The search included the databases MEDLINE, EMBASE, the Cochrane Library, Web of Science, Medion, Aggressive Research Intelligence Facility database, ClinicalTrials.gov, International Standard Randomised Controlled Trial Number Register, and World Health Organization International Clinical Trials Registry Platform. The review included studies of men whose initial prostate biopsies were negative or equivocal and studies using clinical examination or clinical examination plus MRI as comparators. Pooled estimates were not reported due to heterogeneity.

Pecoraro et al performed a search of MEDLINE, EMBASE, Web of Science, Scopus, and the Cochrane Register of Diagnostic Test Accuracy Studies for studies including men with a PSA level between 2 ng/mL and 10 ng/mL.[49] The quality of each study was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist, and the evidence was evaluated using the GRADE approach. Random-effects bivariate models were used to calculate pooled estimates.

Table 3. Characteristics of Systematic Reviews on the Clinical Validity of phi for Diagnosing Prostate Cancer

Study	Dates	Key Inclusion Criteria[a]	Design
Nicholson et al (2015)[48]	2000-2014	Initial prostate biopsy was negative or equivocal, 6+ cores in initial biopsy	Prospective and mixed (prospective/retrospective) OBS
Pecoraro et al (2016)[49]	2003-2014	PSA 2-10 ng/mL	Prospective, retrospective, and mixed (prospective/retrospective) OBS
Bruzzese et al (2014)[50]	2009-2013	TRUS biopsy (6+ cores) for diagnosis; PSA 2-10 ng/mL; first biopsy	Retrospective and prospective OBS
Wang et al (2014)[51]	2000-2014	Biopsy reference standard	Prospective, retrospective, and mixed (prospective/retrospective) OBS
Filella et al (2013)[53]	1990-2011	Biopsy reference standard	Retrospective and prospective OBS

OBS: observational; PSA: prostate-specific antigen; TRUS: transrectal ultrasound.
[a] Results from all studies were with or without digital rectal exam.

Results of the systematic reviews and meta-analyses are shown in Table 4. Pecoraro et al included 17 studies with 6912 men.[49] They rated most of the primary studies as low quality due to the design (most were retrospective), lack of blinding of outcome assessors to reference standard results, lack of clear cutoffs for diagnosis, and lack of explicit diagnostic question. Pooled estimates had high heterogeneity across studies but with generally low specificity of phi at 90% sensitivity.

Nicholson et al included 4 studies with 767 men that included estimates of clinical assessment alone vs clinical assessment plus phi.[48] They concluded that the implication of adding phi to clinical assessment was unclear. Due to heterogeneity in cutoffs used in the primary studies, it was not possible to identify thresholds to use in a clinical setting, and the clinical relevance of many reported outcomes was unclear.

Table 4. Results of Systematic Reviews on the Clinical Validity of phi for Diagnosing Prostate Cancer

Study	Studies	N (Range)	Outcomes	Results (95% CI)
Pecoraro et al (2016)[49]	17	6912 (63-1091)	• Diagnostic performance • Any prostate cancer	Pooled specificity at 90% sensitivity: • phi=31% (29% to 33%) • total PSA=25% (23% to 27%)
Nicholson et al (2015)[48]	4	767 (95-280)	• Diagnostic performance • Any prostate cancer	• No pooled estimates • AUC range: o phi plus clinical assessment, 0.65-0.81 o Clinical assessment alone, 0.62-0.75 • Derived sensitivity (at given specificity) o phi plus clinical assessment, 42% (at 80%), 25% (at 90%), 19% (at 95%) o Clinical assessment alone, 48% (at 80%), 23% (at 90%), 17% (at 95%)
Bruzzese et al (2014)[50]	8	3173 (64-896)	• Diagnostic performance • Any prostate cancer	• Significant heterogeneity • Sensitivity range: o phi, 31%-90% o %fPSA, 12%-90% • Specificity range: o phi, 30%-90% o %fPSA, 11%-90% • AUC: o phi=0.74 (90.70 to 0.77) o %fPSA=0.63 (0.58 to 0.67)
Wang et al (2014)[51]	12		• Diagnostic performance, high-grade (Gleason score ≥7) prostate cancer	• Pooled sensitivity, 90% (87% to 92%) • Pooled specificity, 17% (14% to 19%) • Diagnostic OR=3.06 (1.61 to 5.84) • Pooled AUC=0.67 (0.57 to 0.77)
Filella et al (2013)[53]	13	3928 (63-1091)	• Diagnostic performance • Any prostate cancer	• Significant heterogeneity • Pooled specificity at 90% sensitivity, 32% (29% to 34%)

AUC: area under the curve; CI: confidence interval; OR: odds ratio; PSA: prostate-specific antigen; %fPSA: ratio of free (unbound) PSA to total PSA.

Clinical Studies

The pivotal study described in the FDA SSED included men 50 years and older with nonsuspicious DRE and PSA levels between 4 ng/mL and 10 ng/mL who had a histologically confirmed diagnosis.[47] The null hypothesis was that the phi specificity at 95% sensitivity would be no greater than the specificity of percent free PSA. Seven sites in the United States enrolled 658 men between 2008 and 2009 (97% enrolled prospectively, 3% enrolled retrospectively). Eighty-

one percent of participants were white, 5% were African American, and 1% was Asian. At 95% sensitivity, using a phi cutoff of 22.1, the specificity was 14.1% (precision not reported) for phi compared with 9.9% for percent free PSA. AUC was 0.71 (95% CI, 0.67 to 0.75) for phi compared with 0.65 (95% CI, 0.61 to 0.69) for percent free PSA.

Additional studies have been published since the systematic reviews. In 2015 Fossati et al conducted a case-control study with 1036 European men younger than 60 years of age.[54] They reported that phi had a higher AUC (0.70; 95% CI, 0.64 to 0.76) than total PSA (0.55; 95% CI, 0.48 to 0.61) in men younger than 60 years for detecting any prostate cancer. At 91% sensitivity, phi and total PSA had similar specificity (11.1% [95% CI, 6.8% to 16.8%] vs 10.5% [95% CI, 6.4% to 16.1%]) and an NPV (76.0% [95% CI, 59.3% to 92.7%] vs 75.0% [95% CI, 57.7% to 92.3%]), respectively. At the best combination of sensitivity and specificity (phi cutoff ≥41.2, total PSA cutoff ≥5.72), phi had a sensitivity of 64.2% (95% CI, 51.5% to 75.5%), a specificity of 63.2% (95% CI, 55.5% to 70.4%), and an NPV of 81.8% (95% CI, 75.2% to 88.4%) while total PSA had a sensitivity of 52.2% (95% CI, 39.7% to 64.6%), a specificity of 52.0% (95% CI, 44.3% to 59.7%), and an NPV of 73.6% (95% CI, 65.7% to 81.4%). A decision curve analysis found that using a model with age, prostate volume, total PSA, free PSA, percent free PSA, and phi with a probability cutoff of 10% would avoid 13% of biopsies while missing 0% of cancers; a cutoff of 20% would avoid 51% of biopsies while missing 18% of cancers; and a cutoff of 50% would avoid 94% of biopsies while missing 66% of cancers.

In 2016 Boegemann et al reported on results of a study of 769 European men ages 65 years and younger scheduled for initial or repeat prostate biopsy who were prospectively and retrospectively enrolled.[55] The investigators compared phi with other PSA measures for detecting clinically significant vs insignificant cancer (PRIAS-criteria: T-stage T1c/T2; Gleason score ≤6; number of positive cores per biopsies ≤2; total PSA ≤10 ng/mL; PSA density <0.2 ng/mL). The AUC for phi (0.72; 95% CI, 0.68 to 0.76) was higher than that for total PSA (0.62; 95% CI, 0.58 to 0.66) or percent free PSA (0.64; 95% CI, 0.60 to 0.68).

Morote et al (2016) reported numerically higher but not statistically significantly higher AUC for phi compared with total PSA or total free PSA for detecting aggressive prostate cancer in 357 men with PSA levels between 3 ng/mL and 10 ng/mL scheduled for first biopsy in a retrospective study in Spain.[56] Similarly, Yu et al (2016) reported numerically but not statistically significantly higher AUC for phi vs total PSA in 114 men in China with PSA levels between 2 ng/mL and 10 ng/mL and negative DRE.[57]

Porpiglia et al (2016) reported on the results of an observational retrospective study of 120 men with prostate cancer who received radical prostatectomy but were eligible for active surveillance.[58] Multiparametric MRI (mpMRI), phi, and PCA3 were performed on the single cohort. The base model for predicting pathologically confirmed significant prostate cancer (PCSPCa) had an AUC of 0.71. Relative to the base model for predicting PCSPCa, phi increased the AUC by 4% (0.75; p<0.01), PCA3 increased the AUC by 1% (0.72; p<0.01), and mpMRI increased the AUC by 7% (0.78; p<0.01). Results of mpMRI provided the greatest net benefit in predicting the presence of PCSPCa while phi provided a small incremental benefit in prediction.

Subsection Summary: Clinical Validity
Many studies and systematic reviews of these studies have reported on the clinical validity of phi. In general, the comparator was a component of PSA (total PSA, free PSA) but have not included

other risk factors from a standard clinical exam. Most of the primary studies included men with positive, negative, and inconclusive DRE and men with PSA levels outside of the 4- to 10-ng/mL range. African Americans have a high burden of morbidity and mortality but were not well represented in the study populations. There is no standardization of cutoffs used in a clinical setting for diagnosis and data on the diagnostic accuracy of phi for distinguishing clinically significant from insignificant cancer are lacking. A direct comparison between phi and mpMRI demonstrated a higher net benefit for mpMRI in predicting PCSPCa.

Clinical Utility

No studies directly measuring the effect of phi on clinical outcomes were found. A chain of evidence might be used to demonstrate clinical utility if each link in the chain is intact. The phi test is associated with a diagnosis of prostate cancer, although data on association with a diagnosis of aggressive prostate cancer are lacking. The phi test provided better diagnostic information than other measures of PSA alone, but decisions made with phi result plus other risk factors from clinical examination were not provided in most studies. Optimal cutoffs for classifying men into risk groups have not been standardized. No studies were found describing differences in management based on phi risk assessment. The chain of evidence is incomplete.

Section Summary: proPSA and Prostate Health Index

The analytic validity of phi has been established. At least 4 systematic reviews including a NICE assessment have been reported and included many primary studies. In general, selected studies included some men outside of the intended use population (PSA levels outside of the 4- to 10-ng/mL range and abnormal DRE). Comparisons with diagnosis with clinical examination were lacking. The cutoffs for categorizing men into risk groups in clinical practice have not been standardized, and therefore there is heterogeneity in reporting of performance characteristics and decision curve analyses.

Biomarker testing for selection of men for repeat prostate biopsy

Clinical Context and Test Purpose

The purpose of genetic and protein biomarker testing for prostate cancer is to inform the selection of men who should undergo repeat biopsy. The conventional decision-making tools for identifying men for prostate biopsy include DRE, serum PSA, and patient risk factors such as age, race, and family history of prostate cancer are previously described in the section for selection of men for initial prostate biopsy.

Given the risk, discomfort, burden of biopsy, and the low diagnostic yield, there is a need for noninvasive tests that distinguish potentially aggressive tumors that should be referred for rebiopsy from clinically insignificant localized tumors or other prostatic conditions that do not need rebiopsy with the goal of avoiding low-yield biopsy.

The following PICOTS were used to select literature that provides evidence relevant to this review.

Patients

The relevant populations are men for whom a rebiopsy is being considered because the results of an initial prostate biopsy were negative or equivocal and other clinical symptoms remain suspicious.

Interventions

For assessing future prostate cancer risk, numerous studies have demonstrated the association between many genetic and protein biomarker tests and prostate cancer. Commercially available tests for selection of men for repeat prostate biopsy include those described in Table 5.

Table 5. Commercially Available Tests to Determine Candidate for Prostate Repeat Biopsy

Test	Manufacturer	Description
4Kscore	OPKO lab	Blood test that measures 4 prostate-specific kallikreins, which are combined into an algorithm to produce a score
Progensa	• Hologic Gen-Probe • Many labs offer *PCA3* tests (eg, ARUP Laboratories, Mayo Medical Laboratories, LabCorp)	Urine test that measures *PCA3* mRNA
ConfirmMDx	MDxHealth	Measures hypermethylation of 3 genes in tissue sample
Prostate Health Index (phi)	Beckman Coulter	Blood test that combines several components of PSA with an algorithm to produce a score
Prostate Core Mitomics Test (PCMT)	Mitomics (formerly Genesis Genomics)	Measures deletions in mitochondrial DNA by polymerase chain reaction in tissue sample

PSA: prostate-specific antigen.

In addition to commercially available tests, SNV testing as part of genome-scanning tests for prostate cancer risk assessment is offered by a variety of laboratories, such as Navigenics (now Life Technologies), LabCorp (23andme), and ARUP Laboratories (deCODE), as laboratory-developed tests.

Comparators

Standard clinical examination for determining who goes to biopsy might include DRE, review of history of PSA values, along with consideration of risk factors such as age, race, and family history. The ratio of free (unbound) PSA to total PSA is lower in men who have prostate cancer than in those who do not. A percent free PSA cutoff of 25% has been shown to have a sensitivity and specificity of 95% and 20%, respectively, for a group of men with total PSA levels between 4.0 ng/mL and 10.0 ng/mL.[24]

The best way to combine all of the risk information to determine who should go to biopsy is not standardized. Risk algorithms have been developed that incorporate clinical risk factors into a risk score or probability. Two examples are the PCPT predictive model[25] and the ERSPC-RC).[26] The AUA and the Society of Abdominal Radiology recently recommended that high-quality prostate MRI, if available, should be strongly considered in any patient with a prior negative biopsy who has persistent clinical suspicion for prostate cancer and who is under evaluation for a possible repeat biopsy.[27]

Outcomes

The beneficial outcome of the test is to avoid a negative biopsy for prostate cancer. A harmful outcome is failure to undergo a biopsy that would be positive for prostate cancer, especially when disease is advanced or aggressive. Thus the relevant measures of clinical validity are the

sensitivity and negative predictive value. The appropriate reference standard is biopsy, though prostate biopsy is an imperfect diagnostic tool. Biopsies can miss cancers and repeat biopsies are sometimes needed to confirm the diagnosis. Detection rates vary by biopsy method and patient characteristics, with published estimates between 10% and 28% for a second biopsy and 5% and 10% for a third biopsy.[28,29]

Timing

The timeframe of interest for calculating performance characteristics is time to biopsy result. Men who forgo biopsy based on test results could miss or delay diagnosis of cancer. Longer follow-up would be necessary to determine effects on overall survival.

Setting

Screening using PSA levels and DRE may be performed in the primary care setting with referral to specialty (urologist) care for suspicious findings and biopsy. Clinical practice on screening methods and frequency vary widely.

Progensa PCA3 Assay

PCA3 is overexpressed in prostate cancer, and *PCA3* messenger RNA can be detected in urine samples collected after prostate massage. When normalized using PSA to account for prostate cells released into the urine (PCA3 score), the test has significantly improved specificity compared with serum PSA and may better discriminate patients with benign findings on (first or second) biopsy from those with malignant biopsy results.

The Progensa PCA3 assay (Hologic Gen-Probe) has been approved by FDA to aid in the decision for repeat biopsy in men 50 years or older who have had 1 or more negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care. The Progensa PCA3 assay should not be used for men with atypical small acinar proliferation on their most recent biopsy. The manufacturer's website states that the test is intended to identify men who have negative first biopsy results to determine who needs a follow-up biopsy and that a PCA3 score less than 25 is associated with a decreased likelihood of a positive biopsy.[59]

Analytic Validity

The analytic validity of the Progensa PCA3 has been reviewed by FDA[60] and NICE.[48] Limit of blank was reported as 0.50 pg/mL, limit of detection was 0.69 pg/mL, and limit of quantitation was 3.23 pg/mL. No assay interference was recorded in the SSED report. The SSED report included carryover studies with a 0% false-positive rate for negative samples interspersed with high-titer samples. Accuracy was calculated by percent recovery of PCA3 compared with ultraviolet-determined copies per milliliters of an 8-member panel of female urine spiked with in vitro transcript; the minimum was 90% and the maximum was 118%. Precision as measured by percent coefficient of variation for within- and between-laboratory variation ranged from 12.3 to 25 for PCA3 score in 3 control samples. Linearity was assessed with 11 samples with in vitro transcripts in processed female urine, and the deviation from linearity for PCA3 was less than 9%.

Clinical Validity

Systematic Reviews

Several systematic reviews and meta-analyses have described the clinical validity of Progensa. The characteristics of the reviews are in Table 6. The reviews cover studies reported up to 2014.

All primary studies selected were observational, although 1 study used the placebo arm from a randomized controlled trials and a validation trial not included in the reviews are described below. Reviewers selected studies of men with positive, negative, or inconclusive DRE without restrictions on PSA levels. The 2 most recent reviews (Cui et al [2016],[61] Nicholson et al [2015][48]) are detailed below.

In 2016 Cui et al reported on results of a systematic review that searched PubMed and EMBASE for case-control or cohort studies.[61] Quality was assessed using the QUADAS tool. Pooled estimates were calculated using random-effects models and summarized ROCs when evidence of threshold effect was detected. Nicholson et al was described in the section on phi.[48] In brief, the NICE assessment included studies with men for whom initial prostate biopsy results were negative or equivocal.[52]

Table 6. Characteristics of Systematic Reviews on the Clinical Validity of Progensa for Diagnosing Prostate Cancer

Study	Dates	Key Inclusion Criteria	Design
Cui et al (2016)[61]	Up to 2014	Biopsy as reference standard	Prospective, retrospective (case-control or cohort) OBS
Nicholson et al (2015) (NICE)[48]	2000-2014	Initial prostate biopsy negative or equivocal, 6+ cores in initial biopsy, with/without DRE	Prospective and mixed (prospective/retrospective) OBS (1 included a cohort from an RCT)
Bradley et al (2013) (AHRQ)[62]	1990-2012	Comparative data	Cohort studies
Ruiz-Aragon et al (2010)[63]	2000-2009	Men undergoing prostate cancer screening, biopsy reference standard	Prospective and retrospective OBS

AHRQ: Agency for Healthcare Research and Quality; DRE: digital rectal exam; NICE: National Institute for Health and Care Excellence; OBS: observational; RCT: randomized controlled trial.

Results from the systematic reviews are shown in Table 7. Cui et al included 46 studies with over 12,000 men.[61] The quality of the selected studies was rated as moderate to high. The most common PCA3 cutoff for categorizing low and high risk was 35; 25 studies had a PCA3 cutoff of 35. Most were performed in the United States and Europe; five were conducted in Asia. The estimates of AUC were lower for studies including men having repeated (0.68; 95% CI, 0.67 to 0.70) vs initial (0.80; 95% CI, 0.78 to 0.82) biopsies. AUC values were 0.74 (95% CI, 0.73 to 0.76) for studies with a cutoff value of 35 and 0.77 (95% CI, 0.75 to 0.79) for studies with a cutoff value equal to 35, although the group with varying cutoff (≠35) had a greater range and more variable performance estimates.

Nicholson et al included 13 studies describing 11 cohorts including one from the placebo arm of a randomized controlled trial.[48] Reviewers found that referral criteria for repeat biopsy were often unclear and varied across studies. The criteria also differed based on whether normal or abnormal DREs were included, and the mean or median PSA, when reported, ranged from 4.9 to 11.0 ng/mL. The prevalence of cancer on repeat biopsy varied from 11.4% to 68.3%. Meta-analyses were not performed due to heterogeneity. Some studies used PCA3 scores as a continuous variable and others created risk categories. The addition of PCA3 to clinical assessment, as a continuous or categorical variable, generally led to improvement in AUC. The comparisons were mixed concerning the diagnostic odds ratio (OR), although most studies found increased diagnostic accuracy for PCA3 plus clinical assessment compared with clinical

assessment alone. Studies that fixed sensitivity and derived specificity and those that reported decision curve analysis had mixed results. Reviewers concluded that the clinical benefit of PCA3 in combination with clinical assessment was not confirmed.

Table 7. Results of Systematic Reviews on the Clinical Validity for PCA3 or Progensa for Diagnosing Prostate Cancer

Study	Studies	N (Range)	Outcomes	Results (95% CI)
Cui et al (2016)[61]	46	12,295 (NR)	• Diagnostic performance • Any prostate cancer	• Sensitivity range, 47%-95% o Significant heterogeneity and threshold effect o Pooled estimate, 0.65 (0.63 to 0.66) • Specificity range, 22%-100% o Significant heterogeneity and threshold effect o Pooled estimate: 73% (72% to 74%) • Negative LR: o Pooled estimate, 48% (44% to 52%) • AUC=0.75 (0.74 to 0.77)
Nicholson et al (2015)[48] (NICE)	11	3336 (41-1072)	• Diagnostic performance • Any prostate cancer	• AUC range: o Clinical assessment alone, 0.55-0.75 o Clinical assessment plus PCA3, 0.61-0.76 • Derived sensitivity (at given specificity) range: o Clinical assessment alone, 44%-48% (at 80%) o Clinical assessment plus PCA3, 39%-46% (at 80%)
Bradley et al (2013)[62] (AHRQ)	43	9719 biopsies or prostatectomies (32-1246)	• Diagnostic performance • Any prostate cancer and aggressive prostate cancer	• Derived sensitivity (at given specificity) o tPSA=91% (at 20%) o PCA3=96% (at 20%) • Unable to compare performance for aggressive prostate cancer
Ruiz-Aragon et al (2010)[63]	14	3467 (30-563)	• Diagnostic performance • Any prostate cancer	• Sensitivity range, 47%-82% • Pooled sensitivity, 85% (84% to 87%) • Specificity range, 56%-89% • Pooled specificity, 96% (96% to 97%) • Pooled negative LR=0.15 (0.13 to 0.18)

AHRQ: Agency for Healthcare Research and Quality; AUC: area under the curve; CI: confidence interval; LR: likelihood ratio; NICE: National Institute for Health and Care Excellence; NR: not reported; tPSA: total prostate-specific antigen.

Randomized Controlled Trials

In 2014, the National Cancer Institute conducted a prospective trial to validate the diagnostic use of the PCA3 assay to complement PSA-based detection of prostate cancer.[64] The target population included men who had been screened for prostate cancer, primarily with a PSA test, some of whom had undergone a previous prostate biopsy. The study included 859 men from 11 centers in the United States. The primary study end point was the diagnosis of prostate cancer on biopsy, and the secondary study end point was the diagnosis of high-grade prostate cancer, defined as a Gleason score greater than 6. The primary analyses, including PCA3 thresholds, were determined a priori, and statistical power was based on independent analyses of prevalidation data from similar cohorts. Of the men in the study, 562 were presenting for their initial prostate biopsy. PPV was 80% (95% CI, 72% to 86%), and using a PCA3 score of more than 60, diagnostic sensitivity and specificity of PCA3 score were 42% (95% CI, 36% to 48%) and 91% (95% CI, 87% to 94%), respectively. For patients who underwent a repeat biopsy, the

NPV was 88% (95% CI, 81% to 93%), and, by using a PCA3 score of less than 20, sensitivity and specificity were 76% (95% CI, 64% to 86%) and 52% (95% CI, 45% to 58%), respectively. For the detection of high-grade cancer, performance of PCPT risk calculator was improved by adding PCA3 results to the risk calculator factors, with an AUC improvement of 0.74 to 0.78 for initial biopsy and 0.74 to 0.79 on repeat biopsy ($p \leq 0.003$).

Clinical Studies

The pivotal study describing in the FDA SSED for Progensa included 495 men from 15 clinical sites who had at least 1 negative prostate biopsy and whose urologist recommended repeat biopsy.[60] Prostate biopsy was performed using each site's local standard procedure. A total of 433 (87.5%) were white, 45 (9.1%) were African American or black, and 11 (2.2%) were Asian. A valid PCA3 score and biopsy result were available for 466 men. Using a PCA3 cutoff score of 25, the performance characteristics for positive biopsy were as follows: sensitivity, 77.5% (95% CI, 68.4% to 84.5%); specificity, 57.1% (95% CI, 52.0% to 62.1%); PPV, 33.6% (95% CI, 30.0% to 37.2%); and NPV, 90.0% (95% CI, 86.5% to 93.1%). In other words, 208 men in the study might have been spared an unnecessary repeat biopsy if a cutoff of 25 was used to recommend repeat biopsy. On the other hand, 23 of the men who had a biopsy positive for prostate cancer might have had their diagnosis delayed due to negative PCA3 result.

Clinical studies have compared PCA3 results with clinical examination and risk calculators; those focused on distinguishing between aggressive and indolent cancer are particularly relevant. Ankerst et al (2008) reported that incorporating the PCA3 score into the PCPT risk calculator improved the diagnostic accuracy of the calculator (from an AUC of 0.653 to 0.696).[65] Chun et al (2009), using a multivariate nomogram, demonstrated a 5% gain in predictive accuracy when *PCA3* was incorporated with other predictive variables such as age, DRE results, PSA levels, prostate volume, and biopsy history.[66] In a 2011 study of 218 patients with PSA levels of 10 ng/mL or less, Perdona et al performed a head-to-head comparison of these 2 risk assessment tools and suggested both might have value in clinical decision making.[67]

Several studies have evaluated the PCA3 score as a tool for distinguishing between patients with indolent cancers who may need only active surveillance and those with aggressive cancers who warrant aggressive therapy. Three studies from 2008—Haese et al,[68] Nakanishi et al,[69] and Whitman et al[70]—demonstrated an association between PCA3 scores and evidence of tumor aggressiveness. However, these findings were not confirmed in a 2006 study by Bostwick et al[71] or a 2008 study by vans Gils et al.[72] Auprich et al (2011) reported that PCA3 scores appeared to enhance identification of indolent disease but not pathologically advanced or aggressive cancer.[73]

Tosoian et al (2010) reported on a short-term prospective cohort study evaluating the PCA3 score in relation to outcomes in an active surveillance program involving 294 patients.[74] The PCA3 score did not distinguish between patients who had stable disease and those with more aggressive features.

Clinical Utility

Clinical utility studies using assay results for decision making for initial biopsy, repeat biopsy, or treatment have not been reported, nor have studies of the effects of using assay results on clinical outcomes. Several studies using decision analysis to estimate the cost-benefit tradeoff between reduction in unnecessary biopsies and missed prostate cancers have been published. One group reported potential reductions in unnecessary biopsies of 48% to 52%, with attendant

increases in missed prostate cancers of 6% to 15% using either a PCA3-based nomogram[75] or PCA3 level corrected for prostate volume (PCA3 density).[76] Although both studies were prospective, neither assessed utility of the test for clinical decision making because all patients underwent biopsy. Merdan et al (2015) used decision analysis to simulate long-term outcomes associated with use of the PCA3 score to trigger repeat biopsy compared with the PCPT risk calculator in men with at least 1 previous negative biopsy and elevated PSA levels.[77] They estimated that incorporating the PCA3 score of 25 (biopsy threshold) into the decision to recommend repeat biopsy could avoid 55.4% of repeat biopsies, with a 0.93% reduction in the 10-year survival rate.

Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The PCA3 test is associated with a diagnosis of prostate cancer, although data on its association with a diagnosis of aggressive prostate cancer are lacking. The PCA3 test provided better diagnostic information than other measures of PSA, but comparison with decisions made using risk factors from clinical examination was not provided in most studies. No prospective studies were found describing differences in management based on PCA3 risk assessment. The chain of evidence is incomplete.

Section Summary: Progensa PCA3 Assay

The analytic validity of Progensa has been established. At least 4 systematic reviews, including a health technology assessment, have been reported and included many primary studies. Studies of the PCA3 score as a diagnostic test for prostate cancer have reported sensitivities and specificities in the moderate range. In general, these studies are preliminary and report on clinical performance characteristics in different populations and with various assay cutoff values, reflecting the lack of standardization in performance and interpretation of PCA3 test results. Cutoffs for recommending repeat biopsy with the Progensa test have been suggested by the manufacturer and were used in a validation study for FDA approval. The clinical utility of the PCA3 test is uncertain because there is no evidence that its use can change management in ways that improve outcomes.

Gene Hypermethylation and ConfirmMDx

Epigenetic changes—chromatin protein modifications that do not involve changes to the underlying DNA sequence but can change gene expression—have been identified in specific genes. An extensive literature has reported significant associations between epigenetic DNA modifications and prostate cancer. Several investigators have evaluated detection of hypermethylation products in biologic fluids for early detection of prostate cancer.[78,79] Promoters of 3 genes (adenomatous polyposis coli [*APC*], *GSTP1*, *RARβ2*) were identified early as potentially involved in prostate carcinogenesis.[80] *GSTP1* is the most widely studied methylation marker for prostate cancer, usually as a diagnostic application. Studies in the late 2000s of *GSTP1* hypermethylation using tissue samples reported conflicting results.[81-85] Sunami et al (2009) assayed blood from 40 healthy individuals and 83 men with prostate cancer using a 3-gene cohort (*GSTP1*, *RASSF1*, *RARβ2*) and demonstrated sensitivity of 28% for cancer patients.[86] Trock et al (2012) conducted a small (86-patient) diagnostic exploratory cohort study and showed that hypermethylation of *APC* was associated with high sensitivity and specificity for cancer on repeat biopsy.[87]

In a 2012 meta-analysis by Van Neste et al, 30 peer-reviewed studies of hypermethylation of *GSTP1* and other genes in prostate tissue were evaluated.[88] The pooled estimates of sensitivity

for *GSTP1* to distinguish prostate cancer from normal in biopsies (328 cases, 263 controls) was 82%, with 95% specificity, 95% NPV, and 85% PPV. The combination of *GSTP1*, *APC*, and *RARβ* had a sensitivity of 95%, specificity of 95%, NPV of 99%, and PPV of 95%. Reviewers suggested that a valuable first step in diagnostic use might be to test for methylated genes to select patients undergoing prostate biopsy who might not require repeat biopsy.

Following the 2012 meta-analysis, several studies reported on associations between DNA hypermethylation at various gene loci (*RASSF1A*, *APC*, *GSTP1*, *PTGS2*, *RARβ*, *TIG1*, *AOX1*, *C1orf114*, *GAS6*, *HAPLN3*, *KLF8*, *MOB3B*) and prostate cancer.[89-91] In contrast, Kachakova et al (2013) found that *HIST1H4K* hypermethylation was more likely due to aging than to prostate carcinogenesis.[92]

ConfirmMDx (MDxHealth) is a commercially available test for gene methylation intended to distinguish true- from false-negative prostate biopsies to avoid the need for repeat biopsy in cases of a true negative and to identify men who may need a repeat biopsy. The test measures methylation of the genes *GSTP1*, *APC*, and *RASSF1*.

Analytic Validity

Goessl et al (2002) confirmed in 26 patients that neoplastic transformation could be identified in washings of prostate biopsies by *GSTP1* promoter hypermethylation using methylation-specific polymerase chain reaction (PCR).[93] Chu et al (2002) described a protocol for real-time, quantitative, methylation-sensitive PCR for detecting the methylation change in the 5′ regulatory sequence flanking the *GSTP1* gene that was more sensitive than conventional nested PCR (DNA test limitations were 0.048 ng and 0.64 ng, respectively).[94] Mehrotra et al (2008) confirmed that a field effect was detectable for *APC*, *RARβ2*, and *RASSF1A* up to 3 mm from the malignant core.[95] Van Neste et al (2012) described a study evaluating a multiplex assay consisting of 3 genes: *GSTP1*, *APC*, and *RASSF1*.[96] Thirty cancer-positive tissue samples and 12 cancer-free controls were analyzed with 4 singleplex vs 1 multiplex assay. A control gene (*ACTB*) was used to estimate DNA quantity and quality in 2 replicates. The ratio of *ACTB* copies ranged from 0.73 to 1.17 (outlier removed) for the multiplex assay, with a median ratio of 1.0. *ACTB* copy numbers were higher for the multiplex assay than for a singleplex assay (median, 1.5-fold copy increase). A linear regression model yielded amplification factors of 1.57, 1.19, 4.13, and 1.25 for the *ACTB*, *GSTP1*, *APC*, and *RASSF1* assays, respectively, with consistently high R^2 values (>0.90). Biopsies consisting of 10, 20, and 40 µm from formalin-fixed, paraffin-embedded tissue blocks from the minimization cohort were tested and compared (outliers were removed). The effect of the original sample volume on the relative DNA yield was minor, indicating that samples as small as 20 µm can be used to detect methylation. Older samples showed lower relative DNA yields ($p<0.001$), indicating that the age of formalin-fixed, paraffin-embedded samples does have a negative impact on DNA quality and quantity. Other measures of analytic validity were not found in the literature or the ConfirmMDx website. The laboratory that performs the analyses for ConfirmMDx is certified under the Clinical Laboratory Improvement Amendments.

Clinical Validity

Two blinded multicenter validation studies of the ConfirmMDx test have been performed.[97,98] Partin et al (2014) reported on results of the DOCUMENT study; it evaluated archived, cancer-negative prostate biopsy core tissue samples from 350 men from 5 U.S. urology centers.[98] All patients underwent repeat biopsy within 24 months. Men with 2 consecutive negative biopsies were classified as controls and men with a negative biopsy followed by a positive biopsy were

classified as cases. Thirty (9%) men were excluded from analysis because of noneligibility (n=2), insufficient DNA (n=1), insufficient biopsy cores (n=23), or detection of adenocarcinoma in the first biopsy based on central pathology review (n=4); 320 men were included in analysis (92 cases, 228 controls). Median age was 62 years (range, not given). Median PSA level was 5.3 ng/mL; 23% of men had PSA levels less than 4 ng/mL and 10% had a PSA level of 10 ng/mL or higher. Sixty percent of men had a normal DRE. Forty-two (13%) of the men were black, 232 (73%) were white, and 13 (4%) were Asian. The ConfirmMDx test, performed on the first biopsy, resulted in a NPV of 88% (95% CI, 85% to 91%), sensitivity of 62% (95% CI, 51% to 72%), and specificity of 64% (95% CI, 57% to 70%). The study was not powered to determine accurately the performance characteristics in a subgroup of black patients, but the estimated sensitivity was 77% (95% CI, 46% to 95%), specificity was 66% (95% CI, 46% to 82%), and NPV was 93% (85% CI, 82% to 97%). Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for age, PSA, DRE, first biopsy histopathology characteristics, and race, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome (OR=2.69; 95% CI, 1.60 to 4.51).

The MATLOC study, reported by Stewart et al (2013), tested archived cancer-negative prostate biopsy needle core tissue samples from 498 men from the U.K. and Belgium.[97] Patients underwent repeat biopsy within 30 months; cases had a positive second biopsy while controls had a negative second biopsy. A total of 483 men were included in the analysis (87 cases, 396 controls). The median PSA level was 5.9 ng/mL; 21% of men had PSA levels less than 4 ng/mL and 18% had PSA levels of 10 ng/mL or higher. Seventy-three percent of men had benign DRE. The ConfirmMDx test, performed on the first biopsy, resulted in a NPV of 90% (95% CI, 87% to 93%), sensitivity of 68% (95% CI, 57% to 77%), and specificity of 64% (95% CI, 59% to 69%). Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for patient age, PSA, DRE, and first biopsy histopathology characteristics, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome (OR=3.17; 95% CI, 1.81 to 5.53).

In 2016, Van Neste et al reported on results of combined data from the DOCUMENT and MATLOC studies to investigate whether DNA methylation intensities were associated with high-grade (Gleason score, ≥7) prostate cancer.[99] DNA methylation was the most significant and important predictor of high-grade cancer, resulting in an NPV of 96% (precision not reported).

Clinical Utility

In 2014, Wojno et al reported on a field observation study in which practicing urologists at 5 centers used the ConfirmMDx test to evaluate at least 40 men with previous cancer-negative biopsies who were considered at risk for prostate cancer.[100] Centers reported whether patients who had a negative test assay result had undergone a repeat biopsy at the time of the analysis. Median patient follow-up after the assay results were received was 9 months. A total of 138 patients were included in the analysis. The median PSA level was 4.7 ng/mL. Repeat biopsies had been performed in 6 (4.3%) of the 138 men with a negative ConfirmMDx test, in which no cancer was identified.

In 2013, Aubry et al analyzed the expected reduction in biopsies associated with ConfirmMDx use.[101] Using the MATLOC estimates of performance characteristics for ConfirmMDx, the authors estimated that 1106 biopsies per 1 million people would be avoided. The study did not include a decision analysis comparing the tradeoff in reduction in biopsies and missed cancers.

MDxHealth completed enrollment into the PASCUAL trial in April 2015. The PASCUAL trial is described as an observational trial of ConfirmMDx to evaluate the impact of the test (clinical utility) on physician decisions for repeat biopsy. Results have not yet been published.
No studies were found that directly show the effects of using ConfirmMDx results on clinical outcomes. Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The ConfirmMDx test is associated with a diagnosis of prostate cancer and aggressive prostate cancer. The validity studies of the ConfirmMDx test included men in the intended use population but did not compare performance characteristics with clinical examination plus percent free PSA. One survey of urologists who had previously used the ConfirmMDx test found that most ConfirmMDx negative patients did not have a biopsy. Prospective data on utility should be available after completion of PASCUAL. No data are available on the longer term clinical outcomes of the men who did not have biopsy based on ConfirmMDx results. The chain of evidence is incomplete.

Section Summary: Gene Hypermethylation and ConfirmMDx

Two clinical validation studies have reported on the clinical validity of the ConfirmMDx score in the intended use population. The studies did not provide estimates of validity compared with a standard clinical examination with percent free PSA. No data are available on the long-term clinical outcomes or clinical utility of the test. The indirect chain of evidence is incomplete due to the limitations of evidence on the comparative clinical validity and utility.

TMPRSS Fusion Genes and Mi-Prostate

*TMPRSS*2 is an androgen-regulated transmembrane serine protease that is preferentially expressed in normal prostate tissue. In prostate cancer, it may be fused to an E26 transformation-specific (ETS) family transcription factor (*ERG*, *ETV1*, *ETV4*, *ETV5*), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis. The result of gene fusion with an ETS transcription gene is that the androgen-responsive promoter of *TMPRSS*2 upregulates expression of the ETS gene, suggesting a mechanism for neoplastic transformation. Fusion genes may be detected in tissue, serum, or urine.

TMPRSS2-ERG gene rearrangements have been reported in 50% or more of primary prostate cancer samples.[102] Although *ERG* appears to be the most common ETS family transcription factor involved in the development of fusion genes, not all are associated with *TMPRSS*2. About 6% of observed rearrangements are seen with *SLC45A3*, and about 5% appear to involve other types or rearrangement.[63]

In 2014, Yao et al published a systematic review with meta-analysis of *TMPRSS2-ERG* for the detection of prostate cancer.[103] Literature was searched through July 2013, and 32 articles were included. Pooled sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were 47% (95% CI, 46% to 49%), 93% (95% CI, 92% to 94%), 8.9 (95% CI, 5.7 to 14.1), and 0.49 (95% CI, 0.43 to 0.55), respectively. Statistical heterogeneity was high (I^2>85%). It was unclear whether studies in screening populations were pooled with enriched patient samples (eg, elevated PSA and/or negative biopsy). There also was variability in the type of tissue samples analyzed (urine, prostatic secretions, biopsy, surgical specimens); the type of *TMPRSS2-ERG* assays used (fluorescence in situ hybridization, immunohistochemistry, real-time reverse transcriptase PCR, transcription-mediated amplification); and in *TMPRSS2-ERG* threshold cutoff values.

The Mi-Prostate (MiPS) is a test using the *TMPRSS2-ERG* gene to produce a risk probability for detection of prostate cancer and aggressive prostate cancer by standard biopsy. The probability score is calculated with logistic regression models that incorporate serum PSA, or the PCPT version 1.0, and urine *T2-ERG* and PCA3 scores. The test was developed by, and is only available from, the University of Michigan MLabs, and may be used to decide about monitoring PSA levels or pursuing a prostate biopsy.

Analytic Validity

The MiPS test uses results from the Progensa PCA3 test that demonstrated analytic validity in the FDA submission. The amounts of urine *TMPRSS2-ERG* are determined using transcription-mediated amplification assays. No peer-reviewed, full-length publications describing the analytic validity of the *TMPRSS2-ERG* assays were identified.

Clinical Validity

Tomlins et al (2011) developed a transcription-mediated amplification assay to measure *TMPRSS2-ERG* fusion transcripts in parallel with PCA3.[104] Combining results from the *TMPRSS2-ERG* and PCA tests and incorporating them into the multivariate PCPT risk calculator appeared to improve identification of patients with clinically significant cancer using Epstein criteria and high-grade cancer on biopsy. Although the study was large (1312 men at multiple centers), it was confounded by assay modifications during the study and by use of cross-validation rather than independent validation, using independent training and testing sets.

In 2013, this same group evaluated 45 men using a multivariable algorithm that included serum PSA plus urine *TMPSS2-ERG* and PCA3 from a post-DRE sample.[105] Samples were collected before prostate biopsy at 2 centers. For cancer prediction, sensitivity and specificity were 80% and 90%, respectively. AUC was 0.88.

In 2016, Tomlins et al published results of a validation study of the MiPS score in 1244 prospectively collected, post-DRE urine samples from 7 U.S. clinics.[106] A total of 1225 of the specimens had sufficient materials for both *TMPSS2-ERG* and PCA3 analysis and were included. Eighty percent of patients were presenting for initial biopsy. Seventy-three percent were white; the percentage of African Americans was not given. Approximately 25% of the men were older than 70. Twenty-three percent had an abnormal DRE, and the median PSA level was 4.7 ng/mL. The AUCs for predicting any cancer using PSA alone, PCPT risk calculator alone, and the MiPS score alone were 0.59, 0.64, and 0.76, respectively (CIs not given, $p<0.001$ for MiPS vs PCPT). The AUCs for predicting high-grade cancer were 0.65, 0.71, and 0.78, respectively ($p<0.001$ for MiPS vs PCPT). A MiPS score threshold for recommending biopsy has not been provided, and so sensitivity and NPV were not calculated.

Clinical Utility

Tomlins et al (2016) also used decision-curve analysis to estimate the number of biopsies that would have been performed and cancers that would have been missed using a MiPS risk cutoff for biopsy in their cohort.[106] Compared with a biopsy-all strategy, using a MiPS cutoff for aggressive cancer of 15% would have avoided 36% of biopsies while missing 7.0% of any prostate cancer and 1.6% of high-grade prostate cancer diagnoses. Using the PCPT risk calculator cutoff of 15% for aggressive cancer would have avoided 68% of biopsies while missing 25% of any cancer and 8% of high-grade cancer.

No studies were found that directly show the effects of using MiPS results on clinical outcomes. Given the lack of direct evidence of utility, a chain of evidence would be needed to demonstrate clinical utility. The MiPS test is associated with a diagnosis of prostate cancer and aggressive prostate cancer. The clinical validity study of the MiPS test included men with relevant PSA levels but also included men with positive DRE who would not likely forgo biopsy. The clinical validation study included comparison of performance characteristics with standard risk calculators; comparison with percent free PSA was not provided. Confirmation of performance characteristics is needed. No prospective data are available on using the MiPS score for decision making. No data are available on the longer term clinical outcomes of the men who did not have biopsy based on MiPS results. The chain is incomplete.

Section Summary: TMPRSS Fusion Genes and Mi-Prostate

Concomitant detection of *TMPRSS2-ERG* and PCA3 may more accurately identify men with prostate cancer. However, current evidence is insufficient to support its use. Estimated accuracy varies across available studies. The MiPS test has preliminary data suggesting improved clinical validity compared with the PCPT risk calculator in a validation study, but independent confirmation of clinical validity and comparison with percent free PSA is needed. Data on analytic validity and clinical utility are lacking.

Prostate Core Mitomics Test

The Prostate Core Mitomics Test (PCMT; Mitomics; formerly Genesis Genomics) is a proprietary test intended to determine whether a patient has prostate cancer, despite a negative prostate biopsy, by analyzing deletions in mitochondrial DNA by PCR to detect "tumor field effect." The test is performed on the initial negative prostate biopsy tissue. According to the company website, a negative PCMT result confirms the result of the negative biopsy (ie, the patient does not have prostate cancer) and that the patient can avoid a second biopsy, but a positive PCMT means that the patient is at high risk of undiagnosed prostate cancer. The website also states that physicians should consider using PCMT for patients who have a negative initial biopsy but continue to have elevated PSA, rising PSA, irregular DRE, atypical small acinar proliferation, high-grade prostatic intraepithelial neoplasia, or inconclusive biopsy.[107]

Analytic Validity

No peer-reviewed, full-length publications on the analytic validity of the commercially available PCMT test were identified.

Clinical Validity

A 2006 study retrospectively analyzed mitochondrial DNA variants from 3 tissue types from 24 prostatectomy specimens: prostate cancer, adjacent benign tissue, and benign tissue distant to the tumor (defined as tissue from a nondiseased lobe or at least 10-cell diameters from the tumor if in the same lobe).[108] Prostate needle biopsy tissue (from 12 individuals referred for biopsy) that were histologically benign were used as controls. Results from the prostatectomy tissue analysis showed that 16 (66.7%) of 24 had variants in all 3 tissue types, 22 (91.7%) of 24 had variants in malignant samples, 19 (79.2%) of 24 in adjacent benign samples, and 22 of 24 in distant benign glands. Overall, 273 somatic variants were observed in this sample set. In the control group, 7 (58.3%) patients had between 1 and 5 genetic alterations, mainly in noncoding regions. The authors concluded that the variants found in the malignant group vs the control group differed significantly and that mitochondrial DNA variants are an indicator of malignant transformation in prostate tissue.

In 2008, Maki et al reported on the discovery and characterization of a 3.4-kilobase mitochondrial genome deletion and its association with prostate cancer.[109] A pilot study analyzed 38 benign biopsy specimens from 22 patients, 41 malignant biopsy specimens from 24 patients, and 29 proximal to malignant (PTM) biopsy specimens from 22 patients. All patients with malignant biopsies had a subsequent prostatectomy, and the diagnosis of cancer was confirmed. The PTM biopsy samples were negative for cancer and were from the cohort that underwent prostatectomy. A confirmation study used 98 benign biopsy specimens from 22 patients, 75 malignant biopsy specimens from 65 patients, and 123 PTM biopsy specimens from 96 patients. In the confirmation study, patients had to have at least 2 successive negative biopsies; the first negative biopsy was used for analyses. For both the pilot and confirmation studies, samples for analysis were selected based on a review of pathology reports. The levels of the variation were measured by quantitative PCR and, based on PCR cycle threshold, data were used to calculate a score for each biopsy sample. In the pilot study, the scores were statistically significant between benign and malignant ($p<0.000$) and benign and proximal ($p<0.003$) samples. The PTM samples closely resembled the malignant sample, with no statistically significant resolution between the scores ($p<0.833$), to which the authors attributed to a field cancerization phenomenon. Results from the larger confirmation study were similar. Compared with histopathologic examination of the benign and malignant samples, the sensitivity and specificity were 80% and 71%, respectively, and the AUROC was 0.83 for the deletion. A blinded, external validation study showed a sensitivity and specificity of 83% and 79% and the AUROC of 0.87.

In 2010, Robinson et al[110] assessed the clinical value of the 3.4-kilobase deletion described in the Maki study in predicting rebiopsy outcomes. Levels of the deletion were measured by quantitative PCR in prostate biopsies negative for cancer from 101 patients who underwent repeat biopsy within 1 year and had known outcomes. Of the 101 first biopsies, the diagnosis was normal in 8, atypical and/or had prostatic intraepithelial neoplasia in 50, and hyperplasia or inflammation in 43. The clinical performance of the deletion was calculated with the use of an empirically established cycle threshold cutoff, the lowest cycle threshold as diagnostic of prostate cancer, and the histopathologic diagnosis on second biopsy. Final data were based on 94 patients, who on second biopsy had 20 malignant and 74 benign diagnoses. The cycle cutoff gave a sensitivity and specificity of 84% and 54%, respectively, with an AUROC of 0.75. NPV was 91%.

Clinical Utility

No peer-reviewed, full-length publications on the clinical utility of the commercially available PCMT test was identified.

Section Summary: Prostate Core Mitomics Test

The PCMT test has preliminary data on performance characteristics in a small validation study, but independent confirmation of clinical validity is needed. The studies did not provide estimates of validity compared with a standard clinical examination. No data are available on the long-term clinical outcomes. Data on analytic validity and clinical utility are lacking.

Candidate Gene Panels and SNV Testing

Because no single gene marker that is both highly sensitive and highly specific for diagnosing prostate cancer has been found, particularly in men already known to have elevated PSA levels, some investigators are combining several markers into a single diagnostic panel. Although

promising in concept, only single studies of various panels have been published, and none apparently is offered as a clinical service.

SNVs occur when a single nucleotide is replaced with another, and they are the most common type of genetic variation in humans. They occur normally throughout the genome and can act as biologic markers for disease association. Genome-wide association studies have identified correlations between prostate cancer risk and specific SNVs. However, it is widely accepted that individually, SNV-associated disease risk is low and of no value in screening for disease, although multiple SNVs in combination may account for a higher proportion of prostate cancer. Investigators have begun to explore the use of algorithms incorporating information from multiple SNVs to increase the clinical value of testing.

Ma et al (2014) examined various algorithms for cancer diagnosis and prognosis using urine and plasma levels of multiple genes, including *PCA3*, *PSA*, *TMPRSS2*, and *ERG*.[111] One algorithm distinguished prostate cancer from benign prostatic hypertrophy with an AUC of 0.78. Another algorithm distinguished men with a Gleason score 7 or higher for men with a Gleason score less than 7 (AUC=0.88). Combination of these 2 algorithms into a scoring system predicted the presence of a Gleason score 7 or higher in 75% of men. Qu et al (2013) reported on preliminary results of a 3-gene panel (androgen receptor [*AR*], *PTEN*, *TMPRSS2-ERG*) analyzed by fluorescence in situ hybridization.[112] Thirty-one percent of 110 archived primary tumor samples and 97 metastatic tumor samples from a separate cohort of patients were analyzable. Chromosomal abnormalities were detected in 53% of primary prostate cancers and in 87% of metastatic tumors (p<0.001).

In 2015, Leyten et al reported on the development of a gene panel using specimens from 133 patients that included 3 urinary biomarkers (*HOXC6*, *TDRD1*, *DLX1*).[113] When the gene panel was used with PSA, the combined AUC for predicting high-grade prostate cancer was 0.81 (95% CI, 0.75 to 0.86), which was higher than the concurrently measured Progensa AUC of 0.68 (95% CI, 0.62 to 0.75). Xiao et al (2016) reported the development of an 8-gene panel (*PMP22*, *HPN*, *LMTK2*, *FN1*, *EZH2*, *GOLM1*, *PCA3*, *GSTP1*) that was able to distinguish high-grade prostate cancer from indolent prostate cancer with a sensitivity of 93% (95% CI, 88% to 97%), a specificity of 70% (95% CI, 36% to 104%), a PPV of 98% (95% CI, 95% to 100%), and an NPV of 61% (95% CI, 25% to 97%) in a specimen cohort of 158 men.[114]

A 2012 Agency for Healthcare Research and Quality report on multigene panels in prostate cancer risk assessment reviewed the literature on SNV panel tests for assessing risk of prostate cancer.[115] All studies included in the review had poor discriminative ability for predicting the risk of prostate cancer, had a moderate risk of bias, and none of the panels had been evaluated in routine clinical settings. Reviewers concluded that the evidence on the available SNV panels did not permit a meaningful assessment of analytic validity, that the limited evidence on clinical validity was insufficient to conclude that SNV panels would perform adequately as a screening test, and that there was no evidence on the clinical utility of current panels.

Kader et al (2012) evaluated a panel of 33 prostate cancer–associated SNVs identified from genome-wide association studies in 1654 men.[116] Genetic score was a significant (p<0.001) independent predictor of prostate cancer (OR=1.72; 95% CI, 1.44 to 2.09) after adjusting for clinical variables and family history. Addition of genetic markers to the classification of prostate cancer risk resulted in 33% of men by reclassified into a different risk quartile. Approximately half

of these (n=267) were downgraded to a lower risk quartile, and the other half (n=265) were upgraded into a higher risk quartile. The net reclassification benefit was 10% (p=0.002). The authors concluded that, with the additional information of genetic score, the same number of cancers could be detected but with 15% fewer biopsies.

In a 2010 review by Ioannidis et al, 27 gene variants across a large range of chromosomal locations were identified that increased risk for prostate cancer, although, in all cases, the observed incremental risk was modest (OR≤1.36).[117]

Lindstrom et al (2011), in a study of 10,501 cases of prostate cancer and 10,831 controls, identified 36 SNVs showing association with prostate cancer risk, including two (rs2735893, rs266849) that showed differential association with Gleason score.[118] Per allele odds ranged from 1.07 to 1.44.

Ishak and Giri (2011) reviewed 11 replication studies involving 30 SNVs (19 in men of African descent, 10 in men with familial prostate cancer).[119] Odds ratios were positively associated with prostate cancer, although the magnitude of association was small (range, 1.11-2.63).

Section Summary: Candidate Gene Panels and SNV Testing

Numerous studies have demonstrated the association of many gene panels and SNVs with prostate cancer. These studies, in early stages of development, have shown a modest degree of association with future risk for prostate cancer. The clinical utility of these tests is uncertain; there is no evidence that information obtained from gene panels or SNV testing can be used to change clinical management in ways that will improve outcomes.

Summary of Evidence

For individuals who are being considered for an initial prostate biopsy or a repeat biopsy who receive testing for genetic and protein biomarkers of prostate cancer, the evidence includes systematic reviews and meta-analyses and primarily observational studies. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, other test performance measures, resource utilization, and quality of life. The evidence supporting clinical utility varies by test but has not been directly shown for any biomarker test. In general, the performance of biomarker testing for predicting biopsy referrals compared with clinical examination, including the ratio of free or unbound PSA to total PSA, is lacking. Procedures for referrals for biopsy based on clinical examination vary, making it difficult to quantify performance characteristics for this comparator. There is also considerable variability in biopsy referral practices based on clinical examination alone, and many biomarker tests do not have standardized cutoffs to recommend a biopsy. Therefore, to determine whether the tests improve the net health outcome, prospective, comparative data are needed on how test results are expected to be used vs how they are being used in practice, because of information about the associated effects on outcomes. Many test validation populations have included men with a positive digital rectal exam, PSA level outside of the gray zone (between 3 or 4 ng/mL and 10 ng/mL), or older men for whom the information from PSA test results are less likely to be informative. African American men have a high burden of morbidity and mortality, but have not been well represented in these study populations. It is unclear how to monitor men with low biomarker risk scores who continue to have symptoms or high or rising PSA levels. Comparative studies of the many biomarkers are lacking, and it is unclear how to use the tests in practice, particularly when test results are contradictory. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements

American Urological Association et al

In 2013, the American Urological Association published guidelines on the early detection of prostate cancer.[120] Based on a systematic review of the literature to 2013, the Association recognized that novel urinary markers, such as PCA3 protein biomarker and *TMPRSS2-ERG* fusion gene, may be "used as adjuncts for informing decisions about the need for a prostate biopsy—or repeat biopsy—after PSA [prostate-specific antigen] screening," but emphasized the lack of evidence "that these tests will increase the ratio of benefit to harm."

The American Urological Association and the Society of Abdominal Radiology published joint guidelines in 2016 on prostate magnetic resonance imaging (MRI) and MRI-targeted biopsy.[27] The associations recommended:

> "In patients with negative or low suspicion magnetic resonance imaging (PI-RADS [Prostate Imaging Reporting and Data System] assessment category of 1 or 2, respectively), other ancillary markers (ie PSA [prostate-specific antigen], PSAD [PSA density], PSAV [PSA velocity], PCA3, PHI, 4K) may be of value in identifying patients warranting repeat systematic biopsy, although further data are needed on this topic."

Evaluation of Genomic Applications in Practice and Prevention

In 2013, the Evaluation of Genomic Applications in Practice and Prevention working group published the following recommendations for *PCA3* testing in prostate cancer,[121] based on the Agency for Healthcare Quality and Research comparative effectiveness review,[62] summarized earlier:

- Evidence was insufficient to recommend "PCA3 testing to inform decisions for when to rebiopsy previously biopsy-negative patients for prostate cancer, [or] to inform decisions to conduct initial biopsies for prostate cancer in at-risk men (e.g., previous elevated PSA or suspicious DRE [digital rectal examination])...."
- Evidence was "insufficient ... to recommend PCA3 testing in men with cancer-positive biopsies to determine if the disease is indolent or aggressive in order to develop an optimal treatment plan."
- "...[T]he overall certainty of clinical validity to predict the diagnosis of prostate cancer using PCA3 is deemed 'low.'... [C]linical use for diagnosis is discouraged unless further evidence supports improved clinical validity."
- "...[T]he overall certainty of net health benefit is deemed 'low.'... [C]linical use [is discouraged] unless further evidence supports improved clinical outcomes."

National Comprehensive Cancer Network

National Comprehensive Cancer Network (NCCN) guidelines (v.1.2017) recommend that any man with a PSA level greater than 3 ng/mL undergo workup for benign disease, repeat PSA, and digital rectal examination.[122] The guidelines also recommend consideration of percent free PSA, phi, and 4Kscore in patients with a PSA level greater than 3 ng/mL who have not yet had a biopsy, and consideration of percent free PSA, phi, 4Kscore, PCA3, and ConfirmMDx in men who had a negative biopsy but are thought to be at higher risk (category 2A evidence). NCCN noted that these tests may be especially useful in men with PSA levels between 3 ng/mL and 10 ng/mL. NCCN indicated that:

"... no biomarker test can be recommended over any other at this time The optimal order of biomarker tests and imaging is unknown; and it remains to unclear how to interpret results of multiple tests in individual patients – especially when results are contradictory."

U.S. Preventive Services Task Force Recommendations

The **U.S. Preventive Services Task Force** published recommendations for prostate cancer screening in 2012.[18] Genetic and protein biomarkers addressed in this evidence review, including *PCA3*, were not mentioned.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 8.

Table 8. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT00773773	A Study to Assess if a Combination of Serum Measurements of Molecular Biomarkers and Serum Protein Profiling Can be Used to Predict Which Patients Undergoing Prostatic Biopsy Will be Diagnosed With Cancer	500	Oct 2017
NCT02241122	Improved Prostate Cancer Diagnosis - Combination of Magnetic Resonance Imaging Targeted Biopsies and Biomarkers (Multi-IMPROD)	400	Nov 2017
NCT02250313[a]	PASCUAL (Prostate Assay Specific Clinical Utility at Launch) Study	600	Mar 2018
NCT01739062	Prostate Cancer Risk Assessment Using Genetic Markers in General Practice	4500	Jan 2021
NCT01632930	Medical Economics of Urinary PCSA3 Test for Prostate Cancer Diagnosis	900	Dec 2021

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

81313	PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)
81479	Unlisted molecular pathology procedure
81539	Oncology (high-grade prostate cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA, and human kallikrein-2 [hK2]), utilizing plasma or serum, prognostic algorithm reported as a probability score (Effective 01-01-2017)
81551	Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy (Effective 01-01-2017).
81599	Unlisted multianalyte assay with algorithmic analysis
0053U	Oncology (prostate cancer), FISH analysis of 4 genes (ASAP1, HDAC9, CHD1 and PTEN), needle biopsy specimen, algorithm reported as probability of higher tumor grade

- There are specific CPT codes for PCA3 and 4Kscore® testing: 81313, 81539.
  - Effective January 1, 2017, the code for the 4Kscore test changed to a category 1 multianalyte assay with algorithmic analysis code 81539 with the same wording as 0010M, which was discontinued 01-01-2017.
- For the other types of testing mentioned above, there are no specific CPT codes. The unlisted molecular pathology code 81479 would be used. If the test includes multiple assays, uses an algorithmic analysis, and is reported as a numeric score or a probability, the unlisted multianalyte assay with algorithmic analysis (MAAA) code 81599 would be reported.

DIAGNOSES

Experimental / investigational for all diagnoses related to this policy.

REVISIONS	
12-01-2011	Policy added to the bcbsks.com web site.
04-10-2012	In Coding section: Added HCPCS code: S3721 (effective 04-01-2012).
06-29-2012	Description section updated
	Rationale section updated
	References updated
01-01-2013	In Coding section: ▪ Added CPT code: 81479 (effective 01-01-2013) ▪ Removed CPT codes: 83890, 83891, 83892, 83893, 83894, 83896, 83897, 83898, 83900, 83901, 83902, 83903, 83904, 83905, 83906, 83907, 83908, 83909, 83912 (effective 12-31-2012)
08-20-2013	Description section reviewed with no changes made.

REVISIONS	
	Rationale section updated
	In Coding section: ▪ Coding instructions added.
	References updated
01-01-2015	Policy posted 01-16-2015
	In Coding section: ▪ Added CPT Codes: 81313 (Effective January 1, 2015)
01-01-2016	In Coding section: ▪ Removed HCPCS Code: S3721 (Effective January 1, 2016) ▪ Updated Coding notations.
01-20-2016	▪ Title revised to "Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer" from "Gene-Based Tests for Screening, Detection, and/or Management of Prostate Cancer" ▪ Added "See Also: Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management"
	Description section updated
	In Policy section: ▪ In Item A removed "Genetic tests for the screening, detection, and management" and "This includes, but is not limited to the following:" and added "The following genetic and protein biomarkers for the diagnosis" to read "The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered experimental / investigational:" ▪ In Item A added the following E/I protein biomarkers: "1. Kallikrein markers (eg, 4Kscore™ Test) 2. Metabolomic profiles (eg, Prostarix™) 6. Mitochondrial DNA mutation testing (eg, Prostate Core Mitomics Test™)" ▪ In Item A 3 added "testing" and removed "for disease diagnosis and prognosis" to read "PCA3 testing" ▪ In Item A 4 removed "for diagnosis and prognosis" to read "TMPRSS fusion genes" ▪ In Item A 5 added "Candidate" and removed "multiple gene tests" and "for prostate cancer diagnosis" to read "Candidate gene panels" ▪ In Item A 7 added "testing (eg, ConfirmMDx®)" and removed "for diagnosis and prognosis" to read "Gene hypermethylation testing (eg, ConfirmMDx®)" ▪ In Item A relocated "single-nucleotide polymorphisms (SNPs) for risk assessment" to stand-alone Item B to read "Single nucleotide polymorphisms (SNPs) testing for cancer risk assessment of prostate cancer is considered experimental / investigational."
	Rationale section updated
	In Coding section: ▪ Added CPT Codes: 81599, 0010M ▪ Updated Coding notations.
	References updated
12-20-2017	Description section updated
	In Policy section: ▪ In Item A added "Prostate Health Index (phi)" and removed "Metabolomic profiles (eg, Prostarix™)" ▪ In Item B revised "polymorphisms (SNPs)" to "variant". ▪ Added Policy Guidelines – Information on Genetics Nomenclature Update and Genetic Counseling
	Rationale section updated
	In Coding section: ▪ Added CPT Codes: 81539 (Effective 01-01-2017), 81551 (Effective 01-01-2018)

REVISIONS	
	▪ Removed CPT Code: 0010M (Terminated 01-01-2017) ▪ Coding notations updated
	References updated
07-01-2018	In Coding section: ▪ Added PLA Code: 0053U

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Appendix

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.33

Category	Addressed
1. Testing of an affected individual's germline to benefit the individual	
1a. Diagnostic	
1b. Prognostic	
1c. Therapeutic	
2. Testing cancer cells from an affected individual to benefit the individual	
2a. Diagnostic	X
2b. Prognostic	X
2c. Therapeutic	
3. Testing an asymptomatic individual to determine future risk of disease	
4. Testing of an affected individual's germline to benefit family members	
5. Reproductive testing	
5a. Carrier testing: preconception	
5b. Carrier testing: prenatal	
5c. In utero testing: aneuploidy	
5d. In utero testing: familial variants	
5e. In utero testing: other	
5f. Preimplantation testing with in vitro fertilization	