The 4Kscore® Test Accurately Identifies Risk for Aggressive Prostate Cancer and Reduces Unnecessary Prostate Biopsies (April 2017)

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Executive Summary

Widespread screening for prostate cancer with the prostate-specific antigen (PSA) test began in the United States in the late 1980s, and subsequently a 45% decline in prostate cancer mortality has been observed. Owing to the low specificity of PSA, unnecessary prostate biopsies and over treatment of indolent cancer have led to significant morbidity and harm. Prostate biopsy is a painful procedure associated with up to 4% of men experiencing significant complications such as bleeding, infection and bacterial sepsis. Prostate biopsy can also lead to over treatment of indolent (Gleason score 6) prostate cancer with surgery or radiation therapy.

As a result of this and the failure of a U.S. randomized clinical study of PSA screening to demonstrate benefit, the United States Preventative Services Task Force (USPSTF) in 2012 advised against routine PSA screening for prostate cancer. Consequently, fewer numbers of men are being screened and diagnosed, but proportionally more men are being diagnosed with more advanced disease, reversing past trends.

In order to avoid the dilemma caused by the low specificity of the PSA test, of either over diagnosing prostate cancer or missing aggressive prostate cancer due to reduced screening, a more accurate and noninvasive test is needed. This test would be performed as a second decision point after finding an abnormal PSA result and/or digital rectal examination (DRE), but before a decision to proceed with a prostate biopsy.

The 4Kscore test is designed to minimize the potential harms stemming from unnecessary prostate biopsies, while still allowing for the accurate detection of aggressive, high-grade (Gleason score 7 and higher) prostate cancer in time to intervene with effective treatment. The 4Kscore test is an algorithm-based test combining the blood levels of four kallikrein proteins and a patient’s clinical information to provide a man’s risk for aggressive prostate cancer on prostate biopsy. It is intended to be used in men aged 45-75 years with a PSA between 1.5 and 10 ng/mL and/or an abnormal digital rectal examination prior to prostate biopsy.

The clinical validity of the 4Kscore test was demonstrated in a prospective, double blinded, 1012 patient clinical trial conducted in 2013-14, at 26 centers across the United States. All 1012 men submitted their blood samples prior to a prostate biopsy and the investigators and pathologists were blinded to the 4Kscore results. The area under the receiver operator curve (AUC) for discriminating the presence of Gleason score 7 and higher prostate cancer was 0.821 for the 4Kscore test vs. 0.694 for total PSA and 0.713 for % free PSA. The data also indicated that use of the 4Kscore test could result in a significant reduction of the number of unnecessary biopsies (30-58%), while still identifying a very high percentage of those men who may be at higher risk for aggressive prostate cancer.
Further sub-analysis of this cohort showed that in men 45-75 years of age with a PSA between 1.5-10 ng/mL, the 4Kscore demonstrated an AUC of 0.774 vs 0.676 for % free PSA and 0.607 for tPSA. In this group, there was a potential biopsy reduction of 35% using a 4Kscore cutoff of 7.5%.

A recent prospective study within the Veteran’s Affairs (VA) Health System has further validated the 4Kscore within a cohort including a large subset of African American men. African American men have a higher incidence and higher risk of aggressive prostate cancer, making it imperative to have an accurate test to evaluate the risk for this population. Of 366 men at 8 sites with 4Kscore and complete data, 205 (56%) were African American. The AUC of 4Kscore (0.81) outperformed the base model (0.74) and demonstrated higher utility on decision analysis. There was no significant difference in the AUC of the 4Kscore test between African American and non-African American men (0.80 and 0.84 respectively, p = 0.32).

The clinical utility of the 4Kscore test was confirmed in a retrospective 611 patient study performed at U.S. based community and academic urology centers. The results demonstrated a 64.6% overall reduction in prostate biopsies. Men with a low risk 4Kscore (< 7.5%) had a 94% reduction in prostate biopsies, while men with a high risk 4Kscore (20% or higher) had only a 19% reduction.

The 4Kscore test has been included in the NCCN 2015 and 2016 guidelines for Prostate Cancer Early Detection with level 2A evidence as a means to improve the selection of men at higher risk for clinically significant, aggressive prostate cancer who should undergo prostate biopsy due to an abnormal PSA test and/or DRE finding. It is also included in the 2016 European Association of Urology Prostate Cancer Guidelines. The American Medical Association (AMA) has approved the 4Kscore test for a Category I CPT code, effective January 2017.

The scientific basis of research on the use of the four kallikrein proteins to enhance the sensitivity and specificity of detecting aggressive prostate cancer was led by a team of investigators from Memorial Sloan Kettering Cancer Center. The commercially available 4Kscore test incorporated their scientific knowledge, and was further refined, developed and validated by OPKO in its own clinical laboratory and wholly owned subsidiary, BioReference Laboratories.

The 4Kscore test is a non-invasive blood based test that provides a second decision point prior to a prostate biopsy for men aged 45-75 years, with a PSA between 1.5 and 10 ng/mL and/or an abnormal DRE. It provides an accurate risk of aggressive prostate cancer, allowing for early detection of high risk men, while reducing the number of unnecessary prostate biopsies. It correlates with long term risk of distant metastasis in men with an elevated PSA, and is included in NCCN guidelines for Prostate Cancer Early Detection. Use of the 4Kscore will reduce the costs and complications of unnecessary prostate biopsies, while identifying men at a higher risk of aggressive cancer, who would benefit from more extensive evaluation.
**Intended Use and Target Population**

The 4Kscore test provides a man’s percentage risk (reported on a scale of less than 1% to greater than 95%) of having a Gleason score 7 or higher prostate cancer diagnosed if he were to have a prostate biopsy performed. **The 4Kscore test is intended to be used as a second decision point in men aged 45-75 years with a PSA between 1.5 and 10 ng/mL and/or an abnormal DRE, prior to performing a prostate biopsy in biopsy naïve men and men with a previous history of benign (negative) biopsies.**

The 4Kscore test is not a screening test. The information provided by the 4Kscore test allows the clinician and patient to make a more informed decision on whether or not a prostate biopsy is warranted. The evidence to support this intended use is provided in this dossier.

The 4Kscore test incorporates both laboratory blood test data and the patient’s clinical data. The laboratory blood tests are performed on either serum or plasma to measure the concentrations of four prostate specific kallikrein proteins that are prostate specific. Two of the blood tests are routinely performed in the clinical laboratory: total PSA (tPSA) and free PSA (fPSA), while the other two tests, intact PSA (iPSA) and human kallikrein-related peptidase 2 (hK2), are proprietary to OPKO and essential for the integrity and predictive accuracy of the 4Kscore test for high-grade (Gleason score 7 and higher) prostate cancer. Among the clinical data incorporated in the 4Kscore test are the patient’s age, DRE findings if available, and consideration of any previous benign (negative) prostate biopsy finding.

OPKO Health, Inc. developed the 4Kscore test and offers the 4Kscore test service through BioReference Laboratories, Inc., its wholly owned subsidiary. The 4Kscore test has been developed and validated at BioReference in strict compliance with CLIA regulations, and is performed in a single facility in accordance with the Laboratory Developed Test (LDT) regulations.

**Clinical Scenario**

**Current Standard of Care**

Current clinical practice for prostate cancer early detection is based on PSA testing and/or DRE, typically performed by a primary care physician (PCP). The discovery of an abnormal PSA test and/or DRE is the primary indication for referral to a urologist for further evaluation that could lead to a prostate biopsy to diagnose prostate cancer. This current paradigm leads to an estimated 1 million prostate biopsies being performed each year in the United States. However, 75% of these prostate biopsies performed are unnecessary, as they show either no cancer or low grade (Gleason score 6) prostate cancer, an indolent form of prostate cancer that is age-associated and highly unlikely to cause harm. Because of concern that a more serious, high-grade cancer could be missed by the biopsy, many men with a diagnosis of Gleason score 6 prostate cancer choose to have treatment (radical
prostatectomy or radiation). However, 66% of Gleason score 6 cancers detected by biopsy are confirmed as Gleason score 6 in the radical prostatectomy surgical specimen, rendering the aggressive treatment for these men unnecessary. Consequently, in the current paradigm with an over reliance on the PSA test, many men are needlessly exposed to the harms of biopsy (bleeding, infection, and hospitalization) and suffer long term impaired urological function from unnecessary radical prostatectomy or radiation therapy.

The ultimate goal of prostate cancer screening is to diagnose and treat high-grade, aggressive prostate cancer when it is organ confined and when successful treatment is possible, while minimizing the harms of unnecessary biopsies and over treatment of indolent cancers. It is apparent that to achieve this goal, physicians require a test that is more sensitive and specific for identification of the subset of men with higher risk for aggressive prostate cancer. A new test that could better select those men who should have a prostate biopsy would be extremely valuable. The 4Kscore test was developed to fulfill this clinical need.

Public Health Importance

Prostate cancer is the second leading cause of cancer deaths in men, with 26,000 deaths projected in the US in 2016. Since the advent of PSA screening in 1991, a 45% decline in overall prostate cancer mortality has been observed, with much of this decline attributable to PSA screening. In 2009, the European Randomized Study of Screening for Prostate Cancer (ERSPC) reported a 29% reduction in death from prostate cancer in men undergoing routine PSA screening. However, no benefit to screening was observed in the U.S. based Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), but this was likely due to contamination of the control group (who were to have no PSA screening) with PSA testing. A review of the study determined that 80% of the men in the PLCO control group had at least one PSA test during the trial. Nevertheless, because of the PLCO study results, the net physical and psychological harms that result from unnecessary prostate biopsies, the uncertainty of prostate biopsies under grading or missing prostate cancer, and the over treatment of non-life threatening prostate cancer, in 2012 the USPSTF advised against using the PSA test for routine prostate cancer screening in men.

A 2012 systematic analysis of available literature on active surveillance of prostate cancer suggested that up to 60% of prostate cancers diagnosed in contemporary studies might be safely observed without a need for immediate intervention. Currently, about 40% of low risk patients are on active surveillance, while the rest undergo more aggressive therapy.

The harms associated with unnecessary prostate biopsy and over treatment of low-grade prostate cancer are well documented. A population-based study revealed a four-fold increase from 1996 to 2005 in the incidence of hospital admissions after
prostate biopsy, to an overall rate of 4.1%, with 72% of these admissions due to biopsy-related bacterial septicemia. The primary postsurgical and post-radiation therapy complications include erectile dysfunction, urinary incontinence, and decline in health-related quality of life in the domains of sexual, urinary, and psychological function.

The impact of the USPSTF has been a decrease in overall biopsy rates, and as a consequence, a decrease in the detection of the aggressive Gleason score 7-10 prostate cancers. Evidence is now mounting that with reduced levels of PSA screening, men who would have been found to have aggressive prostate cancer following an abnormal PSA level, and subsequently referred to a urologist, are remaining undetected longer, and presenting with more advanced disease and with reduced likelihood for cure.

In summary, there is a need to address the limitations of PSA screening without abandoning this very effective tool for the early identification of men at risk for prostate cancer. The 4Kscore test offers a second decision point after an abnormal PSA test and/or DRE, using a non-invasive blood sample, to reduce the number of unnecessary prostate biopsies in men at a lower risk for aggressive prostate cancer (Gleason score 7 and higher). The use of the 4Kscore test will distinguish those men at risk for aggressive disease from men at low risk, thereby reducing unnecessary prostate biopsies, unnecessary treatment, and the harms that result from both.

**Threshold PSA Value of 1.5 ng/mL for Further Evaluation**

The 4Kscore is a follow up test after an abnormal PSA and/or DRE. One of the difficulties of using PSA as a screening tool for prostate cancer is the lack of consensus on what PSA threshold is appropriate for further evaluation. Several studies have examined the risk of prostate cancer at a threshold of 1.5 ng/mL, showing an increased risk above this value.

A prospective study of 5,855 men examined the cancer detection rate at various PSA values to determine at what point more frequent PSA testing could be recommended. Of these men, 539 (9.2%) developed prostate cancer, with a median follow up of 7.6 years. They found a very low risk of detection for men with a PSA below 1.0 ng/mL (0 - 0.9%). The rate showed a significant rise for men with a PSA of 1.50-1.99 ng/mL (12.3%) versus for 1.00-1.49 ng/mL (4.7%). Regular, shorter intervals were recommended for PSA testing in men with a PSA greater than 1.5 ng/mL.

A retrospective study of 21,502 men with PSA of 0-4 ng/mL, who in many cases will be considered normal risk, evaluated the prostate cancer risk over a four-year period. Prostate cancer rates were 15-fold higher (19-fold for African American men) in patients with a PSA of 1.5-4.0 ng/mL versus those with a PSA < 1.5 ng/mL. Men with a baseline PSA of < 1.5 ng/mL showed little progression over the study period, with a starting PSA mean of 0.70 ng/mL and an ending mean of 0.88 ng/mL. Men with a PSA between 1.5-4.0 ng/mL, however, progressed from a mean of 2.44...
ng/mL to 3.24 ng/mL. The authors concluded that men with a PSA of 1.5-4.0 ng/mL should be considered in an Early-Warning PSA Zone, with an increased risk as opposed to men with a lower PSA.

A recent discussion of how to approach men with a PSA greater than 1.5 ng/mL discussed the use of follow up tests, including the 4Kscore to better stratify men.\textsuperscript{25} Recognizing the difficulties with PSA screening, especially in minimizing unnecessary procedures, the authors proposed utilizing next generation tests following an abnormal PSA, to improve the specificity of detecting high-risk disease. This combination reduces the unnecessary evaluation of low-risk men, while providing a simple algorithm that identifies higher risk men for further workup, including a prostate biopsy.

**Evidence Supporting the Intended Use of the 4Kscore Test**

**Analytical Validity of the 4Kscore Test**

The different molecular forms of prostate specific antigen (PSA) and human kallikrein related peptidase 2 (hK2) are derived from the prostate.\textsuperscript{26} In men with no or benign conditions of the prostate, most PSA is found as the enzymatically inactive forms of either complexed PSA or degraded (nicked) free PSA, with very low levels of hK2 and the enzymatically active intact PSA form (iPSA).\textsuperscript{27} A significant increase in levels of hK2 and iPSA in serum is associated with aggressive cancer pathology at biopsy and radical prostatectomy.\textsuperscript{28-30}

The 4Kscore test utilizes the laboratory results of four blood biomarkers: total PSA (tPSA), free PSA (fPSA), intact PSA (iPSA) and human kallikrein-related peptidase 2 (hK2). The tPSA and fPSA assays are FDA-approved and purchased from Roche Diagnostics (Indianapolis, IN). The specific kits used are the Elecsys® total PSA (FDA PMA P990056) and the Elecsys® free PSA (FDA PMA P000027). Both assays are performed on the Roche cobas® analyzer in accordance with the instructions provided by Roche Diagnostics. The plasma or serum concentrations of iPSA and hK2, which are 1/100\textsuperscript{th} to 1/1000\textsuperscript{th} of tPSA, are determined using laboratory methods developed by OPKO Health, and designed to run on the PerkinElmer Diagnostics (Waltham, MA) AutoDELFIA® instrument.\textsuperscript{31} The AutoDELFIA immunoassay system, which utilizes time-resolved fluorescence technology, is FDA cleared for use in several IVD kits sold in the U.S. by PerkinElmer.

The analytical validation for iPSA and hK2 assays was performed at BioReference Laboratories and included the analytical limit of quantitation (LoQ), reporting range, precision (intra-lab and inter-lab), reference range for each in benign prostate conditions versus prostate cancer, in vitro stability, in vivo stability of the analytes, and interfering substances (exclusion criteria).

The Roche cobas instrument and the PerkinElmer AutoDELFIA instrument are validated for use with the 4Kscore test according to the BioReference Laboratories “4Kscore Analytical Performance Verification Plan”, which are designed to be in
substantial compliance with FDA standards as well. Appropriate validation reports are maintained at BioReference Laboratories.

In summary, the laboratory measurement of the four kallikrein biomarkers that are part of the 4Kscore test have been fully validated and are robust laboratory assays for use under the conditions employed for the 4Kscore test.

**Clinical Validity in the Decision to Perform Prostate Biopsy**

**2015 US Validation Study:**

The 2015 U.S. validation trial for 4Kscore was a double blinded, prospective study that assessed the accuracy of the 4Kscore test to predict the presence of high-grade (Gleason score 7 and higher) prostate cancer prior to prostate biopsy.\(^1\) Patients were enrolled in the clinical trial at 26 sites across the United States from October 2013 to April 2014. The clinical trial was designed to target the intended use population of men aged 40 to 80 years old who were scheduled to receive a diagnostic prostate biopsy, and as such, placed no restriction on PSA levels or DRE results. Furthermore, the study enrolled men regardless of whether this was their first prostate biopsy, or a repeat prostate biopsy after previous biopsies were benign (negative) for prostate cancer. As this cohort represented a contemporary U.S. population of men, at least a 10-core trans rectal ultrasound guided (TRUS) prostate biopsy was performed on all patients and current Gleason grading criteria were used for the histology.

The study was a double-blinded design, i.e. OPKO performed the four kallikrein immunoassays and generated the 4Kscore result blinded to the biopsy outcome, the urologist and patient did not know the 4Kscore test results prior to the biopsy, and the anatomic pathologist grading the biopsy specimen was blinded to the 4Kscore test result. An independent biostatistician then analyzed the combined data to assess the AUC, sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), risk calibration and clinical utility by decision curve analysis of the 4Kscore test in this population.

The study was IRB-approved and all men were previously scheduled for and received both a prostate biopsy and a 4Kscore test result. The 4Kscore test was performed within 30 days prior to the prostate biopsy. The study was conducted in two parts: first, a 300 patient calibration cohort confirmed the discrimination and correlation of the OPKO 4Kscore test result against the actual biopsy data obtained for these 300 patients. The statistical algorithm used to estimate the risk of high-grade cancer on the basis of the kallikrein markers was “locked down” before analysis of the 1012 patient validation cohort. The 4Kscore test result showed a near perfect correlation with the actual biopsy data for the 1012 patient clinical validation cohort (Figure 1).
Figure 1. The 4Kscore test is accurately calibrated to predict the risk for Gleason score 7 and higher prostate cancer. X Axis: Each point on the curve represents 10% of the 1012 men (ranked by deciles according to their 4Kscore result), Y Axis: Height on Y axis shows the frequency of Gleason score 7 and higher prostate cancer found upon biopsy.

Table 1A summarizes the AUC, sensitivity and specificity, NPV and PPV for the 4Kscore test compared with total PSA, % free PSA, and age adjusted PSA for high-grade (Gleason score 7 and higher) prostate cancer, determined in the U.S. validation study. The AUC of the 4Kscore test for high-grade prostate cancer (Gleason score 7 and higher) was 0.821, and superior compared to PSA alone (AUC=0.694) or % free PSA (AUC=0.712). The 4Kscore test also showed the superior specificity and negative predictive value at sensitivities that were comparable or superior to PSA, % free PSA, or age adjusted PSA. A subanalysis in men aged 45-75 years with a PSA between 1.5-10 ng/mL showed the 4Kscore retained high accuracy and outperformed comparative tests (Table 1B) in this group.

The study also found that the potential reduction in biopsies would have been 30% to 58% depending on the 4Kscore test threshold chosen to perform a prostate biopsy. The decision analysis for clinical utility (see Figure 3 below) showed the 4Kscore test to be superior across all the relevant 4Kscore result risk thresholds compared to the standard of care (all men receive a biopsy), or a PSA-based risk model.

Further sub-analysis of this cohort examined the pathology of radical prostatectomy specimens for the 51 men with Gleason 6 prostate cancer on prostate biopsy. The radical prostatectomy specimen was upgraded (Gleason 7-10) in 67% (22/33) of those men with a 4Kscore greater than 7.5%. Of those with a 4Kscore below 7.5%, only 33% (6/18) had a higher grade in the prostatectomy specimen.
Table 1: The AUC, and sensitivity, specificity, NPV, and PPV of patients enrolled in the OPKO 4Kscore U.S. validation study. A) The data compares the 4Kscore, total PSA, % free PSA, and age adjusted PSA at the cut point listed for the entire cohort of 1,012 men. B) A similar comparison for men aged 45-75 years with a PSA of 1.5-10 ng/mL.

A)

<table>
<thead>
<tr>
<th>Test and Cut Point</th>
<th>Subgroup</th>
<th>N</th>
<th>N Gleason Score ≥ 7</th>
<th>AUC</th>
<th>Sens.</th>
<th>Spec.</th>
<th>NPV</th>
<th>PPV</th>
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</thead>
<tbody>
<tr>
<td>4Kscore 7.5%</td>
<td>All Patients</td>
<td>1012</td>
<td>231 (22.8%)</td>
<td>0.821</td>
<td>93%</td>
<td>44%</td>
<td>95%</td>
<td>33%</td>
</tr>
<tr>
<td>PSA 3.0 ng/mL</td>
<td>All Patients</td>
<td>1012</td>
<td>231 (22.8%)</td>
<td>0.694</td>
<td>93%</td>
<td>24%</td>
<td>92%</td>
<td>27%</td>
</tr>
<tr>
<td>PSA 4.0 ng/mL</td>
<td>All Patients</td>
<td>1012</td>
<td>231 (22.8%)</td>
<td>0.694</td>
<td>84%</td>
<td>40%</td>
<td>89%</td>
<td>29%</td>
</tr>
<tr>
<td>% fPSA 25%</td>
<td>All Patients</td>
<td>1012</td>
<td>231 (22.8%)</td>
<td>0.712</td>
<td>91%</td>
<td>26%</td>
<td>91%</td>
<td>27%</td>
</tr>
<tr>
<td>Age adjusted PSA</td>
<td>All Patients</td>
<td>1012</td>
<td>231 (22.8%)</td>
<td>NA</td>
<td>66%</td>
<td>49%</td>
<td>83%</td>
<td>28%</td>
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</table>

B)

<table>
<thead>
<tr>
<th>Test and Cut Point</th>
<th>Subgroup</th>
<th>N</th>
<th>N Gleason Score ≥ 7</th>
<th>AUC</th>
<th>Sens.</th>
<th>Spec.</th>
<th>NPV</th>
<th>PPV</th>
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</thead>
<tbody>
<tr>
<td>4Kscore 7.5%</td>
<td>45-75 Years PSA 1.5-10</td>
<td>784</td>
<td>159 (20.3%)</td>
<td>0.774</td>
<td>91%</td>
<td>42%</td>
<td>95%</td>
<td>28%</td>
</tr>
<tr>
<td>PSA 3.0 ng/mL</td>
<td>45-75 Years PSA 1.5-10</td>
<td>784</td>
<td>159 (20.3%)</td>
<td>0.607</td>
<td>92%</td>
<td>17%</td>
<td>89%</td>
<td>22%</td>
</tr>
<tr>
<td>PSA 4.0 ng/mL</td>
<td>45-75 Years PSA 1.5-10</td>
<td>784</td>
<td>159 (20.3%)</td>
<td>0.607</td>
<td>80%</td>
<td>35%</td>
<td>87%</td>
<td>24%</td>
</tr>
<tr>
<td>% fPSA 25%</td>
<td>45-75 Years PSA 1.5-10</td>
<td>784</td>
<td>159 (20.3%)</td>
<td>0.676</td>
<td>92%</td>
<td>21%</td>
<td>91%</td>
<td>23%</td>
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<tr>
<td>Age adjusted PSA</td>
<td>45-75 Years PSA 1.5-10</td>
<td>784</td>
<td>187 (23.9%)</td>
<td>NA</td>
<td>59%</td>
<td>46%</td>
<td>81%</td>
<td>21%</td>
</tr>
</tbody>
</table>

In summary, this prospective, double-blinded validation study reports a near perfect calibration of 4Kscore risk prediction of high grade cancer to prostate biopsy results, a superior accuracy of 4Kscore for predicting high grade cancer vs. PSA, % free PSA, or age adjusted PSA; and a potential reduction in unnecessary biopsies by 30-58%.

Veterans Affairs Validation Study:

To further evaluate the validity of the 4Kscore, especially in African American men, a recent multi-institutional study in the Veterans Affairs Health System prospectively enrolled 403 men who were referred for prostate biopsy. Of these, 366 received a
4Kscore test and had complete data available for analysis. There were 205 (56%) African American men, of which 82 (40%) had high grade prostate cancer. There were 161 (44%) non-African American men, of whom 49 (31%) had high grade prostate cancer.

The 4Kscore was compared to a base model consisting of PSA, age, and DRE findings. The performance of 4Kscore was also compared in African American men and non-African American men. The AUC of 4Kscore in the cohort was 0.81 vs 0.74 for the base model (p = 0.011), and 4Kscore demonstrated higher utility on decision curve analysis than the base model. There was no significant difference between discrimination of 4Kscore for aggressive prostate cancer between African American men (0.80) versus non-African American men (0.84) (p = 0.32). The calibration plot for the cohort demonstrated that 4Kscore risk closely matches prostate biopsy findings for high grade cancer (Figure 2).

Figure 2: The 4Kscore test is accurately calibrated to predict the risk for Gleason score 7 and higher prostate cancer in a cohort with significant African American representation.

The 4Kscore has previously been shown to accurately provide the risk of aggressive prostate cancer in large multi-institutional studies. This study shows that in a cohort with high representation of African Americans, the test remains highly correlated with prostate biopsy results, and retains high discrimination for aggressive prostate cancer.

Clinical Utility in the Decision to Perform a Prostate Biopsy

The 4Kscore test improves health care outcomes by providing risk information that leads to a change in behavior of the physician utilizing the test. The 2015 Konety et al. study is a retrospective decision-impact study that examined prostate biopsy reduction at 35 clinical practices that were using the 4Kscore test. The study
involved a total of 611 men with an abnormal PSA test and/or DRE. Each urologist used the 4Kscore test and his or her own clinical judgment to decide how best to manage (i.e. to proceed with a prostate biopsy or not) the patient. The urologists were asked via a questionnaire to evaluate the impact of the 4Kscore test on their patient management decisions.

The overall results showed a 64.6% reduction in prostate biopsies. When the patients were stratified into groups based on the 4Kscore test results (Table 2), the biopsy reduction ranged from 19% to 94%. The three groups are: Low Risk (4Kscore less than 7.5%), Intermediate Risk (4Kscore 7.5%–19.9%), and High Risk (4Kscore 20% and higher). A higher 4Kscore test was associated with a greater likelihood of having a prostate biopsy (p < 0.001). Only 6.0% of men with a low-risk 4Kscore test (less than 7.5%) opted for prostate biopsy, representing a 94% biopsy reduction. In the intermediate risk group 47.1% of men had a prostate biopsy, and 81.0% of men with a high-risk 4Kscore test result group underwent a prostate biopsy.

Table 2. Biopsy utilization in all patients enrolled in Konety et al. 4Kscore clinical utility study.

<table>
<thead>
<tr>
<th>4Kscore Test Category</th>
<th>No Biopsy (Reduction) (n = 395; 64.7%)</th>
<th>Received Biopsy (n = 216; 35.4%)</th>
<th>Total (n = 611)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Risk</strong> (4Kscore test less than 7.5%)</td>
<td>283 (94.0%)</td>
<td>18 (6.0%)</td>
<td>301</td>
</tr>
<tr>
<td><strong>Intermediate Risk</strong> (4Kscore test 7.5%–19.9%)</td>
<td>83 (52.9%)</td>
<td>74 (47.1%)</td>
<td>157</td>
</tr>
<tr>
<td><strong>High Risk</strong> (4Kscore test 20% and higher)</td>
<td>29 (19.0%)</td>
<td>124 (81.0%)</td>
<td>153</td>
</tr>
</tbody>
</table>

The authors concluded that across the 35 urology practice settings, the 4Kscore test had significantly impacted prostate biopsy decisions, and reduced the rate of prostate biopsies by 64.6% overall, and notably, among men with a low risk result (4Kscore 7.5% or less), a 94.0% reduction in prostate biopsy rate was observed.

**Clinical Utility by Decision Curve Analysis (DCA)**

A decision curve analysis (DCA) allows physicians and patients to determine the net clinical benefit (or harm) the use of a particular test or procedure will have when compared to standard of care at their own acceptable threshold for risk. As applied to the 4Kscore test, the DCA estimates a net benefit for the 4Kscore test and other prediction models by summing the benefits (true positives) and subtracting the harms (false positives) where the latter is weighted by a factor so as to reflect the relative harm of a missed cancer compared with an unnecessary biopsy. Figure 3 shows the DCA from the 4Kscore U.S. validation study. At all relevant thresholds likely to be utilized with the 4Kscore test, the DCA showed a favorable net benefit when compared to standard of care (biopsy all) or a PSA predictive model known as the Prostate Cancer Prevention Trial Risk Calculator 2.0 (PCPTRC 2.0).
Figure 3: Decision curve analysis comparing the 4Kscore to other strategies.\textsuperscript{1} Red line, biopsy no patients; orange line, biopsy all patients; green line, a PSA-based risk predictor (PCPTRC 2.0) as criterion for biopsy; and blue line, 4Kscore as criterion for biopsy. The line with the highest net benefit (y-axis) at any particular threshold probability for biopsy (x-axis) will result in the best clinical results.

Reviews in Medical Literature

- A systemic review of the four kallikrein panel in 2016 by McDonald and Parsons examined the benefits and limitations of PSA screening, and the potential utility of the 4Kscore test to improve the specificity of PSA for detecting clinically significant prostate cancer.\textsuperscript{35} The review detailed the improvements in health outcomes, including a 45% reduction in prostate cancer mortality since the widespread use of PSA screening, but also the associated high cost of over diagnosis and over treatment of indolent prostate cancer. The authors describe several studies where the 4Kscore test was evaluated in differing populations and was shown to have superior discrimination versus PSA and PSA risk calculators. The review also pointed out that iPSA and hK2 assays require sophisticated expertise to perform and are not widely available.

The authors stress the goal of prostate cancer screening is “…to maximize the early diagnosis of potentially aggressive but curable disease while minimizing both the detection of indolent disease and the number of invasive confirmatory tests. However, the relatively poor specificity of PSA has
contributed to the over-detection of indolent disease. One potential solution is to focus on the detection of clinically significant prostate cancer by using tests with enhanced specificity.

The authors found that the 4Kscore test is effective by "...providing an individualized prediction of clinically significant cancer regardless of prior screening or previous biopsy." They concluded that it would have utility in the process of shared decision making for prostate biopsies and in the early detection of prostate cancer. This could lead to a 41-71% reduction in prostate biopsies, with a minimal risk for delayed diagnosis of significant prostate cancer.

- In another systematic review of the 4Kscore test, the authors reviewed the clinical studies for ten cohorts consisting of over 15,000 men from peer-reviewed medical journals. The authors found that the 4Kscore test is effective for accurately identifying men at high risk for aggressive disease and is appropriate to select those men who would benefit from a prostate biopsy and prevent a potentially lethal outcome from prostate cancer. The authors conclude that 30-58% of men with a low 4Kscore test result could safely defer prostate biopsy because of having a negligible risk of having missed aggressive prostate cancer.

- A recent review by Bratt et al. examined the potential for blood-based biomarkers to detect prostate cancer. The authors highlighted the importance of PSA screening followed by a four kallikrein panel for men with even modestly elevated PSA values prior to considering a biopsy. The authors noted that statistical models based on the biomarkers in the 4Kscore test improve the specificity of elevated PSA (2.0-10.0 ng/mL), reduce unnecessary prostate biopsies, and better identify men at risk of aggressive prostate cancer who should have a biopsy or additional imaging.

- Filella et al. published a comprehensive review of emerging biomarkers for detection of aggressive prostate cancer in blood and urine. The review included a discussion of the clinical work underlying the 4Kscore as well as other blood and urine tests including PCA3 and phi. It concluded that these biomarkers outperform the specificity of tPSA and percent fPSA.

**Independent Recommendations and Guidelines**

**NCCN Guidelines for Prostate Cancer Early Detection**

The 4Kscore test is included in the most recent 2016 NCCN Guidelines for Prostate Cancer Early Detection based on level 2A evidence, and is indicated as a test to be used after a finding of an abnormal PSA test or DRE. The specific NCCN panel recommendations highlight the 4Kscore test in the following way, consistent with...
its intended use in biopsy naïve patients or in patients with previous benign (negative) biopsies:

“Biomarkers that improve the specificity of (prostate cancer early) detection are not recommended as first line screening tests. However, there may be some patients who meet either PSA standards for consideration of prostate biopsy, but for whom the patient and/or the physician wish to further define the probability of high-grade cancer. 4Kscore (which provides an estimate of the probability of prostate cancer) is potentially informative in patients who have never undergone biopsy or after a negative biopsy”.

The NCCN guidelines go on to further comment on the management of biopsy results:

“It is well known that a negative prostate biopsy does not preclude a diagnosis of prostate cancer on subsequent biopsy. Those patients with negative biopsies should be followed with DRE and PSA. Tests which improve specificity in the post-biopsy state—including 4Kscore should be considered in patients thought to be at a higher risk despite a negative biopsy.”

European Association of Urology (EAU) Prostate Cancer Guidelines

The 4Kscore is included in the 2016 EAU-ESTRO-SIOG Guidelines on Prostate Cancer, with the following recommendation:

“Use the additional diagnostic options in asymptomatic men with a normal DRE and a PSA between 2.0 and 10 ng/mL (risk calculator, or an additional serum or urine-based test [4Kscore] or imaging).”

The guidelines also mention that 4Kscore has been shown to out-perform free/total PSA prostate cancer detection, “with an improved prediction of clinically significant prostate cancer, in men with a PSA between 2-10 ng/mL.”

Regulation and CPT Code

Federal Laboratory Regulation

The 4Kscore test has been developed and validated at BioReference Laboratories, Inc., a wholly owned subsidiary of OPKO Health, Inc. The 4Kscore test was validated in strict compliance with CLIA regulations, and is performed in a single facility in accordance with the federal regulations regarding Laboratory Developed Tests (LDTs).

State Permits

In addition to New Jersey, BioReference Laboratories is licensed by all states requiring out-of-state diagnostic labs to have permits, including California, Connecticut, Florida, Maryland, New York, Pennsylvania, Rhode Island, Vermont, and West Virginia.
The 4Kscore test also has conditional approval by New York State under its Clinical Laboratory Evaluation Program. Most clinical pathologists consider this program as representing the highest standard for evaluating the performance of new LDTs.

**Laboratory Accreditation**

BioReference Laboratories is licensed by CMS and is CLIA certified as a high complexity laboratory under CLIA 88 regulations. The BioReference Laboratories team of board-certified, fellowship-trained pathologists is licensed in all 50 states. The BioReference Laboratories facility is also a College of American Pathologists (CAP)-accredited laboratory.

**4Kscore Test Category I CPT Code**

In October 2015, the American Medical Association (AMA) determined that the 4KScore met the requirements for a Category I CPT code. Below is the CPT code assigned and the description of the test in the 2017 AMA CPT book effective January 2017. The test descriptor is as follows:

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

**Discussion**

**Development of the 4Kscore Test**

Scientists and clinicians from Memorial Sloan Kettering Cancer Center led the initial clinical research on the four kallikrein biomarkers, and the development of algorithms that combined the biomarkers with clinical information, later finalized as the 4Kscore test. This team published a series of articles[^41[^45] that demonstrated the repeated ability of a four kallikrein biomarker and clinical information algorithm to predict the risk of high-grade (Gleason score 7 and higher) prostate cancer and reduce unnecessary prostate biopsies. The men were all enrolled in the European Randomized Study of Screening for Prostate Cancer (ERSPC) and underwent prostate biopsy due to an elevated PSA level (3.0 ng/mL and higher) detected by PSA screening. Multiple cohorts were studied, including men with no prior PSA screening, prior PSA screening, and men who had a prior benign (negative) prostate biopsy. This pioneering effort served as the scientific basis for the development of the commercially available OPKO 4Kscore test.

In the years following the collection of the ERSPC samples, the prostate biopsy standard procedure changed from a 6-core (sextant) biopsy to a 10-core biopsy and new criteria for Gleason grading were also introduced that broadened the criteria for inclusion into Gleason grade 4.[^46] Also, there was growing evidence that Gleason score 6 prostate cancer, though having the histopathological features of adenocarcinoma, was unlikely to metastasize and cause death.[^2[^3] These changes in clinical practice were reflected in the development of the commercialized 4Kscore
test, validated by OPKO in a double blinded, prospective study of a contemporary U.S. population of 1012 men at 26 urology centers across the U.S. in 2013-14, and described in detail below.

The Prostate Testing for Cancer and Treatment (ProtecT) study, an ongoing prospective, randomized, controlled clinical trial, provided an additional opportunity to evaluate the diagnostic accuracy and biopsy reduction potential of the 4Kscore test in a 2015 published report. All available cryopreserved blood samples from those men who agreed to undergo prostate biopsy were retrieved, resulting in a sample set consisting of EDTA plasma from 4765 men, serum from 1860 men, and both plasma and serum from 496 men who had undergone a prostate biopsy. The laboratory performing the testing of the four kallikreins biomarkers was blinded to the biopsy pathology data and vice versa.

The plasma samples assessed with the 4Kscore test against prostate biopsy histology had an AUC=0.820 for discrimination of high-grade (Gleason score 7 and higher) prostate cancer and superior performance compared to a PSA-based model (AUC=0.738). The AUC in this cohort was virtually identical compared with the U.S. validation study of Parekh et al. (discussed above), and the authors reported that the plasma and serum samples provided equivalent AUC performance for the 4Kscore test. A decision curve demonstrated clinical utility across relevant risk thresholds for high-grade (Gleason score 7 and higher) prostate cancer, with a biopsy reduction potential of 43% at a 6% risk threshold.

The 4Kscore was further shown to accurately predict risk of high-grade disease in a population screened with free to total PSA ratio. Braun et al. studied the 4Kscore test in a cohort of 749 who all received a prostate biopsy due to a low free to total PSA ratio (<20%) or a suspicious DRE. The authors analyzed a range of 4Kscore cut points (4-12%) and projected that up to 38% of prostate biopsies could be avoided.

**The Ability of the 4Kscore Test to Predict the Risk of Distant Metastasis**

The 4Kscore test was also evaluated by Stattin et al. in a long-term clinical outcomes study published in 2015, with an endpoint of distant prostate cancer metastasis. PSA and 4Kscore test results were obtained from banked plasma samples in a group of men who had more than 15 years of follow up. During this timeframe, the men were not subject to PSA screening, thus this study examined the natural history of metastatic prostate cancer occurrence observed in men as a function of their PSA and the 4Kscore test results measured over 15 years earlier.

The plasma samples from these men were tested with the 4Kscore test in addition to PSA. The data showed that after 20 years, a 4Kscore test performed at age 50 or 60 on a man with a PSA level of 2.0 ng/mL and higher provided clear risk stratification for subsequent development of metastatic prostate cancer (see Figure 4).
The 50 and 60-year old men with a low 4Kscore test (less than 7.5%) had a 1.3% and 3.0% risk, versus 10.7% and 14.2% risk of metastatic prostate cancer for men with a high 4Kscore test result (7.5% and higher). The short term risk for being diagnosed with metastatic prostate cancer in men with an elevated PSA and a low 4Kscore test results (less than 7.5%) was 0.3% for both groups at 10 years, meaning a decision not to biopsy the prostate at the time of performing the 4Kscore test is safe and reasonable. This is especially true because this study design was a natural history study. Men in this study with elevated PSA had no follow up for prostate cancer. In practice, a man with an elevated PSA and a low 4Kscore would be subjected to regular monitoring with PSA testing that would very likely diagnose the presence of prostate cancer that was destined to become metastatic at an early, successfully treatable stage.

**Figure 4. The 4Kscore test at a 7.5% cut point provides 20-year predictive risk stratification for 50 and 60-year-old men with elevated PSA of 2.0 ng/mL and higher.**

The result of this study shows: 1) the 4Kscore test can discriminate the long term outcome of prostate cancer metastasis in men with an elevated PSA 2) a decision to avoid a prostate biopsy in a man with an elevated PSA, but low 4Kscore test result would be a safe and reasonable clinical judgment.

**Conclusions**

Widespread PSA screening for prostate cancer has led to a 45% decline in prostate cancer mortality. However, this has come at a high cost in terms of the number of men subject to prostate biopsy and over treatment of indolent disease with surgery and radiation therapy. There is a need for a noninvasive test that can provide better sensitivity and specificity than PSA as a decision point prior to prostate biopsy. The 4Kscore test has been developed to fill this medical need, and is intended to be used as a second decision test by providing accurate information on the risk for a man to
be harboring high-grade (Gleason score 7 and higher) prostate cancer before proceeding to a prostate biopsy.

**The 4Kscore test:**

- Is a non-invasive, blood-based test, offered as an LDT by BioReference Laboratories, a wholly owned subsidiary of OPKO Health
- Intended to be used as a second decision tool before performing a prostate biopsy in men aged 45-75 years with a PSA between 1.5-10 ng/mL and/or an abnormal DRE.
- Provides the necessary discrimination for high-grade, Gleason Score 7 and higher prostate cancer (AUC=0.821) determined in a prospective, double blinded, 26-center clinical trial of 1012 men in the U.S.
- Has superior accuracy to PSA, %fPSA, and PSA based risk calculators for predicting high grade prostate cancer
- Has been shown to minimize the risk of over-diagnosis and over treatment of prostate cancer by reducing unnecessary prostate biopsies as much as 64.6%
- Is included in the 2016 NCCN guidelines for prostate cancer early detection in both biopsy naïve and men with a prior benign (negative) biopsy
- Is approved for a Category I CPT code by the AMA
References


