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Biomarkers in prostate cancer surveillance and screening: past, present, and future

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Abstract: The use of biomarkers for prostate cancer (PCa) screening, detection, and prognostication have revolutionized the diagnosis and management of the disease. Current clinical practice has been driven largely by the utilization of prostate-specific antigen (PSA). The lack of specificity of PSA for PCa has led to both unnecessary biopsies and overdiagnosis of indolent cancers. The recent controversial recommendation by the United States Preventive Services Task Force against PCa screening has highlighted the need for novel clinically useful biomarkers. We review the literature on PCa biomarkers in serum, urine, and tissue. While these markers show promise, none seems poised to replace PSA, but rather may augment it. Further validation and consideration of how these novel markers improve clinical outcome is necessary. The discovery of new genetic markers shows promise in stratifying men with aggressive PCa.

Keywords: Biomarkers, prostate cancer, cancer screening

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Introduction

A biomarker can be defined as a laboratory measurement that is associated with a pathologic process and has both diagnostic and prognostic utility [Lesko and Atkinson, 2001]. The diagnosis of prostate cancer (PCa) has relied heavily on the use of such biomarkers, in particular, prostate-specific antigen (PSA), for over 20 years. Recently, the use of PSA has been thrust into the public spotlight following the publication of two large, randomized PCa-screening trials [Andriole *et al.* 2009; Schröder *et al.* 2009a], and the recent, highly controversial recommendation against PCa screening released by the United States Preventive Services Task Force (USPSTF) [Moyer, 2012].

Regardless of the ultimate impact of this recommendation on screening practices, PCa is, and will remain, a major public-health concern. Indeed, it is estimated that in the USA in 2013 there will be 238,590 incident PCa cases and 29,720 deaths [Siegel *et al.* 2013]. While a relatively small proportion of men still present with high-risk, high-grade disease [Abdollah *et al.* 2011; Brawley, 2012], there has been a definite stage migration toward low-risk, low-grade disease in the years since PSA was introduced [Cooperberg *et al.* 2004, 2007]. Earlier detection as a result of screening is the major cause of this stage migration over time.

PSA has provided significant advancement in the diagnosis and prognosis of PCa. However, it does have limitations, including its lack of specificity and no seemingly safe level that confers a zero risk of a PCa diagnosis [Thompson *et al.* 2004]. Further, its indiscriminate use has allowed for overdiagnosis and overtreatment of low-risk PCa that would not have affected the longevity or quality of life had screening not been performed [Walter *et al.* 2006]. These shortcomings have led many to investigate more optimized uses of PSA [Greene *et al.* 2013], in addition to the development of novel biomarkers using various tissue media. This review describes the use of current biomarkers for PCa screening, surveillance, and future directions using blood, urine, and tissue-based media.

Screening markers

Total PSA

Prior to the introduction of PSA, human prostatic acid phosphatase (PAP) was the first serum

biomarker used for PCa. The use of preoperative PAP levels allowed risk stratification for the likelihood of lymph node-positive disease and the development of metastatic disease [Whitesel *et al.* 1984]. However, once PSA use was initiated in the 1980s, it was shown that PAP was inferior to PSA in regard to PCa screening, staging, and prognosis, thus, it became obsolete [Lowe and Trauzzi, 1993].

In 1991, Catalona and colleagues demonstrated that the addition of PSA was a useful adjunct to rectal examination and prostate ultrasonography in screening for PCa. Although all three of these tools exhibited the ability to predict PCa, the predictive value of PSA was the greatest [Catalona *et al.* 1991]. The US Food and Drug Administration officially approved the use of PSA for PCa screening in 1994, and defined 4.0 ng/ml as the upper limit of normal. It was later discovered that around 20% of PCas with PSA values below 4.0 ng/ml are present [Catalona *et al.* 1997]. In addition, data from the Prostate Cancer Prevention Trial failed to demonstrate a PSA threshold that provided both a high sensitivity and specificity, demonstrating rather a continuum of PCa risk at all PSA values [Thompson *et al.* 2005]. This led to the incorporation of PSA in various nomograms and risk calculators to better counsel patients regarding their individual risk of PCa diagnosis, recurrence, and progression [Cooperberg *et al.* 2005, 2009; D'Amico, 2003; D'Amico *et al.* 1998; Kattan *et al.* 1998; Kranse *et al.* 2008; Nam *et al.* 2007b; Thompson *et al.* 2006; van den Bergh *et al.* 2008].

Despite the lack of precise PSA-screening thresholds, PSA has evolved as a useful biomarker for assessing future risk of PCa. Several studies have demonstrated that a baseline PSA level can predict future risk of both PCa diagnosis and, more importantly, lethal PCa [Antenor *et al.* 2004; Fang *et al.* 2001; Lilja *et al.* 2011; Loeb *et al.* 2006; Stenman *et al.* 1994; Vickers *et al.* 2010a]. Men with an initial PSA value above the median age-adjusted PSA level of 0.7–0.9 in younger men (i.e. < 60 years old) predicts for an increased risk of PCa [Antenor *et al.* 2004]. Indeed, a greater baseline PSA is also associated with more aggressive tumor features and a greater biochemical progression rate following treatment [Loeb *et al.* 2006]. Vickers and colleagues observed that a PSA level at age 60 years not only predicts a lifetime risk of clinically detected PCa, but also metastasis, and death from the disease. This suggests that men aged 60 years with a PSA level below the median of 1 ng/ml might harbor PCa; however, it is unlikely to become life threatening.

The efficacy of PSA as a widespread screening tool was recently assessed by two large population-based trials. The Prostate, Lung, Colon, and Ovarian (PLCO) trial showed no benefit in PCa mortality from screening, while the European Randomized Study of Prostate Cancer (ERSPC) trial found a 20% relative reduction in PCa death, albeit with a high incidence of overdiagnosis [Andriole *et al.* 2009; Schröder *et al.* 2009a]. It must be stressed that the PLCO trial had an extremely high contamination rate of PSA testing upwards of 70% in the control arm [Pinsky *et al.* 2010]. Therefore, this trial demonstrated that annual screening is not better than *ad hoc* screening, but did not actually shed light on the question of screening *versus* no screening. Updated results from the European trial using longer follow-up data suggest this benefit to screening persists while decreasing the number of PCas needed to be detected to prevent one death from PCa [Schröder *et al.* 2012].

These trial results were followed by the recent controversial USPSTF recommendation, which relied heavily on a flawed interpretation of the PLCO trial and an overestimation of the harms of screening against any routine use of PSA screening [Moyer, 2012]. The USPSTF recommendation has brought the value of PSA-based screening for PCa under wide scrutiny.

In addition, the release of the Prostate Cancer Intervention *Versus* Observation Trial (PIVOT) results showing no effect of treatment for men with PSA-detected localized PCa on all cause or PCa mortality compared with observation further fueled these questions. However, a closer review of the trial suggests that PIVOT in fact offers strong evidence for risk-based treatment decisions. In the low-risk group, no benefit was noted; however, a clear, early benefit of treatment was demonstrated in those patients with high-risk disease. Moreover, there was a significant reduction in the risk of bone metastasis when treated with radical prostatectomy. Further, when stratified by risk, men at higher risk treated with radical prostatectomy had a 60% relative reduction in PCa mortality compared with men on observation.

So how has PSA screening affected mortality and where does this leave PSA as a biomarker for PCa

screening? Since the early 1990s, age-adjusted PCa mortality in the USA has seen a > 40% decline [Siegel *et al.* 2013]. The Cancer Intervention and Surveillance Modeling Network attempted to quantify the potential contribution of PSA screening to this decline using mathematical modeling of population-based data. Etzioni and colleagues demonstrated that approximately 45–70% of the observed decline in PCa mortality could plausibly be attributed to PSA screening [Etzioni *et al.* 2007].

In a follow-up study evaluating the effect of local treatment advances on PCa mortality, it was projected that changes in treatment over time explained only a modest 22–33% of the decline in mortality, with the remaining decline likely attributable to PSA screening and advances in treatment of recurrence or advanced disease [Etzioni *et al.* 2012]. Despite the lack of specificity of PSA for detecting PCa and the potential harms of overdiagnosis and overtreatment, PSA has been shown to be the single most significant predictive factor for identifying men at increased risk of PCa to date [Fleshner and Lawrentschuk, 2009; Schröder *et al.* 2009b].

Free PSA

PSA circulates in the bloodstream either in a bound or unbound 'free' form. In its bound form PSA is complexed with serine antiproteases such as α1-antichymotrypsin, α1-protease inhibitor, α2-macroglobin, etc. [Christensson *et al.* 1990]. The portion of PSA that remains unbound is termed free PSA [Lilja *et al.* 1991]. The ratio of free PSA to total PSA (percentage-free PSA) is lower in men with PCa compared with those without [Christensson *et al.* 1993]. Catalona and colleagues first demonstrated its usefulness in 773 men with PSA values of 4–10 ng/ml and normal digital rectal examination (DRE). Using a cutoff of < 25% free PSA yielded a sensitivity of 95% and its association was independent of total PSA [Catalona *et al.* 1998]. The correlation of lower percentage-free PSA with higher probability of PCa on biopsy has since been shown in additional studies [Djavan *et al.* 1999; 2000; Woodrum *et al.* 1998].

More recent studies of percentage-free PSA performance have been less impressive than the initial studies [Djavan *et al.* 2002; Khan *et al.* 2004]. This could be related to the instability of free PSA compared with complexed PSA, which may lead to greater analytic variability. This requires strict sample handling and processing within a few hours of collection; otherwise, it should be kept frozen to provide optimal analysis [Piironen *et al.* 1996b]. Further, free PSA measurements of the same specimen using kits from different manufacturers are not reproducible accurately. Whatever the extent that these issues explain the inconsistent performance of percentage-free PSA, this marker has not become widely used as a primary screening tool.

Proenzyme PSA

Proenzyme PSA (proPSA) is an inactive isoform of PSA found in the circulation and in the peripheral zone of the prostate, which has shown to be associated with PCa [Mikolajczyk *et al.* 2000]. Several isoforms of proPSA exist and the nomenclature of these is based on the length of the proleader peptide. The 7-amino acid proPSA isoform is cleaved by human kallikrien 2 (hK2) and trypsin to yield active PSA. Truncated forms containing various lengths of this leader sequence (i.e. 2 [-2] or 4 [-4] amino acids) can be measured in serum using immunoassays [Chan *et al.* 2003; Mikolajczyk *et al.* 2001].

The [-2] proPSA isoform has emerged as a promising biomarker for PCa screening due to its correlation with PCa rather than benign prostatic hypertrophy, in addition to its accuracy in detection of PCa compared with other isoforms [Chan *et al.* 2003; Mikolajczyk *et al.* 2000]. Using serum prospectively stored and retrospectively analyzed in 123 men prior to prostate biopsy, Sokoll and colleagues demonstrated that percentage [-2] proPSA was the best predictor of PCa detection, particularly in the 2–10 ng/ml PSA range. The area under curve (AUC) was greatest for percentage [-2] proPSA at 0.73 compared with 0.52 for PSA and 0.53 for percentage-free PSA [Sokoll *et al.* 2008]. In a larger follow-up study, Sokoll and colleagues showed an improved specificity of 44.9% for percentage [-2] proPSA compared with that of PSA (30.8%) and percentage-free PSA (34.6%) at an 80% sensitivity. In addition, percentage [-2] proPSA increased with increasing Gleason score ($p < 0.001$), and was higher in aggressive cancers ($p = 0.03$), although this requires further study [Sokoll *et al.* 2010].

In a prospective cohort of men with PSA values ranging from 2.5 ng/ml to 10 ng/ml, Le and colleagues assessed the predictive accuracy of total

PSA and its isoforms (e.g. percentage-free PSA, [-2] proPSA), as well as the Beckman Coulter Prostate Health Index (PHI®) [Le *et al.* 2010]. The PHI is calculated for each patient as PHI = ([-2] proPSA/freePSA) × √PSA. On receiver operating characteristic (ROC) analysis, the percentage [-2] proPSA and PHI outperformed both PSA and percentage-free PSA with AUCs of 0.76 and 0.77, respectively. Moreover, at a set sensitivity of 88.5%, percentage [-2] proPSA led to a substantial improvement in specificity as well as positive and negative predictive values. More recently, the superior predictive ability of PHI and percentage [-2] proPSA over total PSA and free PSA was demonstrated separately in prospective Italian and French cohorts of 268 and 452 patients, respectively [Guazzoni *et al.* 2011; Houlgatte *et al.* 2012]. In multivariate accuracy analyses in the Italian study, both PHI (+11%) and percentage [-2] proPSA (+10%) significantly improved the accuracy of established predictors in determining the presence of PCa at biopsy ($p < 0.001$). The results of these analyses are promising and if confirmed in larger multicenter studies may increase the ability to detect PCa while lowering the rate of unnecessary biopsies.

hK2

hK2 and PSA are closely related serine proteases belonging to the human tissue kallikrein family and have 80% sequence homology [Schedlich *et al.* 1987]. In blood, hK2 is present in concentrations of 1–2% compared with PSA [Finlay *et al.* 1998; Piironen *et al.* 1996a]. Immunoassays used to measure hK2 must have low detection limits because of these very low circulating levels. In fact, the first published hK2 assay using a detection limit of 0.1 µg/L found that 57% of the samples had hK2 concentrations below the detection limit [Piironen *et al.* 1996a].

Interest in this marker has increased as it has been found that hK2 expression incrementally increases during development from benign epithelium to primary cancer and lymph-node metastases using a cohort of radical prostatectomy specimens [Darson *et al.* 1999]. Using the serum of referral and screening cohorts, there has been improved discrimination of patients with or without cancer by using a combination of kallikrien-family markers (i.e. total PSA, free PSA, and hK2) compared with that of total PSA alone [Becker *et al.* 2000; Recker *et al.* 1998].

More recent reports evaluating the predictive ability of a panel of four kallikrein markers in men with elevated PSA (i.e. total PSA, free PSA, intact PSA, and hK2) indicated that this panel of markers showed promise, which could lead to reduced unnecessary biopsy rates. In the French arm of the ERSPC trial, Benchikh and colleagues demonstrated the diagnostic accuracy for detection of PCa, as measured by ROC analysis, improved from 0.63 in the base model including PSA, age, and DRE to 0.78 with the addition of the panel of markers in men with PSA ≥ 3 [Benchikh *et al.* 2010]. The addition of this panel of four kallikrein markers seems to add complementary information to PSA when evaluated in both the Malmö Diet and Cancer cohort and in the Rotterdam arm of the ERSPC. In both cohorts, the predictive accuracy to detect PCa improved with the addition of these markers and would potentially decrease unnecessary biopsies by nearly 50% if the predicted probability of PCa was 20% or higher on decision curve analysis [Vickers *et al.* 2011, 2010b]. The hK2-marker panel and proPSA/PHI are both promising markers and it is unclear if one will prove more useful than the other. Future studies might compare these markers' predictive ability head to head or in combination in addition to total PSA.

TMPRSS2-ERG

Tomlins, using a novel bioinformatics approach, first reported on the recurrent chromosomal rearrangement in PCa involving the *TMPRSS2* gene. The author identified recurrent gene fusions involving the 5' untranslated region of the androgen-regulated gene *TMPRSS2* with *ERG* or *ETV1*, two transcription factors of the ETS family [Tomlins, 2005]. TMPRSS2-ERG gene fusions have been reported in approximately 50% of 1500 clinically localized PCa cases in a recent review of over 25 published studies from PSA-screened cohorts from North America, Europe, and Asia [Kumar-Sinha *et al.* 2008]. A 2006 study demonstrated the feasibility of detecting the gene-fusion transcripts in urinary sediments post-DRE [Laxman *et al.* 2006]. The possibility of a noninvasive urinary marker has generated further studies on the clinical applicability of this test.

Detection of the TMPRSS2-ERG gene fusion in urine has been reported to yield a specificity of 93% and a positive predictive value of 94% in a small study of 78 men with PCa [Hessels *et al.* 2007]. A larger, prospective, multicenter study

evaluated the diagnostic accuracy of TMPRSS2-ERG to that of the ERSPC risk calculator using area under the ROC analysis. The ERSPC risk calculator is composed of several calculators that can be used by both patients and physicians to aid in the prediction of a PCa diagnosis and whether it is aggressive or indolent using PSA, prostate volume, previous biopsy results, etc. The gene fusion offered an additional predictive value to the ERSPC risk calculator on multivariate analysis ($p = 0.002$). The addition of TMPRSS2-ERG and *prostate cancer antigen 3* gene (PCA3) (see below) improved diagnostic accuracy to 0.84 from 0.79 using only the ERSPC risk calculator [Leyten *et al*. 2012].

Results of the potential prognostic value of TMPRSS2-ERG gene fusion are conflicting. Several studies have reported a worse prognosis in fusion-positive patients [Nam *et al.* 2007a; Wang *et al.* 2006], while others have been unable to validate these findings [Gopalan *et al.* 2009; Minner *et al.* 2011]. Most recently, Leyton and colleagues showed that TMPRSS2-ERG independently predicted biopsy Gleason score (odds ratio [OR]: 7.16; $p < 0.001$) and clinical tumor stage (OR: 2.60; $p = 0.023$), adjusting for the ERSPC parameters on multivariate logistic regression analysis [Leyten *et al.* 2012].

PCA3

PCA3 is a PCa-specific gene located on chromosome 9q21–22. It is a long noncoding RNA highly overexpressed in prostate tumors relative to nonmalignant prostate tissue [Bussemakers *et al.* 1999]. PCA3 is detectable in the urine and prostatic fluid of men with PCa. This led to the development of a precise molecular urinary assay in which the first 30 ml of urine are collected after an attentive DRE. This assay yielded a sensitivity of 69% and specificity of 79% for predicting PCa on biopsy [Groskopf *et al.* 2006]. In contrast, serum PSA assay sensitivity was 28% for the same group. Current assays report the ratio of PCA3 RNA/PSA mRNA [Hessels *et al.* 2003; Tinzl *et al.* 2004]. PSA mRNA is not upregulated in PCa and it is used to normalize for the amount of prostate-specific RNA in the molecular test sample. Additionally, PCA3 RNA levels are independent of prostate volume and serum PSA [Haese *et al.* 2008; Nakanishi *et al.* 2008]. This allows the potential to add significantly more diagnostic potential than PSA derivatives.

The most studied role of PCA3 has been in men who have an elevated PSA and prior negative biopsy. The clinical rationale of this aims at reducing the number of potentially unnecessary biopsies. Marks and colleagues first evaluated the use of PCA3 in 226 consecutive patients undergoing repeat biopsy. They demonstrated an improved AUC comparing PCA3 *versus* PSA (0.68 *versus* 0.52; $p = 0.008$, respectively) in predicting prostate biopsy outcome. The PCA3 cutoff of 35 had the greatest diagnostic accuracy yielding a sensitivity and specificity of 58% and 72%, respectively. In addition, the risk of positive biopsy findings was correlated with PCA3 score. Indeed, men with a PCA3 score of > 100 had a 50% probability of positive biopsy compared with a probability of only 12% for men with scores < 5 [Marks *et al.* 2007]. Subsequently, several multicenter studies have evaluated the diagnostic ability of urine PCA3 [Ankerst *et al.* 2008; Deras *et al.* 2008; van Gils *et al.* 2007; Wu *et al.* 2012]. In general, these have demonstrated a superior diagnostic accuracy to that of PSA, and combining PCA3 with established biopsy risk factors (i.e. age, PSA, DRE, prostate volume, etc.) improved diagnostic accuracy in multivariable regression models.

Despite attempts to define an optimal cutoff for PCA3, one must understand the trade-offs of using it as a threshold test. Both PCA3 and PSA are continuous variables and as such, defining a precise threshold level to trigger additional action has its limitations. Crawford and colleagues recently demonstrated that using a PCA3 level of 35 resulted in a 77% reduction in the number of false positives found with PSA testing, however, this level also increased the percentage of missed cancers (false negatives) by 2300%. Conversely, using a PCA3 level of 10 as the cutoff reduced the false positives 35.4% and false negatives only increased 5.6% [Crawford *et al.* 2012]. Further, Deras and colleagues showed that the sensitivity of PCA3 ranged from 96% to 20% across various cutoffs of 5 to 90, respectively [Deras *et al.* 2008]. These studies illustrate the trade-offs in defining a specific cutoff on the test characteristics and predictive accuracy.

Additional genetic markers

Interest in various genetic markers of PCa risk is growing exponentially. A heritable component of PCa aggressiveness and survivorship has been demonstrated [Hemminki *et al.* 2008], and several studies have described an association between

risk-allele status with PSA level and prognosis [Gudmundsson *et al.* 2010; Penney *et al.* 2009; Salinas *et al.* 2009]. Appealing features of these genetic markers include their accessibility at any age and no fluctuation over time or in particular conditions. The relative increase in the risk of developing PCa associated with any one particular single nucleotide polymorphism (SNP) is small, generally < 1.5-fold, but appears to increase with increasing number of risk alleles carried. A 2008 case-control study of > 4000 subjects correlated five risk SNPs located on chromosomes 8 and 17 with the risk of developing PCa. They found that carriers of all five of the risk SNPs demonstrated an OR of 9.46 for developing PCa compared with men with none of the risk alleles [Zheng *et al.* 2008]. However, this group of men carrying all five SNPs represented a very small subset of the entire cohort. In addition, Helfand and colleagues evaluated the association between 17 risk alleles and disease detection in men with PSA ≤ 4 ng/ml and normal DRE. Increased risk of PCa was associated with increasing number of risk alleles carried; men with ≥ 10 risk alleles had an OR of 10.6 compared with men with 0–4 risk alleles [Helfand *et al.* 2011]. Conversely, in a separate study, Helfand and colleagues demonstrated having ≤ 1 risk allele was associated with insignificant PCa, defined as organ-confined, Gleason < 4, and tumor volume < 0.5 ml [Helfand *et al.* 2010]. Unfortunately, while some of these risk alleles may confer an increased risk of PCa, most have not been validated in independent cohorts and fail to improve prediction models once known risk or prognostic factors are taken into account.

Epigenetic changes in PCa may be another promising arena for potential biomarkers. Changes in DNA methylation, histone acetylation status, or microRNAs can lead to gene silencing or amplification resulting in gene-expression alterations without altering the DNA sequence. The most studied gene with methylation change associated with PCa is *glutathione S-transferase P1* (GSTP1). This gene encodes for an enzyme involved in the detoxification of carcinogens and is frequently silenced by promoter hypermethylation in PCa [Lee *et al.* 1994; Nelson *et al.* 2001]. However, GSTP1 promoter hypermethylation is not tumor specific as it is also present in about 70% of high-grade prostatic intraepithelial neoplasia [Nakayama *et al.* 2003]. GSTP1 hypermethylation can be detected in serum and urine, therefore it may have utility as a marker for screening. Many initial studies of GSTP1 methylation have exhibited high sensitivity and specificity for PCa in both urine and plasma [Hoque, 2005; Jerónimo *et al.* 2002; Woodson *et al.* 2008]. As more understanding of epigenetic changes in PCa continues, the potential for these changes to serve as a biomarker may also increase.

Surveillance markers

The role of biomarkers in active surveillance is in the early stages. The most robust research in this arena involves PSA and/or PSA kinetics. The use of PSA kinetics has been explored as a possible replacement for repeat prostate biopsy during active surveillance in low-risk patients. Unfortunately, this has not been reliably shown to be an independent predictor of progression while on surveillance, at least not over the short term. Whitson and colleagues revealed that PSA velocity was not associated with risk of biopsy progression, defined as increase in grade and/or volume [Whitson *et al.* 2011]. In addition, Ross and colleagues, using a cohort of surveillance patients from Johns Hopkins Hospital, Baltimore, MD, USA demonstrated that neither PSA velocity nor PSA doubling time predicted adverse pathology and should not be used to replace annual surveillance biopsy for men on active surveillance [Ross *et al.* 2010]. Currently, significant increases in PSA while on surveillance may prompt repeat biopsies, but in many cases PSA increases alone are unreliable markers of progression and should not necessarily be considered as a reliable trigger for treatment.

In an attempt to discover a role for PCA3 in men on active surveillance, Tosoian and colleagues studied the association of PCA3 with biopsy progression, defined as an increase in Gleason grade and/or volume. The mean PCA3 score of men who progressed was similar to that of men without progression (60.0 *versus* 50.8; $p = 0.131$). Further, the AUC for predicting progression was 0.59, which was no more statistically different than a toss of a coin ($p = 0.076$) [Tosoian *et al.* 2010]. While some have found PCA3 levels to correlate with Gleason score and thus PCa aggressiveness [Aubin *et al.* 2010; Haese *et al.* 2008], others have found no association with PCa aggressiveness at biopsy [Deras *et al.* 2008; Hessels *et al.* 2003; Marks *et al.* 2007]. As a result of these conflicting findings, the role of PCA3 in risk assessment during active surveillance requires further study.

Another attractive genetic marker involves quantifying gene expression profiles by measuring mRNA levels of many genes at once. This may allow for a better overall representation of cancer compared with a specific genetic variant. One commercially available example of this has been developed by Myriad Genetics (Salt Lake City, UT, USA) called Prolaris®. This test measures the gene expression of 46 cell-cycle genes from radical prostatectomy specimens to develop a cell-cycle progression (CCP) score. This CCP score has been shown to independently risk stratify men for 10-year PCa death beyond that of PSA and Gleason score for men managed conservatively [Cuzick *et al.* 2012], and most recently has been shown to add independent prognostic information to a standard clinical risk score in a contemporary prostatectomy cohort [Cooperberg *et al.* 2013]. This recent validation study by Cooperberg and colleagues may prove useful in helping to guide decisions regarding adjuvant treatment and in stratifying men for future adjuvant therapy studies. With further validation in studies of prostate biopsy specimens, this test could help men on active surveillance make more informed decisions. In addition, Genomic Health (Redwood City, CA, USA) is set to release a multigene genomic prostate score test later this year. This test, using prostate biopsy tissue, has been prospectively validated as a predictor of adverse pathology for patients with early stage PCa and has the potential to improve treatment decisions for men on active surveillance [Klein *et al.* 2012].

While candidate-marker development is beyond the scope of this review, Pepe and colleagues' description of the prospective-specimen collection, retrospective-blinded evaluation design provides a framework for candidate-marker development, which uses rigorous research standards [Pepe *et al.* 2008]. This type of design is advantageous due to the relative availability of biorepositories. A shortcoming in biomarker development in the active surveillance setting relates to defining an appropriate endpoint. Current definitions of 'progression' on surveillance include changes in PSA kinetics and/or tumor grade/volume on subsequent biopsies. Unfortunately, these definitions are surrogates for meaningful biologic progression and their shortcomings have been demonstrated in the literature. For example, changes in PSA kinetics have not consistently predicted adverse pathology, and serial biopsies may represent more accurate sampling of a stable tumor rather that tumor dedifferentiation [Porten *et al.* 2011; Ross *et al.* 2010; Whitson *et al.* 2011]. In short, biomarker development is challenging and advances are being made to further research methodology [McShane *et al.* 2005; Pepe *et al.* 2008].

Conclusion

While many novel PCa biomarkers have shown promise, none seem currently poised to replace the utility of PSA. The recent USPSTF recommendations against PCa screening have highlighted the limitations of the PSA test and invigorated interest in more specific biomarkers for PCa. However, to halt PCa screening until improved biomarkers become available would be a disservice to all men, especially the near 30,000 men who die of PCa each year. The goal of finding more specific biomarkers is to avoid overdiagnosis and overtreatment associated with PSA screening. To be sure, it must be noted that PSA has been one of most successful tumor markers to date and remains the single most predictive marker for identifying men at increased risk for PCa. Many of the current biomarkers modestly increase the operating characteristics relative to PSA; however, no individual marker is ideal. Moving forward, further validation of promising markers and continued discovery of novel markers is needed. These new biomarkers must show improvement in clinical outcome, which can be accomplished by incorporating decision analytic methods in the validation process. Given the heterogeneity of PCa, perhaps a combination of markers will further improve the predictive accuracy and be the path going forward. While it will be determined which of these markers will play an important role in screening, the fundamental goal is to decrease the number of unnecessary biopsies, and differentiate between indolent and aggressive PCa.

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References

Abdollah, F., Sun, M., Thuret, R., Jeldres, C., Tian, Z., Briganti, A. *et al.* (2011) A competing-risks analysis of survival after alternative treatment

modalities for prostate cancer patients: 1988–2006. *Eur Urol* 59: 88–95.

Andriole, G., Crawford, E., Grubb, R., Buys, S., Chia, D., Church, T. *et al.* (2009) Mortality results from a randomized prostate-cancer screening trial. *N Engl J Med* 360: 1310–1319.

Ankerst, D., Groskopf, J., Day, J., Blase, A., Rittenhouse, H., Pollock, B. *et al.* (2008) Predicting prostate cancer risk through incorporation of prostate cancer gene 3. *J Urol* 180: 1303–1308.

Antenor, J., Han, M., Roehl, K., Nadler, R. and Catalona, W. (2004) Relationship between initial prostate specific antigen level and subsequent prostate cancer detection in a longitudinal screening study. *J Urol* 172: 90–93.

Aubin, S., Reid, J., Sarno, M., Blase, A., Aussie, J., Rittenhouse, H. *et al.* (2010) PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial. *J Urol* 184: 1947–1952.

Becker, C., Piironen, T., Pettersson, K., Björk, T., Wojno, K., Oesterling, J. *et al.* (2000) Discrimination of men with prostate cancer from those with benign disease by measurements of human glandular kallikrein 2 (HK2) in serum. *J Urol* 163: 311–316.

Benchikh, A., Savage, C., Cronin, A., Salama, G., Villers, A., Lilja, H. *et al.* (2010) A panel of kallikrein markers can predict outcome of prostate biopsy following clinical work-up: an independent validation study from the European Randomized Study of Prostate Cancer screening, France. *BMC Cancer* 10: 635.

Brawley, O. (2012) Trends in prostate cancer in the United States. *J Natl Cancer Inst Monogr* 2012: 152–156.

Bussemakers, M., van Bokhoven, A., Verhaegh, G., Smit, F., Karthaus, H., Schalken, J. *et al.* (1999) DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res* 59: 5975–5979.

Catalona, W., Partin, A., Slawin, K., Brawer, M., Flanigan, R., Patel, A. *et al.* (1998) Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA* 279: 1542–1547.

Catalona, W., Smith, D. and Ornstein, D. (1997) Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements. *JAMA* 277: 1452–1455.

Catalona, W., Smith, D., Ratliff, T., Dodds, K., Coplen, D., Yuan, J. *et al.* (1991) Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med* 324: 1156–1161.

Chan, T., Mikolajczyk, S., Lecksell, K., Shue, M., Rittenhouse, H., Partin, A. W. *et al.* (2003) Immunohistochemical staining of prostate cancer with monoclonal antibodies to the precursor of prostate-specific antigen. *Urology* 62: 177–181.

Christensson, A., Björk, T., Nilsson, O., Dahlén, U., Matikainen, M., Cockett, A. *et al.* (1993) Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. *J Urol* 150: 100–105.

Christensson, A., Laurell, C. and Lilja, H. (1990) Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur J Biochem* 194: 755–763.

Cooperberg, M., Broering, J. and Carroll, P. (2009) Risk assessment for prostate cancer metastasis and mortality at the time of diagnosis. *J Natl Cancer Inst* 101: 878–887.

Cooperberg, M., Broering, J., Kantoff, P. and Carroll, P. (2007) Contemporary trends in low risk prostate cancer: risk assessment and treatment. *J Urol*: S14–S19.

Cooperberg, M., Lubeck, D., Meng, M., Mehta, S. and Carroll, P. (2004) The changing face of low-risk prostate cancer: trends in clinical presentation and primary management. *J Clin Oncol* 22: 2141–2149.

Cooperberg, M., Pasta, D., Elkin, E., Litwin, M., Latini, D., Du Chane, J. *et al.* (2005) The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. *J Urol* 173: 1938–1942.

Cooperberg, M., Simko, J., Cowan, J., Reid, J., Djalilvand, A., Bhatnagar, S. *et al.* (2013) Validation of a cell-cycle progression gene panel to improve risk stratification in a contemporary prostatectomy cohort. *J Clin Oncol* 31: 1428–1434.

Crawford, E., Rove, K., Trabulsi, E., Qian, J., Drewnowska, K., Kaminetsky, J. *et al.* (2012) Diagnostic performance of PCA3 to detect prostate cancer in men with increased prostate specific antigen: a prospective study of 1,962 cases. *J Urol* 188: 1726–1731.

Cuzick, J., Berney, D., Fisher, G., Mesher, D., Møller, H., Reid, J. *et al.* (2012) Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. *Br J Cancer* 106: 1095–1099.

D'Amico, A. (2003) Cancer-specific mortality after surgery or radiation for patients with clinically localized prostate cancer managed during the prostate-specific antigen era. *J Clin Oncol* 21: 2163–2172.

D'Amico, A., Whittington, R., Malkowicz, S., Schultz, D., Blank, K., Broderick, G. *et al.* (1998) Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 280: 969–974.

Darson, M., Pacelli, A., Roche, P., Rittenhouse, H., Wolfert, R., Saeid, M. *et al.* (1999) Human glandular kallikrein 2 expression in prostate adenocarcinoma and lymph node metastases. *Urology* 53: 939–944.

Deras, I., Aubin, S., Blase, A., Day, J., Koo, S., Partin, A. *et al.* (2008) PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol* 179: 1587–1592.

Djavan, B., Remzi, M., Schulman, C., Marberger, M. and Zlotta, A. (2002) Repeat prostate biopsy: who, how and when? A review. *Eur Urol* 42: 93–103.

Djavan, B., Zlotta, A., Kratzik, C., Remzi, M., Seitz, C., Schulman, C. *et al.* (1999) PSA, PSA density, PSA density of transition zone, free/total PSA ratio, and PSA velocity for early detection of prostate cancer in men with serum PSA 2.5 to 4.0 ng/mL. *Urology* 54: 517–522.

Djavan, B., Zlotta, A., Remzi, M., Ghawidel, K., Basharkhah, A., Schulman, C. *et al.* (2000) Optimal predictors of prostate cancer on repeat prostate biopsy: a prospective study of 1,051 men. *J Urol* 163: 1144–1149.

Etzioni, R., Gulati, R., Tsodikov, A., Wever, E., Penson, D., Heijnsdijk, E. *et al.* (2012) The prostate cancer conundrum revisited. *Cancer* 118: 5955–5963.

Etzioni, R., Tsodikov, A., Mariotto, A., Szabo, A., Falcon, S., Wegelin, J. *et al.* (2007) Quantifying the role of PSA screening in the US prostate cancer mortality decline. *Cancer Causes Control* 19: 175–181.

Fang, J., Metter, E., Landis, P., Chan, D., Morrell, C. and Carter, H. (2001) Low levels of prostate-specific antigen predict long-term risk of prostate cancer: results from the Baltimore Longitudinal Study of Aging. *Urology* 58: 411–416.

Finlay, J., Evans, C., Day, J., Payne, J., Mikolajczyk, S., Millar, L. *et al.* (1998) Development of monoclonal antibodies specific for human glandular kallikrein (hK2): development of a dual antibody immunoassay for hK2 with negligible prostate-specific antigen cross-reactivity. *Urology* 51: 804–809.

Fleshner, N. and Lawrentschuk, N. (2009) Risk of developing prostate cancer in the future: overview of prognostic biomarkers. *Urology* 73(Suppl.): S21–S27.

Gopalan, A., Leversha, M., Satagopan, J., Zhou, Q., Al-Ahmadie, H., Fine, S. *et al.* (2009) TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. *Cancer Res* 69: 1400–1406.

Greene, K., Albertsen, P., Babaian, R., Carter, H., Gann, P., Han, M. *et al.* (2013) Prostate specific antigen best practice statement: 2009 update. *J Urol* 189(Suppl.): S2–S11.

Groskopf, J., Aubin, S., Deras, I., Blase, A., Bodrug, S., Clark, C. *et al.* (2006) APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem* 52: 1089–1095.

Guazzoni, G., Nava, L., Lazzeri, M., Scattoni, V., Lughezzani, G., Maccagnano, C. *et al.* (2011) Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. *Eur Urol* 60: 214–222.

Gudmundsson, J., Besenbacher, S., Sulem, P., Gudbjartsson, D., Olafsson, I., Arinbjarnarson, S. *et al.* (2010) Genetic correction of PSA values using sequence variants associated with PSA levels. *Sci Transl Med* 2: 62ra92.

Haese, A., de la Taille, A. and Van Poppel, H. (2008) Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol* 54: 1081–1088.

Helfand, B., Kan, D., Modi, P. and Catalona, W. (2011) Prostate cancer risk alleles significantly improve disease detection and are associated with aggressive features in patients with a 'normal' prostate specific antigen and digital rectal examination. *Prostate* 71: 394–402.

Helfand, B., Loeb, S., Kan, D. and Catalona, W. (2010) Number of prostate cancer risk alleles may identify possibly 'insignificant' disease. *BJU Int* 106: 1602–1606.

Hemminki, K., Ji, J., Försti, A., Sundquist, J. and Lenner, P. (2008) Concordance of survival in family members with prostate cancer. *J Clin Oncol* 26: 1705–1709.

Hessels, D., Klein Gunnewiek, J., van Oort, I., Karthaus, H., van Leenders, G., van Balken, B. *et al.* (2003) DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 44: 8–16.

Hessels, D., Smit, F., Verhaegh, G., Witjes, J., Cornel, E. and Schalken, J. (2007) Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res* 13: 5103–5108.

Hoque, M. (2005) Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. *J Clin Oncol* 23: 6569–6575.

Houlgatte, A., Vincendeau, S., Desfemmes, F., Ramirez, J., Benoist, N., Bensalah, K. *et al.* (2012) Use of [-2] pro PSA and phi index for early detection of prostate cancer: a prospective of 452 patients. *Prog Urol* 22: 279–283.

Jerónimo, C., Usadel, H., Henrique, R., Silva, C., Oliveira, J., Lopes, C. *et al.* (2002) Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer. *Urology* 60: 1131–1135.

Kattan, M., Eastham, J., Stapleton, A., Wheeler, T. and Scardino, P. (1998) A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *J Natl Cancer Inst* 90: 766–771.

Khan, M., Sokoll, L., Chan, D., Mangold, L., Mohr, P., Mikolajczyk, S. *et al.* (2004) Clinical utility of proPSA and 'benign' PSA when percent free PSA is less than 15%. *Urology* 64: 1160–1164.

Klein, E., Maddala, T., Millward, C., Cherbavaz, D., Falzarano, S., Knezevic, D. *et al.* (2012) Development of a needle biopsy-based genomic test to improve discrimination of clinically aggressive from indolent prostate cancer. ASCO Meeting Abstracts. *J Clin Oncol* 30(Suppl.): 4560.

Kranse, R., Roobol, M. and Schröder, F. (2008) A graphical device to represent the outcomes of a logistic regression analysis. *Prostate* 68: 1674–1680.

Kumar-Sinha, C., Tomlins, S. and Chinnaiyan, A. (2008) Recurrent gene fusions in prostate cancer. *Nat Rev Cancer* 8: 497–511.

Laxman, B., Tomlins, S., Mehra, R., Morris, D., Wang, L., Helgeson, B. *et al.* (2006) Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. *Neoplasia* 8: 885–888.

Le, B., Griffin, C., Loeb, S., Carvalhal, G., Kan, D., Baumann, N. *et al.* (2010) [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. *J Urol* 183: 1355–1359.

Lee, W., Morton, R., Epstein, J., Brooks, J., Campbell, P., Bova, G. *et al.* (1994) Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proc Natl Acad Sci U S A* 91: 11733–11737.

Lesko, L. and Atkinson, A. (2001) Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. *Annu Rev Pharmacol Toxicol* 41: 347–366.

Leyten, G., Hessels, D., Jannink, S., Smit, F., de Jong, H., Cornel, E. *et al.* (2012) Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *Eur Urol*: doi:10.1016/j.eururo.2012.11.014 [Epub ahead of print]

Lilja, H., Christensson, A., Dahlén, U., Matikainen, M., Nilsson, O., Pettersson, K. *et al.* (1991) Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem* 37: 1618–1625.

Lilja, H., Cronin, A., Dahlin, A., Manjer, J., Nilsson, P., Eastham, J. *et al.* (2011) Prediction of significant prostate cancer diagnosed 20 to 30 years later with a single measure of prostate-specific antigen at or before age 50. *Cancer* 117: 1210–1219.

Loeb, S., Roehl, K., Antenor, J., Catalona, W., Suarez, B. K. and Nadler, R. (2006) Baseline prostate-specific antigen compared with median prostate-specific antigen for age group as predictor of prostate cancer risk in men younger than 60 years old. *Urology* 67: 316–320.

Lowe, F. and Trauzzi, S. (1993) Prostatic acid phosphatase in 1993. Its limited clinical utility. *Urol Clin North Am* 20: 589–595.

Marks, L., Fradet, Y., Deras, I., Blase, A., Mathis, J., Aubin, S. *et al.* (2007) PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* 69: 532–535.

McShane, L., Altman, D., Sauerbrei, W., Taube, S., Gion, M. and Clark, G.; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. (2005) Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol* 23: 9067–9072.

Mikolajczyk, S., Marker, K., Millar, L., Kumar, A., Saedi, M., Payne, J. *et al.* (2001) A truncated precursor form of prostate-specific antigen is a more specific serum marker of prostate cancer. *Cancer Res* 61: 6958–6963.

Mikolajczyk, S., Millar, L., Wang, T., Rittenhouse, H., Marks, L., Song, W. *et al.* (2000) A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. *Cancer Res* 60: 756–759.

Minner, S., Enodien, M., Sirma, H., Luebke, A., Krohn, A., Mayer, P. *et al.* (2011) ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. *Clin Cancer Res* 17: 5878–5888.

Moyer, V. for the US Preventive Services Task Force. (2012) Screening for prostate cancer: US Preventive Services Task Force recommendation statement. *Ann Intern Med* 157: 120–134.

Nakanishi, H., Groskopf, J., Fritsche, H., Bhadkamkar, V., Blase, A., Kumar, S. *et al.* (2008)

PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol* 179: 1804–1810.

Nakayama, M., Bennett, C., Hicks, J., Epstein, J., Platz, E., Nelson, W. *et al.* (2003) Hypermethylation of the human glutathione S-transferase-pi gene (GSTP1) CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: a detailed study using laser-capture microdissection. *Am J Pathol* 163: 923–933.

Nam, R., Sugar, L., Yang, W., Srivastava, S., Klotz, L., Yang, L. *et al.* (2007a) Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. *Br J Cancer* 97: 1690–1695.

Nam, R., Toi, A., Klotz, L., Trachtenberg, J., Jewett, M., Appu, S. *et al.* (2007b) Assessing individual risk for prostate cancer. *J Clin Oncol* 25: 3582–3588.

Nelson, C., La Creis R. Kidd, Sauvageot, J., Isaacs, W., De Marzo, A., Groopman, J. *et al.* (2001) Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. *Cancer Res* 61: 103–109.

Penney, K., Salinas, C., Pomerantz, M., Schumacher, F., Beckwith, C., Lee, G. *et al.* (2009) Evaluation of 8q24 and 17q risk loci and prostate cancer mortality. *Clin Cancer Res* 15: 3223–3230.

Pepe, M., Feng, Z., Janes, H., Bossuyt, P. and Potter, J. (2008) Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. *J Natl Cancer Inst* 100: 1432–1438.

Piironen, T., Lövgren, J., Karp, M., Eerola, R., Lundwall, A., Dowell, B. *et al.* (1996a) Immunofluorometric assay for sensitive and specific measurement of human prostatic glandular kallikrein (hK2) in serum. *Clin Chem* 42: 1034–1041.

Piironen, T., Pettersson, K., Suonpää, M., Stenman, U., Oesterling, J., Lövgren, T. *et al.* (1996b) *In vitro* stability of free prostate-specific antigen (PSA) and prostate-specific antigen (PSA) complexed to alpha 1-antichymotrypsin in blood samples. *Urology* 48(6A Suppl.): 81–87.

Pinsky, P., Black, A., Kramer, B., Miller, A., Prorok, P. and Berg, C. (2010) Assessing contamination and compliance in the prostate component of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. *Clin Trials* 7: 303–311.

Porten, S., Whitson, J., Cowan, J., Cooperberg, M., Shinohara, K., Perez, N. *et al.* (2011) Changes in prostate cancer grade on serial biopsy in men undergoing active surveillance. *J Clin Oncol* 29: 2795–2800.

Recker, F., Kwiatkowski, M., Piironen, T., Pettersson, K., Lümmen, G., Wernli, M. *et al.* (1998) The importance of human glandular kallikrein and its correlation with different prostate specific antigen serum forms in the detection of prostate carcinoma. *Cancer* 83: 2540–2547.

Ross, A., Loeb, S., Landis, P., Partin, A., Epstein, J., Kettermann, A. *et al.* (2010) Prostate-specific antigen kinetics during follow-up are an unreliable trigger for intervention in a prostate cancer surveillance program. *J Clin Oncol* 28: 2810–2816.

Salinas, C., Koopmeiners, J., Kwon, E., FitzGerald, L., Lin, D., Ostrander, E. *et al.* (2009) Clinical utility of five genetic variants for predicting prostate cancer risk and mortality. *Prostate* 69: 363–372.

Schedlich, L., Bennetts, B. and Morris, B. (1987) Primary structure of a human glandular kallikrein gene. *DNA* 6: 429–437.

Schröder, F., Hugosson, J., Roobol, M., Tammela, T., Ciatto, S., Nelen, V. *et al.* (2012) Prostate-cancer mortality at 11 years of follow-up. *N Engl J Med* 366: 981–990.

Schröder, F., Hugosson, J., Roobol, M., Tammela, T., Ciatto, S., Nelen, V. *et al.* (2009a) Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 360: 1320–1328.

Schröder, F., Roobol, M., Andriole, G. and Fleshner, N. (2009b) Defining increased future risk for prostate cancer: evidence from a population based screening cohort. *J Urol* 181: 69–74.

Siegel, R., Naishadham, D. and Jemal, A. (2013) Cancer statistics, 2013. *CA Cancer J Clin* 63: 11–30.

Sokoll, L., Sanda, M., Feng, Z., Kagan, J., Mizrahi, I., Broyles, D. *et al.* (2010) A prospective, multicenter, National Cancer Institute early detection research network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiol Biomarkers Prev* 19: 1193–1200.

Sokoll, L., Wang, Y., Feng, Z., Kagan, J., Partin, A., Sanda, M. *et al.* (2008) [-2]proenzyme prostate specific antigen for prostate cancer detection: a National Cancer Institute early detection research network validation study. *J Urol* 180: 539–543.

Stenman, U., Hakama, M., Knekt, P., Aromaa, A., Teppo, L. and Leinonen, J. (1994) Serum concentrations of prostate specific antigen and its complex with alpha 1-antichymotrypsin before diagnosis of prostate cancer. *Lancet* 344: 1594–1598.

Thompson, I., Ankerst, D., Chi, C., Goodman, P., Tangen, C., Lucia, M. *et al.* (2006) Assessing

prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 98: 501–501.

Thompson, I., Ankerst, D., Chi, C., Lucia, M., Goodman, P., Crowley, J. *et al.* (2005) Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. *JAMA* 294: 66–70.

Thompson, I., Pauler, D., Goodman, P., Tangen, C., Lucia, M., Parnes, H. *et al.* (2004) Prevalence of prostate cancer among men with a prostate-specific antigen level. *N Engl J Med* 350: 2239–2246.

Tinzl, M., Marberger, M., Horvath, S. and Chypre, C. (2004) DD3 RNA analysis in urine? A new perspective for detecting prostate cancer. *Eur Urol* 46: 182–187.

Tomlins, S. (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 310: 644–648.

Tosoian, J. J., Loeb S., Kettermann, A., Landis, P., Elliot, D. J., Epstein, J. I., et al. (2010) Accuracy of PCA3 measurement in predicting short term biopsy progression in an active surveillance program. *J Urol*, 183 (2), 534–538.

van den Bergh, R., Roobol, M., Wolters, T., van Leeuwen, P. and Schröder, F. (2008) The Prostate Cancer Prevention Trial and European Randomized Study of Screening for Prostate Cancer risk calculators indicating a positive prostate biopsy: a comparison. *BJU Int* 102: 1068–1073.

van Gils, M., Hessels, D., van Hooij, O., Jannink, S., Peelen, W., Hanssen, S. *et al.* (2007) The time-resolved fluorescence-based PCA3 test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin Cancer Res* 13: 939–943.

Vickers, A., Cronin, A., Björk, T., Manjer, J., Nilsson, P., Dahlin, A. *et al.* (2010a) Prostate specific antigen concentration at age 60 and death or metastasis from prostate cancer: case-control study. *BMJ* 341: c4521.

Vickers, A., Cronin, A., Roobol, M., Savage, C., Peltola, M., Pettersson, K. *et al.* (2010b) Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. *J Clin Oncol* 28: 2493–2498.

Vickers, A., Gupta, A., Savage, C., Pettersson, K., Dahlin, A., Bjartell, A. *et al.* (2011) A panel of kallikrein marker predicts prostate cancer in a large, population-based cohort followed for 15 years without screening. *Cancer Epidemiol Biomarkers Prev* 20: 255–261.

Walter, L., Bertenthal, D., Lindquist, K. and Konety, B. (2006) PSA screening among elderly men with limited life expectancies. *JAMA* 296: 2336–2342.

Wang, J., Cai, Y., Ren, C. and Ittmann, M. (2006) Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. *Cancer Res* 66: 8347–8351.

Whitesel, J., Donohue, R., Mani, J., Mohr, S., Scanavino, D., Augspurger, R. *et al.* (1984) Acid phosphatase: its influence on the management of carcinoma of the prostate. *J Urol* 131: 70–72.

Whitson, J., Porten, S., Hilton, J., Cowan, J., Perez, N., Cooperberg, M. *et al.* (2011) The relationship between prostate specific antigen change and biopsy progression in patients on active surveillance for prostate cancer. *J Urol* 185: 1656–1660.

Woodrum, D., Brawer, M., Partin, A., Catalona, W. and Southwick, P. (1998) Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 159: 5–12.

Woodson, K., O'Reilly, K., Hanson, J., Nelson, D., Walk, E. and Tangrea, J. (2008) The usefulness of the detection of GSTP1 methylation in urine as a biomarker in the diagnosis of prostate cancer. *J Urol* 179: 508–512.

Wu, A., Reese, A., Cooperberg, M., Sadetsky, N. and Shinohara, K. (2012) Utility of PCA3 in patients undergoing repeat biopsy for prostate cancer. *Prostate Cancer Prostatic Dis* 15: 100–105.

Zheng, S., Sun, J., Wiklund, F., Smith, S., Stattin, P., Li, G. *et al.* (2008) Cumulative association of five genetic variants with prostate cancer. *N Engl J Med* 358: 910–919.

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