

# Special Report: Recent Developments in Prostate Cancer Genetics and Genetic Testing



Assessment  
Program  
Volume 23, No. 7  
January 2009

## Executive Summary

### Background

Prostate cancer is the most common malignancy and second-leading cause of cancer death among males in the U.S. In 2007, there were approximately 218,000 new cases of prostate cancer and 27,000 deaths, with a man's lifetime risk of prostate cancer being 1 in 6. Prostate cancer is a heterogeneous disease with some cancers remaining asymptomatic and others behaving in an aggressive, often fatal, manner. A better understanding is needed of the biologic and genetic differences between these indolent and aggressive forms, as well as of the risk factors for development. Recent advances in the sequencing of the human genome and high-throughput analysis techniques have led to the identification of many potential biomarkers of prostate disease and risk assessment that are currently under investigation. Although proteomic profiling is also an emerging research field, it is not currently as far advanced as gene expression profiling and there have been issues of reproducibility; therefore, this Report focuses on nucleic acid based markers.

### Objective

This Special Report is intended to be a horizon-scanning catalog of nucleic acid-based tests related to prostate cancer risk, detection, or prognosis that are currently available or are likely to be available in the near future. It does not address the question as to whether the TEC criteria are met.

### Search Strategy

Gray literature during 2007 through May 2008 was routinely scanned for articles related to genetic testing and prostate cancer. MEDLINE® (via PubMed) was searched over the last 2 years for articles on genetic biomarkers and prostate cancer.

### Selection Criteria

News articles from the gray literature were organized into specific topic groups.

Titles and abstracts of recent (2006–2008) journal publications were searched for studies of nucleic acid-based biomarkers investigated in clinical populations for use in prostate cancer risk assessment, diagnosis, or prognosis. Special attention was paid to the topic groups determined by the gray literature search. Topics not already identified by the gray literature search with a concentration of publications were also determined from the literature searches. Information on these additional topics was sought via Internet searches (e.g., for evidence of commercialization). Finally, full-length papers were requested for potentially relevant citations for each of the topic areas.



An Association  
of Independent  
Blue Cross and  
Blue Shield Plans



NOTICE OF PURPOSE: TEC Assessments are scientific opinions, provided solely for informational purposes. TEC Assessments should not be construed to suggest that the Blue Cross Blue Shield Association, Kaiser Permanente Medical Care Program or the TEC Program recommends, advocates, requires, encourages, or discourages any particular treatment, procedure, or service; any particular course of treatment, procedure, or service; or the payment or non-payment of the technology or technologies evaluated.

## Discussion

Prostate cancer is a complex, heterogeneous disease. At the extremes of the spectrum, if left untreated, some prostate cancers behave aggressively, metastasize quickly, and cause mortality, while others are indolent and never progress to cause harm. While it would be desirable to screen for aggressive prostate cancer that is likely to cause morbidity and mortality, currently available biomarkers are incompletely sensitive, have poor specificity, and do not distinguish aggressive disease from indolent disease that need only be monitored. It is also not currently possible to predict in advance who is likely to develop aggressive disease. For those given curative treatment (e.g., prostatectomy for clinically localized disease), no biomarkers are available that accurately predict the risk of recurrence.

In response to the need for better biomarkers for risk assessment, diagnosis, and prognosis, a variety of exploratory research is ongoing. Some products of this work have already or are in the process of being translated into commercially available tests. This Special Report examines the state of the evidence for some of these tests. In general, the evidence addresses clinical validity i.e., the association of the test result with outcomes of interest, expressed in terms of clinical performance characteristics such as sensitivity, specificity, predictive value, and comparisons to current standards using receiver-operating curve (ROC) analysis and/or logistic regression. There is no evidence of clinical utility, i.e., that using a test will improve outcomes. Evidence was reviewed for the following tests:

- **Single-nucleotide polymorphisms (SNPs) for risk assessment.** Several large population studies have identified SNPs that are repeatably highly significant predictors of prostate cancer risk, although the genes and biologic mechanisms behind these associations are as yet unknown. Several SNPs combined explain a significant proportion of prostate cancer, but by no means all. A few different groups are commercializing specific SNP panels, combined in one case with family history, as risk assessment tools presumably to identify those men who should start disease surveillance early and be monitored frequently. However, these tests do not predict certainty of disease, nor do they clearly predict aggressive versus indolent disease. While the monitoring of high-risk men may improve outcomes, it is also possible that these could be offset by the harms of identifying and treating additional indolent disease. Recent evidence regarding the safety of prevention with finasteride has not yet been incorporated into guidelines for cancer prevention in high-risk or general-risk populations.
- **PCA3 for disease and diagnosis.** PCA3 is overexpressed in prostate cancer and PCA3 mRNA can be detected in urine samples collected after prostate massage. When normalized using PSA to account for the amount of prostate cells released into the urine (“PCA3 Score”) the test has significantly improved specificity compared to PSA and may better discriminate patients with eventual benign biopsies from those with malignant biopsy results.

One study suggests that PCA3 Score may also have value in identifying patients with less aggressive cancer who may only need surveillance. In general, however, PCA3 assay results to date are preliminary; interpretation of results has not been standardized and clinical utility studies of decision-making for initial biopsy, repeat biopsy or treatment have not been reported.

- **TMPRSS fusion genes for diagnosis and prognosis.** TMPRSS2 is an androgen-regulated transmembrane serine protease that is preferentially expressed in normal prostate tissue. In prostate cancer, it may be fused to an ETS family transcription factor (ERG, ETV1, or ETV4), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis. The result of gene fusion with an ETS transcription gene is that the androgen-responsive promoter of TMPRSS2 positively dysregulates expression of the ETS gene, suggesting a mechanism for neoplastic transformation. Fusion genes may be detected in tissue or urine. Evidence suggests that assays for fusion genes may offer specific disease detection, and that fusion genes are associated with a greater likelihood of biochemical recurrence. However, accurate fusion gene detection is complex, assays have not been standardized, and once they are, larger studies will be needed to determine clinical utility.

- **Candidate gene panels for prostate cancer diagnosis.** Because no single gene markers have been found that are both highly sensitive and highly specific for diagnosing prostate cancer, particularly in men already known to have elevated PSA levels, some investigators are combining several promising markers into a single diagnostic panel. While promising in concept, only very limited evidence is available for these applications.
- **Gene hypermethylation for diagnosis and prognosis.** Epigenetic changes, chromatin protein modifications that do not involve changes to the underlying DNA sequence but which can result in changes in gene expression, have been identified in specific genes in relation to prostate cancer. A review of recently published studies reveals an area of clinical research that has not yet identified the best markers for diagnosis and prognosis, nor the best way to measure them and in which sample type. Standardized assays and interpretation criteria have not yet been agreed upon to enable consistency and comparison of results across studies.

While these studies generate much useful information that may help elucidate the biologic mechanisms of prostate cancer and eventually help design treatments, the reviewed assays are in a developmental phase, currently without evidence of clinical utility.

## Contents

|                           |           |                                  |           |
|---------------------------|-----------|----------------------------------|-----------|
| <b>Objective</b>          | <b>4</b>  | <b>References</b>                | <b>21</b> |
| <b>Introduction</b>       | <b>4</b>  | <b>Glossary of Genetic Terms</b> | <b>25</b> |
| <b>Methods</b>            | <b>10</b> | <b>Appendices</b>                |           |
| <b>Review of Evidence</b> | <b>13</b> | Appendix A                       | <b>28</b> |
| <b>Discussion</b>         | <b>14</b> | Appendix B                       | <b>33</b> |
|                           |           | Appendix C                       | <b>40</b> |
|                           |           | Appendix D                       | <b>46</b> |
|                           |           | Appendix E                       | <b>49</b> |

## Published in cooperation with Kaiser Foundation Health Plan and Southern California Permanente Medical Group.

### TEC Staff Contributors

**Author**—Margaret A. Piper, Ph.D., M.P.H.; **TEC Executive Director**—Naomi Aronson, Ph.D.; **Director, Clinical Science Services**—Kathleen M. Ziegler, Pharm.D.; **Research/Editorial Staff**—Claudia J. Bonnell, B.S.N., M.L.S.; Maxine A. Gere, M.S.

### Blue Cross and Blue Shield Association Medical Advisory Panel

**Allan M. Korn, M.D., F.A.C.P.**—Chairman, *Senior Vice President, Clinical Affairs/Medical Director, Blue Cross and Blue Shield Association*; **Alan M. Garber, M.D., Ph.D.**—Scientific Advisor, *Staff Physician, U.S. Department of Veterans Affairs*; **Henry J. Kaiser, Jr., Professor, and Professor of Medicine, Economics, and Health Research and Policy, Stanford University; **Steven N. Goodman, M.D., M.H.S., Ph.D.**—Scientific Advisor, *Associate Professor, Johns Hopkins School of Medicine, Department of Oncology, Division of Biostatistics (joint appointments in Epidemiology, Biostatistics, and Pediatrics)*—American Academy of Pediatrics Appointee. ■ **Panel Members** **Peter C. Albertsen, M.D.**, *Professor, Chief of Urology, and Residency Program Director, University of Connecticut Health Center*; **Joan M. Bathon, M.D.**, *Professor of Medicine, Division of Rheumatology, Department of Medicine, Johns Hopkins University School of Medicine*; **Sarah T. Corley, M.D., F.A.C.P.**, *Physician Consultant, NexGen Healthcare Information Systems, Inc.*—American College of Physicians Appointee; **Helen Darling, M.A.**, *President, National Business Group on Health*; **Josef E. Fischer, M.D., F.A.C.S.**, *William V. McDermott Professor of Surgery, Harvard Medical School and Chair, Department of Surgery, Beth Israel Deaconess Medical Center*—American College of Surgeons Appointee; **I. Craig Henderson, M.D.**, *Adjunct Professor of Medicine, University of California, San Francisco*; **Mark A. Hlatky, M.D.**, *Professor of Health Research and Policy and of Medicine (Cardiovascular Medicine), Stanford University School of Medicine*; **Walter A. Hollinger, M.D., M.M., M.H.P.E., F.A.C.P.**, *Senior Medical Director, Care Management, Blue Cross and Blue Shield of Florida*; **Bernard Lo, M.D.**, *Professor of Medicine and Director, Program in Medical Ethics, University of California, San Francisco*; **Barbara J. McNeil, M.D., Ph.D.**, *Ridley Watts Professor and Head of Health Care Policy, Harvard Medical School, Professor of Radiology, Brigham and Women's Hospital*; **William R. Phillips, M.D., M.P.H.**, *Clinical Professor of Family Medicine, University of Washington*—American Academy of Family Physicians' Appointee; **Alan B. Rosenberg, M.D.**, *Vice President, Medical Policy, Technology Assessment and Credentialing Programs, WellPoint, Inc.*; **Maren T. Scheuner, M.D., M.P.H.**, *Natural Scientist in the Division of Behavioral and Social Sciences, RAND Corporation; Adjunct Associate Professor, UCLA School of Public Health*—American College of Medical Genetics Appointee; **J. Sanford Schwartz, M.D.**, *Professor of Medicine, Department of Medicine, University of Pennsylvania School of Medicine and Professor, Health Care Systems, Health Management & Economics, The Wharton School*; **Earl P. Steinberg, M.D., M.P.P.**, *President and CEO, Resolution Health, Inc.*; **Robert T. Wanovich, Pharm.D.**, *Vice-President, Pharmacy Affairs, Highmark, Inc.*; **A. Eugene Washington, M.D., M.Sc.**, *Executive Vice Chancellor and Provost, University of California, San Francisco*; **James Weinstein, D.O., M.Sc.**, *Professor and Chair, Department of Orthopaedic Surgery, Dartmouth-Hitchcock Medical Center/Dartmouth Medical School*; **Jed Weissberg, M.D.**, *Associate Executive Director for Quality and Performance Improvement, The Permanente Federation.***

CONFIDENTIAL: This document contains proprietary information that is intended solely for Blue Cross and Blue Shield Plans and other subscribers to the TEC Program. The contents of this document are not to be provided in any manner to any other parties without the express written consent of the Blue Cross and Blue Shield Association.

## Objective

Current needs in prostate cancer assessment and management:

- assays to identify high-risk individuals for screening (risk assessment)
- screening assays that are sensitive (like PSA) and specific (unlike PSA), to reduce the number of unnecessary biopsies (diagnosis)
- assays that can discriminate between indolent and aggressive cancer, to identify patients who most need treatment (prognosis)
- assays to determine likely response to different types of treatment (prognosis of treatment or pharmacogenomics)

This Special Report is intended to be a horizon-scanning catalog of nucleic-acid-based tests related to prostate cancer risk, detection, or prognosis that are currently commercially available or are likely to be available in the near future. It does not address the question as to whether the TEC criteria are met.

## Introduction

### Prostate Cancer

Prostate cancer is the most common malignancy and second-leading cause of cancer death among men in the U.S. In 2007, there were approximately 218,000 new cases of prostate cancer and 27,000 deaths (Hahn et al. 2007), with a man's lifetime risk of prostate cancer being 1 in 6. Prostate cancer is a heterogeneous disease with some cancers remaining asymptomatic and others behaving in an aggressive, often fatal manner. A better understanding is needed of the biologic and genetic differences between these indolent and aggressive forms, as well as of the risk factors for development. Recent advances in the sequencing of the human genome and high-throughput analysis techniques have led to the identification of many potential biomarkers of prostate disease and risk assessment that are currently under investigation. Although proteomic profiling is also an emerging research field, it is not currently as far advanced as gene expression profiling and there have been issues of reproducibility; therefore, this Report focuses on nucleic-acid-based markers.

### Current Methods for Diagnosing Prostate Cancer and Assessing Risk

Currently, the principal screening tests for prostate cancer are the digital rectal examination (DRE) and measurement of the serum marker prostate specific antigen (PSA), recommended on an annual basis beginning at age 50.

The discovery of serum prostate PSA and its widespread use since the late 1980s as an early detection screening test has led to a significant increase in the number of diagnosed cases and has led to a drastic reduction in the number of men with metastatic disease at the time of initial diagnosis. Most men are now diagnosed with prostate cancer in its early stages (localized disease) because of an elevated or rising PSA upon screening. Serum PSA as a screening tool uses 4.0 ng/mL as the upper limit of normal, and levels above this typically prompt a prostate biopsy, to confirm or rule out the presence of cancer. Used as an adjunct to total PSA is percent-free PSA, which measures nonprotein-bound PSA as a percentage of the total level. A higher percentage of free PSA indicates a lower probability of cancer, and raises the likelihood that the total PSA is elevated due to a benign cause. The test for percent-free PSA is approved by the U.S. Food and Drug Administration (FDA) for patients with total PSA levels between 4 and 10 ng/mL, considered a "diagnostic gray zone" (National Comprehensive Cancer Network 2007). Percent-free PSA levels less than 10% are associated with a high risk of prostate cancer, and one multi-institution study showed that a 25% free PSA cutoff detects 95% of prostate cancers (Partin et al. 1998).

Established risk factors for the development of prostate cancer include age, race, and family history. Age is an important risk factor for prostate cancer. It is rarely seen in men younger than 40 years of age, and the risk of developing prostate cancer rises rapidly with each decade thereafter. It has been estimated that 15–30% of men over the age of 50 and 80% of men older than 80 years of age harbor microscopic, undiagnosed prostate cancer (Taichman et al. 2007).

Race is another important risk factor, with African-American men in the U.S. having increased incidence and mortality rates compared with white men, even after adjusting for socioeconomic factors (Hahn et al. 2007). The

increased incidence and aggressiveness of prostate cancer in African-American men suggests a genetic predisposition not only to the development of the disease, but also to more aggressive forms.

Familial clustering of prostate cancer has been reported, although only about 5–10% of cases of prostate cancer are inherited. An inherited susceptibility to prostate cancer may be likely in families with early onset prostate cancer, although what age is early onset has been inconsistently defined (Physician Data Query 2008). Other, lesser well-defined risk factors include endogenous hormones (androgens and estrogens), and some dietary influences (including fat and some vitamins).

Although PSA shows some of the characteristics of an ideal biomarker (reproducible, objective, relatively low cost, can be performed on an easily accessible specimen, etc.), it has several shortcomings. PSA is a tissue-specific marker, but because it can be produced by both benign and malignant prostate tissue, it is not cancer specific. Common benign conditions of the prostate, including hyperplasia and inflammation can lead to an elevated serum PSA. In some reports, only approximately 30% of men with an elevated serum PSA have a biopsy positive for cancer (Parekh et al. 2007; Troyer et al. 2004).

Conversely, using the accepted total PSA value of 4 ng/mL as the upper limit of normal fails to detect a certain percentage of cancers. In one study, 15% of men with a PSA level less than 4.0 ng/mL had prostate cancer, with 14% of them with high-grade disease (Steuber et al. 2007).

Screening for prostate cancer with PSA has resulted in early detection and treatment of early stage disease; however, of great concern is that many of these men are being overtreated for cancers that may never have become clinically relevant. PSA cannot distinguish indolent from aggressive disease, and it is estimated that PSA detects clinically irrelevant cancers 30–50% of the time (Taichman et al. 2007). Overtreatment can result in significant and long-lasting adverse effects (including urinary incontinence and impotence), and therefore, there is a clear need for novel biomarkers that not only detect prostate cancer, but identify the subgroups with aggressive disease that would benefit from treatment.

### Guidelines for Screening and Prevention

Professional organizations vary in their recommendations regarding PSA screening for prostate cancer, summarized in Table 1. In general, most recommend providing information and individualizing the decision to test based on patient preferences regarding disease risk versus screening consequences.

Assessment of future disease risk for the general population (as opposed to those with a strong family history of prostate cancer or of gene mutations associated with increased risk) may become possible with the discovery and evaluation of new biomarkers. The value of such risk assessment depends on the ability to intervene and improve outcomes for high-risk individuals. However, the value of PSA for monitoring high-risk individuals is debatable since it does not discriminate between aggressive and indolent disease. Nor is an effective, safe preventive agent available and generally recommended. Finasteride has been investigated for disease prevention; in the Prostate Cancer Prevention Trial. Finasteride, compared to placebo, reduced incident prostate cancer from 24.4% to 18.4%, an absolute reduction of 6%, in 18,882 men randomized to treatment (Thompson et al. 2004). However, use of finasteride as a preventive agent has not been adopted due to a possible increase in high-grade cancer diagnoses in the treated patients. More recently, these trial data have been further analyzed (Lucia et al. 2008; Redman et al. 2008; Pinsky et al. 2008). The results indicate no significant increase in high-grade tumors as a result of finasteride treatment. Redman et al. (2008) estimated that clinically significant cancers (i.e., those likely to be treated in the U.S.) were reduced from 8.2% (placebo) to 6.0% (finasteride). However, as shown in Table 1, no professional organizations have as yet endorsed the use of finasteride for cancer prevention.

### Molecular Pathology of Prostate Cancer

The molecular pathology of prostate cancer is complex and not completely understood, and appears to be a multistep process with both genetic and epigenetic influences. The mapping of the human genome has stimulated the development of a variety of new technologies to study disease at the molecular level, including microarrays for profiling gene expression and rapid sequencing methods for identifying gene polymorphisms that could be associated

**Table 1.** Summary of PSA Screening and Prostate Cancer Prevention Recommendations from Various Groups

| Organization                               | PSA Screening Recommendation   | Prostate Cancer Prevention Recommendation  |
|--|--|--|
| American Academy of Family Physicians      | Not listed in "Recommended Clinical Preventive Services for Adult Men"   |  |
| US Preventive Services Task Force (USPSTF) | Insufficient evidence for or against routine PSA screening in men <75 years old; recommends against PSA screening in men ≥75   |  |
| American College of Physicians             | Inactive guideline (1997) recommends against general screening, but suggests individualized decision based on patient preferences;<br>Refers generally to USPSTF   |  |
| American Cancer Society                    | PSA screening should be offered; decision should be individualized based on patient preferences  | No general agreement on use of finasteride for cancer prevention   |
| American College of Preventive Medicine    | Recommends against routine screening; information should be provided if life expectancy is >10 years   |  |
| American Urological Association (AUA)      | "The American Urological Association (AUA) encourages men to have annual PSA testing starting at age 50." (over the age of 40 for African-Americans and men with family history); Decision should be individualized based on patient preferences |  |
| National Cancer Institute                  | "The evidence is insufficient to determine whether screening for prostate cancer with prostate-specific antigen (PSA) ... reduces mortality from prostate cancer."   | "...chemoprevention with finasteride reduces the incidence of prostate cancer, but the evidence is inadequate to determine whether chemoprevention with finasteride reduces mortality from prostate cancer." |
| American Society of Clinical Oncology      | No official statement  |  |

with increased risk for prostate cancer or more aggressive disease.

Prostate cancer can be epidemiologically divided into hereditary and sporadic forms, with approximately 5–10% of cases of prostate cancers thought to be due to high-risk inherited genetic factors or susceptibility genes.

**Familial Prostate Cancer.** Genome-wide linkage analyses within families with highly penetrant, early onset prostate cancer have mapped site-specific prostate cancer susceptibility loci and subsequent genes to several different chromosomes. The relationship of the function of these genes to prostate carcinogenesis has not been clearly defined. Some of the more common genes identified include hereditary prostate cancer 1 (HPC1), located on chromosome 1, which accounts for approximately one-third of highly penetrant, early onset cases of familial prostate cancer, hereditary prostate cancer, X-linked (HPCX), on the X chromosome, *elaC* homolog 2 (*ELAC2*) and predisposing for prostate cancer (*PCAP*). However, none of the candidate susceptibility genes has been unequivocally associated with prostate cancer predisposition (Physician Data Query 2008). An additional confounding factor is that because prostate cancer is common in the general population, it is not always possible to distinguish clustering of sporadic prostate cancers within families from true heritable forms.

Some cancer susceptibility syndromes include prostate cancer as a component tumor (Hereditary Breast/Ovarian Cancer syndrome [associated with mutations in *BRCA1* and *BRCA2*]) (Physician Data Query 2008). Studies of male *BRCA1* and *BRCA2* mutation carriers demonstrate that these individuals have an increased risk of prostate cancer, as well as other cancers (Physician Data Query 2008). There is also evidence that men with a *BRCA1* or *BRCA2* mutation develop prostate cancer at a younger age (i.e., younger than 65 years) (Physician Data Query 2008).

**Sporadic Prostate Cancer.** Somatic changes in single, candidate genes have been investigated based upon what is known or suspected of the molecular pathogenesis of prostate cancer. Studies have focused on genes in the categories of tumor suppressor genes, oncogenes, growth factors, oxidative stress response, metabolism, and hormonal control (Mendiratta et al. 2007). While such studies have helped define the

common events related to malignant transformation, no single-gene polymorphisms have emerged as clinically useful biomarkers.

Gene expression arrays have consistently identified a few, single genes that are overexpressed in the majority of prostate cancers. Hepsin and alpha-methylacyl-coenzyme A racemase (*AMACR*) both can be overexpressed 30- to 40-fold above normal controls (Troyer et al. 2004), and *PCA3*, which is a prostate-specific noncoding RNA, is overexpressed in 95% of prostate cancers (Schiffer 2007). Chromosomal translocation results in overexpression of the ETS transcription family members *ERG* and *ETV1* in a substantial proportion of prostate cancers. Activation of *ERG* or *ETV1* by genetic rearrangement occurs with fusion of either gene to the androgen-regulated *TMPRSS2* to generate an androgen-responsive fusion oncoprotein. *TMPRSS2* codes for a prostate specific serine protease that is overexpressed in many prostate cancers. Additional detail on the use of these genes as biomarkers of prostate cancer is reviewed in the Evidence section of this Report.

Single-nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide in the genome sequence is altered. For a genetic variation to be considered a SNP, it must occur in at least 1% of the population. Many SNPs have no effect on cell function, but may help determine an individual's risk of developing a particular disease, or influence response to a certain therapy. A number of chromosomal loci have been identified as associated with an increased risk of prostate cancer; however, these loci have not been consistently linked to genes that consistently predict an increased risk. Potential candidate genes have been identified at three chromosomal loci (8q24, 17q12, and 17q24.3). In the future, SNP microarrays may be useful to predict risk, differentiate benign from malignant prostate, and distinguish indolent from aggressive forms of prostate cancer. Additional detail on this topic is provided in the Evidence section of this Report.

#### **Epigenetic Changes in Prostate Cancer**

Together with changes in the somatic DNA sequence, epigenetic changes have also been associated with prostate cancer development. Epigenetic changes refer to DNA or chromatin protein modifications that do not involve changes to the underlying DNA sequence, but which can result in changes in gene

expression. One of the epigenetic mechanisms thought to be involved in the development of prostate cancer is DNA methylation. Hypermethylation within the promoter region of tumor suppressor genes is an important mechanism of gene inactivation and has been described for many different tumor types. In prostate cancer, hypermethylation of glutathione S-transferase  $\pi$  (GSTP1) is one of the earliest and most consistent findings associated with prostate cancer development, seen in up to 90% of cases (Reynolds et al. 2007). In addition, increased GSTP1 methylation has been found associated with histologic changes in the prostate that may precede cancer development (termed prostatic intraepithelial neoplasia or PIN), and therefore, GSTP1 methylation has emerged as a potential early biomarker.

Epigenetic alterations are also potentially reversible, unlike genetic alterations such as mutations, making them possible targets for gene therapy. Additional detail on the potential use of gene methylation identification tests for prostate cancer can be found in the Evidence section of this Report.

### The Ideal Biomarker

Ideal biomarkers for prostate cancer are needed for both diagnostic and prognostic purposes. In particular, diagnostic tests that are more specific to cancer would improve upon PSA testing and potentially reduce the need for biopsy in many men. Additionally, biomarkers that can distinguish between aggressive cancers that are likely to progress and require treatment from those that are indolent and safely manageable with surveillance could greatly decrease current levels of overtreatment and associated morbidity. Several biomarkers are currently being investigated and validated on a number of different specimens, including tissue and body fluids (serum, urine, and ejaculate).

In contrast to using one biomarker (e.g., PSA), a panel of markers may be more suitable to sensitive and specific detection of a cancer with a high degree of molecular heterogeneity. Examples of panels will be reviewed in the Evidence section of this Report.

### Biomarker Discovery and Selection

There are two major pathways for discovery of genetic (and epigenetic) biomarkers. Table 2 outlines the advantages and limitations of the candidate gene approach versus the genome-

wide approach. Both of these methods have been applied in studies of genetic biomarkers related to prostate cancer and examples of each will follow in the Review of Evidence.

### Developing Evidence to Support the Utility of New Biomarkers

Different conceptual frameworks have been described to organize the evidence development process for diagnostic tests. For example, Pepe et al. (2001), motivated by the goals of the National Cancer Institute's Early Detection Research Network (EDRN), formulated 5 phases of development for biomarkers to be used as cancer screening tests:

- Phase 1: Preclinical exploratory studies to identify potentially discriminating biomarkers. Patient sample populations are highly selected for known outcomes. Independent training and test sample sets are advisable. The potential for spurious results due to testing large numbers should be addressed.
- Phase 2: Clinical assay development and finalization to estimate clinical sensitivity and specificity. Assess potential confounders and clinical characteristics that significantly affect the relationship.
- Phase 3: Retrospective longitudinal studies of clinical specimens collected from cancer cases before their clinical diagnosis, evaluated and compared with those from similar patients who did not develop cancer to determine the capacity of the biomarker to detect preclinical disease.
- Phase 4: Prospective screening studies of the clinically relevant population, with diagnostic follow-up of those who test positive to determine the stage or nature of the cancer at the time it can be detected.
- Phase 5: Ideally, randomized, controlled trials in the clinically relevant population, in which one arm is subjected to screening and appropriate intervention if screen-positive while the other arm is not screened, are undertaken to determine the effect of screening on outcomes, e.g., mortality.

Holtzman and Watson (1999), reporting for the National Institutes of Health (NIH)-Department of Energy (DOE) Task Force on Genetic Testing, identified 3 criteria that need to be satisfied to ensure the safety and effectiveness

**Table 2.** Advantages and Limitations of Two Major Methods of Discovering and Validating Genetic Markers of Disease

| Approach  | Advantages  | Limitations   |
|---|---|---|
| Candidate genes or other biomarkers chosen a priori             | <ul style="list-style-type: none"> <li>- Uses existing knowledge about the disease biology to choose genes and gene polymorphisms or other kinds of biomarkers for study</li> <li>- Hypothesis-driven</li> <li>- Fastest approach</li> <li>- Least expensive</li> </ul>   | Only helpful if: <ul style="list-style-type: none"> <li>- Biomarker-outcome association is very strong</li> <li>- A priori choices and hypotheses are correct</li> </ul>  |
| Genomic or proteomics pattern analysis, e.g., from microarrays* | <ul style="list-style-type: none"> <li>- No a priori hypothesis or choice of markers</li> <li>- Broad scope, e.g., genome-wide array or complete gene-expression profile of sample of interest</li> <li>- May provide information on previously unsuspected associations</li> <li>- Provides a large amount of data</li> <li>- Likely to explain interindividual variation</li> </ul> | <ul style="list-style-type: none"> <li>- Does not use information on known biological associations</li> <li>- Optimal data management and data analysis methods are complex and evolving</li> <li>- False-positive associations more likely</li> <li>- Large sample sizes and replication in independent populations required</li> <li>- Expensive</li> <li>- Assay format less practical in a clinical setting; results of initial scan may need to be developed into a simpler test format</li> </ul> |

\*Microarrays can be based on SNPs, DNA oligonucleotides, cDNA from a library, cDNA from expressed mRNA, or comparative genomic hybridization.

of new genetic tests. Since then, these criteria have been more fully described (Burke et al. 2002) and adopted by several groups, including the Health and Human Services Secretary's Advisory Committee on Genomics, Health and Society, and the Centers for Disease Control's Evaluation of Genomic Applications in Practice and Prevention project. The evidence required for each of the criteria applies regardless of clinical application; in fact, these criteria are appropriate for all types of laboratory tests. They are:

- **Clinical validity:** clinical sensitivity, specificity, and predictive value, derived from patients representative of the clinical population for whom the test is intended. Clinical validity describes the strength of association between the test result and the clinical outcome of interest.
- **Analytic validity:** analytic sensitivity and specificity; expanded by others to include several elements contributing to accurate technical performance. Analytic validity is given particular attention in genetic testing since the majority of tests to date have been offered as laboratory-developed services, and thus do not undergo the extensive technical evaluation given manufactured test kits submitted to the FDA.
- **Clinical utility:** the clinical benefits and risks that accrue from both positive and negative results, i.e., net impact on patient outcomes when the test results are used to influence management decisions.

These two conceptual evidence frameworks are not mutually exclusive; rather, they can be merged and applied to all test applications. Table 3 shows the EDRN phases of development applied to the various types of genetic biomarkers for cancer, correspondence to the NIH-DOE Task Force criteria, and the types of comparisons to be made in each phase of development.

## Methods

### Search Methods

Genetic tests developed and offered in academic medical center clinical laboratories often have supportive peer-reviewed journal

publications. However, many new genetic tests are developed and offered as a commercial service by small, biomedical industry laboratories, or are developed by industry and licensed to established clinical laboratories; supportive publications may be less common in these circumstances. Moreover, there is no comprehensive database that lists all commercially available genetic tests and the laboratories that offer them. Although the GeneTests website (<http://www.geneclinics.org/servlet/access?id=8888892&key=i5omQrwtYYUCA&fcn=y&fw=YtGw&filename=/>) lists clinical laboratories offering genetic tests for inherited genetic diseases, the list is not comprehensive for all types of genetic tests nor does it reflect recently introduced tests. Review articles may also be out of date by the time they are published. Therefore, to obtain information on genetic tests currently available or in later stages of development for a particular disease or application, it is necessary to search both published and gray literature.

Gray literature is information that is not available through the usual bibliographic sources such as MEDLINE®. Broadly, for purposes of this report, gray literature may include information regarding tests newly or soon to be introduced, or announcements of clinical trial results (e.g., medical news articles, press releases, etc.). Online medical news services and general internet searches (e.g., for “prostate cancer” AND genetic) will return several leads for investigation. Gray literature sources of data include meeting abstracts, which are incomplete as evidence, but indicate possible publications in the near future. Tests kits that are submitted to the FDA include kit inserts that contain much FDA-reviewed data on analytic validity and possibly some on clinical validity. However, few new genetic tests are submitted to the FDA for clearance; rather, most are offered as laboratory-developed tests and validation data is often not publicly available. Finally, FDA Advisory Committee meeting materials may be a source of data if, rarely, a genetic test happens to be addressed.

For this report, gray literature (Table 4) during 2007 through May 2008 was routinely scanned for articles related to genetic testing and prostate cancer.

Also, MEDLINE® (via PubMed) was searched using the following strategies:

**Table 3.** Development Process for Different Types of Cancer-related Genetic Tests (Adapted from Pepe et al. 2001, who addressed only diagnostic screening)

|   | Test Type   |  |  |   |  |
|---|---|--|--|---|--|
|   | Risk Assessment   | Diagnostic Screening   | Differential Diagnosis   | Prognosis   | Prediction<br>(Pharmacogenomic)  |
| Test application  | Determine probability of future disease by evaluating heritable, germline DNA variants associated with increased risk | Population or subgroup screening for existing disease by evaluating acquired, somatic tumor DNA mutations associated with cancer | Confirm/reject possible disease diagnosis by evaluating acquired, somatic tumor DNA mutations associated with cancer | Predict course of disease by evaluating acquired, somatic tumor DNA mutations associated with aggressive vs. non-aggressive cancer                      | Predict response to therapy and/or therapy-related adverse events by evaluating acquired somatic tumor DNA mutations associated with cancer response OR by evaluating germline DNA variants associated with poor drug metabolism or with adverse events              |
| Outcome of interest   | Disease frequency and/or related morbidity/mortality  | Disease morbidity, mortality   | Disease morbidity, mortality   | Disease recurrence, metastasis, mortality   | Treatment response or adverse event rate   |
| <p><b>Phase 1:</b> Develop initial assay configuration and estimate clinical validity (strength of the association between the test result and the outcome of interest). Note, where multiple genetic markers are tested, assay includes an algorithm (e.g., multiplicative or other) for combining the individual marker results into a single, informative result or interpretation</p> <p><b>Phase 2:</b> Finalize assay configuration and establish analytic validity (technical performance characteristics of the assay); refine clinical validity estimates in study populations representing the clinical population of interest.</p> |   |  |  |   |  |
| Phase 1 & 2 study comparison  | Germline DNA of patients who did vs. who did not develop disease  | Tumor tissue vs. corresponding normal tissue, OR blood-derived or urine samples from cases vs. from non-cases                    | Tumor tissue vs. corresponding normal tissue, OR blood-derived or urine samples from cases vs. from non-cases        | Tumor tissue at diagnosis or after initial treatment from similarly managed patients who later did vs. did not develop the clinical outcome of interest | Pretreatment tumor tissue from patients treated with therapy of interest and who responded vs. did not respond; OR Germline DNA of patients who did vs. did not experience adverse events/response (where outcome may be affected by variability in drug metabolism) |

**Table 3.** Development Process for Different Types of Cancer-related Genetic Tests (Adapted from Pepe et al. 2001, who addressed only diagnostic screening) (cont'd)

|   | Test Type   |   |  |   |   |
|---|---|---|--|---|---|
|   | Risk Assessment   | Diagnostic Screening  | Differential Diagnosis   | Prognosis   | Prediction<br>(Pharmacogenomic)   |
| <b>Phase 3:</b> Add additional longitudinal detection information to clinical validity.   |   |   |  |   |   |
| Phase 3 study comparison  |   | Banked longitudinal series of clinical specimens from cancer cases prior to diagnosis, vs. specimens from initially similar patients who did not develop cancer |  | Banked longitudinal series of case clinical specimens between diagnosis/initial treatment and outcome of interest, vs. specimens from initially similar patients who did not develop the outcome                                |   |
| <b>Phase 4:</b> Determine initial aspects of clinical utility by comparing stage of cancer detected with test use vs. without. Note: comparison may use historical controls.                                |   |   |  |   |   |
| Phase 4 study comparison  | Prospectively monitored high-risk patients; in those who develop cancer, stage of cancer at diagnosis vs. general population  | Prospectively screened test-positives; stage of cancer detected at time of test vs. at time of diagnosis in a similar, unscreened population                    | Prospectively detected test-positives; stage of cancer detected at time of test vs. at time of diagnosis in a similar, untested population | Prospectively monitored test-positives; extent of recurrence, metastases at time of test vs. at time of detection in a similar population given usual monitoring  |   |
| Modeling may be used to estimate clinical utility based on accumulated clinical data and relevant historical information.   |   |   |  |   |   |
| <b>Phase 5:</b> Determine clinical utility of test (final configuration) i.e., impact on outcomes of interest of using test results to direct patient management. Note: may use indirect chain of evidence. |   |   |  |   |   |
| Phase 5 study comparison  | Compare cancer frequency and related morbidity/mortality in patients tested (high-risk patients given preventive treatment and/or monitoring, as available) vs. initially similar patients not tested | Compare cancer outcomes in test-screened population vs. similar unscreened population   | Compare management decisions and cancer outcomes in tested population vs. similar untested population                                      | Compare recurrence and/or metastasis rates and disease-related mortality in tested (test-positives receive additional therapy regimens and/or additional monitoring, as available) vs. untested, otherwise similar populations; | Compare response or adverse event rates when test results are used to select therapy, dose, etc. vs. usual methods for therapy selection and modification |

**Table 4.** Internet Newsletters Scanned for Relevant News Articles

| Newsletter                                    | Frequency | URL   |
|---|-----------|---|
| GenomeWeb Daily News                          | daily     | <a href="http://www.genomeweb.com/">http://www.genomeweb.com/</a>   |
| Genome Technology Online                      | daily     | <a href="http://www.genome-technology.com/">http://www.genome-technology.com/</a>                           |
| Medscape Daily News                           | daily     | <a href="http://www.medscape.com/home">http://www.medscape.com/home</a>                                     |
| Medscape Pathology & Lab Medicine News        | weekly    | <a href="http://www.medscape.com/pathology">http://www.medscape.com/pathology</a>                           |
| Medscape Hematology-Oncology News             | weekly    | <a href="http://www.medscape.com/hematology-oncology">http://www.medscape.com/hematology-oncology</a>       |
| Genomics & Health Weekly Update               | weekly    | <a href="http://www.cdc.gov/genomics/update/current.htm">http://www.cdc.gov/genomics/update/current.htm</a> |
| Genetic Alliance Weekly Bulletin              | weekly    | <a href="http://www.geneticalliance.org/weekly.bulletin">http://www.geneticalliance.org/weekly.bulletin</a> |
| The Genetic & Public Policy Center Newsletter | monthly   | <a href="http://www.dnapolicy.org/">http://www.dnapolicy.org/</a>   |
| PHG Foundation Genomics and Policy News       | monthly   | <a href="http://www.phgfoundation.org/news/">http://www.phgfoundation.org/news/</a>                         |

- Search 1: “Prostatic Neoplasms”[MeSH®] AND (“Diagnosis”[MeSH®] OR “Prognosis”[MeSH®] OR “Risk”[MeSH®]). Reviewed first 560 of 29,333 hits obtained on April 23, 2008.

- Search 2: “Prostatic Neoplasms”[MeSH®] AND (“Tumor Markers, Biological”[MeSH®] OR “Genomics”[MeSH®] OR “genetics”[Subheading]). Limited to English, Humans, last 3 years, clinical trial. Reviewed all 424 hits obtained on April 28, 2008.

— Search 2 did not return many relevant articles already retrieved from Search 1. Therefore, the “clinical trial” limit was removed.

- Search 3: Same as Search 2 without “clinical trial” limit. Reviewed 1,960 of 3,871 hits obtained on April 28, 2008.

### Study Selection

News articles from the gray literature were organized into specific topic groups.

Titles and abstracts of recent (2006–2008) journal publications, including review articles, were searched for studies of nucleic-acid-based biomarkers investigated in clinical populations for use in prostate cancer risk assessment, diagnosis, or prognosis. Special attention was paid to the topic groups determined by the gray literature search. Topics not already identified by the gray literature search with a concentration of publications were also determined from the formal literature searches. Information on these additional topics was sought via Internet

searches (e.g., for evidence of commercialization). Finally, full-length papers were requested for potentially relevant citations for each of the topic areas.

### Medical Advisory Panel Review

This Special Report was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on September 16, 2008. In order to maintain the timeliness of the scientific information in this Special Report, literature searches were performed subsequent to the Panel’s review (see “Search Methods”). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the tables and text where appropriate. There were no studies that would change the conclusions of this Special Report.

### Review of Evidence

#### Genetic Biomarkers of Sporadic Prostate Cancer

This review is focused on nucleic acid-based biomarkers with potential clinical applications for the risk assessment, diagnosis, or prognosis of prostate cancer. One category of nucleic-acid-based biomarkers is “genetic,” i.e., dependent on DNA sequence changes. Single-nucleotide polymorphisms (SNPs), insertions, deletions, or variations in number of repeat sequences are examples of genetic changes that might be variably associated with disease risk, diagnosis, or prognosis. In contrast, “epigenetic” biomarkers are unchanged in DNA sequence, but contain other kinds of

modifications to DNA or associated chromatin proteins that alter gene expression. This Report will describe examples of new genetic and epigenetic biomarkers and summarize the current state of evidence development. Several of these biomarker tests are already available as commercial testing services.

### Overview of New Genetic Biomarkers for Prostate Cancer

The search and study selection process described in Methods resulted in 5 major topic areas:

- Single nucleotide polymorphisms derived from genome-wide association studies
- Prostate cancer gene 3 (PCA3; formerly DD3)
- TMPRSS-ETS Fusion Genes
- Candidate gene panels
- Epigenomic biomarkers (gene methylation)

Table 5 provides a summary for each topic area, identifying the biomarker application (risk assessment, diagnosis, prognosis), key publications, study populations and a brief summary of results. Commercial services currently available or known to be in development are described in separate rows. Phases of development were estimated by comparing the descriptions from Table 3 to the existing studies (see Introduction). Detail for each topic area can be found in the Appendices cited in the Table subheadings.

The diversity of biomarkers studied and the volume of studies published within the last few years indicates a highly active field of study that is focused on identifying high-risk individuals, improving early diagnosis and targeting biopsy to those most likely to harbor disease, or detecting aggressive disease that needs additional treatment. However, in all cases, phase of development was estimated to be phase 2 at best, and in several cases only phase 1. This may be because specimen type (e.g., gene methylation) or final assay configuration has not been determined (e.g., TMPRSS-ETS fusion genes), because results are conflicting (e.g., GSTP1 methylation measured in tissue for disease prognosis) or because clinical validity was determined for highly selected cases and controls, rather than for an unselected population representing the clinical population of interest (several examples of candidate gene panels). Most importantly, for none of the biomarkers has evidence been gathered to directly or indirectly support use in conjunction

with currently applied clinical parameters to improve health outcomes (phases 4-5).

## Discussion

Prostate cancer is a complex, heterogeneous disease. At the extremes of the spectrum, if left untreated some prostate cancers behave aggressively, metastasize quickly and cause mortality while others are indolent and never progress to cause harm. While it would be desirable to screen for aggressive prostate cancer that is likely to cause morbidity and mortality, currently available biomarkers are incompletely sensitive, have poor specificity, and do not distinguish aggressive disease from indolent disease that need only be monitored. Nor is it currently possible to predict in advance who is likely to develop aggressive disease. For those given curative treatment (e.g., prostatectomy for clinically localized disease), no biomarkers are available that accurately predict risk of recurrence.

In response to the need for better biomarkers for risk assessment, diagnosis, and prognosis, a variety of exploratory research is ongoing. Some products of this work have already or are in the process of being translated into commercially available tests. This Report examines the state of the evidence for some of these tests. In general, the evidence addresses clinical validity i.e., the association of the test result with outcomes of interest, expressed in terms of clinical performance characteristics such as sensitivity, specificity, predictive value, and comparisons to current standards using receiver operating curve (ROC) analysis and/or logistic regression. There is no evidence of clinical utility, i.e., that using a test will improve outcomes. Evidence was reviewed for the following tests:

- **Single nucleotide polymorphisms (SNPs) for risk assessment (Appendix A).** Several large population studies have identified SNPs that are repeatably highly significant predictors of prostate cancer risk, although the genes and biologic mechanisms behind this association are as yet unknown. Several SNPs combined explain a significant proportion of prostate cancer, but by no means all. A few different groups are commercializing specific SNP panels, combined in one case with family history, as risk assessment tools presumably to identify those men who should start disease surveillance early and

**Table 5.** Overview of New Genetic Biomarkers for Prostate Cancer Diagnosis or Prognosis

| Type; Purpose  | Key Publication(s)                     | Available Test (Source)                 | Genetic Markers   | Developmental Phase                       | Study Population   | Target Patient Population   | Results  |
|--|--|---|---|---|--|---|--|
| <b>Single Nucleotide Polymorphisms Derived from Genome-wide Association Studies (Appendix A)</b> |  |   |   |   |  |   |  |
| Risk assessment; identify high-risk patients for follow-up and monitoring                        | Zheng et al. 2008                      | Focus 5 (ProActive Genetics, U.S.)      | 5 previously reported SNPs  | 1–2                                       | Cases from regional Swedish cancer registries; matched controls from Swedish Population Registry | General population, no indication of disease or strong family history | 5 SNPs along with family history were cumulatively associated with prostate cancer (OR for having $\geq 5$ risk markers 9.46, 95% CI, 3.62–24.72, $p=1.29 \times 10^{-8}$ ); AUC for all 6 markers 0.633; PAR for all 6 markers 46%<br><i>Baseline risk of 6% by age 70 changes to 57% for the &lt;2% of patients who had <math>\geq 5</math> risk markers</i> |
|  | Gudmundsson et al. 2008                | deCODE ProCa (deCODE Genetics, Iceland) | 2 SNPs reported in key publication and 6 previously reported SNPs | 1–2                                       | Various large population studies   | General population, no indication of disease or strong family history | Individual ORs for SNP association with cancer ranged from 0.78 to 1.79; p-values ranged from $2.7 \times 10^{-7}$ to $6.4 \times 10^{-18}$ ; test assumes individual markers are independent and risks can be multiplied<br><i>Baseline risk of 6% by age 70 changes to as low as 4.3% or to as high as 36.8%</i>   |
|  | Eeles et al. 2008                      | (U.K., in development)                  | 7 SNPs reported in key publication and 5 previously reported SNPs | 1<br>large UK screening trial in progress | Enriched, high-risk case series  | Men with family history of prostate cancer                            | 12 SNPs explain 15% of familial prostate cancer risk; Individual SNP p-values for association with cancer ranged from $2.7 \times 10^{-8}$ to $8.7 \times 10^{-29}$<br><i>Test in development; included SNPs not clear</i>   |
|  | Thomas et al. 2008; Yeager et al. 2007 | Not available (U.S.-NCI)                | 4 SNPs reported in key publication and 3 previously reported SNPs | 1   | Various large population studies   | General population, no indication of disease or strong family history | Individual PARs ranged from 8–20% in populations of European ancestry; OR for prostate cancer 2.70 comparing low-risk (10th percentile) to high-risk (90th percentile)<br><i>Test in development; included SNPs not clear</i>  |

**Table 5.** Overview of New Genetic Biomarkers for Prostate Cancer Diagnosis or Prognosis (cont'd)

| Type; Purpose  | Key Publication(s)   | Available Test (Source)   | Genetic Markers   | Developmental Phase  | Study Population  | Target Patient Population   | Results  |
|--|--|---|---|--|---|---|--|
| <b>Prostate Cancer Gene 3 (PCA3; formerly DD3) (Appendix B)</b>                                |  |   |   |  |   |   |  |
| Diagnostic; avoid unnecessary biopsies in patients with clinical or PSA indications for biopsy | Marks et al. 2007; Groskopf et al. 2006; van Gils et al. 2007a, 2007b; Deras et al. 2008; Groskopf et al. 2007 | PROGENSA™ PCA3 (Europe) or manufactured reagents (U.S.) (Gen-Probe, U.S.) | PCA3 mRNA overexpression, measured in first catch urine after an “attentive” digital rectal exam  | 1–2<br>Being measured in Glaxo-SmithKline REDUCE trial of preventive therapy | Patients scheduled for first or second biopsy based on clinical evaluation and/or PSA results | Patients with clinical or PSA results suggestive of prostate cancer | Average sensitivity for PCA3 Score and positive biopsy result 61%; average specificity 74%; comparable values for PSA 81% and 28%, respectively; PCA3 AUC 0.7; PSA AUC 0.55; PCA3 remained significant in multivariable models   |
| Prognostic; discriminate low-volume/low-grade cancer from significant cancer                   | Nakanishi et al. 2008  | PROGENSA™ PCA3 (Europe) or manufactured reagents (U.S.) (Gen-Probe, U.S.) | PCA3 mRNA overexpression, measured in first catch urine after an “attentive” digital rectal exam  | 1  | Patients scheduled for prostatectomy; median serum PSA >2.5 ng/mL                             | Patients with biopsy-positive prostate cancer                       | For predicting low volume/grade cancer, PCA3 alone vs. with Gleason score (respectively):<br>Sensitivity = 63%; 70%<br>Specificity = 81%; 73%<br>PPV = 57%; 23%<br>NPV = 84%; 96%  |
| <b>TMPRSS-ETS Fusion Genes (Appendix C)</b>  |  |   |   |  |   |   |  |
| Prognostic; identify aggressive disease, estimate likelihood of recurrence                     | Various, see Appendix C  | (in development; exclusively licensed to Gen-Probe, U.S.)                 | ETS transcription factor gene fusion with the TMPRSS2 gene (codes for an androgen-regulated transmembrane serine protease); to date, primarily measured in excised tissue | 1<br>Being measured in Glaxo-SmithKline REDUCE trial of preventive therapy   | Patients with early, localized disease, biopsied or treated with surgery                      | Patients with early, localized disease                              | Conflicting evidence regarding association of TMPRSS2 fusion gene detection and biochemical recurrence or survival outcomes; subtypes of gene fusion may have more significant associations with biochemical recurrence; strong association with higher stage but conflicting associations with Gleason scores. Fusion gene structure is variable and complex, making it a difficult assay target. |

**Table 5.** Overview of New Genetic Biomarkers for Prostate Cancer Diagnosis or Prognosis (cont'd)

| Type; Purpose                                | Key Publication(s)  | Available Test (Source)   | Genetic Markers  | Developmental Phase                | Study Population   | Target Patient Population   | Results   |
|--|---|---|--|------------------------------------|--|---|---|
| <b>Candidate Gene Panels (Appendix D)</b>    |   |   |  |                                    |  |   |   |
| Diagnostic and/or prognostic                 | Various, see Appendix D   | None currently available; see below for test known to be in development                           | Various combinations of genes derived from previously identified candidate genes, or from gene expression analyses | 1                                  | Patients with elevated PSA or with known cancer vs. known healthy controls | Patients with clinical or PSA results consistent with prostate cancer | Better discrimination using gene combinations compared to each gene alone   |
| Diagnostic; Improve accuracy of first biopsy | None; meeting abstract and publication submissions reportedly in progress | ?-test name unknown; Q3 2008 release per HDC (Health Discovery Corp., licensed to Clariant, U.S.) | 4 genes, not identified  | 1–2, per press release description | Not described  | ? Patients with clinical or PSA results suggestive of prostate cancer | Per press release, In biopsy tissue:<br>91.3% sensitivity for Grade 3+ cancer<br>100% specificity for normal cells<br>90% specificity for BPH |

**Table 5.** Overview of New Genetic Biomarkers for Prostate Cancer Diagnosis or Prognosis (cont'd)

| Type; Purpose  | Key Publication(s)  | Available Test (Source)  | Genetic Markers  | Developmental Phase | Study Population  | Target Patient Population   | Results   |
|--|---|--|--|---------------------|---|---|---|
| <b>Epigenomic Biomarkers (gene methylation) (Appendix E)</b>   |   |  |  |                     |   |   |   |
| Diagnostic; Prognostic (predict PSA recurrence)  | Extensive literature; see Appendix E for study examples by sample type        | (see below for specific commercial tests)                            | Various methylated genes or methylated gene combinations; most often GSTP1 | 1                   | Highly variable study populations; variety of specimens evaluated                     | Unclear   | Studies largely underpowered to detect statistically significant associations; Some diagnostic markers also positive in BPH; Prognostic markers tested primarily vs. PSA recurrence rather than disease-specific survival   |
| Diagnostic; Sample is tissue; Test is adjunct to histology evaluation, especially where PSA and histology do not agree | None specifically conducted by original patent holder or subsequent licensees | GSTP1 Methylation Assay (LabCorp, U.S.; service based on sublicense) | GSTP1 methylation  | 1                   | Various small patient populations with or suspicious for prostate cancer vs. controls | Patients with clinical or PSA results suggestive of prostate cancer | Two studies of GSTP1 hypermethylation using tissue samples reported significant results for identifying cancer with a percent sensitivity of 92, a percent specificity of 85, and an AUC of about 0.9. However, two other studies did not find significant associations with disease. |

**Table 5.** Overview of New Genetic Biomarkers for Prostate Cancer Diagnosis or Prognosis (cont'd)

| Type; Purpose   | Key Publication(s)   | Available Test (Source)  | Genetic Markers             | Developmental Phase | Study Population   | Target Patient Population  | Results   |
|---|----------------------|--|-----------------------------|---------------------|--|--|---|
| <b>Epigenomic Biomarkers (gene methylation) (Appendix E) (cont'd)</b> |                      |  |                             |                     |  |  |   |
| Prognostic; sample is tissue; inform treatment decisions              | Cottrell et al. 2007 | In development, available ?late 2008 (Epigenomics, Inc., U.S.) | ABHD9, Chr3-EST methylation | 1                   | Sample tissue from archived prostatectomies; patients received no neo-adjuvant or adjuvant therapy prior to PSA recurrence | Patients initially treated for prostate cancer, monitored for recurrence | Gene methylation levels independently and significantly discriminated between recurrence and non-recurrence after at least 4 years of follow-up in univariate and multivariable models; AUC without methylation markers, 0.75; with either methylation marker, 0.79 or 0.81 |
| Diagnostic; intended sample is urine; early detection                 | None known           | In development, available ?late 2008 (Epigenomics, Inc., U.S.) | Not specified               | 1                   | Tissue samples from patients with prostate cancer, benign prostate conditions, and age-matched normal controls             | ?General population screening  | AACR 2008 poster reports novel methylation "biomarkers" that "specifically discriminate prostate cancer from benign prostate conditions such as BPH"; developmental work will focus on urine samples  |

be monitored frequently. However, these tests do not predict certainty of disease, nor do they clearly predict aggressive versus indolent disease. While the monitoring of high-risk men may improve outcomes, it is also possible that these could be offset by the harms of identifying and treating additional indolent disease. Recent evidence regarding the safety of prevention with finasteride (see Background, Guidelines for Screening and Prevention) has not yet been incorporated into guidelines for cancer prevention in high-risk or general-risk populations.

- **PCA3 for disease and diagnosis (Appendix B).** PCA3 is overexpressed in prostate cancer and PCA3 mRNA can be detected in urine samples collected after prostate massage. When normalized using PSA to account for the amount of prostate cells released into the urine (PCA3 Score) the test has significantly improved specificity compared to PSA and may better discriminate patients with eventual benign biopsies from those with malignant biopsy results.

One study suggests that PCA3 Score may also have value in identifying patients with less-aggressive cancer who may only need surveillance. In general, however, PCA3 assay results to date are preliminary; interpretation of results has not been standardized and clinical utility studies of decision-making for initial biopsy, repeat biopsy or treatment have not been reported.

- **TMPRSS fusion genes for diagnosis and prognosis (Appendix C).** TMPRSS2 is an androgen-regulated transmembrane serine protease that is preferentially expressed in normal prostate tissue. In prostate cancer, it may be fused to an ETS family transcription factor (ERG, ETV1, or ETV4), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis. The result of gene fusion with an ETS transcription gene is that the androgen-responsive promoter of TMPRSS2 positively

dysregulates expression of the ETS gene, suggesting a mechanism for neoplastic transformation. Fusion genes may be detected in tissue or urine. Evidence suggests that assays for fusion genes may offer specific disease detection, and that fusion genes are associated with a greater likelihood of biochemical recurrence. However, accurate fusion gene detection is complex, assays have not been standardized, and once they are, larger studies will be needed to determine clinical utility.

- **Candidate gene panels for prostate cancer diagnosis (Appendix D).** Because no single gene markers have been found that are both highly sensitive and highly specific for diagnosing prostate cancer, particularly in men already known to have elevated PSA levels, some investigators are combining several promising markers into a single diagnostic panel. While promising in concept, only very limited evidence is available for these applications.
- **Gene hypermethylation for diagnosis and prognosis (Appendix E).** Epigenetic changes, chromatin protein modifications that don't involve changes to the underlying DNA sequence but which can result in changes in gene expression, have been identified in specific genes in relation to prostate cancer. A review of recently published studies reveals an area of clinical research that has not yet identified the best markers for diagnosis and prognosis, nor the best way to measure them and in which sample type. Standardized assays and interpretation criteria have not yet been agreed upon to enable consistency and comparison of results across studies.

While these studies generate much useful information that may help elucidate the biological mechanisms of disease and eventually help design treatments, the reviewed assays are in a developmental phase, currently without evidence of clinical utility.

---

NOTICE OF PURPOSE: TEC Assessments are scientific opinions, provided solely for informational purposes. TEC Assessments should not be construed to suggest that the Blue Cross Blue Shield Association, Kaiser Permanente Medical Care Program or the TEC Program recommends, advocates, requires, encourages, or discourages any particular treatment, procedure, or service; any particular course of treatment, procedure, or service; or the payment or non-payment of the technology or technologies evaluated.

CONFIDENTIAL: This document contains proprietary information that is intended solely for Blue Cross and Blue Shield Plans and other subscribers to the TEC Program. The contents of this document are not to be provided in any manner to any other parties without the express written consent of the Blue Cross and Blue Shield Association.

# References

- Aitchison A, Warren A, Neal D et al. (2007). RASSF1A promoter methylation is frequently detected in both pre-malignant and non-malignant microdissected prostatic epithelial tissues. *Prostate*, 67(6):638-44.
- Alumkal JJ, Zhang Z, Humphreys EB et al. (2008). Effect of DNA Methylation on identification of aggressive prostate cancer. *Urology*; 2(6):1254-9.
- Amundadottir LT, Sulem P, Gudmundsson J et al. (2006). A common variant associated with prostate cancer in European and African populations. *Nat Genet*, 38(6):652-8.
- Anonymous. (2008). Prostate cancer screening "hope." BBC News Channel (February 10, 2008). Available online at <http://news.bbc.co.uk/1/hi/health/7234922.stm>. Last accessed May 2008.
- Arora R, Koch MO, Eble JN et al. (2004). Heterogeneity of Gleason grade in multifocal adenocarcinoma of the prostate. *Cancer*, 100(11):2562-6.
- Attard G, Clark J, Ambrosine L, et al. Transatlantic Prostate Group. (2008a). Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. *Oncogene*, 27(3):255-65.
- Attard G, Clark J, Ambrosine L, et al. Transatlantic Prostate Group. (2008b). Heterogeneity and clinical significance of ETV1 translocations in human prostate cancer. *Br J Cancer*, 99(2):514-20.
- Barry M, Perner S, Demichelis F et al. (2007). TMPRSS2-ERG fusion heterogeneity in multifocal prostate cancer: clinical and biologic implications. *Urology*; 70(4):630-5.
- Bastian PJ, Palapattu GS, Yegnasubramanian S et al. (2008). CpG island hypermethylation profile in the serum of men with clinically localized and hormone refractory metastatic prostate cancer. *J Urol*, 179(2):529-54; discussion 534-5.
- Bibikova M, Chudin E, Arsanjani A et al. (2007). Expression signatures that correlated with Gleason score and relapse in prostate cancer. *Genomics*, 89(6):666-72.
- Burke W, Atkins D, Gwinn M, et al. (2002). Genetic test evaluation: information needs of clinicians, policy makers, and the public. *Am J Epidemiol*, 156(4):511-8.
- Bussemakers MJ, van Bokhoven A, Verhaegh GW et al. (1999). DD5: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res*, 59(23):5975-9.
- Christensen GB, Camp NJ, Farnham JM et al. (2007). Genome-wide linkage analysis for aggressive prostate cancer in Utah high-risk pedigrees. *Prostate*, 67(6):605-15.
- Chuang CK, Chu DC, Tzou RD et al. (2007). Hypermethylation of the CpG islands in the promoter region flanking GSTP1 gene is a potential plasma DNA biomarker for detecting prostate carcinoma. *Cancer Detect Prev*, 51(1):59-65.
- Clark J, Merson S, Jhavar S, et al. (2007). Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. *Oncogene*, 26(18):2667-75.
- Costa VL, Henrique R, Jeronimo C. (2007). Epigenetic markers for molecular detection of prostate cancer. *Dis Markers*, 25(1-2):51-41.
- Cottrell S, Jung K, Kristiansen G et al. (2007). Discovery and validation of 5 novel DNA methylation markers of prostate cancer prognosis. *J Urol*, 177(5):1753-8.
- de Kok JB, Verhaegh GW, Roelofs RW et al. (2002). DD5(PCA5), a very sensitive and specific marker to detect prostate tumors. *Cancer Res*, 62(9):2695-8.
- de la Taille A. (2007). Progensa PCA5 test for prostate cancer detection. *Expert Rev Mol Diagn*, 7(5):491-7.
- Demichelis F, Fall K, Perner S et al. (2007). TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene*, 26(51):4596-9.
- Deras IL, Aubin SM, Blase A et al. (2008). PCA5: a molecular urine assay for predicting prostate biopsy outcome. *J Urol*, 179(4):1587-92.
- Duggan D, Zheng SL, Knowlton M et al. (2007). Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. *J Natl Cancer Inst*, 99(24):1856-44.
- Eeles RA, Kote-Jarai Z, Giles GG et al. (2008). Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet*, 40(5):516-21.
- Eilers T, Machtens S, Tezval H et al. (2007). Prospective diagnostic efficiency of biopsy washing DNA GSTP1 island hypermethylation for detection of adenocarcinoma of the prostate. *Prostate*, 67(7):757-65.
- Ellinger J, Bastian PJ, Jurgan T et al. (2008a). CpG island hypermethylation at multiple gene sites in diagnosis and prognosis of prostate cancer. *Urology*; 71(1):161-7.
- Ellinger J, Haan K, Heukamp LC et al. (2008b). CpG island hypermethylation in cell-free serum DNA identifies patients with localized prostate cancer. *Prostate*, 68(1):42-9.
- Fradet Y, Saad F, Aprikian A et al. (2004). uPM5, a new molecular urine test for the detection of prostate cancer. *Urology*; 64(2):511-5; discussion 515-6.

- Freedman ML, Haiman CA, Patterson N et al. (2006).** Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc Natl Acad Sci USA*, 103(58):14068-75.
- Fuessel S, Sickert D, Meye A et al. (2005).** Multiple tumor marker analyses (PSA, hK2, PSCA, trp-p8) in primary prostate cancers using quantitative RT-PCR. *Int J Oncol*, 25(1):221-8.
- Gelmann EP. (2008).** Complexities of prostate-cancer risk. *N Engl J Med*, 358(9):961-3.
- Groskopf J, Aubin SM, Deras IL et al. (2006).** APTIMA PCA5 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem*, 52(6):1089-95.
- Groskopf J, Deras IL, Blase A et al. (2007).** The PCA5 score is independent of prostate gland volume and can synergize with other patient information for predicting biopsy outcome. American Urological Association Annual Meeting, Anaheim, CA; Abstract No. 1709.
- Gudmundsson J, Sulem P, Manolescu A et al. (2007a).** Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet*, 39(5):651-7.
- Gudmundsson J, Sulem P, Rafnar T et al. (2008).** Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet*, 40(5):281-5.
- Gudmundsson J, Sulem P, Steinthorsdottir V et al. (2007b).** Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet*, 39(8):977-83.
- Hahn NM, Kelley MR, Klaunig JE. (2007).** Constitutional polymorphisms of prostate cancer: prognostic and diagnostic implications. *Future Oncol*, 3(6), 665-82.
- Haiman CA, Patterson N, Freedman ML et al. (2007).** Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet*, 39(5):638-44.
- Helfand BT, Loeb S, Cashy J et al. (2008).** Tumor characteristics of carriers and noncarriers of the deCODE 8q24 prostate cancer susceptibility alleles. *J Urol*, 179(6):2197-201; discussion 2202.
- Henrique R, Ribeiro FR, Fonseca D et al. (2007).** High promoter methylation levels of APC predict poor prognosis in sextant biopsies from prostate cancer patients. *Clin Cancer Res*, 13(20):6122-9.
- Hessels D, Klein Gunnewiek JM, van Oort I et al. (2005).** DD5(PCA5)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol*, 44(1):8-15; discussion 15-6.
- Hessels D, Smit FP, Verhaegh GW et al. (2007).** Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 5 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res*, 13(17):5105-8.
- Holtzman NA, Watson MS. (1999).** Promoting safe and effective genetic testing in the United States. Final report of the Task Force on Genetic Testing. *J Child Fam Nurs*, 2:588-90.
- Hopkins TG, Burns PA, Routledge MN. (2007).** DNA methylation of GSTP1 as biomarker in diagnosis of prostate cancer. *Urology*, 69(1):11-6.
- Klezovitch O, Risk M, Coleman I et al. (2008).** A causal role for ERG in neoplastic transformation of prostate epithelium. *Proc Natl Acad Sci USA*, 105(6):2105-10.
- Kolata G. (2008).** \$500 to learn risk of cancer of the prostate. *The New York Times* (January 17, 2008). Available online at <http://query.nytimes.com/gst/fullpage.html?res=9806E5DF153FF954A25752C0A96E9C8B65>. Last accessed May 2008.
- Lapointe J, Kim YH, Miller MA et al. (2007).** A variant TMPRSS2 isoform and ERG fusion product in prostate cancer with implications for molecular diagnosis. *Mod Pathol*, 20(4):467-75.
- Lapointe J, Li C, Higgins JP et al. (2004).** Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proc Natl Acad Sci USA*, 101(3):811-6.
- Laxman B, Morris DS, Yu J et al. (2008).** A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res*, 68(3):645-9.
- Laxman B, Tomlins SA, Mehra R et al. (2006).** Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. *Neoplasia*, 8(10):885-8.
- Liu W, Chang BL, Cramer S et al. (2007).** Deletion of a small consensus region at 6q15, including the MAP3K7 gene, is significantly associated with high-grade prostate cancers. *Clin Cancer Res*, 13(17):5028-35.
- Lucia MS, Darke AK, Goodman PJ, et al. (2008).** Pathologic characteristics of cancers detected in the prostate cancer prevention trial: implications for prostate cancer detection and chemoprevention. *Cancer Prev Res*, 1:167-175.
- Marks LS, Fradet Y, Deras IL et al. (2007).** PCA5 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology*, 69(5):532-5.
- Mehra R, Tomlins SA, Shen R et al. (2007).** Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer. *Mod Pathol*, 20(5):538-44.
- Mendiratta P, Febbo PG. (2007).** Genomic signatures associated with the development, progression, and outcome of prostate cancer. *Mol Diagn Ther*, 11(6):345-54.
- Mitelman F. (2000).** Recurrent chromosome aberrations in cancer. *Mutat Res*, 462(2-3):247-55.
- Nakagawa T, Kollmeyer TM, Morlan BW, et al. (2008).** A tissue biomarker panel predicting systemic progression after PSA recurrence post-definitive prostate cancer therapy. *PLoS One*, 3(5):e2518.

- Nakanishi H, Groskopf J, Fritsche HA et al. (2008).** PCA5 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol*, 179(5):1804-9; discussion 1809-10.
- Nam RK, Sugar L, Wang Z et al. (2007a).** Expression of TMPRSS2:ERG gene fusion in prostate cancer cells is an important prognostic factor for cancer progression. *Cancer Biol Ther*, 6(1):40-5.
- Nam RK, Sugar L, Yang W, et al. (2007b).** Expression of the TMPRSS2:ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. *Br J Cancer*, 97(12):1690-5.
- National Comprehensive Cancer Network. (2007).** Clinical Practice Guidelines in Oncology. V.2.2007; Prostate cancer early detection. Available online at [http://www.nccn.org/professionals/physician\\_gls/PDF/prostate\\_detection.pdf](http://www.nccn.org/professionals/physician_gls/PDF/prostate_detection.pdf). Last accessed May 2008.
- Parekh DJ, Ankerst DP, Troyer D et al. (2007).** Biomarkers for prostate cancer detection. *J Urol*, 178(6):2252-9.
- Partin AW, Brawer MK, Subong EN et al. (1998).** Prospective evaluation of percent-free PSA and complexed-PSA for early detection of prostate cancer. *Prostate Cancer Prostatic Dis*, 1:197-203.
- Pepe MS, Etzioni R, Feng Z, et al. (2001).** Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst*, 93(14):1054-61.
- Perner S, Demichelis F, Beroukhim R et al. (2006).** TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res*, 66(17):8337-41.
- Perner S, Mosquera JM, Demichelis F, et al. (2007).** TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. *Am J Surg Pathol*, 31(6):882-8.
- Petrovics G, Liu A, Shaheduzzaman S et al. (2005).** Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. *Oncogene*, 24(23):5847-52.
- Physician Data Query. (2008).** Genetics of prostate cancer. Available at <http://www.cancer.gov/cancertopics/pdq/genetics/prostate/healthprofessional>.
- Pinsky P, Parnes H, Ford L. (2008).** Estimating rates of true high-grade disease in the prostate cancer prevention trial. *Cancer Prev Res*, 1:182-186.
- Rajput AB, Miller MA, De Luca A et al. (2007).** Frequency of the TMPRSS2:ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. *J Clin Pathol*, 60(11):1238-43.
- Redman MW, Tangen CM, Goodman PJ, et al. (2008).** Finasteride does not increase the risk of high-grade prostate cancer: A bias-adjusted modeling approach. *Cancer Prev Res*, 1: 174-181.
- Reibenwein J, Pils D, Horak P et al. (2007).** Promoter hypermethylation of GSTP1, AR, and 14-5-3sigma in serum of prostate cancer patients and its clinical relevance. *Prostate*, 67( 4):427-32.
- Reynolds MA, Kastury K, Groskopf J et al. (2007).** Molecular markers for prostate cancer. *Cancer Lett*, 249(1):5-15.
- Rogers CG, Gonzalgo ML, Yan G et al. (2006).** High concordance of gene methylation in post-digital rectal examination and post-biopsy urine samples for prostate cancer detection. *J Urol*, 176(5):2280-4.
- Roupret M, Hupertan V, Catto JW et al. (2008).** Promoter hypermethylation in circulating blood cells identifies prostate cancer progression. *Int J Cancer*, 122(4):952-6.
- Roupret M, Hupertan V, Yates DR et al. (2007).** Molecular detection of localized prostate cancer using quantitative methylation-specific PCR on urinary cells obtained following prostate massage. *Clin Cancer Res*, 15(6):1720-5.
- Rubin MA, Zhou M, Dhanasekaran SM et al. (2002).** alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. *JAMA*, 287(15):1662-70.
- Schalken JA, Hessels D, Verhaegh G. (2005).** New targets for therapy in prostate cancer: differential display code 3 (DD3(PCA5)), a highly prostate cancer-specific gene. *Urology*, 62(5 suppl 1):54-45.
- Schiffer E. (2007).** Biomarkers of prostate cancer. *World J Urol*, 25:55762.
- Schmidt U, Fuessel S, Koch R et al. (2006).** Quantitative multi-gene expression profiling of primary prostate cancer. *Prostate*, 66(14):1521-34.
- Schulz W. (2005).** Qualified promise: DNA methylation assays for the detection and classification of human cancers. *J Biomed Biotechnol*, 2005(5):227-9.
- Schumacher FR, Feigelson HS, Cox DG et al. (2007).** A common 8q24 variant in prostate and breast cancer from a large nested case-control study. *Cancer Res*, 67(7):2951-6.
- Seth A, Watson DK. (2005).** ETS transcription factors and their emerging roles in human cancer. *Eur J Cancer*, 41(16):2462-78.
- Severi G, Hayes VM, Padilla EJ et al. (2007).** The common variant rs1447295 on chromosome 8q24 and prostate cancer risk: results from an Australian population-based case-control study. *Cancer Epidemiol Biomarkers Prev*, 16(5):610-2.
- Sokoll LJ, Ellis W, Lange P et al. (2008).** A multicenter evaluation of the PCA5 molecular urine test: pre-analytical effects, analytical performance, and diagnostic accuracy. *Clin Chim Acta*, 389(1-2):1-6.
- Steuber T, Helo P, Lilja H. (2007).** Circulating biomarkers for prostate cancer. *World J Urol*, 25:111-19.

- Suuriniemi M, Agalliu I, Schaid DJ et al. (2007).** Confirmation of a positive association between prostate cancer risk and a locus at chromosome 8q24. *Cancer Epidemiol Biomarkers Prev*, 16(4):809-14.
- Taichman RS, Loberg RD, Mehra R et al. (2007).** The evolving biology and treatment of prostate cancer. *J Clin Invest*, 117:2551-61.
- Thomas G, Jacobs KB, Yeager M et al. (2008).** Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*, 40(5):510-5.
- Thompson IM, Pauler DK, Goodman PJ, et al. (2004).** Prevalence of prostate cancer among men with a prostate-specific antigen level <4.0 ng per milliliter. *N Engl J Med*, 350:2259-46.
- Tinzl M, Marberger M, Horvath S et al. (2004).** DD3PCA5 RNA analysis in urine--a new perspective for detecting prostate cancer. *Eur Urol*, 46(2):182-6; discussion 187.
- Tomlins SA, Laxman B, Varambally S et al. (2008).** The role of SPINK1 in ETS rearrangement-negative prostate cancers. *Cancer Cell*, 13:519-28.
- Tomlins SA, Laxman B, Dhanasekaran SM et al. (2007).** Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature*, 448:595-9.
- Tomlins SA, Mehra R, Rhodes DR et al. (2006).** TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. *Cancer Res*, 66(7):5596-400.
- Tomlins SA, Rhodes DR, Perner S et al. (2005).** Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*, 310(5748):644-8.
- Troyer DA, Mubiru J, Leach RJ et al. (2004).** Promise and challenge: Markers of prostate cancer detection, diagnosis and prognosis. *Dis Markers*, 20(2):117-28.
- Tu JJ, Rohan S, Kao J, et al. (2007).** Gene fusions between TMPRSS2 and ETS family genes in prostate cancer: frequency and transcript variant analysis by RT-PCR and FISH on paraffin-embedded tissues. *Mod Pathol*, 20(9):921-8.
- van Gils MP, Cornel EB, Hessels D et al. (2007a).** Molecular PCA5 diagnostics on prostatic fluid. *Prostate*, 67(8):881-7.
- van Gils MP, Hessels D, van Hooij O et al. (2007b).** The time-resolved fluorescence-based PCA5 test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin Cancer Res*, 13(5):959-45.
- Varambally S, Dhanasekaran SM, Zhou M et al. (2002).** The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*, 419(6907):624-9.
- Wang J, Cai Y, Ren C et al. (2006).** Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. *Cancer Res*, 66(17):8547-51.
- Wang L, McDonnell SK, Slusser JP et al. (2007).** Two common chromosome 8q24 variants are associated with increased risk for prostate cancer. *Cancer Res*, 67(7):2944-50.
- Winnes M, Lissbrant E, Damber JE et al. (2007).** Molecular genetic analyses of the TMPRSS2-ERG and TMPRSS2-ETV1 gene fusions in 50 cases of prostate cancer. *Oncol Rep*, 17(5):1055-6.
- Woodson K, O'Reilly KJ, Hanson JC et al. (2008).** The usefulness of the detection of GSTP1 methylation in urine as a biomarker in the diagnosis of prostate cancer. *J Urol*, 179(2):508-11; discussion 511-2.
- Woodson K, O'Reilly KJ, Ward DE et al. (2006).** CD44 and PTGS2 methylation are independent prognostic markers for biochemical recurrence among prostate cancer patients with clinically localized disease. *Epigenetics*, 1(4):185-6.
- Xu J, Kalos M, Stolk JA et al. (2001).** Identification and characterization of protein, a novel prostate-specific protein. *Cancer Res*, 61(4):1565-8.
- Yeager M, Orr N, Hayes RB et al. (2007).** Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet*, 39(5):645-9.
- Zheng SL, Sun J, Cheng Y et al. (2007).** Association between two unlinked loci at 8q24 and prostate cancer risk among European Americans. *J Natl Cancer Inst*, 99(20):1525-33.
- Zheng SL, Sun J, Wiklund F et al. (2008).** Cumulative association of five genetic variants with prostate cancer. *N Engl J Med*, 358(9):910-9.

# Glossary of Genetic Terms

These definitions are derived primarily from the Genetics Home Reference (<http://ghr.nlm.nih.gov/glossary>), the National Library of Medicine's web site for consumer information about genetic conditions and the genes or chromosomes related to those conditions, and also from the National Cancer Institute Dictionary of Cancer Terms (<http://www.cancer.gov/dictionary/>), with minor edits or additions.

**Array:** see *DNA microarray*

**Base:** The bases are the "letters" that spell out the genetic code. In DNA, the code letters are A, T, G, and C, which stand for the chemicals adenine, thymine, guanine, and cytosine, respectively. In base pairing, adenine always pairs with thymine, and guanine always pairs with cytosine.

**Biomarker:** see *genetic marker*

**Candidate gene:** Gene selected for study based on existing knowledge about the disease biology.

**Chip:** see *DNA microarray*

**Chromatin:** The material of chromosomes. It is a complex of DNA, histones, and nonhistone proteins found within the nucleus of a cell. Chromatin occurs in two forms during the phase between mitotic divisions: 1) as heterochromatin, seen as condensed, readily stainable clumps; 2) as euchromatin, dispersed lightly staining or nonstaining material. During mitotic division the chromatin condenses into chromosomes.

**Chromosome:** Structure found in the nucleus of a cell, which contains the genes. Chromosomes come in pairs, and a normal human cell contains 46 chromosomes.

**Chromosomal translocation:** see *translocation*

**Coding region:** Sequence of DNA consisting of a series of nucleotide bases (code) giving rise to the mature messenger RNA that will be translated into the specific amino acids of the protein product.

**DNA:** Deoxyribonucleic acid. The molecules inside cells that carry genetic information and pass it from one generation to the next.

**DNA microarray:** A process that allows thousands of pieces of DNA that are fixed to a glass slide to be analyzed at one time. It is used to identify the genes (pieces of DNA) in specific cells or tissue that are actively used to make RNA, which then may be used to make proteins. It is also used to detect single nucleotide polymorphisms within a population.

**Epigenetics:** Changes in the regulation of the expression of gene activity without alteration of DNA sequence. *Methylation* is one epigenetic mechanism.

**Expression:** see *gene expression*

**Frame:** see *reading frame*

**Gene:** The functional and physical unit of heredity passed from parent to offspring. Genes are pieces of DNA, and most genes contain the information for making a specific protein.

**Genes, tumor-suppressing:** Genes that normally restrain cell growth but, when missing or inactivated by mutation, allow cells to grow uncontrolled.

**Gene expression:** The process by which a gene's coded information is translated into the structures present and operating in the cell (either proteins or RNAs).

**Gene mapping:** Any method used for determining the location of and relative distances between genes on a chromosome.

**Gene sequencing:** Process by which the nucleotide sequence is determined for a segment of DNA.

**Genetic marker:** An identifiable segment of DNA with enough variation between individuals that its inheritance and co-inheritance with alleles of a given gene can be traced; used in linkage analysis.

**Genetic profile:** Information about specific genes, including variations and gene expression, in an individual or in a certain type of tissue. A genetic profile may be used to help diagnose a disease or learn how the disease may progress or respond to treatment with drugs or radiation.

**Genetics:** The branch of science concerned with the means and consequences of transmission and generation of the components of biological inheritance.

**Genetic susceptibility:** Increased susceptibility to a particular disease due to the presence of one or more gene mutations, and/or a combination of alleles (haplotype), not necessarily abnormal, that is associated with an increased risk for the disease, and/or a family history that indicates an increased risk for the disease.

**Genetic testing:** Examining a sample of blood or other body fluid or tissue for biochemical, chromosomal, or genetic markers that indicate the presence or absence of genetic disease.

**Genome:** All the DNA contained in an organism or a cell, which includes both the chromosomes within the nucleus and the DNA in mitochondria.

**Genome-wide association study (GWAS):** A method of searching the genome for single nucleotide polymorphisms (SNPs) that occur more frequently in people with a particular disease than in people without the disease. Each study can look at hundreds or thousands of SNPs at the same time (e.g., using DNA microarray technology). Researchers use data from this type of study to pinpoint genes that may contribute to a person's risk of developing a certain disease.

**Genotype:** see *genetic profile*

**Fusion gene, protein:** A protein created after two genes are joined together (e.g., as a result of a translocation).

**Hereditary mutation:** see *mutation, hereditary*

**Histone:** A type of protein found in chromosomes. Histones bind to DNA, help give chromosomes their shape, and help control the activity of genes.

**Hypermethylation:** see *methylation*

**Inversion:** A chromosomal rearrangement in which a segment of genetic material is broken away from the chromosome, inverted from end to end, and re-inserted into the chromosome at the same breakage site.

**Karyotype:** A photographic representation of the chromosomes of a single cell, cut and arranged in pairs. After appropriate staining, each chromosome has a characteristic banding pattern that helps to identify them; both chromosomes in a pair will have the same banding pattern.

**Locus (plural, loci):** The physical site or location of a specific gene or DNA sequence on a chromosome. Locus naming conventions at the macroscopic level include the chromosome number (e.g., 10), designation of the short (p) or long (q) chromosomal arm, and the band (e.g., 11) plus sub-band if appropriate (e.g., 23), written as e.g., 10q11.23. See *karyotype*.

**Malignant transformation:** The genetic change(s) that a normal cell undergoes as it becomes malignant.

**Mapping:** see *gene mapping*

**Messenger RNA (mRNA):** RNA that serves as a template for protein synthesis.

**Methylation:** The attachment of methyl groups to DNA at cytosine bases; correlated with reduced transcription of the gene and thought to be the principal mechanism in X-chromosome inactivation and imprinting.

**Microarray:** see *DNA microarray*

**Mitochondria:** Any of various round or long cellular organelles of most eukaryotes that are found outside the nucleus, produce energy for the cell through cellular respiration, and are rich in fats, proteins, and enzymes.

**Mitochondrial DNA:** Double-stranded DNA of mitochondria. In eukaryotes, the mitochondrial genome is circular and codes for ribosomal RNAs, transfer RNAs, and about 10 proteins.

**Mutation:** Any alteration in a gene from its natural state; may be disease causing or a benign, normal variant. See also *polymorphism*.

**Mutation, hereditary:** The presence of an altered gene within the egg and/or sperm (germ cell) such that the altered gene can be passed to subsequent generations.

**Mutation, somatic:** Alterations in DNA that occur after conception. Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. These alterations can (but do not always) cause cancer or other diseases.

**Mutation, sporadic:** Denoting either a genetic disorder that occurs for the first time in a family due to a new mutation.

**Nucleic acid:** Any of various acids (as an RNA or a DNA) composed of nucleotide chains.

**Nucleotide:** One of the structural components, or building blocks, of DNA and RNA. A nucleotide consists of a base (one of four chemicals: adenine, thymine, guanine, and cytosine) plus a molecule of sugar and one of phosphoric acid.

**Oncogene:** A gene that normally directs cell growth. If altered, an oncogene can promote or allow the uncontrolled growth of cancer. Alterations can be inherited or caused by an environmental exposure to carcinogens.

**Penetrance:** The probability of a gene or genetic trait being expressed. "Complete" penetrance means the gene or genes for a trait are expressed in all the population who have the genes. "Incomplete" penetrance means the genetic trait is expressed in only part of the population. The percent penetrance also may change with the age range of the population.

**Polymerase chain reaction (PCR):** A procedure that produces multiple copies of a short segment of DNA through cycles of: 1) denaturation (heat-induced separation of double-stranded DNA into single strands); 2) annealing (binding of specific primers on either end of the target segment); and 3) elongation (extension of the primer sequences over the target segment with DNA polymerase). The amplified product, doubled each cycle for 30 or more cycles, can then be subjected to further testing. RT-PCR stands for reverse transcription PCR, in which the starting material is RNA, which is transcribed by RT into DNA and then the DNA is subjected to PCR.

**Polymorphism:** Difference in DNA sequence among individuals. See also *mutation*.

**Polymorphism, single nucleotide (SNP):** A single nucleotide variation in a genetic sequence that occurs at appreciable frequency in the population. SNPs are archived in the NIH Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation. SNPs are mapped to a specific location in the human genome DNA sequence; multiple SNPs submitted to the database that map to the same position are called a reference SNP cluster. Each reference SNP cluster is given a unique rs ID number.

**Proteomics:** Field that utilizes protein sequences, expression and structure to determine how proteins relate, interact and function in an organism; includes characterizing and cataloguing proteins and protein libraries, comparing variations in protein expression levels under different conditions, studying protein interactions and functional roles; techniques are performed in an automated, large scale manner; may also involve bioinformatic analysis and storage of data.

**Proto-oncogene:** A gene having the potential for change into an active oncogene.

**Reading frame:** A sequence of messenger RNA that is translated into an amino acid chain, three bases at a time, each triplet sequence coding for a single amino acid. **In-frame mutations** do not cause a shift in the triplet reading frame; **out-of-frame mutations** involving a number of base pairs that is not a multiple of three disrupts the triplet reading frame for mRNA transcription and protein translation.

**Rearrangement:** A structural alteration in a chromosome, usually involving breakage and reattachment of a segment of chromosome material, resulting in an abnormal configuration; examples include inversion and translocation.

**Sequencing:** see *gene sequencing*

**Single nucleotide polymorphism:** see *polymorphism, single nucleotide*

**Somatic mutation:** see *mutation, somatic*

**Sporadic mutation:** see *mutation, sporadic*

**Susceptibility:** see *genetic susceptibility*

**Transcription:** The process of copying information from DNA into new strands of messenger RNA (mRNA). The mRNA then carries this information to the cytoplasm, where it serves as the blueprint for the manufacture of a specific protein.

**Translation:** The process of turning instructions from mRNA, base by base, into chains of amino acids that then fold into proteins. This process takes place in the cytoplasm, on structures called ribosomes.

**Translocation:** A chromosome alteration in which a whole chromosome or segment of a chromosome becomes attached to or interchanged with another whole chromosome or segment, the resulting hybrid segregating together at meiosis.

**Tumor-suppressing genes:** see *genes, tumor-suppressing*

# Appendix A

## Single Nucleotide Polymorphisms Derived from Genome-wide Association Studies

### Focus 5 Assay (ProActive Genetics, U.S.)

| Type;<br>Purpose  | Key Publication(s) | Genetic Markers            | Developmental<br>Phase |
|---|--------------------|----------------------------|------------------------|
| Risk assessment;<br>Identify high-risk patients for<br>follow-up and monitoring | Zheng et al. 2008  | 5 previously reported SNPs | 1–2                    |

Zheng et al. (2008) studied the effect of family history and SNP variants from 5 chromosomal regions that had been previously associated with a risk of prostate cancer. The genes in these regions have not been identified. The study design was a population-based, case-control study, conducted in Sweden. Cases (n=3,161) were recruited from 4 of 6 regional cancer registries; controls (n=2,149) were randomly selected from the Swedish Population Registry and matched by age and geographic region.

Family history and each SNP were independent, significant predictors of prostate cancer in a multivariable regression model adjusting for age and geographic location (Table A1), and together accounted for 46% of prostate cancer cases in the population studied (population attributable risk). Family history and the 5 SNPs had a cumulative association with prostate cancer after adjustment for age and geographic region (tests for interaction among SNPs were not significant). Men who had a family history and carried all five SNP variants were nearly 10 times more likely to have prostate cancer as men with no family history and no variants (Table A2). By ROC analysis, the AUC for the model with all variables was 0.633, greater than that for a model with only age, geographic region, and family history (AUC=0.608). None of the SNPs were associated with other clinical characteristics of prostate cancer when considered individually or simultaneously.

**Table A1.** Odds Ratios and Population Attributable Risks for Five SNPs and Family History, Adjusted for Age and Geographic Region (adapted from Zheng et al. 2008)

| Variable or SNP     | SNP<br>Chromosomal<br>Location | Frequency of<br>Variant Allele in<br>Cases/Controls (%) | Odds Ratio<br>(95% CI) | p value                | PAR <sup>1</sup> (%) |
|---------------------|--------------------------------|---|------------------------|------------------------|----------------------|
| Family history (FH) |                                | 19/9  | 2.22 (1.83–2.68)       | $1.15 \times 10^{-17}$ | 9.89                 |
| rs4430796           | 17q12                          | 38/30   | 1.38 (1.21–1.57)       | $1.62 \times 10^{-6}$  | 10.23                |
| rs1859962           | 17q24.3                        | 30/25   | 1.28 (1.11–1.47)       | $5.49 \times 10^{-4}$  | 6.54                 |
| rs16901979          | 8q24 (region 1)                | 31/26   | 1.22 (1.06–1.40)       | $5.31 \times 10^{-3}$  | 3.58                 |
| rs6983267           | 8q24 (region 2)                | 10/7  | 1.53 (1.22–1.92)       | $1.83 \times 10^{-4}$  | 22.17                |
| rs1447295           | 8q24 (region 3)                | 82/77   | 1.37 (1.18–1.59)       | $3.44 \times 10^{-5}$  | 5.41                 |
| All 5 SNPs          |                                |   |                        |                        | 40.45                |
| All 5 SNPs + FH     |                                |   |                        |                        | 46.34                |

<sup>1</sup> Population attributable risk, European ancestry

**Table A2.** Cumulative Effect of Family History and Five SNPs on the Risk of Prostate Cancer (adapted from Zheng et al. 2008)

| Number of Risk Factors Detected | Cases/Controls with Number of Factors (%) | Odds Ratio (95% CI) | p value                | Risk of Cancer* |
|---------------------------------|---|---------------------|------------------------|-----------------|
| 0                               | 5.0/10                                    | 1.00                |                        | 6.0%            |
| 1                               | 27/34                                     | 1.62 (1.27–2.08)    | $1.27 \times 10^{-4}$  | 9.7%            |
| 2                               | 36/36                                     | 2.07 (1.62–2.64)    | $5.86 \times 10^{-9}$  | 12.4%           |
| 3                               | 22/17                                     | 2.71 (2.08–3.53)    | $9.54 \times 10^{-14}$ | 16.3%           |
| 4                               | 8.2/3.5                                   | 4.76 (3.31–6.84)    | $9.17 \times 10^{-19}$ | 28.6%           |
| ≥5                              | 1.4/0.3                                   | 9.46 (3.62–24.72)   | $1.29 \times 10^{-8}$  | 56.8%           |
|                                 |   |                     | for trend:             |                 |
|                                 |   |                     | $4.78 \times 10^{-28}$ |                 |

\*Relative to baseline risk by age 70=6% (approximate average age of study population); from <http://apps.nccd.cdc.gov/uscs/Table.aspx?Group=TableAll&Year=2004&Display=n>

According to an article published January 17, 2008, in the *New York Times* (Kolata 2008), the research team, which included researchers from Sweden's Karolinska Institute, Johns Hopkins University, and Wake Forest University's School of Medicine, has formed a company called ProActive Genetics. The company will further develop and commercialize the test under the name Focus 5; according to the article, the test is expected to cost "less than \$300."

Limitations to the study include a very homogeneous population, and, as the authors note, the possibility of model overfitting influencing the results. Additional study of this model in other populations, including those of different ethnic backgrounds, will be needed for validation. Using this model to develop a test for risk of prostate cancer is unlikely to be helpful at this time. As Gelmann (2008) points out in an accompanying editorial, screening recommendations are already available for men with a family history. For those without, high-risk carriers of 4 or 5 variant SNPs (odds ratio for cancer about 4.5) account for 5.4% of the cases (true positives) but also 2.2% of the controls (false positives; data not shown). True risks could be determined by analyzing banked samples from completed, prospective trials in relevant populations. Additionally, this study determined risk across the spectrum of disease; it has been more difficult to identify markers of aggressive versus indolent disease, but these are the markers that would be of greatest clinical benefit.

#### deCODE ProCa Assay (deCODE Genetics, Iceland)

| Type; Purpose   | Key Publication(s)      | Genetic Markers   | Developmental Phase |
|---|-------------------------|---|---------------------|
| Risk assessment; Identify high-risk patients for follow-up and monitoring | Gudmundsson et al. 2008 | 2 SNPs reported in key publication and 6 previously reported SNPs | 1–2                 |

deCODE Genetics has studied genetic markers of prostate cancer in the Icelandic population and in other populations mainly of European descent. Recently, Gudmundsson et al. (2008; lead author from deCODE Genetics) reported the discovery and confirmation of 2 new SNP variants significantly associated with prostate cancer in populations of European ancestry. The variant A allele of the SNP rs5945572 located on chromosome Xp11.22 and the variant A of the SNP rs721048 on chromosome 2p15 had significant odds ratios for prostate cancer of 1.23 and 1.15, respectively. Moreover, rs721048 was significantly associated with aggressive prostate cancer at an odds ratio of 1.11 ( $p=2.6 \times 10^{-3}$ ).

Shortly after publication of the Gudmundsson et al. (2008) paper, deCODE Genetics (Reykjavik, Iceland) launched deCODE ProCa, a reference laboratory test to estimate the risk of prostate cancer in white European males based on the 2 recently reported SNPs and 6 others identified by prior research (Table A3). Performed exclusively by deCode's CLIA-licensed reference lab, the test is priced at \$500. For the individual patient, the test result consists of the individual SNP genotypes, and the cumulative lifetime risk compared to the general population risk, presumably similar to the proposed Focus 5 test based on Zheng et al. (2008).

According to information on the deCODE Genetics website (<http://www.decodediagnostics.com>), interpretation of the test results are “based on the presumption that these markers are independent, and the individual risks therefore multiply.” Also according to the website information, “about 10% of the population has a genotype combination that confers an average two-fold relative risk and about 1% have relative risk above 3.” The risk of the highest-risk genotype is considerably lower than the highest-risk genotype reported by Zheng et al. (2008), which also incorporates family history. A sample report suggests that, “Patients with higher risk for prostate cancer may benefit from earlier and more frequent screening by digital rectal examination, PSA, and ultrasound. Patients who have a higher risk together with a borderline PSA test may benefit from additional evaluation or earlier follow-up PSA testing.” The limitations of this test are similar to those outlined for Zheng et al. (2008).

**Table A3.** SNPs Included in the deCODE ProCa Test for Risk of Prostate Cancer

| SNP        | SNP Chromosomal Location | Approximate Frequency of Variant Allele in Cases/Controls (%) | Unadjusted Odds Ratio (95% CI) | p value                | PAR <sup>1</sup> (%) | References               |
|------------|--------------------------|---|--------------------------------|------------------------|----------------------|--------------------------|
| rs2710646  | 2p15                     | 22/19   | 1.15 (1.10–1.21)               | $7.66 \times 10^{-9}$  | 5                    | Gudmundsson et al. 2008  |
| rs5945572  | Xp11.22                  | 40/35   | 1.23 (1.16–1.30)               | $3.95 \times 10^{-13}$ | 7                    | Gudmundsson et al. 2008  |
| rs16901979 | 8q24                     | 5.6/3.1   | 1.79 (1.53–2.11)               | $1.1 \times 10^{-12}$  | 13 (joint)           | Gudmundsson et al. 2007a |
| rs1447295  | 8q24                     | 13/9.2  | 1.60 (1.43–1.77)               | $6.4 \times 10^{-18}$  |                      |                          |
| rs6983267  | 8q24                     | 56/50   | 1.26 (1.13–4.41)               | $9.42 \times 10^{-13}$ | 21                   | Yeager et al. 2007       |
| rs10896450 | 11q13.3                  | 52 (overall)  | 0.78 (0.69–0.88)               | $1.76 \times 10^{-9}$  | 19                   | Thomas et al. 2008       |
| rs1859962  | 17q24                    | 51/46   | 1.20 (1.14–1.27)               | $2.5 \times 10^{-10}$  | 36 (joint)           | Gudmundsson et al. 2007b |
| rs4430796  | 17q12                    | 49/52   | 0.91 (0.87–0.94)               | $2.7 \times 10^{-7}$   |                      |                          |

<sup>1</sup> Population attributable risk, European ancestry

**United Kingdom studies (commercial test in development)**

| Type;<br>Purpose  | Key Publication(s) | Genetic Markers  | Developmental<br>Phase                              |
|---|--------------------|--|---|
| Risk assessment;<br>Identify high-risk patients for<br>follow-up and monitoring | Eeles et al. 2008  | 7 SNPs reported in key publication<br>and 5 previously reported SNPs | 1<br><br>large UK<br>screening trial<br>in progress |

Eeles et al. (2008) and a U.K.-based research group studied an enriched case series of white men diagnosed with prostate cancer at 60 years of age or younger or with a family history. The authors confirmed the previously reported association of 3 SNPs at chromosome 8q24 and 2 SNPs at 17q12 and 17q24 with prostate cancer. The authors also identified 7 new loci on chromosomes 3, 6, 7, 10, 11, 19 and X. These latter 7 loci together explain about 6% of familial risk; together, the 12 loci explain 15% of familial prostate cancer risk. According to a BBC news item on February 10, 2008, “a trial is starting later this year to screen for [those] genes in men with a family history of the cancer ... We’re doing the trial because we need to see who would come forward for the test, who would benefit, what kind of results do they get on their biopsies and what kind of cancer develops” (Anonymous 2008). The article also said that the researchers planned to produce a test based on the reported genetic variations, so that men with the highest level of risk can be offered regular prostate screening.

**NCI studies**

| Type;<br>Purpose  | Key Publication(s)                        | Genetic Markers  | Developmental<br>Phase |
|---|---|--|------------------------|
| Risk assessment;<br>Identify high-risk patients for<br>follow-up and monitoring | Thomas et al. 2008;<br>Yeager et al. 2007 | 4 SNPs reported in key publication<br>and 3 previously reported SNPs | 1                      |

Investigators from the National Cancer Institute (NCI) and their partners in the Cancer Genetic Markers of Susceptibility (CGEMS) initiative recently updated an earlier study (Thomas et al. 2008; Yeager et al. 2007). Together, the studies confirmed 2 independent loci at 8q24 and another at 17q12, and reported 4 new risk loci on chromosomes 7, 10, and 11. All 7 loci remained strongly associated ( $p \leq 3.24 \times 10^{-7}$ ) with risk of prostate cancer after mutual adjustment for other SNPs; individual population attributable risks ranged from 8–20% in populations of European ancestry. Using a single SNP per chromosomal region, the odds ratio for prostate cancer was 2.70 comparing low-risk (10th percentile) to high-risk (90th percentile) groups.

**Supporting Studies**

The studies described in the preceding text build on a number of studies published within the last few years that tested many large patient and control populations for thousands of SNPs using SNP microarrays that span the entire genome (i.e., SNPs located at roughly equal intervals across each chromosome). The purpose of such studies (genome-wide association studies or GWAS) is to identify common, inherited gene variations that are present in patients with prostate cancer, but not in controls. Because so many SNPs are tested at one time, there is a large potential for false-positive results. Therefore, large population studies and replication of potential risk marker associations in additional, independent populations are extremely important to establish confidence in the identified marker. In the past, risk markers for prostate cancer have been described in linkage studies of families with an extensive history of disease, but have been difficult to validate. GWAS studies, however, can be applied to the general population, or limited to patients with family histories, to patients with more aggressive disease versus less aggressive disease, to patients of a specific ethnicity, etc., to search for various types of risk markers.

In 2006, studies first described GWAS-derived and relatively common SNPs at 8q24 that were strongly associated with prostate cancer in both populations of European and of African-American descent (Amundadottir et al. 2006; Freedman et al. 2006). Following those initial reports, several GWAS confirmed and reported additional risk SNPs that delineated 3 apparently independent regions within a 600-kilobase segment of 8q24 (Severi et al. 2007; Wang et al. 2007; Schumacher et al. 2007; Suuriniemi et al. 2007; Haiman et al. 2007; Zheng et al. 2007; Helfand 2008). Of particular interest, SNPs found in region 2 were more common in African-Americans and were strongly associated with disease in that population. However, the majority of studies have been conducted in populations of European ancestry.

Additional studies, including those in Table 5 and others described in the preceding text have identified and confirmed risk marker SNPs in several other chromosomal locations. Some of these have clear or potential links to genes that may help explain the biology of the disease or direct new treatments. For example, the proto-oncogene MYC is located approximately 260 kilobases telomeric (toward the end of the chromosome arm) to region 1 of 8q24. SNP rs10993994 at 10q11.23 is near MSMB, the gene for microseminoprotein, beta- (MSP). MSP is synthesized by the epithelial cells of the prostate gland; loss of expression has been associated with recurrence after radical prostatectomy. SNP rs4430796 is located in the gene HNF1B at 17q12; HNF1B codes for transcription factor 2 (TCF2). Depending on the TCF2 isoform produced, the result may be to activate or inhibit transcription of target genes. Mutation of HNF1B that disrupts normal function has been identified as the cause of MODY5 (Maturity-Onset of Diabetes, Type 5). SNP rs2735839 is located between KLK2 (encoding kallikrein 2) and KLK3 (encoding PSA) on chromosome 19; both gene products are serine proteases. Kallikrein 2 has also been investigated as a screening and prognostic marker.

Some studies have identified potential markers specific for aggressive disease (e.g., Gudmundsson et al. 2008, as described in the preceding text; Christensen et al. 2007; Liu et al. 2007). Duggan et al. (2007) conducted an exploratory GWAS for markers of aggressive disease in two independent populations, and identified a SNP located in the DAB2IP gene. DAB2IP encodes a Ras GTPase-activating protein, which is expressed in normal prostate epithelial cells and decreased in prostate cancer cells; induced expression in prostate cancer cell lines suppresses their growth. However, additional study and validation needed to determine the clinical usefulness of SNP markers of aggressive disease.

The identification and confirmation of several SNP markers of disease risk and aggressiveness at different chromosomal locations, the independent importance of family history (Zheng et al. 2008), and the fact that not all disease is explained by the combination of these markers along with family history underscores the complexity of prostate cancer, and the difficulty of developing panels of markers that are clinically useful predictors.

# Appendix B

## PCA3: PROGENSA™ PCA3 (Europe) or Manufactured Reagents (Gen-Probe, U.S.)

| Type;<br>Purpose  | Key Publication(s)   | Genetic Markers  | Developmental<br>Phase   |
|---|--|--|--|
| Diagnostic;<br>Avoid unnecessary biopsies in patients with clinical or PSA indications for biopsy | Marks et al. 2007;<br>Groskopf et al. 2006;<br>van Gils et al. 2007a, 2007b;<br>Deras et al. 2008;<br>Groskopf et al. 2007 | PCA3 mRNA overexpression, measured in first catch urine after an “attentive” digital rectal exam | 1–2<br>Being measured in Glaxo-SmithKline REDUCE trial of preventive therapy |

Bussemakers et al. (1999) were the first to describe an mRNA that was overexpressed in prostate cancer tissue compared to normal prostate tissue. First named DD3, now formally known as PCA3 (prostate cancer gene 3), the gene maps to chromosome 9q21-22; the gene product and biological function are unknown. Early studies (de Kok et al. 2002; Schalken et al. 2003) described the clinical specificity of PCA3 mRNA, its detection in urine (particularly concentrated urine sediment), and the need to normalize the PCA3 mRNA quantitative result to background epithelial cell nuclear material (usually represented by PSA mRNA quantitation). A prototype urine assay was developed at DiagnoCure (Quebec, Canada) and called uPM3 (Fradet 2004). Additional studies were conducted with promising clinical results, but unacceptably high sample failure rates (Hessels et al. 2005; Tinzi et al. 2004).

More recently, Gen-Probe (San Diego, CA) acquired exclusive worldwide diagnostic rights to the PCA3 gene, and improved the detection limit in urine (nonsedimented) by employing mRNA target capture, transcription-mediated amplification (TMA) of target mRNA, and hybridization protection to detect amplified PCA3 (Groskopf et al. 2006). In this assay, both PCA3 and PSA mRNA are separately quantified and the ratio of PCA3 to PSA mRNA is called the PCA3 Score. Using the Gen-Probe assay format, studies determined that:

- the sample failure rate is 1.8 to 4.5% (Groskopf et al. 2006; Marks et al. 2007; Sokoll et al. 2008; Nakanishi et al. 2008);
- the PCA3 Score is poorly (Nakanishi et al. 2008), or not at all (Deras et al. 2008; Groskopf et al. 2007), correlated with prostate volume; and
- the higher the PCA3 Score, the higher the likelihood of prostate cancer (Deras et al. 2008; Marks et al. 2007).

The test requires that the urine specimen be a first catch after an “attentive” digital rectal exam, consisting of 3 strokes of each prostate lobe with firm pressure to release cells into the urine. To avoid mRNA degradation, a specimen collection kit, containing a stabilizing buffer, can be used for transport and storage at ambient or freezing temperatures; PCA3 and PSA mRNA in such processed specimens has been demonstrated to be stable for at least 5 days at 2–8°C (Groskopf et al. 2006).

The Gen-Probe assay obtained a CE-Mark in late 2006 and is available in Europe as the PROGENSA™ PCA3 Assay. The assay has not been cleared by the FDA. A recent publication (de la Taille 2007) states that “In the USA, the assay is enabled in various laboratories via purchase of analyte specific reagents [ASR] from the manufacturer.” ASRs are reagents “intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.” ASRs are regulated by FDA and are subject to general controls, including current Good Manufacturing Practices, but are exempt from premarket notification. According to a recent FDA guidance, ASRs can only be sold as individual reagents, not in combination with other ASRs, reagents, or equipment, and cannot be promoted by the manufacturer with specific analytical or clinical performance claims (<http://www.fda.gov/cdrh/oivd/guidance/1590.html>). Thus, the laboratory purchasing ASRs must establish the use of ASRs and interpretation of results of any assays that incorporate ASRs; this includes establishing cutoff values for determining normal versus abnormal results, or probabilities of risk. The Gen-Probe website re-directs U.S. inquires regarding PCA3 to <http://www.pca3.org/pro/labs> for information on laboratories offering PCA3 testing in the U.S.

Evidence regarding the assay technical performance (analytic validity), association of results with outcomes (clinical validity) and improvement of outcomes with test use (clinical utility) is summarized in Table B1. Additional information, based at least in part on some of these studies, can be found in the package insert for the European-marketed PROGENSA™ PCA3 Assay at <http://www.gen-probe.com/pdfs/pi/501377-EN-RevA.pdf>.

In general, assay results are reproducible with overall average variability of about 20%. Percent recovery of mRNA from samples with known quantities ranged from 94 to 120. Correlation of results on the same samples assayed at different sites is very high (e.g.,  $R=0.968$ , Sokoll et al. 2008).

Several studies address clinical validity in patients scheduled for an additional biopsy (Marks et al. 2007) or first biopsy (Groskopf et al. 2006; van Gils et al. 2007a, 2007b; Deras et al. 2008; Groskopf et al. 2007; Table B1). Note that two studies (van Gils et al. 2007a and 2007b) used sedimented urine; van Gils et al. (2007b) used a different assay format. Groskopf et al. (2006), van Gils et al. (2007a), van Gils et al. (2007b), and Deras et al. (2008) report significantly greater mean PCA3 Scores for patients with follow-up biopsy results indicating malignancy than for those with benign results. Within each biopsy category, however, individual PCA3 Score results vary widely and benign and malignant categories overlap considerably (van Gils et al. 2007a, 2007b; Deras et al. 2008). Average percent sensitivities for PCA3 Score and serum PSA were 61 (Table B1) and 81 (at a cutoff of 2.5 ng/mL; Groskopf et al. 2006); average percent specificities were 74 (Table B1) and 28 (at a cutoff of 2.5 ng/mL; Groskopf et al. 2006). However, studies chose different cutoff values for interpreting the PCA3 assay results, so these calculations are not truly comparable across studies.

Receiver-operating curve (ROC) analysis for discriminating benign versus malignant disease results in an area under the curve (AUC) of approximately 0.7 for PCA3 Score (Groskopf et al. 2006; Marks et al. 2007; van Gils et al. 2007a, 2007b; Groskopf et al. 2007; Deras et al. 2008) versus about 0.55 for serum PSA (Marks et al. 2007; van Gils et al. 2007; Deras et al. 2008). Multivariable models indicated significantly improved AUCs when PCA3 was added to serum PSA and other clinically significant variables (Groskopf et al. 2007; Deras et al. 2008).

Taken together, study results suggest that the PCA3 Score provides incremental improvement over serum PSA measurement in discriminating patients with eventual benign biopsies from those with malignant biopsy results, and markedly improves upon serum PSA specificity. PCA3 Score may also have value in identifying patients with less aggressive cancer who may only need surveillance. However, results to date are preliminary; interpretation of assay results has not been standardized (i.e., cutoff value) and clinical utility studies of decision-making for initial biopsy, repeat biopsy or treatment have not been reported.

PCA3 has also been incorporated into multiplex assays of genetic markers for diagnosis of prostate cancer (see following section, “Candidate Gene Panels”).

PCA3 measurement has been incorporated into at least one ongoing clinical trial being conducted by GlaxoSmithKline (GSK). PCA3 will be tested in up to 6,800 clinical samples obtained from patients enrolled in GSK's REDUCE ("Reduction by Dutasteride of Prostate Cancer Events") clinical trial, which is designed to determine the efficacy and safety of GSK's drug dutasteride (AVODART®) in reducing the risk of prostate cancer in men at increased risk of this disease.

PCA3 has also come to the attention of the National Cancer Institute's Early Detection Research Network (EDRN) Prostate and Urologic Cancer Collaboration Group. The EDRN 2008 annual report ([http://www.compass.fhcrc.org/edrnneci/files/pdf/edrn\\_4th-report\\_200801.pdf](http://www.compass.fhcrc.org/edrnneci/files/pdf/edrn_4th-report_200801.pdf)) notes that additional studies of PCA3 for prostate cancer diagnosis are in progress.

| Type;<br>Purpose   | Key Publication(s)    | Genetic Markers  | Developmental<br>Phase |
|--|-----------------------|--|------------------------|
| Prognostic;<br>Discriminate low volume/ low<br>grade cancer from significant<br>cancer | Nakanishi et al. 2008 | PCA3 mRNA overexpression,<br>measured in first catch urine after<br>an "attentive" digital rectal exam | 1                      |

Nakanishi et al. (2008; Table B1) applied the PCA3 Score to the discrimination of low volume/ low grade prostate cancer (defined as organ-confined cancer with a dominant tumor volume less than 0.5 cc and the absence of any Gleason grade 4 or 5 disease) from other, "significant" prostate cancer. Average PCA3 scores differed significantly between these groups (p-value not reported) and PCA3 was a significant predictor of low volume/grade cancer in a multivariable model with serum PSA and other clinical predictors. PCA3 Score AUC was 0.757 compared to serum PSA AUC of 0.632. PCA3 Score sensitivity was 65%, specificity was 81%, and negative predictive value (NPV) was 84%. Adding Gleason score to PCA3 improved sensitivity to 70%, reduced specificity to 73%, and improved NPV to 96%. Additional study of this application will be necessary to show that patients with low PCA3 levels can be safely observed without treatment.

**Table B1.** Analytic Validity and Clinical Validity Studies of Testing for PCA3 mRNA in Urine Samples

| Study                             | Sample             | PCA3 mRNA method              | Patients   | No. Pts.                      | Results  |
|-----------------------------------|--------------------|-------------------------------|--|-------------------------------|--|
| <b>Analytic Validity Studies</b>  |                    |                               |  |                               |  |
| Groskopf et al. 2006 <sup>1</sup> | Urine <sup>2</sup> | TMA <sup>3</sup><br>cutoff=50 | Biopsy patients, mean serum PSA 7.7 ng/mL;<br>Men 45 yrs old with no risk factors; | 70                            | Precision determined in 18 assay runs performed by 3 different operators using 3 different reagent lots and instrument systems:<br>Inter-run PCA3 Score CV, 15–24%<br>Stability: mRNA copy number constant ≤5 days at 4°C; PCA3 Score within 20% of initial value up to 2 weeks at 4°C |
|                                   |                    |                               | Men after radical prostatectomy<br>(U.S., 1 site)                                  | 52                            |  |
|                                   |                    |                               |  | 21                            |  |
| Sokoll et al. 2008 <sup>1</sup>   | Urine <sup>2</sup> | TMA <sup>3</sup><br>cutoff=35 | Controls of known PCA3 and PSA mRNA copy numbers;                                  | 3 each<br>(low, medium, high) | PCA <sup>3</sup> Intra-assay %CV: 4–14<br>Inter-assay %CV: 4–10<br>Inter-site %CV: 0–3   |
|                                   |                    |                               | Men with known biopsy outcomes, median serum PSA 7.0 ng/mL                         | 72<br>(24% Pr Ca)             | PSA <sup>4</sup> Intra-assay %CV: 6–9<br>Inter-assay %CV: 6–8<br>Inter-site %CV: 2–8   |
|                                   |                    |                               | (Canada, 2 sites)  |                               | Both sites correctly classified the same number of blinded specimens as negative (49/72, 68%)<br><br>Individual specimens correlated between sites, R=0.968<br><br>AUCs by site, 0.703 and 0.706   |

**Table B1.** Analytic Validity and Clinical Validity Studies of Testing for PCA3 mRNA in Urine Samples (cont'd)

| Study                             | Sample                                  | PCA3 mRNA method              | Patients  | No. Pts.           | Results   |
|-----------------------------------|---|-------------------------------|---|--------------------|---|
| <b>Clinical Validity Studies</b>  |   |                               |   |                    |   |
| Groskopf et al. 2006 <sup>1</sup> | Urine <sup>2</sup>                      | TMA <sup>3</sup><br>cutoff=50 | Biopsy patients, mean serum PSA 7.7 ng/mL;  | 70                 | Mean PCA3 Scores:<br>Normal: 4.5<br>Biopsy-negative: 27<br>Biopsy-positive: 82, p<0.01<br>After prostatectomy: 20 of 21 had background values;<br>1 sample PCA3 Score=55, follow-up resulted in positive biopsy<br>ROC analysis:<br>Pre-biopsy: AUC=0.746 (95% CI: 0.574–0.918)<br>PCA3 Score sensitivity=69%, specificity=79%<br>Serum PSA sensitivity=81%, specificity=28% (cutoff 2.5 ng/mL) |
|                                   |   |                               | Men 45 years old with no risk factors;  | 52                 |   |
|                                   |   |                               | Men after radical prostatectomy   | 21                 |   |
| Marks et al. 2007 <sup>1</sup>    | Urine <sup>2</sup>                      | TMA <sup>3</sup><br>cutoff=35 | Consecutive biopsy patients with history of ≥1 negative biopsy; serum PSA >2.5 ng/mL (U.S. and Canada, 3 sites) | 233<br>(26% Pr Ca) | ROC analysis:<br>PCA3 Score, AUC=0.678 (95% CI: 0.597–0.759)<br>Serum PSA, AUC=0.524 (95% CI: 0.438–0.610); p=0.008<br>PCA3 Score, sensitivity=58%, specificity=72%, OR=3.6   |
| van Gils et al. 2007a             | Urine <sup>2</sup> ,<br>sedi-<br>mented | TMA <sup>3</sup><br>cutoff=43 | Men scheduled for biopsy; mean serum PSA 8.7 ng/mL (Netherlands, 1 site)  | 67<br>(34% Pr Ca)  | Median PCA3 Score for:<br>Patients with negative biopsy, 19<br>Patients with positive biopsy, 48, p<0.006<br>ROC analysis:<br>PCA3 Score, AUC=0.70 (95% CI: 0.58–0.83)<br>Serum PSA, AUC=0.66 (95% CI: 0.53–0.79)<br>PCA3 Score, sensitivity=61%, specificity=80%   |

**Table B1.** Analytic Validity and Clinical Validity Studies of Testing for PCA3 mRNA in Urine Samples (cont'd)

| Study   | Sample                                  | PCA3 mRNA method              | Patients  | No. Pts.                                 | Results   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
|---|---|-------------------------------|---|--|---|-----------|-------------|-----------|---------------|----|----|--------------|----|----|------|----|----|-----|----|----|-------|----|----|
| <b>Clinical Validity Studies (cont'd)</b>       |   |                               |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| van Gils et al. 2007b                           | Urine <sup>2</sup> ,<br>sedi-<br>mented | RT-PCR<br>cutoff=58           | Biopsy patients; serum PSA<br>3–15 (mean 7.5) ng/mL<br><br>(Netherlands, 5 sites)     | 583<br>(33% Pr Ca)                       | Average PCA3 Score for:<br>Patients with negative biopsy, 24 (range: 0–1,862)<br>Patients with positive biopsy, 90 (range:, 0–4,088); $p=1 \times 10^{-9}$<br>ROC analysis:<br>PCA3 AUC=0.66 (95% CI: 0.61–0.71)<br>Serum PSA AUC=0.57 (95% CI: 0.52–0.63)<br>PCA3 Score sensitivity=65%, specificity=66%, NPV=80%  |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| Groskopf et al. 2007 <sup>1</sup><br>[Abstract] | Urine <sup>2</sup>                      | TMA <sup>3</sup>              | Men scheduled for prostate biopsy   | 244 training<br>243 test<br>(randomized) | Risk of Pr Ca=69% when PCA3 Score >100<br>Multivariable analysis with PCA3, age, tumor volume, serum PSA:<br>AUC=0.774<br>PCA3 AUC=0.734<br>At sensitivity of 60%, PCA3 Score specificity=75%<br>At sensitivity of 60%, multivariable specificity=82–85%  |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| Deras et al. 2008 <sup>1</sup>                  | Urine <sup>2</sup>                      | TMA <sup>3</sup><br>cutoff=35 | Consecutive biopsy patients; median serum<br>PSA 5.6 ng/mL (U.S. and Canada, 4 sites) | 570<br>(36% Pr Ca)                       | <table border="1"> <thead> <tr> <th>Condition</th> <th>PCA3 median</th> <th>PCA3 mean</th> </tr> </thead> <tbody> <tr> <td>Normal or BPH</td> <td>15</td> <td>30</td> </tr> <tr> <td>Inflammation</td> <td>13</td> <td>24</td> </tr> <tr> <td>ASAP</td> <td>27</td> <td>33</td> </tr> <tr> <td>PIN</td> <td>24</td> <td>35</td> </tr> <tr> <td>Pr Ca</td> <td>38</td> <td>63</td> </tr> </tbody> </table> <p>PCA3 Score for Pr Ca vs. all others, <math>p &lt; 0.0001</math></p> <p>ROC analysis:<br/>Multivariable (PCA3, prostate volume, DRE result, serum PSA)<br/>AUC=0.752<br/>PCA3 AUC=0.686<br/>PCA3 AUC=0.703 in men undergoing first biopsy<br/>PCA3 AUC=0.684 in men with a previous negative biopsy<br/>Serum PSA AUC=0.547<br/>PCA3 Score sensitivity=54%, specificity=74%</p> | Condition | PCA3 median | PCA3 mean | Normal or BPH | 15 | 30 | Inflammation | 13 | 24 | ASAP | 27 | 33 | PIN | 24 | 35 | Pr Ca | 38 | 63 |
| Condition                                       | PCA3 median                             | PCA3 mean                     |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| Normal or BPH                                   | 15                                      | 30                            |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| Inflammation                                    | 13                                      | 24                            |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| ASAP  | 27                                      | 33                            |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| PIN   | 24                                      | 35                            |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |
| Pr Ca   | 38                                      | 63                            |   |  |   |           |             |           |               |    |    |              |    |    |      |    |    |     |    |    |       |    |    |

**Table B1.** Analytic Validity and Clinical Validity Studies of Testing for PCA3 mRNA in Urine Samples (cont'd)

| Study                                     | Sample             | PCA3 mRNA method              | Patients  | No. Pts. | Results  |
|---|--------------------|-------------------------------|---|----------|--|
| <b>Clinical Validity Studies (cont'd)</b> |                    |                               |   |          |  |
| Nakanishi et al. 2008 <sup>1</sup>        | Urine <sup>2</sup> | TMA <sup>3</sup><br>cutoff=25 | Consecutive men scheduled for biopsy, serum PSA $\geq 2.5$ ng/mL;                 | 59       | PCA3 Score not correlated with serum PSA ( $p=0.797$ )<br>Average PCA3 Score for:<br>low volume/low grade cancer, 24.5<br>significant cancer, 56.5   |
|   |                    |                               | Men scheduled for prostatectomy, median serum PSA 4.8 ng/mL<br><br>(U.S., 1 site) | 83       | Multivariable analysis (also including prostate volume, Gleason score, % positive biopsy cores, log serum PSA) to predict low volume cancer:<br>PCA3 OR=0.022, $p=0.001$ Serum PSA OR=0.004, $p=0.004$<br>ROC analysis for predicting low volume/grade cancer:<br>PCA3 Score AUC=0.757      Serum PSA AUC=0.632<br>For predicting low volume/grade cancer, PCA3 alone vs. with Gleason score (respectively):<br>Sensitivity=63%; 70%<br>Specificity=81%; 73%<br>PPV=57%; 23%<br>NPV=84%; 96% |

**Abbreviations**

ASAP: Atypical small acinar proliferation; AUC: area under the curve; BPH: benign prostatic hyperplasia; CV: coefficient of variation; DRE: digital rectal exam; NPV: negative predictive value; PIN: prostate intraepithelial neoplasia; PPV: positive predictive value; Pr Ca: prostate cancer; PSA: prostate-specific antigen; ROC: receiver operating curve; RT-PCR: reverse transcription polymerase chain reaction

<sup>1</sup> Includes author(s) affiliated with Gen-Probe

<sup>2</sup> Collected following DRE, 3 strokes per lobe

<sup>3</sup> Transcription-Mediated Amplification, normalized to PSA mRNA for PCA3 Score

<sup>4</sup> PSA mRNA in urine, also measured by TMA

# Appendix C

## TMPRSS-ETS Fusion Genes

| Type;<br>Purpose   | Key Publication(s) | Genetic Markers  | Developmental<br>Phase  |
|--|--------------------|--|---|
| Prognostic;<br>Identify aggressive disease,<br>estimate likelihood of recurrence | Various, see text  | ETS transcription factor gene<br>fusion with the TMPRSS2 gene<br>(codes for an androgen-regulated<br>transmembrane serine protease);<br>to date, primarily measured in<br>excised tissue | 1<br><br>Being<br>measured<br>in Glaxo-<br>SmithKline<br>REDUCE trial<br>of preventive<br>therapy |

**Biochemistry.** The members of the ETS family of transcription factors share a conserved DNA binding domain, the erythroblast transformation specific (ETS) domain, so called because the founding member of the ETS family was discovered by virtue of overexpression in erythroleukemia cells. ETS transcription factors modulate (positively or negatively) transcription of target genes involved in cell proliferation, differentiation, development, transformation, and apoptosis (Petrovics et al. 2005). ETS genes are frequently amplified, overexpressed, or rearranged as in the fusion products characteristic of Ewing's sarcoma and certain leukemias (Seth et al. 2005).

Epithelial tumors (carcinomas) display many nonspecific but few recurrent chromosomal rearrangements (Mitelman 2000). Prostate cancer is a recently discovered exception. The ETS-related gene (ERG), a member of the ETS family of transcription factors, is overexpressed in a subset of prostate cancers; overexpression is correlated with cancer recurrence (Petrovics 2005). Overexpression of ERG is most commonly seen in conjunction with TMPRSS2 translocations creating TMPRSS2-ERG fusion genes (Tomlins et al. 2005). Less commonly, other ETS family transcription factors have been discovered in TMPRSS2 fusion genes in prostate cancer tissue (TMPRSS2-ETV1 and TMPRSS2-ETV4); these 3 fusion genes appear to be mutually exclusive (Tomlins et al. 2005, 2006).

TMPRSS2 is an androgen-regulated transmembrane serine protease that is preferentially expressed in normal prostate tissue. The result of gene fusion with ETS transcription genes is that the androgen-responsive promoter of TMPRSS2 positively dysregulates expression of ERG or ETV, suggesting a mechanism for neoplastic transformation. In vivo studies in transgenic mice have shown that targeted expression of the ERG transcript in prostate epithelial cells results in the development of focal precancerous prostatic intraepithelial neoplasia (Klezovitch et al. 2008).

**Clinical Studies.** TMPRSS2-ERG or -ETV fusion genes have been detected in 40% or more of prostate cancer tissue samples in several studies (e.g., Lapointe et al. 2007; Mehra et al. 2007; Nam et al. 2007a; Perner et al. 2006; Rajput et al. 2007a; Tu et al. 2007). For TMPRSS2 and ERG genes, which are both located closely together on chromosome 21, a fusion gene can be formed either by translocation and insertion of genetic sequences, or by deletion of intervening sequences (Perner 2006; Barry et al. 2007). Various methods of detection have been used in clinical research studies, including, most commonly, indirect detection of fusion using fluorescence in situ hybridization (FISH) and direct detection using reverse transcription polymerase chain reaction (RT-PCR). Tu et al. (2007) report good correlation between these two methods.

A number of studies have reported on the association of TMPRSS2 fusion genes with prostate cancer characteristics or outcomes, summarized in Table C1. Multivariable analysis of biochemical failure outcomes by fusion status were statistically significant in two separate studies by Nam et al. (2007a and b). Demichelis et al. (2007), however, reported no significant association of fusion status with metastases or disease-specific death in a multivariable analysis. In univariate analyses Winnes et al. (2007), Wang et al. (2006), and Mehra et al. (2006) also reported no significant association between fusion genes and progression-free survival or biochemical recurrence.

Fusion gene subtype may be of importance in assessing prognosis. Attard et al. (2008a) evaluated the subset of fusion isoforms containing only a portion of the ERG and found significant associations with cause-specific and overall survival after adjustment for other clinical-pathologic factors. Similarly, in univariate analysis Wang et al. (2006) found a significant association between fusion genes containing the native TMPRSS2 translation initiation codon in frame with the ERG gene and biochemical recurrence and seminal vesicle invasion. Perner et al. (2006) reported that the subtype of fusion genes created by deletion was more common in cases with biochemical recurrence (statistical significance not reported).

Perner et al. (2007) hypothesize that TMPRSS2-ETS gene fusion is an early event, in that it was detected most often in clinically localized cancers, less often in hormone-naïve or -refractory metastases, and least in high grade prostatic intraepithelial neoplasia. Yet it also is reported to be significantly associated with higher stage disease (Mehra et al. 2007; Nam et al. 2007b; Perner 2006). Data regarding association with Gleason score are conflicting, with some relatively large studies reporting significant associations (Rajput et al. 2007b, Demichelis et al. 2007; Nam et al. 2007b) but others reporting no significant association (Perner et al. 2006; Perner et al. 2007; Mehra 2006; Winnes et al. 2007)

**Genetic Complexity.** Fusion genes appear to be heterogeneous in composition and joining sequences. For example, in the most common configuration of TMPRSS2-ERG, the first exon of TMPRSS2 is fused to the fourth exon of ERG in the expressed mRNA. However, Lapointe et al. (2007) report a novel mRNA transcript in which an alternative TMPRSS2 exon 1 of different size is found. Wang et al. (2006) reported 8 different sizes of fusion gene mRNA, and found multiple fusion mRNAs in one sample. They also categorized 6 different types of TMPRSS2-ERG variants; in 3, the native TMPRSS2 translation initiation codon is in frame with the ERG gene but is out of frame in the other 3 variants. Clark et al. (2007) characterized 14 distinct combinations of sequences from the TMPRSS2 and ERG genes and also reported finding distinct fusions in different regions of a single prostate. Any assays designed for clinical use must take known variants into account to ensure accuracy of detection.

The majority of cases of prostate cancer are multifocal at diagnosis with variable Gleason score across foci (Arora et al. 2004). In a study of higher-stage cases, Barry et al. (2007) evaluated tissue samples from multiple, distinct foci for TMPRSS2-ERG fusion genes using in situ probes. In 41% of cases that were positive for fusion in at least one focus, the authors found that fusion status was homogeneous within a given locus, but heterogeneous across foci from a single case. In some cases, some foci were negative for fusion genes while others were positive; some cases revealed fusion by deletion in some foci and fusion by insertion in others, suggesting clonal diversity. Thus, when evaluating TMPRSS2 fusion genes, biopsy strategies that do not sample all foci could be falsely negative. Therefore, noninvasive means of detection that are not restricted to tissue samples from individual cancer foci are very important in the clinical setting.

Laxman et al. (2006) explored the possibility of detecting TMPRSS2-ERG transcripts in the urine of patients after digital rectal exam. The authors amplified mRNA in urine by quantitative polymerase chain reaction, and determined the presence of fusion transcripts in the urine of 19 men known to have prostate cancer. Patients had been scheduled for biopsy or radical prostatectomy; PSA values ranged from 0.2 to 19 and averaged 6.7 ng/mL. Forty-two percent of patients had fusion mRNA detected in urine. Matched prostate tissue was tested in 3 fusion-positive patients and in 2 fusion-negative patients; tissue results correlated with urine results. The authors noted that their assay did not detect all reported isoforms.

**Table C1.** Studies of the Association of TMPRSS2 Fusion Genes with Prostate Cancer Diagnosis or Prognosis

| Study                  | Patients   | Specimen   | n                             | Results  |
|------------------------|--|--|-------------------------------|--|
| Attard et al. 2008a    | Retrospective cohort of men whose cancers were conservatively managed            | Tissue from radical prostatectomy  | 1,062 cores from 445 patients | Multivariate analysis, compared to no gene fusion:<br>Samples positive for fusion including only a portion of the ERG gene:<br>HR for cause-specific survival=1.72, 95% CI 1.02–2.89<br>HR for overall survival=1.43, 95% CI 1.04–1.97<br>Samples positive for fusion including only a portion of the ERG gene and with more than one copy of the fusion sequence:<br>HR for cause-specific survival=2.66, 95% CI 1.39–5.11<br>HR for overall survival=1.84, 95% CI 1.15–2.94<br>Samples positive for fusion including the entire ERG gene did not exhibit significantly poorer survival |
| Rajput et al. 2007     | Radical prostatectomy patients; no additional description reported               | Tissue from radical prostatectomy, single institution  | 672 cores from 196 patients   | Final number of cases with sectioning and FISH assay success with Gleason pattern data, 106<br>Percent of patients with fusion, by Gleason score:<br>BPH 0%<br>Gleason 2 6.7%<br>Gleason 3 41.3%<br>Gleason 4 37.5%<br>Gleason 5 43.8%<br>p=0.017 for fusion, Gleason 2 vs. 3-5  |
| Mehra et al. 2007      | Hospital-based cohort of men surgically treated for clinically localized disease | Tissue from radical prostatectomy; from University of Michigan Prostate Specialized Programs of Research Excellence (SPORE) Tissue Core (U.S.) | 96 (360 cores)                | 75 cases qualified for assessment ( $\geq 50$ cancer cells per case);<br>TMPRSS2-ERG fusion genes detected in 54%, TMPRSS2-ETV1 in 2% of cases (none had TMPRSS1-ETV4 fusion);<br>TMPRSS2 rearranged in 11% of cases without identifiable fusion gene partner;<br>Fusion gene significantly associated with high pathologic stage (p=0.04) but not with biochemical recurrence or Gleason score (<7 vs. $\geq 7$ )   |
| Demichelis et al. 2007 | Men with early prostate cancer in a population-based watchful waiting cohort     | Tissue from radical prostatectomy (Orebro University, Sweden)  | 111 (of total 223 in cohort)  | TMPRSS2-ERG fusion genes detected in 15%;<br>86% of cases with high ERG expression were fusion gene-positive;<br>Fusion-positive tumors more likely to have a higher Gleason score (p=0.01);<br>Association between fusion and metastases or disease-specific death: cumulative incidence ratio (CIR)=2.7, p<0.01; CIR=1.8, p=0.2 after adjustment for Gleason score   |

**Table C1.** Studies of the Association of TMPRSS2 Fusion Genes with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study              | Patients  | Specimen  | n                             | Results   |
|--------------------|---|---|-------------------------------|---|
| Nam et al. 2007a   | Men surgically treated for clinically localized disease   | Tissue from radical prostatectomy (Toronto, Canada)                           | 26                            | <p>TMPRSS2-ERG fusion genes detected in 42% of cases;<br/>Fusion not significantly associated with cancer stage;<br/>For fusion-positive cases vs. fusion-negative cases, OR for biochemical failure=11.4 (95% CI: 1.7–78.4, p=0.008);<br/>Five-year biochemical recurrence-free survival for patients with and without fusion was 20.5% (95% CI: 1.3–55.9%) and 62.5% (95% CI: 27.3–89.3%), p=0.009;<br/>In a multivariable Cox model, fusion was the only significant predictor of biochemical failure at HR=7.0 (95% CI: 1.1–44.6; p=0.03)</p> |
| Nam et al. 2007b   | Men surgically treated for clinically localized disease; no other treatment given   | Tissue from radical prostatectomy   | 165                           | <p>49% positive for TMPRSS2-ERG fusion gene;<br/>OR for biochemical recurrence, fusion-positive vs. fusion-negative: 10.9, 95% CI, 4.3–27.9, p=10<sup>-7</sup><br/>Adjusted HR for disease recurrence 8.6, 95% CI, 3.6–20.6 (only grade and fusion status significant)<br/>Fusion status significantly associated with increased risk for recurrence for all Gleason Score categories (5–6, 7, 8–10) and all stages</p>   |
| Winnes et al. 2007 | Patients presenting with clearly palpable prostate tumors   | Biopsy tissue   | 50                            | <p>TMPRSS2-ERG fusion genes detected in 36% of cases;<br/>No significant differences in cause-specific or progression-free survival between fusion-positive and fusion-negative patients;<br/>21% of patients with progression-free survival ≤1 year were fusion-positive vs. 41% with progression-free survival &gt;1 year fusion-positive (not significant)</p>   |
| Perner et al. 2007 | Patient samples representing localized cancer, high-grade lesions, hormone-naïve and hormone refractory metastases, a variety of benign conditions, and normal prostate | Prostatectomy tissue samples from 2 hospital-based cohorts (U.S. and Germany) | 397 samples from 300 patients | <p>Fusion detected in:<br/>48.5% of clinically localized samples (n=237)<br/>30% of hormone-naïve metastases (n=34)<br/>33% of hormone-refractory metastases (n=9)<br/>19% of high grade prostatic intraepithelial neoplasia (n=26)<br/>0% of a variety of benign lesions or normal epithelium (n=100)<br/>No significant association of fusion with Gleason score</p>  |

**Table C1.** Studies of the Association of TMPRSS2 Fusion Genes with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study              | Patients   | Specimen   | n                          | Results  |
|--------------------|--|--|----------------------------|--|
| Wang et al. 2006   | Men undergoing radical prostatectomy for clinically localized cancer             | Tissue samples from Baylor Prostate Specialized Programs of Research Excellence (SPORE) Tissue Core (U.S.) | 59 (cancer)<br>28 (benign) | 59% of cancer tissue samples TMPRSS2-ERG fusion-positive;<br>0% of benign prostate samples fusion-positive;<br>8 sizes of fusion gene mRNA; multiple fusion mRNAs in one sample common;<br>Fusion mRNAs containing the native TMPRSS2 translation initiation codon in frame with the ERG gene are associated with early PSA recurrence (p=0.035) and seminal vesicle invasion (p=0.005);<br>Expression level of in-frame fusion genes was also lower than with other, out of frame fusion genes (p=0.041)  |
| Perner et al. 2006 | Men with clinically localized prostate cancer or with hormone-refractory disease | Prostatectomy tissue samples from 2 hospital-based cohorts (U.S. and Germany)                              | 211 (897 cores)            | 49% of primary prostate cancer samples and 41% of hormone-naïve metastatic lymph node samples were fusion-positive;<br>60% of fusion-positive samples had a discrete deletion (of variable size) between TMPRSS2 and the ERG loci;<br>Fusion with deletion associated with high tumor stage (p=0.03) and metastases (p=0.02) but not with Gleason grade; more common in hormone-refractory than in hormone-naïve metastatic samples (71% vs. 25%, statistical significance not reported);<br>Fusion with deletion more common in cases with biochemical recurrence compared to fusion-negative cancers (statistical significance not reported) |

Thus, while reports suggest that TMPRSS2 fusion genes may be markers for diagnosis and possibly for prognosis, much work remains to explore fusion gene complexity, including the exploration of ETS fusion gene partners other than TMPRSS2 (Tomlins et al. 2007; Attard et al. 2008b). In addition, it is necessary to design accurate non-invasive assays for diagnosis, and determine clinical validity for prognosis. Once standardized assays are available, larger studies will be needed to determine clinical utility. TMPRSS2 fusion genes have also been incorporated into multiplex assays of genetic markers for diagnosis of prostate cancer (see following section, “Candidate Gene Panels”).

**Commercial Development.** Currently, the patent rights to TMPRSS2-ETS translocations are shared between the University of Michigan and the Brigham and Women’s Hospital. Gen-Probe (San Diego, CA) has exclusively licensed the rights to commercially develop urine- and tissue-based tests.

Like PCA3, TMPRSS2-ETS fusion genes have come to the attention of the National Cancer Institute’s Early Detection Research Network (EDRN) Prostate and Urologic Cancer Collaboration Group. The EDRN 2008 annual report ([http://www.compass.fhcrc.org/edrnci/files/pdf/edrn\\_4th-report\\_200801.pdf](http://www.compass.fhcrc.org/edrnci/files/pdf/edrn_4th-report_200801.pdf)) briefly describes TMPRSS2 fusion to ERG and ETV genes and notes ongoing development of assays to detect fusion indicators in urine.

# Appendix D

## Candidate Gene Panels

| Type;<br>Purpose             | Key Publication(s) | Genetic Markers  | Developmental<br>Phase |
|------------------------------|--------------------|--|------------------------|
| Diagnostic and/or prognostic | Various, see text  | Various combinations of genes derived from previously identified candidate genes, or from gene expression analyses | 1                      |

Because no single gene markers have been found that are both highly sensitive and highly specific for identifying prostate cancer, particularly in men already known to have elevated PSA levels, some investigators are combining several promising markers into a single diagnostic panel. Some examples and preliminary results are shown in Table D1. In interviews, Laxman et al. (2008) indicated their intent to improve and commercialize their panel, initially as a supplement to PSA testing.

Bibikova et al. (2007; Table D1) reported a gene expression study initially of 512 candidate genes, reduced to 16 gene makers correlated with Gleason score. Combining the results of the 16-gene panel into a single gene expression score (GEX), the authors reported statistically significant discrimination between patients with and without cancer, and between cancer patients with and without relapse. According to ROC analysis, the GEX improved upon Gleason score in predicting relapse. The study was funded by Illumina, Inc. (San Diego, CA) but no information regarding this assay could be found on their website.

Several additional panels have been studied and reported in a single publication (Table D1); no additional follow-up or indication of commercialization was found for any of these panels.

### Candidate Gene Panel: Health Discovery Corporation

| Type;<br>Purpose                             | Key Publication(s) | Genetic Markers         | Developmental<br>Phase             |
|--|--------------------|-------------------------|------------------------------------|
| Diagnostic; improve accuracy of first biopsy | None               | 4 genes, not identified | 1–2, per press release description |

In April 2008, Health Discovery Corporation (Savannah, GA) announced the successful clinical trial results for a new molecular diagnostic test for prostate cancer licensed exclusively to Clariant, Inc. (Aliso Viejo, CA). Study samples of prostate tissue were obtained in collaboration with M.D. Anderson Cancer Center. According to a press release (available at <http://www.healthdiscoverycorp.com/>), the test “is based on a unique combination of 4 genes that accurately identify the presence of Grade 3 or higher (clinically significant cancer) prostate cancer cells in prostate tissue.” No publication or meeting presentation was found in relation to this study. The press release indicates that additional clinical studies will be conducted to increase the number of specimens tested, then the assay will be offered commercially.

**Table D1.** Candidate Gene Panels

| Study                 | Samples  | n          | Gene Panel  | Results  |
|-----------------------|--|------------|---|--|
| Laxman et al. 2008    | Urine collected after DRE in patients with elevated PSA levels (59% positive for cancer)                                     | 234        | PCA3, TMPRSS2-ERG, GOLPH2 <sup>1</sup> , SPINK1 <sup>2</sup>                  | AUC of each marker greater than that for PSA in same patients (p=0.0002)<br>Leave-one-out cross-validation analysis of the same samples used to generate a multivariable model: AUC=0.736<br>Sensitivity=62%; Specificity=75%<br>PPV=78%; NPV=58%<br>"Limited" discrimination among clinical risk groups |
| Nakagawa et al. 2008  | Cases and controls within a cohort with rising PSA post-surgery  | 213<br>213 | 17 genes selected from previously reported marker panels tested in this study | AUC for systemic progression 0.88 (95% CI, 0.84-0.92)<br>HR for prostate cancer death in cases 2.5<br>HR for progression after 5 yrs in controls 4.7 (p=0.0007 and 0.0005, respectively)   |
| Hessels et al. 2007   | Urinary sediments from:<br>– men with prostate cancer-positive biopsies, and<br>– men with prostate cancer-negative biopsies | 78<br>30   | TMPRSS2-ERG, PCA3   | Panel sensitivity: 73% (better than either marker alone)<br>Panel positive predictive value: 94% (In the subset of patients with a history of negative biopsies but persistently elevated PSA)   |
| Petrovics et al. 2005 | Matched cancerous and benign prostatectomy tissue  | 55         | ERG, PCA3, AMACR <sup>3</sup>   | Overexpression of at least 1 of the 3 genes was detected in 54 of 55 cancerous samples compared to matched benign tissue   |

**Table D1.** Candidate Gene Panels (cont'd)

| Study                | Samples   | n           | Gene Panel   | Results  |
|----------------------|---|-------------|--|--|
| Schmidt et al. 2006  | Matched cancerous and benign prostatectomy tissue                                       | 106         | PCA3, EZH2 <sup>4</sup> , prostatein <sup>5</sup> , TRPM8 <sup>6</sup>               | AUC=0.90<br>Prostein, PSA, and TRPM8 were more highly expressed in organ-confined tumors compared to those not organ-confined  |
| Bibikova et al. 2007 | Archived formalin-fixed prostate cancer tissue samples;<br>Noncancerous prostate tissue | 71+79<br>47 | 16 genes <sup>7</sup> derived from a gene expression array of<br>512 candidate genes | 16 genes with strong correlation to Gleason grade selected in study of 71 samples<br>Summary gene expression score (GEX) calculated as an "expression analogy of Gleason grade"<br>Follow-up study on 79 cancerous and 47 noncancerous specimens:<br>Mean GEX, cancer: 7.38±0.35<br>Mean GEX, non-cancer: 7.2±0.16, p=0.0013<br>GEX AUC, relapse: 0.73<br>Gleason AUC, relapse: 0.65<br>GEX significantly distinguished between patients with a Gleason score of 7 who did and did not relapse (p=0.005) |

<sup>1</sup> SPINK1: Kazal type 1; overexpressed in prostate cancer (Tomlins et al. 2008)

<sup>2</sup> GOLPH2: golgi phosphoprotein 2; overexpressed in prostate cancer (Lapointe et al. 2004)

<sup>3</sup> AMACR: x-methylacyl-CoA racemase; commonly overexpressed in prostate cancer (Rubin et al. 2002)

<sup>4</sup> EZH2: zeste homolog 2; reported to be more active in metastatic than localized disease (Varambally et al. 2002)

<sup>5</sup> Prostein: a prostate-specific transmembrane protein (Xu et al. 2001)

<sup>6</sup> TRPM8: transient receptor potential melastatin member 8; a potential discriminator of high vs. low grade disease (Fuessel et al. 2003)

<sup>7</sup> Positively correlated: CCNE2, CDC6, FBP1, HOXC6, MKI67, MYBL2, PTTG1, DTL, UBE2C, WNT5A, ALCAM; Negatively correlated: AZGP1, CCK, MYLK, PPAP2B, PROK1

# Appendix E

## Epigenetic Biomarkers of Prostate Cancer: Gene Methylation

| Type;<br>Purpose   | Key Publication(s)                | Genetic Markers  | Developmental<br>Phase |
|--|-----------------------------------|--|------------------------|
| Diagnostic;<br><br>Prognosis (predict PSA<br>recurrence) | Extensive literature;<br>see text | Various methylated genes or<br>methylated gene combinations;<br>most often GSTP1 | 1                      |

There is an extensive literature reporting significant associations of epigenetic DNA modifications with prostate cancer. Studies are primarily small, retrospective pilot evaluations of hypermethylation status of various candidate genes for discriminating prostate cancer from benign conditions (diagnosis) or for predicting disease recurrence and association with clinicopathologic predictors of aggressive disease (prognosis). Table E1 gives examples of recently published studies identified from the gray literature and/or in PubMed search results (see Methods). Please note that these are only examples, not the universe of studies on this topic, the retrieval and synthesis of which is beyond the scope of this report. Several points can be made from these recent studies:

- Populations in diagnostic applications are highly variable, with percentage of patients with prostate cancer ranging from 24 to 70, where indicated.
- Several different sample types are being investigated, with urine or urine sediment of particular interest for diagnostic applications, but tissue, tissue washings, whole blood, serum, and plasma studied for both diagnostic and prognostic applications.
- With the exception of GSTP1, which is included in several studies, a wide variety of methylation markers are under study.
- Nearly all studies chose candidate genes for methylation studies based on prior study and/or biologic plausibility; one study conducted a genome-wide association study (Cottrell et al. 2007).
- Sample sizes are largely underpowered to detect statistically significant associations, particularly if corrected for multiple hypothesis testing (i.e., multiple methylation markers independently considered). Only one study in Table E1 made such a correction (Cottrell et al. 2007).
- Some studies use quantitative methods to measure gene methylation, others use only qualitative methods. Although not indicated in the table, a variety of detection methods and interpretation criteria are used across studies.
- In terms of prognosis, only one study evaluated the direct outcome of disease-specific survival rather than only the indirect biochemical (PSA) recurrence outcome (Henrique et al. 2007).

These observations regarding the most recently published studies suggest an area of clinical research that has not yet identified the best sample, the best markers for diagnosis and prognosis, nor the best way to measure them. Some markers appear to be positive not only in prostate cancer, but in benign cases, complicating their use as cancer-specific markers (e.g., GSTP1 methylation has been reported in BPH). Rather than single markers, panels of markers may need to be identified and validated for different applications in order to achieve clinical validity and utility. Nor does it appear that the more commonly studied markers have been measured reliably and with clinically useful performance in non-invasive, readily collectable samples. The degree of methylation rather than only presence or absence at specific gene sites may be necessary for

**Table E1.** Recent Publications Describing Gene Hypermethylation and Association with Prostate Cancer Diagnosis or Prognosis

| Study   | Purpose              | Study Type   | Sample   | n  | Genes Tested for Hypermethylation   | Results  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
|---|----------------------|--|--|--|---|--|--|-------|-------|------|------|------|-------|----|----|----|----|-------|------|----|----|----|----|-------|----------------|----|----|----|----|-------|----------------|----|----|----|----|-------|------------------|----|----|----|----|-------|
| <b>Sample = prostate tissue or tissue washing</b> |                      |  |  |  |   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| Eilers et al. 2007                                | Diagnosis            | Candidate gene evaluation compared to histologic diagnosis                               | Washings from multiple biopsy cores, from patients suspicious for Pr Ca (Germany, 1 site)  | 86 (42% positive for Pr Ca)                    | GSTP1<br>(quantitative assay)   | Positive Pr Ca samples had a higher mean hypermethylation vs. those with benign histology (28% vs. 6.5%, p<0.001).<br>AUC for Pr Ca diagnosis=0.90 (95% CI, 0.82–0.98; p<0.001)<br>%Sensitivity 92, %Specificity 86, %PPV 82, %NPV 94  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| Aitchison et al. 2007                             | Diagnosis            | Candidate gene evaluation in pre-malignant vs. matched normal epithelial prostate tissue | Laser-capture microdissected PIN and matched normal epithelium from archived needle biopsies from cases with no Pr Ca (UK, 1 site) | 14   | RASSF1A<br>(qualitative assay)  | RASSF1A was present in both pre-malignant and benign epithelia and could not distinguish between the two   |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| Ellinger et al. 2008a                             | Diagnosis, prognosis | Candidate gene evaluation comparing Pr Ca to BPH; no correction for multiple tests       | Prostatectomy tissue from:<br>1. patients with clinically localized Pr Ca or<br>2. BPH<br>(Germany, 1 site)                        | 80<br><br>26<br><br>(follow-up info on 41 pts) | GSTP1, APC, PTGS2, MDR1, RARBeta, Reprimo, TIG1<br><br>(quantitative assay) | <p><b>Diagnosis:</b> Examples of best-performing hypermethylated single genes or gene combinations:</p> <table border="1"> <thead> <tr> <th></th> <th>%Sens</th> <th>%Spec</th> <th>%PPV</th> <th>%NPV</th> <th>%AUC</th> </tr> </thead> <tbody> <tr> <td>GSTP1</td> <td>92</td> <td>85</td> <td>95</td> <td>79</td> <td>0.917</td> </tr> <tr> <td>TIG1</td> <td>96</td> <td>88</td> <td>96</td> <td>88</td> <td>0.951</td> </tr> <tr> <td>GSTP1 and TIG1</td> <td>89</td> <td>96</td> <td>99</td> <td>73</td> <td>0.925</td> </tr> <tr> <td>TIG1 and EDNRB</td> <td>96</td> <td>92</td> <td>98</td> <td>89</td> <td>0.943</td> </tr> <tr> <td>TIG1 and RARBeta</td> <td>91</td> <td>92</td> <td>97</td> <td>77</td> <td>0.918</td> </tr> </tbody> </table> <p><b>Prognosis:</b><br/>Several combinations of 2 hypermethylation loci significantly correlated with capsular penetration, extra-prostatic extension, positive surgical margins, or Gleason score<br/>Hypermethylation at APC and Reprimo correlated significantly with PSA recurrence (p=0.008)<br/>More than 5 hypermethylated genes correlated with PSA-free survival (p=0.007)</p> |  | %Sens | %Spec | %PPV | %NPV | %AUC | GSTP1 | 92 | 85 | 95 | 79 | 0.917 | TIG1 | 96 | 88 | 96 | 88 | 0.951 | GSTP1 and TIG1 | 89 | 96 | 99 | 73 | 0.925 | TIG1 and EDNRB | 96 | 92 | 98 | 89 | 0.943 | TIG1 and RARBeta | 91 | 92 | 97 | 77 | 0.918 |
|   | %Sens                | %Spec  | %PPV   | %NPV   | %AUC  |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| GSTP1   | 92                   | 85   | 95   | 79   | 0.917   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| TIG1  | 96                   | 88   | 96   | 88   | 0.951   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| GSTP1 and TIG1                                    | 89                   | 96   | 99   | 73   | 0.925   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| TIG1 and EDNRB                                    | 96                   | 92   | 98   | 89   | 0.943   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |
| TIG1 and RARBeta                                  | 91                   | 92   | 97   | 77   | 0.918   |  |  |       |       |      |      |      |       |    |    |    |    |       |      |    |    |    |    |       |                |    |    |    |    |       |                |    |    |    |    |       |                  |    |    |    |    |       |

**Table E1.** Recent Publications Describing Gene Hypermethylation and Association with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study  | Purpose                    | Study Type   | Sample   | n                                  | Genes Tested for Hypermethylation                        | Results   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
|--|----------------------------|--|--|------------------------------------|--|---|--|-------|-------|------|------|--------------------|----|----|----|----|------------------|----|----|----|----|
| <b>Sample = prostate tissue or tissue washing (cont'd)</b> |                            |  |  |                                    |  |   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
| Cottrell et al. 2007                                       | Prognosis (PSA recurrence) | Genome-wide association study for regions methylated only in aggressive Pr Ca; corrected for multiple testing                                | Prostatectomy tissue samples (US & Europe, 5 sites)  | discovery: ~400<br>validation: 223 | ABHD9, Chr3-EST<br><br>(final assay, quantitative)       | Validation population: ABHD9 and Chr3-EST significantly discriminated between patients with early recurrence vs. nonrecurrence ( $p < 0.001$ , both markers), including in the subpopulation with intermediate Gleason scores ( $p < 0.01$ , both markers) and between Gleason 2-6 vs. Gleason 8-10 ( $p < 0.001$ , both markers).<br>Multivariate model for PSA recurrence including ABHD9 or Chr3-EST, Gleason score, pathological stage, surgical margin status: AUC with either methylation marker better than with none (0.81 or 0.79 vs. 0.75). |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
| Alumkal et al. 2008  | Prognosis (PSA recurrence) | Nested case-control study of methylation status of 15 candidate genes which methylation known to silence; no correction for multiple testing | Prostatectomy tissue samples from patients followed 5 yrs (US, 1 site)   | 151                                | CDH13, ASC<br><br>(qualitative assay)                    | CDH13 odds ratio (OR) for PSA recurrence=5.5, 95% CI 1.34–22.7 ( $p=0.02$ ), adjusted for clinicopathologic risk factors. CDH13 and/or ASC OR = 5.64, 95% CI, 1.47–21.7 ( $p=0.01$ ).<br><br><table border="1"> <thead> <tr> <th></th> <th>%Sens</th> <th>%Spec</th> <th>%PPV</th> <th>%NPV</th> </tr> </thead> <tbody> <tr> <td>Clinico-pathologic</td> <td>55</td> <td>95</td> <td>84</td> <td>82</td> </tr> <tr> <td>CDH13 and/or ASC</td> <td>72</td> <td>48</td> <td>39</td> <td>79</td> </tr> </tbody> </table>                                 |  | %Sens | %Spec | %PPV | %NPV | Clinico-pathologic | 55 | 95 | 84 | 82 | CDH13 and/or ASC | 72 | 48 | 39 | 79 |
|  | %Sens                      | %Spec  | %PPV   | %NPV                               |  |   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
| Clinico-pathologic   | 55                         | 95   | 84   | 82                                 |  |   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
| CDH13 and/or ASC   | 72                         | 48   | 39   | 79                                 |  |   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |
| Woodson et al. 2006  | Prognosis (PSA recurrence) | Candidate gene evaluation at prostatectomy, correlation with PSA recurrence; no correction for multiple tests                                | Prostatectomy tissue from patients with node-negative clinically localized Pr Ca, not otherwise treated (US, 1 site) | 60                                 | GSTP1, RARbeta1, CD44, PTGS2<br><br>(quantitative assay) | In multivariable analysis adjusting for Gleason grade, the presence of both CD44 and PTGS2 methylation predicted PSA recurrence (OR=8.9, 95% CI 1.85-42.6, $p=0.006$ ).<br>Patients with tumor methylation of both CD44 and PTGS2 had significantly shorter time to PSA recurrence, after adjustment for Gleason grade.   |  |       |       |      |      |                    |    |    |    |    |                  |    |    |    |    |

**Table E1.** Recent Publications Describing Gene Hypermethylation and Association with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study  | Purpose   | Study Type   | Sample  | n                              | Genes Tested for Hypermethylation                             | Results  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
|--|---|--|---|--------------------------------|---|--|---|---------------------------|------------------------------|----|-----------------|-----------------|---------|----|----|-----|---------|------|-------|----|----|-----------------|---------|------|-------|----|----|----|---------|------|-----------|----|---|----|---------|------|
| <b>Sample = prostate tissue or tissue washing (cont'd)</b> |   |  |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| Henrique et al. 2007                                       | Prognosis (Disease-specific survival; PSA recurrence) | Candidate gene evaluation compared to histologic diagnosis; no correction for multiple tests | Biopsy tissue from men referred for elevated PSA (Portugal, 1 site)   | 83                             | APC, CCND2, GSTP1, RARB2, RASSF1A<br><br>(quantitative assay) | High-level methylation of APC associated with worse disease-specific survival over median 45 months (p=0.01), independent of clinical variables.<br><br>High-level methylation of APC (p=0.002), GSTP1 (p=0.047), or RASSF1A (p=0.019) associated with shorter time to PSA recurrence, independent of clinical variables.  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| <b>Sample = urine or urine sediment</b>                    |   |  |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| Rogers et al. 2006   | Diagnosis   | Candidate gene evaluation compared to histologic diagnosis; no correction for multiple tests | Urine collected after attentive DRE in men referred for diagnostic biopsy (US, 1 site)                      | 17<br>(70% positive for Pr Ca) | GSTP1, APC, EDNRB<br><br>(qualitative assay)                  | <table border="1"> <thead> <tr> <th colspan="3">% of patients positive for hypermethylation</th> </tr> <tr> <th></th> <th>Biopsy-positive</th> <th>Biopsy-negative</th> </tr> </thead> <tbody> <tr> <td>GSTP1</td> <td>25</td> <td>20</td> </tr> <tr> <td>APC</td> <td>17</td> <td>20</td> </tr> <tr> <td>EDNRB</td> <td>85</td> <td>60</td> </tr> <tr> <td>At least 1 gene</td> <td>100</td> <td>60</td> </tr> </tbody> </table>  | % of patients positive for hypermethylation |                           |                              |    | Biopsy-positive | Biopsy-negative | GSTP1   | 25 | 20 | APC | 17      | 20   | EDNRB | 85 | 60 | At least 1 gene | 100     | 60   |       |    |    |    |         |      |           |    |   |    |         |      |
| % of patients positive for hypermethylation                |   |  |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
|  | Biopsy-positive                                       | Biopsy-negative  |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| GSTP1  | 25  | 20   |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| APC  | 17  | 20   |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| EDNRB  | 85  | 60   |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| At least 1 gene  | 100   | 60   |   |                                |   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| Roupret et al. 2007  | Diagnosis   | no correction for multiple tests   | Urine sediment from patients with localized Pr Ca who underwent radical prostatectomy; age-matched controls | 95<br><br>38                   | RASSF1A, APC, GSTP1, RARBeta-2                                | <table border="1"> <thead> <tr> <th></th> <th>% Pts Methylatn-pos Pr Ca</th> <th>% Pts Methylatn-pos Controls</th> <th>OR</th> <th>p-value</th> <th>AUC</th> </tr> </thead> <tbody> <tr> <td>RASSF1A</td> <td>78</td> <td>8</td> <td>41</td> <td>&lt;0.0001</td> <td>0.85</td> </tr> <tr> <td>APC</td> <td>50</td> <td>5</td> <td>18</td> <td>&lt;0.0001</td> <td>0.74</td> </tr> <tr> <td>GSTP1</td> <td>83</td> <td>13</td> <td>33</td> <td>&lt;0.0001</td> <td>0.86</td> </tr> <tr> <td>RARBeta-2</td> <td>62</td> <td>3</td> <td>61</td> <td>&lt;0.0001</td> <td>0.80</td> </tr> </tbody> </table> |   | % Pts Methylatn-pos Pr Ca | % Pts Methylatn-pos Controls | OR | p-value         | AUC             | RASSF1A | 78 | 8  | 41  | <0.0001 | 0.85 | APC   | 50 | 5  | 18              | <0.0001 | 0.74 | GSTP1 | 83 | 13 | 33 | <0.0001 | 0.86 | RARBeta-2 | 62 | 3 | 61 | <0.0001 | 0.80 |
|  | % Pts Methylatn-pos Pr Ca                             | % Pts Methylatn-pos Controls   | OR  | p-value                        | AUC   |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| RASSF1A  | 78  | 8  | 41  | <0.0001                        | 0.85  |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| APC  | 50  | 5  | 18  | <0.0001                        | 0.74  |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| GSTP1  | 83  | 13   | 33  | <0.0001                        | 0.86  |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |
| RARBeta-2  | 62  | 3  | 61  | <0.0001                        | 0.80  |  |   |                           |                              |    |                 |                 |         |    |    |     |         |      |       |    |    |                 |         |      |       |    |    |    |         |      |           |    |   |    |         |      |

**Table E1.** Recent Publications Describing Gene Hypermethylation and Association with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study  | Purpose              | Study Type  | Sample   | n                            | Genes Tested for Hypermethylation  | Results   |   |                |              |                         |                         |            |            |
|--|----------------------|---|--|------------------------------|--|---|---|----------------|--------------|-------------------------|-------------------------|------------|------------|
| <b>Sample = urine or urine sediment (cont'd)</b> |                      |   |  |                              |  |   |   |                |              |                         |                         |            |            |
| Woodson et al. 2008                              | Diagnosis            | Candidate gene evaluation compared to histologic diagnosis                                  | Urine sediments, collected after attentive DRE in men referred for diagnostic biopsy; matched biopsy tissue (U.S., 1 site)                 | 100 (24% positive for Pr Ca) | GSTP1 (quantitative assay)   | <b>% Pts GSTP1 methylation</b>  |   |                |              |                         |                         |            |            |
|  |                      |   |  |                              |  |   | <b>BPH</b>  | <b>PIN</b>     | <b>Pr Ca</b> | <b>Sens<sup>1</sup></b> | <b>Spec<sup>1</sup></b> | <b>PPV</b> | <b>NPV</b> |
|  |                      |   |  |                              |  | Urine   | 2   | 13             | 75           | 75                      | 97                      | 90         | 90         |
|  |                      |   |  |                              |  | Biopsy  | 9   | 50             | 88           | 88                      | 87                      | 68         | 96         |
|  |                      |   |  |                              |  |   |   |                |              |                         |                         |            |            |
|  |                      |   |  |                              |  | <b>% Pts GSTP1 methylation</b>  |   | <b>p-value</b> |              |                         |                         |            |            |
|  |                      |   |  |                              |  | Pathol stage  | II  | 20             |              |                         |                         |            |            |
|  |                      |   |  |                              |  |   | III   | 100            |              | 0.05                    |                         |            |            |
|  |                      |   |  |                              |  | Gleason grade   | ≤6  | 50             |              |                         |                         |            |            |
|  |                      |   |  |                              |  |   | 7-10  | 87             |              | 0.10                    |                         |            |            |
| <b>Sample = whole blood, plasma, or serum</b>    |                      |   |  |                              |  |   |   |                |              |                         |                         |            |            |
| Chuang et al. 2007                               | Diagnosis            | Candidate gene evaluation compared to histologic diagnosis                                  | Plasma from<br>1. Pr Ca Pts, and<br>2. BPH Pts<br>Tissue from Pr Ca Pts (Taiwan, 1 site)   | 36                           | GSTP1 (quantitative assay)   | Pr Ca samples: 30% positive for GSTP1 methylation<br>BPH samples: 7.4% weakly positive. |   |                |              |                         |                         |            |            |
|  |                      |   |  | 27                           |  |   |   |                |              |                         |                         |            |            |
|  |                      |   |  | 22                           |  |   | Tissue from 22 of 36 Pr Ca Pts: 100% positive for GSTP1 methylation; 9 of 22 positive for both tissue and plasma DNA; of 9, 6 were high-grade |                |              |                         |                         |            |            |
| Bastian et al. 2008                              | Diagnosis, prognosis | Candidate gene evaluation compared to clinical evaluation; no correction for multiple tests | Serum from men with:<br>1. clinically localized disease;<br>2. metastatic hormone refractory disease;<br>3. benign biopsies (U.S., 1 site) | 192                          | GSTP1, NEP, MDR1, CD44, EDNRB, ESR1, RAR-beta, PTGS2, RASSF1A (quantitative assay) | <b>% of pts positive for hypermethylation</b>   |   |                |              |                         |                         |            |            |
|  |                      |   |  |                              |  | <b>Group</b>  | <b>GSTP1</b>  | <b>MDR1</b>    | <b>EDNRB</b> | <b>RAR-beta</b>         |                         |            |            |
|  |                      |   |  |                              |  | Neg biopsy  | 0   | 0              | 0            | 0                       |                         |            |            |
|  |                      |   |  |                              |  | Pr Ca:  |   |                |              |                         |                         |            |            |
|  |                      |   |  |                              |  | No recurrence   | 5   | 38             | 0            | 0                       |                         |            |            |
|  | Recurrence           | 20  | 16   | 0                            | 0  |   |   |                |              |                         |                         |            |            |
|  | Metastasis           | 28  | 89   | 50                           | 39   |   |   |                |              |                         |                         |            |            |

**Table E1.** Recent Publications Describing Gene Hypermethylation and Association with Prostate Cancer Diagnosis or Prognosis (cont'd)

| Study   | Purpose                    | Study Type  | Sample  | n                      | Genes Tested for Hypermethylation                         | Results  |               |                    |              |            |
|---|----------------------------|---|---|------------------------|---|--|---------------|--------------------|--------------|------------|
| <b>Sample = whole blood, plasma, or serum (cont'd)</b>                          |                            |   |   |                        |   |  |               |                    |              |            |
| Ellinger et al. 2008b   | Diagnosis, prognosis       | Candidate gene evaluation compared to histologic diagnosis; no correction for multiple tests  | Serum from patients with:<br>1. Pr Ca undergoing prostatectomy<br>2. BPH diagnosed after TURP   | 168<br><br>42          | GSTP1, PTGS2, TIG1, Reprimo<br><br>(quantitative assay)   | Pr Ca vs. BPH:   |               |                    |              |            |
|   |                            |   |   |                        |   | <b>%Sens</b>   | <b>%Spec</b>  | <b>%NPV</b>        | <b>%PPV</b>  | <b>AUC</b> |
|   |                            |   |   |                        |   | GSTP1  | 42            | 93                 | 29           | 96         |
| Any methyl.   | 47                         | 93  | 30  | 96                     | 0.699   |  |               |                    |              |            |
| PSA (4 ng/mL cutoff)  | 90                         | 36  | 54  | 86                     | 0.653   |  |               |                    |              |            |
| No correlation between methylation at any site and clinicopathologic parameters |                            |   |   |                        |   |  |               |                    |              |            |
| Roupret et al. 2008   | Prognosis (PSA recurrence) | Candidate gene evaluation at diagnosis and relapse (or corresponding time point), correlation with PSA recurrence; no correction for multiple tests | Whole blood samples from:<br>1. Pr Ca pts with PSA recurrence<br>2. Pr Ca pts with no PSA recurrence<br>3. Pts with PSA 3ng/mL but biopsy-negative (UK, 1 site) | 20<br><br>22<br><br>22 | GSTP1, APC, RASSF1a, RARbeta2<br><br>(quantitative assay) | GSTP1, RASSF1a, RARbeta2, and APC highly significant (p<0.0001) for methylation at diagnosis in Pr Ca patients compared to biopsy-negative patients. |               |                    |              |            |
|   |                            |   |   |                        |   | At relapse time point, significantly more methylation of GSTP1, RASSF1a, RARbeta2, and APC in Group 1 vs. Group 2 or 3 (p<0.001).                    |               |                    |              |            |
|   |                            |   |   |                        |   | At relapse time point, the level of methylation was significantly higher compared to diagnosis time point in Group 1 (p<0.001) but not in Group 2    |               |                    |              |            |
| Reibenwein et al. 2007  | Prognosis (PSA recurrence) | Candidate gene evaluation compared to clinical evaluation; no correction for multiple tests   | Serum from patients with:<br>1. hormone refractory (HR) Pr Ca<br>2. early-stage Pr Ca, treated with RT<br>3. healthy controls (Austria, 1 site)                 | 62<br><br>14<br><br>49 | GSTP1, AR, 14-3-3sigma<br><br>(qualitative assay)         | <b>% of patients positive for hypermethylation</b>   |               |                    |              |            |
|   |                            |   |   |                        |   | <b>GSTP1</b>   | <b>AR</b>     | <b>14-3-3sigma</b> |              |            |
|   |                            |   |   |                        |   | HR Pr Ca   | 32 (p<0.001*) | 40                 | 87 (p=0.03*) |            |
| Early Pr Ca   | 21 (p=0.006*)              | 36  | 86  |                        |   |  |               |                    |              |            |
| LN mets   | 53 (p=0.02**)              |   |   |                        |   |  |               |                    |              |            |
| No mets   | 18                         |   |   |                        |   |  |               |                    |              |            |
| Controls  | 0                          | 26  | 55  |                        |   |  |               |                    |              |            |
| *compared to controls   |                            |   |   |                        |   |  |               |                    |              |            |
| **compared to no mets   |                            |   |   |                        |   |  |               |                    |              |            |

<sup>1</sup> BPH + PIN vs. Cancer

useful discrimination. Recent advances in assay methods have moved to more accurate, quantitative assays that can be automated for high volume (Schulz 2005; Hopkins et al. 2007; Costa et al. 2007). However, standardized assays and interpretation criteria have not yet been agreed upon to enable consistency and comparison of results across studies.

Some hypermethylation markers are being commercially developed or are already being marketed for clinical use, in the form of laboratory-developed tests offered by CLIA-licensed private laboratories (FDA clearance not required).

In a study conducted by Epigenomics, Inc. (Seattle, WA) and partners, prostate cancer tissue samples from archived prostatectomies collected at 5 sites in the U.S. and Europe were scanned using genome-wide arrays for chromosomal regions methylated only in aggressive prostate cancer (Cottrell et al. 2007). Two hypermethylated DNA sequences, in the gene abhydrolase domain containing 9 (ABHD9) and the expressed sequence tag on chromosome 3 (Chr3-EST) were strongly associated with recurrence. Little is known about the relevance of these sequences to prostate cancer. A follow-up study with assays specific for hypermethylation of these sequences and conducted in an independent population of tissue samples found that ABHD9 and Chr3-EST significantly discriminated between patients with early recurrence vs. nonrecurrence. Both markers also significantly discriminated between patients with Gleason 2–6 versus Gleason 8–10 disease. Combined use of ABHD9 or Chr3-EST, Gleason score, pathological stage, and surgical margin status resulted in AUCs of 0.81 or 0.79, better than the AUC for clinicopathologic characteristics alone (AUC=0.75). However, sensitivity and specificity of either marker for recurrence were not specified, and it is not clear that an AUC of about 0.8 is sufficient to change surveillance or treatment for those patients without hypermethylated markers. This study represents developmental work in a program to design and validate better tests for prostate cancer diagnosis and prognosis.

Epigenomics Inc. has also funded studies of “Proprietary gene methylation biomarkers using DMH (Differential Methylation Hybridization) technology” reported at recent meetings, stating plans to “optimize and validate the most promising candidate biomarkers in a clinical study on urine samples” (Epigenomics Inc. press release, Monday, April 14, 2008; available at [http://www.epigenomics.com/en/Newsroom/Press\\_Releases/2008/datednews/080414\\_PCS\\_AACR.html](http://www.epigenomics.com/en/Newsroom/Press_Releases/2008/datednews/080414_PCS_AACR.html)). The specific markers, however, were not identified.

### Epigenetic Biomarkers of Prostate Cancer: GSTP1 Methylation

| Type;<br>Purpose   | Key Publication(s)   | Genetic Markers   | Developmental<br>Phase |
|--|--|-------------------|------------------------|
| Diagnostic; sample is tissue;<br>test is adjunct to histopathology<br>evaluation, especially where PSA<br>and histology do not agree | None specifically<br>conducted by original<br>patent holder or<br>subsequent licensees | GSTP1 methylation | 1                      |

OncoMethylome Sciences (Durham, NC) licensed technology to detect hypermethylation of GSTP1 to Veridex LLC (North Raritan, NJ), a Johnson & Johnson company, who subsequently issued a sublicense to LabCorp (Burlington, NC). The test is currently available from LabCorp (“Glutathione S-transferase Gene (GSTP1, pi-class) Methylation Assay”) and the required specimen is formalin-fixed, paraffin-embedded tissue. The test is stated to be an adjunct to histopathology. Two studies of GSTP1 hypermethylation using tissue samples reported significant results for identifying cancer with a percent sensitivity of 92, a percent specificity of 85, and an AUC of about 0.9 (Eilers et al. 2007; Ellinger et al. 2008). However, two other studies did not find significant associations with disease (Woodson et al. 2006; Henrique et al. 2007). In studies of other specimen types, generally poorer sensitivities for cancer were reported (Table 9); no evidence was found that this test is commercially available for specimens other than tissue.



**Technology  
Evaluation  
Center**

**Blue Cross and  
Blue Shield Association**  
225 North Michigan Avenue  
Chicago, Illinois 60601-7680  
[www.bcbs.com/tec](http://www.bcbs.com/tec)