Update: Use of Serum PSA for Diagnosis and Monitoring of Prostate Cancer

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Disclosure
Conflict of Interest

• Beckman Coulter Corporation – manufacturer of serum PSA test; grant support for PSA and pro-PSA studies,

• Gen Probe, Inc - manufacturer of urine PCA3 gene test for prostate cancer; paid member of the Gen Probe Scientific Advisory Board for 2003-2010; received grant support for PCA3 studies

• Health Discovery Corporation - developing urine gene test for prostate cancer; received grant support for a test validation study; currently an employee (CSO)
### 2011 Estimated US Cancer Cases*

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate</strong></td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td><strong>Lung &amp; bronchus</strong></td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td><strong>Colon &amp; rectum</strong></td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td><strong>Urinary bladder</strong></td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td><strong>Melanoma of skin</strong></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td><strong>Non-Hodgkin</strong></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td><strong>lymphoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td>3%</td>
<td></td>
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<tr>
<td><strong>Oral Cavity</strong></td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td><strong>Leukemia</strong></td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td><strong>All Other Sites</strong></td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>699,560</strong></td>
<td><strong>668,470</strong></td>
</tr>
</tbody>
</table>

|                      |          |          |
| **Prostate**         | 32%     |          |
| **Breast**           |         |          |
| **Lung & bronchus**  | 12%     |          |
| **Colon & rectum**   | 11%     |          |
| **Uterine corpus**   | 6%      |          |
| **Ovary**            | 4%      |          |
| **Non-Hodgkin**      | 4%      |          |
| **lymphoma**         |         |          |
| **Melanoma**         | 4%      |          |
| **Thyroid**          | 3%      |          |
| **Pancreas**         | 2%      |          |
| **Urinary bladder**  | 2%      |          |
| **All Other Sites**  | 20%     |          |

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.

Source: American Cancer Society, 2011.
2011 Estimated US Cancer Deaths*

Men 290,890

Women 272,810

- Lung & bronchus 32%
- Prostate 10%
- Colon & rectum 10%
- Pancreas 5%
- Leukemia 5%
- Non-Hodgkin lymphoma 4%
- Esophagus 4%
- Liver & intrahepatic bile duct 3%
- Urinary bladder 3%
- All other sites 21%

- Lung & bronchus 25%
- Breast 15%
- Colon & rectum 10%
- Ovary 6%
- Pancreas 6%
- Leukemia 4%
- Non-Hodgkin lymphoma 3%
- Uterine corpus 3%
- Multiple myeloma 2%
- All other sites 24%

ONS=Other nervous system.
Source: American Cancer Society, 2011
Serum PSA For Early Detection of Prostate Cancer: Current Status

- Positive predictive value is 25% when PSA = 4-10 ng/ml and 2.5-4.0 ng/ml
- The high false positive rate (75%) leads to many unnecessary biopsies
- Serum PSA cannot identify indolent tumors, which leads to unnecessary treatments for many subjects with damaging side effects
The PSA test, commonly used as a screening tool for detecting prostate cancer, is now all but useless for predicting prostate cancer risk, according to Stanford University School of Medicine researchers. A study of prostate tissues collected over 20 years -- from the time it first became standard to remove prostates in response to high PSA levels to the present:
- "Reveals that as a screen, the PSA test now indicates nothing more than the size of the prostate gland”
- “Only 226 out of every 100,000 men over the age of 65 dies of prostate cancer, which is a rate of 0.3 percent”
- "Our job now is to stop removing every man's prostate who has prostate cancer"
Prostate Cancer – In 2008 Recommendations for Detection and Management

1. Cancer screening in the U.S. was recommended for males >50 years (baseline at age 40), or for those with a family history of prostate cancer

2. Serum PSA testing recommended on an annual basis, along with a digital rectal exam (DRE)

3. Biopsy was considered when:
   ~ Serum PSA was greater than 4.0 or 2.5 ng/ml
   ~ DRE was positive
   ~ PSA velocity was greater than 0.75 ng/ml/yr
4. Biopsy (sextant, 10 core, 12 core) leads to cancer detection in 25% of cases. Rebiopsy: 2\textsuperscript{nd} has a 10-20\% PPV, and 3\textsuperscript{rd} bx has 1-2\% PPV.

5. Treatment options were prostatectomy or ablative treatment of the prostate with either radiation or cryotherapy.
The benefit of screening for prostate cancer with serum prostate-specific–antigen (PSA) testing, digital rectal examination, or any other screening test is unknown. There has been no comprehensive assessment of the trade-offs between benefits and risks. Despite these uncertainties, PSA screening has been adopted by many patients and physicians in the United States and other countries. The use of PSA testing as a screening tool has increased dramatically in the United States since 1988.

“We now know that prostate-cancer screening provided no reduction in death rates at 7 years and that no indication of a benefit appeared with 67% of the subjects having completed 10 years of follow-up”
Screening and Prostate-Cancer Mortality in a Randomized European Study

Fritz H. Schröder, M.D., Jonas Hugosson, M.D., Monique J. Roobol, Ph.D., Teuvo L.J. Tammela, M.D., Stefano Ciatto, M.D., Vera Nelen, M.D., Maciej Kwiatkowski, M.D., Marcos Lujan, M.D., Hans Lilja, M.D., Marco Zappa, Ph.D., Louis J. Denis, M.D., Franz Recker, M.D., Antonio Berenguer, M.D., Liisa Määttänen, Ph.D., Chris H. Bangma, M.D., Gunnar Aus, M.D., Arnauld Villers, M.D., Xavier Rebillard, M.D., Theodorus van der Kwast, M.D., Bert G. Blijenberg, Ph.D., Sue M. Moss, Ph.D., Harry J. de Koning, M.D., Anssi Auvinen, M.D., for the ERSPC Investigators

“The rate of overdiagnosis of prostate cancer has been estimated to be as high as 50% in the screening group…. Overdiagnosis and overtreatment are probably the most important adverse effects of prostate-cancer screening and are vastly more common than in screening for breast, colorectal, or cervical cancer”
Last week, two major studies from the United States and Europe found that P.S.A. testing — the annual blood test used to screen men for prostate cancer— “saves few if any lives, while exposing patients to aggressive and unnecessary treatments that can leave them impotent and incontinent.”

And there is an important tradeoff. P.S.A. testing increases a man’s risk of being treated for a cancer that would never have harmed him in the first place. The European study found that for every man who was helped by P.S.A. screening, at least 48 received unnecessary treatment that increased risk for impotency and incontinence.

Dr. Otis Brawley, chief medical officer of the American Cancer Society, summed up the European data this way: “The PSA test is about 50 times more likely to ruin your life than it is to save your life.”
Prostate Cancer
Dilemmas and Questions

1. Do we need to screen for early detection of prostate cancer?
2. Who needs to be screened?
3. Who needs to have a biopsy? And, if negative, is a second, third, or fourth biopsy warranted?
4. Who needs to be treated and when?
Recommendations of the US Preventive Services Task Force on Prostate Cancer Screening - 2009
The U.S. Preventive Services Task Force (USPSTF)

• Independent panel of nationally renowned, non-federal experts in primary care and evidence-based medicine

• Charged by U.S. Congress to review the scientific evidence for clinical preventive services and develop evidence-based recommendations for the health care community
## Grades of Recommendation

<table>
<thead>
<tr>
<th>Certainty of net benefit</th>
<th>Magnitude of net benefit</th>
<th>Substantial</th>
<th>Moderate</th>
<th>Small</th>
<th>Zero/Negative</th>
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<tr>
<td>High</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
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<tr>
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<td>B</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>I – Insufficient Evidence</td>
<td></td>
<td></td>
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</tbody>
</table>
Figure 1. Analytic Framework for Screening for Prostate Cancer

Analytic Framework

False positives

Effects of treatment 16
Benefits of Early Detection and Rx

• In men younger than age 75 years, the USPSTF found inadequate evidence to determine whether treatment for prostate cancer detected by screening improves health outcomes compared with treatment after clinical detection.

• In men age 75 years or older, the USPSTF found adequate evidence that the incremental benefits of treatment for prostate cancer detected by screening are small to none.
Harms of Detection and Early Treatment

• The USPSTF found convincing evidence that treatment for prostate cancer detected by screening causes moderate-to-substantial harms, such as erectile dysfunction, urinary incontinence, bowel dysfunction, and death. These harms are especially important because some men with prostate cancer who are treated would never have developed symptoms related to cancer during their lifetime.
USPSTF Conclusions

• Evidence is insufficient to assess the balance of benefits and harms of prostate cancer screening in men younger than age 75 years.
  Grade: I statement.

• The USPSTF recommends against screening for prostate cancer in men age 75 years or older.
  Grade: D recommendation.
Helping Men Decide

- Almost assuredly, some men who are screened and treated will live longer because of that treatment – we will have found a cancer that would have caused them harm some time in the future (most likely 10-15 years from now)
- Equally certain is that some men who were screened suffer or die needlessly from the treatment for a cancer that would never have caused them any problem in their lifetime
AUA: PSA Best Practice Statement
2009 Update

• Early detection and risk assessment of prostate cancer should be offered to asymptomatic men, 40 years of age or older when the estimated life expectancy is more than 10 years.

• Patients should be informed of the known risks and the potential benefits of cancer screening
American Cancer Society -2010
Prostate Cancer Screening Guidelines

• Men should be informed of risks and benefits through informed decision making process
• Process should begin at age 50 for men with average risk, but as early as 40 years for high-risk factors
• A serum PSA of 4.0 ng/ml should be the threshold for further action for men with average risk, but 2.5 ng/ml is appropriate if individual risk assessment is done (race, family hx, prior biopsies, DRE results)
“Latest” Recommendation from the US Preventive Services Task Force on Prostate Cancer Screening - 2011

Serum PSA based screening of men under the age of 74:
• Results in little to no reduction in prostate cancer-specific mortality at 10 years
• Presents significant harms related to over diagnosis and over treatment
Screening For Prostate Cancer

- Consensus - Early detection is the key to cure
- A new detection test and strategy is needed
  ~ to reduce the false positive rate
  ~ to identify those men with aggressive disease who actually need to be treated
PREVIOUS UNSUCCESSFUL ATTEMPTS TO IMPROVE THE SPECIFICITY OF THE SERUM PSA TEST

- Age Adjusted Reference Ranges
- PSA Velocity/Density
- Free PSA and F/T Ratio
- Complexed PSA
Current Proposed Improvements to Serum PSA

1. Serum Pro-PSA precursor forms
2. Other markers: Kallikreins (HK-2), EPCA, auto-antibodies and sarcosine
3. Gene detection in prostate cells released into urine
   ~ Panels of “selected” genes
   ~ Gene patterns defined by “supervised learning” mathematical analysis
Molecular Forms of PSA

Free PSA

PSA

proPSA

BPSA

intactPSA

AA
Complexed Disease

237
yes

239 - 244
no
Cancer

237
no
BPH

232 - 237
no
benign

*active PSA not present in serum

Mikolajczyk et al, Urology 59, 797-802, 2002
Disease-Associated Forms of Free PSA

- **BPSA** (nicked PSA)
- **BPH**
  - Transition Zone
- **pPSA** (precursor PSA)
  - Prostate
    - Peripheral Zone
  - Cancer
Multiple Forms of proPSA

APLILSR - PSA (native proPSA)

[-7]Pro

ILSR - PSA

[-4]Pro

SR - PSA

[-2]Pro
Typical Proportions of proPSA Forms in Cancer Serum with PSA 4-10 ng/ml

intact non-native
PSA 40%
proPSA 33%
BPSA 27%

[-4]pPSA 30%
[-2]pPSA 20%
[-5/-7]pPSA 50%
Pro PSA and BPSA Immunoassays


CAPTURE Biotinylated anti-PSA mAb

Streptavidin Coated Microtiter Plate
PSA Isoforms: The Next Generation of Prostate Cancer Detection

William J. Catalona, MD
Professor of Urology
Director of Clinical Prostate Cancer Program
of Northwestern’s Robert H Lurie Comprehensive Cancer Center
Northwestern University
Chicago, IL
Early Clinical Studies with Prototype Research Assays

- 1091 serum samples from men enrolled in prostate cancer screening studies
  - Innsbruck PSA 2-4: 75 No Ca 71 Ca
  - PSA 1-10: 71 No Ca 77 Ca
  - St. Louis PSA 2.5–4: 245 No Ca 164 Ca
  - PSA 4-10: 241 No Ca 144 Ca

42% cancer. Extra-prostatic tumor in 12% with PSA 2.5-4 ng/ml and 25% with PSA 4-10 ng/ml
Sensitivity and Specificity of % Pro-PSA in PSA 4-10 ng/ml Range

• In the 4-10 ng/ml range, % pro-PSA detected 90% of cancers while avoiding 31% of unnecessary biopsies
• % Free PSA avoided 20% and complexed PSA avoided 19%
Sensitivity and Specificity of Pro-PSA: 2-4 ng/ml PSA Range

• Using a ratio of pro-PSA/free-PSA for recommending biopsy, 90% of cancers were detected while avoiding 19% of unnecessary biopsies

• % Free PSA avoided 10%, complexed PSA avoided 11%
NCI-EDRN Study of % -2 proPSA
Sokoll, L et al. Ca Epid Bio Prev 2010:19;1193

- N=566 subjects screened, 245 (43%) cancers were detected

<table>
<thead>
<tr>
<th>Serum PSA</th>
<th>2.0-4.0 ng/ml</th>
<th>2.0-10.0 ng/ml</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>Specificity</td>
<td>52%</td>
<td>45%</td>
</tr>
<tr>
<td>PPV</td>
<td>~50%</td>
<td></td>
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</table>
-2proPSA and Prostate Health Index
PHI = (-2proPSA/Free PSA) (sqrt T-PSA)

• Le, B et al. J Urol 2010: 183; 1355
• N=2034 subjects screened (PSA>2.5 or +DRE)
• 74 men biopsied, 30 (41%) cancers detected
• %proPSA: spec = 48% at sens = 89%; PPV=55%
• PHI = (-2proPSA/Free PSA) (sqrt T-PSA)
• PHI: spec = 65% at sens = 89%; PPV = 54%
Proposed Improvements

1. Serum Pro-PSA precursor forms
2. Other markers: Kallikreins (HK-2), EPCA, auto-antibodies and sarcosine
3. Gene detection in prostate cells released into urine
   ~ Panels of “selected” genes
   ~ Gene patterns defined by “supervised learning” mathematical analysis
Serum Kallikreins

• HK2 – A prognostic or diagnostic marker?
  Martin et al J Urol 175:104, 2006

• HK11 – A diagnostic marker?
  No significant differences were observed for HK 11, HK 11/PSA ratio and HK11 density in men with PCa (n =36) vs without cancer (n=78)
EPCA (Epitope 2.22 and 2.19)

- Early Prostate Cancer Antigen is a nuclear matrix protein, described by R. Getzenberg, reported to be a highly sensitive and specific test for prostate cancer.
- Onconome, Inc licensed the test, but has failed to validate the clinical claims and has initiated legal action for misrepresentation against the inventor, the Univ of Pittsburgh and Johns Hopkins.
Auto-Antibody Tests for PCa

Wang, X. Lab Med 2008:39;165-171 (review article)

Panels and autoantibody signatures for early detection of PCa

• Koziol J et al. Clin Ca Res 2003:9;5120-6
  AB panel to antigens: c-myc, cyclin B1, IMP1, Koc, p53, p62, survivin

  Phage microarray defined 22 peptides for AAB test for PCa

• Oncimmune, LTD – 8 to 10 serum biomarker panel (TBD)
• Oxford Gene Technology – 15 serum biomarker panel

• Question of organ cancer specificity for each of these panel tests

• Assay issues - lot to lot consistency of recombinant ag production, reference materials and long-term assay standardization and QC materials for routine use
Sarcosine

- Sarcosine is an amino acid (glycine) metabolite, reported to be elevated in the urine of ~40% of PCa patients and not present in healthy controls.
- Early validation studies have not confirmed that claim!
  ~Jentmik et al. J Urol 2011: 185; 385
Proposed Improvements

1. Serum Pro-PSA precursor forms
2. Other markers: Kallikreins (HK-2), EPCA, auto-antibodies and sarcosine
3. Gene detection in prostate cells released into urine
   - Panels of “selected” genes
   - Gene patterns defined by “supervised learning” mathematical analysis
mRNA Based Detection of Prostate Cancer Cells in Urine

- Prostatic massage causes release of prostate cancer cells into the urine

- Detection of prostate specific genes in urine, such as DD3 (PCA3), has potential use for detection of prostate cancer.

Bussemakers, M, Ca Res 59:5975, 1999
PCA3 – Prostate Cancer Antigen

- Prostate-specific, non-coding mRNA
- Low expression level in normal prostate tissue
- Over-expressed in ~90% of prostate tumors (~60 to 100-fold), which allows for discrimination
- Feasibility of quantitative urine test first demonstrated in Schalken laboratory
- PCA3 Score: PCA3 mRNA levels normalized to prostate-specific housekeeping gene (PSA mRNA)

PCA3 Test Procedure – Gen Probe

Digital Rectal Exam (3 strokes per lobe) → Urine Specimen → PCA3 and PSA mRNA concentrations measured in separate tubes

Quantitative ratio of PCA3/PSA mRNA = PCA3 Score

- PCA3 Score < cutoff → Lower risk of positive biopsy
- PCA3 Score ≥ cutoff → Higher risk of positive biopsy

PCA3 Score Correlates with Probability of Positive Biopsy

Overall 34% biopsy positive

Source: CE-marked European package insert
Subject group = 529 men scheduled for prostate biopsy
Recent PCA3 Validation Studies

  N=583 men screened, PSA 3.0-15.0, 534 (~90%) were informative (mRNA extracted), sensitivity = 65% and specificity = 66%
  N=72 (17 PCa; 55 bx neg controls), informative (?), sensitivity=60%, specificity=70%
- Roobol, M et al. Eur Uro 2010:58;475
  N=721 screened, PSA>3.0, informative (?), sensitivity = 68%; specificity = 60%. PPV PCA3=24% and PPV PSA=20%.
- Report from European Multi Center Study of PCA3-Apr 16, 2010
  N=516 screened, PSA 2.5-10.0, informative (?), sensitivity = 64% and specificity = 76%
Summary for PCA 3 Test

- PCA 3 score improves the detection of prostate cancer. At a cutoff score of 35, the sensitivity is ~ 60% and specificity ~ 70%). The test predicts the probability of a positive biopsy, e.g. score > 50 has PPV of greater than 50% - but too many cancers are missed if it used as a stand alone test and its main use is for selecting men for re-biopsy.
- Does PCA3 score have prognostic significance and can it identify indolent cancers?
A Four Gene Expression Signature for Prostate Cancer Cells Consisting of UAP1, PDLIM5, IMPDH2, and HSPD1

Isabelle Guyon, Herbert A. Fritsche, Paul Choppa, Li-Ying Yang and Stephen D. Barnhill,

Health Discovery Corporation, Savannah, GA

In collaboration with Thomas A. Stamey, MD
Department of Urology, Stanford University School of Medicine

Research effort to discover biomarkers for “aggressive” prostate cancer

1. Initiative of Dr. Thomas Stamey
   Head, Dept of Urology
   Stanford University Medical Center
2. Radical Prostatectomy Specimens, n=87
3. Fresh frozen, laser microdissected, and carefully labeled cell types
4. Analyzed with Affymetrix U133A Microarray Chip (>20,000 genes)
### Quality data

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<th>Zone</th>
<th>Histological classification</th>
<th>Num</th>
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<tr>
<td>CZ</td>
<td>Normal (NL)</td>
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<tr>
<td></td>
<td>Dysplasia (Dys)</td>
<td>4</td>
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<tr>
<td></td>
<td>Grade 4 cancer (G4)</td>
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<td>PZ</td>
<td>Normal (NL)</td>
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<tr>
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<td>Grade 3 cancer (G3)</td>
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<td>Grade 4 cancer (G4)</td>
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<td>TZ</td>
<td>Benign Prostate Hyperplasia (BPH)</td>
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<td>Grade 4 cancer (G4)</td>
<td>8</td>
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<tr>
<td>Total</td>
<td></td>
<td>87</td>
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Affymetrix **U133A** microarray, ~20,000 genes
AUC ranking (N = 140)
Support Vector Machines (SVM)

Linear SVM

Boser-Guyon-Vapnik 1992

Non-linear SVM

Test error rate 14.6%

Test error rate 9.7%
Genes Overexpressed in PCa

RFE Selected Genes

UMPK F5 POV1 PP1B RGS10 IMPDH2 MRPL12 MACMARCK PYCR1 HPN LIM EIF3S8 HOXC6 EZH2 BAZ1A MTHFD1 L3P1 CLDN8 DKFZp564 AMACR GA17 SAICAR HSPD1 CCT3

Italic: known PCa biomarkers

Figure 3: Heat map of 19 genes, free of IP rights according to our records (red means over-expressed, blue means under-expressed). In color: the panel of complementary genes selected by SVM-RFE. In gray italic: other top ranking genes bound by third party IP rights.
### Biological Significance of Best Classifier Genes Selected by the SVM RFE

<table>
<thead>
<tr>
<th>Name</th>
<th>UAP1</th>
<th>PDLIM5</th>
<th>IMPDH2</th>
<th>HSPD1</th>
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<td>Unigene</td>
<td>Hs.492859</td>
<td>Hs.480311</td>
<td>Hs.476231</td>
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<td>Chromosome</td>
<td>1q23.3</td>
<td>4p527.0</td>
<td>3p21.2</td>
<td>2q33.1</td>
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<td>Genbank</td>
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<td>BC002676.1</td>
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<td>EC #</td>
<td>2.7.7.23</td>
<td>NA</td>
<td>1.1.1.205</td>
<td>NA</td>
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<td>Pathway</td>
<td>Aminosugar metabolism</td>
<td>Unknown, but LIM domain interacts with Cytoskeleton</td>
<td>De novo guanine nucleotide biosynthesis</td>
<td>Mitochondrial Chaperonin HSP 60</td>
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<td>Comment</td>
<td>Androgen responder-assoc with PCa and male infertility</td>
<td>Over-expressed in PCa. Similar to LIM domain protein</td>
<td>Anti-tumor drug target for inhibition</td>
<td>Upregulated in prostate cancer</td>
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A New Molecular Signature for Prostate Cancer

A four-gene panel test was developed on a PCR platform and shown to detect cancer cells in both formalin-fixed and fresh frozen tissues with high accuracy and the findings were published in the peer-reviewed publication – UroToday International Journal: 2, Aug, 2009
Validation In A Blinded Clinical Trial

N = 32 cancer, 19 BPH, 18 Normal

AUC = 0.97; sensitivity = 90%, specificity = 96%
Is there a role for a prostate tissue bx test?

- 1 million prostate biopsies are performed annually in the USA
- ~75% of these are reported as negative, but 20-30% of these are “false” negative, as shown by second and third biopsies
- Therefore many additional biopsies are performed on those men who are identified as high risk for PCA
- Can a genomic test better identify men who require additional biopsy by detection of a cancer “field effect”?  
- The area surrounding a cancer foci is not normal tissue!

Nonn L, et al. Evidence for Field Cancerization of the Prostate, Prostate DOI.10.1002/pros.20983

A validation study of the 4-gene tissue test for detection a cancer field effect is being designed with collaborators. The study objectives are:
1. How far from the tumor foci can gene abnormality be detected?
2. Can these gene biomarkers better identify who needs a 2nd bx?
New Diagnostic Approach

• HDC 4-gene prostate cancer test in urine for cancer detection
• Triad of Prostate Cancer Screening Tests
• DRE or PSA Reflex to Gene Test for BX

Elevated PSA  Abnormal Rectal Exam  HDC Genomic Test on Urine  Biopsy
Can the prostate genes be detected in urine?

We split a single female urine specimen into three specimens and spiked each one with RNA extract from PC3 cancer cells, a high grade prostate cancer cell line.
Results of the Urine Cell Spiking Experiment
We were able to get a signal that seemed to be concentration dependent!
Summary of HDC Studies to Establish the Urine 4-Gene Profile Test

1. Optimized RT-PCR reaction conditions, prepared standard curves, selected positive (PC3) and negative (leukocyte) controls and validated the assays using PC3 RNA extract spiked into urine from healthy subjects.

3. Established the sample collection conditions to ensure RNA stability in urine (RNase inhibitor added).

4. Selected 6 reference genes to evaluate, as B2M alone was not a reliable reference gene in urine.

5. Developed an algorithm to normalize target gene expression and for summing the normalized target gene expression (S-score).

6. Performed initial clinical studies on urine sediment obtained from cancer subjects and men scheduled for biopsy.
Validation Study of Urine Gene Test

- N=49 men who had serum PSA > 2.5 ng/ml or a positive DRE and were scheduled for biopsy
- Urine was collected without DRE, coded and sent to lab for testing in a blinded manner
- Urine sediment was obtained by centrifugation, extracted for RNA, subjected to PT-PCR assays and S score calculated (positive value = cancer and negative value = non-cancer)
Prospective, Blinded Validation Study

N= 49 men with PSA> 2.5 or +DRE

- The HDC urine four-gene profile test (with six reference genes) showed a sensitivity of 68% in 20/29 cancer cases and a specificity of 75% in 15/20 for the non-cancer subjects.

The sensitivity and specificity of the HDC gene test was almost as good as the PCA3 test (65%/83%), and the HDC test was performed on urine collections obtained without a prostatic massage being performed, as is required in the Gen Probe assays.

While the prostatic massage may increase the detection rate of the HDC gene test as it does for the PCA3 test, it limits the test’s use in general screening.
Clinical Study Results

• The HDC urine four-gene profile test (with six reference genes) correctly identified all 4 men who had cancer in the group of 12 men who were scheduled for a biopsy.

• The test was negative in 5/8 (60%) men who had a negative biopsy, which means that the test could be used to significantly reduce the rate of unnecessary biopsies.

• The apparent false positive rate of 3/8 in this group might be an overestimation, since second or third biopsies might be needed to detect the prostate cancer (there is a 20% cancer detection rate for men undergoing repeat biopsy).
The invention provides methods for isolating RNA from the soluble fraction of urine. The methods can be used for detecting the presence or absence of an RNA, or quantifying the amount of an RNA. The methods are useful for diagnosing an individual suspected of having a disease by detecting the level of RNA associated with the disease in the soluble fraction of urine. The methods are also useful for prognosing an individual diagnosed with a disease by detecting the level of RNA associated with the disease in the soluble fraction of urine.
Patent from Quest

• The published patent application from Quest for isolating the soluble fraction of RNA from urine demonstrates that a significant amount of mRNA from the four target genes and reference genes that are used in the HDC Urine Gene Test is present in the supernatant!
New One Step Urine Assay for Sediment and Supernatant

• We have developed a new assay for “whole” urine that is equivalent to assays of sediment and supernatant
• No centrifugation is required
• Entire urine sample is filtered through a 22 micron filter, which captures both tumor cells and free RNA. The filter device is subjected to RNA extraction.
ROC for the “Whole Urine” Assay
At sensitivity=80%, specificity=84%
At sensitivity=90%, specificity=60%
Summary

• The HDC urine four-gene profile test appears to have excellent sensitivity (~80%) and specificity (~80%) for prostate cancer detection.

• The test accuracy may improve with urine samples collected after prostatic massage.

• A larger clinical trial is being planned to confirm the test performance and to compare the HDC test with the PCA3 test (Gen-Probe).
Other Gene Tests for PCA?

Gene test (AMACR, PCA3 and PSA ref gene) performed on urine sediment after prostatic massage in 43 Ca and 49 non Ca.
Sensitivity = 81% and specificity = 84%.

AMACR is alpha-methylacyl-CoA racemase
Other Gene Tests for PCA?

- ASCO Meeting 2009 Abstract Ross et al, Dana Farber. Blood test of six genes, 5 decreasing and one increasing in PCa. In 204 Ca, 110 BPH and 170 normals, sensitivity = 86% and specificity = 83%
- Test licensed to Source MDx, and clinical trial of 1000 men is reported to be underway.
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Conclusions

I. Current serum PSA-based cancer detection strategies must be enhanced to significantly reduce the false positive rate and the number of unnecessary biopsies.

II. Various “reflex test” opportunities to improve the clinical specificity while maintaining the 80-90% detection rate include:
   - Serum Pro-PSA variants and PHI
   - Urine PCA3 gene test
   - Urine 4-gene panel
   - Other gene expression tests in development

III. The next challenge in prostate cancer detection is to detect only the clinically significant cancers and to prove the efficacy of prostate cancer screening for improved survival.
Serum PSA for Clinical Management of Prostate Cancer

NCCN Guidelines for Prostate Cancer - Version 4.2011

~ Serum levels should drop to “undetectable” (0.2 ng/ml) after prostatectomy or ablative radiotherapy
~ Detectable serum PSA levels after “curative” therapy indicates the presence of residual disease
~ Detectable serum levels observed during long term follow up indicate recurrence or metastasis
~ Status of recurrent disease can be assessed with serum PSA, until the cancer cells become hormone resistant
Thank You!