Prostate Cancer Detection Biomarkers “Non-Genomic”

27th Annual International Prostate Cancer Update

- Prostate Health Index (phi) (Blood)
- PCA3 (Urine)
- ConfirmDx (Tissue)
- Exo106 (Exosome-Urine)

- Disclosure – Genprobe/Hologics, Exosome, Beckman, MDxHealth (Investigator)

- Alan W. Partin, MD, PhD
  The Johns Hopkins School of Medicine
  Baltimore, MD

TWITTER - @alan_partin
Other Important Biomarkers NOT planning to discuss

- 4KScore
- Decipher (Genomic Classifier)
- Prolaris
- SNP’s (deCODE)
- PTEN (ProMark)
- OncotypeDx (GPS)
A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range.


Northwestern University, Johns Hopkins University, Harvard University, University of Michigan, Mayo Clinic, Erasmus University, Vanguard Urologic Institute, University of California Los Angeles, University of Utah, Beckman Coulter, Inc,

\[ \phi = \left( \frac{p2PSA}{fPSA} \right) \times \text{square root } PSA \]
## Individual Risk Assessment

Higher \( \phi \) values associated with increased risk

<table>
<thead>
<tr>
<th>( \phi )</th>
<th>&lt;25</th>
<th>25-34.9</th>
<th>35-54.9</th>
<th>&gt;55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Risk PCa</td>
<td>Ref.</td>
<td>1.6</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>% Prob. PCa</td>
<td>11</td>
<td>18</td>
<td>33</td>
<td>52</td>
</tr>
</tbody>
</table>
Development and Internal Validation of a *Prostate Health Index Based Nomogram* for Predicting Prostate Cancer at Extended Biopsy

Giovanni Lughezzani,* † Massimo Lazzeri, † Alessandro Larcher, Giuliana Lista, Vincenzo Scattoni, Andrea Cestari, Nicoló Maria Buffi, Vittorio Bini and Giorgio Guazzoni

Journal of Urology, Vol. 188, 1144-1150, October 2012

**Table 3. Specificity at 90% sensitivity of variables predicting PCA at biopsy**

<table>
<thead>
<tr>
<th>Predictors</th>
<th>% Specificity at 90% Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPSA</td>
<td>9.3 (6.8–12.4)</td>
</tr>
<tr>
<td>% fPSA</td>
<td>17.8 (14.4–21.7)</td>
</tr>
<tr>
<td>p2PSA</td>
<td>15.6 (12.4–19.3)</td>
</tr>
<tr>
<td>PHI</td>
<td>27.0 (22.1–30.4)</td>
</tr>
<tr>
<td>Base multivariable model:*</td>
<td>33.8 (29.1–38.8)</td>
</tr>
<tr>
<td>+ tPSA</td>
<td>34.6 (29.9–39.6)</td>
</tr>
<tr>
<td>+ % fPSA</td>
<td>35.1 (30.4–40.1)</td>
</tr>
<tr>
<td>+ p2PSA</td>
<td>35.6 (30.9–40.7)</td>
</tr>
<tr>
<td>+ PHI</td>
<td>41.6 (36.6–46.7)</td>
</tr>
</tbody>
</table>

* Including age, prostate volume, DRE and biopsy history.
PHI for prediction of Significant cancers

The Prostate Health Index Selectively Identifies Clinically Significant Prostate Cancer

ALL  Epstein Significant  GS > 7
Prostate Health Index (PHI) Predicts High-Stage Pathology in African-American Men


Reference

Area under ROC curves

Baseline (DRE): 0.602
Baseline + %p2PSA: 0.739
Baseline + PSA: 0.635
Baseline + PHI: 0.750

Urology. 2016 Apr;90:136-40
PCA3

- PCA3 is a prostate-specific noncoding RNA that is over-expressed 60-100 fold more in prostate tumors.
- PCA3 can be detected in the urine.
- The over-expression of PCA3 mRNA can be quantified and expressed relative to the PSA gene.
**PCA3 Urine Assay Procedure**

**Quantitative ratio of PCA3/PSA mRNA = PCA3 Score**

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

PCA3: a molecular urine assay for predicting prostate biopsy outcome.

Deras IL, Aubin SM, Blase A, Day JR, Koo S, Partin AW, Ellis WJ, Marks LS, Fradet Y, Rittenhouse H, Groskopf J.
Gen-Probe, Inc., San Diego, California 92121, USA.
USE OF PCA3 URINARY ASSAY TO SUPPLEMENT PSA-BASED PROSTATE CANCER SCREENING

EDRN GU Group
John Wei, MD, MS (PI)
Martin Sanda, MD
Ian Thompson, MD
Alan Partin, MD
Ziding Feng, PhD
Lori Sokol, PhD
Jacob Kagan, PhD

Genprobe
Jack Groskopf, PhD

Study Sites
• Johns Hopkins (Alan Partin)
• Harvard BIDMC (Martin Sanda)
• U of Michigan (John Wei, Montie)
• UT San Antonio (Ian Thompson)
• Wash Univ St Louis (Adam Kibel)
• Fox Chase Cancer Center (Rosalia Verterbo)
  • NYU (Samir Taneja)
• San Diego Clinical Trials (Mohamed Bidair)
  • Univ. of Washington (Dan Lin)
  • UT Southwestern (Yair Lotan)
  • Univ. of Alabama (Erik Busby)

N=568   J Clin Oncol. 2014 Dec 20;32(36):4066-72
### Initial Biopsy: High PPV @ PCA3 score > 60

<table>
<thead>
<tr>
<th>Test result (PCA3 score &gt; 60)</th>
<th>Cancer</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (%)</td>
<td>Absent (%)</td>
</tr>
<tr>
<td>Positive (&gt; 60)</td>
<td>112 (20)</td>
<td>28 (5)</td>
</tr>
<tr>
<td>Negative (≤ 60)</td>
<td>153 (27)</td>
<td>275 (48)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>265 (47)</td>
<td>303 (53)</td>
</tr>
</tbody>
</table>

SENS: \( \frac{112}{265} = 0.42 \) (95% CI: 0.36 – 0.48)
SPEC: \( \frac{275}{303} = 0.90 \) (95% CI: 0.87 – 0.94)
PPV: \( \frac{112}{140} = 0.80 \) (95% CI: 0.72 – 0.86)
NPV: \( \frac{275}{428} = 0.64 \) (95% CI: 0.60 – 0.69)

If cancer is present, PCA3 will be positive 80% of the time.

### Repeat Biopsy: High NPV @ PCA3 score < 20

<table>
<thead>
<tr>
<th>Test result (PCA3 score &lt; 20)</th>
<th>Cancer</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (%)</td>
<td>Absent (%)</td>
</tr>
<tr>
<td>Positive (≥ 20)</td>
<td>51 (17)</td>
<td>116 (38)</td>
</tr>
<tr>
<td>Negative (&lt; 20)</td>
<td>16 (5)</td>
<td>119 (40)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>67 (22)</td>
<td>235 (78)</td>
</tr>
</tbody>
</table>

SENS: \( \frac{51}{67} = 0.76 \) (95% CI: 0.64 – 0.86)
SPEC: \( \frac{120}{235} = 0.51 \) (95% CI: 0.44 – 0.57)
PPV: \( \frac{51}{167} = 0.31 \) (95% CI: 0.24 – 0.38)
NPV: \( \frac{119}{135} = 0.88 \) (95% CI: 0.81 – 0.93)

If cancer is not present, PCA3 will be negative 88% of the time.
Epigenetics: DNA Methylation
From Molecular Biology to Medical Diagnostics
DNA Methylation

Methylation present in all cancers:
- Most efficient way to shutdown gene expression
- Frequency of methylation in different cancer tissues is attractive
- Small number of biomarkers provides clinically relevant information

Highly stable relative to mRNA and proteins

Sensitive detection in all sample types:
- Including FFPE and serum
- Detects 1 cancer cell among 10,000 normal cells

Methylation-specific PCR runs on any PCR machine
- Automatable, reproducible, reliable
Prostate Cancer Diagnosis

Prostate Cancer in the USA

30 million
PSA screenings\(^1,2\)

1.5 million
abnormal PSA test results\(^3\)

1,000,000
prostate biopsy procedures\(^4\)

250,000
diagnosed with prostate cancer\(^5\)

30,000
deaths\(^5\)

\(~750,000\) negative biopsy procedures

Sampling Error

Challenge with current methods:
- Standard of care for biopsy = 12 cores
- The needle may miss the tumor focus
- Pathologists can only interpret what is on the slide

A biopsy procedure samples less than 1% of the entire gland

Potential application

- PSA Screening
  - Prostate DRE

- Elevated PSA
  - Abnormal DRE

- Biopsy
  - Pathology

- Negative Histology

- Prostate ConfirmMDx

- Continued routine screening

- Repeat Biopsy
MATLOC Study

Methylation Analysis To Locate Occult Cancer
Detection Of Cancer Using methylated Events in Negative Tissue
Clinically, the epigenetic profile of prostatic biopsy tissue, resulted in a negative predictive value (NPV) of 90%, identifying a large procedure. The epigenetic profile of GSTP1, RASSF1, RASSF1, and APC are all significant predictors for PCa risk, especially to identify those men who should forego a repeat biopsy following a negative initial biopsy. An integrated risk model, which included both clinical and epigenetic parameters, resulted in a sensitivity of 74%, a specificity of 63% and an NPV of 91%:

\[
\text{OR} = \frac{\text{Positive cases}}{\text{Negative cases}} = \frac{59}{28} = 2.107
\]

\[
\text{OR} = \frac{\text{Positive cases}}{\text{Negative cases}} = \frac{143}{253} = 0.565
\]

68% sensitivity, 64% specificity, 90% negative predictive value.

**Relative Contribution**

- **GSTP1**: 30%
- **APC**: 42%
- **RASSF1**: 28%

Partin AW *et al.* (submitted). Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies.
**Background:** Epigenetic Assay is Most Significant Independent Predictor of PCa Detection on Repeat Biopsy

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.1981</td>
</tr>
<tr>
<td>PSA</td>
<td>1.02</td>
<td>0.2191</td>
</tr>
<tr>
<td>HGPIN</td>
<td>1.13</td>
<td>0.5674</td>
</tr>
<tr>
<td>Atypia</td>
<td>2.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>Epigenetic Assay</td>
<td>3.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Risk Stratification of Aggressive Prostate Cancer Based on Combined Epigenetic and Clinical Data of Men with Initial Cancer-Negative Biopsies**

**AUA 2015 – New Orleans**

Prostate. 2016 April

<table>
<thead>
<tr>
<th>The Johns Hopkins School of Medicine</th>
<th>University of Edinburgh</th>
<th>Maastricht University Medical Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alan W Partin</td>
<td>Grant D Stewart</td>
<td>Leander Van Neste (*)</td>
</tr>
<tr>
<td>Jonathan I Epstein</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>MDxHealth (*)</th>
<th>University of St Andrews</th>
<th>Ghent University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph Bigley</td>
<td>David J Harrison</td>
<td>Wim Van Criekinge (*)</td>
</tr>
</tbody>
</table>
**Objective**: Development of an Epigenetic Risk Score for Clinically Significant PCa

- Negative Biopsy
- Persistent Risk Factors

DNA Methylation

- NPV ~96% Significant PCa
- NPV ~90% All PCa

- Clinically Significant or Indolent PCa?

Develop Algorithm for Significant PCa Risk
Materials and Methods:

Epigenetic Multiplex DNA Methylation Test (ConfirmMDx)
- *GSTP1* – DNA detoxification
- *APC* – apoptosis
- *RASSF1* – cell cycle regulation

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>PSA (mean)</th>
<th>Age (mean)</th>
<th>DRE</th>
<th>Histopathology</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>Low Risk</td>
<td>226</td>
<td>6.8</td>
<td>64</td>
<td>63%</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>benign</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67%</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HGPIN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67%</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>atypia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8%</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>High Risk</td>
<td>43</td>
<td>8.2</td>
<td>67</td>
<td>57%</td>
<td>65%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14%</td>
</tr>
</tbody>
</table>
Results: Multivariate Logistic Regression
Epigenetic Health Index (EHI)

Odds Ratio for GS >= 7

Risk Factor

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGPIN</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
</tr>
<tr>
<td>% positive cores</td>
<td>1</td>
</tr>
<tr>
<td>PSA</td>
<td>2</td>
</tr>
<tr>
<td>Atypia</td>
<td>2</td>
</tr>
<tr>
<td>Methylation Intensity</td>
<td>16</td>
</tr>
</tbody>
</table>
Validation of a Novel Non-Invasive Urine Exosome Gene Expression Assay (EXO106) to Predict High-Grade Prostate Cancer in Patients Undergoing Initial Biopsy with an Equivocal PSA

American Urological Association (AUA) Annual Meeting 2015
JAMA Oncol. 2016 Mar 31 - [Epub ahead of print]
Authors

- James McKiernan*
- Michael Donovan, NYC, NY
- Vince O'Neill, Cambridge, MA
- Stefan Bentink, Martinsried, Germany
- Mikkel Noerholm, Martinsried, Germany
- Susan Belzer, St. Paul, MN
- Johan Skog, Cambridge, MA
- Alan Partin, Baltimore, MD
- Gerald Andriole, St. Louis, MO
- Gordon Brown, Vorhees, NJ
- James Cochran, Dallas, TX
- John Wei, Livonia, MI
- Ian Thompson, San Antonio, TX
- Peter Carroll, San Francisco, CA
Exosomes: Rich Source of Molecular Details

- Exosomes and microvesicles are secreted by virtually all cells into all biofluids, as an active process of cellular communication.
- Exosomes are lipid bilayer protected vesicles, which makes them stable under varying conditions and protects their contents from degradation.
- Exosomes contain RNA (mRNA, microRNA, tRNA, rRNA, lncRNA, and other RNA species), DNA and protein.

Miranda K et al., Kidney International 2010; 78:191-199.
EXO106 Urine-Based Liquid Biopsy Test

3 gene signature on exosomal RNA

ERG qPCR
(ETS transcription factor, partner of TMPRSS2 fusion)

PCA3 qPCR
(LncRNA, Prostate Cancer Antigen 3)

SPDEF qPCR
(SAM pointed domain containing ETS transcription factor; androgen independent transactivator of PSA)

Multivariate Algorithm

High grade cancer (GS>6)
EXO106 risk score

Intended Use:
The EXO106 risk score is used to predict the presence of high grade (GS ≥ 7) prostate cancer for men 50 years or older with a PSA 2-10ng/mL presenting for an initial biopsy.
Discrimination for High-Grade Cancer

EXO106 + SOC:
AUC: 0.725

EXO106:
AUC: 0.711

SOC:
AUC: 0.631

PSA:
AUC: 0.545

AUC_{EXO106+SOC} > AUC_{SOC} \quad P < 0.00004
Performance of the EXO106 Cut-Point to Identify High-Grade Disease in Validation Cohort

<table>
<thead>
<tr>
<th>Biopsy Result</th>
<th>Gleason≥7</th>
<th>Negative + Gleason=6</th>
<th>Total</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXO106 &gt; cut point</td>
<td>136</td>
<td>245</td>
<td>381</td>
<td>Sensitivity % 91.89</td>
</tr>
<tr>
<td>EXO106 ≤ cut point</td>
<td>12</td>
<td>126</td>
<td>138</td>
<td>Specificity % 33.96</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>371</td>
<td>519</td>
<td>PPV % 35.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NPV % 91.30</td>
</tr>
</tbody>
</table>

High Grade Biopsy Prevalence % 28.52
EXO106 Negative (%) 26.59

Majority of false negatives (9/12) were GS 3+4 with < 3 positive cores

High grade disease defined as Gleason ≥4+3, false negative rate <5%
Conclusions

- Exosomal mRNA can be isolated and analyzed using a first-catch, random urine
- The EXO106 test (combining PCA3, ERG and SPDEF) provides a high NPV for high-grade cancer
- Of 148 cases of Gleason 7, test only missed 3 cases of dominant pattern 4
  - False negative rate of <5%
- 2016 now CLIA available/approved assay
## Summary

<table>
<thead>
<tr>
<th>Marker</th>
<th>Source</th>
<th>FDA</th>
<th>CLIA</th>
<th>Diagnosis</th>
<th>Prognosis</th>
<th>Cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phi</td>
<td>Beckman</td>
<td>6/2012</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>70-400</td>
</tr>
<tr>
<td>PCA3</td>
<td>Hologics</td>
<td>2/2012</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>440</td>
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<tr>
<td></td>
<td>Gen-probe</td>
<td></td>
<td></td>
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<tr>
<td>Exo106</td>
<td>Exosome</td>
<td>PEND</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>400-500</td>
</tr>
<tr>
<td>ConfirmDx</td>
<td>MDxHealth</td>
<td>2014</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>2400</td>
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