Current and future biomarkers in prostate cancer

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Experimental Urology
Radboud University Nijmegen
Challenges generated by clinical studies

• ERSPC (population based screening for prostate cancer)
  - Evidence that prostate cancer related mortality can be reduced by at least 20%!
  - BUT at the cost of significant over-treatment

• Challenge for translational research
  - Biomarkers for clinically significant prostate cancer
New markers

• **Phase 1:**
  - Exploratory study using home made first generation test

• **Phase 2:**
  - Establishment of a reproducible assay; both inter- and intra-assay variability should be assessed

• **Phase 3:**
  - Retrospective-or prospective analysis of biomarker using standardised/second generation test

• **Phase 4:**
  - Prospective multi-centre evaluation of biomarker

• **Phase 5:**
  - Clinical implementation / commercialisation of a biomarker

• **Phase 6:**
  - CE-marked test (EC)/FDA-approval of test (US)
The current evaluation status of biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Substrate</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
<th>Phase 5</th>
<th>Phase 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1+ methylation</td>
<td>Urine/Bx</td>
<td>yes</td>
<td>yes</td>
<td>ongoing</td>
<td>ongoing</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>PCA3&lt;sup&gt;DD3&lt;/sup&gt;</td>
<td>Urine</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>End 2006 PROGENSA™</td>
<td>2007/2012 PROGENSA™ CE-marked FDA</td>
</tr>
<tr>
<td>T2-erg</td>
<td>Urine</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
Target discovery; identify differentially expressed genes
‘..the methods change, the ‘song’ remains the same..’”

- Differential screening of cDNA libraries (‘85)
- Subtraction hybridization (‘89)
- Differential display (Pardee et al-1992)
- Array technology (~2000)
  - cDNA
  - Oligo
- Next Generation Sequencing (2010)
Molecular profiling: DD3...DD3 and PCA3

PCA3^{DD3}

(Bussemakers et al., Cancer Res. 59: 5975-79, 1999)

Overexpressed in >95% of PrCa

DD3: A New Prostate-specific Gene, Highly Overexpressed in Prostate Cancer


University Hospital Nijmegen, Nijmegen, the Netherlands; M. J. G. B., A. V. B., G. W. V., F. P. S., J. A. S., F. M. J. D. I., and James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore.
PCA3, a non-coding (nc)RNA (1996..controversial...)

- Non-coding RNA
  - No large open-reading frames (protein coding regions)
    - Random 3\textsuperscript{rd} base composition (no codon pref.)
ncRNAs as biomarkers
(2010….of course…)

- miRNAs can be detected in plasma of patients with prostate cancer
- Yet, the first PrCa ncRNAs are ‘long’ ncRNAs, PCA3 and PCGEM
- PCA3 is still the most PrCa specific gene described to date
PCA3 is **66-fold** over-expressed in malignant prostate tissue

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>PCA3 mRNA copies/μg tissue (x 10^5): range</th>
<th>PCA3 mRNA copies/μg tissue (x 10^5): median</th>
<th>Fold over-expression: median</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH (n=12)</td>
<td>0.2-10.1</td>
<td>2.4</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 10% PCa (n=13)</td>
<td>6.6-166.0</td>
<td>25.3</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 10% PCa (n=27)</td>
<td>7.0-994.0</td>
<td>158.4</td>
<td>66</td>
</tr>
</tbody>
</table>
Cells in prostatic urethra

Digital Rectal Exam (DRE)
PCA3 clinical studies

• Post-DRE urine-analysis of PCA3 mRNA to help in the diagnosis of PCa
Clinical implementation requires a validated molecular diagnostic technology platform

- PROGENSA quantitative PCA3 Assay

Simple, non-invasive, urine specimen collected post-DRE

DRE (3 strokes per lobe)

First catch urine specimen (20-30 mL)

Transport tube with urine (2 mL) for 7 days/ambient temperature

What is measured in the urine post-DRE?

PCA3 mRNA
Measuring PCA3 mRNA to calculate the PCA3 Score

Using transcription-mediated amplification technology, PCA3 mRNA molecules are amplified

PCA3 Assay

Quantitative PCA3 Score
Studies using quantitative PCA3 Assay for diagnosing PCa show consistent results

<table>
<thead>
<tr>
<th>Study: technology</th>
<th>N</th>
<th>Mean PSA</th>
<th>Pos Bx</th>
<th>Sens</th>
<th>Spec</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: TRF</td>
<td>108</td>
<td>11.3</td>
<td>22%</td>
<td>67%</td>
<td>83%</td>
<td>90%</td>
</tr>
<tr>
<td>2: TRF</td>
<td>534</td>
<td>33%</td>
<td>65%</td>
<td>66%</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>3: PROGENSA</td>
<td>68</td>
<td>7.7</td>
<td>24%</td>
<td>69%</td>
<td>79%</td>
<td>89%</td>
</tr>
<tr>
<td>4: PROGENSA</td>
<td>226</td>
<td>7.4</td>
<td>27%</td>
<td>58%</td>
<td>72%</td>
<td>83%</td>
</tr>
<tr>
<td>5: PROGENSA</td>
<td>199</td>
<td>8.2</td>
<td>25%</td>
<td>57%</td>
<td>73%</td>
<td>84%</td>
</tr>
<tr>
<td>6: PROGENSA</td>
<td>463</td>
<td></td>
<td></td>
<td>56%</td>
<td>76%</td>
<td>85%</td>
</tr>
<tr>
<td>Total</td>
<td>1598</td>
<td></td>
<td>63 %</td>
<td>74 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCA3 clinical studies

- Post-DRE urine-analysis of PCA3 mRNA to help in the diagnosis of Pca
- ‘..What can PCA3 add when we have used serum PSA?..’
Studies in men scheduled for repeat biopsy

Elevated Serum PSA

Initial Biopsy

PCa

Biopsy Positive

Biopsy Negative

Repeat Biopsy

OR

Use PCA3

WHAT TO DO NEXT?
Two comparable studies performed, with very consistent outcomes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>US study</th>
<th>European study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men</td>
<td>233</td>
<td>463</td>
</tr>
<tr>
<td>• mean serum PSA</td>
<td>7.4 ng/mL</td>
<td>8.2 ng/mL</td>
</tr>
<tr>
<td>Informative rate</td>
<td>97%</td>
<td>100%</td>
</tr>
<tr>
<td>Number of men with positive Bx</td>
<td>27%</td>
<td>25%</td>
</tr>
</tbody>
</table>

A PCA3 Score cut-off of 35 provides the greatest diagnostic accuracy for repeat biopsy.

US study (n=233)
Sensitivity: 57%
Specificity: 73%

European study (n=463)
Sensitivity: 56%
Specificity: 75%

AUC ROC
Serum PSA: 0.524
PCA3 Score: 0.678

AUC ROC
% free PSA: 0.572
PCA3 Score: 0.698
The higher the PCA3 Score, the greater the likelihood of a positive repeat biopsy

US study (n=233) 27% positive Bx

European study (n=463) 25% positive Bx
PCA3 clinical studies

• Post-DRE urine-analysis of PCA3 mRNA to help in predicting the **prognosis** of PCa: preliminary data
Hypothesis

- PCA3 measures PrCa cells in urine
- Urine contains a mixture of normal and cancer cells
- PSA mRNA in ratio normalizes for cells of prostatic origin (normal and malignant)
- High tumor volume + increased potential to invade into prostate ductal system (grade ↑) results in higher fraction of cancer cells → PCA3 ↑
The PCA3 Score in low volume / low grade PCa is significantly lower than in significant PCa.

Low volume: tumour volume < 0.5 mL; Low grade: Gleason Score ≤ 6

P = 0.004

PCA3 and significant prostate cancer

- PCA3 can discriminate insignificant from significant cancer
  - 8 studies confirm this observation (7 independent cohorts)

- Preliminary conclusions:
  - PCA3 aids in the discrimination ‘insignificant-’ from ‘significant’
Studies in men scheduled for first biopsy (de la Taille, EAU 2011)
Number of men with first Bx- or Bx+
40% of men had PCA3 below a cut-off of 20, 60% below 35
**Number of men with first Bx+**

*Probability of Bx+ increases with increasing PCA3 Score*

![Bar chart showing the probability of Bx+ (PPV) for different PCA3 Score ranges. The chart indicates that the probability increases with higher PCA3 scores, with 17% in the 0-20 range, 38% in the 20-35 range, 53% in the 35-75 range, and 78% in the >75 range.]*
Number of men with first Bx+

Probability of Bx+ increases with increasing PCA3 Score

Probability of Bx+ (PPV)
Number of men with first Bx+

Probability of Bx+ increases with increasing PCA3 Score

95% of men with PCa Gleason ≥ 7 have PCA3 Score above 20
87% of men with PCa Gleason ≥ 7 have PCA3 Score above 35
Clinical implications of PCA3 testing

- This European study showed that PCA3, in patients with a moderately elevated PSA level, increases prostate cancer detection while unnecessary prostate biopsies can be avoided.

- PCA3 was superior to serum total PSA, PSAD and % free PSA for predicting initial Bx outcome.

- Using a cut-off of 20, 40% of biopsies could be avoided, while continuing to detect 95% of PCa with Gleason Score $\geq 7$.

- The PCA3 Score may be indicative for aggressiveness of PCa.

- The PROGENSA® PCA3 Assay is a simple urine test that can be of additional help for urologists in counselling patients whether or not to perform an initial Bx for selected cases.
As the PCA3 score increases the likelihood for positive biopsy increases. As the PCA3 score decreases, the likelihood for a positive biopsy decreases. The greatest diagnostic utility occurs at a cut-off of 35.
Conclusions

• Molecular diagnosis of prostate cancer (Progensa® PCA3) is available for the clinic

• The higher the PCA3 Score, the greater the likelihood of a positive biopsy

• The higher the PCA3 Score, the greater the risk of significant Pca
## PCA3 indications-2012

<table>
<thead>
<tr>
<th>Marker/Producer</th>
<th>Status</th>
<th>1. Clinical indication</th>
<th>2. Clinical indication</th>
<th>Indications under study</th>
</tr>
</thead>
</table>
| PCA3 Progensa®PCA3/Genprobe | CE marked product- 2007 FA approved product- 2012 | Urine test: Repeat Bx | Urine test: First Bx | • Active Surveillance  
• Screening  
• Screening familial PrCa  
• Therapy follow up |
Cancer is a heterogeneous disease way forward; biomarker panels

- TMPRSS2-erg!
- AMACR
- PCGEM
- ...

Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer

Scott A. Tomlins, Daniel R. Rhodes, Sven Perner, Saravana M. Dhana Sekaran, Rohit Mehra, Xiao-Wei Sun, Sooryanarayanan Parambally, Xuhong Cao, Joelle Tchinda, Rainer Kuefer, Charles Lee, James E. Montie, Rajal B. Shah, Kenneth J. Pienta, Mark A. Rubin, Arul M. Chinnaiyan
Recurrent gene fusions in PCa

ERG
Overexpressed in PCa

TMPRSS2

TMPRSS2:ERGa
Aberrant gene expression under androgenic control

T2:ERG Urine Test:
Validation of initial study Hessels et al *in SDVA/Laval cohort*

- High specificity vs. biopsy outcome agrees with previous publication.*
- Sensitivity consistent with T2:ERG prevalence in prostate tumors

<table>
<thead>
<tr>
<th></th>
<th>T2:ERG assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (95% C.I.)</td>
<td>41% (34-45)</td>
</tr>
<tr>
<td>Specificity (95% C.I.)</td>
<td>95% (90-98)</td>
</tr>
<tr>
<td>Accuracy (95% C.I.)</td>
<td>71% (65-75)</td>
</tr>
</tbody>
</table>

**Panel: PCA3 and T2:ERG**

*SDVA/Laval Study*

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA3 Score (cutoff = 35)</td>
<td>52%</td>
<td>86%</td>
</tr>
<tr>
<td>T2:ERG (urine sediment)</td>
<td>41%</td>
<td>95%</td>
</tr>
<tr>
<td>PCA3 + T2:ERG</td>
<td>75%</td>
<td>83%</td>
</tr>
</tbody>
</table>

**PCA3 + T2:ERG**

- 23% increase in sensitivity with only 3% loss in specificity.
- Similar results obtained by Hessels, et al.
Urine TMPRSS2:ERG for risk in men with elevated...
Challenge for **all** PrCa biomarkers (serum and urine)

- **Poor golden standard**
  - US guided prostate biopsies
    - Miss the cancer 10-25 %
    - Underestimation of stage/grade 25-50 %
  - Gleason Grading for assessment of aggressiveness
    - Does not qualify as biomarker (‘..Gleason Grading is an art..’)
Candidate panel for prediction of biological potential

- Immunohistochemistry
  - E-cadherin/EZH2
  - KI 67
  - erg

- FISH
  - PTEN
  - TMPRSS2-erg

- Molecular signature test
  - Prolaris, CCCP
  - Genomic Health
  - ....
Tissue tests…first things first...

• Improve imaging to sample the cancer adequately
The challenge for tissue tests

- Standardization
- Standardization
- Standardization
- Standardization
- Standardization
Conclusion

- Urinary prostate cancer tests are reliable and can be clinically implemented
- Urinary tests and improvements thereof should be included in clinical studies
- The golden standard needs to be improved
  - Better imaging
  - Molecular pathology
Conclusion & Perspectives

- First molecular diagnostic test for PCa is available
- ‘Serious’ candidates complementing PCA3 to a panel of 2-4 markers are in clinical evaluation
  - PCA3 + T2-erg; sens 73-75 %; spec 69-83 % !!
- These biomarkers should have a significant impact on the diagnosis and staging of prostate cancer
- These biomarkers are candidates to serve as intermediate end-point for clinical trials
What time is it?

It’s … biomarker time!
DISCUSSION SLIDES
Demands for a screening biomarker; PCA3?

- Easy and non-invasive ✅
- Significantly more specific than PSA ✅
- Significantly less over diagnosis ✅
- (Less under diagnosis of significant prostate cancer, how does PCA3 perform @ PSA< 3?) ✅
- Cost effective (☑️)
Performance of the Prostate Cancer Antigen 3 (PCA3) Gene and Prostate-Specific Antigen in Prescreened Men: Exploring the Value of PCA3 for a First-line Diagnostic Test

Monique J. Roobol *, Fritz H. Schröder, Pim van Leeuwen, Tineke Wolters, Roderick C.N. van den Bergh, Geert J.L.H. van Leenders, Daphne Hessels

Erasmus MC, University Medical Centre, Department of Urology, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Conclusions: PCA3 as a first-line screening test shows improvement of the performance characteristics and identification of serious disease compared with PSA in this prescreened population.

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### Table 4 – Prostate cancer detection with different prostate-specific antigen and PCA3 cut-off values as biopsy indication

<table>
<thead>
<tr>
<th>PSA, ng/ml</th>
<th>Biopsied men, No. (%)</th>
<th>PCa cases, No. (%)</th>
<th>PPV</th>
<th>Missed PCa, No. (%) (n = 122)</th>
<th>Missed serious PCa, No. (%) (n = 19)</th>
<th>Biopsies saved, No. (%) (n = 721)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cut-off</td>
<td>721</td>
<td>122</td>
<td>16.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>≥2.0</td>
<td>347 (48.1)</td>
<td>70 (57.4)</td>
<td>20.2</td>
<td>52 (42.6)</td>
<td>9 (47.4)</td>
<td>374 (51.9)</td>
</tr>
<tr>
<td>≥3.0</td>
<td>229 (31.8)</td>
<td>43 (35.3)</td>
<td>18.8</td>
<td>79 (64.7)</td>
<td>11 (57.9)</td>
<td>492 (68.2)</td>
</tr>
<tr>
<td>≥4.0</td>
<td>146 (20.3)</td>
<td>29 (23.8)</td>
<td>19.9</td>
<td>93 (76.2)</td>
<td>13 (68.4)</td>
<td>575 (79.8)</td>
</tr>
<tr>
<td>≥10.0</td>
<td>21 (2.9)</td>
<td>4 (3.3)</td>
<td>19.1</td>
<td>118 (96.7)</td>
<td>18 (94.7)</td>
<td>700 (97.1)</td>
</tr>
<tr>
<td>PCA3 score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>689 (95.6)</td>
<td>118 (96.7)</td>
<td>17.1</td>
<td>4 (3.3)</td>
<td>0 (0.0)</td>
<td>32 (4.4)</td>
</tr>
<tr>
<td>≥20</td>
<td>533 (73.9)</td>
<td>103 (84.4)</td>
<td>19.3</td>
<td>19 (15.6)</td>
<td>1 (5.3)</td>
<td>188 (26.1)</td>
</tr>
<tr>
<td>≥35</td>
<td>348 (48.3)</td>
<td>83 (68.0)</td>
<td>23.9</td>
<td>39 (32.0)</td>
<td>5 (26.3)</td>
<td>373 (51.7)</td>
</tr>
<tr>
<td>≥100</td>
<td>90 (12.5)</td>
<td>28 (23.0)</td>
<td>31.1</td>
<td>94 (77.0)</td>
<td>12 (63.2)</td>
<td>631 (87.5)</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PPV = positive predictive value; PSA = prostate-specific antigen.

* 721 men biopsied with 122 PCa cases detected, of which 19 cases were classified as serious (T2a or higher and/or Gleason score ≥3 + 3).
<table>
<thead>
<tr>
<th>PSA, ng/ml</th>
<th>Missed serious PCa, No. (%) (n = 19)</th>
<th>Biopsies saved, No. (%) (n = 721)</th>
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<tr>
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<td>PCA3 score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>0 (0.0)</td>
<td>32 (4.4)</td>
</tr>
<tr>
<td>≥20</td>
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<td>≥100</td>
<td>12 (63.2)</td>
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</tr>
</tbody>
</table>
Conclusion

- Established role for PCA3 in the diagnosis of PrCa
- Great potential for combination with other PrCa specific (progression) markers; ets gene fusions!
- Carefully designed study for utility of PSA, PCA3 and ets gene fusions in population based screening needs to be prioritized (combined with CE MRI)