Urinary biomarkers for the early diagnosis of PrCa

Jack A Schalken
The biomarker ‘identity crisis’

…I think I am a biomarker…
...Therefore I am a biomarker...
...I think...

‘Freeley’ adapted from Descartes’ cogito ergo sum
• ‘classical’ triage that leads to the diagnosis PrCa

Serum PSA

Ultrasound-guided biopsy

Gleason grading

‘Golden’ standard

sPSA⁺ (4K PSA panel, PHI)
Urine MDx (PCA3, QUATTRO)

• PrCa diagnosis 2.0

Multiparametric MRI (/ image fusion ultrasound)-guided biopsy

Histopathology including a molecular classifier
- OncotypeDx, InformDx, Confirm Dx, Prolaris, Decipher, (ProMark)

MRI=magnetic resonance imaging; PHI=Prostate Heath Index; PSA=prostate-specific antigen; SNP=single nucleotide polymorphism.
The clinical unmet need
Early diagnosis of prostate cancer

Identification of a biomarker (panel) to identify patients with clinically significant prostate cancer using a diagnostic substrate that can be obtained non-invasively (e.g., urine).

(Ideally suitable for primary care physician and screening)

( Particularly in the sPSA “grey zone” 1.5-10 ng/ml)
PCA3- ‘..the front runner urine test..’

quick summary 1999-2015 (300+ ‘SCI papers’)

• 1994; Marion Bussemakers continues work on molecular profiling at Johns Hopkins (Dr William B Isaacs)
• 1995; PCA3 formerly known as Differential Display code 3 (DD3) identified, work continued in Nijmegen
• Complex locus, no protein; non coding RNA (ncRNA); work funded by PCF (1997-99)
• 2002; concept of molecular uroscopy (molecular test on urine)
• 2003; proof of concept
• 2006; CE marked test launched in Europe (HologicGenprobe)
• 2012; FDA repeat Bx indication
PrCa tests should move ‘upstream’ in the diagnostic triage

Age: 50  Elevated PSA
The basis for both urine assays: Tissue gene expression profile of PCA3 and erg

![PCA3 'prototypical' high fold change/high p value](image_url)

- NP
- BPH
- LG
- HG
- CRPC
- Meta
A stepwise approach for the identification and validation of a prognostic gene panel.

**Molecular profiling**
- **Platform**: GeneChip® 1.0 ST array (Affymetrix)
- **Specimen**: mRNA from well annotated snap frozen normal prostate and PCa tissue (n=99)
- **Biomarkers selected**: 39

**Testing biomarkers on another cohort of tissue**
- **Platform**: qPCR
- **Specimen**: mRNA from well annotated snap frozen normal prostate and PCa tissue (n=107)
- **Biomarkers selected**: 34

**Testing biomarkers on a cohort of urinary sediments**
- **Platform**: qPCR
- **Specimen**: mRNA from urinary sediments (n=16)
- **Biomarkers selected**: 8

**Testing biomarkers in intention-to-treat cohort of urinary sediments**
- **Platform**: Laboratory Diagnostic Test/Roche LC480
- **Specimen**: mRNA from urinary sediments (n=358)
- **Biomarkers selected**: 3
Example of result from gene selection algorithm

RRM2

- NP
- BPH
- LG
- HG
- CRPC
- Meta
Clinical study NG 0901

- 8 new markers were tested in a clinical study
- Urine sediments (post DRE) as diagnostic substrate
- PCA3 as comparator
- Identification of GS ≥ 7 as primary end point
## Diagnostic potential

### Table 3A Biomarker characteristics in urinary sediments of the clinical intend-to-treat population

<table>
<thead>
<tr>
<th></th>
<th>No (n=201)</th>
<th>Prostate cancer</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (Q1-Q3)</td>
<td>median (Q1-Q3)</td>
<td></td>
</tr>
<tr>
<td>Serum PSA (ng/ml)</td>
<td>6.8 (5.1-9.4)</td>
<td>9.2 (6.1-13.7)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>PCA3 score</td>
<td>24 (12-57)</td>
<td>60 (31-107)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>HOXC4</td>
<td>5260 (1560-9930)</td>
<td>12600 (4140-24100)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>HOXC6</td>
<td>321 (84-838)</td>
<td>962 (390-2760)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>DLX1</td>
<td>1 (1-1)</td>
<td>1 (1-231)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>TDRD1</td>
<td>124 (1-383)</td>
<td>367 (60-1560)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>ONECUT2</td>
<td>776 (259-2020)</td>
<td>1280 (570-2860)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>NKAIN1</td>
<td>162 (37-440)</td>
<td>291 (94-891)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>MS4A8B</td>
<td>168 (1-592)</td>
<td>612 (126-2100)</td>
<td>&lt;0.001(^a)</td>
</tr>
<tr>
<td>PPFIA2</td>
<td>167 (1-684)</td>
<td>534 (111-1240)</td>
<td>&lt;0.001(^a)</td>
</tr>
</tbody>
</table>

\(^a\) = Mann Whitney test.
### Table 3B: Biomarker characteristics in urinary sediments of the clinical intend-to-treat population

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>≤6 (n=64)</th>
<th>≥7 (n=93)</th>
<th>p-value</th>
<th>REST (n=265)</th>
<th>≥7 (n=93)</th>
<th>p-value</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA (ng/ml)</td>
<td>8 (5.3-10.1)</td>
<td>10.8 (7.2-20.1)</td>
<td>&lt;0.001²</td>
<td>6.9 (5.2-9.5)</td>
<td>10.8 (7-20.1)</td>
<td>&lt;0.001²</td>
<td>0.72 (0.65-0.78)</td>
</tr>
<tr>
<td>PCA3 score</td>
<td>55.5 (29-93)</td>
<td>61 (32-111)</td>
<td>0.278⁵</td>
<td>31 (15-65)</td>
<td>61 (32-111)</td>
<td>&lt;0.001⁶</td>
<td>0.68 (0.62-0.75)</td>
</tr>
<tr>
<td>HOXC4</td>
<td>8120 (8600-21525)</td>
<td>14700 (4820-30700)</td>
<td>0.034⁴</td>
<td>5940 (1880-12300)</td>
<td>14700 (4820-30700)</td>
<td>&lt;0.001⁴</td>
<td>0.69 (0.62-0.75)</td>
</tr>
<tr>
<td>HOXC6</td>
<td>633 (309-1410)</td>
<td>1550 (520-3970)</td>
<td>&lt;0.001²</td>
<td>392 (110-985)</td>
<td>1550 (520-3970)</td>
<td>&lt;0.001²</td>
<td>0.76 (0.70-0.82)</td>
</tr>
<tr>
<td>DLX1</td>
<td>1 (1-22)</td>
<td>35 (1-758)</td>
<td>&lt;0.001²</td>
<td>1 (1-1)</td>
<td>35 (1-758)</td>
<td>&lt;0.001²</td>
<td>0.70 (0.63-0.77)</td>
</tr>
<tr>
<td>TDRD1</td>
<td>159 (1-481)</td>
<td>843 (146-8065)</td>
<td>&lt;0.001⁴</td>
<td>130 (1-416)</td>
<td>843 (146-8065)</td>
<td>&lt;0.001⁴</td>
<td>0.73 (0.67-0.80)</td>
</tr>
<tr>
<td>ONECUT2</td>
<td>1020 (355-1802)</td>
<td>1790 (710-5270)</td>
<td>&lt;0.001²</td>
<td>804 (276-1950)</td>
<td>1790 (710-5270)</td>
<td>&lt;0.001²</td>
<td>0.69 (0.62-0.75)</td>
</tr>
<tr>
<td>NKA1N</td>
<td>192 (77-438)</td>
<td>392 (128-1900)</td>
<td>0.006²</td>
<td>163 (41-440)</td>
<td>392 (128-1900)</td>
<td>&lt;0.001²</td>
<td>0.66 (0.59-0.73)</td>
</tr>
<tr>
<td>MS4A8B</td>
<td>472 (69-1070)</td>
<td>1010 (196-3250)</td>
<td>0.001³</td>
<td>204 (1-775)</td>
<td>1010 (196-3250)</td>
<td>&lt;0.001³</td>
<td>0.70 (0.63-0.76)</td>
</tr>
<tr>
<td>PPRA2</td>
<td>353 (56-722)</td>
<td>713 (147-1790)</td>
<td>0.004</td>
<td>210 (1-704)</td>
<td>713 (147-1790)</td>
<td>&lt;0.001³</td>
<td>0.67 (0.51-0.74)</td>
</tr>
</tbody>
</table>

REST = no prostate cancer + Gleason score ≤6, AUC = Area Under the Curve, 95% CI = 95% Confidence Interval. ² = Mann Whitney test.
Multivariate analysis and bootstrapping

- HOXC6
- DLX1
- TDRD1
- (KLK3 mRNA for normalization)

- Project name ‘..QUATTRO..’
Figure 2. The ROC curves for four models: Progensa PCA3 (purple line, AUC = 0.688; 95% CI 0.63 – 0.75), QUATTRO (red line, AUC = 0.78; 95% CI 0.72 – 0.84) and sPSA + QUATTRO (green line, AUC = 0.82; 95% CI 0.73 – 0.87) for the prediction of Gleason score ≥7 PCa diagnosis upon biopsy.
'Quattro' performance at low PSA values

![Graph showing AUC (GS ≥ 7) for different PSA levels]

- Serum PSA ≤ 7.5: 179 subjects
- Serum PSA ≤ 10: 246 subjects
- Serum PSA ≤ 12.5: 292 subjects
- Serum PSA ≤ 15: 305 subjects
- All cases: 358 subjects

AUC (GS ≥ 7):
- sPSA
- QUATTRO: NG0901 (sediments)
Quattro development

- Publication Q1 2015
- Quattro from RND substrate (cell pellet) to whole urine
- Quattro LDT
- Quattro CE-IVD (August 4th 2015)
Assay flow scheme

50 ml urine Post-DRE

Fixative Tube (1:1)

1 ml Magnapure96

RNA

RT-qPCR In One-step

Amplification Curves
Validation study NG1401

- Whole urine test (LDT) performs similar to cell pellet assay (RUO)
- Whole urine assay performs optimal using three mRNAs
- INDEPENDENT Validation study confirms initial test cohort study
# Diagnosis of GS ≥ 7 upon biopsy

## Low Risk (HOXC6/DLX1 – 27.5)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG0901 (N=490)</td>
<td>92%</td>
<td>32%</td>
<td>93%</td>
<td>27%</td>
</tr>
<tr>
<td>NG1201 (N=371)</td>
<td>92%</td>
<td>37%</td>
<td>94%</td>
<td>31%</td>
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## High Risk (HOXC6/DLX1 – 115.5)

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<tbody>
<tr>
<td>NG0901 (N=490)</td>
<td>36%</td>
<td>89%</td>
<td>84%</td>
<td>48%</td>
</tr>
<tr>
<td>NG1201 (N=371)</td>
<td>27%</td>
<td>90%</td>
<td>80%</td>
<td>47%</td>
</tr>
</tbody>
</table>
Validation study
Quattro is significantly higher in GS\(\geq 7\) PrCa

A

B

No PCa  GS\(^{\ast}\) 6  GS \(\geq 7\)

HOXC6-DLX1 score

\[ p < 0.001^* \]

\[ p = 0.002^* \]
Clinical utility

- Patients with a serum PSA 2.5-10 ng/ml and a Quattro score <27.5 have a 7% risk for GS ≥ 7 PrCa
- 35% of biopsies can be saved
Conclusion

• Molecular urine tests are particularly useful in the sPSA grey zone with potential for even lower sPSAs

• Need for carefully designed study for utility of sPSA, and urine biomarker tests for early diagnosis with improved ‘golden standard’

• We need to agree on the state of the art risk profile of serum PSA (PCPT 6-9 %; Lucia 9-12) to miss significant cancer
PCPT; risk for significant cancer low PSA range

- State of the art risk profile for missing high grade/significant PrCa using a serum PSA threshold value of 3 ng/ml is 5,7/10.5 %

- State of the art risk profile for missing high grade/significant PrCa using a serum PSA threshold value of 4 ng/ml is 9,4/15,1 %

- The QUATTRO assay positions 31% of the cases with 3-10 ng/ml sPSA at low risk for PCa. In this low risk group for PCa, 7% high grade PCa is present (or 4% in the 3-8 ng/ml sPSA range only, data not shown)

- The state of the art risk profile of Quattro is lower than that for serum PSA and this provides a rationale for studies in patients with a serum PSA 1,5/2-10 ng/ml
## Summary: Clinical Validation ‘Quattro’

<table>
<thead>
<tr>
<th>Study</th>
<th>Substrate</th>
<th>Genes</th>
<th>Informative samples</th>
<th>Informative Rate (%)</th>
<th>AUC (95% CI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG0901</td>
<td>Urinary Sediments (RUO)</td>
<td>HOXC6, TDRD1, DLX1</td>
<td>358</td>
<td>81</td>
<td>0.77 (0.71-0.83)</td>
<td>90</td>
<td>39</td>
<td>92</td>
<td>34</td>
</tr>
<tr>
<td>NG0901</td>
<td>Whole Urine (LDT)</td>
<td>HOXC6, DLX1</td>
<td>490</td>
<td>95</td>
<td>0.75 (0.70-0.80)</td>
<td>92</td>
<td>32</td>
<td>93</td>
<td>27</td>
</tr>
<tr>
<td>NG1201</td>
<td>Whole Urine (LDT, validation)</td>
<td>HOXC6, DLX1</td>
<td>371</td>
<td>96</td>
<td>0.73 (0.67-0.78)</td>
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- ‘classical triage that leads to the diagnosis PrCa

- ‘Golden’ standard

  - Serum PSA
  - Ultrasound-guided biopsy
  - Gleason grading

- sPSA+ (4K PSA panel, PHI)
- Urine MDx (PCA3, QUATTRO)

- PrCa diagnosis 2.0

  - Multiparametric MRI (image fusion ultrasound)-guided biopsy
  - Histopathology including a molecular classifier
    - OncotypeDx, InformDx, Confirm Dx, ProLaris, Decipher, (ProMark)

MRI=magnetic resonance imaging; PHI=Prostate Health Index; PSA=prostate-specific antigen; SNP=single nucleotide polymorphism.