Urinary biomarkers for the early diagnosis of PrCa

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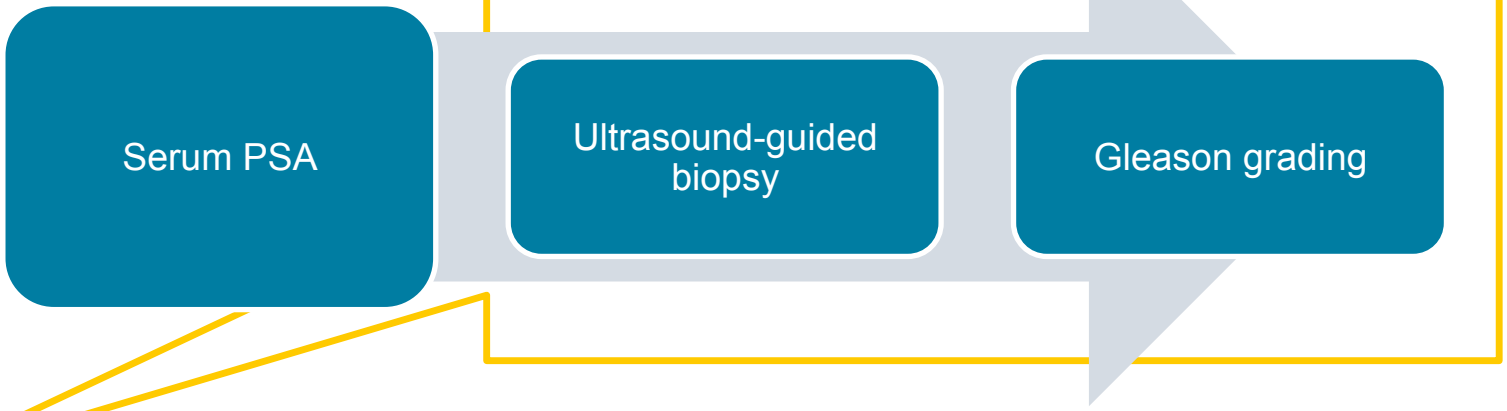
The biomarker ‘identity crisis’

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

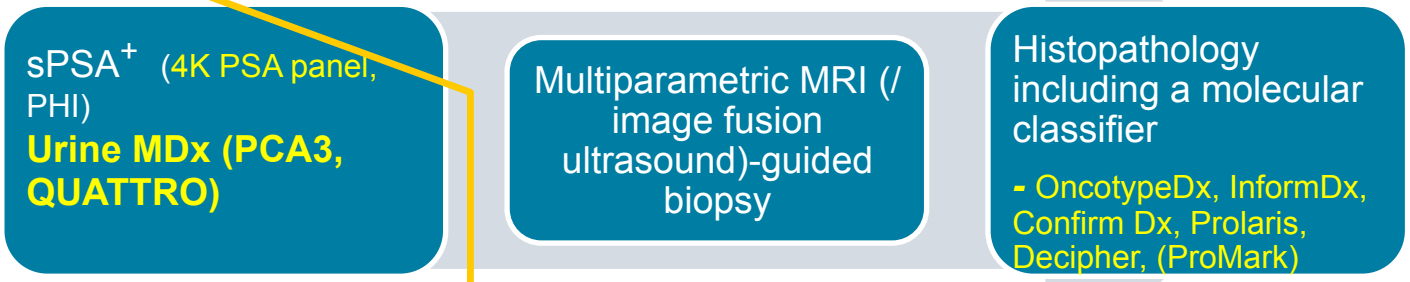
‘Freeley’ adapted from Descartes’ ***cogito ergo sum***

- ‘classical’ triage that leads to the diagnosis PrCa



‘Golden’ standard

- PrCa diagnosis 2.0



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 (eg urine).

(Ideally suitable for primary care physician and screening)

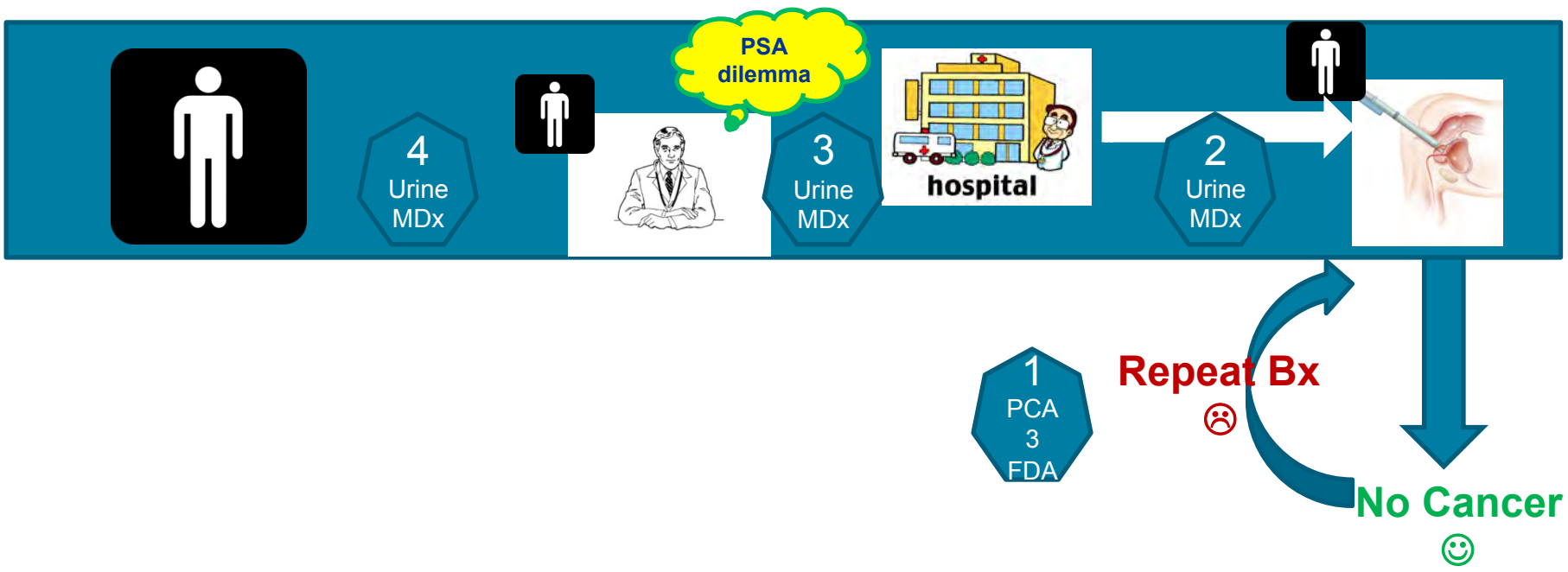
(Particularly in the sPSA ''grey zone" 1,5-10 ng ml)

PCA3- '..the front runner urine test..'

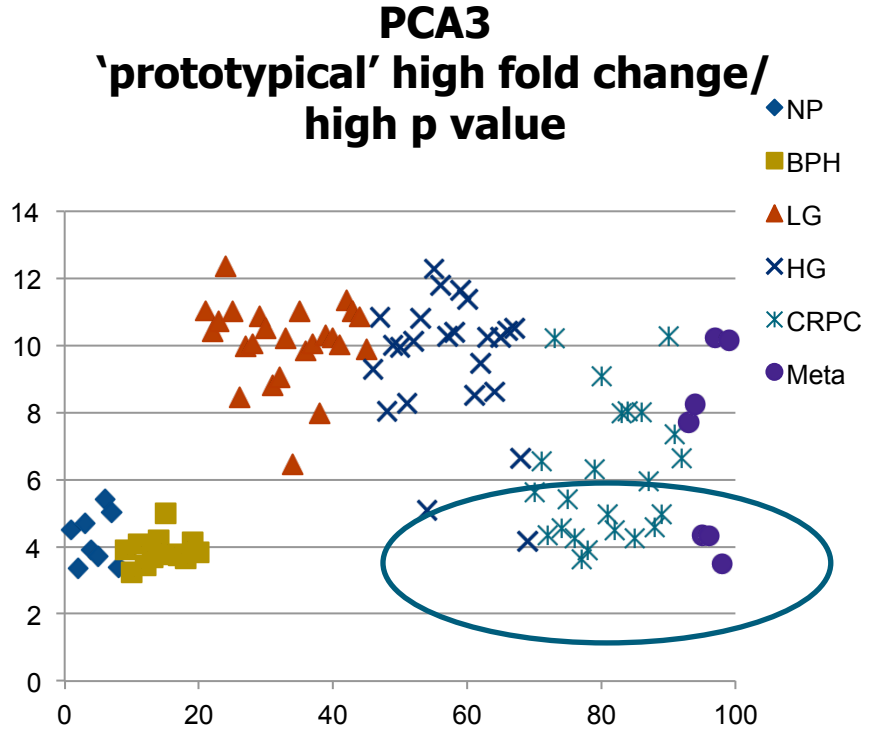
quick summary 1999-2015 (300+ 'SCI papers')

- 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

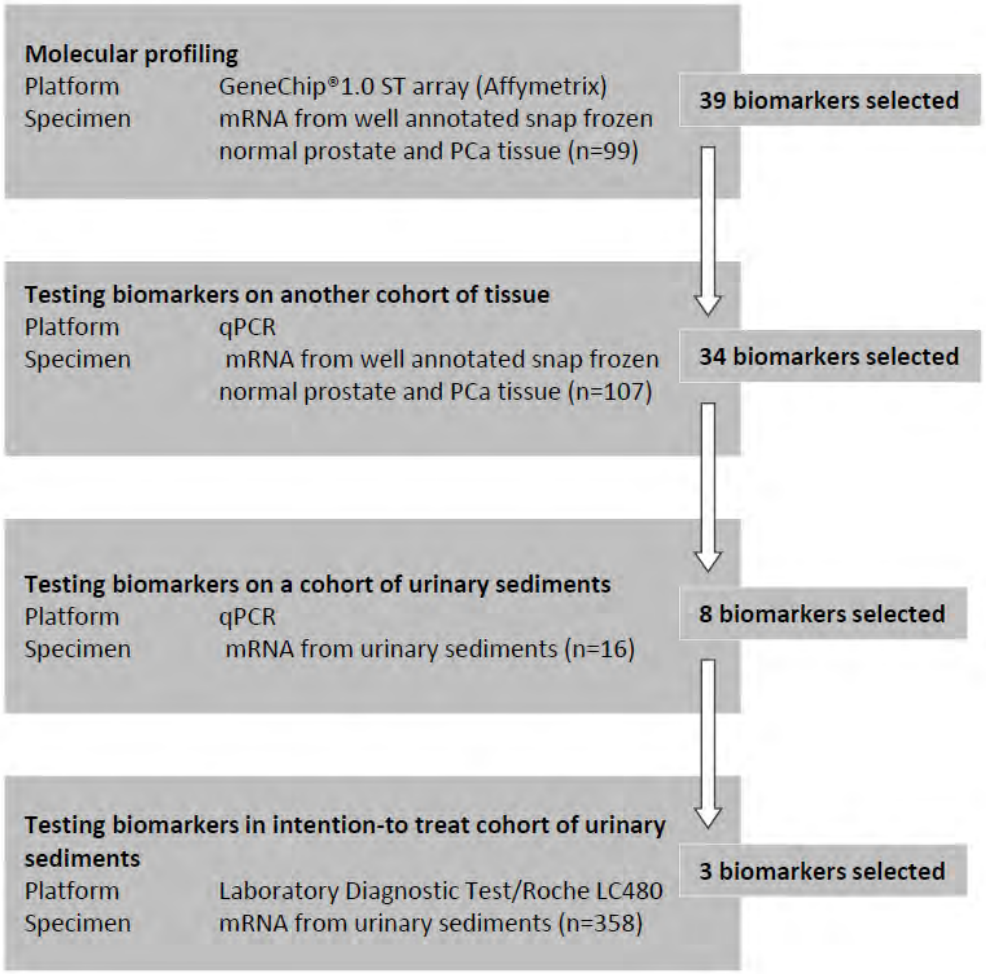
PrCa tests should move ‘upstream’ in the diagnostic triage



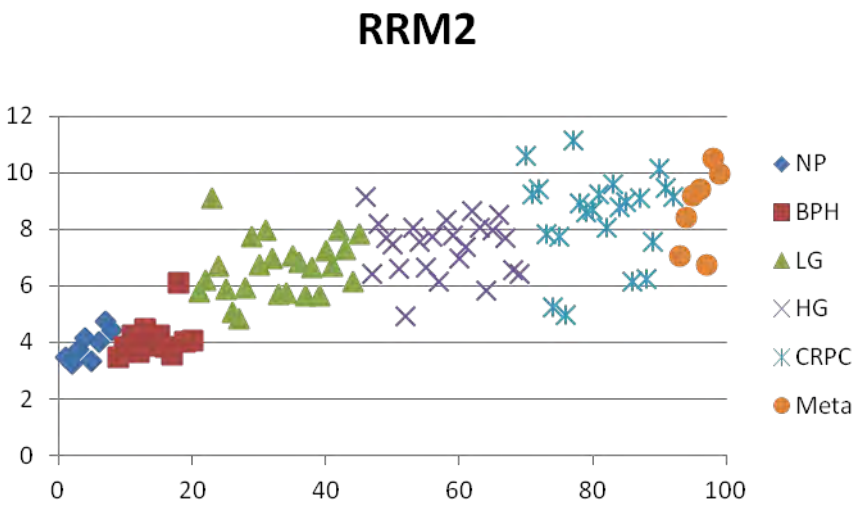
~~The basis for both urine assays:~~ Tissue gene expression profile of PCA3 and erg



A stepwise approach for the identification and validation of a prognostic gene panel.



Example of result from gene selection algorithm



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

	Prostate cancer		
	No (n=201) median (Q1-Q3)	**Yes (n=157)** median (Q1-Q3)	**p-value**
Serum PSA (ng/ml)	6.8 (5.1-9.4)	9.2 (6.1-13.7)	<0.001[a]
PCA3 score	24 (12-57)	60 (31-107)	<0.001[a]
HOXC4	5260 (1560-9930)	12600 (4140-24100)	<0.001[a]
HOXC6	321 (84-838)	962 (390-2760)	<0.001[a]
DLX1	1 (1-1)	1 (1-231)	<0.001[a]
TDRD1	124 (1-383)	367 (60-1560)	<0.001[a]
ONECUT2	776 (259-2020)	1280 (570-2860)	<0.001[a]
NKAIN1	162 (37-440)	291 (94-891)	<0.001[a]
MS4A8B	168 (1-592)	612 (126-2100)	<0.001[a]
PPFIA2	167 (1-684)	534 (111-1240)	<0.001[a]

[a] = Mann Whitney test.

Prognostic potential

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

	Gleason score			Gleason score			
	≤6 (n=64) median (Q1-Q3)	≥7 (n=93) median (Q1-Q3)	p-value	REST (n=265) median (Q1-Q3)	≥7 (n=93) median (Q1-Q3)	p-value	AUC (95% CI)
Serum PSA (ng/ml)	8 (5.3-10.1)	10.8 (7-20.1)	<0.001[a]	6.9 (5.2-9.5)	10.8 (7-20.1)	<0.001[a]	0.72 (0.65-0.78)
PCA3 score	55.5 (29-93)	61 (32-111)	0.278[a]	31 (15-65))	61 (32-111)	<0.001[a]	0.68 (0.62-0.75)
HOXC4	8120 (3600-21525)	14700 (4820-30700)	0.034[a]	5940 (1880-12300)	14700 (4820-30700)	<0.001[a]	0.69 (0.62-0.75)
HOXC6	633 (309-1410)	1550 (520-3970)	<0.001[a]	392 (110-985)	1550 (520-3970)	<0.001[a]	0.76 (0.70-0.82)
DLX1	1 (1-22)	35 (1-758)	<0.001[a]	1 (1-1)	35 (1-758)	<0.001[a]	0.70 (0.63-0.77)
TDRD1	159 (1-481)	843 (146-8065)	<0.001[a]	130 (1-416)	843 (146-8065)	<0.001[a]	0.73 (0.67-0.80)
ONECUT2	1020 (355-1802)	1790 (710-5270)	<0.001[a]	804 (276-1950)	1790 (710-5270)	<0.001[a]	0.69 (0.62-0.75)
NKAIN1	192 (77-438)	392 (128-1900)	0.006[a]	163 (41-440)	392 (128-1900)	<0.001[a]	0.66 (0.59-0.73)
MS4A8B	472 (69-1070)	1010 (196-3250)	0.001[a]	204 (1-775)	1010 (196-3250)	<0.001[a]	0.70 (0.63-0.76)
PPFIA2	353 (56-722)	713 (147-1790)	0.004	210 (1-704)	713 (147-1790)	<0.001[a]	0.67 (0.61-0.74)

REST = no prostate cancer + Gleason score ≤6. AUC = Area Under the Curve. 95% CI = 95% Confidence Interval.
[a] = Mann Whitney test

Multivariate analysis and bootstrapping

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

- Project name '..QUATTRO..'

‘Quattro’ outperforms PCA3

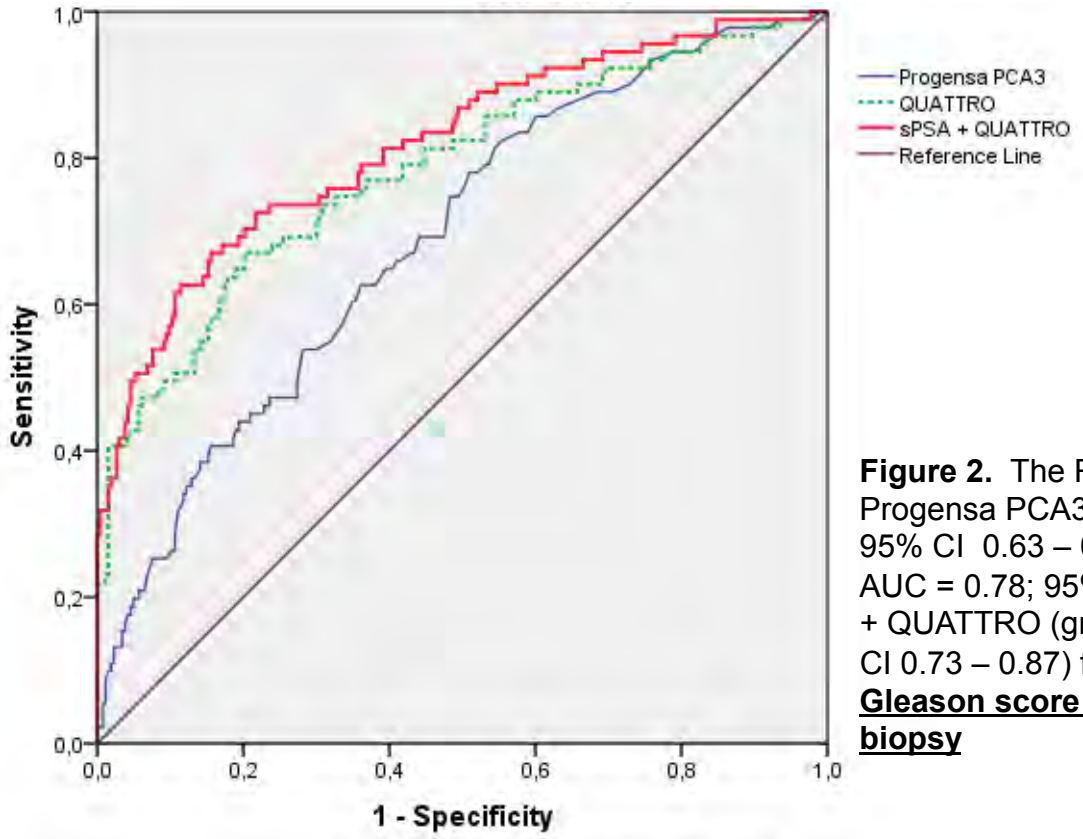
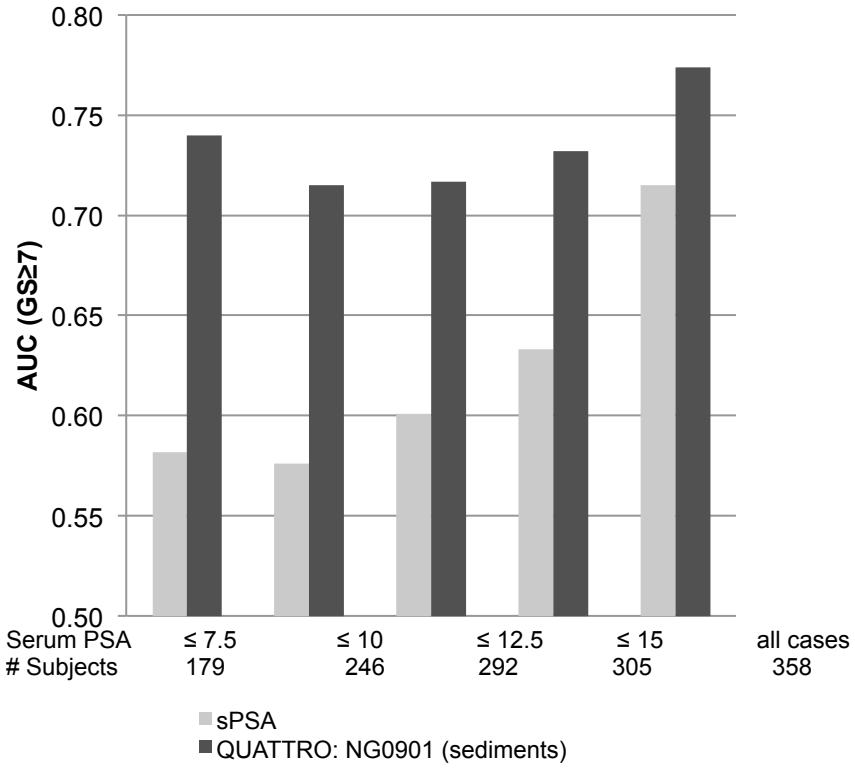


Figure 2. The ROC curves for four models: Progensa PCA3 (purple line, AUC = 0.688; 95% CI 0.63 – 0.75), QUATTRO (red line, AUC = 0.78; 95% CI 0.72 – 0.84) and sPSA + QUATTRO (green line, AUC = 0.82; 95% CI 0.73 – 0.87) for **the prediction of Gleason score ≥7 PCa diagnosis upon biopsy**

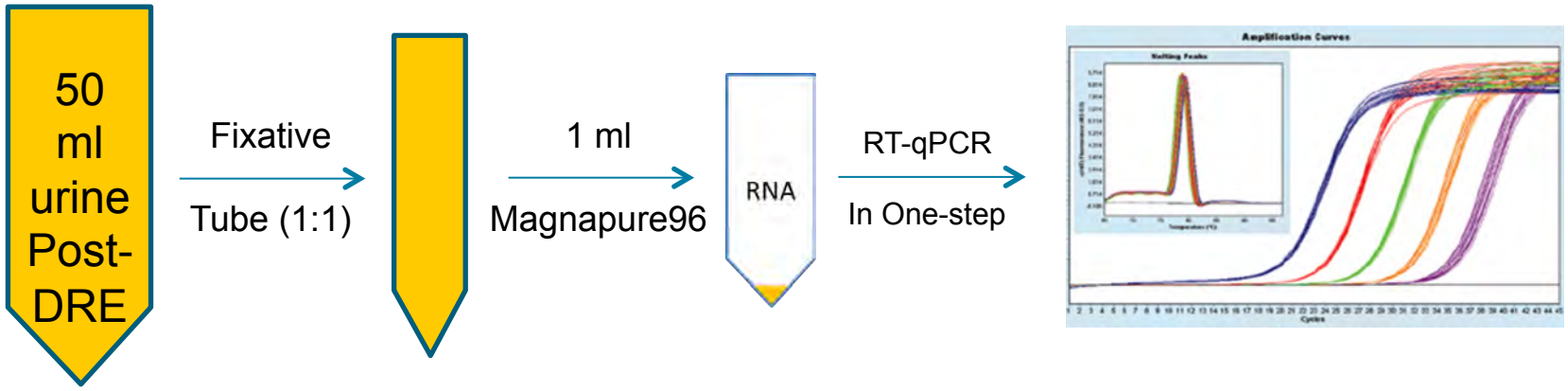
'Quattro' performance at low PSA values



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



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

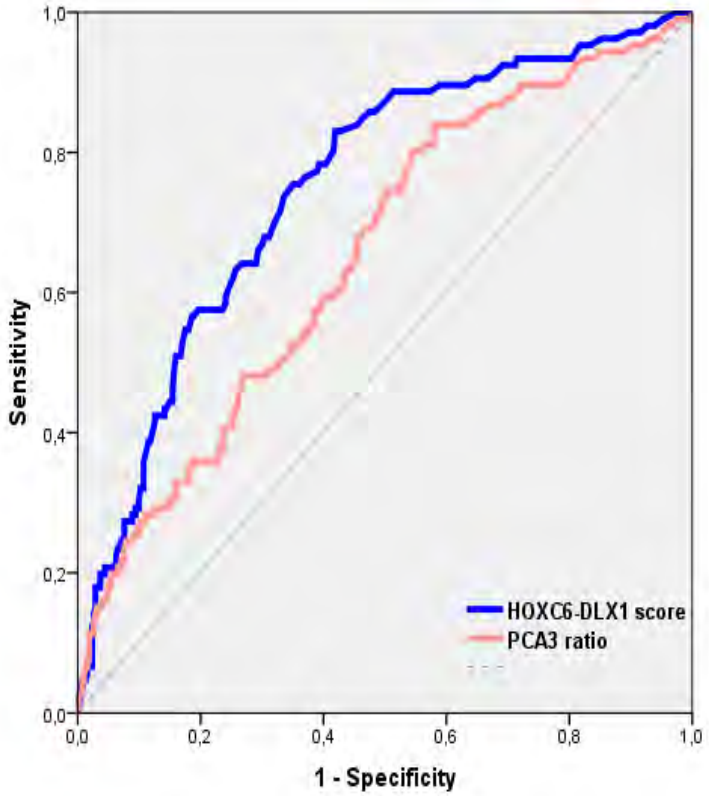
Low Risk (HOXC6/DLX1 – 27,5)

	Sensitivity	Specificity	NPV	PPV
NG0901 (N=490)	92%	32%	93%	27%
NG1201 (N=371)	92%	37%	94%	31%

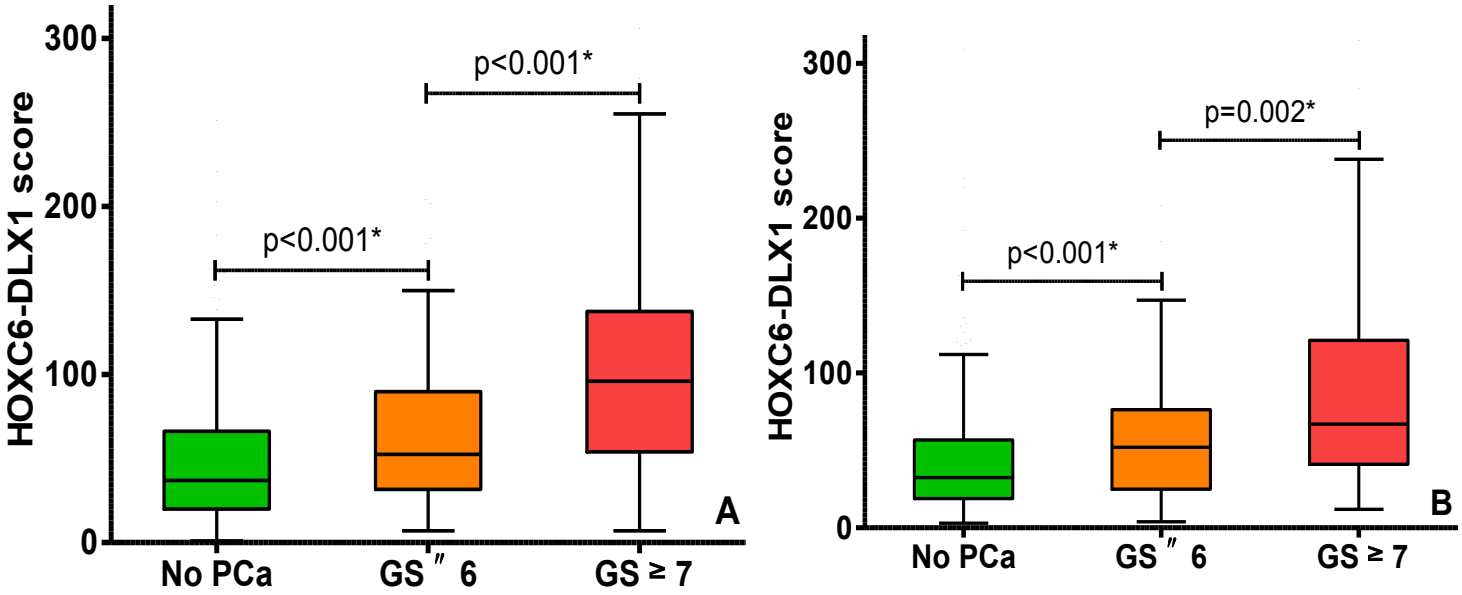
High Risk (HOXC6/DLX1 – 115,5)

	Sensitivity	Specificity	NPV	PPV
NG0901 (N=490)	36%	89%	84%	48%
NG1201 (N=371)	27%	90%	80%	47%

Validation study



Quattro is significantly higher in GS≥7 PrCa



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
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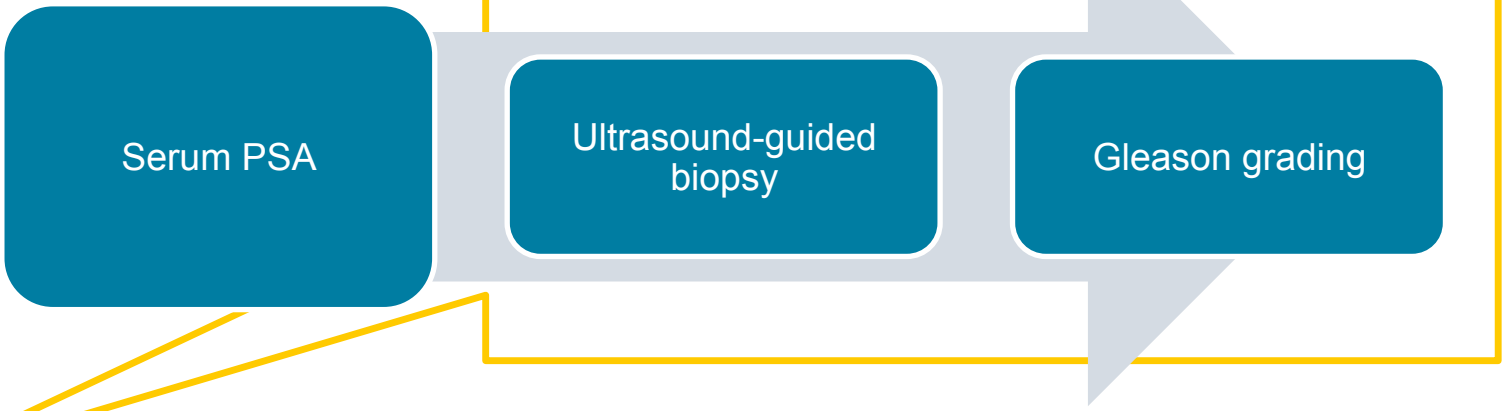
Summary: Clinical Validation ‘Quattro’

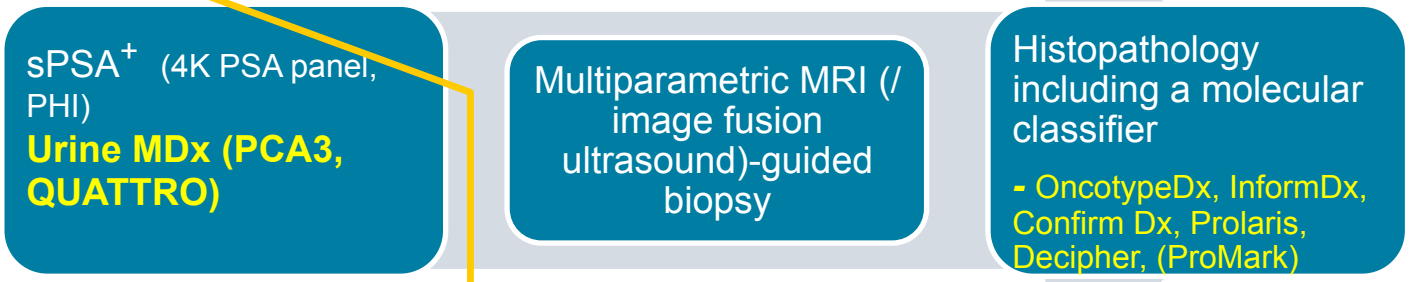
Study	Substrate	Genes	Informative samples	Informative Rate (%)	AUC *(95% CI)*	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
NG0901	Urinary Sediments (RUO)	HOXC6, TDRD1, DLX1	358	81	0,77 *(0,71-0,83)*	90	39	92	34
NG0901	Whole Urine (LDT)	HOXC6, DLX1	490	95	0,75 *(0,70-0,80)*	92	32	93	27
NG1201	**Whole Urine (LDT, validation)**	**HOXC6, DLX1**	**371**	**96**	**0,73 *(0,67-0,78)***	**92**	**37**	**94**	**31**

- ‘classical triage that leads to the diagnosis PrCa



‘Golden’ standard

- PrCa diagnosis 2.0



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

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