# Derivation and validation of blood mRNA expression signatures to stratify CRPC cancer patients and predict poor outcome

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# Disclosures

- Authors  do not have any disclosure to discuss

# Introduction (I)

- Prostate cancer (PCa) prognosis is very heterogeneous: Survival for CRPC patients is variable from months to > 8 years

- Need to develop biomarkers to better stratify patient risk and individualize patient management

- PSA easily evaluated and is highly reproducible, but levels do not always correlate with disease burden, does not always reflect anti-tumour activity and does not predict overall survival

# Introduction (II)

- Novel high-throughput technologies has allowed the identification of promising tumour-tissue markers…

- In PCa  it is difficult to obtain tumor-tissue from patients…… this makes the identification and validation of blood- and/or urine-based biomarkers critical

- Circulating tumor cells (CTCs) in PCa is a promising biomarker, but may be difficult to isolate in patients with early or low volume metastatic disease

# Introduction (III)

- In addition to CTCs, other changes in normal peripheral blood cell populations can be found in cancer:
  - chromosomal aberrations in normal blood cells have been described in patients with solid tumors, including PCa
  - Blood cells express 16000-20000 gene transcripts in response to micro- and macro-environmental changes

**Hypotheses:**

- **Can we derive prognostic genetic signatures in the whole blood of prostate cancer patients?**
- **Can these signatures improve the risk stratification of our patients?**

# Methods (I): Two Stage Study

- **Stage I**: Derivation of gene-expression signatures

| Cases | Control |
|---|---|
| ~2/3: 60-70 pts | ~1/3: 30-40 pts |
| Stage IV CPRC (PWG2):<br>3 rising PSA values 1 week apart each. | T1 o T2 localised PCa<br>PSA ≤20 ng/mL<br>Gleason ≤3+4<br><50% of core biopsies involved |
| No active PCa treatment within 4 weeks of sampling | |

- **Stage II**: Validation of a gene-expression signature
  - **Advanced CRPC** (similar criteria to Stage I)

# Methods (II)

- Five mLs of peripheral blood collected in PAXgene tubes (2.5mL): ≥1 month after any PCa-related therapy (apart from LHRHa)
  - In a proportion of CRPC patients CTC enumeration was also carried out using the CellSearch™ system (Veridex, Raritan, NJ)
  - Diagnostic and baseline characteristics as well as previous treatment and evolution data were collected

- RNA extraction from whole blood extracted using the PAXgene Blood RNA isolation kit (PreAnalitiX)
  - Stage I: RNA amplified, labelled, fragmented (NuGen Ovation system), and hybridised with Affymetrix U133Plus 2.0 GenChip oligonucleotide arrays
  - Stage I-II: First strand cDNA synthesis for qRT-PCR using TaqMan assays

# Stage I: patients characteristics

| Active Surveillance |
|---|
| 30 out of 31 patients were evaluable |

| CRPC | |
|---|---|
| 64 out of 69 patients were evaluable | |
| **Performance Status** | |
| - ECOG 0 | 19 (30%) |
| - ECOG 1 | 42 (65%) |
| - ECOG ≥2 | 3 (5%) |
| **Gleason score** | |
| - Gleason ≤6 | 10 (17%) |
| - Gleason 7 | 13 (23%) |
| - Gleason ≥8 | 34 (60%) |
| **Metastasis** | |
| -Bone | 55 (89%) |
| - Visceral | 9 (14%) |

| CRPC (continued) | |
|---|---|
| **Baseline PSA** | |
| - median ng/mL | 177 |
| - (range) | (122-2684) |
| **PSA doubling time** | |
| - PSADT <3 months | 37 (58%) |
| - PSADT ≥3 & <6 months | 17 (26%) |
| - PSADT ≥6 months | 10 (16%) |
| **CTC counts** | |
| - <5 CTC/7.5 mL | 13 (42%) |
| - ≥5 CTC/7.5 mL | 18 (58%) |
| - Unknown | 33 |
| **Previous chemotherapy** | |
| - Docetaxel | 19 (30%) |
| **Concomitant steroids** | |
| - Yes | 22 (34%) |

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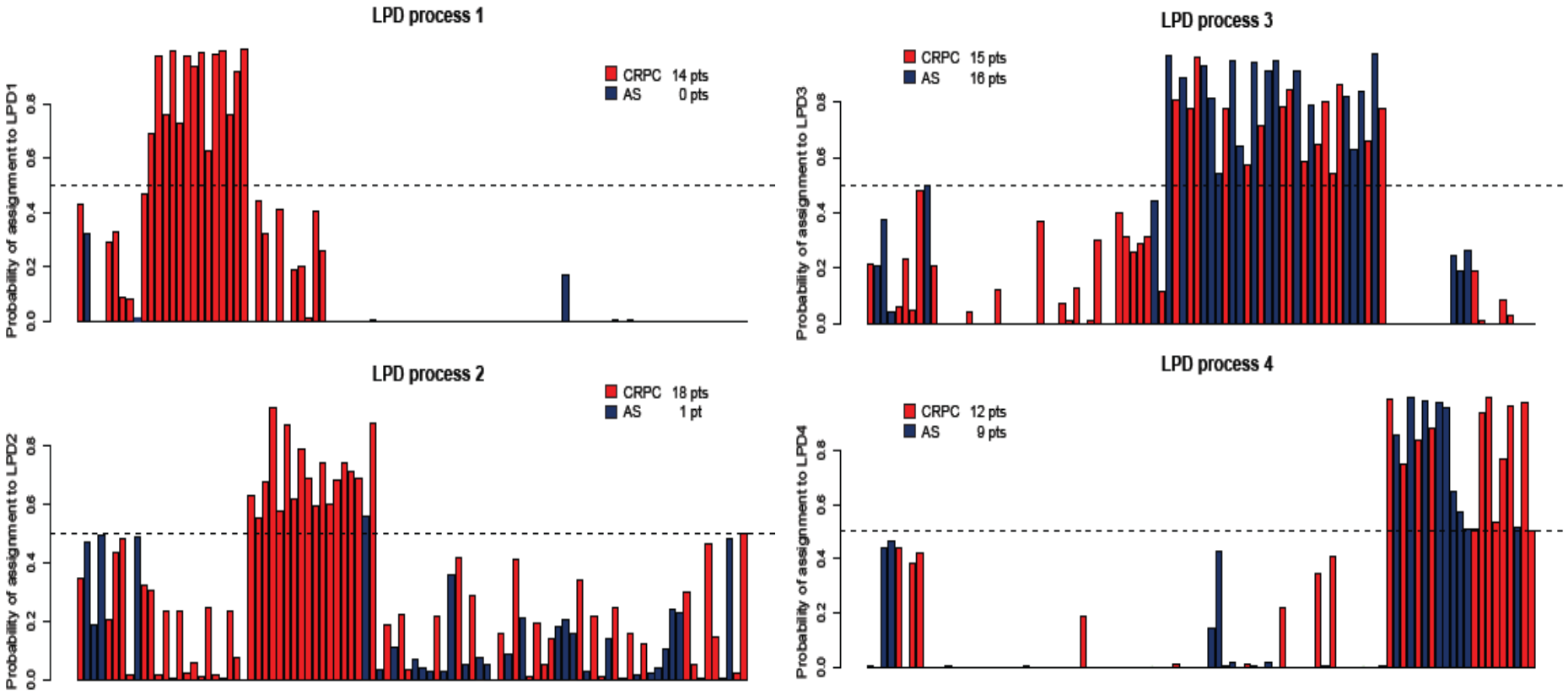
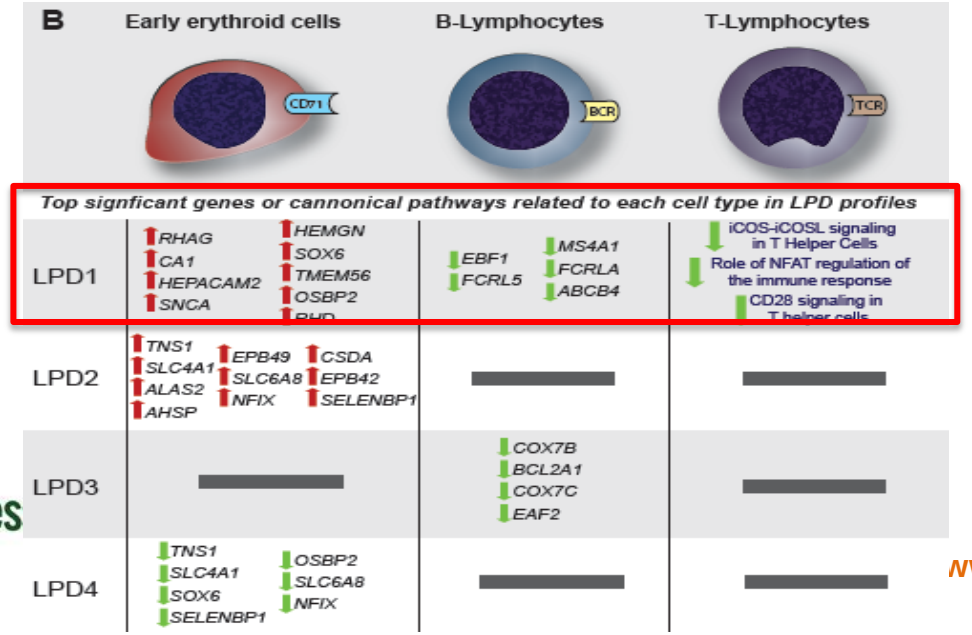
# Stage I: Gene Expression Analyses

- Latent Process Decomposition (LPD): an unsupervised Bayesian approach to classifying samples:
  1. Defines an optimal number of groups in the structure of the data
  2. Assigns a probability that each sample belongs to a group

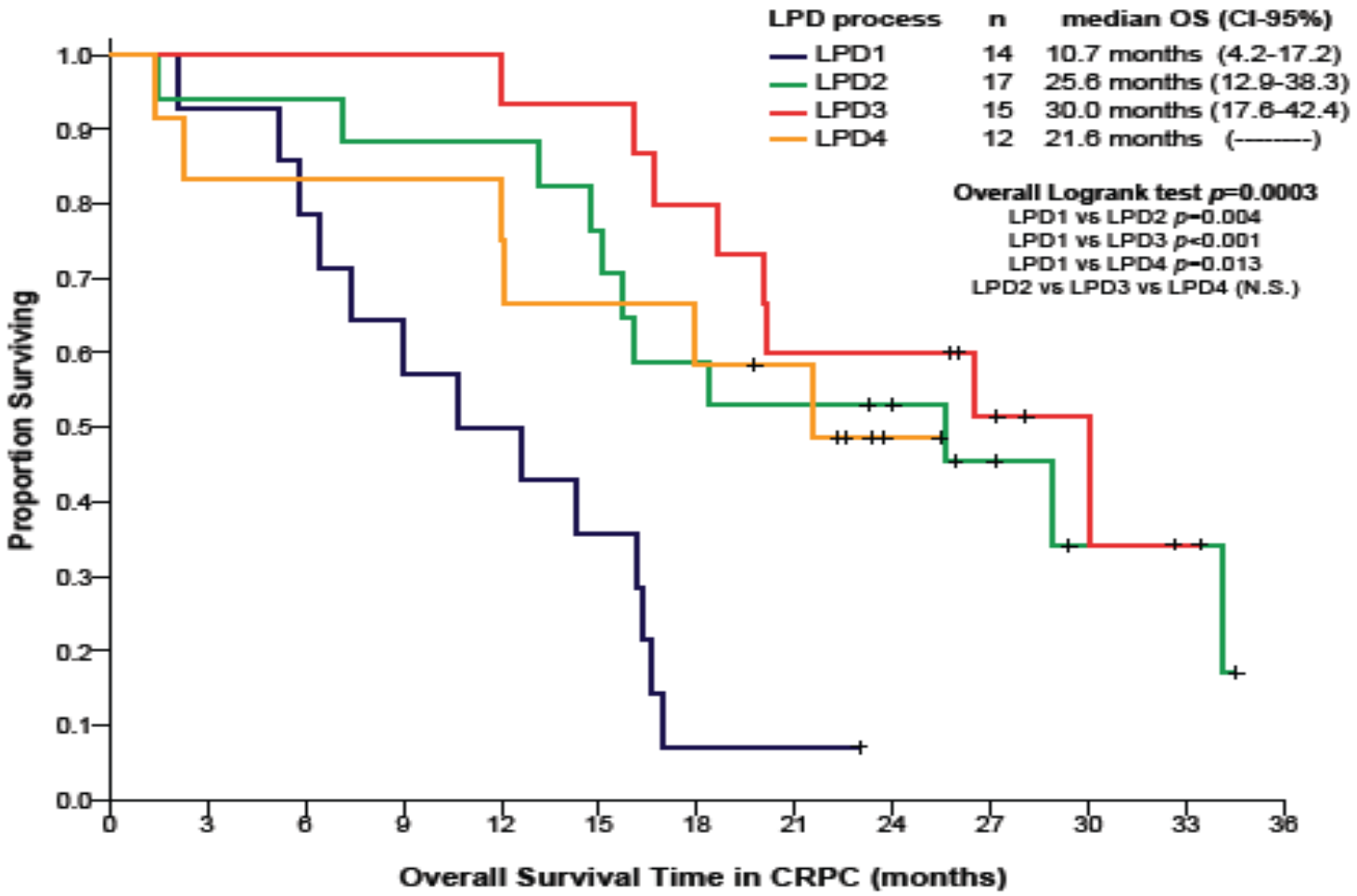


# Stage I: differentially expressed probe-sets

| Group | CRPC | Surveillance | Dif. Expr. probesets |
|---|---|---|---|
| **LPD 1** | 14 | 0 | 2740 |
| **LPD 2** | 17 | 1 | 541 |
| **LPD 3** | 15 | 16 | 2179 |
| **LPD 4** | 12 | 9 | 10063 |
| 10 Unclassified | | | |



# Stage I: LPD groups and survival in CRPC



# Stage I: Derivation of a signature for LPD1

**10 probe-set (9 genes ) Classifier**

- The random forest machine learning algorithm was used to identify a 10 probeset (9-genes) signature to be used as a test for each LPD group membership

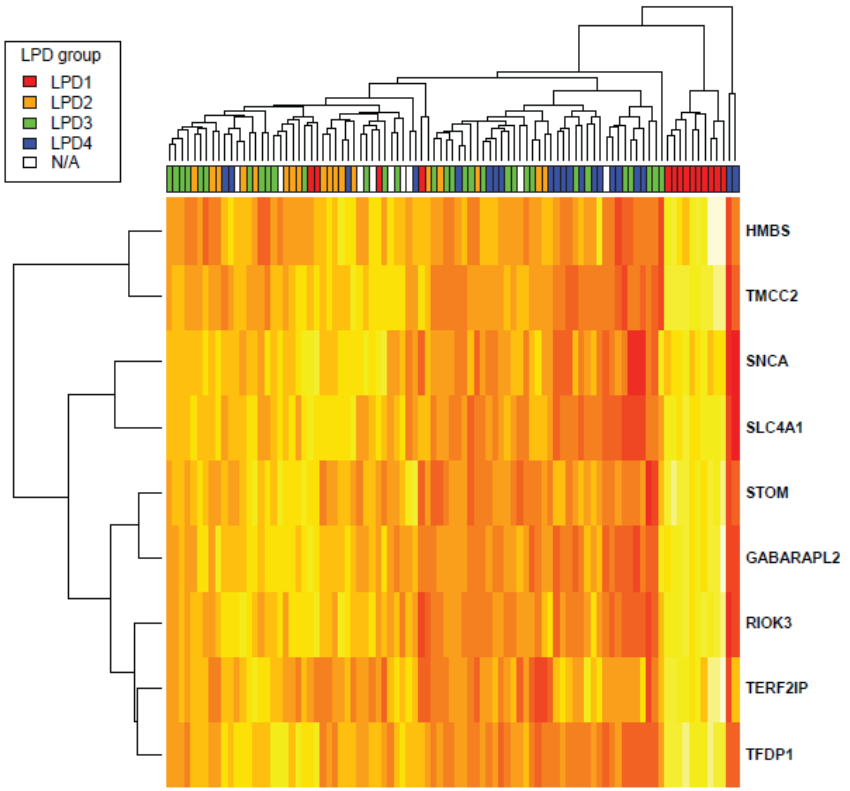
| Probeset | Symbol | Gini importance metric |
|---|---|---|
| 201174_s_at | TERF2IP | 5.3984552 |
| 213096_at | TMCC2 | 5.1047048 |
| 204467_s_at | SNCA | 3.5190276 |
| 209046_s_at | GABARAPL2 | 2.5084932 |
| 207827_x_at | SNCA | 1.8047234 |
| 202129_s_at | RIOK3 | 1.3102857 |
| 212330_at | TFDP1 | 1.0905238 |
| 203040_s_at | HMBS | 0.9611742 |
| 1552713_a_at | SLC4A1 | 0.8626886 |
| 201060_x_at | STOM | 0.4762092 |

- Internal Cross-validation for LPD 1 membership
  - Sensitivity 93%
  - Specificity 100%
  - Misclassification rate 1.2%

# Stage I: verification of LPD1 signature

**Gene expression verification**

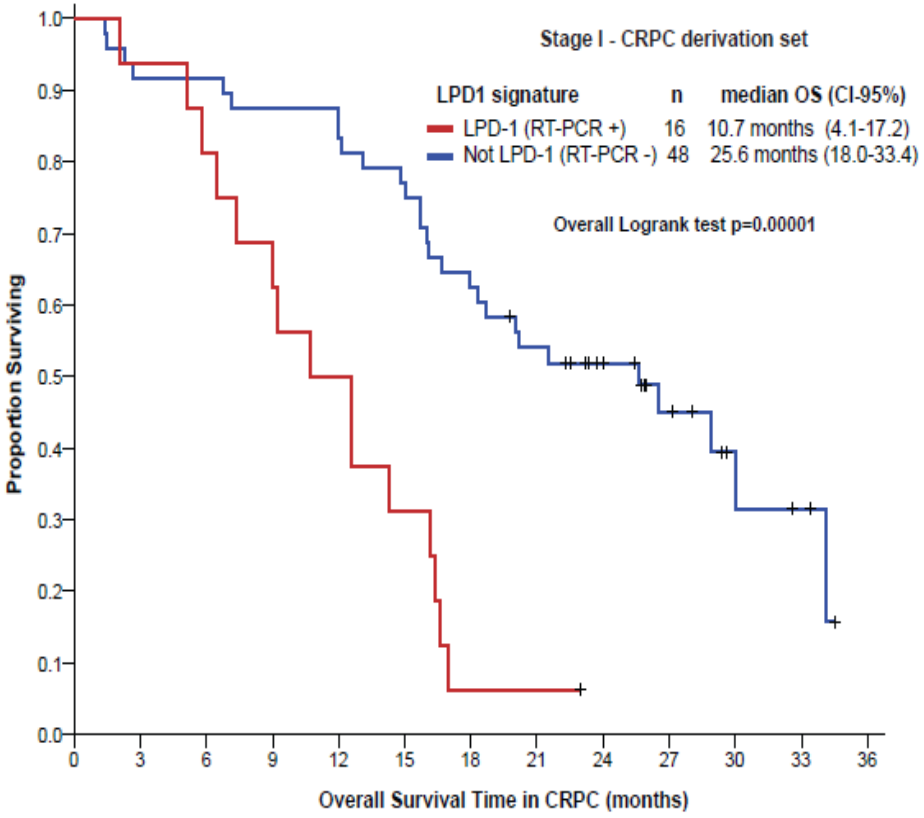
- High correlation between TaqMan and Affy Expression levels ($r^2$=0.7-0.9)
- Using this formula we can classify the 10 previously unclassified patients
  - including 6 CRPC




$$f(x)=\frac{e^z}{e^z+1}\ ,where\ z=6656.3-22.9*TMCC2(\Delta Ct)+388.6*SLC4A1(\Delta Ct)+483.3*TDFP1(\Delta Ct)+129.6*STOM(\Delta Ct)+$$
$$+110*HMBS(\Delta Ct)-301.7*RIOK3(\Delta Ct)+173.5*SNCA(\Delta Ct)+54.5*TERF2IP(\Delta Ct)-524.9*GABARAPL2(\Delta Ct)$$

$f(x) \geq 0.5$ for LPD1 membership

# Stage I: LPD1 signature and survival




## *Multivariate analyses (Cox)*

| Factor | HR | P-value |
|---|---|---|
| **LPD 1** | 3.05 | 0.017 |
| **Albumin*** | 0.91 | 0.037 |
| **Docetaxel** | 12.09 | 0.010 |
| **ECOG ≥1** | 2.01 | 0.093 |
| **ALP (*UNL)** | 1.06 | 0.146 |

* As continuous; UNL= upper normal limit

# Stage II: Validation

**Stage II Sample Size**

**Fisher-Irwin Test to compare 2 proportions**

- CRPC survival ~18 months

**Based on Stage I results:**

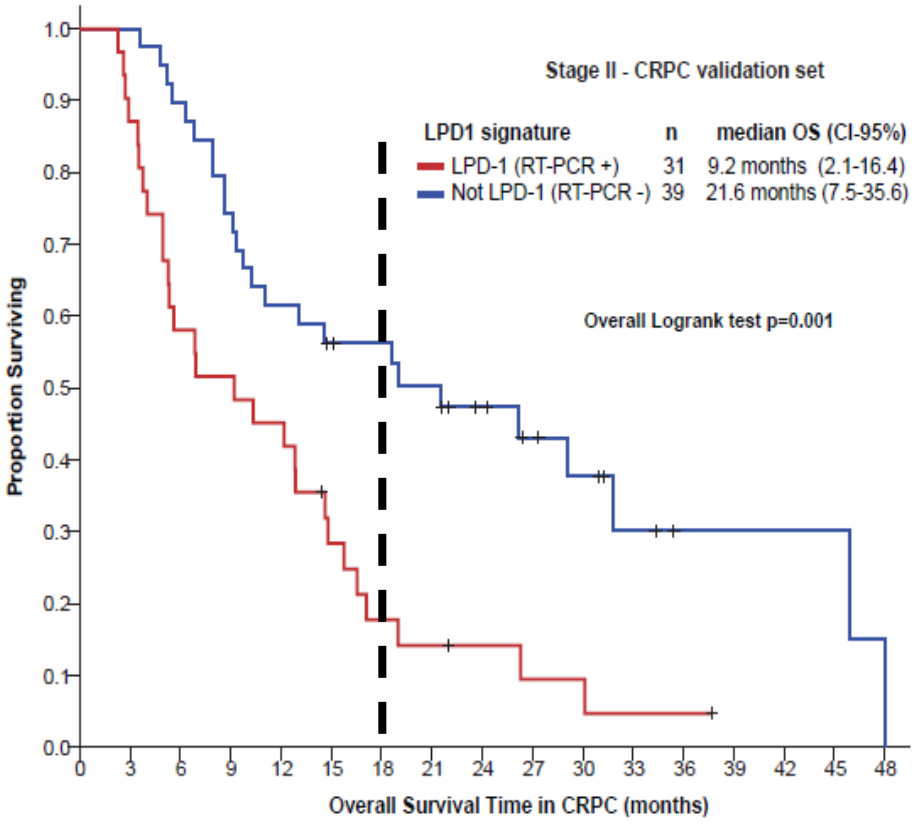
- 18-months LPD1/non-LPD1 OR = 5.0
- LPD 1 frequency ≥ 25%
- Alpha-error = 0.05 & Power =0.80

**Minimum size 66 CRPC pts**

| CRPC | |
|---|---|
| 70 patients were evaluable | |
| **Performance Status** | |
| - ECOG 0 | 21 (30%) |
| - ECOG 1 | 47 (67%) |
| - ECOG ≥2 | 2 (3%) |

| **CRPC** (continued) | |
|---|---|
| **Baseline PSA** | |
| - median ng/mL | 70 |
| - (range) | (3-5219) |
| **Gleason Score** | |
| -Gleason ≤6 | 12 (17%) |
| - Gleason 7 | 21 (30%) |
| - Gleason ≥8 | 37 (53%) |
| **Metastasis** | |
| -Bone | 68 (97%) |
| - Visceral | 11 (16%) |
| **Circulating tumour cells** | |
| - <5 CTC/7.5 mL | 29 (41%) |
| - ≥5 CTC/7.5 mL | 41 (59%) |
| **Previous chemotherapy** | |
| - Docetaxel | 47 (67%) |
| **Concomitant steroids** | |
| - Yes | 36 (51%) |

# Stage II: LPD1 signature validation




**18-months OS**

LPD 1: 16% **vs** Non-LPD1: 58%

18-months Mortality OR **5.6**

***Multivariate analyses (Cox)***

| Factor | HR | P-value |
|---|---|---|
| **LPD 1** | 1.84 | 0.047 |
| **Albumin*** | 0.85 | 0.003 |
| **Docetaxel** | 2.44 | 0.020 |
| **ECOG ≥1** | 2.60 | 0.016 |

* As continuous; UNL= upper normal limit

# Conclusions

- **In this study we demonstrated the feasibility of analyzing gene expression data from peripheral blood in PCa patients**
- **Using Bayesian unsupervised analysis we identified a robust expression profile associated with poor prognosis CRPC**
- **We derived and validated a 9-gene gene expression signature from whole blood with prognostic value in CRPC**
- **Future studies will include a prospective validation in a multi-institutional study and evaluation as predictive and pharmacodynamic biomarkers**

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