A metastatic biology gene expression assay to predict the risk of distant metastases in patients with localized prostate cancer treated with primary radical treatment

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BACKGROUND

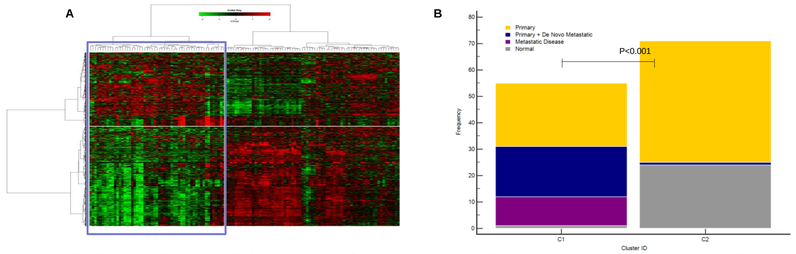
- Approximately 20% of patients with organ-confined prostate cancer (PCa) will develop disease recurrence following radical treatment (surgery or external beam radiotherapy (EBRT)).
- We hypothesized that a molecular subgroup of early PCa may have metastatic potential at presentation, resulting in disease recurrence.
- These patients may benefit from intensification of treatment such as pelvic nodal irradiation, extended lymph node dissection, extended adjuvant androgen deprivation therapy (ADT), chemotherapy or novel agents.

METHODS

- Using unsupervised hierarchical clustering of gene expression from a Discovery PCa dataset of 126 formalin-fixed and paraffin embedded (FFPE) radical prostatectomy resections including samples with known concomitant metastases, we identified a novel molecular subgroup with a transcriptional profile similar to metastatic disease (Fig. 1).
- We developed a 70-gene expression assay (Metastatic Assay) to prospectively identify patients within the subgroup from FFPE. Initial assessment found the assay to be prognostic in three independent publicly available prostatectomy datasets (Glinsky, Erho, Taylor).
- We therefore assessed the prognostic value of the assay in FFPE radical prostatectomy samples collected from multiple international sites and FFPE biopsy samples collected from patients treated with radical EBRT.
- Tumor resections and tumor biopsy specimens were obtained from 322 surgical patients (n = 61, IPCRC, Republic of Ireland; n = 142, Oslo, Norway; n = 34, Surrey, UK; n = 85, Wales Cancer Bank, UK) and 248 patients treated with radical EBRT (n = 248, Belfast, UK). The regions of highest Gleason grade were identified for macrodissection, RNA extraction and gene expression analysis.
- Samples were dichotomized as Metastatic Assay positive or negative using a pre-specified cut-off. The association of assay results with biochemical failure (BF) and distant metastases (DM) was tested on multivariate analysis (MVA).

RESULTS – Assay Development

Figure 1. *Molecular subtyping and identification of the metastatic subgroup*



A. Hierarchical clustering of transcriptional profiles from the discovery cohort. Specific genes which are upregulated (red) or downregulated (green) are labelled on the vertical axis within gene clusters.
B. Bar chart representing the number and type of each tumor mapping to each of the two identified sample clusters within the discovery cohort.

- A proportion of primary prostate cancer clustered with the primary cancers with known concomitant metastatic disease (‘Metastatic like subgroup’, highlighted in purple) whilst the normal samples clustered together with the remaining primary prostate cancer (‘Non-metastatic like subgroup’).

RESULTS – Surgical Cohort

- On MVA, the metastatic assay was significantly associated with BF (HR 1.59 [1.11-2.29], p=0.0128) and DM (HR 3.09 [1.70-5.61], p=0.0002) in the independent surgical cohort (n=322).
- In a combined model with CAPRA-S, the assay identified patients at high risk of BF (HR 2.67 [1.90-3.75], p<0.0001) and DM (HR 7.53 [4.13-13.73, p<0.0001) better than either model alone.

Figure 2. *Validation of the Metastatic Assay using an independent resection validation dataset*

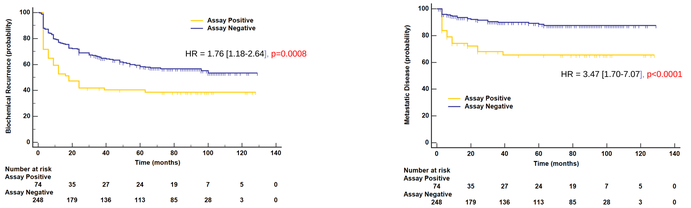


Figure 3. *Validation of the Metastatic Assay in radical resections using a combined model with CAPRA-S to stratify high- and low-risk*

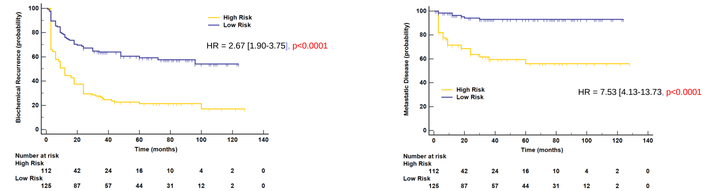


Table 1. *Validation of Metastatic Assay in primary prostate cancer resection dataset*

BIOCHEMICAL RECURRENCE

Multivariate Model 1

Covariate	HR	95% CI	*p*
Metastatic Assay	1.59	1.11 to 2.29	0.0128
Gleason Score (3 + 4)			
< 6	2.39	0.58 to 9.87	0.2295
6	0.70	0.40 to 1.23	0.2173
4 + 3	1.96	1.29 to 2.96	0.0016
8 – 10	2.80	1.82 to 4.30	<0.0001
Age	1.00	0.97 to 1.03	0.8733
PSA	1.01	1.00 to 1.01	0.0319

Multivariate Model 2

Covariate	HR	95% CI	*p*
Metastatic Assay	1.72	1.19 to 2.48	0.0042
CAPRA-S	2.52	1.79 to 3.54	<0.0001
Combined Model	**HR**	**95% CI**	***p***
Metastatic Assay + CAPRA-S	2.67	1.90 to 3.75	<0.0001

METASTATIC RECURRENCE

Multivariate Model 1

Covariate	HR	95% CI	*P*
Metastatic Assay	3.09	1.70 to 5.61	0.0002
Gleason Score (3 + 4)			
< 6	5.20	0.65 to 41.86	0.1231
6	0.50	0.11 to 2.36	0.3850
4 + 3	4.37	1.90 to 10.01	0.0005
8 – 10	6.88	2.94 to 16.13	<0.0001
Age	0.97	0.92 to 1.02	0.2550
PSA	1.00	0.99 to 1.02	0.6413

Multivariate Model 2

Covariate	HR	95% CI	*P*
Metastatic Assay	2.94	1.60 to 5.40	0.0005
CAPRA-S	4.76	2.46 to 9.23	<0.0001
Combined Model	**HR**	**95% CI**	***p***
Metastatic Assay + CAPRA-S	7.53	4.13 to 13.73	<0.0001

Abbreviations: HR, hazard ratio; CI, confidence intervals; PSA, prostate specific antigen; CAPRA-S, Cancer of the Prostate Risk Assessment post-surgical

RESULTS – Radiotherapy Cohort

- On MVA, the metastatic assay was significantly associated with BF (HR 1.86 [1.07-3.22], p=0.0277) and DM (HR 2.83 [1.13-7.11], p=0.0273) in the radiotherapy cohort (n=248).

Figure 4. *Validation of the Metastatic Assay using an independent biopsy dataset*

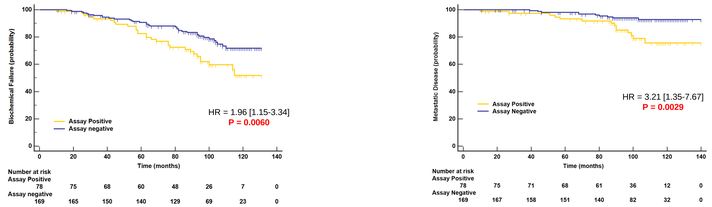


Figure 5. *Stratification of patients based on the Metastatic Assay with a High Gleason score (Gleason score ≥ 4 + 3)*

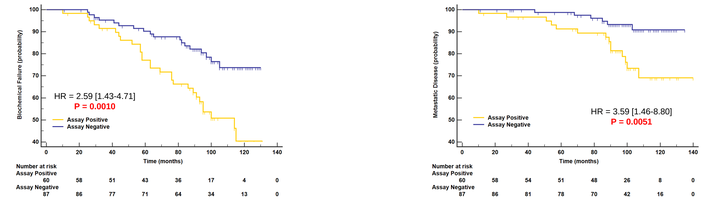


Table 2. *Performance of Metastatic Assay in independent prostate cancer radiotherapy dataset*

BIOCHEMICAL RECURRENCE

Covariate	HR	95% CI	*p*
Metastatic Assay	1.86	1.07 to 3.22	0.0277
Gleason Score			
6 (vs 3 + 4)	0.63	0.24 to 1.66	0.3506
4 + 3 (vs 3 + 4)	1.81	0.84 to 1.66	0.1290
8 – 10 (vs 3 + 4)	1.39	0.62 to 3.10	0.4290
Age	0.97	0.93 to 1.00	0.0872
PSA	1.00	1.00 to 1.00	<0.0001
ADT	1.27	0.64 to 2.55	0.4985

METASTATIC RECURRENCE

Covariate	HR	95% CI	*p*
Metastatic Assay	2.83	1.13 to 7.11	0.0273
Gleason Score			
6 (vs 3 + 4)	0.51	0.05 to 4.93	0.5666
4 + 3 (vs 3 + 4)	2.72	0.65 to 11.41	0.1747
8 – 10 (vs 3 + 4)	2.68	0.61 to 11.69	0.1924
Age	0.99	0.94 to 1.06	0.8331
PSA	1.00	1.00 to 1.00	<0.0001
ADT	2.05	0.66 to 6.35	0.2173

CONCLUSIONS

- The Metastatic Assay predicts BF and DM in PCa patients treated with either radical surgery or EBRT.
- This assay may help to select patients at low risk of relapse, who may benefit from an active surveillance approach, and to identify those patients at high risk of metastatic disease for additional treatment aimed at preventing disease recurrence.

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