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Molecular Subgroup of Primary Prostate Cancer Presenting with Metastatic Biology

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Abstract

Background: Approximately 4–25% of patients with early prostate cancer develop disease recurrence following radical prostatectomy.
Objective: To identify a molecular subgroup of prostate cancers with metastatic potential at presentation resulting in a high risk of recurrence following radical prostatectomy.
Design, setting, and participants: Unsupervised hierarchical clustering was performed using gene expression data from 70 primary resections, 31 metastatic lymph nodes, and 25 normal prostate samples. Independent assay validation was performed using 322 radical prostatectomy samples from four sites with a mean follow-up of 50.3 months.
Outcome measurements and statistical analysis: Molecular subgroups were identified using unsupervised hierarchical clustering. A partial least squares approach was used to generate a gene expression assay. Relationships with outcome (time to biochemical and metastatic recurrence) were analysed using multivariable Cox regression and log-rank analysis.
Results and limitations: A molecular subgroup of primary prostate cancer with biology similar to metastatic disease was identified. A 70-transcript signature (metastatic assay) was developed and independently validated in the radical prostatectomy samples. Metastatic assay positive patients had increased risk of biochemical recurrence (multivariable hazard ratio [HR] 1.62 [1.13–2.33]; $p = 0.0092$) and metastatic recurrence (multivariable HR = 3.20 [1.76–5.80]; $p = 0.0001$). A combined model with Cancer of the Prostate Risk Assessment post surgical (CAPRA-S) identified patients at an increased

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risk of biochemical and metastatic recurrence superior to either model alone (HR = 2.67 [1.90–3.75]; $p < 0.0001$ and HR = 7.53 [4.13–13.73]; $p < 0.0001$, respectively). The retrospective nature of the study is acknowledged as a potential limitation.
Conclusions: The metastatic assay may identify a molecular subgroup of primary prostate cancers with metastatic potential.
Patient summary: The metastatic assay may improve the ability to detect patients at risk of metastatic recurrence following radical prostatectomy. The impact of adjuvant therapies should be assessed in this higher-risk population.
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1. Introduction

Although prognosis for localised prostate cancer patients following radical prostatectomy is very good, 4–25% (dependent upon disease stage and use of population prostate-specific antigen [PSA] screening) will develop metastatic disease within 15 years [1,2]. In addition, patients with low- and some intermediate-risk prostate cancers are best treated by active surveillance; however, there is clinical uncertainty about progression in this population [3]. Progression in low/intermediate risk may be due to a more biologically aggressive genotype of primary tumours, whilst in clinically higher risk groups there may be undetected micrometastatic disease at presentation [4]. This could be treated by adjuvant approaches including pelvic radiotherapy [5], extended lymph node dissection [6], adjuvant hormone therapy [7], or chemotherapy [8].

Presently, metastatic risk is estimated from histopathologic grade (Gleason score [GS] and clinical grade grouping), tumour stage, and presenting PSA level. These prognostic factors have limitations;15% of lower-grade prostate cancer patients (Gleason $\leq$7) experience disease recurrence [9], whereas 74–76% of higher-grade patients (Gleason $>$7) do not develop metastatic disease following surgery [10]. For Gleason 7 tumours, dominant lesion grade affects prognosis, 40% of Gleason 4 + 3 patients developing recurrence by 5 years compared with 15% for Gleason 3 + 4 [11]. Clearly, there is a need to identify additional prognostic factors to guide adjuvant treatment. Current approaches can broadly be classified as mathematical risk models using clinical factors such as Cancer of the Prostate Risk Assessment (CAPRA) [12] and CAPRA-surgery (CAPRA-S) [13] scoring, or biomarkers measured from tumour tissue. Regarding biomarkers, researchers have taken immunohistochemical approaches such as high Ki67 expression [14] or PTEN loss to indicate metastatic potential [15]. Others have used multiplexing approaches where a gene expression [16–18] or proteomic signature [19] has been trained against known outcomes to predict high- and low-risk disease using archived material.

It is recognised that malignancies originating from the same anatomical site can represent different molecular entities [20]. We hypothesised that a unique molecular subgroup of primary prostate cancers may exist that has a gene expression pattern associated with metastatic disease. We took an unsupervised hierarchical clustering approach using primary localised prostate cancer, primary prostate cancer presenting with concomitant metastatic disease, lymph node metastasis, and normal prostate samples to identify a novel "metastatic subgroup". A 70-transcript signature (metastatic assay) was developed using this approach and independently validated in a cohort of radical prostatectomy samples for biochemical and metastatic recurrence.

2. Patients and methods

2.1. Study design

Study design followed the reporting recommendations for tumour marker prognostic studies (REMARK) guidelines as outlined in the criteria checklists (Supplementary Table 1 and Appendix A) and REMARK study design diagram (Supplementary Fig. 1).

2.2. Patients

Formalin-fixed paraffin-embedded (FFPE) sections from 126 samples (70 primary prostate cancer specimens from radical prostatectomy resections including those with known concomitant metastases, 31 metastatic disease in lymph nodes, and 25 histologically confirmed normal prostate samples that did not display hypertrophy, sourced from bladder resections) were collected from the University of Cambridge and the Institute of Karolinska for molecular subgroup identification (Supplementary Table 2). A secondary training dataset of 75 primary resection samples was collected, of which 20 were profiled in duplicate, to aid in the selection of the final signature length (Supplementary Table 3). For independent *in silico* validation, three public datasets were identified [17,21,22]: GSE25136 (n = 79; Supplementary Table 4), GSE46691 (n = 545; Supplementary Table 5), and GSE21034 (n = 126; Supplementary Table 6). A total of 322 FFPE prostatectomy samples from four sites were collected for independent validation of the assay (Supplementary Table 7). Biochemical recurrence was defined as a post-prostatectomy rise in PSA of $>$0.2 ng/ml followed by a subsequent rise. Metastatic recurrence was defined as radiologic evidence of any metastatic disease, including lymph node, bone, and visceral metastases. Inclusion criteria were T1a–T3c NX M0 prostate cancers treated by radical prostatectomy, no previous systemic adjuvant or neoadjuvant treatment in non-recurrence patients, and at least 3-yr follow-up. Ethical approval was obtained from East of England Research Ethics Committee (Ref: 14/EE/1066).

2.3. Metastatic subgroup and assay discovery

The 126 discovery samples were analysed for gene expression using a cDNA microarray platform optimised for FFPE tissue. Unsupervised hierarchical clustering, an unbiased statistical method to discover structure in data, was applied to the gene expression profiles. Genes were selected using variance-intensity ranking and then an iterative procedure of clustering with different gene lists to determine the optimal set for reproducibility. Data matrices were standardised to median gene expression and agglomerative two-dimensional hierarchical clustering was performed, using Euclidean

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distance and Ward's linkage. The optimal number of sample and gene clusters were identified using the GAP statistic [23].

Gene ontology biological processes determined biological significance of the gene clusters. Chi-square or analysis of variance tests were used to assess association of sample clusters with clinical data. Class labels were assigned to samples, classifying the subgroup enriched with metastatic tumours as the "metastatic-subgroup" and the subgroup enriched with normal prostate samples as the "non-metastatic-subgroup".

A signature to identify the metastatic-subgroup was developed using partial-least-squares (PLS) regression. All model development steps (preprocessing, gene filtering/selection, model parameter estimation) were nested within 10 × 5-fold cross validation (CV), including assessment of signature score reproducibility in 5× separate FFPE sections and repeatability across 20 resection samples from the secondary training dataset with technical duplicates. In sum, area under the receiver operating characteristic curve (AUC), C-index performance for metastatic recurrence in the additional dataset of 75 resections, and assay stability across replicates were used to guide the final number of transcripts detected by the assay. Thresholds for dichotomising predictions were selected at the point where sensitivity and specificity for detecting the metastatic subgroup reached a joint maximum.

2.4. Statistical assessment of metastatic assay performance

The performance of the metastatic assay regarding biochemical and metastatic progression was assessed by sensitivity and specificity. Cox regression was used to investigate prognostic effects of the assay with respect to time to recurrence endpoints. The estimated effect of the assay was adjusted for PSA, age, and GS in a multivariable model. A second multivariable analysis was performed to investigate the prognostic effect of the assay when adjusting for CAPRA-S [13], whilst further assessing additional prognostic effect of a combined model generated for the assay and CAPRA-S together. Verification of proportional hazard assumptions was assessed using a statistical test based on the Schoenfeld residuals [24]. Samples with unknown clinical factors were excluded. All tests of statistical significance were two sided at 5% level of significance.

2.5. Combined model development and application (metastatic assay and CAPRA-S)

A combined model using metastatic assay dichotomised calls and CAPRA-S dichotomised into low risk (CAPRA-S: 0–5) and high risk (CAPRA-S: 6–10) was assessed in the resection validation cohort independently against biochemical and metastatic endpoints using Cox regression analysis. Participants were classified as the "low risk" group given a combined model result of assay negative/CAPRA-S low risk; otherwise, they were labelled as the "high risk" group (ie, samples that were classified as assay negative/CAPRA-S high risk, assay positive/CAPRA-S low risk, or assay positive/CAPRA-S high risk).

See the Supplementary material for additional experimental detail.

3. Results

3.1. Molecular subtyping and identification of a metastatic subgroup in the discovery cohort

We hypothesised that a molecular subgroup of poor prognosis primary prostate cancers would be transcriptionally similar to metastatic disease. To identify this subgroup, we measured gene expression in primary prostate cancers, primary prostate cancers with known concomitant metastases, metastatic lymph node samples, and histologically confirmed normal prostate tissue (Supplementary Table 2).

Unsupervised hierarchical clustering identified two sample groups and two gene clusters (Fig. 1A). Importantly, one of the molecular subgroups (C1) demonstrated significant enrichment for primary cancers with known concomitant metastatic disease (Fig. 1A and 1B, chi-square $p < 0.0001$). In addition, the C1 group contained all metastatic lymph node samples and no normal prostate samples. We defined this subgroup as the "metastatic subgroup" and the other (C2) as the "non-metastatic subgroup".

3.2. Identifying metastatic-subgroup biology

A feature of the metastatic subgroup was loss of gene expression observed in gene cluster 1 (G1) (Fig. 1A and Supplementary Table 8). To investigate whether loss of gene expression was due to epigenetic silencing, we measured DNA methylation in eight metastatic- and 14 non-metastatic-subgroup samples (Supplementary Table 9). Semi-supervised hierarchical clustering of the methylation data of downregulated genes (G1) separated the samples into two groups (Supplementary Fig. 2 and Supplementary Table 10), with 7/8 samples (88%) from the metastatic subgroup (M2) and 10/14 samples (71%) from the nonmetastatic subgroup clustering together (M1) (chi-square, $p = 0.02$). Functional analysis demonstrated that the metastatic subgroup had higher levels of methylation in genes that negatively regulate pathways known to be involved in aggressive prostate cancer such as WNT and growth signalling (Supplementary Table 11) [25]. Together these data suggest that epigenetic silencing is a feature of the metastatic subgroup and may therefore be important in metastases.

To better understand the molecular processes upregulated in the metastatic subgroup, we performed differential gene analysis, identifying 222 genes that were overexpressed. Ingenuity Pathway Analysis (www.ingenuity.com) identified two upregulated pathways in the metastatic subgroup (False Discovery Rate (FDR) $p < 0.05$). The ToppGene Suite [26] identified 18 upregulated pathways (FDR $p < 0.05$) (Supplementary Table 12). These pathways represented mitotic progression and Forkhead Box M1 (FOXM1) pathways. Consistently, FOXM1 was 2.80-fold overexpressed in the metastatic subgroup.

3.3. Development of a metastatic assay

Next, we developed an assay that could identify metastatic-subgroup tumours (Supplementary Fig. 3). Computational classification using PLS regression resulted in a 70-transcript metastatic assay. In the training set, the AUC under CV for detecting the metastatic-subgroup was 99.1 (98.5–99.8). The standard deviation (SD) in assay scores using five separate sections from the same tumour was 0.06, representing 6.9% of the assay range and 100% agreement in assay call. In a secondary training dataset of 75 primary resections, the C-index for detecting the metastatic subgroup was 90.4, with an SD in assay scores using 20 patient samples with technical replicates of 0.02 representing 2.9% of assay range (Supplementary Fig. 4).

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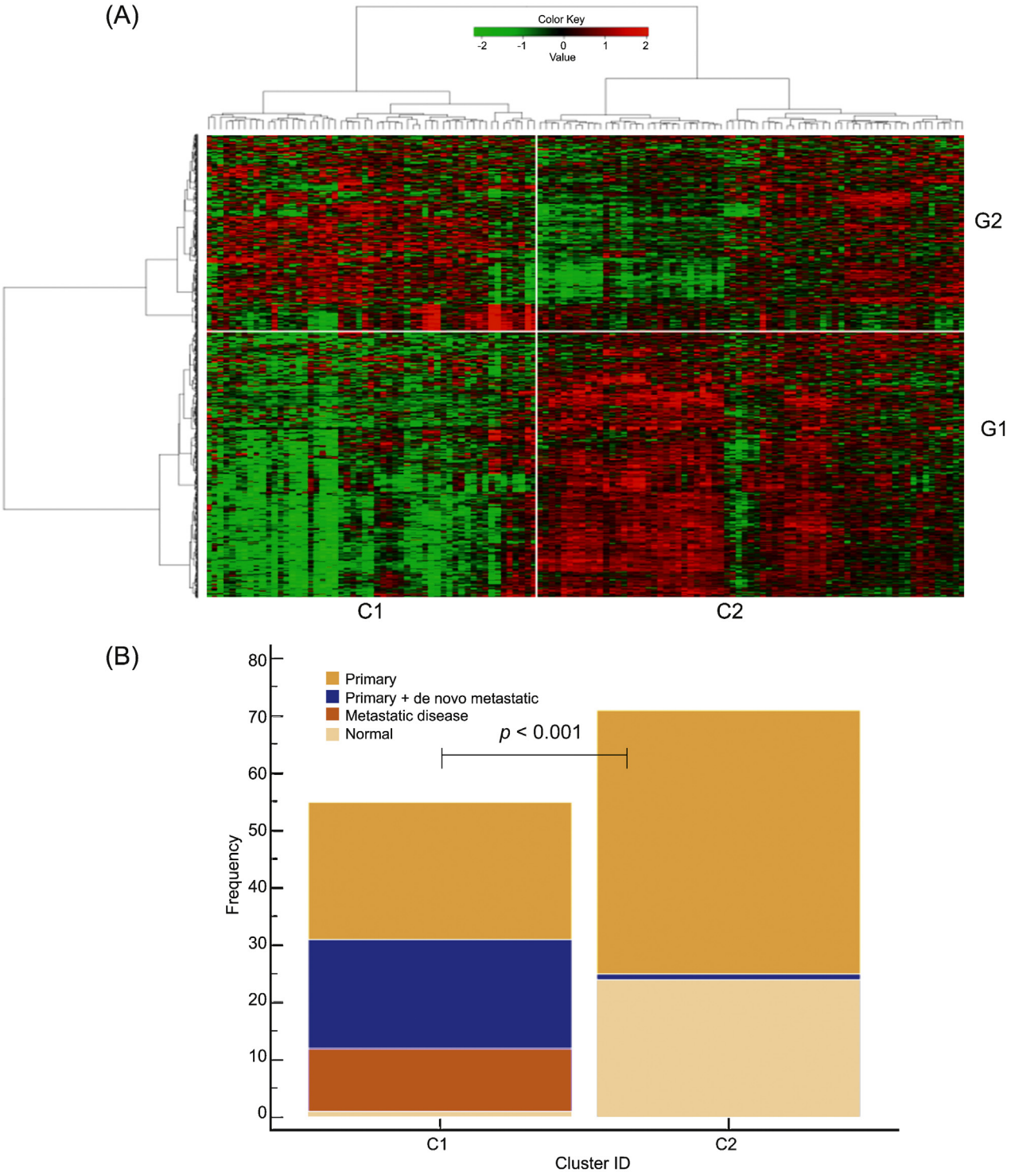

Fig. 1 – Molecular subtyping and identification of the metastatic subgroup. (A) Hierarchical clustering of transcriptional profiles from the discovery cohort. Specific genes that are upregulated (red) or downregulated (green) are labelled on the vertical axis within gene clusters. Sample cluster C1 represents the "metastatic subgroup" characterised by a shutdown of gene expression (G1) compared with sample cluster C2. (B) Bar chart representing the number and type of each tumour mapping to each of the two identified sample clusters within the discovery cohort.

Importantly, as the assay was trained against a distinct molecular subgroup rather than clinical outcome, there was a bimodal distribution of scores (Supplementary Fig. 5). The metastatic assay gene list and weightings are listed in Supplementary Table 13.

3.4. *Metastatic assay performance in public datasets*

The assay was applied to three independent public prostate cancer resection gene expression datasets. Assay scores were calculated using the PLS model and dichotomised into

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Table 1 – Validation of metastatic assay in the Memorial Sloan Kettering Cancer Centre (MSKCC) cohort

Biochemical recurrence				Metastatic recurrence			
Covariate	HR	95% CI	*p*	Covariate	HR	95% CI	*p*
Multivariate model 1				Multivariate model 1			
Metastatic assay	3.03	1.43–6.41	0.0040	Metastatic assay	2.53	0.67–9.54	0.1735
Gleason (3 + 4)				Gleason (3 + 4) [a]			
<7	0.38	0.10–1.37	0.1409	<7	0.00	0.00	0.9658
4 + 3	2.04	0.76–5.43	0.1579	4 + 3	22.61	2.34–218.06	0.0073
8–10	8.09	2.74–23.91	0.0002	8–10	187.79	16.52–2134.99	<0.0001
Age	0.99	0.94–1.04	0.6564	Age	0.88	0.80–0.97	0.0110
PSA	1.00	0.96–1.04	0.9857	PSA	0.94	0.89–0.98	0.0106
Multivariate model 2				Multivariate model 2			
Metastatic assay	3.35	1.62–6.94	0.0012	Metastatic assay	3.95	1.15–13.53	0.0298
CAPRA-S	3.92	1.92–7.99	0.0002	CAPRA-S	3.50	1.13–10.80	0.0302

HR = hazard ratio; CI = confidence intervals; PSA = prostate-specific antigen; CAPRA-S = Cancer of the Prostate Risk Assessment postsurgical.
Multivariable analysis of the MSKCC cohort for biochemical recurrence (right) and metastatic recurrence (left), *p* values, HRs and 95% CIs of the HR are outlined within the table (multivariate model 1). Covariate analysis of the metastatic assay adjusting for CAPRA-S within the MSKCC cohort is also included with *p* values, HRs and 95% CIs of the HR are outlined (multivariate model 2).
[a] Absence of metastatic events in patients with Gleason score <3 + 4.

assay positive and assay negative. In the first ($n = 79$) [21], the assay was significantly associated with biochemical recurrence with a sensitivity of 70.3% and specificity of 66.7% (chi-square $p = 0.0049$). In a second ($n = 545$) [17], the assay was significantly associated with metastatic recurrence with a sensitivity of 67.0% and specificity of 54.6% (chi-square $p < 0.0001$) (Supplementary Table 14). Using a third dataset with time to event data ($n = 126$) [22], multivariable analysis adjusting for Gleason (grades represented in four subgroups), age, and PSA demonstrated an increased risk of biochemical recurrence (hazard ratio [HR] = 3.03 [1.43–6.41]; $p = 0.0040$; Table 1 and Fig. 2A). However, possibly due to the small number of metastatic events (11%), the association with outcome in multivariable analysis did not reach statistical significance (HR = 2.53 [0.67–9.54]; $p = 0.1735$; Table 1 and Fig. 2B).

3.5. *Metastatic assay performance in an independent primary prostate cancer resection dataset*

The assay was then applied to 322 FFPE prostatectomy samples from four clinical sites with a median follow-up of 50.3 months using predefined inclusion/exclusion criteria per REMARK guidelines (Supplementary Fig. 1). A predefined assay cut-off of 0.3613 was used to define metastatic assay positivity in a blinded manner. On multivariable analysis, a positive assay result was associated with an increased risk of biochemical recurrence (HR = 1.62 [1.13–2.33]; $p = 0.0092$;

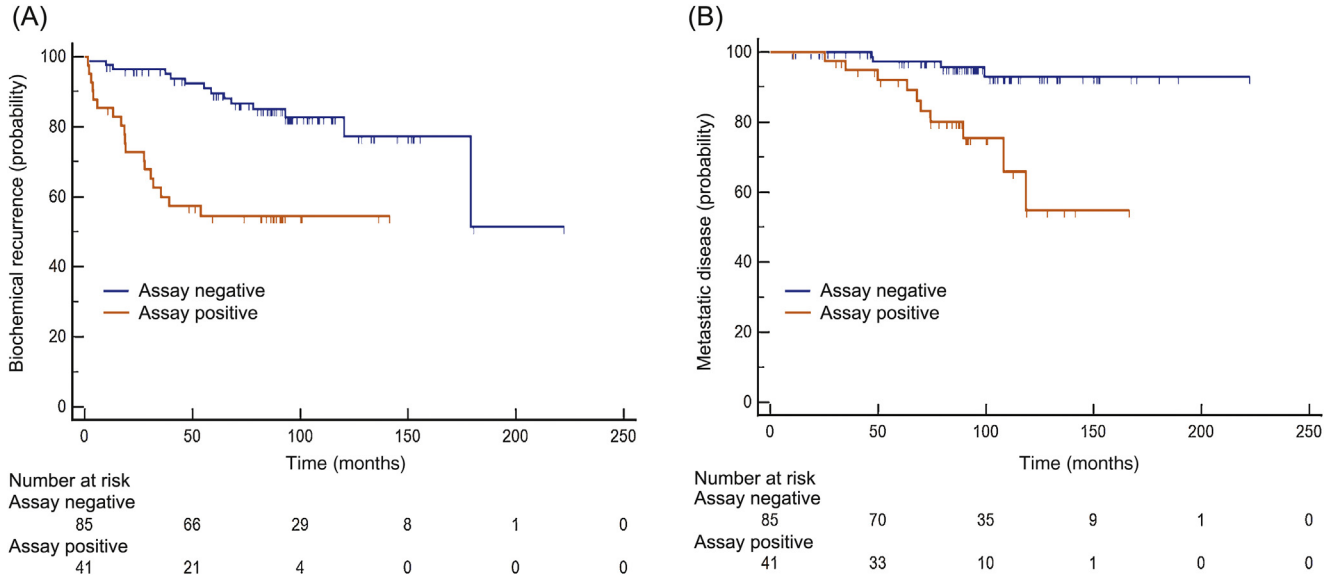

Fig. 2 – Validation of the metastatic assay in resections using the MSKCC *in silico* dataset. Kaplan–Meier survival analysis for association of the metastatic assay at predicting (A) time to biochemical recurrence and (B) metastatic recurrence in the MSKCC *in silico* cohort. Survival probability (%) showed reduced progression-free survival in months of the "assay positive" (orange) of 41 patients when compared with the "assay negative" (blue) of 85 patients for biochemical and metastatic disease respectively (HR = 3.76 [1.70–8.34]; $p < 0.0001$ and HR = 6.00 [1.90–18.91]; $p = 0.0005$, respectively). HR = hazard ratio.

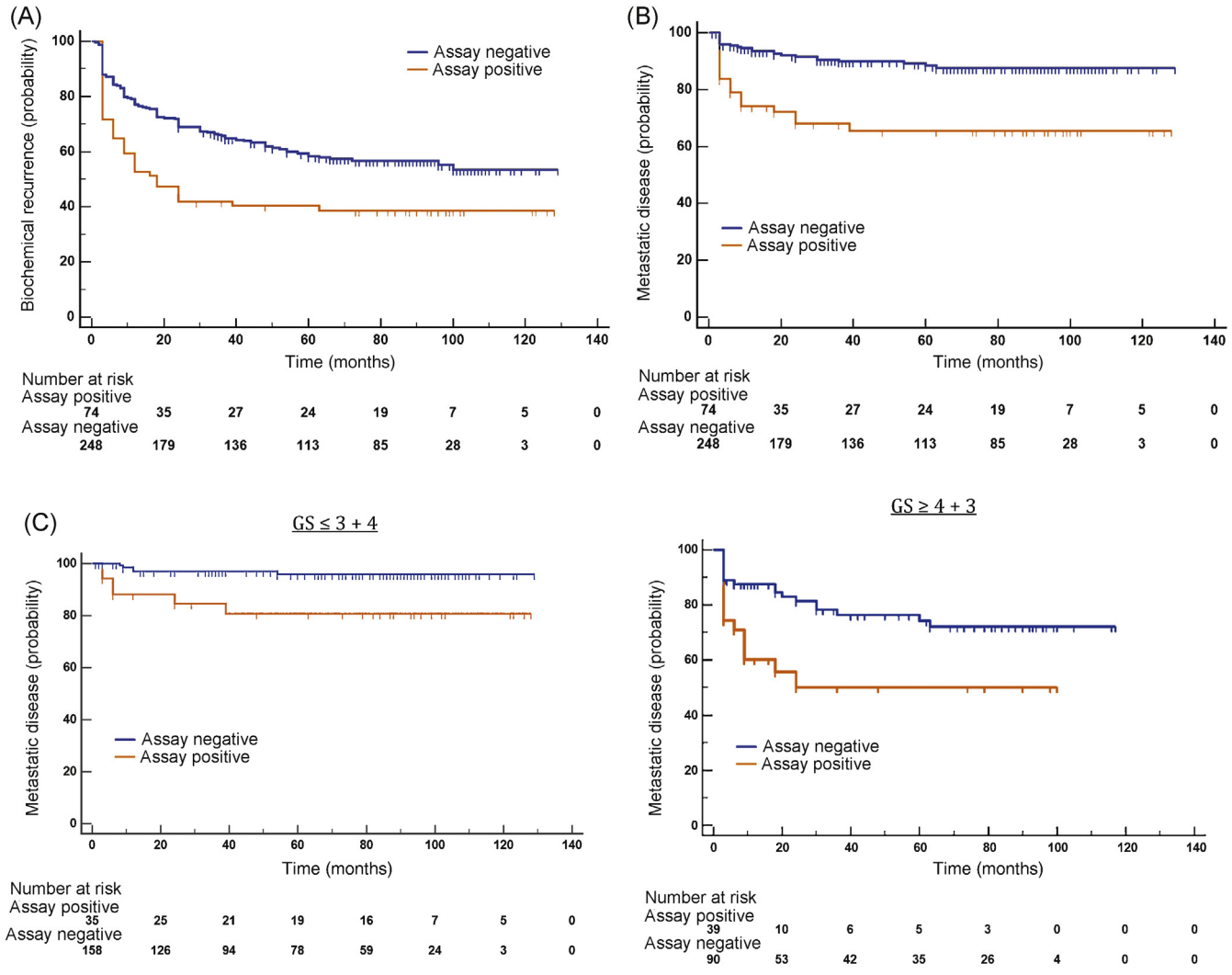

Fig. 3 – Validation of the metastatic assay in the retrospective independent resection validation dataset. Kaplan–Meier survival analysis for association of the metastatic assay at predicting (A) time to biochemical recurrence and (B) metastatic recurrence in the resection validation cohort. Survival probability (%) showed reduced progression-free survival in months of the "assay positive" (orange) of 74 patients when compared with the "assay negative" (blue) of 248 patients for biochemical and metastatic disease respectively (HR = 1.76 [1.18–2.64]; $p = 0.0008$ and HR = 3.47 [1.70–7.07]; $p < 0.0001$, respectively). (C) Association of the metastatic assay at predicting metastatic recurrence stratified into low-risk (GS ≤ 3 + 4) and high-risk (GS ≥ 4 + 3) tumours (HR = 5.61 [1.19–26.47]; $p = 0.0013$ and HR = 2.43 [1.14–5.17]; $p = 0.0036$ respectively). GS = Gleason score; HR = hazard ratio.

Fig. 3A and Table 2) and metastatic recurrence (HR = 3.20 [1.76–5.80]; $p = 0.0001$; Fig. 3B and Table 2). Although the assay was designed to provide information as a dichotomous result, it was also an independent predictor of both biochemical and metastatic recurrence when assessed as a continuous variable in multivariate analysis (HR = 1.16 [1.03–1.30]; $p = 0.0155$ and HR = 1.52 [1.24–1.85]; $p < 0.0001$ [per 0.1 unit change in assay score]; Supplementary Table 15).

3.6. Comparison of the metastatic assay with clinical risk stratification

To test assay independence from approaches used in the clinic, we assessed its performance within risk groups defined by GS and the CAPRA-S model in the independent resection validation cohort. When separated by Gleason (high-risk GS ≥4 + 3 and low-risk GS ≤3 + 4), the metastatic assay identified patients at higher risk of metastatic recurrence with an HR of 2.43 (1.14–5.17; $p = 0.0036$) and HR of 5.61 (1.19–26.47; $p = 0.0013$) in the high- and low-risk GS groups, respectively (Fig. 3C).

The CAPRA-S prognostic model uses PSA at presentation, age, GS, T-stage, seminal vesicle invasion, extracapsular extension, lymph node invasion, and surgical margins [13]. In a multivariable analysis adjusted for CAPRA-S, both the metastatic assay and the CAPRA-S were significantly associated with biochemical recurrence (HR = 1.72 [1.19–2.48]; $p = 0.0042$ and HR = 2.52 [1.79–3.54]; $p < 0.0001$) and development of metastatic disease (HR = 2.94 [1.60–5.40]; $p = 0.0005$ and HR = 4.76 [2.46–9.23]; $p < 0.0001$; Table 2). Given the independence of the metastatic assay result and CAPRA-S score, a combined model was assessed. Patients classified within the high-risk subgroup were

Table 2 – Validation of metastatic assay in the independent resection validation dataset

Biochemical recurrence				Metastatic recurrence			
Covariate	HR	95% CI	*p*	Covariate	HR	95% CI	*p*
Multivariate model 1				Multivariate model 1			
Metastatic assay	1.62	1.13–2.33	0.0092	Metastatic assay	3.20	1.76–5.80	0.0001
Gleason (3 + 4)				Gleason (3 + 4)			
<7	0.76	0.44–1.30	0.3224	<7	0.72	0.19–2.73	0.6358
4 + 3	1.95	1.29–2.95	0.0017	4 + 3	4.33	1.89–9.93	0.0006
8–10	2.79	1.82–4.30	<0.0001	8–10	6.85	2.92–16.04	<0.0001
Age	1.00	0.97–1.03	0.9027	Age	0.97	0.92–1.02	0.2828
PSA	1.01	1.00–1.01	0.0321	PSA	1.00	0.99–1.01	0.6423
Multivariate model 2				Multivariate model 2			
Metastatic assay	1.72	1.19–2.48	0.0042	Metastatic assay	2.94	1.60–5.40	0.0005
CAPRA-S	2.52	1.79–3.54	<0.0001	CAPRA-S	4.76	2.46–9.23	<0.0001
Combined model				Combined model			
Metastatic assay + CAPRA-S	2.67	1.90–3.75	<0.0001	Metastatic assay + CAPRA-S	7.53	4.13–13.73	<0.0001

HR = hazard ratio; CI = confidence intervals; PSA = prostate-specific antigen; CAPRA-S = Cancer of the Prostate Risk Assessment postsurgical.
Multivariable analysis of the metastatic assay in the independent resection validation cohort for biochemical recurrence (right) and metastatic recurrence (left), *p* values, HRs and 95% CIs of the HR are outlined within the table (multivariate model 1). Covariate analysis of the metastatic assay adjusting for CAPRA-S within the independent resection validation cohort is also included with *p* values, HRs and 95% CIs of the HR outlined (multivariate model 2). Analysis from a combined model of the metastatic assay and CAPRA-S within the independent resection validation cohort was also assessed, outlining *p* values, HRs, and 95% CIs for biochemical and metastatic disease recurrence (combined model).

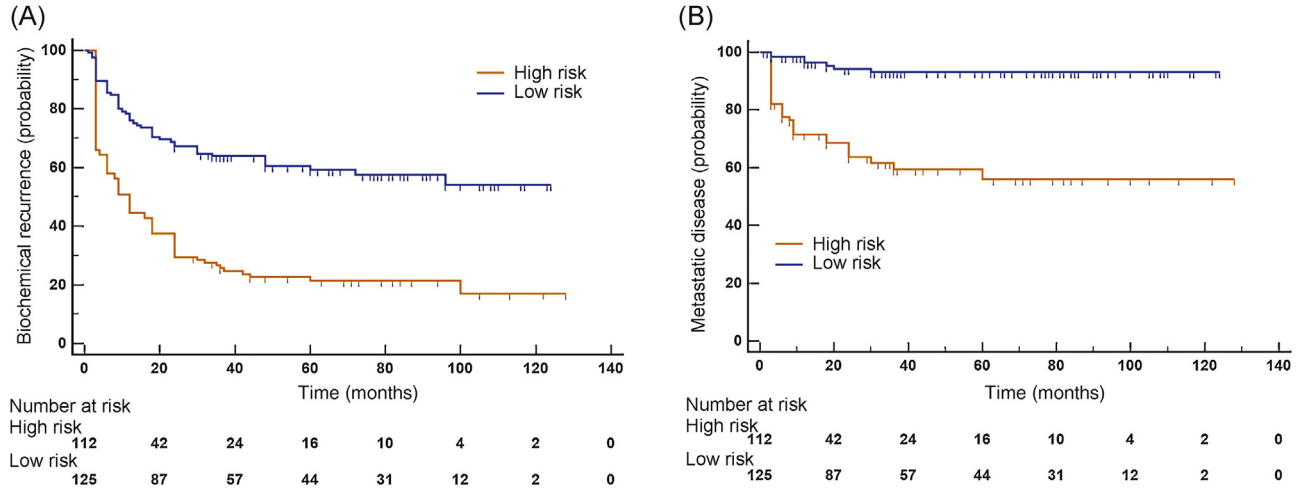

Fig. 4 – Validation of the metastatic assay in resections using a combined model with CAPRA-S to stratify high and low risk. (A) Association of a combined model (metastatic assay + CAPRA-S) at predicting time to biochemical recurrence of high/low-risk disease in the resection cohort. Reduced progression-free survival in months of the "high-risk" subgroup (orange) of 112 patients when compared with the "low-risk" subgroup (blue) of 125 patients (HR = 2.67 [1.90–3.75]; $p < 0.0001$). (B) Association of a combined model (metastatic assay + CAPRA-S) at predicting time to metastatic disease recurrence of high/low-risk disease in the resection cohort. Reduced progression-free survival in months of the "high-risk" subgroup (orange) of 112 patients compared with the "low-risk" subgroup (blue) of 125 patients (HR = 7.53 [4.13–13.73]; $p < 0.0001$). CAPRA-S = Cancer of the Prostate Risk Assessment postsurgical; HR = hazard ratio.

significantly associated with both biochemical and metastatic recurrence (HR = 2.67 [1.90–3.75]; $p < 0.0001$ and HR = 7.53 [4.13–13.73]; $p < 0.0001$, respectively), demonstrating superiority to either model alone (Fig. 4 and Table 2, combined model).

To assess the clinical impact of the combined model of metastatic assay plus CAPRA-S, additional performance metrics were assessed for the metastatic endpoint in the independent resection validation cohort. As the assay was dichotomous, the comparison of sensitivity and specificity between the metastatic assay alone, CAPRA-S alone, and the combined model were investigated. Whilst the sensitivity of CAPRA-S (70.5%) was greater than that of the metastatic assay alone (47.7%), there was an increase in sensitivity to 80.1% in the combined model. There was, however, a decrease in specificity from 81.9% (metastatic assay) and 71.5% (CAPRA-S) to 61.1% in the combined model, which may indicate patients who have not yet experienced recurrence within the 50.3-months median follow-up (Supplementary Table 16).

Assessment as a continuous predictor using AUC and decision curve analysis demonstrated an improvement in discrimination power of metastatic events and a greater net benefit for the combined model at a representative risk

threshold of 25% (AUC = 0.80 and net benefit = 0.052), compared with either metastatic assay (AUC = 0.71 and net benefit = 0.035) or CAPRA-S alone (AUC = 0.76 and net benefit = 0.021) (Supplementary Table 17 and Supplementary Fig. 6). This suggests that for patients with a 25% risk of developing metastatic recurrence, a greater net benefit is achieved using the metastatic assay in conjunction with CAPRA-S. In addition, the continuous combined model had a C-index of 0.82 (0.76–0.86) compared with a C-index of 0.71 (0.64–0.78) for metastatic assay and a C-index of 0.73 (0.66–0.79) for CAPRA-S alone (Supplementary Table 17).

4. Discussion

The majority of early prostate cancer patients treated by radical resection are cured. However, up to 25% of patients develop metastatic disease within 15 years [1,2]. In surveillance for low/intermediate-risk disease, there is concern about risks of clinical undergrading and disease progression, with a proportion of patients needing treatment within 5 years [3]. This engenders clinical uncertainty in modern practice in two key areas: firstly, in the appropriate and safe selection of patients for active surveillance, particularly in the Gleason 3 + 4 intermediate group, and secondly, in patients undergoing radical local treatment for intermediate- and higher-grade tumours, where adjuvant locoregional and systemic treatment may improve outcome. A test that helps select patients at a higher risk of progression in these settings will have significant clinical utility.

Several prognostic gene expression assays have been developed by comparing gene expression data between good and poor outcome patients [16–18]. In contrast, we identified a molecular subgroup of primary prostate cancer samples that shared biology with metastatic disease. We developed an assay for this molecular subgroup, which identified patients at risk of biochemical and metastatic recurrence in three publicly available and one prospectively collected multicentre dataset.

Consistent with the molecular subgroup representing metastatic biology, the assay was better at predicting metastatic recurrence rather than biochemical recurrence. The latter does not necessarily predict metastatic development; only one-third of patients with biochemical recurrence develop measurable metastatic disease 8 years after resection [27]. In addition, the HR of 3.20 for metastatic recurrence compares favourably to the reported hazard ratios for other prognostic assays to predict metastatic disease, with HRs ranging between 1.40 and 3.30 [16–18]. A significant feature of assay performance was independence from CAPRA-S, allowing the development of a combined risk model with superior performance to either CAPRA-S or the metastatic assay individually.

An interesting feature of the metastatic subgroup was methylation and loss of gene expression such as OLFM4 known to inhibit metastatic processes including WNT signalling [28]. It is therefore possible that novel therapies aimed at reversing epigenetic silencing or targeting WNT signalling may act against the metastatic biology in this molecular subgroup [29]. Regarding upregulated genes in the metastatic-subgroup, a significant proportion was regulated by FOXM1 known to promote prostate cancer progression [30]. Indeed, others have found increased *FOXM1* gene expression to be prognostic and have included it in a 31-gene expression assay [16]. Interestingly only 6/70 genes in the metastatic assay overlapped with three prognostic signatures that are entering clinical practice (AZGP1 [18], PTTG1, TK1 and KIF11 [16], and ANO7 and MYBPC1 [17])—Oncotype Prostate ($p = 0.16$), Prolaris ($p = 0.06$), and Decipher ($p = 0.06$)—after multiple test correction using a Benjamini–Hochberg correction, likely reflecting the distinct approach of molecular subtyping versus trained endpoint analysis (Supplementary Fig. 7).

A potential limitation of this study is the retrospective validation of the assay in historic datasets. Diagnostic and surgical approaches have improved with time, which may reduce disease recurrence. We expect, however, that the effect of these improvements would mostly be on local recurrence, whereas this assay has been developed to predict metastatic disease progression, likely largely beyond surgical control at presentation.

5. Conclusions

We have identified a molecular subgroup of primary prostate cancer with metastatic capacity. We hypothesise that using this molecular subtyping approach may improve patient stratification considering active surveillance and may benefit patients with higher-risk clinically localised disease by focusing loco-regional and systemic adjuvant therapy in those at the highest risk of regional and systemic failure.

Author contributions: Richard D. Kennedy had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Walker, Harkin, Kennedy.
Acquisition of data: Walker, Knight, Logan, Blayney, McCavigan, Price, Jellema, Steele.
Analysis and interpretation of data: Walker, Knight, Kennedy.
Drafting of the manuscript: Walker, Logan, Knight, Clarke, Kennedy.
Critical revision of the manuscript for important intellectual content: Waugh, Mills, Neal, Clarke, Harkin.
Statistical analysis: McCavigan, Knight, Steele.
Obtaining funding: Kennedy, Harkin.
Administrative, technical, or material support: Sherif, Warren, Neal, Berge, Svindland, Pandha, Mason, McDade, Watson, Davidson, Uprichard, Kay, Eden, Foster.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2017.03.027.

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