Evidence table 1

	Study/Patients' characteristics	Findings	Conclusion & Quality/RoB
Analytical validity			
Warf et al., 2015 Aim: to demonstrate that the CCP score is a robust and reproducible molecular diagnostic tool	Commercial samples of FFPE prostate biopsy or RP tissue, or residual RNA were used for this validation study. Precision of the CCP score was assessed in a set of 6 FFPE biopsy and 12 FFPE RP samples. The linear range for each gene was tested on three "samples", each of which was the combination of RNA from biopsy and RP clinical samples with known CCP scores.	The precision of the signature was determined by testing 18 Samples. Standard deviation (SD) of the signature was 0.1 CCP score units (95% CI, 0.08- 0.13) between measurements. This represents 1.6% of the observed CCP scores from previous validation studies (observed CCP scores varied from -2 to 4.1). Stability of stored RNA: Stability was assessed by testing the reproducibility of CCP scores of 11 samples (5 biopsy and 6 RP) over an 8-week timeframe. Each sample was tested every 2 weeks. CCP was reproducible as SD was ≤ 0.1 CCP score units, which is similar to overall precision of the signature. Yields of RNA extraction: 100% of the RP (952/952) and biopsy samples (6,573/6,573) provided sufficient RNA for testing. Linearity of the signature: The linear range of the signature was 260-fold range of RNA concentrations. **This range surpasses the 20-fold range of RNA concentrations over which the signature was clinically validated, and clinical samples are tested** (40 to 2 ng/µL). Three samples were tested across a range of RNA concentrations from 125 to 0.06 ng/µL. All 3 samples had consistent CCP scores. Amplification efficiency of genes within the CCP gene expression signature: there is no statistical difference in the amplification efficiencies when comparing housekeeper and target genes (P= 0.39).	CCP gene expression signature is precise & reproducible when testing prostate FFPE needle biopsy and FFPE RP samples.
Clinical validity			
Cuzick et al., 2015 Design: retrospective study Aim: to validate the **prognostic value** of a cell cycle progression score (CCP score) and combined clinical-cell-cycle-risk (CCR) score. **Main outcome** was **prostate cancer related mortality.**	N= 585 men. Patients' characteristics: Median age at dx: 71 y; 36% had PSA between 10 and 25; 30% had PSA between 4 and 10. Median follow-up was 9.5 years. Median CCP score: 0.40. 35% had CCP score >0-1; 39% had CCP score >1-2. 31% and 30% had Gleason score 3 + 4 and 4+3 respectively. CAPRA 8-10: 53% CCR >3: 67% Clinical stage 2: 371 (43); 3/4 127 (47) Patients were diagnosed with localized prostate cancer by needle biopsy and treated conservatively 15% had inadequate tumor & 83% failed CCP score quality assurance. Inclusion: age >76 y at dx and had clinically localized prostate cancer diagnosed by needle biopsy.	Univariate analysis (for one unit change in CCP score): CCP score HR 2.08 (1.76, 2.46), P<10 -13). The higher the CCP score, the higher the hazard ratio (>0-1: HR=2.21 (1.15-4.27); >1-2: HR=6.84 (3.57-13.1); >2: HR=14.1 (6.48-30.5)). The 10-year death rates increased with higher CCP score. All clinical variables were significant at the univariate level except for age at diagnosis. In the multivariate analysis including CAPRA, the CCP score hazard ratio was 1.76 (95% CI (1.44, 2.14), P<10 -6) after adjusting for GS, PSA, extent of disease, and clinical stage. No interaction was found between CCP and CAPRA, Gleason score, or PSA The clinical-cell cycle-risk (CCR) score, which is a predefined linear combination of CCP score and CAPRA, was also predictive of mortality [2.17 (95% CI (1.83, 2.57), x2 = 89.0, P<10 -20)]. The CCR score was based on standard clinical variables. No other variable added significant information to the predefined CCR	Among patients with localized prostate cancer by needle biopsy and who were conservatively managed, CCP & CCR scores are predictive of mortality. CCP can provide prognostic information that could not be obtained from clinical data. Limitations: study included symptomatic patients with worse prognosis than contemporary cohorts; Tx change on or after 6 months were unknown except in 170 men (29%). Some

	Exclusion: Patients treated by RP or radiation therapy within 6 months of dx.	Score. In exploratory analysis, there is no statistically significant difference in the predictive value of CCP score for the first 5 years compared to after 10 years.	misclassification of cause of death may have occurred; retrospective study; Some authors were employees of Myriad Genetics, the manufacturer. Quality: low
Cuzick et al., 2012 Design: retrospective study Aim: prostate cancer mortality This study is similar to the previous one (Cuzick et al., 2015). The aim, inclusion & exclusion criteria are similar.	Patients' characteristics: median age 70.5 (range 65.8-73.4). PSA 21.4 (range 11.9-42.0). Median follow-up: 11.8 years. Gleason score was distributed as followed: <7: 30.4%, 7: 43.6%, >7: 26.1%. N= 349 patients. Most were clinical stage T2 (n=106), most had GS 7 (n=152, 43.6%) Comparators: Gleason score, PSA, extent of disease, age at dx, clinical stage, hormone use, Ki67 IHC.	Univariate analysis: For CCP score, HR was 2,56 (1.90, 3.45). The 10-year mortality rate (for 1-unit increase in CCP score) increased as CCP score increased [CCP < 0, death rate was 19.3% and increased to 19.8%, 21.1%, 48.2%, and 74.9% for CCP score groups (0–1, 1–2, 2–3, and >3, respectively)]. In the multivariate analysis: Only CCP score (HR 1.65 (1.31, 2.09)) χ2=17.7; P=2.6×10-5), Gleason score [<7: HR 0.61 (0.32, 1.16) P=5.0x10-4; GS >7: HR 1.90 (1.18, 3.07)], and PSA [log (1+PSA) HR1.37 (1.05, 1.79) P=0.017] were statistically significant. However, CCP score was a stronger prognostic factor than Gleason score or PSA (HR 1.96 (1.43, 2.68)). The predictive effect of CCP score in the first 5 years was strong and significant whereas beyond 5 years, CCP lost its predictive effect (multivariate HR 2.14, (1.55, 2.95) x2=22, P=3x10-6) with a much lesser effect thereafter (multivariate HR=1.27 (0.92, 1.75) x2=2.1, P=0.15).	CCP score is an independent predictor of mortality among patients with localized prostate cancer by needle biopsy and who were conservatively managed. Limitations: Tx change on or after 6 months were unknown; Misclassification of cause of death may have occurred; retrospective study; Some authors were employees of Myriad Genetics, the manufacturer; The assay produced a score in 79% of the samples. Quality: low
Bishoff et al., 2014 Design: retrospective study Aim: to assess the prognostic value (association with biochemical recurrence and metastasis) of the CCP score in men treated with prostatectomy	There were 3 cohorts. All patients were diagnosed with prostate adenocarcinoma without evidence of lymph node or bone metastasis. The CCP score was derived from simulated biopsy or diagnostic biopsy. Clinical characteristics: Median RP CCP score varied from -0.4 (-0.9, 0.2) to 0.3 (-0.9, 0.3). Median age at surgery were similar and ranged from 62 to 63 years. Median PSA varied from 5.5 to 7.2 ng/ml. Gleason score varied but most people had Gleason score < 7 in all the cohorts. The clinical stage of most patients was T1. The median follow-up ranged from 61 to 132 months. The number of BCR events varied. Comparators included PSA, Gleason score, age at dx, clinical stage, adjuvant Tx, percentage positive cores.	CCP was associated with biochemical recurrence and no significant interaction was found between CCP score and other variables (α=0.01). In the first cohort, HR was 2.05 (95% CI 1.49–2.82) (N=283, 48/283 had BCR) for each 1-unit increase in CCP score. In multivariable analysis, CCP score was still a significant predictor of BCR (HR 1.66 (1.19, 2.32) P=0.0033). In the second & third cohorts (N=176, N=123), HR were 1.33 (1.04–1.70, p = 0.027) and 1.86 (1.25–2.78, p = 0.0028) respectively. CCP score remained a significant predictor of BCR in multivariate analysis (HR not shown). CCP score was associated with metastasis in all the cohorts: In the first cohort HR was 7.33 (2.26–23.8), P= 2.7 × 10–4). In the second & third cohorts, HR were 6.14 (2.15–17.5, p = 1.2 × 10–4) and 3.32 (1.10–9.99, p = 0.035).	CCP score, derived from biopsy specimen, may be associated with BCR and predict metastatic disease. Limitations included: retrospective design, small sample size with patients with metastasis, majority of authors have financial ties with the manufacturer. The quality of the study is low.

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<td>Rayford et al., 2018

Aim: to investigate whether CCP score can improve risk stratification in African Americans.</td>
<td>The study included 150 AA and 60 Caucasians. The groups were significantly different in Gleason score, clinical stage, and AUA risk. Median age was 65 years and PSA was 5 ng/ml.
AA vs Caucasians: Most were Intermediate to low risk
GS: 3+4, 78 [52%] vs 25 [42%]; p=0.020
Clinical Stage: T1c, 118 [79%] vs 56 [93%]; p=0.0022
AUA risk:
Low 30 [20%] vs 25 [42%]
Intermediate 60 [40%] vs 25 [42%]
High 60 [40%] vs 10 [17%]
PSA (ng/mL): 5.6 [4.0–8.8] vs 4.8 [3.6–6.9]; p=0.093</td>
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<tr><th></th><th>Clinical Parameters AA vs Caucasians</th><th>CCP score AA vs Caucasians</th><th>CCP score in the AUA risk</th></tr>
<tr><td>Low risk</td><td>20% vs 42%</td><td>30% were more aggressive vs 12% were more aggressive</td><td>3.2 vs. 2.9 (Difference btw AA & Caucasian not significant)</td></tr>
<tr><td>Intermediate risk</td><td>40% vs 42%</td><td>21.67% were more aggressive vs 8%</td><td>3.4 vs. 3.2 (Difference btw AA & Caucasian not significant)</td></tr>
<tr><td>High risk</td><td>40% vs 17%</td><td>23.33% were more aggressive vs 10%
Overall 24% of AA vs 10% of Caucasians were reclassified to higher risk by CCP score</td><td>3.8 vs. 3.5 (Difference btw AA & Caucasian not significant)</td></tr>
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<td>CCP score could improve risk stratification (and perhaps mortality) in AA men beyond clinical parameters.

Limitations: retrospective design, lack of report of follow-up, data is derived from a single practice center, significant difference in clinical characteristics.

Quality: low</td>
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<tr><td>Lin et al., 2018</td><td colspan="4">Very low-quality study concluded CCR score threshold adequately categorized patients into low and high-risk groups for 10-year prostate cancer mortality (PCM).</td></tr>
<tr><td>Canter et al., 2019</td><td colspan="4">Very low-quality study concluded CCP and CCR scores may predict clinical outcomes (progression to metastatic disease) irrespective of ancestry (AA, non-AA).</td></tr>
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<tr><td colspan="4">Clinical utility</td></tr>
<tr><td colspan="4">Please refer to page 8.</td></tr>
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<tr><td colspan="4">FINDINGS BY OUTCOMES</td></tr>
<tr><td>Outcomes</td><td>Study</td><td>Findings</td><td>Quality</td></tr>
<tr><td rowspan="4">Prostate cancer mortality</td><td>Cuzick et al., 2015</td><td>1.76 (95% CI (1.44, 2.14), P<10 -6) after adjusting for GS, PSA, extent of disease, and clinical stage.</td><td rowspan="2">Low</td></tr>
<tr><td>Cuzick et al., 2012</td><td>Only CCP score (HR 1.65 (1.31, 2.09) P=2.6×10-5); GS >7: HR 1.90 (1.18, 3.07), and PSA HR1.37 (1.05, 1.79) P=0.017] were statistically significant. However, CCP score was a stronger prognostic factor than Gleason score or PSA (HR 1.96 (1.43, 2.68)).</td></tr>
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<tr><td>Cuzick et al., 2021</td><td>CCP score: HR 4.36 (2.65, 7.16); P = 1.3 × 10-8.</td><td rowspan="2"></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>BCR, Metastasis</td><td>Bishoff et al,, 2014</td><td>First cohort, CCP score was a significant predictor of BCR (HR 1.66 (1.19, 2.32) P=0.0033).

In the second & third cohorts (N=176, N=123), HR were 1.33 (1.04–1.70, p = 0.027) and 1.86 (1.25–2.78, p = 0.0028) respectively. CCP score remained a significant predictor of BCR in multivariate analysis (HR not shown).</td><td></td></tr>
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	Canter et al., 2019	CCP score: HR 2.04 (1.47–2.79); p < 0.001 after adjusting for CAPRA score, ancestry, treatment. CCR score: HR 3.86 (2.91–5.23); p < 0.001).	
Risk stratification	Rayford et al., 2018	Overall, 24% of AA vs 10% of Caucasians were reclassified to higher risk by CCP score	
	Lin et al., 2018	CCR score threshold adequately categorized patients into low and high-risk groups for 10-year prostate cancer mortality (PCM).	

Evidence table 2: PROLARIS POST-PROSTATECTOMY

Study authors, design, aim	Patients' Characteristics	Findings	Conclusion, Quality
Clinical validity			
Swanson et al., 2021 Design: retrospective cohort study Primary outcome: Ability to identify patients at risk for progression to metastatic disease and DSM after RP	Patients: men with radical prostatectomy (RP) N=360, Age: 67.5 (63.3, 71.5); median f/u 16 years; most prevalent Pre-RP PSA was <10; surgical margin+: 24%; CAPRA-S low (0-2) 46%; CCP 0.2 (−0.3, 0.7); CCR 1.140 (0.494, 2.033). Inclusion: treatment with radical prostatectomy; cell-cycle progression score; preoperative prostate specific antigen; no neoadjuvant therapy; and clinical follow-up. 80% of the cohort were deceased at time of analysis. The 73 patients were alive at the time of analysis and had a median follow-up of 23.5 years.	Based on CAPRA-S, 167 (46%) patients were considered low risk of disease progression, 126 (35%) were intermediate risk, and 67 (19%) were high risk Post RP: The combined cell-cycle risk score (CCR) was a predictor of metastases (HR = 3.03 (1.49, 6.20); **p = .003**) after controlling for CAPRA-S. It was also a significant predictor of disease-specific mortality (HR = 3.40 [95% CI: 1.52, 7.59]; **p = .004**) 11% (41/360) developed metastases and 9% (33/360) experienced disease-specific mortality. Post BCR: CCP was predictive of metastases after BCR (HR 1.70 [95% CI: 1.14, 2.53]; **p = .012**) CCR was also prognostic of metastases post-BCR (HR = 1.56 [95% CI: 1.20, 2.03]; **p = .001**) Cancer of the prostate risk assessment postsurgical score was predictive of metastases post biochemical recurrence (HR 1.15 (1.03, 1.28) p=0.016) but was improved by the addition of cell cycle progression (HR = 1.70 [95% CI: 1.14, 2.53]; p = .012).	Conclusion: CCP & CCR may predict metastases and mortality post prostatectomy and therefore help find patients at risk of treatment failure who can benefit from early intervention. Limitations: retrospective study, three authors were employees of Myriad. Quality: moderate to low.
(Shangguan et al., 2020) Design: Retrospective study Primary outcome: biochemical recurrence (BCR) after RP	Patients: Men with adverse pathologic features, pT3 or positive surgical margins who underwent RP Age: 68 y; GS 7: 55%; CCP score 0.45 (0.3–1.3); 78% had extracapsular extension. Postoperative PSA < 0.1 ng/mL: 80% CAPRA-S score Low risk (0–2): 10 (10.0%) Intermediate risk (3–5) 44 (44.0%) High risk (6–10) 46 (46.0%) Median f/u: 47 months Median CCP score 0.45 Median initial PSA value 15.3 ng/mL	**CCP score was independent predictor of BCR** (HR 1.37 (1.006-1.874; p = **0.046**)) after RP **5-year BCR-free survival** for: low (< 0) CCP score: 89% intermediate- (0-1) CCP: 39% high- (> 1) CCP score: 12.9%	CCP may risk stratified patients better than the clinical & pathological variables and is a predictor of recurrence after RP in patients with adverse pathology after prostatectomy. Quality: low (insufficient follow-up, retrospective design, selection bias, due to

			monocentric database, compromising generalizability)
(Léon et al., 2018) Design: retrospective study Aim: to compare the ability of the CCP score and the expression of PTEN or Ki-67 to predict BCR in a cohort of patients treated by RP	N=652 but 619 blocks were assayed for CCP expression. 512 patients had passing CCP scores and complete clinical data. Patients were treated by RP. Median time from surgery to the last follow-up among BCR-free patients was 72 months Median age: 63 Median pre-surgical PSA: 8ng/ml 36% had GS <7 Median CAPRA-S score: 3 Median CCP score from RP: 0.08 30% had extracapsular extension Positive surgical margins: 8% Comparators: PSA, positive surgical margins; CAPRA-S score; CCP score; Ki-67; PTEN, Age at surgery; pathologic Gleason score; extracapsular extension	BCR occurred in 41% of patients CCP score may predict BCR after RP: HR of 1.44 (95% CI 1.17-1.75; p = 5.3 × 10-4) Ki-67 may predict BCR after RP: 1.89 (95% CI 1.38-2.57; p = 1.6 × 10-4). PTEN was not associated with BCR risk. In multivariable analysis, only the CCP score remained significantly predictive of BCR (p=0.026). The best model incorporated CAPRA-S and CCP scores as predictors, with HRs of 1.32 and 1.24, respectively.	Prolaris test was a better predictor of BCR after RP than Ki-67 & PTEN, and that it could be used in combination with the CAPRA-S score to identify patients who are at high risk of recurrence. Quality: Low (financial interest, short f/u, 5% had insufficient tumor material)
(Leapman et al., 2018) Design: retrospective cohort study Aim: To compare the discriminating ability gene expression test examining a panel of cell-cycle progression (CCP) genes with the expression status of three markers of genomic alterations in PCa: PTEN, ERG, and Ki-67. Outcomes: biochemical recurrence (BCR), and metastasis or PCa-specific mortality (PCSM).	Participants were dx with localized PCa and treated with radical prostatectomy. N=424 CCP scores were computed and compared with expression status of PTEN, ERG, Ki-67. Inclusion: patients with localized PCa treated with RP, CCP score were performed. F/u after RP: 9.5 years Median Age: 59 y; median PSA: 5.9 Biopsy Gleason grade group: 1: 64% Clinical stage T2: 61% Gleason grade group at RP: 1:43%; 2: 41% Pathologic T stage: T2 stage: 75% Pathologic N stage: N0 50% Nx 48% Negative surgical margins: 84% CAPRA-S: 66% were low (0-2)	At 10 yr after RP, 27% experienced BCR and 4% developed metastasis or PCSM. Controlling for CAPRA-S, CCP was associated with risks of recurrence (hazard ratio [HR] 1.51, (1.08–2.11) and metastasis/PCSM (HR 2.15, 95% CI 1.36–3.39). PTEN loss was **NOT** associated with recurrence but was associated with metastasis/PCSM (HR 5.26, 95% CI 2.57–10.7) **Parameter** / Outcome: biochemical recurrence, Cox proportional hazards regression / Outcome: metastasis/PCSM, Fine and Gray competing risk regression HR (95% CI) p value / HR (95% CI) p value **Univariable models** PTEN loss 1.41 (0.91, 2.21) 0.13 / 5.20 (2.49, 10.8) <0.01 High Ki67 expression (>10) 1.13 (0.78, 1.65) 0.51 / 1.53 (0.72, 3.24) 0.26 Positive ERG 0.69 (0.46, 1.02) 0.06 / 0.48 (0.21, 1.12) 0.09 CCP score 2.35 (1.84, 3.00) <0.01 / 2.55 (1.63, 3.99) <0.01 CAPRA-S 1.41 (1.31, 1.53) <0.01 / 1.36 (1.21, 1.52) <0.01 CAPRA-S/CCP interaction 2.34 (1.98, 2.75) <0.01 / 2.29 (1.74, 3.00) <0.01 **Multivariable models** PTEN loss 1.41 (0.90, 2.20) 0.13 / 5.26 (2.57, 10.7) <0.01 CAPRA-S 1.42 (1.31, 1.53) <0.01 / 1.38 (1.23, 1.55) <0.01 Harrell's concordance index 0.72 / 0.80 CCP score 1.51 (1.08, 2.11) 0.02 / 2.15 (1.36, 3.39) <0.01 CAPRA-S 1.44 (1.25, 1.67) <0.01 / 1.29 (1.16, 1.45) <0.01 Harrell's concordance index 0.72 / 0.81 Positive ERG 0.83 (0.56, 1.23) 0.35 / 0.58 (0.25, 1.37) 0.22 CAPRA-S 1.41 (1.30, 1.52) <0.01 / 1.34 (1.20, 1.49) <0.01 High Ki67 expression 1.11 (0.77, 1.61) 0.58 / 1.64 (0.78, 3.47) 0.19 Surgical CAPRA-S 1.42 (1.31, 1.53) <0.01 / 1.38 (1.23, 1.54) <0.01 PTEN loss, high Ki67, ERG+ 2.27 (1.09, 4.70) 0.03 / 8.47 (3.74, 19.2) <0.01 CAPRA-S 1.43 (1.32, 1.54) <0.01 / 1.40 (1.24, 1.58) <0.01 CAPRA-S = Cancer of the Prostate Risk Assessment score (postsurgical); CCP = cell-cycle progression; CI = confidential interval; HR = hazard ratio; PCSM = prostate cancer–specific mortality.	The prognostic ability of CCP score is comparable to that of PTEN for metastasis or prostate cancer related mortality. Quality: Low (population is skewed toward lower-risk profile patients which have impacted the rate mortality or metastasis; therefore statistical power is decreased for these outcomes; findings cannot

			be generalized to intermediate or high-risk patients; there is COI in the form of research grant)
Bishoff et al., 2014	Refer to above.		
Other low-quality study (J. Cuzick et al., 2011)	Showed that CCP score may be associated with mortality and BCR.		