**Supplementary Figures & Tables**



**Supplementary Figure 1.** EV-RNA yields from samples of different clinical categories collected at the NNUH. NEC – No Evidence of Cancer (*n =* 54), L – Low risk (*n =* 18), I – Intermediate risk (*n =* 55), H – High risk (*n =* 43), Post-RP – Post radical prostatectomy (*n =* 3). Post RP and H are significantly different from all others (*p* < 0.005 Wilcoxon-U test).



|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **CAPRA** | **NEC** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Train** | *64* | *40* | *53* | *54* | *24* | *30* | *20* | *23* | *14* | *10* | *9* |
| **Test** | *30* | *22* | *27* | *28* | *11* | *14* | *12* | *11* | *7* | *1* | *6* |

**Supplementary Figure 2.** Boxplots of PUR signatures relative to no evidence of cancer (NEC) and CAPRA scores 1 – 10 in the Training and Test cohorts. Numbers of samples within each group are as detailed in the table above.



**Supplementary Figure 3.** AUC curves for each of the four PUR signatures (Green – PUR-1, Blue – PUR-2, Yellow – PUR-3, Red – PUR-4) predicting presence of D’Amico Intermediate- or High-risk cancers on initial biopsy in both training and test cohorts.

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**Supplementary Figure 4.** DCAplot depicting the standardised net benefit of adopting PUR-4 as a continuous predictor for detecting significant cancer on initial biopsy, when significant is defined as: D’Amico risk group of Intermediate or greater (teal), Gleason ≥ 7 (orange) or Gleason ≥ 4+3 (red). To assess benefit in the context of cancer arising with a PSA-screened population of men we used data from the intervention arm of the CAP study. Bootstrap analysis was used to adjust the prevalence of Gleason grades to be representative of this population. For full details see Supplementary Methods.

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**Supplementary Figure 5.** **A)** Kaplan-Meier plot of AS progression, including MP-MRI criteria over time in days with respect to PUR thresholds described by the corresponding colours Green - 1° and 2° PUR-1, Blue - 3° PUR-1, Yellow - 3° PUR-4, Orange - 2° PUR-4, Red - 1° PUR-4. Table underneath details the number of patients still at risk of progression within each group. **B)** Kaplan-Meier plot of progression, including MP-MRI criteria, with respect to the dichotomised PUR thresholds described by the corresponding colours Green – PUR-4 < 0.174, Red – PUR-4 ≥ 0.174 and the number of patients within each group at the given time intervals in months from urine collection.

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**Supplementary Figure 6.** Kaplan-Meier plots and risk tables of AS progression with optimised PUR-4 thresholds when the AS cohort is split based on D’Amico risk categories. **A)** D’Amico low-risk men only **B)** Intermediate risk men only.



**Supplementary Figure 7.** PUR signatures from Active Surveillance longitudinal samples: 1° PUR-1 (Green), 2° PUR-1 (Purple), 1° PUR-2 (Blue), 1° PUR-3 (Yellow), 2° PUR-4 (Orange), 1° PUR-4 (Red). Samples within each numbered box are from a single patient with coloured circles underneath indicating primary PUR signature. A) patients that did not reach clinical progression criteria, as described in methods. B) patients that reached clinical progression criteria. Arrows and numbers under coloured circles detail the number of days between consecutive samples from a patient.

**Supplementary Table 1.** Coefficients of the 36 gene probes included as variables in the final PUR model and the intercepts.

|  |  |
| --- | --- |
| PUR variable: | Coefficient |
| *Intercept* | -2.178157 |
| *AMACR* | 0.68299729 |
| *AMH* | 0.33631836 |
| *ANKRD34B* | 0.1673693 |
| *APOC1* | 0.37122737 |
| *AR* (exons 4-8) | -0.4771042 |
| *DPP4* | -1.3364905 |
| *ERG* (exons 4-5) | 0.02561319 |
| *GABARAPL2* | 0.51388528 |
| *GAPDH* | -0.9188083 |
| *GDF15* | 0.27927613 |
| *HOXC6* | 0.65430249 |
| *HPN* | -0.4625853 |
| *IGFBP3* | -1.2101205 |
| *IMPDH2* | 0.45431166 |
| *ITGBL1* | -0.1094984 |
| *KLK4* | -1.5051707 |
| *MARCH5* | -1.4391403 |
| *MED4* | -1.0766399 |
| *MEMO1* | -1.9473755 |
| *MEX3A* | 0.23180719 |
| *MME* | -0.9433935 |
| *MMP11* | 0.99181693 |
| *MMP26* | 0.35495892 |
| *NKAIN1* | 0.03529522 |
| *PALM3* | 0.19549659 |
| *PCA3* | 2.75492107 |
| *PPFIA2* | -0.7369071 |
| *SIM2 (short)* | 0.90314335 |
| *SMIM1* | -0.2209302 |
| *SSPO* | 0.92313638 |
| *SULT1A1* | 1.7614731 |
| *TDRD1* | 0.26666292 |
| *TMPRSS2/ERG fusion* | 0.47922694 |
| *TRPM4* | 0.05947011 |
| *TWIST1* | -0.2593533 |
| *UPK2* | 0.63826112 |
| Cp 1 | 2.42583541 |
| Cp 2 | 1.48559352 |
| Cp 3 | -0.4792212 |

**Supplementary Table 2.** Active surveillance cohort characteristics.

|  |  |
| --- | --- |
| **Characteristics:** |  |
| Patients, *n* | 87 |
| Age, year, mean (median; IQR) | 64 (66, 7) |
| PSA, ng/ml, mean (median; IQR) | 7.8 (7.5, 3.3) |
| D'Amico: |  |
| Low *n* (%) | 55 (63) |
| Intermediate *n* (%) | 32 (37) |
| CAPRA Group: |  |
| Low (0-2) *n* (%) | 59 (68) |
| Intermediate (3-5) *n* (%) | 27 (31) |
| High (≥6) *n* (%) | 1 (1) |
| Gleason Score: |  |
| Gs ≤6, *n* | 79 |
| Gs = 3+4, *n* | 7 |
| Gs = 4+3, *n* | 1 |
| Number of biopsies taken: |  |
| 1 | 14 |
| 2 | 28 |
| ≥3 | 35 |
| NA: | 10 |
| Number of negative biopsies following a positive |  |
| 1 | 26 |
| 2 | 3 |
| NA: | 58 |
| Progressed to treatment due to: |  |
| PSA increase | 17 |
| Adverse histopathology | 6 |
| MP-MRI criteria only | 9 |
| Non-progressed to treatment due to: |  |
| Any criteria | 49 |
| Self-elected for treatment: | 3 |
| Died of other causes: | 3 |
| Gs = Gleason score; IQR = interquartile range; PSA = prostate-specific antigen; NA = not available; MP-MRI = multiparametric magnetic resonance imaging | |