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A locked transcriptomic panel improves prediction of biochemical recurrence after radical prostatectomy

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Article

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Abstract

BACKGROUND

After radical prostatectomy, clinical variables alone incompletely predict biochemical recurrence, and the tissue genomic assays in use are proprietary and priced per patient. This study evaluated whether a transcriptomic panel locked in advance, ProstEx, adds discrimination for biochemical recurrence over the clinical model, and whether it ranks future metastasis from the diagnostic biopsy.

METHODS

Five public prostatectomy cohorts and a Belfast radiotherapy cohort were analyzed under specifications frozen before outcomes were examined. No patient was scored by a model fitted on his own cohort. The primary outcome was biochemical recurrence. Discrimination was measured with the C statistic of Harrell with patient level bootstrap intervals. Added value was measured as the paired change in C, net benefit and reclassification.

RESULTS

In the primary set (288 men, 107 events), evaluated by leave one cohort out validation so that every read is external, the panel raised C from 0.683 to 0.724 (paired change + 0.041, 95% CI 0.011 to 0.070). In the secondary set (795 men, 181 events) the change was + 0.053 (0.033 to 0.074). On the same patients, the leading panel score stood level with the cell cycle progression score, and the panel added net benefit at decision thresholds of 0.40 and 0.50. The panel also ranked future metastasis from the diagnostic biopsy (C 0.511 to 0.631, change + 0.120, internal resampling), and its locked metastasis score read an AUC of 0.627 on 212 external events. On the distinct disease free survival endpoint the panel did not add (change − 0.039, − 0.071 to − 0.011).

CONCLUSIONS

A locked and open transcriptomic panel adds discrimination and net benefit for biochemical recurrence over the clinical model across independent cohorts, with a supporting read for metastasis at the diagnostic biopsy. Prospective validation in a contemporary cohort is the next step.

Introduction

After radical prostatectomy, biochemical recurrence reorganizes care: it sets monitoring intensity, the timing of salvage treatment and entry into adjuvant trials. Grade, stage, prostate specific antigen and margin status carry this decision, combined in scores such as CAPRA S [1]. Their discrimination is incomplete, and identical clinical profiles can follow different courses.

Tissue genomic assays were built to close this gap. The cell cycle progression score [2], the 17 gene Genomic Prostate Score [3] and the Decipher classifier [4, 5] each answer one question with one proprietary test at a price per patient. Cooperberg and colleagues validated the cell cycle progression score in 413 prostatectomy patients (hazard ratio 2.1 per unit for recurrence, 1.7 after CAPRA S adjustment), with concordance and decision curve gains for the combined score [26]. Cullen and colleagues validated the Genomic Prostate Score for recurrence on biopsy tissue in 431 men (hazard ratio per 20 units 2.9; 2.7 after adjustment for clinical risk group) [28]. In a community cohort, Decipher combined with CAPRA S also raised discrimination for recurrence [31]. Progression biology is already described in named public collections, so a panel can be specified once, computed identically anywhere and audited in full. It must prove added value over the full clinical model in unseen cohorts, not mere association.

ProstEx is that panel: eight gene set scores with small prespecified Cox models, frozen and hashed before outcomes were computed. The hypothesis was that tumor biology read in this way carries risk information the clinical variables miss. Four predictions followed, with a fifth as a supporting test. First, the panel would add discrimination for biochemical recurrence over the clinical model, judged out of cohort. Second, the gain would hold in a wider set under a core base. Third, the panel would stand level with the cell cycle progression score on the same patients and hold at decision thresholds. Fourth, the claim would be bounded by endpoint: on disease free survival, a composite endpoint the panel was not specified for, added value would be measured rather than assumed. Fifth and supporting, the same instrument would rank future metastasis from the diagnostic biopsy.

Results

Cohorts and analysis sets

Five public prostatectomy cohorts form the proving ground: TCGA [6], the DKFZ early onset cohort [7], the Long cohort (GSE54460) [8], and the CamCap (GSE70768) and Stockholm (GSE70769) cohorts [9]. Each deposits tumor expression with the clinical components and recurrence outcome an out of cohort added value test requires. The primary set drew 288 men with 107 recurrences from the three cohorts depositing every full base component; the secondary set drew 795 men with 181 recurrences from all five under a core base (Table 1; Supplementary Table S1). Long and colleagues derived a 24 gene recurrence signature from the GSE54460 cohort itself, reporting improvement over clinical parameters and over proliferation based panels [8]; the present panel was prespecified from public gene sets and derived from no evaluated cohort.

Table 1
Cohorts and analysis sets

Cohort	Accession	Platform	Patients	Events	Role in this study
TCGA	prad_tcga (cBioPortal)	RNA sequencing	414	52 recurrences	Secondary set; benchmark frame
DKFZ early onset	prostate_dkfz_2018 (cBioPortal)	RNA sequencing	82	18 recurrences	Secondary set; sensitivity set
Long	GSE54460	RNA sequencing (FFPE tissue)	100 profiled; 91 in primary set	45 recurrences (primary set)	Primary and secondary sets
CamCap	GSE70768	Illumina expression array	112 profiled; 110 in primary set	19 recurrences	Primary and secondary sets
Stockholm	GSE70769	Illumina expression array	94 profiled; 87 in primary set	43 recurrences	Primary and secondary sets
Pooled primary set	Three cohorts above	Mixed	288	107 recurrences	Headline recurrence read (full clinical base)
Pooled secondary set	Five cohorts above	Mixed	795	181 recurrences	Recurrence read (core clinical base)
Pooled benchmark frame	Five cohorts above	Mixed	799	183 recurrences	Single score benchmark
Belfast radiotherapy cohort	GSE116918	Almac prostate array, diagnostic biopsies	248 profiled; 223 in analysis set	22 distant metastases	Future metastasis model (internal resampling)
Mayo (Erho)	GSE46691	Affymetrix exon array	545	212 metastases (binary)	External transport of the locked

FFPE, formalin fixed paraffin embedded. Event counts are for the analysis set named in the same row. The benchmark frame differs slightly from the model frames because it requires only a deposited recurrence outcome and the expression data needed for a single score.

Cohort	Accession	Platform	Patients	Events	Role in this study
					metastasis score
Ross (Johns Hopkins)	GSE51066	Affymetrix exon array	85	51 metastases (binary)	External transport, post recurrence spectrum

FFPE, formalin fixed paraffin embedded. Event counts are for the analysis set named in the same row. The benchmark frame differs slightly from the model frames because it requires only a deposited recurrence outcome and the expression data needed for a single score.

Added discrimination for biochemical recurrence

The panel added discrimination over the clinical model in the primary set, with a positive gain in every cohort. Under the first prediction, any gain had to appear when no patient was scored by a model fitted on his own cohort; the evaluation was therefore leave one cohort out, and every recurrence C statistic reported here is external. The clinical base read a C statistic of 0.683 (95% CI 0.629 to 0.738). Adding the panel raised C to 0.724 (0.672 to 0.773), a paired change of + 0.041 (0.011 to 0.070), positive in all three cohorts (Fig. 1A). Apoptosis activity alone added + 0.027 (0.008 to 0.047).

The wider set and subgroups

The gain held in the wider set, largest where grade decides hardest. Had it been an artifact of one base definition, it should have faded under the reduced base pooled across all five cohorts. It did not. The core base read 0.621 (0.583 to 0.658), and the panel raised it to 0.674 (0.638 to 0.707), a change of + 0.053 (0.033 to 0.074), positive in all five cohorts (Fig. 1B). The sensitivity set without the margin term agreed (+ 0.047, 0.022 to 0.076). In grade groups 3 to 5 of the primary set the change was + 0.043 (0.004 to 0.091). In grade groups 1 and 2 of the secondary set (416 men, 61 events), C rose from 0.587 to 0.636 (change + 0.049, − 0.002 to + 0.102) (Supplementary Table S6).

Benchmark against the cell cycle progression score

On the same patients, the leading panel score stood level with the cell cycle progression score. Under the third prediction, a new instrument earns its place only by matching the established score where both can be computed; single scores were read head to head on the same 799 patients. The strongest were the ProstEx metastasis row (C 0.6485) and CCP 31 (C 0.6463), ahead of proliferation (0.6064) and angiogenesis (0.5624) (Fig. 2). In fitted models, the base plus CCP 31 read 0.713 in the primary set and the base plus panel plus CCP 31 read 0.725, so CCP 31 added nothing beyond the panel. The pooled adjusted association of CCP 31 resolved at scale (hazard ratio per standard deviation 1.184, 1.057 to

1.325; univariate 1.386, 1.251 to 1.537). A score built from the Decipher gene list read 0.5487; it is not the commercial assay, whose value lies in proprietary weights, and no result here judges the assay.

Decision impact

The panel model added net benefit at the thresholds where treatment is decided. Under the second part of the third prediction, a gain at the thresholds where salvage treatment and trial entry are weighed can change care; a gain on average may never touch a decision. Decision curve analysis on the primary set, using five year recurrence risk, found net benefit added at a threshold of 0.40 (+ 0.052, 0.009 to 0.100) and at 0.50 (+ 0.070, 0.022 to 0.118) over the base (Fig. 3A), corresponding to seven to eight fewer unnecessary interventions per 100 men at the same benefit. The frozen flags gave a net reclassification improvement of + 0.158 (0.080 to 0.236), carried by 40 recurrence free men whom the panel flagged as low risk, with none lost (Supplementary Table S9).

Boundary of the claim: disease free survival

On the distinct disease free survival endpoint, the panel did not add, and no claim is made for that endpoint. The reasoning was the fourth prediction: the panel was specified for biochemical recurrence, and a composite endpoint tests a different question. The sensitivity analysis pooled TCGA (413 men, 85 events) and the MSKCC primaries in their GEO form (GSE21034; 130 men, 27 events) [25], with the core base and the same design, and the endpoint was never pooled with the recurrence reads. The clinical base read C 0.669 (0.621 to 0.715) and the panel read 0.630 (0.578 to 0.685), a change of − 0.039 (− 0.071 to − 0.011) (Supplementary Section S1.3). The recurrence claim is therefore a claim about biochemical recurrence as deposited by each cohort, and it extends no further. One supporting question remains: whether the same instrument carries information at the earliest decision point.

Supporting result: future metastasis from the diagnostic biopsy

From the diagnostic biopsy, the panel ranked future metastasis and defined a rule out group in which almost all men remained free of spread. Under the supporting test, if the scores read progression rather than one endpoint definition, the same instrument should carry metastasis information at diagnosis. The proving ground is the Belfast cohort of Jain and colleagues [10]: 223 of 248 profiled men, with 22 distant metastasis events over a median follow up of 87 months, treated with radical radiotherapy after neoadjuvant hormone therapy and profiled on pretreatment biopsies (internal resampling). The clinical base read C 0.511 (0.390 to 0.641), close to chance, and the panel raised it to 0.631 (0.523 to 0.741), a change of + 0.120 (0.016 to 0.220). The base weakness is documented, not assumed: prostate specific antigen carried no signal (univariate C 0.463), while grade (0.596) and stage (0.601) separated modestly. An audit found no coding defect, and against the strongest defensible respecification the gain held at + 0.102 (0.012 to 0.186) (Fig. 4A; Supplementary Table S7). The frozen rule out flag covered 53.8% of men (120 of 223). Among 108 flagged men evaluable at five years, three developed metastasis, a five year

negative predictive value of 0.972 (Fig. 4B). The panel was the only model with positive net benefit at the 0.03 threshold where the flag operates, gaining + 0.0143 (0.0038 to 0.0294) over the base (Fig. 3B). Carried unchanged and without refitting to two external cohorts, the locked metastasis score read an AUC of 0.627 (0.581 to 0.673) on 212 events in GSE46691 [4] and 0.641 (0.517 to 0.763) on the 51 events of GSE51066 (Fig. 4C). Across 263 external events the ranking held at a consistent and modest level.

Discussion

A locked panel of biology scores added discrimination for biochemical recurrence over the clinical model, in every cohort and in both prespecified sets, and added net benefit at the thresholds where treatment is weighed. Its leading row stood level with the cell cycle progression score, which added nothing beyond the panel in fitted models. The claim is bounded by endpoint: on disease free survival the panel did not add, and no claim is made there. As a supporting result, the same instrument ranked future metastasis from the diagnostic biopsy, defined a rule out group in which 97 of 100 men remained free of metastasis at five years, and read externally on 263 events in two further cohorts. A six gene academic signature likewise added value over clinical scores for recurrence in independent validation [29].

The panel is also open: its gene sets and weights are public, it computes from expression data already held, and it carries no per patient price. The Belfast cohort had previously validated a dedicated metastatic assay [10]. That assay came from Walker and colleagues, whose 70 transcript signature predicted biochemical recurrence (hazard ratio 1.62) and metastatic recurrence (3.20) in 322 prostatectomy samples [27]. A general panel specified for recurrence raised discrimination at diagnostic biopsy in that cohort and transported its core score without refitting. A locked 45 gene metastasis score derived across six cohorts, three used for metastasis here, has since read metastasis in two further cohorts [30]; its endpoint is metastasis, not recurrence. The clinical base there is weak by documentation, not assumption: prostate specific antigen has spent its ranking power in a high risk radiotherapy population where grade and stage plateau, and the gain survives the strongest clinical comparator the cohort supports.

The strengths of the study are its discipline: specifications frozen and hashed before outcomes were examined, a locked instrument never fitted on evaluated patients, and decision impact reported beside discrimination. Its limitations follow. All evidence is retrospective, from public cohorts assembled for other purposes. The metastasis model rests on one resampled cohort, with external support for its core score rather than for the full model, and the recurrence read is a risk score with no rule out claim. A prospective study in a contemporary cohort is the next step toward clinical use.

Materials and methods

Cohorts and endpoints

Public prostatectomy cohorts were analyzed: TCGA [6], the DKFZ early onset cohort [7], the Long cohort (GSE54460) [8], and the CamCap (GSE70768) and Stockholm (GSE70769) cohorts [9]. The primary outcome was biochemical recurrence as defined by each cohort. The primary set required the full clinical base of grade group, pathologic stage in three bands, log transformed preoperative prostate specific antigen and surgical margin status, and pooled the three cohorts depositing every component (288 men, 107 events). The secondary set used a core base of grade group and pathologic stage and pooled all five cohorts (795 men, 181 events). Each set is a complete case set for its own base. A sensitivity set without the margin term (375 men, 127 events) and the disease free survival set are described in the Supplementary Appendix. One record per patient was retained; no two expression profiles met the fingerprint correlation threshold of 0.99. Sample flow is in Table 1 and Supplementary Table S1. The metastasis analyses used a Belfast radiotherapy cohort profiled on pretreatment diagnostic biopsies (GSE116918) [10] and two external cohorts with binary metastasis outcomes, GSE46691 [4] and GSE51066 [11]. CAPRA S [1] is reported in the Supplementary Appendix.

The locked panel

ProstEx computes eight scores with singscore [12], which scores each sample on its own ranks. Five scores come from the Hallmark collection [13]: angiogenesis (36 genes), epithelial mesenchymal transition (200 genes, read as invasion), E2F targets and G2M checkpoint (200 genes each) averaged as proliferation, apoptosis (161 genes, read as apoptosis activity), and androgen response (101 genes). The immune inflammation score is the 18 gene interferon gamma profile of Ayers and colleagues [14]. The resisting cell death score is a curated five gene antiapoptotic core of the BCL2 family. The metastasis score is the 17 gene set of Ramaswamy and colleagues [15], scored as the seven gene up set minus the seven gene down set, with three equivocal genes excluded. The CCP 31 comparator was a singscore over the 31 cell cycle progression genes [2]. Scores were computed on log2 expression as deposited, without renormalization, and standardized within cohort (Supplementary Table S10).

Models, evaluation and decision analysis

Risk models were Cox proportional hazards models [16]. The panel entered as one ridge penalized block of eight scores over the base, with the base linear predictor as offset. The penalizer was chosen by five fold cross validation within the training cohorts, from a frozen grid. Within each rotation the panel score was recalibrated on the training cohorts and applied to the held out cohort [17]. Two comparators were fitted identically: base plus CCP 31, and base plus panel plus CCP 31. The recurrence evaluation was leave one cohort out, so every recurrence C statistic is external. Discrimination was measured with the C statistic of Harrell [18] on pooled held out predictions, and added value as the paired change in C between nested models. Confidence intervals are patient level percentile bootstrap intervals (2000 resamples) [19], resampling paired differences for changes in C. Calibration was assessed by linear predictor slope and observed against predicted five year risk (Supplementary Appendix). Decision curve analysis [20] used five year risk in patients evaluable at five years, with patient level bootstrap intervals on differences. Reclassification used the categorical net reclassification improvement [21] between the frozen base and panel flags on the secondary set.

Future metastasis analyses

In the Belfast cohort, 223 of 248 profiled men formed the complete case set, with 22 distant metastasis events over median follow up of 87 months. The endpoint was distant metastasis free survival. The base contained Gleason sum bands (7 or less, 8, 9 or more), clinical stage (T2 or less, T3 or more) and log transformed prostate specific antigen. Age was not a covariate, by frozen design. Evaluation used ten fold cross validation grouped by patient, so these reads are internal resampling. The panel block was ridge penalized, with the penalizer selected inside the training folds. A rule out flag was fixed from the training folds at the largest predicted five year risk keeping five year sensitivity for metastasis at or above 90% among evaluable training men; its five year negative predictive value was computed among evaluable flagged men. The base was audited field by field against the deposit (Supplementary Table S7). For external transport, the locked metastasis score, fitted on Belfast only, was applied without refitting to GSE46691 (545 men, 212 events) and GSE51066 (85 men, 51 events). Both deposits carry a binary outcome without follow up times. GSE46691 is a nested case control sample, so predictive values are not defined.

Statistical analysis and reporting

Analyses were run in Python (version 3.12.3), with lifelines (version 0.30.3) for the unpenalized Cox fits. Penalized and offset stages used a Newton solver for the Breslow partial likelihood written for this program and verified against lifelines (maximum absolute coefficient difference 0.00000208, with identical concordance). Reporting follows the guidelines for reporting of statistics for clinical research in urology [22], the TRIPOD + AI statement [23] and the REMARK recommendations [24]. Only public deidentified data were used.

Declarations

Data availability

All data analyzed in this study are publicly available. The accessions are listed in Table 1 and the Supplementary Appendix. The frozen specifications and the analysis code are held by the author and will be deposited in a public repository on acceptance.

Competing interests

The author declares no competing interests.

Ethics statement

This study is a secondary analysis of publicly available deidentified data. No participants were recruited and no identifiable information was accessed.

Use of artificial intelligence tools

Artificial intelligence tools were used only in reviewing the manuscript, to check language and internal consistency. The author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Author contributions

VS conceived and designed the study, curated the data, performed the formal analysis, developed the software, and wrote and revised the manuscript. VS 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.

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Figures

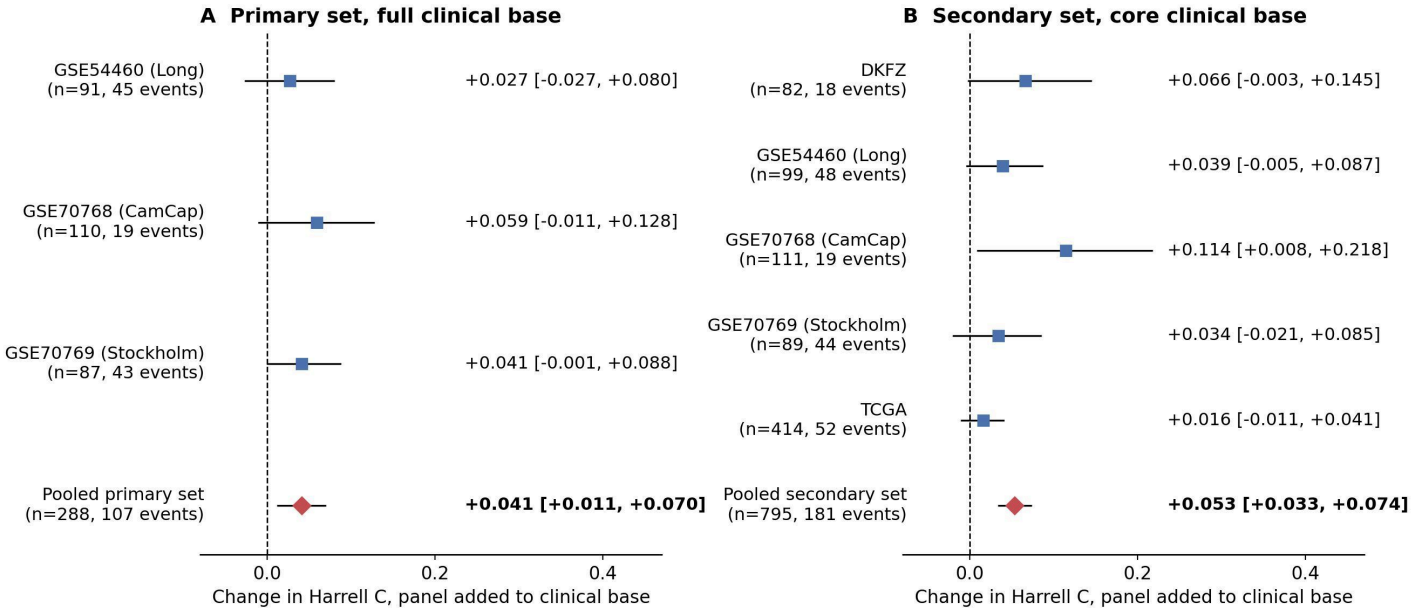

Figure 1

Added discrimination for biochemical recurrence, by cohort and pooled. Change in the C statistic of Harrell when the eight score panel is added to the clinical base, evaluated by leave one cohort out validation so that no patient is scored by a model fitted on his own cohort (external validation). (A) Primary set (full clinical base: grade group, pathologic stage, log prostate specific antigen, margin status). (B) Secondary set (core clinical base: grade group and pathologic stage). Squares mark single cohort estimates and diamonds mark pooled estimates. Whiskers are 95% bootstrap confidence intervals at the patient level. The gain is positive in every cohort of both sets and in both pooled reads.

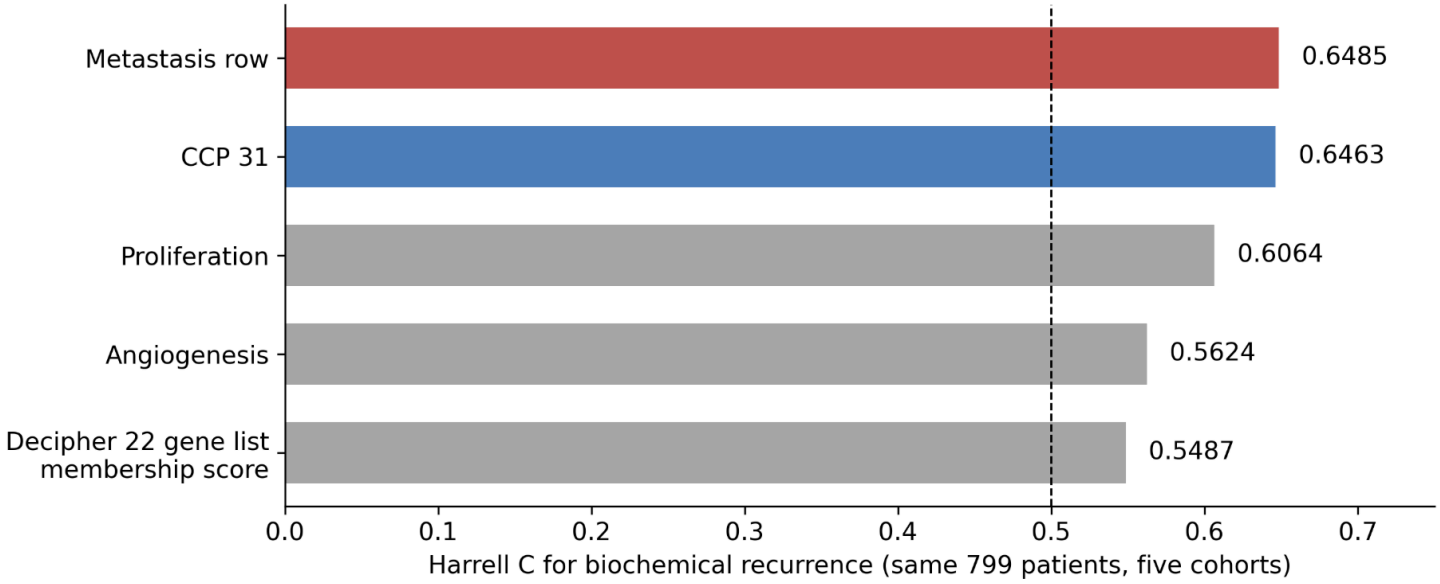

Figure 2

Single score benchmark on the same patients. C statistic of Harrell for biochemical recurrence for individual scores computed on the same 799 prostatectomy patients across five cohorts. The ProstEx metastasis row and the cell cycle progression score (CCP 31) stand at the top together. The Decipher 22 bar is a score built from the published gene list. It is not the commercial assay, whose weights are proprietary. The dashed line marks chance (0.5).

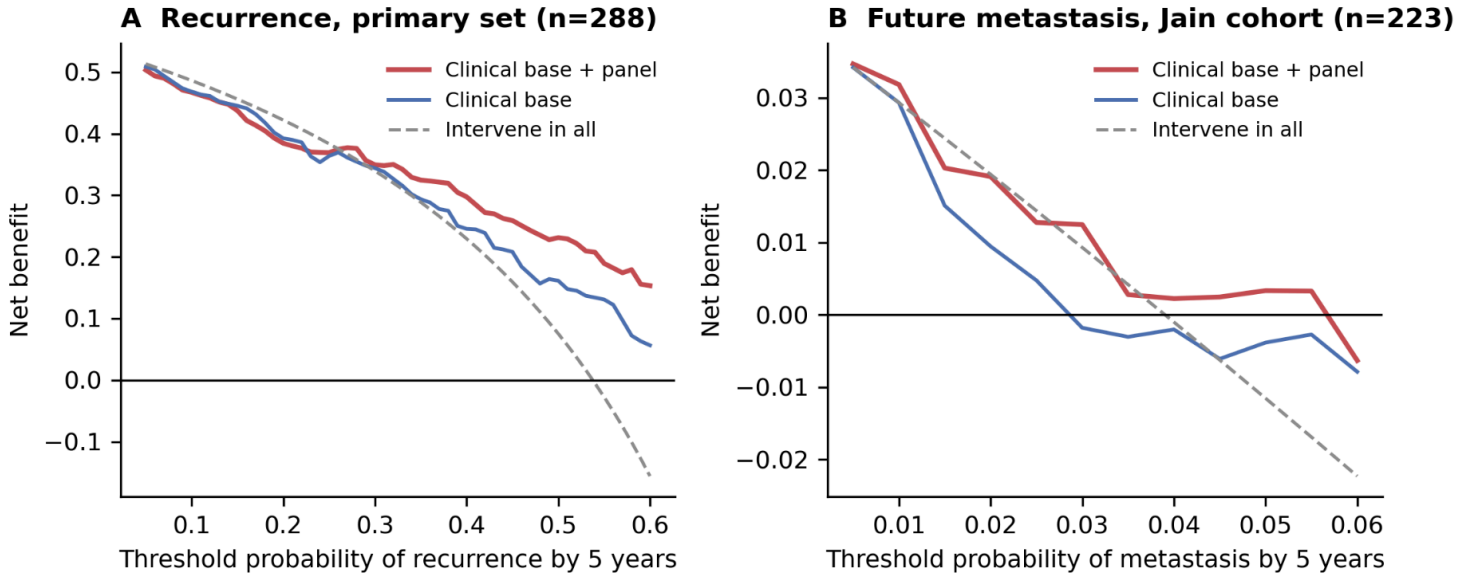

Figure 3

Decision curve analysis. Net benefit of the clinical base and of the base plus panel against the strategies of intervening in all patients or in none, across the threshold ranges shown on the horizontal axes. (A) Recurrence by five years, primary set (186 of 288 men evaluable at five years): the panel model separates upward from the base across the threshold range where postoperative decisions are made, gaining +0.052 net benefit at a threshold of 0.40 and +0.070 at 0.50. (B) Future metastasis by five years, Belfast cohort (205 of 223 men evaluable at five years, internal resampling): at the 0.03 threshold where the rule out flag operates, the panel is the only fitted model with positive net benefit, gaining +0.0143 over the base.

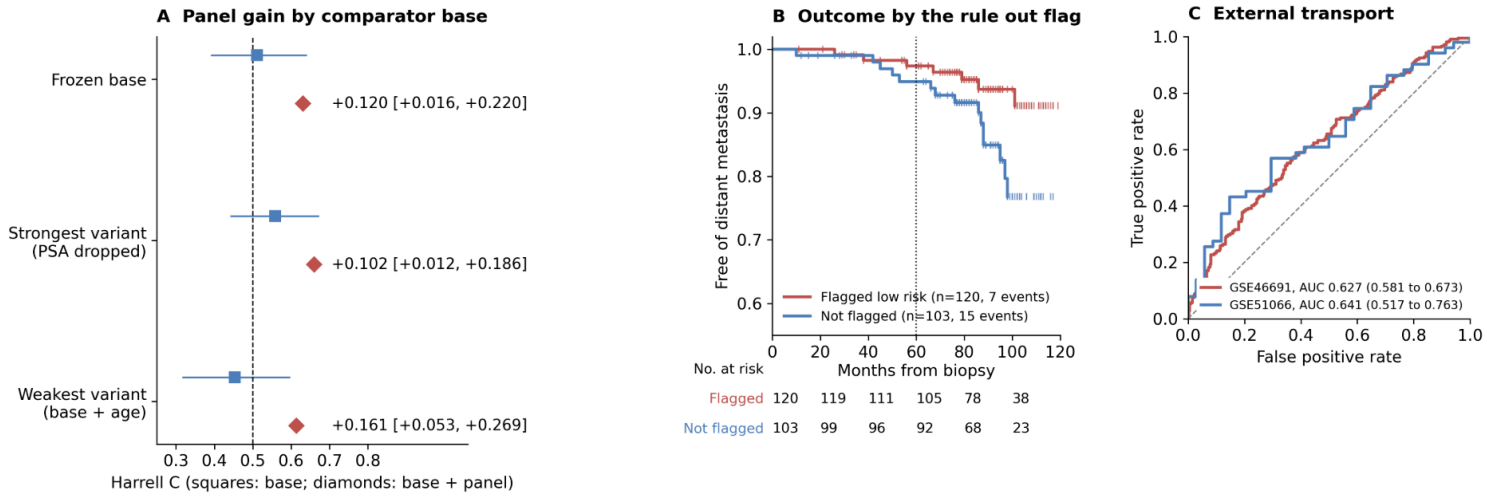

Figure 4

Future metastasis from the diagnostic biopsy and external transport of the locked metastasis score. (A) The panel gain does not depend on a weak comparator: squares are clinical base C statistics with 95% bootstrap confidence interval whiskers and diamonds are the same bases with the panel added, for the frozen base and for its strongest and weakest defensible respecifications, all by internal resampling, with the paired change in C and its bootstrap interval printed at right. (B) Kaplan Meier freedom from distant metastasis by the frozen rule out flag, which was fixed from training folds only. Tick marks show censoring. The flag covered 120 of 223 men. Among flagged men evaluable at five years the negative predictive value was 0.972. The dotted line marks five years. The number of men at risk is shown beneath the panel. (C) Receiver operating characteristic curves of the locked metastasis score, carried unchanged and without refitting, for the binary metastasis outcome in GSE46691 (545 men, 212 events) and GSE51066 (85 men, 51 events). The area under the curve with its 95% confidence interval is given in the curve legend for each cohort. The dashed line marks chance.

Supplementary Files

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- ProstExRecurrencePCPDSupplementary.docx