Development and validation of a 24-gene predictor of response to postoperative radiotherapy in prostate cancer: a matched, retrospective analysis

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Summary

Background Postoperative radiotherapy has an important role in the treatment of prostate cancer, but personalised patient selection could improve outcomes and spare unnecessary toxicity. We aimed to develop and validate a gene expression signature to predict which patients would benefit most from postoperative radiotherapy.

Methods Patients were eligible for this matched, retrospective study if they were included in one of five published US studies (cohort, case-cohort, and case-control studies) of patients with prostate adenocarcinoma who had radical prostatectomy (with or without postoperative radiotherapy) and had gene expression analysis of the tumour, with long-term follow-up and complete clinicopathological data. Additional treatment after surgery was at the treating physician's discretion. In each cohort, patients who had postoperative radiotherapy were matched with patients who had not had radiotherapy using Gleason score, prostate-specific antigen concentration, surgical margin status, extracapsular extension, seminal vesicle invasion, lymph node invasion, and androgen deprivation therapy. We constructed a matched training cohort using patients from one study in which we developed a 24-gene Post-Operative Radiation Therapy Outcomes Score (PORTOS). We generated a pooled matched validation cohort using patients from the remaining four studies. The primary endpoint was the development of distant metastasis.

Findings In the training cohort (n=196), among patients with a high PORTOS (n=39), those who had radiotherapy had a lower incidence of distant metastasis than did patients who did not have radiotherapy, with a 10-year metastasis rate of 5% (95% CI 0–14) in patients who had radiotherapy (n=20) and 63% (34–80) in patients who did not have radiotherapy (n=19; hazard ratio [HR] 0·12 [95% CI 0·03–0·41], p<0·0001), whereas among patients with a low PORTOS (n=157), those who had postoperative radiotherapy (n=78) had a greater incidence of distant metastasis at 10 years than did their untreated counterparts (n=79; 57% [44–67] *vs* 31% [20–41]; HR 2·5 [1·6–4·1], p<0·0001), with a significant treatment interaction ($p_{interaction}$<0·0001). The finding that PORTOS could predict outcome due to radiotherapy treatment was confirmed in the validation cohort (n=330), which showed that patients who had radiotherapy had a lower incidence of distant metastasis compared with those who did not have radiotherapy, but only in the high PORTOS group (high PORTOS [n=82]: 4% [95% CI 0–10] in the radiotherapy group [n=57] *vs* 35% [95% CI 7–54] in the no radiotherapy group [n=25] had metastasis at 10 years; HR 0·15 [95% CI 0·04–0·60], p=0·0020; low PORTOS [n=248]: 32% [95% CI 19–43] in the radiotherapy group [n=108] *vs* 32% [95% CI 22–40] in the no radiotherapy group [n=140]; HR 0·92 [95% CI 0·56–1·51], p=0·76), with a significant interaction ($p_{interaction}$=0·016). The conventional prognostic tools Decipher, CAPRA-S, and microarray version of the cell cycle progression signature did not predict response to radiotherapy ($p_{interaction}$>0·05 for all).

Interpretation Patients with a high PORTOS who had postoperative radiotherapy were less likely to have metastasis at 10 years than those who did not have radiotherapy, suggesting that treatment with postoperative radiotherapy should be considered in this subgroup. PORTOS should be investigated further in additional independent cohorts.

Funding None.

Introduction

Radiotherapy is a mainstay in the treatment of localised prostate cancer.[1] Adjuvant radiotherapy after prostatectomy has been shown to reduce biochemical recurrence by approximately 50% in three randomised controlled trials.[2–4] In the SWOG 8794 study,[5] the only trial with a primary endpoint of metastasis-free survival, adjuvant radiotherapy improved metastasis-free survival and overall survival at 15 years of follow-up. No reported randomised controlled trials have examined salvage radiotherapy after biochemical recurrence to date, but several retrospective studies[1,6–8] have suggested that salvage radiotherapy prevents subsequent biochemical recurrence in a large proportion of patients (24–66% biochemical recurrence-free at 5 years of follow-up). Although radiation after prostatectomy has shown significant clinical benefit, some patients are probably better candidates for radiotherapy than others, and could

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Research in context

Evidence before this study

We searched PubMed for papers published until July 24, 2016, with the search term: "(predictor OR predictive) AND (signature OR model) AND radiation therapy AND prostate cancer AND gene AND clinical". We did not identify any previous molecular signatures that predicted response to radiotherapy in prostate cancer. Although prognostic signatures have become widely established in cancer research, the development of signatures that can predict response to individual therapies has been a difficult proposition.

Added value of this study

We report, to our knowledge, the first molecular signature (Post-Operative Radiation Therapy Outcomes Score [PORTOS]) developed in prostate cancer to predict response to radiotherapy. This model was trained and independently validated on a commercial clinical platform, and can select patients who would benefit most from postoperative radiotherapy.

Implications of all the available evidence

We show that the gene expression signature PORTOS can predict response to postoperative radiation. Future work should focus on independent validation in additional cohorts, ideally in randomised controlled trials.

benefit from improved selection based on individual tumour characteristics.

Gene signatures have been successfully used in various cancer types to develop prognostic and predictive tools that allow clinicians to direct therapeutic agents to the patients who will benefit the most. Although prognostic signatures are designed to risk-stratify patients independent of treatment, predictive signatures select subsets of patients who would benefit from a particular intervention. Oncotype Dx is an example of a commercially available gene signature that was initially designed as a prognostic tool,[9] but was subsequently found to be useful in establishing which patients benefit from adjuvant chemotherapy in breast cancer.[10] Predictive signatures have also been developed in breast cancer to predict response to radiation[11] and chemoradiation,[12] and treatment-predictive signatures have been developed in other cancers such as lung cancer[13] and colon cancer.[14] However, no gene signatures have been shown to specifically predict response to radiotherapy in prostate cancer.

We aimed to use clinical and genomic databases to develop and validate a gene expression signature that can predict response to radiotherapy. We used matched samples run on a commercial clinical platform with long-term clinical follow-up to train a ridge-penalised Cox model that was independently validated on a matched cohort of patients to predict response to postoperative radiotherapy.

Methods

Study design and participants

This matched, retrospective study included patients with prostate adenocarcinoma who had radical prostatectomy with or without postoperative radiotherapy at four US medical centres and were included in five studies (four cohort or case-cohort studies, and one case-control study): Mayo Clinic I (Rochester, MN; year of radical prostatectomy 1987–2001),[15,16] Mayo Clinic II (Rochester, MN; 2000–06),[17] Johns Hopkins University (Baltimore, MD; 1992–2010),[18] Thomas Jefferson University (Philadelphia, PA; 1999–2009),[19] and Durham VA Medical Center (Durham, NC; 1991–2010).[20]

Patients were selected for this analysis if they had complete data for all clinicopathological variables used in matching. Patients of any age were eligible for this analysis. There were no other inclusion or exclusion criteria. Patients were treated with postoperative radiotherapy or systemic therapy according to the treating physician's discretion.

Exact matching (1:1) of patients who had received postoperative radiotherapy with patients who had not was done in the Mayo Clinic I cohort to construct the training cohort, and in a pooled cohort consisting of the Mayo Clinic II, Johns Hopkins University, Thomas Jefferson University, and Durham VA Medical Center cohorts to construct the validation cohort (figure 1). Patients included in the radiotherapy group were those that received adjuvant or salvage postoperative radiotherapy after radical prostatectomy and before the primary endpoint of metastasis.

Matching was done on the basis of surgical Gleason score, preoperative prostate-specific antigen (PSA) concentration, surgical margin status, extracapsular extension, seminal vesicle invasion, lymph node invasion, and androgen deprivation therapy. Gleason score was categorised into low (≤6), intermediate (7), and high (8–10). Similarly, PSA concentration was stratified into low (<10 ng/dL), intermediate (10–20 ng/dL), and high (>20 ng/dL). Surgical margin status, extracapsular extension, seminal vesicle invasion, and lymph node invasion were treated as binary variables and defined by each institution. We defined androgen deprivation therapy as treatment after radical prostatectomy, but before metastasis. We did not have data for target volume, radiotherapy dose range, androgen deprivation therapy modalities, duration of androgen deprivation therapy, PSA concentration at time of radiation, time from surgery to PSA recurrence, or PSA doubling times, because data for these variables had not been collected

for these cohorts at the time of the study. All patients who received radiotherapy were given radiotherapy to the postoperative bed, but we did not have information about pelvic nodal radiotherapy.

The primary clinical endpoint was incidence of distant metastasis after prostatectomy confirmed by CT scan or bone scan in all cohorts. In the Johns Hopkins University cohort, MRI was also used to assess metastasis.

Procedures

Affymetrix Human Exon 1.0 ST microarray (Affymetrix, Santa Clara, CA) data from formalin-fixed, paraffin-embedded radical prostatectomy samples were obtained for all cohorts. Pathology was reviewed separately at each institution. Microarray hybridisation was done in a Clinical Laboratory Improvement Amendments (CLIA)-certified clinical laboratory (GenomeDx Biosciences, San Diego, CA). Informed consent protocols were approved by local Institutional Review Boards. Microarray preprocessing and normalisation were done as previously described.[15–19,21] Consent was not needed because the tissues were previously archived. Microarray data are available from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus with accession numbers GSE46691, GSE62116, GSE72291, GSE79956, GSE79957, and GSE79915.

To develop the radiotherapy predictive signature, we started with gene compilations from Gene Ontology[22] and Gene Set Enrichment Analysis[23] that were related to response to DNA damage and radiation. The Gene Set Enrichment Analysis gene list names were Amundson Gamma Radiation Resistance, Response, and Poor Survival; Ghandhi Bystander and Direct Irradiation; Smirnov Response to Irradiation 2 h and 6 h; Warters Response to 5 Gy IR and Response to Irradiation Skin; and Zhou Cell Cycle Genes in Irradiation Response 2 h, 6 h, and 24 h. The Gene Ontology gene lists were Cellular Response to DNA Damage, Response to Ionizing Radiation, and Response to Radiation. 1800 unique DNA damage repair and radiation response genes were available on our microarray platform.[22,23] Using the training cohort, each of the 1800 genes was ranked in order of its univariate $p_{interaction}$ value in a Cox proportional hazards model. We used this ranked gene list to train a ridge-penalised Cox model[24] with distant metastasis as the endpoint and with treatment, and the interaction terms of radiotherapy and expression of each gene, as the variables.[25] Lambda was selected using 10-fold cross-validation to minimise the mean cross-validation partial-likelihood error rate. In the training cohort, 93 patients had a metastatic event. Thus, selection of the variables included in the model was done by varying the number of variables from ten to 25 (nine to 24 genes plus treatment), to range from roughly ten to four events per variable. The final gene list was the model that minimised the $p_{interaction}$ of radiotherapy and score in the training cohort. The predictions from the model are calculated by taking the difference between the prediction without radiotherapy and that with radiotherapy and converting to binary scores, which we have termed the Post-Operative Radiation Therapy Outcomes Score (PORTOS), using a cutoff of 0, where patients with scores greater than 0 (high PORTOS) benefit from radiotherapy, and patients with scores less than or equal to 0 (low PORTOS) do not benefit from radiotherapy. The natural cutoff point is 0 because it represents no difference in predicted outcomes irrespective of treatment with radiotherapy. The model was locked before it was applied to the independent validation cohort.

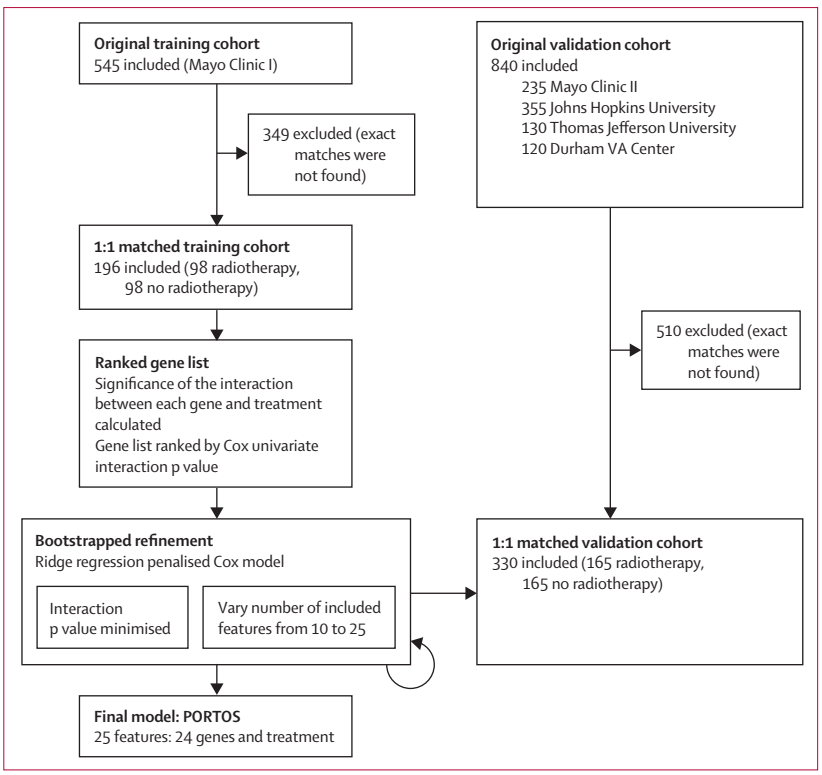


Figure 1: **Study design**

Statistical analysis

Cumulative incidence curves were generated for the primary endpoint of distant metastasis. We present metastasis rate at 10 years as 1 minus the Kaplan-Meier estimates. All hazard ratios (HRs) and 10-year metastasis rates are reported with 95% CIs. We used Greenwood's formula to obtain the 95% CI of the 10-year survival probability and converted them to metastasis rates. We used a Wald test of the interaction term between radiotherapy and the predictive scores in a Cox model including the main effects of treatment and score to assess the significance of predicting treatment response. We also used this method to assess whether three published clinical scores prognostic for outcomes after radical prostatectomy could predict response to

postoperative radiotherapy: Decipher,[15] mCCP (the microarray version of the cell cycle progression signature),[21,26] and CAPRA-S[27] in the validation cohort only. High versus low scores for these models were split either by the median or the 25th and 75th percentiles. ANOVA and the χ^2 test were used to assess differences between continuous and categorical variables between patient groups. Significance was set as a two-tailed p value of less than 0·05. All statistical analyses were done in R 3.1.2.

***Panel*: Post-Operative Radiation Therapy Outcomes Score genes**

- *DRAM1* DNA damage regulated autophagy modulator 1; GSEA: Amundson, Smirnov, Warters
- *KRT14* keratin 14; GO: response to ionising RT, response to radiation
- *PTPN22* protein tyrosine phosphatase, non-receptor type 22; GSEA: Amundson
- *ZMAT3* zinc finger matrin-type 3; GO: cellular response to DNA damage, GSEA: Ghandi, Smirnov, Warters
- *ARHGAP15* Rho GTPase activating protein 15; GSEA: Amundson
- *IL1B* interleukin 1 beta; GSEA: Ghandi
- *ANLN* anillin actin binding protein; GSEA: Zhou
- *RPS27A* ribosomal protein S27a; GO: cellular response to DNA damage
- *MUM1* melanoma associated antigen (mutated) 1; GO: cellular response to DNA damage
- *TOP2A* topoisomerase (DNA) II alpha; GO: cellular response to DNA damage, GSEA: Zhou
- *GNG11* G protein subunit gamma 11; GSEA: Amundson
- *CDKN3* cyclin dependent kinase inhibitor 3; GSEA: Zhou
- *HCLS1* haematopoietic cell-specific Lyn substrate 1; GSEA: Amundson
- *DTL* denticleless E3 ubiquitin protein ligase homologue; GO: cellular response to DNA damage, response to radiation, GSEA: Zhou
- *IL7R* interleukin 7 receptor; GSEA: Ghandi
- *UBA7* ubiquitin like modifier activating enzyme 7; GO: cellular response to DNA damage
- *NEK1* NIMA related kinase 1; GO: cellular response to DNA damage, response to ionising RT, GO: response to radiation
- *CDKN2AIP* CDKN2A interacting protein; GO: cellular response to DNA damage
- *APEX2* apurinic/apyrimidinic endonuclease 2; GO: cellular response to DNA damage
- *KIF23* kinesin family member 23; GSEA: Amundson, Smirnov, Zhou
- *SULF2* sulfatase 2; GSEA: Zhou
- *PLK2* polo like kinase 2; GSEA: Amundson, Smirnov
- *EME1* essential meiotic structure-specific endonuclease 1; GO: cellular response to DNA damage
- *BIN2* bridging integrator 2; GSEA: Amundson

GO=Gene Ontology.[22] GSEA=Gene Set Enrichment Analysis.[23] RT=radiotherapy.

See Online for appendix

Role of the funding source
There was no funding source for this study. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Matched cohorts were generated for the training cohort (Mayo Clinic I; n=545) and for the validation cohort (pooled Mayo Clinic II, Johns Hopkins University, Thomas Jefferson University, and Durham VA Center; n=840; figure 1). The final training cohort consisted of 196 patients with a median follow-up time of 10·8 years (IQR 6·2–15·0) and the final validation cohort had 330 patients with a median follow-up time of 7·0 years (4·5–10·0). Patients excluded from the final cohorts did not have a match or did not have complete data to find a match. No data were missing for the clinicopathological variables used for matching, time to metastasis, or metastatic events. We used the training cohort to generate a 24-gene signature of DNA damage-related and radiation-related genes that predicted response to postprostatectomy radiotherapy. Most of the genes in the signature (18 of 24) were curated from the Gene Ontology or Gene Set Enrichment Analysis radiation response sets (panel; appendix p 2). Six genes were related only to DNA damage response and four genes were related to both DNA damage and radiation response (appendix p 2). Several genes involved in immune response are included in the 24-gene signature, including *IL1B*,[28] *IL7R*,[29] *PTPN22*,[30] and *HCLS1*.[31]

In the matched training cohort, 39 (20%) of 196 patients had a high PORTOS (>0) and 157 (80%) had a low PORTOS (table). In the high score group, 12 (63%) of 19 patients who did not have radiotherapy and one (5%) of 20 patients who did have radiotherapy had a distant metastatic event by 10 years of follow-up. In the low score group, 24 (30%) of 79 patients who did not have radiotherapy and 44 (56%) of 78 patients who did have radiotherapy had a distant metastatic event by 10 years of follow-up. In the training cohort, PORTOS predicted response to radiotherapy (interaction between radiotherapy and score, $p_{interaction}<0·0001$; appendix p 3). In patients with a high PORTOS, those who had radiotherapy had a lower incidence of distant metastasis than did patients who did not have radiotherapy, with a 10-year metastasis rate of 5% (95% CI 0–14) in patients who had radiotherapy and 63% (34–80) in patients who did not have radiotherapy (HR 0·12 [95% CI 0·03–0·41], p<0·0001; figure 2A, B; appendix p 3). In patients with a low PORTOS, those who had radiotherapy had a higher incidence of distant metastasis than did patients who did not have radiotherapy, with a 10-year metastasis rate of 57% (95% CI 44–67) compared with 31% (20–41; HR 2·5 [95% CI 1·6–4·1], p<0·0001; figure 2A, C; appendix p 3).

Results for the training cohort were confirmed in our independent matched validation cohort; PORTOS

predicted response to radiotherapy (interaction between radiotherapy and score, $p_{interaction}$=0·016; appendix p 3). 82 (25%) of 330 patients had a high PORTOS, and 248 (75%) had a low PORTOS. No consistent associations of clinicopathological variables with PORTOS across both the training and validation cohorts were found (table). In the high score group, six (24%) of 25 patients who did not have radiotherapy and two (4%) of 57 patients who did have radiotherapy had a distant metastatic event by 10 years of follow-up. In the low score group, 38 (27%) of 140 patients who did not have radiotherapy and 24 (22%) of 108 patients who did have radiotherapy had a distant metastatic event by 10 years of follow-up. In the high PORTOS group, patients who had radiotherapy had a lower incidence of distant metastasis than patients who did not have radiotherapy (HR 0·15 [95% CI 0·04–0·60], p=0·0020; figure 2D, E), with a 10-year metastasis rate of 4% (95% CI 0–10) in patients who had radiotherapy and 35% (7–54) in patients who did not have radiotherapy. In the low PORTOS group, patients who had radiotherapy had a similar incidence of distant metastasis as patients who did not have radiotherapy (HR 0·92 [95% CI 0·56–1·51], p=0·76; figure 2D, F), with a 10-year metastasis rate of 32% (95% CI 19–43) in patients who had radiotherapy and 32% (22–40) in those who did not have radiotherapy.

Three widely used prognostic scores do not predict response to postoperative radiotherapy within the validation cohort. Using the median score as the cutoff point, the interactions between the Decipher, mCCP, and CAPRA-S prognostic models with radiotherapy were not significant (interaction between radiotherapy and score, Decipher $p_{interaction}$=0·99, mCCP $p_{interaction}$=0·34, CAPRA-S $p_{interaction}$=0·34; appendix p 4). Patients with high Decipher, mCCP, or CAPRA-S scores do worse than do those with a low score regardless of treatment, and patients treated with radiotherapy have improved outcomes regardless of risk score (figure 3). As a sensitivity analysis, we also did an interaction analysis for the three prognostic signatures with the 25th and 75th percentiles as the cutoff points, and found that all $p_{interaction}$ values remained non-significant (appendix p 5). These results illustrate the differences between prognostic and predictive signatures, and while certain prognostic signatures do contain predictive information,[10] these signatures are not predictive for post-prostatectomy radiation therapy.

	Training cohort			Validation cohort		
	Low PORTOS (n=157)	High PORTOS (n=39)	p value	Low PORTOS (n=248)	High PORTOS (n=82)	p value
Age (years, range)	66 (48–79)	64 (49–74)	0·14	62 (40–78)	60·5 (47–76)	0·22
PSA <10 ng/dL	74 (47%)	22 (56%)		159 (64%)	63 (77%)	
PSA 10–20 ng/dL	35 (22%)	9 (23%)	0·44	56 (23%)	14 (17%)	0·076
PSA >20 ng/dL	48 (31%)	8 (21%)		33 (13%)	5 (6%)	
Gleason score ≤6	7 (5%)	5 (13%)		15 (6%)	5 (6%)	
Gleason score 7	64 (41%)	20 (51%)	0·038	164 (66%)	48 (59%)	0·42
Gleason score 8–10	86 (55%)	14 (36%)		69 (28%)	29 (35%)	
Positive surgical margins	105 (67%)	25 (64%)	0·89	173 (70%)	57 (70%)	1·00
Seminal vesicle invasion	65 (41%)	9 (23%)	0·054	67 (27%)	25 (31%)	0·64
Extracapsular extension	87 (55%)	25 (64%)	0·42	149 (60%)	53 (65%)	0·55
Lymph node invasion	23 (15%)	1 (3%)	0·074	10 (4%)	0 (0%)	0·14
Radiotherapy	78 (50%)	20 (51%)	1·00	108 (44%)	57 (70%)	<0·0001
Adjuvant	27 (17%)	9 (23%)	0·54	30 (12%)	11 (13%)	0·90
Salvage	51 (33%)	11 (28%)	0·75	79 (32%)	46 (56%)	<0·0001
ADT	91 (58%)	17 (44%)	0·15	49 (20%)	11 (13%)	0·26
Adjuvant	45 (29%)	8 (21%)	0·41	30 (12%)	8 (10%)	0·71
Salvage	68 (43%)	11 (28%)	0·12	30 (12%)	5 (6%)	0·19

Data are median (range) or n (proportion) unless otherwise specified. PORTOS=Post-Operative Radiation Therapy Outcomes Score. PSA=prostate-specific antigen. ADT=androgen deprivation therapy.

***Table*: Baseline characteristics**

Discussion

In this retrospective study, we used high-throughput gene expression and clinical data to develop and validate PORTOS, a 24-gene expression signature that predicts response to postprostatectomy radiotherapy in matched training and validation cohorts of patients with prostate cancer. We show that of patients who had radiotherapy, patients with high PORTOS had a lower incidence of distant metastasis than that in patients with low scores. We show that in comparison to PORTOS, the widely used genomic and clinical risk tools Decipher, mCCP, and CAPRA-S did not predict response to postoperative radiotherapy.

All biomarkers currently in clinical use for prostate cancer are either prognostic[15,32,33] or diagnostic.[34] Although prognostic biomarkers can identify which patients have aggressive disease and presumably need intensification of therapy, they do not identify which particular therapy should be given. The development of truly predictive (as opposed to prognostic) treatment response biomarkers is a difficult proposition, because it ideally requires balanced datasets from treated versus untreated patients. Although randomised clinical trials would be perfectly suited for this purpose, the scarcity of high-throughput genomic or transcriptomic data from trial samples has precluded this analysis to date. Thus, to our knowledge, PORTOS is the first validated molecular signature developed to predict response to therapy in prostate cancer, and is a potentially clinically useful tool for specifically selecting patients for radiotherapy.

Response to radiotherapy can be affected by a number of different factors, including the intrinsic radiosensitivity or radioresistance of a tumour. However, extrinsic variables can also affect the perceived tumour response. A tumour that has already metastasised before radiotherapy will clinically behave as a non-responder and a tumour that was cured by surgery alone will behave as a responder, but neither of these factors is related to the radiation itself. Although predictive gene models are

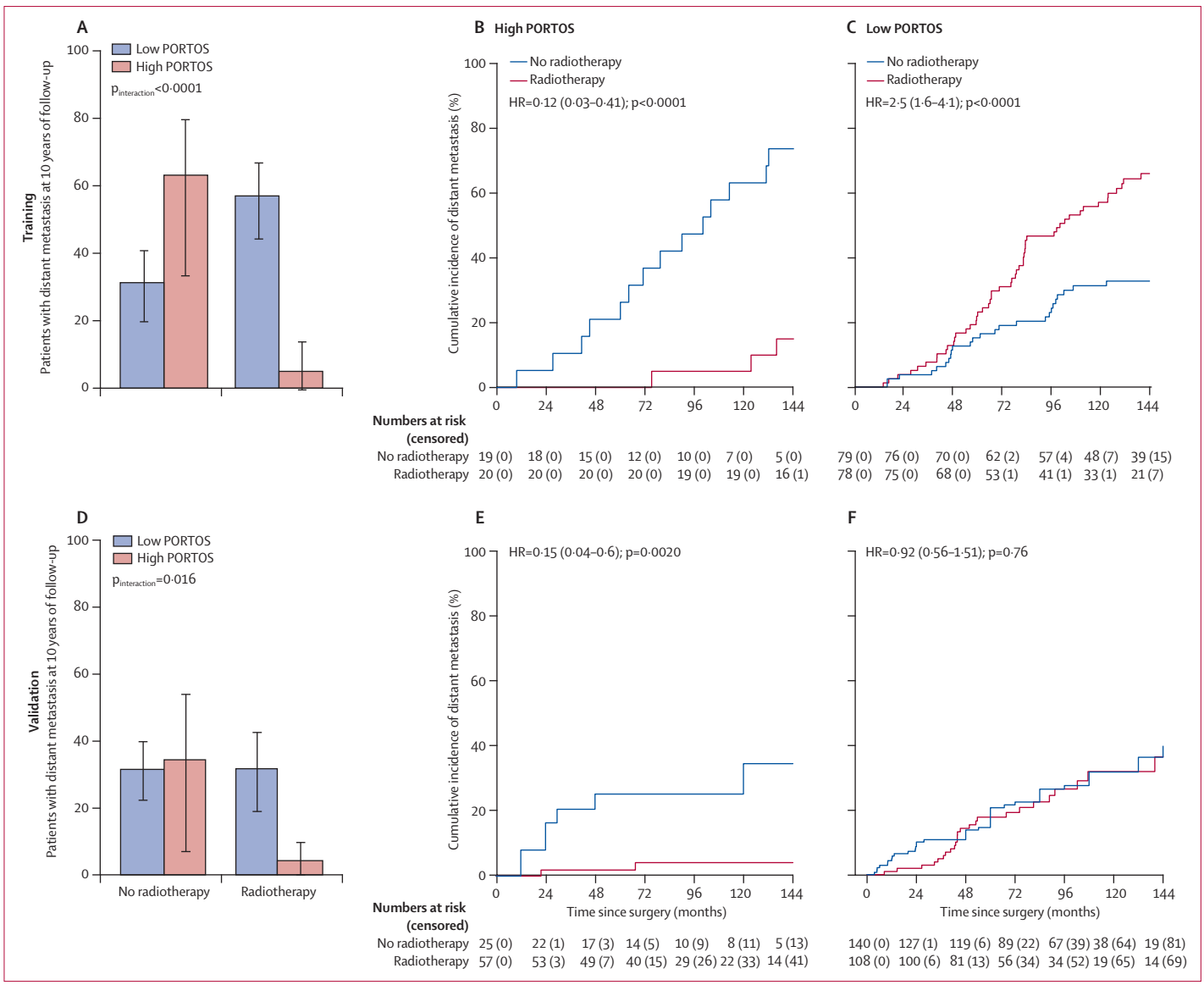
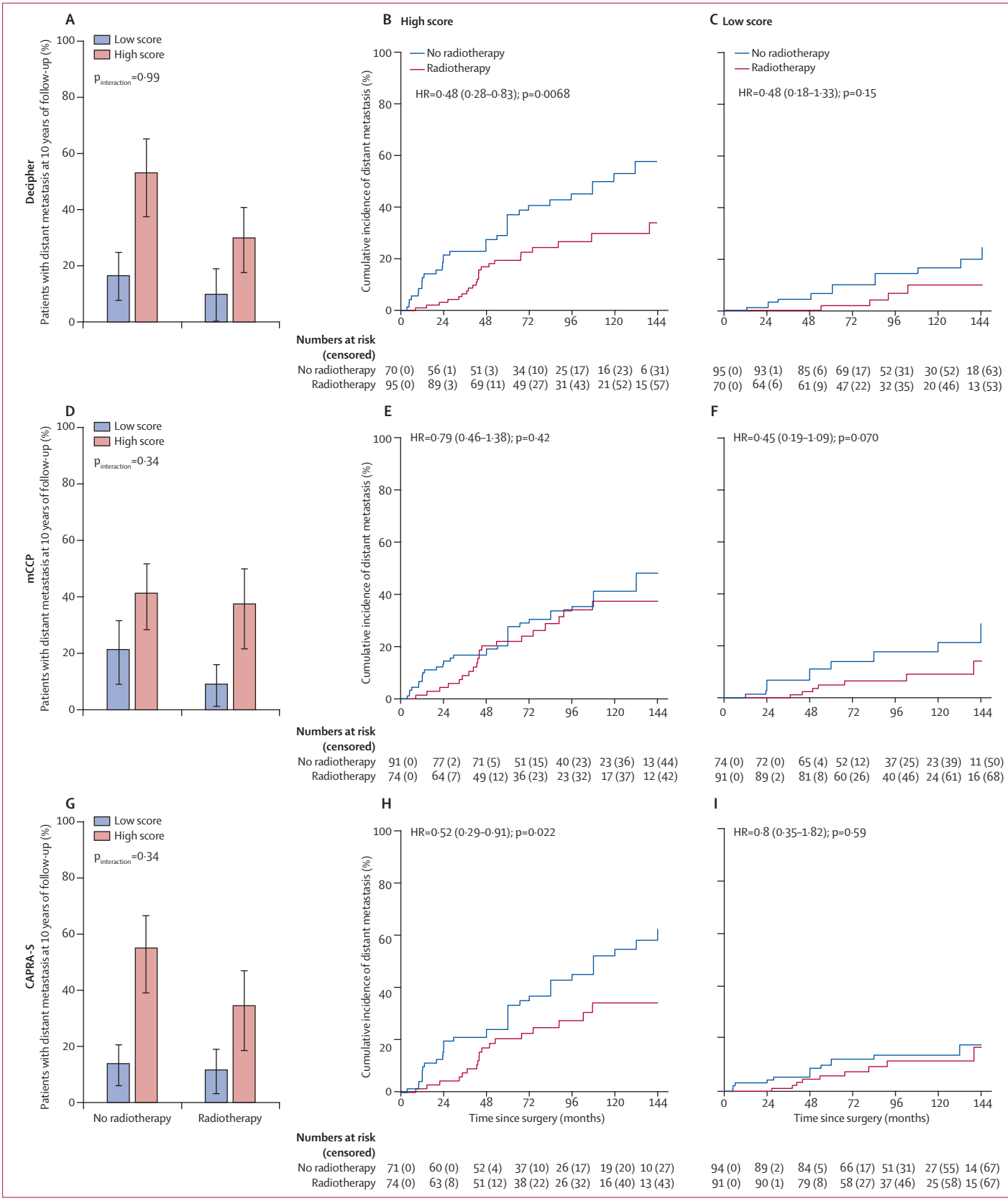


Figure 2: **10-year and cumulative incidence of distant metastasis in the training and validation cohorts stratified by low and high PORTOS**
(A) 10-year metastasis rates for high and low PORTOS with and without postoperative radiotherapy in the training cohort. Kaplan-Meier curves comparing patients with and without radiotherapy for patients with high PORTOS (B) and low PORTOS (C) in the training cohort. (D) 10-year metastasis rates for high and low PORTOS with and without postoperative radiotherapy in the validation cohort. Kaplan-Meier curves comparing patients with and without radiotherapy for patients with high PORTOS (E) and low PORTOS (F) in the validation cohort. Interaction between radiotherapy and score, $p_{interaction}$. Error bars are 95% confidence intervals. HR=hazard ratio. PORTOS=Post-Operative Radiation Therapy Outcomes Score.

not necessarily able to distinguish the tumour intrinsic versus extrinsic reasons for treatment response, PORTOS is designed to enrich for intrinsic radiation response by only including genes that were related to radiation or DNA damage response on the basis of experimental and literature evidence.

This work has a few limitations. First, the clinical cohorts were retrospectively collected. Therefore, treatment selection bias is an inevitable consequence because patients were chosen to receive radiotherapy for specific clinical reasons. In the training cohort, we see that in patients with a low score, those who had

Figure 3: **10-year and cumulative incidence of distant metastasis in the validation cohort stratified by low and high score**
(A) 10-year metastasis rates for high and low Decipher with and without postoperative radiotherapy in the validation cohort. Kaplan-Meier curves comparing patients with and without radiotherapy for patients with high Decipher (B) and low Decipher score (C) in the validation cohort. (D) 10-year metastasis rates for high and low mCCP with and without postoperative radiotherapy in the validation cohort. Kaplan-Meier curves comparing patients with and without radiotherapy for patients with high mCCP (E) and low mCCP score (F) in the validation cohort. (G) 10-year metastasis rates for high and low CAPRA-S with and without postoperative radiotherapy in the validation cohort. Kaplan-Meier curves comparing patients with and without radiotherapy for patients with high CAPRA-S (H) and low CAPRA-S (I) in the validation cohort. All scores were split by the median into high and low score groups. Error bars are 95% confidence intervals. HR=hazard ratio. mCCP= microarray version of the Cell Cycle Progression signature.


A
Low score
High score
$p_{interaction}$=0·99
Decipher
Patients with distant metastasis at 10 years of follow-up (%)
B High score
No radiotherapy
Radiotherapy
HR=0·48 (0·28–0·83); p=0·0068
Cumulative incidence of distant metastasis (%)
C Low score
No radiotherapy
Radiotherapy
HR=0·48 (0·18–1·33); p=0·15
Numbers at risk (censored)
No radiotherapy 70 (0) 56 (1) 51 (3) 34 (10) 25 (17) 16 (23) 6 (31)
Radiotherapy 95 (0) 89 (3) 69 (11) 49 (27) 31 (43) 21 (52) 15 (57)
95 (0) 93 (1) 85 (6) 69 (17) 52 (31) 30 (52) 18 (63)
70 (0) 64 (6) 61 (9) 47 (22) 32 (35) 20 (46) 13 (53)
D
Low score
High score
$p_{interaction}$=0·34
mCCP
Patients with distant metastasis at 10 years of follow-up (%)
E
HR=0·79 (0·46–1·38); p=0·42
Cumulative incidence of distant metastasis (%)
F
HR=0·45 (0·19–1·09); p=0·070
Numbers at risk (censored)
No radiotherapy 91 (0) 77 (2) 71 (5) 51 (15) 40 (23) 23 (36) 13 (44)
Radiotherapy 74 (0) 64 (7) 49 (12) 36 (23) 23 (32) 17 (37) 12 (42)
74 (0) 72 (0) 65 (4) 52 (12) 37 (25) 23 (39) 11 (50)
91 (0) 89 (2) 81 (8) 60 (26) 40 (46) 24 (61) 16 (68)
G
Low score
High score
$p_{interaction}$=0·34
CAPRA-S
Patients with distant metastasis at 10 years of follow-up (%)
No radiotherapy
Radiotherapy
H
HR=0·52 (0·29–0·91); p=0·022
Cumulative incidence of distant metastasis (%)
Time since surgery (months)
I
HR=0·8 (0·35–1·82); p=0·59
Time since surgery (months)
Numbers at risk (censored)
No radiotherapy 71 (0) 60 (0) 52 (4) 37 (10) 26 (17) 19 (20) 10 (27)
Radiotherapy 74 (0) 63 (8) 51 (12) 38 (22) 26 (32) 16 (40) 13 (43)
94 (0) 89 (2) 84 (5) 66 (17) 51 (31) 27 (55) 14 (67)
91 (0) 90 (1) 79 (8) 58 (27) 37 (46) 25 (58) 15 (67)

postoperative radiotherapy had a higher incidence of distant metastasis than those who were not treated, which might be due to various factors. Although we do attempt to match on all major and available clinicopathological variables, we do not capture all possible extrinsic confounders that might affect treatment selection by the physician or clinical outcomes (eg, treatment timing, treatment duration, radiation dose, PSA kinetics, ethnicity, comorbidities, or other medications). Since these and other variables were not accounted for in the matching process, these unmeasured confounders could conceivably affect our results, and are difficult to address in retrospective studies. Therefore, these results should be confirmed in additional cohorts, and, ideally, in a randomised controlled trial. Furthermore, in the training cohort specifically, statistical overfitting contributed to this counter-intuitive result of treated patients having worse outcomes than untreated patients. Overfitting is a well known and unavoidable issue in model development, emphasising the importance of our validation results. The validation results are not affected by overfitting, and show that treated and untreated patients with low scores have similar outcomes and that the patients with high scores with radiotherapy have better outcomes than patients who did not have radiotherapy.

Another limitation of this work is the difference in the populations of the training and validation cohorts. The training cohort included patients with more adverse clinical and pathological features than the validation cohort. However, the high metastasis event rate in the training cohort allowed us to train with a small number of patients. Furthermore, validation in a cohort that is not a perfect match of the training cohort emphasises the generalisability of the model. Clinical tests, such as Decipher, are often validated in somewhat different populations than they were originally trained.[17–20,35] Validation of PORTOS suggests that it will be robust despite the limitations of the data. We are working on acquiring additional cohorts, which will allow us to increase our patient numbers for further investigation. Data from future trials such as RADICALS (NCT00541047) and RTOG 9601 (NCT00002874) could provide potential validation cohorts.

Additionally, although PORTOS predicts response to postoperative radiotherapy, our study design does not distinguish between adjuvant and salvage radiotherapy, primarily because of the difficulty of creating adequately powered matched cohorts for adjuvant or salvage therapy alone. As additional transcriptomic data from larger datasets become available, more specific signatures can be developed for either adjuvant or salvage radiotherapy. Findings from a study[19] have suggested that Decipher might be differentially prognostic for patients receiving adjuvant versus salvage radiotherapy; however, this hypothesis was not formally tested. Our results indicate that Decipher is equally prognostic in patients treated or not with postoperative radiotherapy. However, a combination of Decipher and PORTOS could allow for selection of patients who need postoperative radiotherapy (using PORTOS), and help decide whether to irradiate in the adjuvant or salvage setting (using Decipher).

It is clear that biological data will be used to drive radiotherapy decisions. One study[36] has already reported findings on a prognostic signature in the context of definitive radiation, and we present a predictive signature in the context of postoperative radiation. Ultimately, we envision the field moving towards a biomarker-driven treatment approach, whereby prognostic signatures are used to select patients with aggressive disease, and predictive signatures are used to select specific therapies. PORTOS represents a new tool in this framework, and is embedded in a commercially available, clinical-grade platform. We believe this work will improve the personalisation of therapy for patients with prostate cancer.

Contributors

SGZ, SLC, PLN, and FYF contributed to the study concept and design. SGZ, SLC, MY, HA-DA, and FYF contributed to the study development and methods. SGZ, SLC, NE, HA-DA, MA, ED, APD, SJF, RJK, AER, EMS, RBD, and FYF collected data. All authors analysed and interpreted data. FYF provided administrative, technical, and material support. SGZ, SLC, PLN, and FYF supervised the study. SGZ, SLC, DES, MY, PLN, and FYF wrote the manuscript. All authors approved the final version of the manuscript.

Declaration of interests

SGZ reports grants from Prostate Cancer Foundation and travel expenses from GenomeDx Biosciences, outside the submitted work; and has a patent PORTOS provisional filing United States Patent and Trademark Office (USPTO) pending. SLC reports employment at PFS Genomics, outside the submitted work; and has a patent PORTOS provisional filing USPTO pending. DES reports grants from Prostate Cancer Foundation, during the conduct of the study. NE reports employment at GenomeDx Biosciences, during the conduct of the study. HA-DA reports employment at GenomeDx Biosciences, during the conduct of the study. MA reports employment at GenomeDx Biosciences, during the conduct of the study. SAT reports personal fees from Ventana Medical Systems, Janssen, and Abbvie; grants and personal fees from Medivation and Astellas; travel support from Thermo Fisher; grants from GenomeDx; personal fees and equity in Strata Oncology; and has a patent on ETS gene fusions in prostate cancer with royalties paid to the University of Michigan from Hologic and Ventana Medical Systems, outside of the submitted work. SAT is included in the royalties distribution stream from the University of Michigan. ED reports employment at GenomeDx Biosciences, during the conduct of the study; and has a patent PORTOS provisional filing USPTO pending. MRC reports grants and personal fees from Myriad Genetics; grants from Genomic Health and GenomeDx Biosciences; and personal fees from Dendreon, Astellas, Bayer, and Janssen, outside the submitted work. SJF reports grants and personal fees from GenomeDx, during the conduct of the study. RJK reports grants and royalties from GenomeDx Biosciences, outside the submitted work. AER reports grants and personal fees from GenomeDx Biosciences, during the conduct of the study. EMS reports consulting for GenomeDx Biosciences, outside the submitted work. RBD reports grants from GenomeDx Biosciences, during the conduct of the study. PLN reports personal fees from Medivation, Ferring, and GenomeDx Biosciences, outside the submitted work. FYF reports non-financial support from GenomeDx Biosciences, during the conduct of the study; grants from Varian, personal fees from Medivation and Astellas; grants and personal fees from Celgene, outside the submitted work; has a patent PORTOS provisional filing USPTO pending; and is a founder and serves as president for PFS Genomics. MY, CS, APD, and PRC declare no competing interests.

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References

1 Gunderson LL, Tepper JE. Clinical radiation oncology, 3rd edn. Philadelphia, PA: Elsevier Saunders, 2012.

2 Bolla M, van Poppel H, Collette L, et al. Postoperative radiotherapy after radical prostatectomy: a randomised controlled trial (EORTC trial 22911). *Lancet* 2005; **366:** 572–78.

3 Thompson IM Jr, Tangen CM, Paradelo J, et al. Adjuvant radiotherapy for pathologically advanced prostate cancer: a randomized clinical trial. *JAMA* 2006; **296:** 2329–35.

4 Wiegel T, Bottke D, Steiner U, et al. Phase III postoperative adjuvant radiotherapy after radical prostatectomy compared with radical prostatectomy alone in pT3 prostate cancer with postoperative undetectable prostate-specific antigen: ARO 96–02/AUO AP 09/95. *J Clin Oncol* 2009; **27:** 2924–30.

5 Thompson IM, Tangen CM, Paradelo J, et al. Adjuvant radiotherapy for pathological T3N0M0 prostate cancer significantly reduces risk of metastases and improves survival: long-term followup of a randomized clinical trial. *J Urol* 2009; **181:** 956–62.

6 Neuhof D, Hentschel T, Bischof M, Sroka-Perez G, Hohenfellner M, Debus J. Long-term results and predictive factors of three-dimensional conformal salvage radiotherapy for biochemical relapse after prostatectomy. *Int J Radiat Oncol Biol Phys* 2007; **67:** 1411–17.

7 Pisansky TM, Kozelsky TF, Myers RP, et al. Radiotherapy for isolated serum prostate specific antigen elevation after prostatectomy for prostate cancer. *J Urol* 2000; **163:** 845–50.

8 Stephenson AJ, Shariat SF, Zelefsky MJ, et al. Salvage radiotherapy for recurrent prostate cancer after radical prostatectomy. *JAMA* 2004; **291:** 1325–32.

9 Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; **351:** 2817–26.

10 Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006; **24:** 3726–34.

11 Eschrich SA, Fulp WJ, Pawitan Y, et al. Validation of a radiosensitivity molecular signature in breast cancer. *Clin Cancer Res* 2012; **18:** 5134–43.

12 Weichselbaum RR, Ishwaran H, Yoon T, et al. An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer. *Proc Natl Acad Sci USA* 2008; **105:** 18490–95.

13 Pitroda SP, Pashtan IM, Logan HL, et al. DNA repair pathway gene expression score correlates with repair proficiency and tumor sensitivity to chemotherapy. *Sci Transl Med* 2014; **6:** 229ra42.

14 Roepman P, Schlicker A, Tabernero J, et al. Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. *Int J Cancer* 2014; **134:** 552–62.

15 Erho N, Crisan A, Vergara IA, et al. Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. *PLoS One* 2013; **8:** e66855.

16 Nakagawa T, Kollmeyer TM, Morlan BW, et al. A tissue biomarker panel predicting systemic progression after PSA recurrence post-definitive prostate cancer therapy. *PLoS One* 2008; **3:** e2318.

17 Karnes RJ, Bergstralh EJ, Davicioni E, et al. Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. *J Urol* 2013; **190:** 2047–53.

18 Ross AE, Johnson MH, Yousefi K, et al. Tissue-based genomics augments post-prostatectomy risk stratification in a natural history cohort of intermediate- and high-risk men. *Eur Urol* 2016; **69:** 157–65.

19 Den RB, Feng FY, Showalter TN, et al. Genomic prostate cancer classifier predicts biochemical failure and metastases in patients after postoperative radiation therapy. *Int J Radiat Oncol Biol Phys* 2014; **89:** 1038–46.

20 Freedland SJ, Choeurng V, Howard L, et al. Utilization of a genomic classifier for prediction of metastasis following salvage radiation therapy after radical prostatectomy. *Eur Urol* 2016; **70:** 588–96.

21 Prensner JR, Zhao S, Erho N, et al. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. *Lancet Oncol* 2014; **15:** 1469–80.

22 Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25:** 25–29.

23 Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005; **102:** 15545–50.

24 Benner A, Zucknick M, Hielscher T, Ittrich C, Mansmann U. High-dimensional Cox models: the choice of penalty as part of the model building process. *Biom J* 2010; **52:** 50–69.

25 Tian L, Alizadeh AA, Gentles AJ, Tibshirani R. A simple method for estimating interactions between a treatment and a large number of covariates. *J Am Stat Assoc* 2014; **109:** 1517–32.

26 Cuzick J, Berney DM, Fisher G, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. *Br J Cancer* 2012; **106:** 1095–99.

27 Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. *Cancer* 2011; **117:** 5039–46.

28 Ben-Sasson SZ, Wang K, Cohen J, Paul WE. IL-1beta strikingly enhances antigen-driven CD4 and CD8 T-cell responses. *Cold Spring Harb Symp Quant Biol* 2013; **78:** 117–24.

29 Al-Rawi MA, Mansel RE, Jiang WG. Interleukin-7 (IL-7) and IL-7 receptor (IL-7R) signalling complex in human solid tumours. *Histol Histopathol* 2003; **18:** 911–23.

30 Stanford SM, Bottini N. PTPN22: the archetypal non-HLA autoimmunity gene. *Nat Rev Rheumatol* 2014; **10:** 602–11.

31 Cavnar PJ, Mogen K, Berthier E, Beebe DJ, Huttenlocher A. The actin regulatory protein HS1 interacts with Arp2/3 and mediates efficient neutrophil chemotaxis. *J Biol Chem* 2012; **287:** 25466–77.

32 Klein EA, Cooperberg MR, Magi-Galluzzi C, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. *Eur Urol* 2014; **66:** 550–60.

33 Cuzick J, Swanson GP, Fisher G, et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol* 2011; **12:** 245–55.

34 Tomlins SA, Day JR, Lonigro RJ, et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. *Eur Urol* 2016; **70:** 45–53.

35 Klein EA, Yousefi K, Haddad Z, et al. A genomic classifier improves prediction of metastatic disease within 5 years after surgery in node-negative high-risk prostate cancer patients managed by radical prostatectomy without adjuvant therapy. *Eur Urol* 2015; **67:** 778–86.

36 Lalonde E, Ishkanian AS, Sykes J, et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. *Lancet Oncol* 2014; **15:** 1521–32.