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Utilization of a Genomic Classifier for Prediction of Metastasis Following Salvage Radiation Therapy after Radical Prostatectomy

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Abstract

Background: Despite salvage radiation therapy (SRT) for recurrent prostate cancer (PCa) after radical prostatectomy (RP), some patients still progress to metastases. Identifying these men would allow them to undergo systemic therapy including testing novel therapies to reduce metastases risk.
Objective: To test whether the genomic classifier (GC) predicts development of metastatic disease.
Design, setting, and participants: Retrospective multi-center and multi-ethnic cohort study from two academic centers and one Veterans Affairs Medical Center in the United States involving 170 men receiving SRT for recurrent PCa post-RP.
Outcome measurements and statistical analysis: Time from SRT to development of metastatic disease tested using Cox regression, survival c-index, and decision curve analysis. Performance of GC was compared to the Cancer of the Prostate Risk Assessment Score and Briganti risk models based on these metrics.
Results and limitations: With a median 5.7 yr follow-up after SRT, 20 patients (12%) developed metastases. On multivariable analysis, for each 0.1 unit increase in GC (scaled from 0 to 1), the hazard ratio for metastasis was 1.58 (95% confidence interval 1.16–2.17; $p = 0.002$). Adjusting for androgen deprivation therapy did not materially change the results. The c-index for GC was 0.85 (95% confidence interval 0.73–0.88) versus 0.63–0.65 for published clinico-pathologic risk models. The 5-yr cumulative incidence of metastasis post-SRT in patients with low, intermediate, and high GC scores was 2.7%, 8.4%, and 33.1%, respectively ($p < 0.001$).
Conclusions: While validation in larger, prospectively collected cohorts is required, these data suggest GC is a strong predictor of metastases among men receiving SRT for recurrent PCa post-RP, accurately identifying men who are excellent candidates for systemic therapy due to their very high-risk of metastases.
Patient summary: Genomic classifier and two clinico-pathologic risk models were evaluated on their ability to predict metastases among men receiving salvage radiation therapy for recurrent prostate cancer. Genomic classifier was able to identify candidates for further therapies due to their very high-risk of metastases.

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1. Introduction

Our ability to predict outcomes following salvage radiation (SRT) for recurrent disease after radical prostatectomy (RP) is poor. A prior study evaluated 1540 men treated with SRT at multiple academic centers [1]. The best nomogram predicted 6-yr prostate specific antigen (PSA) control with 69% accuracy. This compares poorly to other prostate cancer (PCa) nomograms, which predict PCa death (a more definitive endpoint) with 80% accuracy or higher at diagnosis [2], post-RP [1,3], or at initial rising PSA post-RP [4]. While intermediate endpoints (eg, rising PSA) are important, assessment of risk factors for hard endpoints such as metastasis are needed.

Advancements in genetics and high throughput "omics" coupled with a robust biomarker discovery program have resulted in several commercially available tissue-based molecular markers for PCa prognosis [5]. While these markers have been evaluated in multiple populations, only one has been examined on patients receiving radiation as primary curative therapy [6] and none in men all receiving SRT. Based upon performance in other populations, a promising test is the Decipher genomic classifier (GC) [7–9]. Unlike other tests that examined a limited number of genes in their discovery [5], GC examined the whole tumor gene expression profile. Thus, GC has the theoretical advantage of capturing the entire tumor biology in one signature. GC was developed among men undergoing RP at the Mayo Clinic to predict metastases using a nested case-control study design [7]. It has subsequently been evaluated in multiple independent populations receiving varying degrees of postoperative radiation [8,10,11], but never in men who all received SRT. Importantly, these prior studies all included men who received adjuvant radiation, some of whom were cured with surgery alone. As such, it is impossible to assess whether GC predicted response to radiation or the likelihood of the surgery being curative, which invariably would also relate to metastases risk. Therefore, it is crucial to assess the ability of GC to identify metastases risk in a more homogenous group of men who all recurred and all received SRT to address whether GC predicts outcomes after SRT.

To test whether GC predicts metastases after SRT, we performed a multi-center study of men undergoing SRT post-RP. Our population included men from two tertiary-care referral centers and a Veterans Affairs (VA) hospital, which contained nearly 50% African-American men. Importantly, no man in this study cohort was included in the GC development. Thus, this study is an independent evaluation of GC's ability to predict metastases in men undergoing SRT. We hypothesized GC would predict metastases with high accuracy, especially compared with standard clinico-pathologic variables and two clinico-pathologic risk models: the Cancer of the Prostate Risk Assessment Post-Surgical (CAPRA-S) model [12] and the Briganti risk model [13], which was developed for predicting biochemical recurrence following early SRT. Neither of these clinico-pathologic risk models were initially designed to predict metastasis. The Briganti model, however, represents a recent and relevant risk model for post-SRT patients while CAPRA-S has been externally validated on a European cohort to predict metastasis [14].

2. Materials and methods

2.1. Study cohort

A total of 170 RP patients who received postoperative SRT without prior neoadjuvant or adjuvant therapy and without lymph node invasion were included. Lymph node invasion was defined by the presence of at least one node with a tumor. Seventy prostatectomy patients treated at Durham VA between 1991 and 2010 with postoperative SRT were obtained for analysis (Supplementary Fig. 1). Patients analyzed from Thomas Jefferson University ($n = 61$; yr of RP 1991–2009) and Mayo Clinic ($n = 39$; yr of RP 2000–2006) were obtained from a prior validation study wherein GC had been performed using RP tumor tissue testing GC for predicting metastases in men undergoing postoperative radiation (adjuvant and salvage) [11]. Of the 188 patients in this prior study, only 100 received radiation with PSA > 0.2 ng/ml (ie, SRT), and were thus included in the current study. Importantly, no patient in the current study was included in the GC development [7,11]. Radiation therapy regimens were as previously described where patients were treated to a median dose of 66.6 Gy [11,15]. At all three institutions, only the prostatic fossa is typically radiated for node negative patients. The primary endpoint for the current study was metastasis (regional or distant) detected using computed tomography and/or a bone scan. SRT was defined as radiation for PSA > 0.2 ng/ml or by radiation following salvage androgen deprivation therapy (ADT). Concurrent ADT with SRT was defined as ADT administered within 3 mo of SRT [16–18]. ADT was delivered at the discretion of the providing physician at each institution with a median administration time of 12 mo post-RP. The study met the REporting recommendations for tumor MARKer prognostic studies criteria for evaluation of prognostic biomarkers [19]. The Institutional Review Boards at Durham VA, Thomas Jefferson University, and Mayo Clinic approved this study.

2.2. Tumor tissue sampling, RNA extraction, and testing

RP tumor specimens from Durham VA patients were selected after restaging and regrading from the original hematoxylin and eosin slides. Formalin-fixed paraffin embedded tumor blocks with the highest Gleason grade, and if present, extraprostatic extension or seminal vesicle invasion were selected. Using a hematoxylin and eosin slide freshly prepared from the formalin-fixed paraffin embedded block, the target region of tumor was selected to additionally have >80% tumor by area to minimize presence of benign glands. The tumor was sampled using a single 1.0-mm diameter disposable biopsy punch tool (Miltex, York, PA, USA). RNA extraction, Affymetrix Human Exon 1.0 ST oligonucleotide microarray (Affymetrix, Santa Clara, CA, USA) data generation and preprocessing were as previously described [11]. The approach for tissue selection and analysis was identical at the other sites, as we have previously described [11].

2.3. Calculation of GC, clinico-pathologic risk models, and combined models

GC is a locked risk model developed on a nested case-control data set consisting of 545 patients from the Mayo Clinic [7] which is independent of the cohorts involved in the current study and thus there are no overlapping patients. The expression values for the 22 prespecified biomarkers constituting GC were extracted from the normalized data matrix and entered into the random forest algorithm that was locked

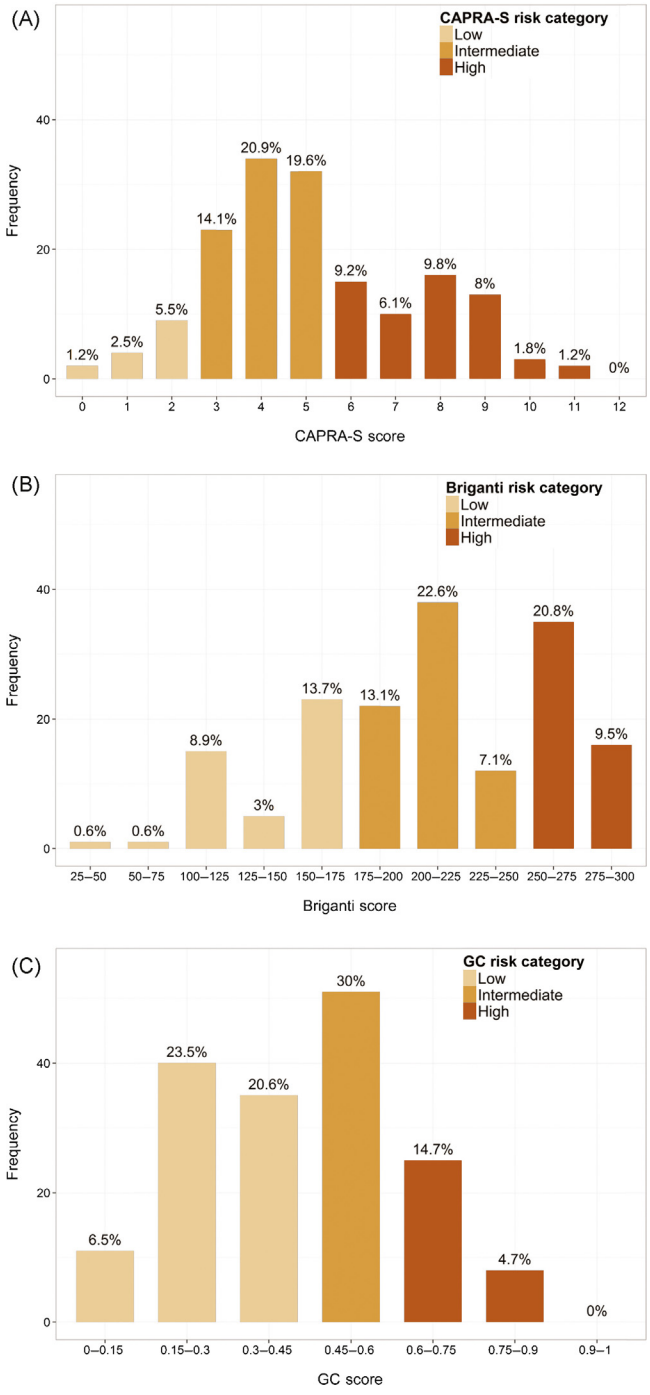

Fig. 1 – Distribution of the study cohort by (A) Cancer of the Prostate Risk Assessment Score, (B) Briganti (categorized by tertiles), and (C) genomic classifier risk scores.
CAPRA-S = Cancer of the Prostate Risk Assessment Score; GC = genomic classifier.

with defined tuning and weighting parameters as reported previously [7]. Thus, each patient received only a single GC score. The GC read-out is a continuous score between 0 and 1, with higher scores indicating greater metastatic potential [7,8]. Previously established and locked cut-points of 0.45 and 0.60 were used to categorize patients into low-, intermediate-, and high-risk groups. These cut-points were selected by optimizing the hazard ratio (HR) between both the intermediate- and high-risk categories versus the low-risk category using an independent data set [10,20].

CAPRA-S scores [12] and Briganti scores were as previously described [13]. The models in which GC was combined with either clinico-pathologic variables or a clinico-pathologic risk model were generated by internally fitting a Cox model on the combined variables of interest.

2.4. Statistical analyses

In time-to-event analyses, event times were defined as time from SRT completion to metastases or date of last follow-up if no metastases.

The prognostic accuracy of CAPRA-S, Briganti, GC, and the combined models was established based on time-dependent receiver operating characteristic curves (survival c-index) [21]. Confidence intervals (CIs) for time-dependent c-indices were computed via bootstrap resampling. Cumulative incidence curves of metastasis risk were constructed using Fine-Gray competing risks analysis with death as a competing risk. To address issues potentially arising due to a few events, penalized likelihood Cox regression methods (least absolute shrinkage and selection operator [LASSO] and Firth) were used for identifying the most prognostic risk factors ensuring robustness of the analyses while avoiding overestimation of risk factors [22]. As the penalty parameter, λ, in a LASSO regression tends to 0, more variables begin to have a nonzero HR in a multivariable model. The order in which variables appear indicates their order of importance in predicting metastasis. An extension of decision curve analysis to survival data was used to determine the net benefit from the use of GC, CAPRA-S, and Briganti risk models [23]. Survival c-indices were considered statistically significant if the lower bound of the 95% exceeded 0.50. The significance level was 0.05 for all tests and analyses were performed in R v3.0.

3. Results

3.1. Patient characteristics

Our cohort was 32% African-American men and 68% Caucasian-American. Consistent with a high-risk cohort who all failed prostatectomy, 53% had extraprostatic extension, 27% seminal vesicle invasion, and 81% had positive margins (Table 1). Eighteen percent had a pathologic Gleason score $\geq$ 8. Median PSA prior to SRT was 0.6 ng/ml (range, 0.1–39; interquartile range, 0.4–1.7). Median time from prostatectomy to SRT was 12 mo (range, 1–160; interquartile range, 5–31). Nineteen percent received concurrent (within 3 mo of SRT) ADT with radiation and 18% received nonconcurrent ADT (ie, >3 mo of SRT delivery). Median follow-up post-RP and post-SRT among patients who did not develop metastases or die was 7.4 yr and 5.7 yr, respectively. During follow-up, 20 (12%) patients developed metastases. Complete clinical data to calculate CAPRA-S and Briganti risk models was available for 163 patients with 18 metastases. CAPRA-S categorized 9.2% of these patients as low-risk while 41.8% were deemed low-risk by GC (Fig. 1).

3.2. GC as a predictor of metastases

Univariable analysis demonstrated that GC, pathologic Gleason score $\geq$ 4+3, extraprostatic extension, and pre-SRT PSA all significantly predicted post-SRT metastasis (Table 2). GC remained an independent predictor after adjusting for other clinical variables including concurrent

Table 1 – Demographic and clinical characteristics of eligible patients.

Variables	Study Cohort
No. patients (%)	170 (100)
Patient age, yr	
Median (range)	61 (39, 75)
IQR (Q1, Q3)	56–66
Race, n (%)	
African-American	54 (31.8)
Caucasian	115 (67.6)
Other	1 (0.6)
Preoperative PSA (ng/ml)	
Median (range)	8.3 (0.4, 80.4)
IQR (Q1, Q3)	5.5–13.5
Pathologic Gleason score, n (%)	
≤6	25 (14.7)
7 (3 + 4)	72 (42.4)
7 (4 + 3)	42 (24.7)
≥8	30 (17.6)
Unknown	1 (0.6)
Extraprostatic extension, n (%)	
	89 (52.7)
Seminal vesicle invasion, n (%)	
	45 (26.6)
Positive surgical margins, n (%)	
	137 (80.6)
Pre-SRT PSA (ng/ml)	
Median (range)	0.6 (0.1, 39.0)
IQR (Q1, Q3)	0.4–1.7
Time from RP to SRT, mo	
Median (range)	12.4 (1.3, 159.7)
IQR (Q1, Q3)	5.0–31.0
Concurrent ADT, n (%)	
	33 (19.4)
ADT administered at any time, n (%)	
	63 (37.1)

ADT= androgen deprivation therapy, IQR= interquartile range; PSA= prostate specific antigen; RP= radical prostatectomy; RT= radiation treatment.

ADT (Table 2). Each 0.1 unit increase in GC score increased the risk of metastasis by 1.58 (95% CI 1.16–2.17; $p = 0.002$). On multivariable analysis (MVA) adjusting for any ADT, whether concurrent or otherwise, GC remained significantly predictive of metastases (HR 1.56; $p = 0.003$; Supplementary Table 1). The estimates of the HRs from a LASSO Cox regression captures the importance of GC in predicting metastasis as it was the first variable to enter the penalized model (Fig. 2A), corroborating results from the MVA. The final and least important variable to enter the model was age at RP. Further, the addition of GC to any individual clinico-pathologic variable or risk model significantly improved their ability to discriminate risk (Fig. 2B). Finally, the 5-yr cumulative incidence of metastasis post-SRT in patients with low, intermediate, and high GC scores using the previously locked cut-points of 0.45 and 0.60 was 2.7%, 8.4%, and 33.1%, respectively ($p < 0.001$; Fig. 3C).

3.3. *Comparison of models to predict metastases*

The survival c-index for GC (0.85; 95% CI 0.73–0.88) at 5 yr post-SRT was substantially higher than for CAPRA-S (0.63; 95% CI 0.49–0.78) or Briganti (0.65; 95% CI 0.54–0.81; Fig. 2C). The sensitivity and specificity for GC (to predict metastasis at 5 yr post-SRT) was 94% and 54% using the 0.45 cut-off compared with 67% and 86% using the 0.60 cut-off (Supplementary Fig. 3). While both Briganti and CAPRA-S models significantly predicted metastases on univariable analysis, neither was significantly correlated with metastases when modeled with GC on MVA (Table 2). Cumulative incidence plots for metastasis by risk-category showed that only GC significantly stratified risk (Fig. 3A–C). Consistent with the better survival c-index, decision curve analysis

Table 2 – Cox univariable and multivariable analysis of clinico-pathologic variables, genomic classifier, Cancer of the Prostate Risk Assessment Post-Surgical, and Briganti risk models for prediction of metastasis.

		UVA		MVA	
	Variables	HR (95% CI)	p-value	HR (95% CI)	p-value
Model I	Patient age, yr	1.00 (0.93 -1.07)	0.992	0.99 (0.91 -1.07)	0.757
	Log2 Preoperative PSA	1.22 (0.76 -1.96)	0.417	1.28 (0.79 -2.11)	0.315
	Pathologic Gleason Score ≤3+4	Ref	1.000	Ref	1.000
	Pathologic Gleason Score ≥4+3	3.45 (1.37 -9.57)	0.008	1.46 (0.47 -4.76)	0.513
	Extraprostatic Extension	3.16 (1.18 -10.34)	0.021	1.34 (0.38 -5.32)	0.655
	Seminal Vesicle Invasion	1.80 (0.68 -4.50)	0.225	0.68 (0.21 -1.99)	0.483
	Positive Surgical Margins	0.50 (0.19 -1.49)	0.197	0.59 (0.21 -1.82)	0.335
	Time from RP to SRT, months	0.98 (0.94 -1.00)	0.105	0.99 (0.95 -1.02)	0.397
	Log2 Pre-SRT PSA	1.08 (1.03 -1.11)	0.004	1.14 (0.88 -1.48)	0.327
	ADT	4.28 (1.66 -12.66)	0.002	2.74 (0.99 -8.54)	0.051
	GC[a]	1.66 (1.25 -2.20)	<0.001	1.56 (1.14 -2.12)	0.003
Model II	CAPRA-S[b]	1.20 (0.99 -1.46)	0.068	1.12 (0.92 -1.37)	0.241
	GC[b]	1.66 (1.25 -2.20)	<0.001	1.63 (1.22 -2.18)	<0.001
Model III	Briganti[c]	1.28 (1.00 -1.65)	0.022	1.14 (0.90 -1.45)	0.244
	GC[a]	1.66 (1.25 -2.20)	<0.001	1.62 (1.21 -2.18)	<0.001

CI = confidence interval; MVA = multivariable analysis; UVA, univariable analysis.
[a] Genomic classifier hazard ratios reported per 0.1 unit increase.
[b] Cancer of the Prostate Risk Assessment Post-Surgical hazard ratios reported per 1 unit increase.
[c] Briganti hazard ratios reported per 25 unit increase.

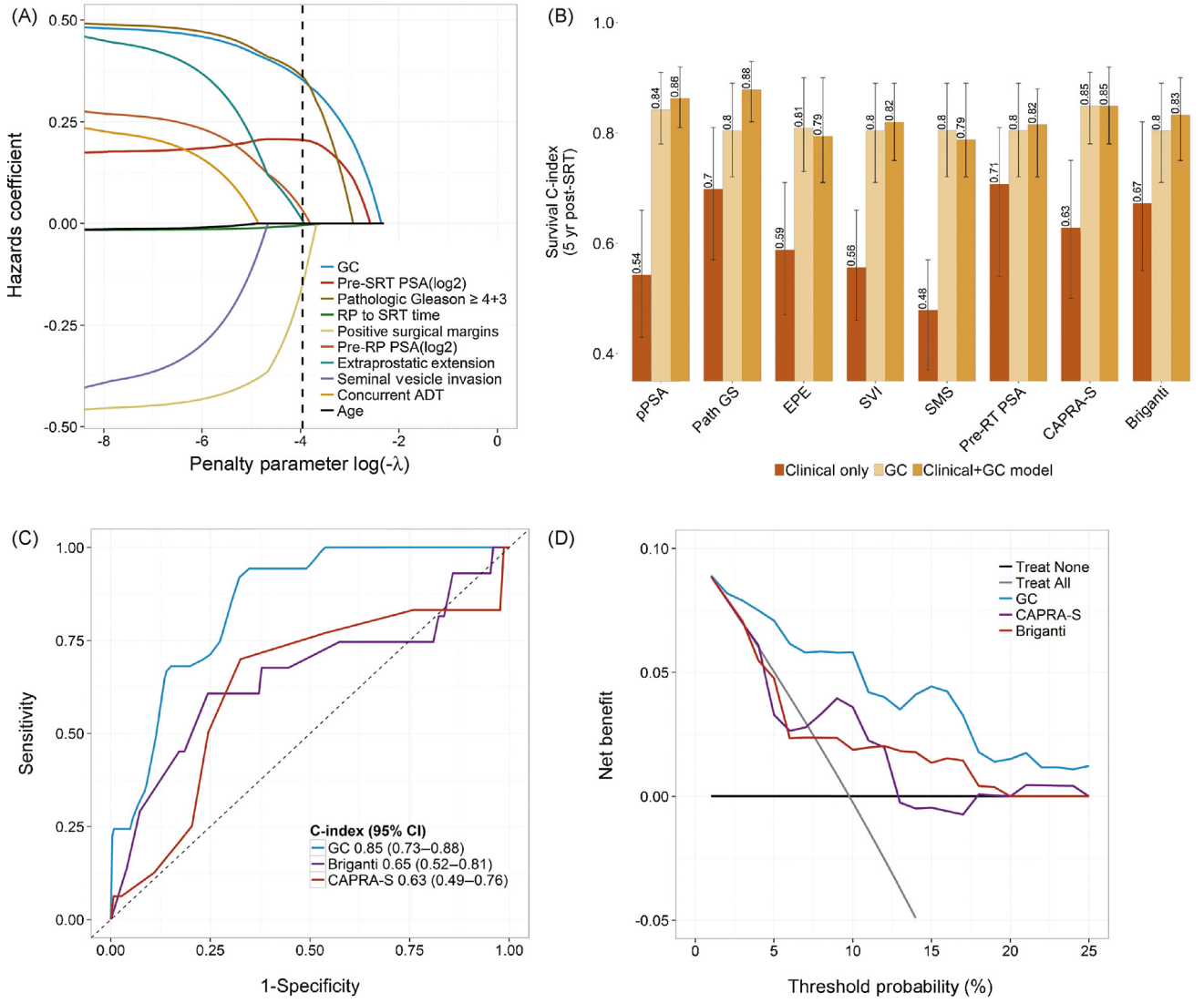

Fig. 2 – (A) Least absolute shrinkage and selection operator coefficient path demonstrating the importance of genomic classifier (GC) and clinico-pathologic variables in the prediction of metastasis. The optimal penalty parameter indicated by a vertical dashed line was found using 5-fold cross-validation. (B) Survival c-indices for prediction of metastasis at 5 yr post-salvage radiation therapy (SRT) of clinico-pathologic variables, GC, and combined GC plus clinico-pathologic models evaluated on individual data sets dependent upon the completeness of the clinico-pathologic variable of interest. Error bars indicate the 95% confidence interval. (C) Survival c-indices at 5 yr post-SRT for GC, Cancer of the Prostate Risk Assessment Score, and Briganti risk models for prediction of metastasis, and (D) decision curve analysis comparing net benefit at 5 yr post-SRT of GC, Cancer of the Prostate Risk Assessment Score, and Briganti risk models across various threshold probabilities for prediction of metastasis.
ADT = androgen deprivation therapy; CAPRA-S = Cancer of the Prostate Risk Assessment Score; CI = confidence interval; EPE = extraprostatic extension; GC = genomic classifier; pPSA = preoperative prostate specific antigen; SRT = salvage radiation therapy; SVI = seminal vesicle invasion; RT = radiation therapy.

showed GC had a higher net-benefit compared with other examined clinico-pathologic models (Fig. 2D) across a wide range of decision threshold probabilities (~0–25% metastasis risk).

3.4. *Impact of concurrent ADT on GC's predictive ability*

Percentages of patients in low-, intermediate-, and high-risk GC groups who received concurrent ADT were 17.4%, 20.8%, and 22.6%, respectively. A subset analysis to evaluate the effect of concurrent ADT on GC risk group outcomes was performed (Fig. 3D). Removing patients that received concurrent ADT with SRT, who tended to be higher-risk, resulted in an overall decreased metastasis risk, although GC remained strongly predictive of metastases post-SRT with 5-yr cumulative incidences for low, intermediate, and high scores of 1.5%, 10.8%, and 28.8%, respectively ($p = 0.009$).

3.5. *GC can reclassify men assigned to risk groups by CAPRA-S or Briganti*

Figure 1 shows the distributions of CAPRA-S, Briganti, and GC scores. Using CAPRA-S, most patients were categorized as intermediate- (55%) or high-risk (36%) for disease progression (Fig. 1A). As Briganti risk model does not specify risk groups, convenience cut-points were used to approximate

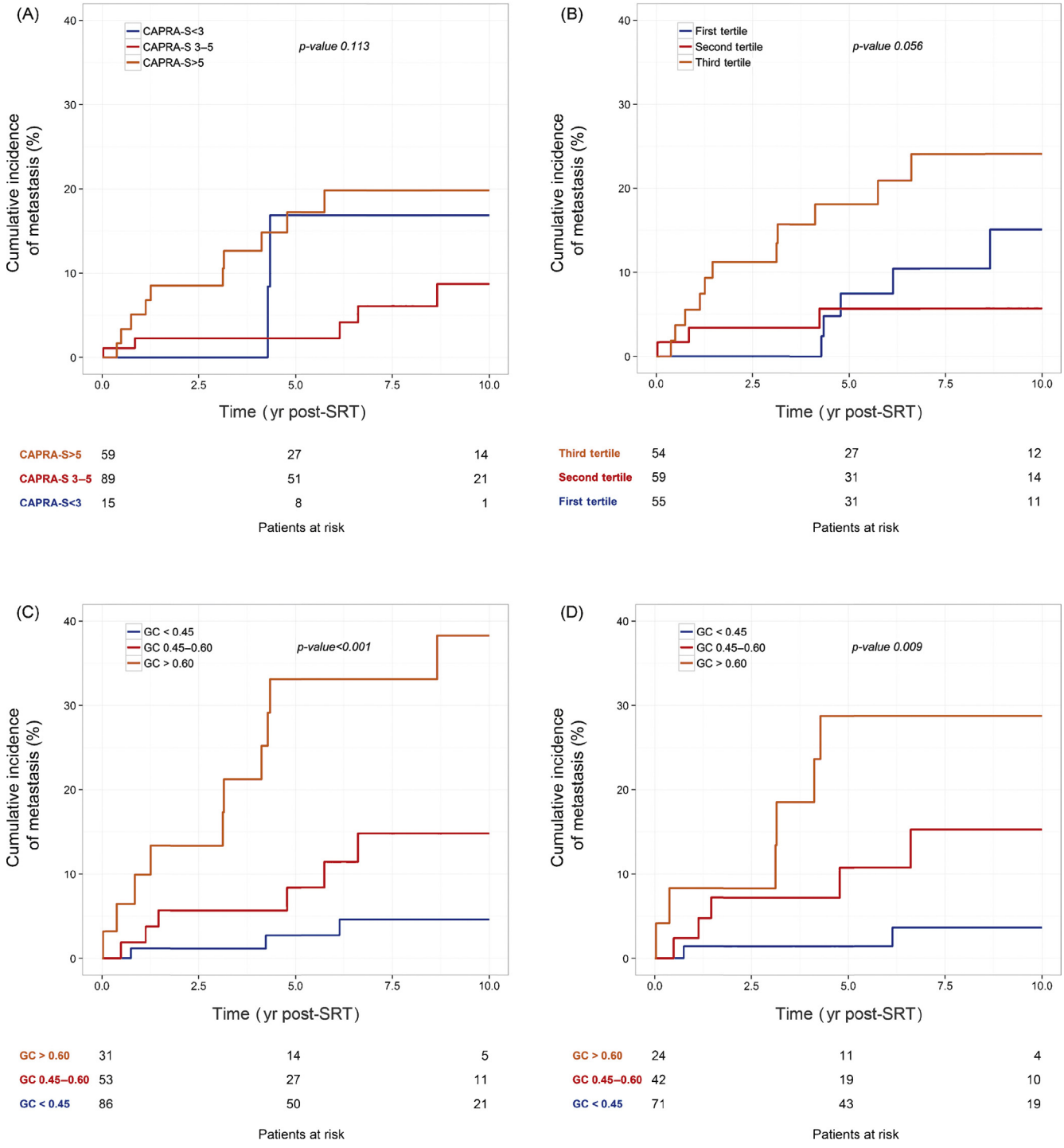

Fig. 3 – Cumulative incidence curves of metastasis in which patients are stratified by (A) Cancer of the Prostate Risk Assessment Score risk categories, (B) tertiles of Briganti risk scores, (C) genomic classifier risk categories, and (D) genomic classifier risk categories after excluding those patients whom received concurrent androgen deprivation therapy.
CAPRA-S = Cancer of the Prostate Risk Assessment Score; GC = genomic classifier; SRT = salvage radiation therapy.

tertiles of risk model-predictions, which were compared with GC tertiles for reclassification analysis. Using Briganti, the distribution of patients grouped approximately by tertiles was 27%, 43%, and 30% for low-, intermediate-, and high-risk respectively (Fig. 1B). Reclassification analysis shows that 31 (39%) patients in the upper two tertiles of risk by Briganti were 'down-graded' to the first tertile by GC and notably 30 (97%) of these patients remained metastasis-free during follow-up (Supplementary Table 2). With regards to CAPRA-S, 73 (49%) patients who were categorized as intermediate- or high-risk were reclassified as GC low-risk of which 70 (96%) remained metastasis-free during follow-up.

3.6. Subset analysis of men receiving early SRT

As Briganti was developed for patients receiving early SRT (pre-SRT PSA $\leq$ 0.5 ng/ml), we performed a secondary analysis to calculate the c-index for this subset (n = 78). Within this subset, only GC had a significant c-index of 0.79 (95% CI 0.72–0.85; Supplementary Fig. 2A) with neither Briganti (c-index 0.44; 95% CI 0.34–0.78) nor CAPRA-S (c-index 0.68; 95% CI 0.46–0.82) performing well. Similar results were obtained in another secondary analysis using PSA $\leq$ 1.0 ng/ml definition for early SRT (Supplementary Fig. 2B).

4. Discussion

Treating patients with biochemical recurrence post-RP is challenging. Several studies found SRT may be a second chance of cure, especially in individuals without distant metastasis [1,24–26]. However, as frequently observed in medicine, a one-size-fits-all strategy is not necessarily optimal. Indeed, while SRT can achieve long-term cancer control in some patients, the response might be poor in others. In this context, the ability to predict long-term cancer control in these individuals is of outmost importance, as it allows for an informed counseling of patients, a more individualized follow-up scheme, and potentially more effective treatment strategies. While some prediction models are available for these patients [1,13], their performance is suboptimal. To address this limitation, we tested the performance characteristics of the previously established and locked GC in predicting metastasis after SRT in men with recurrent disease post-RP. We relied on a multi-institutional, multi-ethnic cohort, of which none were included in the GC development, to maximize generalizability of our findings.

Our study has several important findings. Firstly, on MVA, GC was an independent predictor of metastasis in patients treated with SRT post-RP. Each 0.1-unit increase in GC was associated with a 62% increase in metastasis risk. Indeed, GC separated a group with a 5-yr cumulative metastasis incidence of 2.7% versus 33.1%. This indicates that GC is able to correctly capture and categorize tumor aggressiveness.

Secondly, while the number of events was modest, we explored how GC affected the predictive ability of tools based on clinical tumor characteristics (eg, CAPRA-S and Briganti) and found when GC was added to the multivariable models, these tools were not significantly linked with metastases risk. Moreover, when GC was modeled with individual clinico-pathologic variables, only a minor improvement in discrimination performance was observed versus GC alone. This suggests the examined genomic biomarker signature (GC) explains all the variability captured by routinely available clinical tumor characteristics. Thirdly, GC had the most favorable discrimination accuracy versus CAPRA-S and Briganti risk models, highlighting that this genomic biomarker captures variation in tumor behavior beyond the ability of conventional clinical models.

To put these findings in clinical terms, we tested GC's ability to reclassify men assigned to risk-groups by CAPRA-S and Briganti risk models with the caveat that neither of these clinico-pathologic risk models were initially designed to predict metastasis. Almost 40–50% of patients classified as intermediate- or high-risk by CAPRA-S and/or the Briganti model were reclassified to low-risk by GC. Nearly all of these patients (96–97%) remained metastasis free during follow-up. This implies GC may optimize the prediction in a large proportion of patients that would otherwise be classified incorrectly as intermediate- or high-risk.

Our findings were confirmed, when, in secondary analysis, we limited our inquiry to patients treated with SRT alone (ie, without concurrent ADT), or to patients treated with early SRT. Taken together, these observations imply GC has important clinical implications and incorporating GC in clinical practice can greatly improve our ability to predict outcomes of patients with recurrent PCa undergoing SRT.

Given GC's accuracy to predict metastases risk, the next step is to better understand GC's clinical utility. Despite absence of completed randomized trials comparing adjuvant to salvage radiation, most urologists in both Europe and the United States [27] use SRT as a de facto standard of care. Given the strong prognostication from GC, it appears that patients with high GC should be considered for systemic therapy in addition to SRT. For these men, the recommendation is not to forego SRT, but rather that SRT may not be enough and these patients are good candidates for clinical trials or for additional therapies to combine with SRT including docetaxel, which was recently shown to have a survival benefit as adjuvant treatment for men with high-risk PCa undergoing radiation plus ADT [28] as well as several other settings [29,30].

Our study is not without limitations. Firstly, our results were derived from retrospective observational data and, therefore, the indication and selection of patients to undergo SRT was not standardized nor was the lymph node dissection standardized. This is evidenced by the variable time points at which SRT was received. Secondly, the number of events was modest. Thirdly, not enough follow-up data was available to address more definitive endpoints, such as cancer-specific and overall mortality. Larger sample sizes with longer follow-up are needed. Although most patients were imaged with conventional computed tomography and bone scans, there was a lack of standardization and absence of novel imaging modalities (eg, sodium fluoride positron emission tomography) to detect metastases. Also, imaging intervals were at the discretion of the ordering physician. Finally, validation in larger and more generalized and prospective cohorts is necessary using PCa death as the endpoint.

5. Conclusions

Despite these limitations, our findings are particularly intriguing and provide a unique, more individualized approach to managing men receiving SRT post-RP. Indeed, the GC biomarker provides accurate and comprehensive insight regarding tumor aggressiveness in these individuals. Specifically, this biomarker accurately down-staged almost 50% of patients predicted to harbor very aggressive tumors by clinical features. Most importantly, it accurately identifies a group of men with a 33% 5-yr risk of metastases

despite SRT who may be excellent candidates for inclusion into clinical trials for novel therapies due to their very high-risk of metastases despite local salvage therapy.

Author contributions: Robert B. Den had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Freedland, Buerki, Davicioni, Karnes, Den.
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Analysis and interpretation of data: Freedland, Choeurng, Howard, Yousefi, Buerki, Abdollah, Davicioni, Den.
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Appendix A. Supplementary data

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