

# Development and Validation of a Prostate Cancer Genomic Signature that Predicts Early ADT Treatment Response Following Radical Prostatectomy



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## Abstract

**Purpose:** Currently, no genomic signature exists to distinguish men most likely to progress on adjuvant androgen deprivation therapy (ADT) after radical prostatectomy for high-risk prostate cancer. Here we develop and validate a gene expression signature to predict response to postoperative ADT.

**Experimental Design:** A training set consisting of 284 radical prostatectomy patients was established after 1:1 propensity score matching metastasis between adjuvant-ADT (a-ADT)-treated and no ADT-treated groups. An ADT Response Signature (ADT-RS) was identified from neuroendocrine and AR signaling-related genes. Two independent cohorts were used to form three separate data sets for validation (set I,  $n = 232$ ; set II,  $n = 435$ ; set III,  $n = 612$ ). The primary endpoint of the analysis was postoperative metastasis.

**Results:** Increases in ADT-RS score were associated with a reduction in risk of metastasis only in a-ADT patients. On multivariable analysis, ADT-RS by ADT treatment interaction term

remained associated with metastasis in both validation sets (set I: HR = 0.18,  $P_{\text{interaction}} = 0.009$ ; set II: HR = 0.25,  $P_{\text{interaction}} = 0.019$ ). In a matched validation set III, patients with Low ADT-RS scores had similar 10-year metastasis rates in the a-ADT and no-ADT groups (30.1% vs. 31.0%,  $P = 0.989$ ). Among High ADT-RS patients, 10-year metastasis rates were significantly lower for a-ADT versus no-ADT patients (9.4% vs. 29.2%,  $P = 0.021$ ). The marginal ADT-RS by ADT interaction remained significant in the matched dataset ( $P_{\text{interaction}} = 0.035$ ).

**Conclusions:** Patients with High ADT-RS benefited from a-ADT. In combination with prognostic risk factors, use of ADT-RS may thus allow for identification of ADT-responsive tumors that may benefit most from early androgen blockade after radical prostatectomy. We discovered a gene signature that when present in primary prostate tumors may be useful to predict patients who may respond to early ADT after surgery. *Clin Cancer Res*; 1–9. ©2018 AACR.

## Introduction

Androgen deprivation therapy (ADT) has served as the foundation for advanced prostate cancer management since its discovery by Huggins and Hodges in 1941 (1). In fact, in the United States, approximately half of all prostate cancer patients will receive a course of ADT at some point during treatment (2). In the primary radiotherapy setting, a large body of evidence

supports the radiosensitizing properties of adjuvant ADT and a resultant overall survival benefit (3). Similarly, in men with lymph node-positive disease after radical prostatectomy, adjuvant ADT has demonstrated a survival benefit (4). Furthermore, large retrospective series have also suggested improved cancer specific mortality with adjuvant ADT in the presence of seminal vesicle invasion on surgical pathology (5). Indeed, the adjuvant ADT arm of the SWOG S9921 trial of high-risk prostate cancer

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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## Translational Relevance

Adjuvant ADT after surgery and/or radiation has demonstrated a survival benefit in higher risk prostate cancer (PCa). Despite such benefits, ADT has been associated with side effects and might not benefit every patient. Thus, identification of a subset of patients who are unlikely to receive benefit from ADT is a step in the right direction. Neuroendocrine (NE)PCa has higher rates of ADT resistance and is highly correlated with castration-resistant disease. Stemming from this observation, we reasoned that a score created from gene expression patterns of NEPCa may serve as an early marker of androgen resistance for primary prostate tumors in the localized disease setting. Here, we developed and validated a genomic signature for predicting ADT response after radical prostatectomy using neuroendocrine and ADT resistance genes. This signature allows for earlier identification of ADT-responsive prostate cancers that may be more optimal candidates for multimodal systemic therapy or clinical trials of novel agents.

treated with radical prostatectomy demonstrated a 92% 5-year biochemical recurrence-free survival (6). Furthermore, several institutions have published favorable results with adjuvant ADT after radical prostatectomy for high-risk prostate cancer (7–9). In fact, an analysis of SEER-Medicare data found that 23% of high-risk prostate cancer patients undergoing radical prostatectomy received adjuvant ADT (10). Thus, while the majority of radical prostatectomy patients do not receive adjuvant ADT, there are a significant number of high-risk prostate cancer patients receiving adjuvant ADT in the United States after radical prostatectomy.

Nevertheless, despite its potential benefits, ADT has been associated with cardiovascular, thrombotic, and cognitive side-effects in addition to its detrimental impact on quality of life (11). Thus, it is vital to identify the subset of patients who are likely to receive no benefit from ADT to not only minimize ADT-associated morbidity but also enable early transition to other therapeutic options. Unfortunately, progression during and after adjuvant ADT for clinically localized high-risk disease has been understudied, and investigations have identified only a limited number of clinical predictors in this setting (12).

Herein, we employed multi-institutional radical prostatectomy cohorts to develop and validate the first genomic signature (ADT response signature) predicting progression after adjuvant ADT. The biologic rationale for the ADT-RS stems from the observation that neuroendocrine prostate cancer (NEPCa) has higher rates of ADT resistance than nonneuroendocrine histology and neuroendocrine differentiation is highly correlated with castration-resistant disease (13). We reasoned that a score created from gene expression patterns of NEPCa (including NE differentiation, AR signaling, cell proliferation) may serve as an early marker of androgen resistance for primary prostate tumors in the localized disease setting.

## Materials and Methods

### Study design

Expression profiles for 1,212 patients were retrieved from Decipher GRID database (NCT02609269), from three previously published studies of the Decipher test in men with adverse

pathology and clinical findings after radical prostatectomy (14–16). The training cohort (Mayo Clinic I,  $n = 545$  all Caucasian) is a case–control study. It was stratified into those who received adjuvant androgen deprivation therapy within 1 year of radical prostatectomy and prior to PSA recurrence (a-ADT) and those who did not receive adjuvant ADT or received ADT after PSA recurrence or metastasis (no ADT). During the study period, institutional practice at Mayo Clinic administration of a-ADT was generally intended to be lifelong although it is uncertain whether patients discontinued treatment after a period of ADT as described previously (17). a-ADT and no ADT patients were then matched using propensity scores on pathologic features (preoperative PSA, pathologic Gleason score, extraprostatic extension, seminal vesicle invasion) in a 1-to-1 ratio to define a matched training set of 284 patients that was well-balanced across these variables including adjuvant radiotherapy (Fig. 1; Table 1). Two independent cohorts, with case–cohort design, were used to form three separate datasets for validation. Validation set I consisted of 232 Caucasian men treated with radical prostatectomy at Mayo Clinic (MC) between 2001 and 2006 from a previously reported case–cohort study. The sampling fraction for the MC cohort was 20% (15). Validation set I included high-risk men treated with radical prostatectomy at MC and as per institutional practice during the period of study, adjuvant ADT ( $\pm$  external beam radiotherapy) was commonly administered to men with high Gleason scores and seminal vesicle invasion (SVI) or those harboring lymph node invasion (LNI). Validation set II ( $n = 435$ ) combined all patients from Validation set I with a subset of a second case–cohort designed study of 260 men (21 African Americans, 235 Caucasian, 4 other races) treated with radical prostatectomy between 2001 and 2009 at Johns Hopkins (JHU). The sampling fraction for the JHU cohort was 35% (16). The JHU cohort included intermediate-high risk men treated with radical prostatectomy that per institutional practice during the period of study, received no adjuvant or salvage (i.e., upon PSA rise) therapy prior to metastatic onset ("natural history cohort"). To properly analyze the data using Cox regression for case–cohort studies, the JHU case–cohort study was modified by resampling the subcohort to match the sampling fraction of the MC case-cohort study (18). Using the upweighted Validation Set II ( $n = 1479$ ), Validation set III used 1:1 propensity scores (19) to clinically match a-ADT to no ADT patients, yielding 612 patients for analysis (Fig. 1; Table 1).

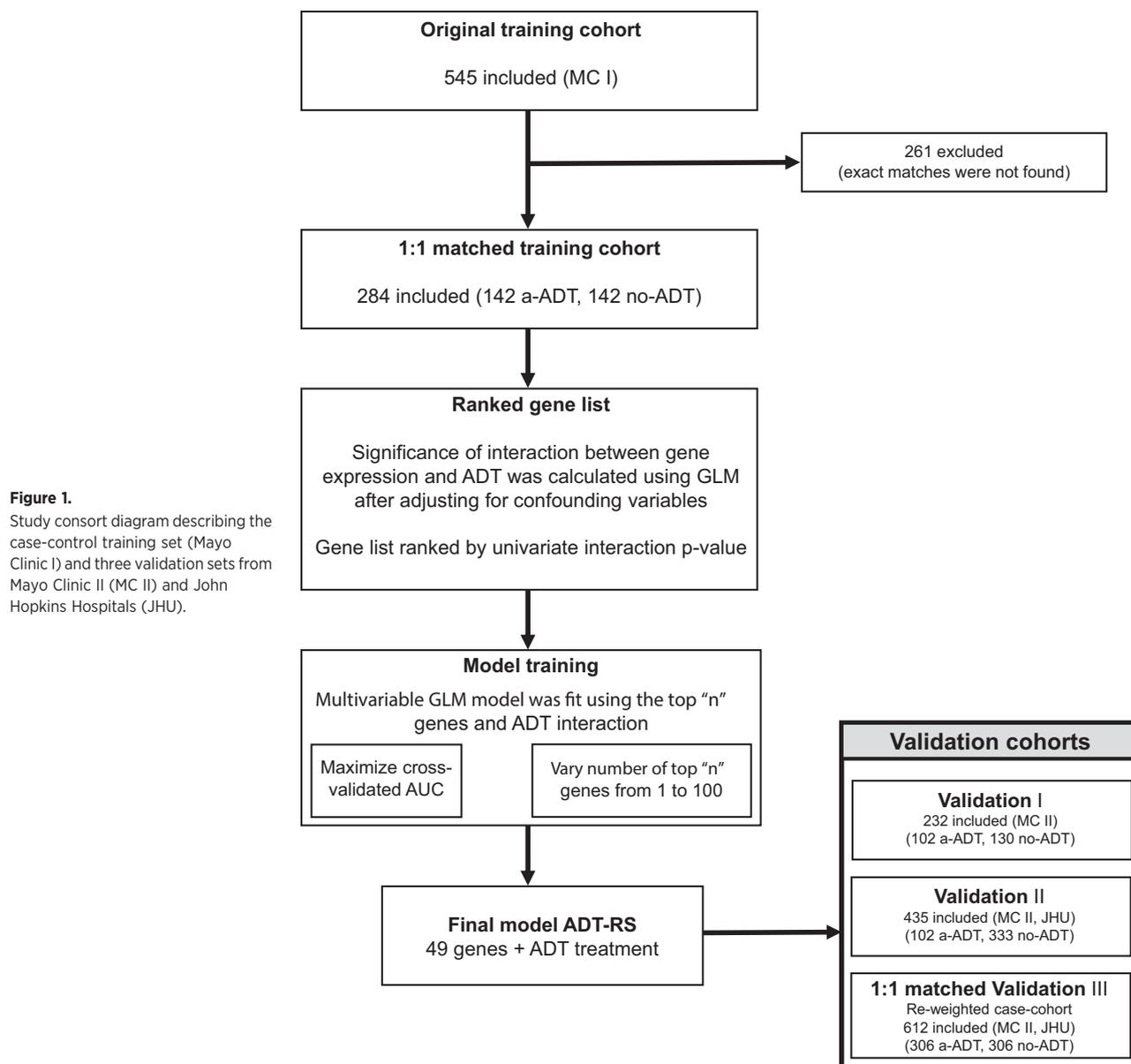
### RNA extraction and data preprocessing

Specimen selection and processing and data normalization has been described previously (16).

### Feature selection and ADT response signature (ADT-RS) development

A systematic literature review identified 1,632 genes extracted from studies investigating the neuroendocrine differentiation, castration resistance models, and resistance to ADT (Supplementary Table S1). This list also included several genes involved in AR signaling and cell proliferation. This list was further filtered using a two-step filtering procedure:

(i) **Feature ranking:** 1,632 features were independently fit using a generalized linear model (GLM) with logit link that incorporated an interaction between ADT treatment status and the expression of each individual feature while adjusting for confounding variables. Then, the features were ranked on the basis of their  $P_{\text{interaction}}$  (univariate feature ranking).



(ii) **Model training:** Using the first " $n$ " number of features from this ranked list (the number of " $n$ " features will be optimized using leave-one-patient-out-cross-validation LOOCV), a GLM was fit using ADT interaction with the " $n$ " features (Multivariable model training).

In our approach, LOOCV was performed on the training set to discover the optimal set of features " $n$ ". Beginning with three features ( $n = 3$ ), the highest ranked three features were used to train the model and calculate the cross-validated AUC. This process was repeated with increasing number of features to train and calculate the cross-validated AUC. In each iteration of LOOCV, the trained model was a logit model for which the optimal parameter " $\lambda$ " was identified using the "`cv.glmnet`" function with 10-fold cross-validation, elastic net mixing parameter set to one, and mean squared error as the optimization metric. This optimization procedure was truncated at 100 features because there was no improvement in the cross-validated AUC.

The set of genes that produced the highest cross-validated AUC among ADT-treated patients was used in training the final and locked model. The ADT-RS score for a given patient is calculated by taking the difference between risk given ADT treatment and risk given no ADT-treatment and has values that range from  $-1$  and  $1$  (20). ADT-RS scores were scaled so that higher scores (closer to  $1$ ) were associated with benefit from adjuvant ADT.

#### Statistical analysis

The primary endpoint of the analysis was metastatic onset following surgery (i.e., positive bone and/or CT scans). Event times were defined as time from radical prostatectomy to metastases or date of last follow up. Prognostic performance of genomic risk models was assessed using the survival  $c$ -index (21) with bootstrapped 95% confidence intervals. For purposes of comparisons with established prognostic models, ADT-RS scores were multiplied by a factor of  $-1$  so that higher scores reflect a

**Table 1.** Demographic and clinical characteristics of eligible patients

Variables	Training set	Validation set I	Validation set II	Validation set III
Patients, <i>n</i> (%)	284 (100%)	232 (100%)	435 (100%)	612 (100%)
Patient age, y				
Median (range)	67 (48–78)	64 (46–78)	62 (38–78)	62 (46–78)
IQR (Q1–Q3)	62–70	58–69	57–66	58–67
Preoperative PSA (ng/mL)				
Median (range)	14.2 (1.2–201)	9.4 (1.8–194)	9.4 (1.8–194)	9.5 (1.8–194)
IQR (Q1–Q3)	7.5–28.0	6.2–15.9	6.2–15.1	5.9–15.8
Pathologic Gleason score, <i>n</i> (%)				
≤6	8 (3%)	18 (8%)	19 (4%)	29 (5%)
7 (3+4)	NA <sup>a</sup>	76 (33%)	141 (32%)	213 (35%)
7 (4+3)	NA <sup>a</sup>	41 (18%)	79 (18%)	93 (15%)
8	57 (20%)	39 (17%)	63 (14%)	76 (12%)
≥9	115 (40%)	58 (25%)	133 (31%)	201 (33%)
Extraprostatic extension, <i>n</i> (%)	208 (73%)	99 (43%)	251 (58%)	284 (46%)
SVI, <i>n</i> (%)	153 (54%)	80 (80%)	151 (35%)	367 (60%)
Positive surgical margins, <i>n</i> (%)	172 (61%)	133 (57%)	192 (44%)	371 (61%)
LNI, <i>n</i> (%)	72 (25%)	33 (14%)	84 (19%)	183 (30%)
Adjuvant radiotherapy, <i>n</i> (%)	43 (15%)	49 (21%)	49 (11%)	101 (17%)
Androgen deprivation therapy, <i>n</i> (%)	142 (50%)	102 (44%)	102 (23%)	306 (50%)
Follow-up time for censored patients, y				
Median (range)	15.2 (5.9–21.9)	7.0 (0.0–11.6)	7.9 (0.0–19.0)	7.4 (0.2–18.0)
IQR (Q1–Q3)	13.0–18.2	4.9–9.1	5.2–10.1	5.2–9.4

<sup>a</sup>Primary Gleason grade unavailable for Training set. A total of 104 patients (37%) had a Gleason score of 7.

higher risk of metastasis. Validation of the response-predictive capability of the model was conducted using univariable and multivariable Cox regression for case-cohort designs (18) to estimate the conditional effect of biomarker by treatment interaction (22). Clinicopathologic variables that were adjusted for in the multivariable analysis (MVA) included treating institution, preoperative PSA (log<sub>2</sub> transformed), pathologic Gleason score (≥4+3 vs. ≤3+4), extraprostatic extension (EPE), SVI, margin status (SM), LNI, and adjuvant radiotherapy (ART) defined as radiotherapy within 12 months of radical prostatectomy. In addition, a matched analysis interrogating the marginal interaction effect (22) was performed for comparison with the unmatched MVA. a-ADT were matched to no ADT patients in a one-to-one ratio, with replacement, on clinicopathologic variables using the MatchIt package in R. Cumulative incidence curves for metastasis were generated for a-ADT and no ADT groups within subsets of patients with low and high predictive model scores using Fine-Gray competing risks analysis (23). Statistical inference was conducted using a weighted version of the log-rank test (24). A Cox regression model was fit to the matched Validation set III where the data were weighted by the number of times a patient was selected and a robust variance (25) calculated for statistical inference to account for the reweighting process. Decipher, mCCP (26) (the microarray version of the cell-cycle progression signature created by taking the mean of the 31 CCP genes), and the ADT-RS were each categorized objectively based on their median scores. Statistical analyses were performed in R v3.2.2, and all tests were performed at the 5% significance level.

## Results

### Development of ADT-RS

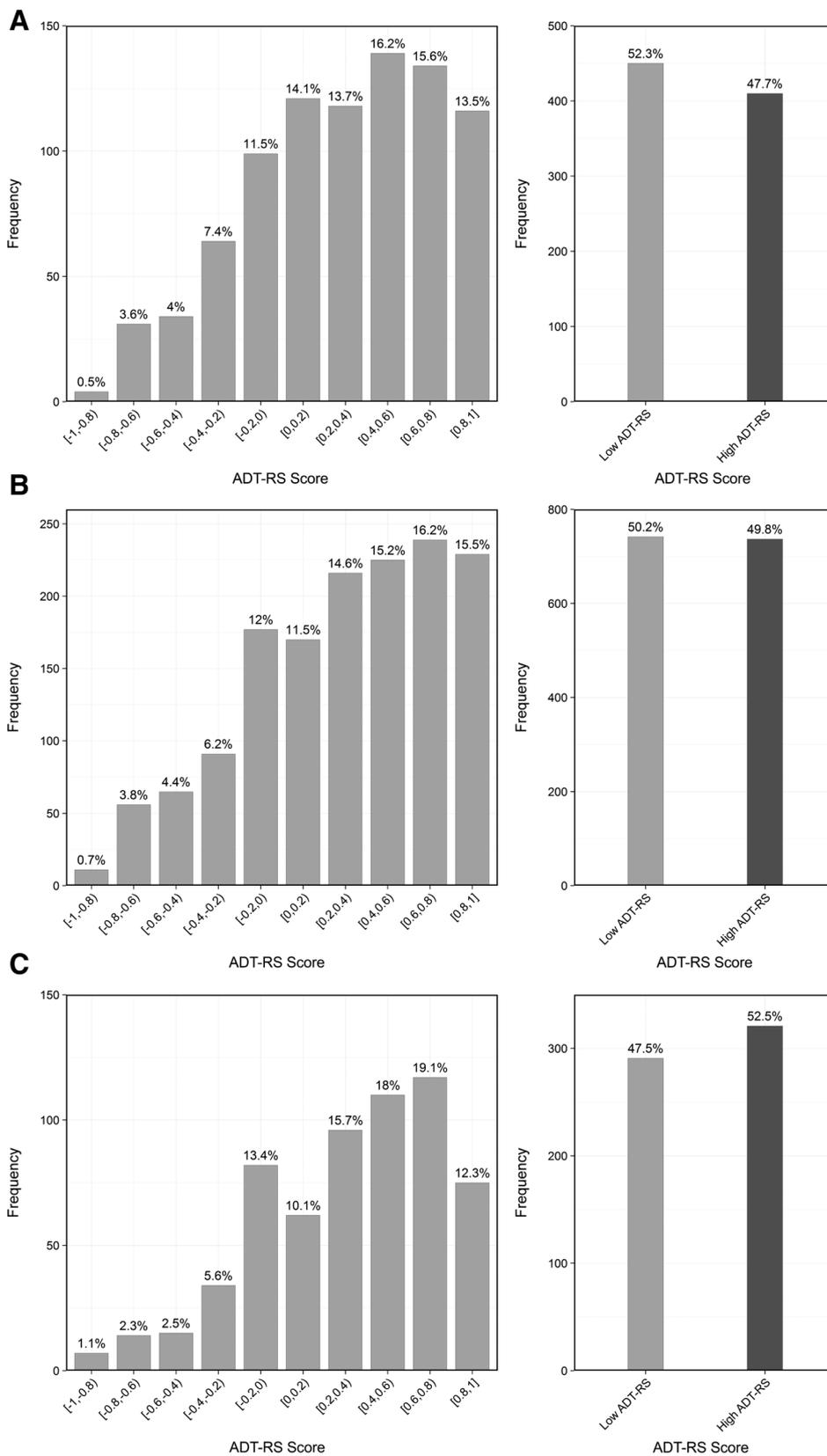
A radical prostatectomy cohort of 545 men with long-term follow up (median 16.9 years) and available genome-wide expression profiles was used to derive a matched set of a-ADT and no ADT patients (*n* = 284), which was used to train the ADT-RS

model. Feature selection was conducted on a curated list of 1,632 genes related to NEPCa, cell proliferation, AR signaling, and castration resistance from a literature review (Supplementary Table S1). Genes (*n* = 84) with a significant interaction with ADT treatment were used to train the model using a generalized linear model (Fig. 1). Only 49 genes had a nonzero coefficient (Supplementary Table S2) and were contributing to the final score. The ADT-RS model has score values ranging from -1 to 1 where 1 is high response to ADT upon treatment (lower likelihood of metastasis post-ADT) and -1 is lower response (higher likelihood of metastasis post-ADT). We used the median score of 0.36 as a cut-off point to categorize patients into either low ADT-RS or high ADT-RS. The cross-validated AUC among a-ADT patients in the training cohort was 0.84.

On the basis of analyzing the biological function of the genes' interactome, we found the ADT-RS genes to be related to key signaling pathways including NOTCH, WNT β-catenin, TNF-α, insulin, and chemokine signaling (Supplementary Fig. S1). In particular, the *REST* and *EZH2* genes were highly connected nodes in the functional network. *SOX2* and *NANOG* were identified as key transcription factors in enrichment analysis (*P* = 0.01). Biological process over-representation analysis found cell-cycle activity, DNA repair, chromatin modification, and immune response as the key gene ontologies in ADT-RS.

### Clinical characteristics of validation sets

With a median follow up of 7 years among censored patients, 50% and 18% of men from Validation set I developed metastasis in the a-ADT (*n* = 102) and no-ADT (*n* = 130) groups, respectively. Fifty-eight percent of patients had a Gleason score ≥ 4+3 and 14% had lymph node-positive disease (Table 1). In Validation set II, 102 patients were in the a-ADT group and 64% of patients had a Gleason score ≥ 4+3 and 19% lymph node-positive disease (Table 1). A total of 174 patients developed metastasis during study follow up with a median 7.9 years of follow up for censored patients. In the validation cohorts, among the treated and metastasized patients, four patients received



**Figure 2.** Frequency of patients across ADT-RS scores and category (low vs. high) within validation set I (A), validation set II (B), and 1:1 matched validation set III (C).

**Table 2.** Results of Cox proportional hazards analysis of ADT-RS and clinicopathologic risk factors

	Variables	UVA		MVA	
		HR (95% CI)	P	HR (95% CI)	P
Validation set I (Mayo Clinic MC II)	Log <sub>2</sub> pretreatment PSA	1.16 (0.91-1.48)	0.227	1.19 (0.91-1.57)	0.203
	Pathologic Gleason $\geq$ 4+3	4.33 (2.27-8.25)	<0.001	3.57 (1.72-7.40)	<b>0.001</b>
	Extraprostatic extension	2.88 (1.66-5.00)	<0.001	1.10 (0.53-2.28)	0.789
	Seminal vesicle invasion	2.39 (1.39-4.12)	<b>0.002</b>	1.68 (0.84-3.34)	0.143
	Positive surgical margins	1.05 (0.61-1.79)	0.868	1.47 (0.75-2.90)	0.260
	Lymph node invasion	2.06 (1.02-4.14)	<b>0.043</b>	0.57 (0.23-1.43)	0.231
	Adjuvant radiation therapy	1.83 (1.00-3.38)	0.052	0.98 (0.44-2.21)	0.966
	ADT-RS	0.91 (0.35-2.34)	0.841	0.91 (0.34-2.39)	0.842
	ADT	5.60 (2.94-10.65)	<0.001	3.93 (1.79-8.62)	<b>0.001</b>
	ADT-RS:ADT interaction	0.20 (0.06-0.71)	<b>0.013</b>	0.18 (0.05-0.65)	<b>0.009</b>
Validation set II (Mayo Clinic MC II + Hopkins JHU)	Institution (ref: Hopkins)	0.67 (0.46-0.96)	<b>0.031</b>	0.42 (0.23-0.77)	<b>0.005</b>
	Log <sub>2</sub> pretreatment PSA	1.17 (0.97-1.41)	0.097	1.14 (0.92-1.40)	0.240
	Pathologic Gleason $\geq$ 4+3	4.91 (3.11-7.76)	<0.001	3.81 (2.32-6.24)	<0.001
	Extraprostatic extension	3.27 (2.16-4.93)	<0.001	1.27 (0.75-2.13)	0.370
	SVI	3.53 (2.41-5.18)	<0.001	2.57 (1.59-4.16)	<0.001
	Positive surgical margins	1.26 (0.87-1.82)	0.220	1.86 (1.17-2.97)	<b>0.008</b>
	LNI	3.71 (2.38-5.80)	<0.001	1.77 (1.01-3.08)	<b>0.045</b>
	Adjuvant radiotherapy	1.35 (0.77-2.38)	0.290	0.86 (0.34-2.15)	0.746
	ADT-RS	0.52 (0.33-0.81)	<b>0.004</b>	0.67 (0.41-1.09)	0.109
	ADT	2.32 (1.45-3.72)	<0.001	1.78 (0.86-3.69)	0.121
ADT-RS:ADT interaction	0.34 (0.13-0.90)	<b>0.031</b>	0.25 (0.08-0.80)	<b>0.019</b>	

NOTE: Boldface indicates statistical significance.

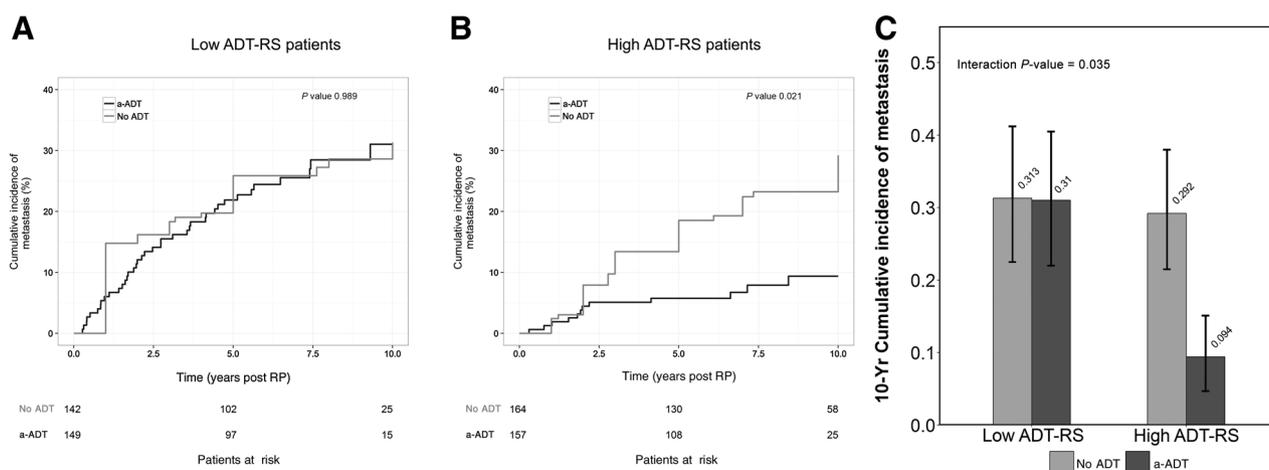
Abbreviations: ADT, androgen deprivation therapy; MVA, multivariable analysis; UVA, univariable analysis.

adjuvant ADT treatment within a month of clinical metastasis as evidenced by imaging and one patient received ADT within 4 months of clinical metastasis. In matched Validation set III, clinicopathologic variables including Gleason score ( $P = 0.18$ ) were balanced, but not the administration of adjuvant radiotherapy, which remained substantially different across treatment arms ( $P < 0.001$ ; Supplementary Table S3).

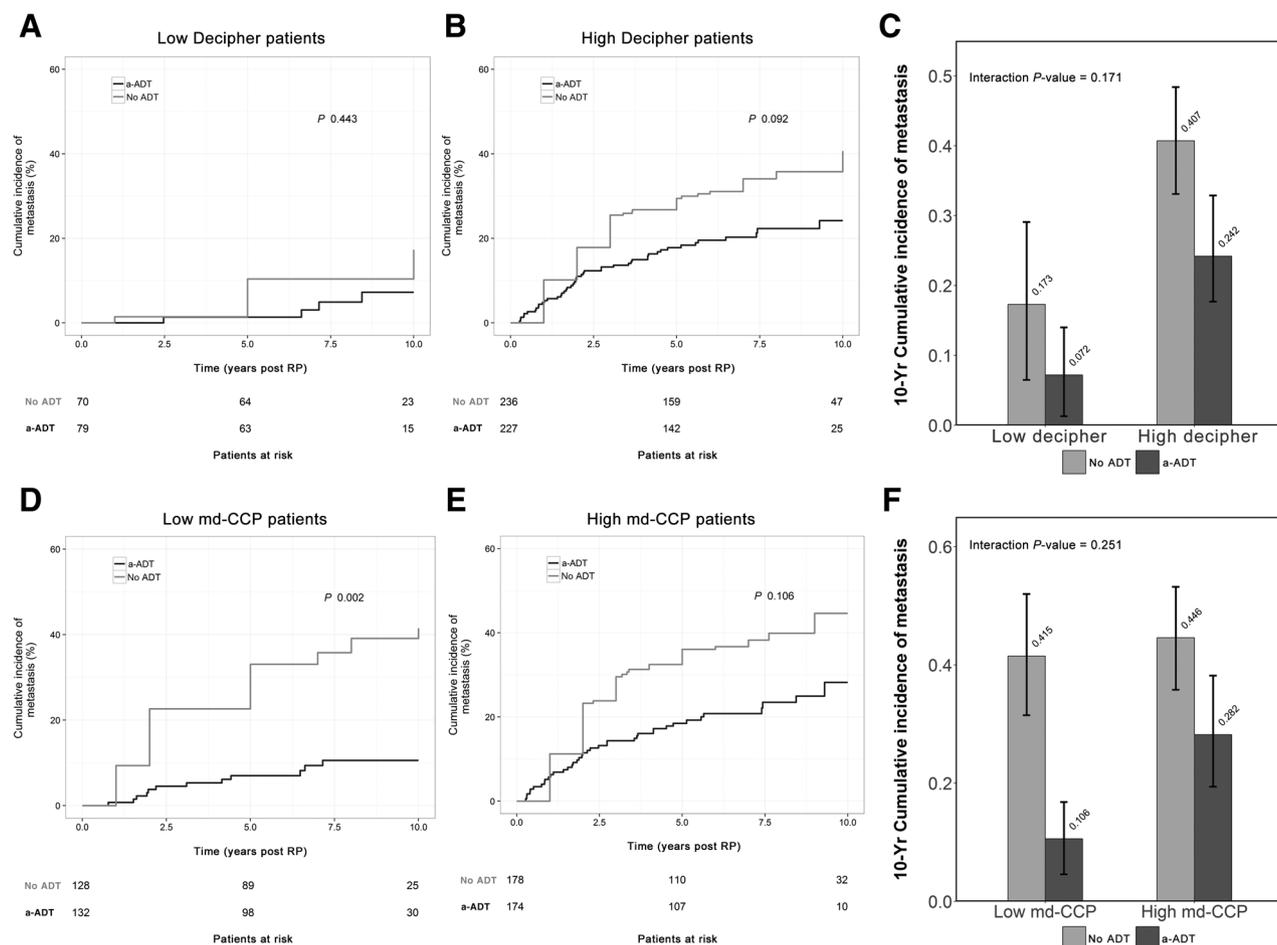
### Analysis of ADT-RS in validation sets I and II

In the validation sets, ADT-RS scores ranged from  $-0.92$  to  $0.97$  (Fig. 2). ADT-RS had survival c-indices at 5 years postsurgery of  $0.63$  in both Validation sets I (95% CI:  $0.54-0.70$ ; Supplementary Table S4) and II (95% CI:  $0.58-0.67$ ), suggesting that indepen-

dent of its interaction with ADT the ADT-RS score itself had a weak prognostic capability. However, the conditional interaction effect between ADT-RS and ADT treatment was significant in multivariable Cox regression for both Validation sets I (HR =  $0.18$ ;  $P_{\text{interaction}} = 0.009$ ) and II (HR =  $0.25$ ;  $P_{\text{interaction}} = 0.019$ ; Table 2). In other words, patients with higher ADT-RS score had lower metastasis rates with adjuvant ADT as compared with men with lower ADT-RS who also received this treatment. The only other significant variable in the multivariable model for Validation set I was pathologic Gleason score (HR =  $3.57$ ;  $P = 0.001$ ). In Validation set II, pathologic Gleason, seminal vesicle invasion, positive margins, and lymph node positivity were also significantly associated with risk of metastasis.

**Figure 3.**

Cumulative incidence of metastasis given ADT and ADT-RS scores. Cumulative incidence curves showing that among patients with low ADT-RS scores (A), incidence of metastasis is not significantly different between treatment arms, and among patients with High ADT-RS scores, a-ADT patients are at significantly reduced risk of metastasis compared with no-ADT patients (B). C, Bar plots illustrating the 10-year cumulative incidence of metastasis rates for each ADT-RS by ADT treatment combination.

**Figure 4.**

Cumulative incidence of metastasis given ADT and prognostic signatures in the matched validation set III. Cumulative incidence curves comparing a-ADT and no-ADT patients for patients with low (**A**) and high Decipher, showing that patients in the a-ADT arm have lower incidence of metastasis in high but not low Decipher (**B**). **C**, Bar plots illustrating the 10-year cumulative incidence of metastasis rates for low and high Decipher (split by median score) by ADT treatment combination. Cumulative incidence curves comparing a-ADT and no-ADT patients for patients with low cell-cycle genes score (**D**) and high cell-cycle genes score (**E**), showing that patients in the a-ADT arm have lower incidence of metastasis in both low and high cell-cycle genes. **F**, Bar plots illustrating the 10-year cumulative incidence of metastasis rates for low and high cell-cycle genes (split by median score) by ADT treatment combination.

#### Analysis of ADT-RS in the propensity-matched Validation set III

ADT-RS scores among the matched validation cohort were dichotomized using the median score of 0.36 obtained from Validation set II as a cut-off point to categorize patients into either Low ADT-RS or High ADT-RS. In the matched Validation set III, patients with low ADT-RS scores had similar incidence rates of metastasis in both treated (31%) and untreated arms (31.3%; Fig. 3A and B). In contrast, as judged by their metastatic outcomes among men with high ADT-RS scores, a-ADT patients had a significant benefit with treatment compared with patients with no treatment where the incidence rates were 9.4% and 29.2%, respectively ( $P = 0.02$ ; Fig. 3C). The marginal ADT-RS by ADT interaction effect was statistically significant with a  $P$  value of 0.035 (Supplementary Table S5) and consistent with the case-cohort validation sets suggesting ADT-RS is predictive of treatment-response from ADT.

While the ADT-RS, Decipher, and cell-cycle genes genomic risk models each exhibited varying but significant prognostic signal in discriminating metastatic risk across all three validation sets

(Supplementary Table S4), Decipher and cell-cycle genes did not predict response to ADT (Fig. 4). Indeed, the marginal interaction effects for both risk models were not significant (Decipher: HR = 1.61,  $P = 0.171$ ; cell-cycle genes: HR = 1.40,  $P = 0.251$ ; Supplementary Table S5), illustrating what appear to be purely prognostic signatures compared with the one that is both prognostic and predictive in ADT-RS.

## Discussion

In this study, we leveraged historically different institutional practices related to the timing of postoperative therapy between Mayo Clinic and Johns Hopkins to develop an 84-gene ADT-response signature. We then validated it in radical prostatectomy cohorts from tertiary referral centers using multivariable Cox regression analysis. At MC, a-ADT was commonly administered to men with high-risk disease (e.g., SVI with high Gleason scores or LNI), whereas at JHU no therapy of any kind was administered prior to metastatic onset ("natural

history cohort"). We observed that the HRs for metastasis of the ADT-RS -ADT interaction term were 0.18 ( $P_{\text{interaction}} = 0.009$ ) and 0.25 ( $P_{\text{interaction}} = 0.019$ ) in the two validation sets, respectively. In the matched Validation set III, ADT-RS was associated with reduced 10-year metastasis in the a-ADT arm, but not in the no-ADT arm, supporting ADT-RS score is predictive of progression with adjuvant ADT. Taken together, these findings support that in localized prostate cancer, an ADT-resistant phenotype (Low ADT-RS) and an ADT-sensitive phenotype (High ADT-RS) can be detected from gene expression analysis of radical prostatectomy tissue.

To gain functional insights on the 84 genes, we extracted first-degree gene partners of ADT-RS from human protein networks (27, 28) and conducted transcription factor enrichment analysis using EnrichR online tool. The 84 genes retained in ADT-RS encompassed several biologically relevant pathways that may explain the androgen-resistant phenotype (Supplementary Fig. S1). For instance, *REST* has been associated with castration resistance and neuroendocrine differentiation due to aberrant splicing patterns (29, 30). Similarly, *EZH2* was found to be the transcriptional mechanism for N-Myc associated neuroendocrine differentiation (31). An earlier study showed that *SOX2* is an AR-regulated genes that promotes castration resistance (32). Recent investigation by Mu and colleagues (33) found that inhibiting *SOX2* could reverse neuroendocrine differentiation, and 8 genes in the ADT-RS were regulated by *SOX2*. Thus, many of the genes in the ADT-RS have strong biologic mechanisms to support their relevance to androgen resistance.

It is critical to distinguish a "predictive" score from a "prognostic" score at this juncture. The importance of this distinction goes beyond nomenclature, as a predictive score for ADT response has significant potential to influence clinical practice. Current guidelines recommend adjuvant ADT after radical prostatectomy only if there is lymph node involvement (34). However, there are large retrospective (5) and prospective (6) series suggesting a benefit to adjuvant ADT in the high-risk pN0 setting. ADT-RS could be used to identify these patients who are most likely to benefit from adjuvant ADT, thereby potentially changing the current paradigm of adjuvant ADT. Furthermore, for patients who are not likely to benefit from ADT, ADT-RS may enable triage of these patients to trials of other therapeutic modalities while preventing the side effects of unnecessary ADT. This holds the potential to change the paradigm of adjuvant ADT delivery after radical prostatectomy for high risk prostate cancer. Recently Zhao and colleagues, reported on the application of the PAM50 breast cancer classifier in prostate cancer and found that while the Luminal B subtype had the worst prognosis (compared with Luminal A and Basal) they had better survival with adjuvant hormones. We have analyzed ADT-RS scores in the context of PAM50 subtypes for 5,239 radical prostatectomy patients and found that Luminal B subtype is enriched with high ADT-RS scores ( $P = 0.0001$ ; Supplementary Fig. S2). While, the genes used for ADT-RS scores and PAM50 do not overlap, these results suggest that the ADT-RS model scores and PAM50 subtypes are picking up similar tumor biology with clinical implications for use of ADT. Ongoing studies led by investigators from the NRG Oncology cooperative group will look at the relationship between ADT-RS scores and PAM50 subtypes in patients treated with first-line radiotherapy and hormones in patients from completed randomized controlled trials.

Our study did have some noteworthy limitations. First, the study was conducted on retrospective cohorts of mostly Caucasians and any marginal estimates obtained in this study may therefore be biased. Further characterization of ADT-RS in the African Americans is needed. Care was taken in using the appropriate statistical methods to account for issues introduced through reweighting and replication of retrospective observational data, but there is no substitute for level 1 evidence. Second, given the retrospective nature of this study it is uncertain whether patients discontinued treatment after a period of ADT. Validation in cohorts from randomized clinical trials obtained either retrospectively or prospectively will be important to validate this predictive gene signature. Moreover, the ADT-RS cut-off point of 0.36 was originally obtained without regard to ADT response, but objectively derived by median split so that bias may be reduced in the comparison of predictive performance with that of Decipher and mCCP. This cut-off point will require further validation as a clinically meaningful cut-off point useful for selection of a-ADT. As additional transcriptomic data from larger datasets becomes available, it is possible that more specific signatures can be developed using the same methodology. We acknowledge that the ADT-RS gene signature derived from neuroendocrine genes does not encompass the complete spectrum of ADT resistance mechanisms and there is potential for improvement with future investigations and additional transcriptomic data. Currently, ongoing studies looking at ADT as first-line therapy will be used to improve the current ADT-RS model. Finally, our findings also do not directly apply to the metastatic prostate cancer or primary radiotherapy setting; however, these are areas of active pursuit.

An ADT response signature validated as a predictive biomarker (as opposed to prognostic) with a significant interaction term for predicting metastasis. Further validation studies on random clinical trials are required to define the role of ADT-RS in predicting benefit or response to early hormone therapy in men with high-risk prostate cancer after surgery. ADT-RS may allow for identification of patients that may be optimal candidates for chemotherapy or trials of novel systemic agents.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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# Clinical Cancer Research

## Development and Validation of a Prostate Cancer Genomic Signature that Predicts Early ADT Treatment Response Following Radical Prostatectomy

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