Supplementary Online Content

Zhao SG, Chang SL, Erho N, et al. Associations of luminal and basal subtyping of prostate cancer with prognosis and response to androgen deprivation therapy. *JAMA Oncol.* Published online May 11, 2017. doi:10.1001/jamaoncol.2017.0751

This supplementary material has been provided by the authors to give readers additional information about their work.

© 2017 American Medical Association. All rights reserved.

eAppendix. Methods

Matched cohort design and predicting response to ADT

To investigate if subtype could predict ADT response, a matched cohort with 2:1 matching for ADT untreated and treated patients was created from the MCI and MCII cohorts in order to select patients from a single institution with a mix of post-operatively treated and untreated patients. This resulted in a cohort of 315 patients, 210 of which did not receive any ADT, and 105 which received ADT treatment. The decision to perform 2:1 matching was to maximize sample size using patients only from the MC cohorts. We chose to only include patients from the MC cohorts for this analysis because patients in these cohorts received a mix of adjuvant and salvage ADT and RT, allowing us to account for the effects of both in our models. JHMI patients did not receive any post-operative treatment. CCF patients did not receive adjuvant treatment, and information about salvage ADT treatment was unavailable in the dataset. All TJU and DVA patients were treated with radiation. We defined androgen deprivation therapy (ADT) as treatment (with LHRH agonist alone or in combination with androgen receptor antagonists) after radical prostatectomy but before the primary endpoint of metastasis. Matching was performed based on Gleason, prostate specific antigen (PSA, ng/mL), positive surgical margins (SM), extracapsular extension (ECE), seminal vesicle invasion (SVI), lymph node invasion (LNI), as well as post-operative radiation therapy (RT). Data on the duration and dose of ADT were not available. Supplementary Table S6 provides details of which patients in this matched cohort received adjuvant, salvage, or both ADT and/or RT. Nearly all lymph node positive patients from the MC cohorts received ADT, as well as some who received ADT for other reasons at the treating physicians' discretion.

Microarray data accession

Microarray data is available on Gene Expression Omnibus with accession numbers GSE46691, GSE62116, GSE72291, GSE62667, GSE79956, GSE79957, and GSE79915. Additional data is also freely available for academic research purposes through a material transfer agreement with GenomeDx Biosciences.

Statistical analysis

In the demographics tables, ANOVA and Chi-squared test were used to evaluate differences between continuous and categorical variables, respectively, between patient groups. Kaplan-Meier curves were generated by pooling clinical data from all available microarray cohorts. Gleason score was stratified into low (<7), intermediate (7), and high risk (8-10). PSA was stratified into low (<10 ng/mL), intermediate (10-20 ng/mL), and high risk (>20 ng/mL) in a similar manner. SM, ECE, SVI, and LNI were considered binary variables and defined by the respective institutions. Cox regression was used for both univariable and multivariable analysis (UVA/MVA). Stratification by cohort was used when performing UVA/MVA analyses to account for baseline differences between cohorts[1]. The interaction term for treatment and subtype in a Cox model was used to evaluate prediction of treatment response, and a significant interaction Wald test p-value indicated that a subtype could predict response to ADT[2-4]. Statistical significance was set as a two-tailed p-value <0.05. All statistical analyses were performed in R 3.1.2.

Gene set enrichment analysis

Functional and biological analyses of the PAM50 subtypes in prostate cancer were investigated using Gene Set Enrichment Analysis (GSEA). First, a T-test was performed on every gene comparing expression in the specified subtype vs. not in that subtype. The T-statistic was used to generate a pre-ranked list which was input into GSEA.

eReferences

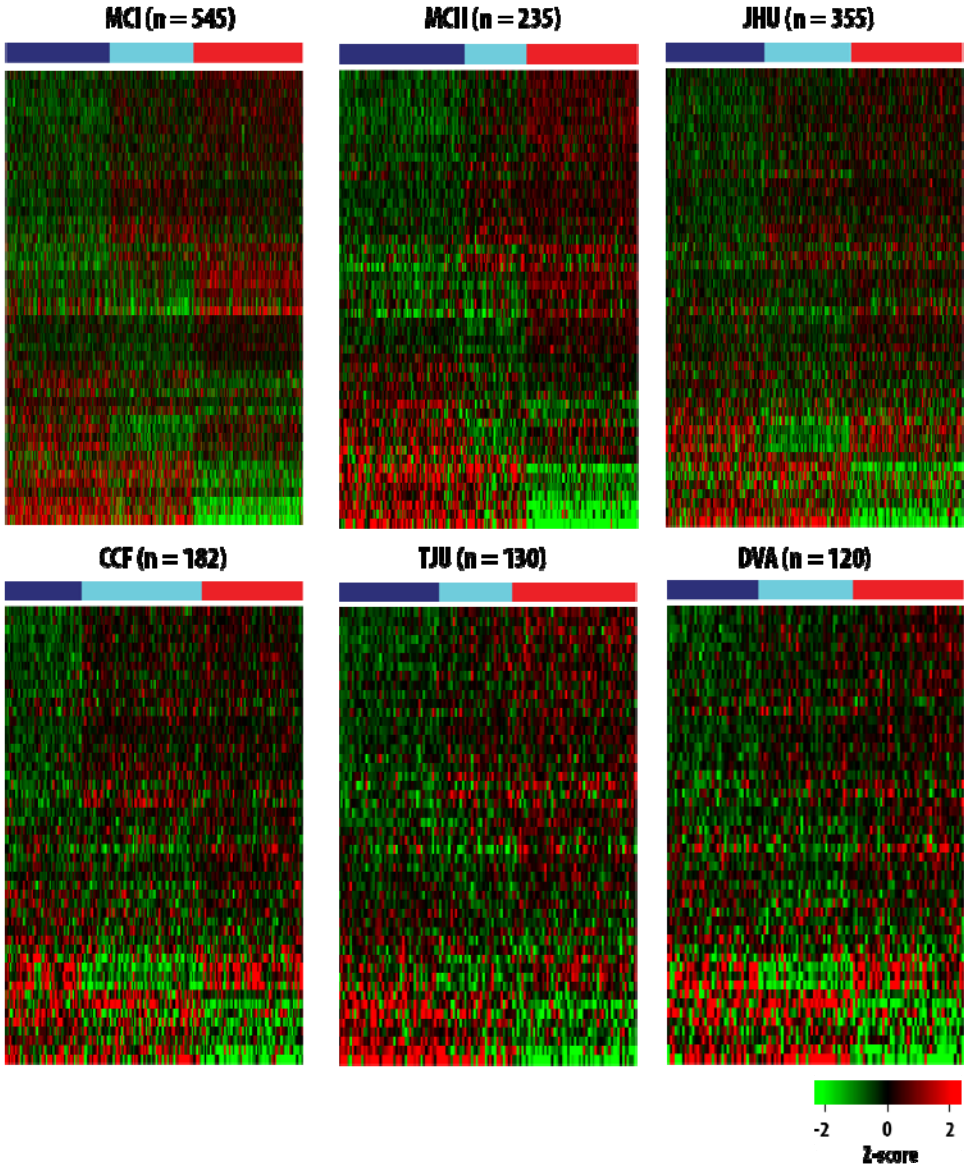
1. Prensner JR, Zhao S, Erho N, et al. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. *Lancet Oncol.* 2014;15(13):1469-1480.
2. Roepman P, Schlicker A, Tabernero J, et al. Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. *Int J Cancer.* 2014;134(3):552-562.

© 2017 American Medical Association. All rights reserved.

3. Yamoah K, Johnson MH, Choeurng V, et al. Novel Biomarker Signature That May Predict Aggressive Disease in African American Men With Prostate Cancer. *J Clin Oncol.* 2015;33(25):2789-2796.
4. Zhao SG, Chang SL, Spratt DE, et al. Development and validation of a 24-gene predictor of response to postoperative radiotherapy in prostate cancer: a matched, retrospective analysis. *Lancet Oncol.* 2016.

© 2017 American Medical Association. All rights reserved.

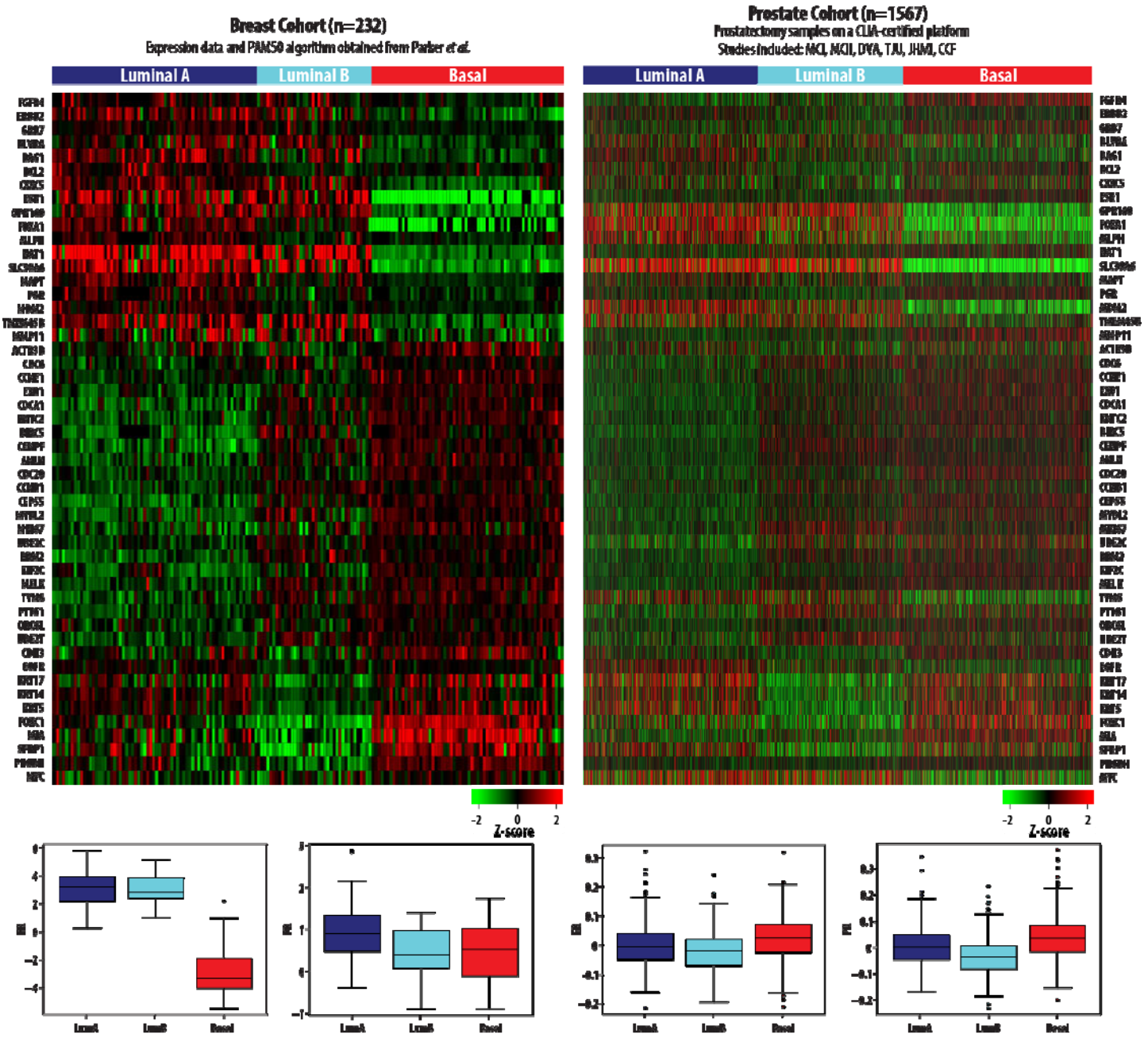
eFigure 1. PAM50 in Retrospective Prostate Cohorts



Heatmaps depicting the PAM50 subtypes (each column represents a sample, each row represents a gene, dark blue = Luminal A, light blue = Luminal B, red = Basal) with genes in the same order as shown in Figure 1.

© 2017 American Medical Association. All rights reserved.

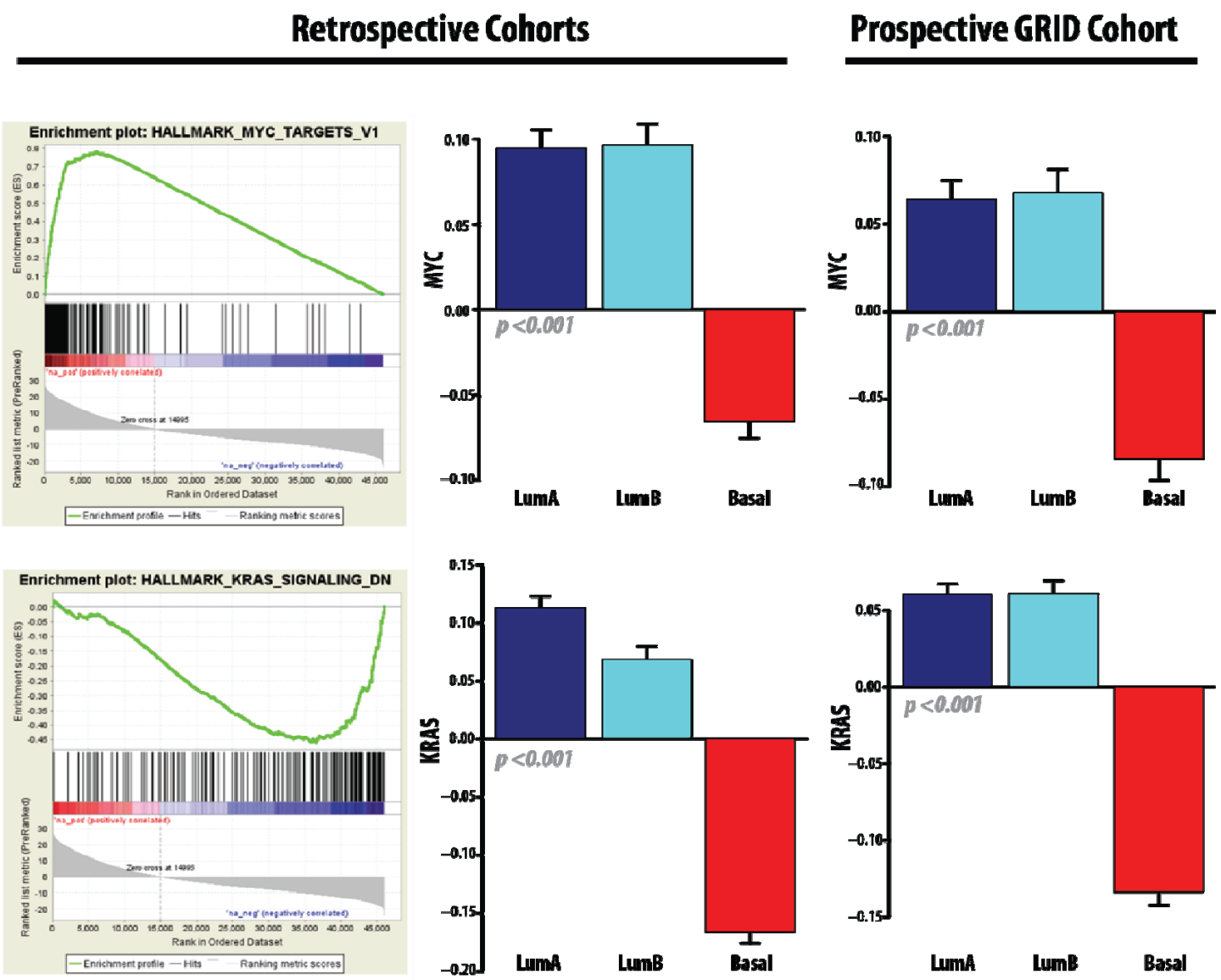
eFigure 2. PAM50 in Breast vs Prostate Cancer



On the left, a heatmap of the PAM50 clusters in the breast cohort from Parker et al. are shown for basal, luminal A, and luminal B. Below are boxplots demonstrating that in breast cancer, ER is higher in luminal versus basal, and that PR is highest in luminal A. On the right, we show a heatmap of the PAM50 clusters in prostate cancer (MCI, II, CC, TJU, JHU, DVA) with the same order of genes as displayed for the breast cancer data, which demonstrates a similar pattern of expression. Below are boxplots showing that ER does not demonstrate the same differences between luminal and basal as in breast, but PR does show lower expression in luminal B compared to luminal A as in breast cancer.

© 2017 American Medical Association. All rights reserved.

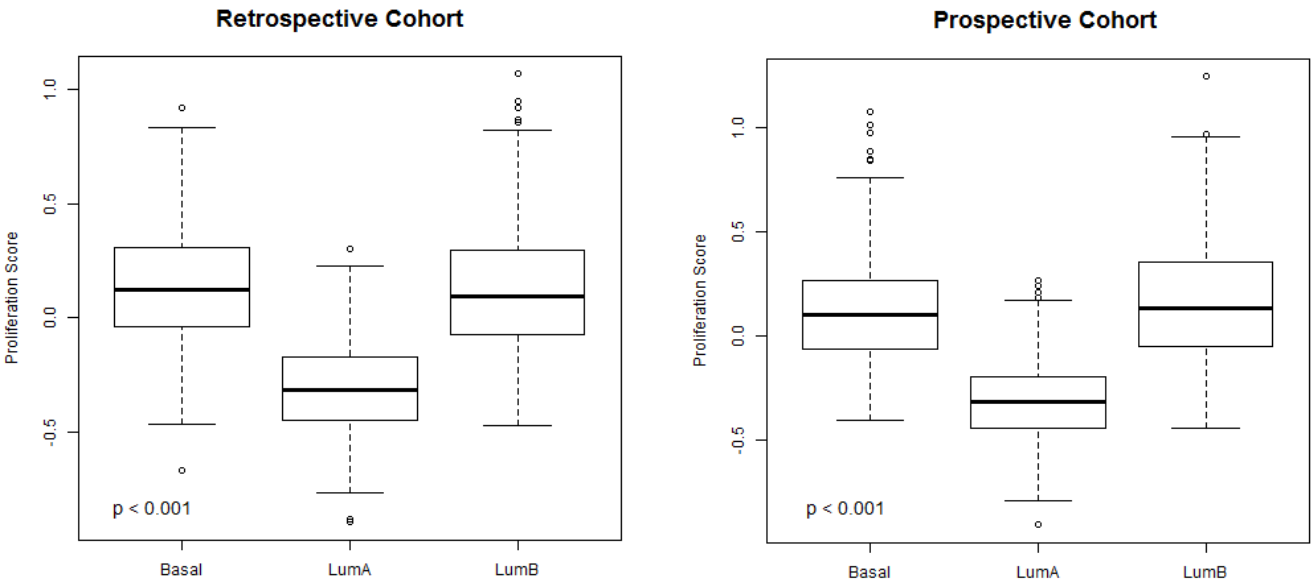
eFigure 3. Luminal Subtypes Are Associated With MYC and KRAS



On GSEA analysis, MYC targets are positively enriched in the luminal-like samples, and MYC also demonstrates increased expression in the luminal-like samples in both the retrospective and prospective cohorts. Genes which are down-regulated by KRAS are negatively enriched in the luminal-like samples, and KRAS expression is also increased in the luminal-like samples in both the retrospective and prospective cohorts.

© 2017 American Medical Association. All rights reserved.

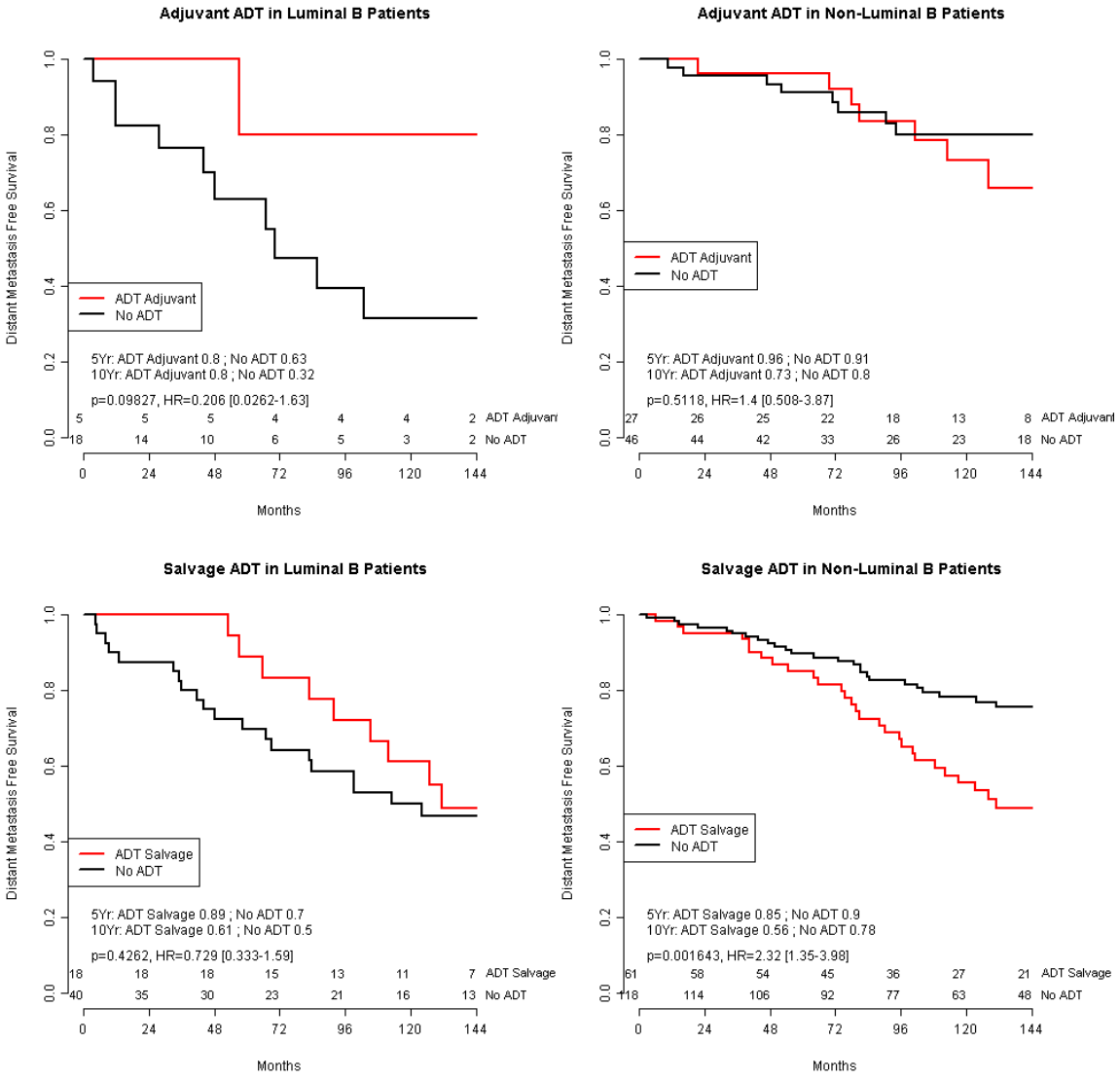
eFigure 4. PAM50 Proliferation Score Across Subtypes



Box plots of the PAM50 proliferation score across the basal, luminal A and luminal B subtypes within the retrospective and prospective cohorts. ANOVA *P*<.001 for both cohorts.

© 2017 American Medical Association. All rights reserved.

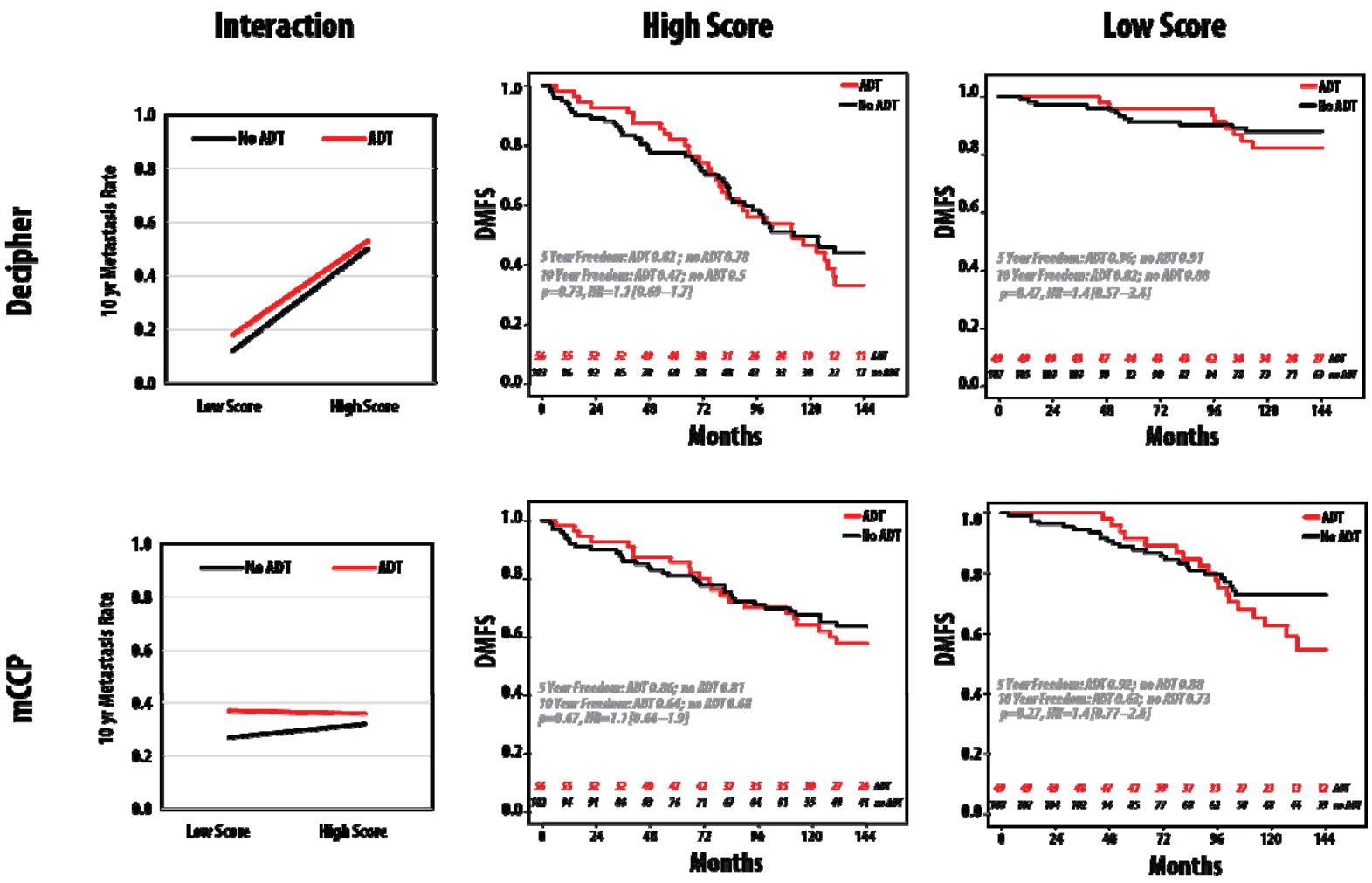
eFigure 5. Survival Outcomes for the Matched Cohort, Separating Patients Receiving Adjuvant and Salvage ADT



Kaplan-Meier curves to visualize effect of adjuvant and salvage ADT separately within the Luminal B and non-Luminal B patients. Top: Patients receiving adjuvant ADT and their matched no ADT samples. Bottom: Patients receiving salvage ADT and their matched no ADT samples. While the numbers are small, the trends show that both adjuvant and salvage ADT may provide benefit in Luminal B patients, but not in non-Luminal B patients, similar to when adjuvant and salvage ADT are pooled.

© 2017 American Medical Association. All rights reserved.

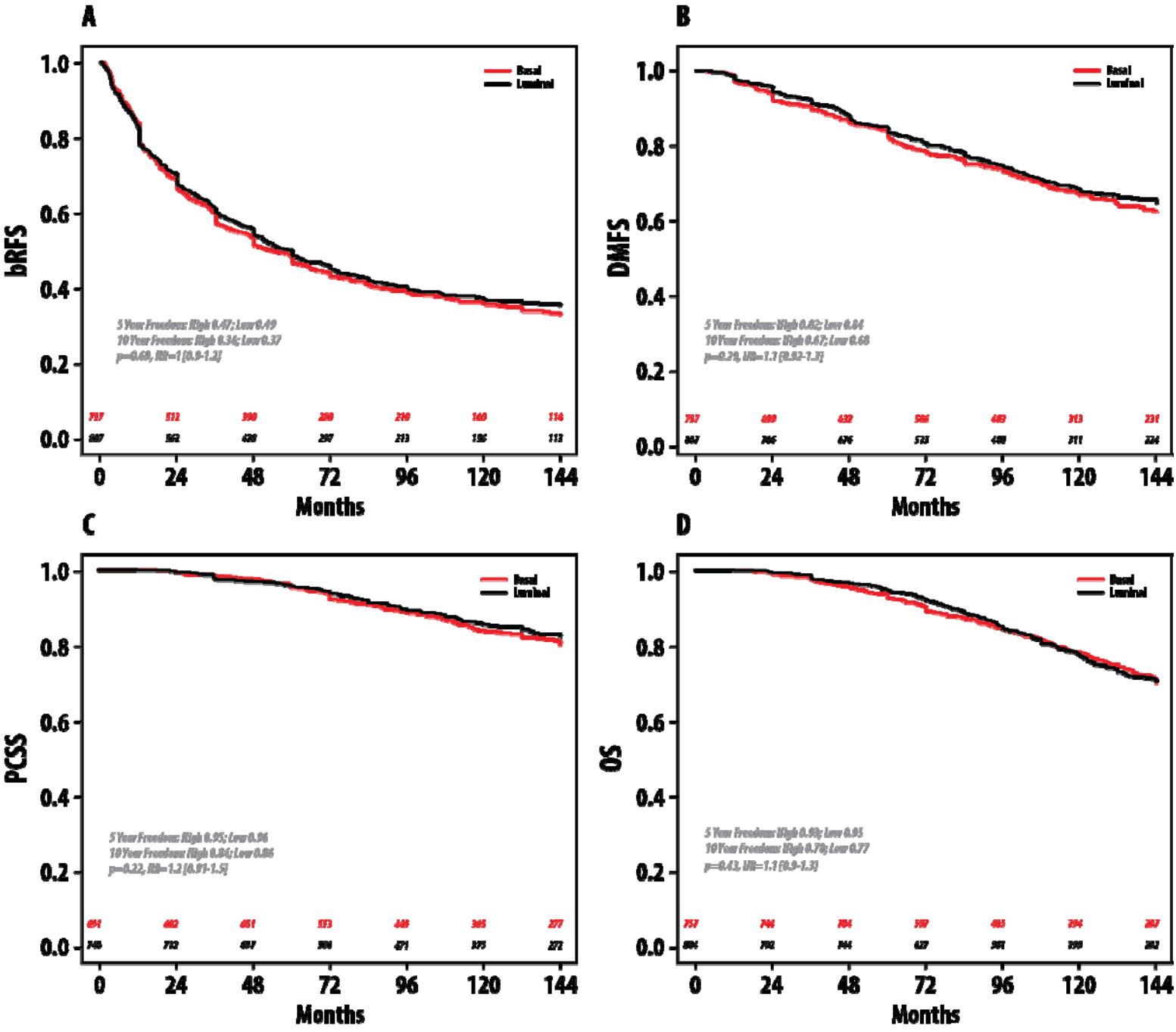
eFigure 6. Decipher and mCCP Are Not Predictive for Response to Postoperative ADT in the Matched Cohort



In the matched ADT cohort, we find that patients with high versus low Decipher or mCCP (the microarray version of the cell cycle progression signature) do not respond differently to post-operative ADT treatment. Interaction terms between treatment and Decipher (*P*=.60) and mCCP (*P*=.57) were nonsignificant.

© 2017 American Medical Association. All rights reserved.

eFigure 7. A Previously Published 100-Gene Set Is Not Associated With Clinical Outcomes in Prostate Cancer



A 100-gene (50 luminal, 50 basal) set was examined in the pooled retrospective cohorts by taking the mean expression after median centering and scaling of the 48 available luminal and 49 available basal genes on our platform, and assigning a group based on the higher of the two scores. We find that there is no association with these groups and DMFS, bRFS, PCSS, or OS within the retrospective prostate cancer cohort of 1567 patients.

© 2017 American Medical Association. All rights reserved.

eTable 1. Demographics for Pooled Retrospective Cohort (n=1567)

		Basal (n=582)	Luminal A (n=538)	Luminal B (n=447)	Total (n=1567)	*P* Value
Age (years)		62.4 +/- 6.98	62.5 +/- 6.71	62.4 +/- 6.92	62.4 +/- 6.87	.98
	NA	1 (0.002)	1 (0.002)	1 (0.002)	3 (0.002)	
PSA (ng/dL)	*< 10*	351 (0.603)	318 (0.591)	238 (0.532)	907 (0.579)	
	10 to 20	131 (0.225)	145 (0.27)	113 (0.253)	389 (0.248)	.002
	> 20	87 (0.149)	64 (0.119)	92 (0.206)	243 (0.155)	
	NA	13 (0.022)	11 (0.02)	4 (0.009)	28 (0.018)	
Gleason	*<6*	68 (0.117)	53 (0.099)	23 (0.051)	144 (0.092)	
	7	328 (0.564)	335 (0.623)	218 (0.488)	881 (0.562)	<.001
	8 to 10	184 (0.316)	149 (0.277)	205 (0.459)	538 (0.343)	
	NA	2 (0.003)	1 (0.002)	1 (0.002)	4 (0.003)	
SM	*No*	297 (0.51)	261 (0.485)	214 (0.479)	772 (0.493)	.55
	Yes	284 (0.488)	276 (0.513)	232 (0.519)	792 (0.505)	
	NA	1 (0.002)	1 (0.002)	1 (0.002)	3 (0.002)	
SVI	*No*	427 (0.734)	388 (0.721)	300 (0.671)	1115 (0.712)	.08
	Yes	153 (0.263)	148 (0.275)	145 (0.324)	446 (0.285)	
	NA	2 (0.003)	2 (0.004)	2 (0.004)	6 (0.004)	
ECE	*No*	272 (0.467)	259 (0.481)	147 (0.329)	678 (0.433)	<.001
	Yes	306 (0.526)	278 (0.517)	298 (0.667)	882 (0.563)	
	NA	4 (0.007)	1 (0.002)	2 (0.004)	7 (0.004)	
LNI	*No*	524 (0.9)	486 (0.903)	383 (0.857)	1393 (0.889)	.03
	Yes	57 (0.098)	50 (0.093)	63 (0.141)	170 (0.108)	
	NA	1 (0.002)	2 (0.004)	1 (0.002)	4 (0.003)	

Abbreviations: PSA: prostate specific antigen, SM: positive surgical margins, SVI: seminal vesicle invasion, ECE: extracapsular extension, LNI: lymph node invasion.

© 2017 American Medical Association. All rights reserved.

eTable 2. Univariable and Multivariable Analysis in Pooled Retrospective Cohort (n=1567)

		UVA		MVA	
		***P* Value**	**HR [95% CI]**	***P* Value**	**HR [95% CI]**
bRFS	Age (yrs)	.36	1 [0.99-1.01]	.97	1 [0.99-1.01]
	PSA 10-20 vs. <10	.13	1.13 [0.97-1.32]	.59	1.04 [0.89-1.22]
	PSA >20 vs. <10	8.7E-06	1.49 [1.25-1.77]	.13	1.16 [0.96-1.4]
	Gleason 7 vs. <7	.12	1.23 [0.95-1.61]	.89	1.02 [0.77-1.34]
	Gleason 8-10 vs. <7	3.0E-11	2.49 [1.9-3.27]	3.9E-05	1.83 [1.37-2.43]
	SM	1.2E-04	1.31 [1.14-1.5]	.03	1.17 [1.01-1.35]
	SVI	0.0E+00	2.01 [1.75-2.31]	4.5E-10	1.65 [1.41-1.93]
	ECE	1.6E-09	1.51 [1.32-1.73]	.10	1.13 [0.98-1.31]
	LNI	4.3E-05	1.49 [1.23-1.8]	.21	0.87 [0.71-1.08]
	Basal vs. LumB	3.2E-06	0.69 [0.59-0.81]	.01	0.81 [0.69-0.96]
	LumA vs. LumB	5.4E-07	0.66 [0.57-0.78]	4.8E-03	0.79 [0.66-0.93]
OS	Age (yrs)	7.6E-05	1.03 [1.01-1.04]	.01	1.02 [1-1.03]
	PSA 10-20 vs. <10	.62	1.06 [0.85-1.31]	.20	0.87 [0.7-1.08]
	PSA >20 vs. <10	.01	1.35 [1.08-1.69]	.15	0.83 [0.65-1.07]
	Gleason 7 vs. <7	1.1E-03	1.98 [1.32-2.98]	.02	1.69 [1.11-2.59]
	Gleason 8-10 vs. <7	1.1E-14	5.01 [3.33-7.53]	5.2E-09	3.65 [2.36-5.63]
	SM	2.4E-03	1.32 [1.1-1.59]	.06	1.2 [1-1.45]
	SVI	1.2E-13	1.96 [1.64-2.33]	1.7E-04	1.49 [1.21-1.83]
	ECE	1.2E-07	1.66 [1.38-2.01]	.34	1.11 [0.9-1.37]
	LNI	6.0E-10	2.1 [1.66-2.65]	.03	1.33 [1.02-1.72]
	Basal vs. LumB	5.1E-04	0.69 [0.56-0.85]	.24	0.88 [0.71-1.09]
	LumA vs. LumB	2.8E-07	0.56 [0.45-0.7]	1.9E-03	0.69 [0.55-0.87]

Abbreviations: PSA: prostate specific antigen, SM: positive surgical margins, SVI: seminal vesicle invasion, ECE: extracapsular extension, LNI: lymph node invasion, bRFS: biochemical recurrence-free survival, OS: overall survival.

© 2017 American Medical Association. All rights reserved.

eTable 3. Univariable and Multivariable Analysis in Pooled Retrospective Cohort (n=1567) to Examine Independence of Subtypes from D'Amico Risk Classification

		UVA		MVA	
		P Value	HR [95% CI]	*P* Value	HR [95% CI]
bRFS	Age	.36	1 [0.99-1.01]	.30	1.01 [0.99-1.02]
	D'Amico	3.49E-10	1.87 [1.54-2.27]	5.71E-08	1.73 [1.42-2.1]
	LNI	4.32E-05	1.49 [1.23-1.8]	2.66E-15	3.46 [2.55-4.71]
	Basal vs. LumB	3.23E-06	0.69 [0.59-0.81]	.01	0.78 [0.65-0.93]
	LumA vs. LumB	5.44E-07	0.66 [0.57-0.78]	1.40E-03	0.74 [0.61-0.89]
DMFS	Age	.88	1 [0.99-1.02]	.30	1.01 [0.99-1.03]
	D'Amico	3.71E-10	2.86 [2.06-3.97]	7.89E-08	2.48 [1.78-3.46]
	LNI	<.001	2.56 [2.06-3.19]	6.78E-10	3.21 [2.22-4.65]
	Basal vs. LumB	8.95E-11	0.5 [0.4-0.61]	2.19E-06	0.54 [0.42-0.7]
	LumA vs. LumB	9.14E-14	0.42 [0.34-0.53]	8.53E-08	0.49 [0.38-0.64]
PCSS	Age	.86	1 [0.98-1.02]	.31	1.01 [0.99-1.04]
	D'Amico	7.47E-06	3 [1.86-4.86]	6.06E-05	2.7 [1.66-4.4]
	LNI	2.22E-15	3.19 [2.4-4.25]	5.72E-04	2.71 [1.54-4.78]
	Basal vs. LumB	3.40E-04	0.59 [0.44-0.79]	.06	0.71 [0.5-1.01]
	LumA vs. LumB	1.84E-08	0.38 [0.27-0.53]	7.95E-05	0.45 [0.3-0.67]
OS	Age	7.62E-05	1.03 [1.01-1.04]	1.09E-05	1.04 [1.02-1.06]
	D'Amico	2.63E-06	1.98 [1.49-2.63]	3.12E-05	1.85 [1.38-2.46]
	LNI	6.02E-10	2.1 [1.66-2.65]	2.28E-03	2.22 [1.33-3.71]
	Basal vs. LumB	5.09E-04	0.69 [0.56-0.85]	.17	0.84 [0.66-1.08]
	LumA vs. LumB	2.77E-07	0.56 [0.45-0.7]	4.23E-05	0.57 [0.43-0.74]

Abbreviations: LNI: lymph node invasion.

Note: D'Amico high-risk was compared to intermediate and low risk combined, as there were only 19 low risk patients.

© 2017 American Medical Association. All rights reserved.

eTable 4. Demographics for GRID (n=2215)

		Basal (n=755)	Luminal A (n=737)	Luminal B (723)	Total (n=2215)	*P* Value
Age (years)		64.1 +/- 7.09	64.4 +/- 6.64	64.9 +/- 6.61	64.4 +/- 6.79	.11
PSA (ng/dL)	*NA*	67 (0.089)	63 (0.085)	59 (0.082)	189 (0.085)	.44
	< 10	382 (0.506)	373 (0.506)	360 (0.498)	1115 (0.503)	
	10 to 20	72 (0.095)	85 (0.115)	90 (0.124)	247 (0.112)	
	> 20	33 (0.044)	28 (0.038)	36 (0.05)	97 (0.044)	
Gleason	*NA*	268 (0.355)	251 (0.341)	237 (0.328)	756 (0.341)	<.001
	<6	61 (0.081)	53 (0.072)	29 (0.04)	143 (0.065)	
	7	453 (0.6)	489 (0.664)	431 (0.596)	1373 (0.62)	
	8 to 10	174 (0.23)	132 (0.179)	204 (0.282)	510 (0.23)	
SM	*NA*	67 (0.089)	63 (0.085)	59 (0.082)	189 (0.085)	.88
	No	310 (0.411)	295 (0.4)	299 (0.414)	904 (0.408)	
	Yes	375 (0.497)	376 (0.51)	365 (0.505)	1116 (0.504)	
SVI	*NA*	70 (0.093)	66 (0.09)	59 (0.082)	195 (0.088)	.003
	No	530 (0.702)	554 (0.752)	499 (0.69)	1583 (0.715)	
	Yes	152 (0.201)	116 (0.157)	165 (0.228)	433 (0.195)	
ECE	*NA*	73 (0.097)	67 (0.091)	59 (0.082)	199 (0.09)	<.001
	No	336 (0.445)	325 (0.441)	260 (0.36)	921 (0.416)	
	Yes	348 (0.461)	345 (0.468)	403 (0.557)	1096 (0.495)	
LNI	*NA*	71 (0.094)	67 (0.091)	60 (0.083)	198 (0.089)	.02
	No	646 (0.856)	628 (0.852)	624 (0.863)	1898 (0.857)	
	Yes	8 (0.011)	9 (0.012)	21 (0.029)	38 (0.017)	
	NA	101 (0.134)	100 (0.136)	78 (0.108)	279 (0.126)	

Abbreviations: PSA: prostate specific antigen, SM: positive surgical margins, SVI: seminal vesicle invasion, ECE: extracapsular extension, LNI: lymph node invasion.

© 2017 American Medical Association. All rights reserved.

eTable 5. Demographics for Matched Cohort (n=315)

		Basal (n=118)	Luminal A (n=124)	Luminal B (n=73)	Total (n=315)	*P* Value
Age (years)		64.4 +/- 6.64	64.2 +/- 7.04	64.8 +/- 6.65	64.4 +/- 6.79	.86
PSA (ng/dL)	*< 10*	75 (0.636)	80 (0.645)	37 (0.507)	192 (0.61)	
	10 to 20	19 (0.161)	24 (0.194)	17 (0.233)	60 (0.19)	.27
	> 20	24 (0.203)	20 (0.161)	19 (0.26)	63 (0.2)	
Gleason	*<6*	13 (0.11)	7 (0.056)	1 (0.014)	21 (0.067)	
	7	72 (0.61)	91 (0.734)	41 (0.562)	204 (0.648)	.002
	8 to 10	33 (0.28)	26 (0.21)	31 (0.425)	90 (0.286)	
SM	*No*	57 (0.483)	49 (0.395)	32 (0.438)	138 (0.438)	.39
	Yes	61 (0.517)	75 (0.605)	41 (0.562)	177 (0.562)	
SVI	*No*	105 (0.89)	100 (0.806)	62 (0.849)	267 (0.848)	.20
	Yes	13 (0.11)	24 (0.194)	11 (0.151)	48 (0.152)	
ECE	*No*	70 (0.593)	79 (0.637)	34 (0.466)	183 (0.581)	.06
	Yes	48 (0.407)	45 (0.363)	39 (0.534)	132 (0.419)	
LNI	*No*	118 (1)	124 (1)	73 (1)	315 (1)	NA

Abbreviations: PSA: prostate specific antigen, SM: positive surgical margins, SVI: seminal vesicle invasion, ECE: extracapsular extension, LNI: lymph node invasion.

© 2017 American Medical Association. All rights reserved.

eTable 6. Number of Patients Receiving ADT and RT in the Matched Cohort (n=315)

	Adjuvant ADT Only	Salvage ADT Only	Both Adjuvant and Salvage ADT	No ADT
Adjuvant RT Only	4	6	2	23
Salvage RT Only	3	14	2	41
Both Adjuvant and Salvage RT	1	0	0	0
No RT	18	53	2	146

Abbreviations: ADT: Androgen deprivation therapy; RT: Radiation therapy

© 2017 American Medical Association. All rights reserved.