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Prostate Cancer

Compound Genomic Alterations of *TP53*, *PTEN*, and *RB1* Tumor Suppressors in Localized and Metastatic Prostate Cancer

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Abstract

Background: *TP53*, *PTEN*, and *RB1* tumor suppressor genes (TSGs) are recurrently altered in treatment-resistant prostate cancer. Cooperative loss of two or more TSGs may drive more aggressive disease.
Objective: To determine clinical outcomes of single and compound TSG alterations across the spectrum of prostate cancer.
Design, setting, and participants: Massively parallel targeted sequencing using castration-sensitive prostate cancer (CSPC; localized [L] and metastatic [M1]) and castration-resistant prostate cancer (CRPC) specimens (n = 285). TSG altered (TSG-alt) was any copy number loss or deleterious mutation of one or more TSGs (*TP53*, *PTEN*, and *RB1*).
Outcome measurements and statistical analysis: For L-CSPC, event-free survival (EFS) and time to CRPC were estimated. For M1-CSPC and M1-CRPC, overall survival (OS) was estimated. Cox regression models assessed the association between cumulative TSG hits (zero hits vs one hit vs two to three hits) and outcomes with multivariable analyses adjusted for clinicopathological factors.
Results and limitations: TSG variants increased with advanced disease (L-CSPC: 39%; M1-CSPC: 63%, M1-CRPC: 92%). TSG-alt L-CSPC had shorter EFS (median 2.6 yr, hazard ratio [HR] 1.95, 95% confidence interval [CI] 1.22–3.13) and time to CRPC (median 9.5 mo, HR 3.36, 95% CI 1.01–11.16). Cumulative gene hits led to an incremental risk of relapse (EFS: one gene, HR 1.69, 95% CI 0.99–2.87; two to three genes, HR 2.70, 95% CI 1.43–5.08; both versus zero genes, p = 0.004). There was evidence of inferior OS with increasing TSG hits in the metastatic cohorts. Only four (8%) patients in the M1-CRPC cohort were TSG-neg, one of whom died after 5.2 yr. Multivariable analyses adjusting for mutational and copy number burden did not demonstrate a significant independent association of increasing gene hits and poorer outcomes.
Conclusions: Deleterious TSG variants are associated with an increased risk of relapse (L) and death (M1) in CSPC. Poorer outcomes are seen with compound gene hits in both early and advanced disease, and this may in part reflect increasing global genomic instability.
Patient summary: Men with prostate tumors with compound tumor suppressor gene mutations have poorer outcomes. These findings help identify patients with aggressive features who may benefit from intensified treatment.

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1. Introduction

Despite advances in the treatment of metastatic prostate cancer, the disease course is almost invariably marked by evolution toward a therapy-resistant and lethal disease phenotype. In recent years, large-scale efforts have interrogated the genome of primary, metastatic, and castration-resistant (CRPC) prostate cancer. This has spurred biomarker discovery and translation, which parallels the increasing availability and application of clinical-grade massively parallel sequencing.

Classical tumor suppressor genes (TSGs), *TP53*, *PTEN*, and *RB1*, are among the most frequently altered genes in primary prostate cancer [1,2], leading to aberrant PI3K/AKT, RAS/RAF, and cell cycle signaling [2]. Through comprehensive genomic profiling of metastatic castration-resistant tumors, *TP53*, *RB1*, and *PTEN* alterations have been shown to be significantly enriched in resistant disease [3,4], which suggests a critical role of the loss of tumor suppressor function in driving aggressive disease biology. Loss of *PTEN* is associated with increased risks of biochemical recurrence [5,6] and metastasis [7] after curative treatment. *TP53* mutations may also confer a poorer response to androgen receptor (AR)-targeted therapy for CRPC [8]. *RB1* loss is nearly universal in neuroendocrine prostate cancer—an aggressive, AR-independent disease variant also marked by frequent concurrent alterations in *PTEN* and *TP53* [9,10].

Preclinical studies have implicated co-operative functional loss of *TP53*, *RB1*, and *PTEN* with the development of lethal prostate cancer [11]. Inactivation of each of mouse *Trp53* and *Pten* alone does not result in rapid development of prostate tumors; however, combined inactivation leads to rapidly invasive, metastatic, and lethal disease [12]. *Rb1* loss appears to facilitate lineage plasticity and metastatic potential on the background of *Pten* deficiency, with additional *Trp53* loss driving antiandrogen resistance and a switch to a transcriptional profile that resembles neuroendocrine prostate cancer [13,14].

Leveraging a targeted multigene massively parallel sequencing assay using clinical tumor specimens, we sought to determine whether loss-of-function (LOF) alteration(s) of *TP53*, *PTEN*, and *RB1* are associated with adverse clinical outcomes in castration-sensitive and castration-resistant prostate cancer cohorts.

2. Patients and methods

2.1. Patients and cohorts

We identified consecutive patients treated at Dana-Farber Cancer Institute (DFCI, Boston, MA, USA) with a diagnosis of prostate cancer between 1998 and 2016, who underwent OncoPanel sequencing of tumor tissue from prostate (primary) or other (metastatic) site. Three retrospective clinical cohorts were formed for the analyses (Supplementary Fig. 1): (1) a localized castration-sensitive prostate cancer (L-CSPC) cohort of nonmetastatic disease at diagnosis and curative-intent local treatment, with or without adjuvant androgen deprivation therapy (ADT), excluding men primarily managed by active surveillance; (2) a metastatic castration-sensitive prostate cancer (M1-CSPC) cohort comprising patients with either de novo metastasis or metastatic relapse after prior local therapy (including salvage radiotherapy), excluding patients who received ADT for biochemical recurrence without a curative intent; and (3) a metastatic CRPC (M1-CRPC) cohort with metastatic tumor biopsy within 6 mo prior to or after the date of castration resistance, as defined by Prostate Cancer Clinical Trials Working Group 3 (PCWG3) criteria [15]. All patients provided informed consent, and the study was approved by the center's Institutional Review Board.

2.2. Tumor specimens and targeted sequencing

Sequencing of formalin-fixed, paraffin-embedded (FFPE) tissue from biopsies of patients with localized or metastatic disease was performed using OncoPanel (versions 1 or 2) as part of our institutional cancer cohort study called *Profile*. OncoPanel is a clinical-grade, targeted, massively parallel sequencing assay of over 300 cancer-related genes (including *TP53*, *PTEN*, and *RB1*, of interest in this study) previously validated in a CLIA laboratory at the Brigham and Women's Hospital [16,17], evaluating somatic mutations, copy number alterations, and structural rearrangements. The methods used to determine the significance of alterations are detailed in the Supplementary material. Additionally, tumor mutational burden (TMB) was estimated by determining the number of nonsynonymous somatic mutations that occur per megabase of exonic sequence data across all genes on the panel. To estimate the percent of genome copy-number altered (PGA), an internally developed tool (RobustCNV) was used to calculate the fractional coverage of specified genomic intervals compared with the median fractional coverage obtained in a panel of 152 FFPE non-neoplastic samples, as previously described [18]. Patients with any significant alteration in *TP53* and/or *PTEN* and/or *RB1* were classified as "tumor suppressor gene altered" (TSG-alt). The primary analysis adopted the definition of TSG-alt (monoallelic/biallelic) as any significant mutation and/or one- or two-copy deletion. The secondary analysis restricted this definition to biallelic inactivation only, defined as significant mutation or homozygous deletion.

2.3. Statistical methods

The analyses assessed the association of TSG biomarker status (TSG-alt vs TSG-neg) and unique gene hits (zero hits, one hit, or two to three hits; TSG0, TSG1, and TSG2–3, respectively) with clinical outcomes. Time to event endpoints in L-CSPC started follow-up from time of biopsy preceding local therapy and included event-free survival (EFS) defined as time to prostate-specific antigen relapse, metastasis, or death; time to CRPC (TTCRPC) per PCWG3 criteria; and overall survival (OS) defined as time to death from any cause. Time from ADT commencement for metastatic disease to CRPC and death (OS) were endpoints in the M1-CSPC cohort. Duration of first-line CRPC therapy and OS (from the date of diagnosis of CRPC) were endpoints in the M1-CRPC cohort. Cox proportional hazard regression models assessed the association of biomarker status or unique gene hits with event-free endpoints, and estimated the hazard ratio (HR) and 95% confidence interval (CI). The proportional hazard assumptions were assessed and confirmed using the scaled Schoenfeld residuals. Multivariable models were adjusted for age at diagnosis, clinical stage, prostate biopsy International Society of Urological Pathology (ISUP) grade, TMB, and PGA for L1-CSPC cohort. Distribution of endpoints according to biomarker status or unique gene hits were estimated using the Kaplan-Meier method. Assuming an analytic cohort of 205 (eg, L1-CSPC) with 73 (36%) EFS events and 39% patients with TSG-alt, there was 80% power (two-sided type I error of 0.05) to detect an HR of 2.0 comparing TSG-alt versus TSG-neg. Two-sided p values were reported for all analyses. The analysis used R programming, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1 – Key patient characteristics across cohorts

	L-CSPC (*N* = 205)	M1-CSPC (*N* = 43)	M1-CRPC (*N* = 48)
Median follow-up (IQR)[a], yr	3.1 (1.6, 4.3)	3.3 (1.4, 5.7)	4.1 (2.0, 6.6)
Age at stage diagnosis, yr			
Median (IQR)	62 (57, 67)	62 (58, 67)	64 (61, 70)
Local therapy, no. (%)			
RP	158 (77)	8 (19)	14 (29)
RT	47 (23)	5 (12)	13 (27)
None	0 (0)	30 (70)	21 (44)
Clinical stage at prostate cancer diagnosis, no. (%)			
Localized	205 (100)	13 (30)	21 (44)
Metastatic	0 (0)	30 (70)	27 (56)

IQR = interquartile range; L-CSPC = localized castration-sensitive prostate cancer; M1-CRPC = metastatic castration-resistant prostate cancer; M1-CSPC = metastatic castration-sensitive prostate cancer; RP = radical prostatectomy; RT = radiotherapy.
[a] Follow-up was estimated using Kaplan-Meier method by counting those still alive as an event.

Table 2 – Frequency and distribution of monoallelic/biallelic *TP53*, *RB1*, and *PTEN* alterations and measures of genomic instability

	L-CSPC (*N* = 205)	M1-CSPC (*N* = 43)	M1-CRPC (*N* = 48)
TSG altered, *N* (%)	80 (39)	27 (63)	44 (92)
Mutation and/or deletion, *N* (%)			
TP53	40 (20)	16 (37)	35 (73)
PTEN	33 (16)	12 (28)	32 (67)
RB1	31 (15)	16 (37)	36 (75)
Coalterations, *N* (%)			
TP53 + *PTEN*	14 (7)	7 (16)	27 (56)
PTEN + *RB1*	5 (2)	7 (16)	28 (56)
TP53 + *RB1*	6 (3)	8 (19)	28 (58)
Unique TSG hits, *N* (%)			
0	125 (61)	16 (37)	4 (8)
1	57 (28)	15 (35)	9 (19)
2	22 (11)	7 (16)	11 (23)
3	1 (0)	5 (12)	24 (50)
TMB (mut/Mb), median (range)	4.0 (0.0, 21.8)	6.0 (1.2, 11.9)	5.3 (0.0, 51.8)
PGA (%), median (range)	1.0 (0.0, 25.0)	8.0 (0.0, 25.0)	24.0 (0.0, 61.0)

L-CSPC = localized castration-sensitive prostate cancer; M1-CRPC = metastatic castration-resistant prostate cancer; M1-CSPC = metastatic castration-sensitive prostate cancer; PGA = percent of genome copy-number altered; TMB = tumor mutational burden; TSG = tumor suppressor gene.

3. Results

Patient characteristics of the analytic cohorts are presented in Table 1. Most L-CSPC ($n = 205$) patients had clinically significant tumors (ISUP grade 2–3: 115 [57%]; ISUP grade 4–5: 53 [26%]) that were organ confined (clinical stage T1: 120 [62%]; T2: 52 [27%]). Thirty men (70%) with M1-CSPC ($n = 43$) presented with de novo metastatic disease and 50% met high-volume disease criteria. Similarly, 53% of M1-CRPC patients (n 48) had high-volume disease.

3.1. *Landscape* TP53, PTEN, *and* RB1 *alterations and genomic instability*

TSG alterations were increasingly frequent in metastatic and castration-resistant disease (monoallelic/biallelic loss: L-CSPC 39% vs M1-CSPC 63% vs M1-CRPC 92%; Table 2 and Supplementary Table 1). Hemizygous loss dominated the alteration landscape of L-CSPC, particularly of *PTEN* and *RB1* (Supplementary Table 1). A high proportion of L-CSPC patients with an alteration in a given TSG harbored coalteration in another TSG: 19/40 of *TP53*-alt, 18/33 of *PTEN*-alt, and 10/31 of *RB1*-alt. TSG coalteration was nearly universal in M1-CRPC (31/35 of *TP53*-alt, 31/32 of *PTEN*-alt, and 32/36 of RB1-alt). The frequency of two or more unique TSG hits was markedly increased in M1-CRPC compared with that in M1-CSPC and L-CSPC (73% vs 28% vs 11%, respectively), with half showing inactivation of all three TSGs, in keeping with a greater alteration burden with advanced disease states. Indeed, median estimated TMB and PGA were significantly higher in M1-CRPC (TMB: 5.3 mut/Mb vs 4.0 [L-CSPC], $p = 0.02$; PGA: 24% vs 1% [L-CSPC], $p < 0.0001$). With respect to biallelic inactivation, the frequency of individual TSG alterations was comparable with historical datasets (Supplementary Table 3). Biallelic loss of two or more TSGs was notably uncommon in L-CSPC (0.5%) and M1-CSPC (4.7%; Supplementary Table 2).

3.2. *Association of TSG alterations and clinical outcomes*

3.2.1. *Localized disease*

Men with any TSG-alt had a nearly two-fold increased risk of relapse (EFS: median 2.6 vs 7.6 yr; HR 1.95, 95% CI 1.22–3.13, $p = 0.005$; Fig. 1A). TSG-alt was associated with shorter TTCRPC (HR 3.36, 95% CI 1.01–11.16, $p = 0.048$; Fig. 1B). Individually, *TP53* and *PTEN* alterations were associated

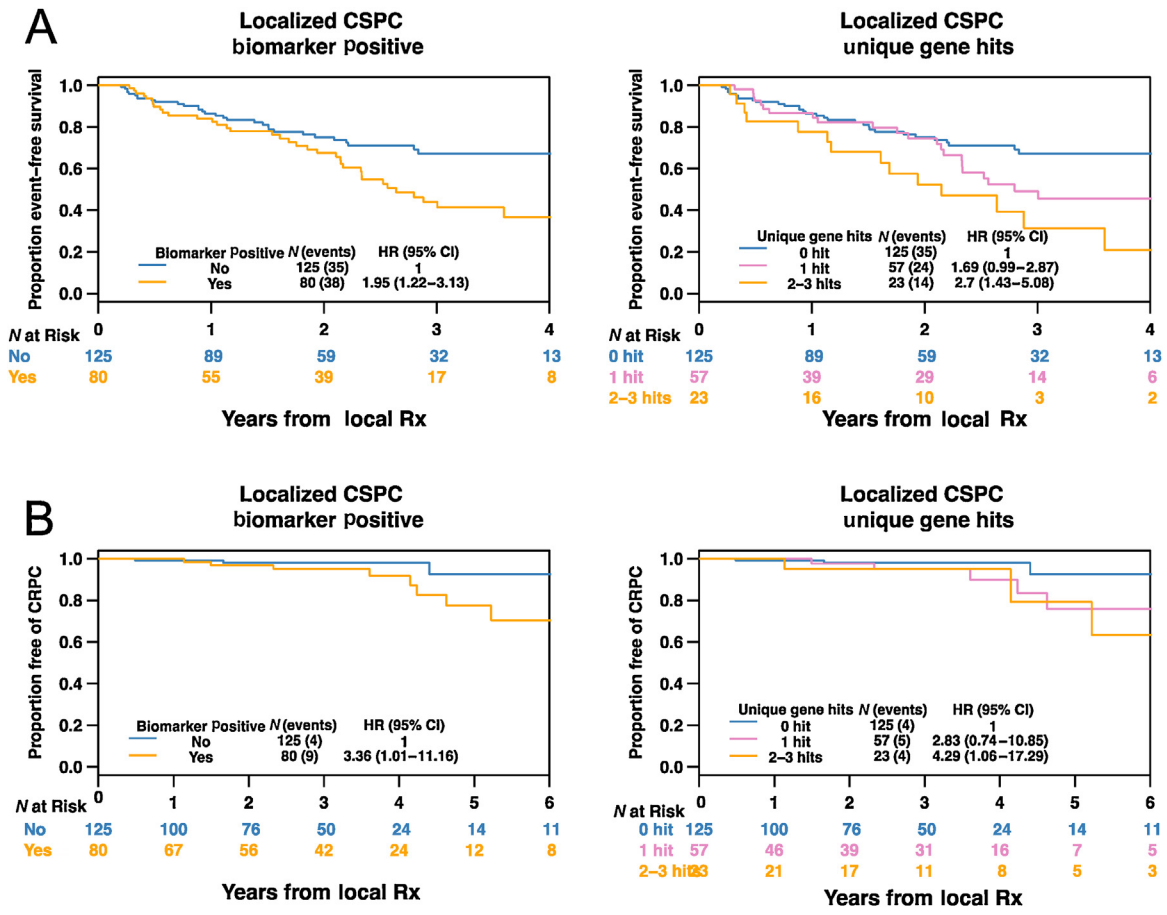

Fig. 1 – Localized disease: Kaplan-Meier curves for (A) event-free survival and (B) time to CRPC, by TSG alteration and unique gene hits (monoallelic/biallelic). Biomarker positive indicates any TSG alteration. CI = confidence interval; CRPC = castration-resistant prostate cancer; CSPC = castration-sensitive prostate cancer; HR = hazard ratio; Rx = recurrence; TSG = tumor suppressor gene.

with relapse (*PTEN*: HR 1.76, $p = 0.04$; *TP53*: HR 1.92, $p = 0.01$; Supplementary Fig. 2). Cumulative TSG hits were associated with an incremental risk of relapse (log rank $p = 0.004$; TSG1 vs TSG0, HR 1.69, 95% CI 0.99–2.87; TSG2–3 vs TSG0, HR 2.70, 95% CI 1.43–5.08) and a risk of CRPC (log rank $p = 0.08$; TSG1 vs TSG0, HR 2.83, 95% CI 0.74–10.85; TSG2–3 vs TSG0, HR 4.29, 95% CI 1.06–17.29; Table 3). PGA and TMB were prognostic for relapse (PGA: HR 1.18, $p < 0.001$; TMB: HR 1.08, $p = 0.017$) and CRPC (PGA: HR 1.36, $p = 0.022$; TMB: not significant at HR 1.13, $p = 0.14$).

When adjusted for clinicopathological variables including markers of genomic instability in multivariable analysis, no independent association of TSG-alt and relapse was observed (HR 1.35, 95% CI 0.71–2.54, $p = 0.358$); however, there was evidence of an association when restricted to TSG biallelic inactivation only, although this did not meet statistical significance (HR 1.90, 95% CI 0.98–3.71, $p = 0.059$; Supplementary Tables 4 and 6). Multivariable analysis of cumulative gene hits showed lower HRs and no significant association with EFS (TSG1 vs TSG0, HR 1.27, 95% CI 0.65–2.45; TSG2–3 vs TSG0, HR 1.90, 95% 0.75–4.83) or TTCRPC (TSG1 vs TSG0, HR 0.47, 95% CI 0.08–2.88; TSG2–3 vs TSG0, HR 0.55, 95% CI 0.07–4.25). The low rate of compound biallelic TSG loss in L-CSPC did not permit an analysis of increasing gene hits.

3.2.2. *Metastatic disease*

In the M1-CSPC cohort, there was evidence of inferior OS with TSG loss (4-yr survival rate: TSG-alt 64% [95% CI 44–94] vs TSG-neg 100%). The presence of a TSG mutation (HR 5.03, $p = 0.02$) or deletion (HR 16.54, $p < 0.001$) was significantly associated with an increased risk of death. Increasing TSG hits lead to incrementally poorer OS (4-yr survival rate: TSG1 85% [95% CI 63–100]; TSG2–3 43% [95% CI 19–97]; both comparisons vs TSG0 100%; Fig. 2B). However, there was no indication of an association between TSG-alt and TTCRPC in this cohort (HR 1.59, 95% CI 0.74–3.39, $p = 0.23$; Fig. 2A).

Median follow-up in the M1-CRPC cohort was 4.1 yr. The median duration of first-line CRPC therapy was short at 6 mo (interquartile range 3–10 mo), with the majority of patients (64%) receiving first-line AR-directed therapy (conventional nonsteroidal antiandrogen: 35%; abiraterone: 19%; enzalutamide: 10%). While the study was not powered to detect a significant difference in OS in the M1-CRPC cohort, there was evidence of an increased risk of death with TSG-alt (median OS: 4.5 yr, HR 3.26, 95% CI 0.43–24.72, $p = 0.23$) and cumulative gene hits (log rank $p = 0.13$; TSG1 vs TSG0, HR 5.89, 95% CI 0.69–50.48; TSG2–3 vs TSG0, HR 2.71, 95% CI 0.35–21.08; Fig. 3); however, these associations did not meet statistical significance. Similar findings were noted on analysis of biallelic inactivation

Table 3 – Time to event analyses by TSG alteration and unique gene hits (monoallelic/biallelic)

L-CSPC				
EFS	*N* (events)	Median EFS (yr), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	125 (35)	7.6 (4.3, NA)	1	0.005
Yes	80 (38)	2.6 (2.2, NA)	1.95 (1.22–3.13)	
Unique gene hits				
0 hit	125 (35)	7.6 (4.3, NA)	1	0.004
1 hit	57 (24)	2.8 (2.3, NA)	1.69 (0.99–2.87)	
2–3 hits	23 (14)	2.1 (1.6, NA)	2.7 (1.43–5.08)	
L-CSPC				
TTCRPC	*N* (events)	Median TTCRPC (mo), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	125 (4)	NA (NA, NA)	1	0.04
Yes	80 (9)	9.5 (9.5, NA)	3.36 (1.01–11.16)	
Unique gene hits				
0 hit	125 (4)	NA (NA, NA)	1	0.08
1 hit	57 (5)	NA (NA, NA)	2.83 (0.74–10.85)	
2–3 hits	23 (4)	9.5 (5.2, NA)	4.29 (1.06–17.29)	
L-CSPC				
OS	*N* (events)	Median OS (yr), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	125 (1)	NA (NA, NA)	1	0.06
Yes	80 (3)	10.3 (7.6, NA)	7.01 (0.71–69.69)	
Unique gene hits				
0 hit	125 (1)	NA (NA, NA)	1	–
1 hit	57 (2)	10.3 (7.6, NA)	NE	
2–3 hits	23 (1)	NA (NA, NA)	NE	
M1-CSPC				
TTCRPC	*N* (events)	Median TTCRPC (mo), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	16 (10)	10 (2.5, NA)	1	0.23
Yes	27 (21)	7 (2.6, 11.3)	1.59 (0.74–3.39)	
Unique gene hits				
0 hit	16 (10)	10 (2.5, NA)	1	0.47
1 hit	15 (13)	6.7 (3.7, NA)	1.65 (0.72–3.77)	
2–3 hits	12 (8)	7 (1.4, NA)	1.5 (0.59–3.82)	
M1-CSPC				
OS	*N* (events)	Median OS (yr), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	16 (0)	NA (NA, NA)	1	–
Yes	27 (10)	4.9 (3.4, NA)	NE	
Unique gene hits				
0 hit	16 (0)	NA (NA, NA)	1	–
1 hit	15 (4)	5.6 (4.9, NA)	NE	
2–3 hits	12 (6)	2.3 (1.1, NA)	NE	
M1-CRPC				
Duration of first-line CRPC therapy	*N* (events)	Median duration (mo), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	4 (4)	12.7 (4.1, NA)	1	0.28
Yes	44 (44)	5.7 (3.8, 8.2)	1.77 (0.63–4.98)	
Unique gene hits				

Table 3 (*Continued*)

M1-CRPC				
Duration of first-line CRPC therapy	*N* (events)	Median duration (mo), (95% CI)	HR (95% CI)	*p* value
0 hit	4 (4)	12.7 (4.1, NA)	1	0.54
1 hit	9 (9)	5.7 (5.6, NA)	1.87 (0.56–6.21)	
2–3 hits	35 (35)	5.1 (3.4, 9.1)	1.74 (0.61–4.97)	

M1-CRPC				
OS	*N* (events)	Median OS (yr), (95% CI)	HR (95% CI)	*p* value
TSG altered				
No	4 (1)	5.2 (5.2, NA)	1	0.23
Yes	44 (18)	4.5 (3.3, NA)	3.26 (0.43–24.72)	
Unique gene hits				
0 hit	4 (1)	5.2 (5.2, NA)	1	0.13
1 hit	9 (6)	2 (1, NA)	5.89 (0.69–50.48)	
2–3 hits	35 (12)	5.9 (3.3, NA)	2.71 (0.35–21.08)	

CI = confidence interval; CRPC = castration-resistant prostate cancer; EFS = event-free survival; HR = hazard ratio; L-CSPC = localized castration-sensitive prostate cancer; M1-CRPC = metastatic castration-resistant prostate cancer; M1-CSPC = metastatic castration-sensitive prostate cancer; NA = not reached the median time yet; NE = not evaluable due to zero or few events in a group; OS = overall survival; TTCRPC = time to castration-resistant prostate cancer; TSG = tumor suppressor gene.

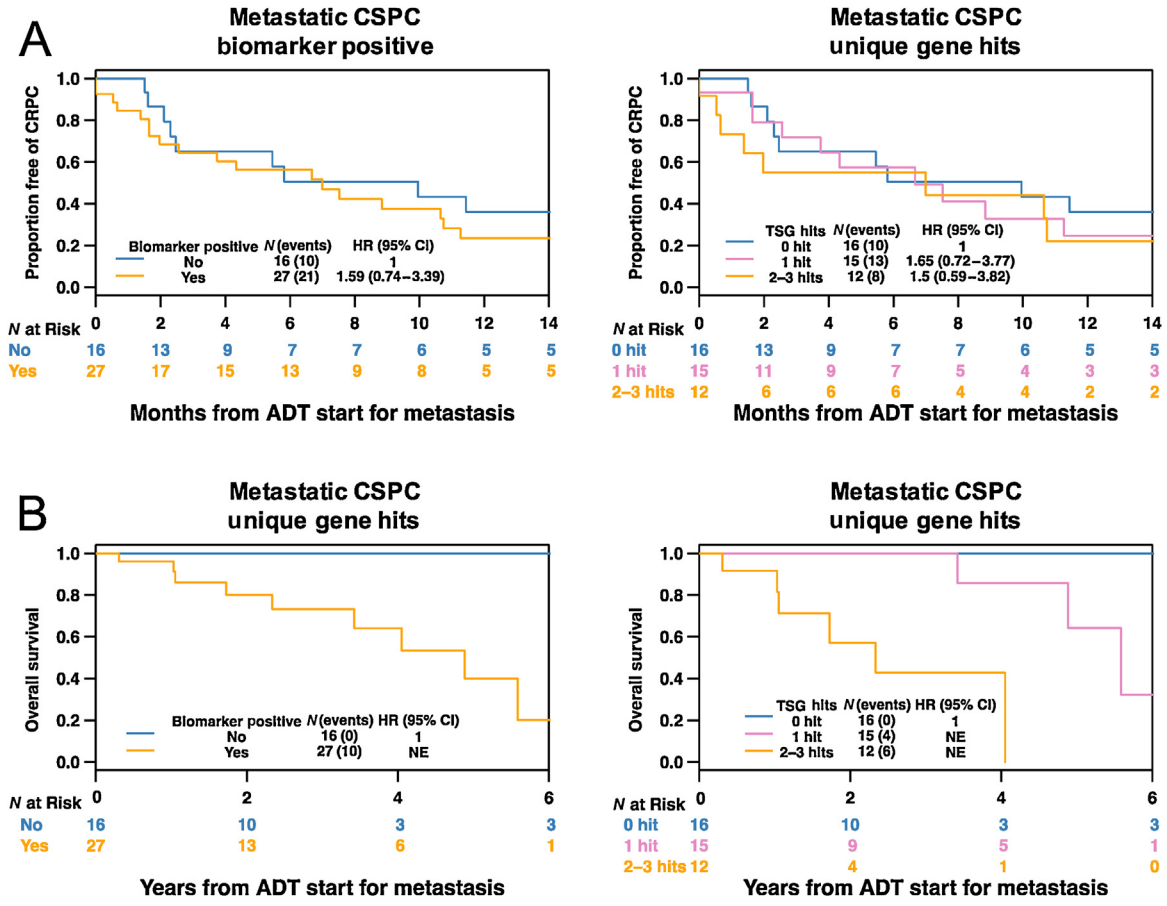

Fig. 2 – Metastatic hormone-sensitive disease: Kaplan-Meier curves for (A) time to CRPC and (B) overall survival, by TSG-alt and unique gene hits (monoallelic/biallelic). Biomarker positive indicates any TSG alteration. ADT = androgen deprivation therapy; CI = confidence interval; CRPC = castration-resistant prostate cancer; CSPC = castration-sensitive prostate cancer; HR = hazard ratio; NE = not evaluable; TSG = tumor suppressor gene; TSG-alt = TSG altered.

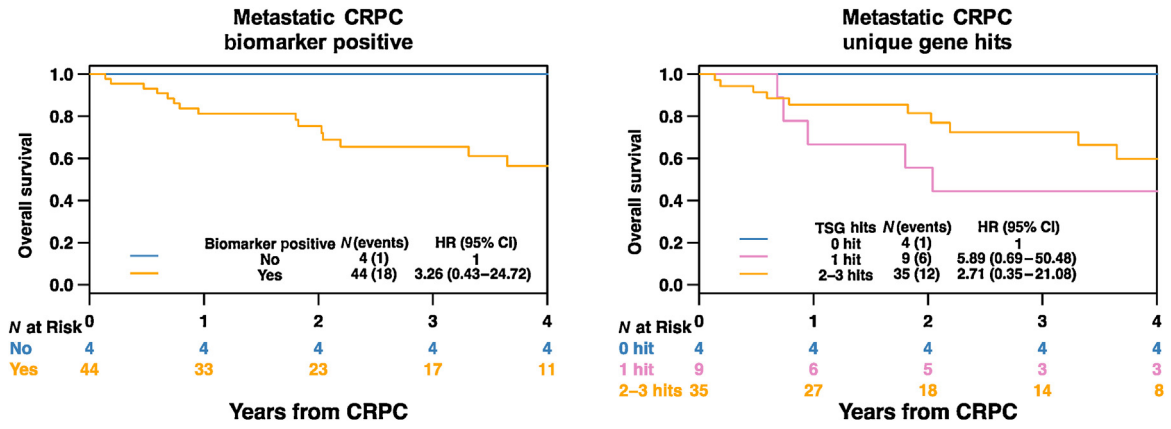

Fig. 3 – Metastatic castration-resistant disease: Kaplan-Meier curves for overall survival by TSG-alt and unique gene hits (monoallelic/biallelic). Biomarker positive indicates any TSG alteration. CI = confidence interval; CRPC = castration-resistant prostate cancer; HR = hazard ratio; NE = not evaluable; TSG = tumor suppressor gene; TSG-alt = TSG altered.

only (Supplementary Table 5). Notably, of the four (8%) men who were TSG unaltered, only one died at 5.2 yr after the diagnosis of CRPC. Multivariate analyses of OS in the metastatic cohorts are included in the Supplementary material (Supplementary Table 7).

3.2.3. *Validation cohort*

To validate the association of TSG alterations and risk of relapse in L-CSPC, we analyzed a cohort of localized prostate cancer [2] (n = 157) profiled for copy number alterations by array comparative genomic hybridization (and for *PTEN* and *TP53* mutations by exon sequencing in a subset). The distribution of TSG alterations (monoallelic/biallelic) was similar to the DFCI cohort (Supplementary Table 8). Forty-four percent had TSG-alt, and 14% harbored two or more TSG hits. Increasing gene hits were significantly associated with relapse (disease-free survival: log rank $p < 0.001$; TSG1 vs TSG0, HR 1.09, 95% CI 0.50–2.36; TSG2–3 vs TSG0, HR 3.93, 95% CI 1.87–8.24; Supplementary Fig. 3).

4. Discussion

In this study, we demonstrate that genomic alterations of *TP53*, *PTEN*, or *RB1* are common events across the entire clinical spectrum of significant prostate cancer. Leveraging the application of a clinical-grade targeted sequencing assay (OncoPanel) and using clinical FFPE tumor specimens, we show that men who harbor tumor alterations in these TSGs are at a significantly increased risk of poorer clinical outcomes. Cumulative TSG loss leads to incrementally poorer outcomes and furthermore identifies a subset of adverse-risk patients who may be prospectively identified for investigational therapies.

Our findings demonstrate frequent *TP53*, *PTEN*, and *RB1* aberrations in advanced prostate cancer [1], in keeping with an evolutionary model of selective pressure for molecularly and phenotypically resistant clones that are present early and become increasingly frequent with advanced disease. The prognostic impact of compound TSG loss (present before testosterone suppression in a minority of patients) is consistent with preclinical modeling, suggesting that compound loss leads to more aggressive disease biology through cooperative cellular mechanisms. Loss of *TP53*, *PTEN*, and *RB1* drives transdifferentiation to a non-AR–driven anaplastic tumor phenotype akin to neuroendocrine prostate cancer [19,20]. *Pten*/*TP53* null prostate progenitor/stem cells results in the development of neuroendocrine cells and a tumor-initiating phenotype [21]. In mouse models, either *Rb1* or *Trp53* inactivation develops prostatic intraepithelial neoplasia; however, dual inactivation leads to neuroendocrine differentiation with rapid development of metastasis [11], antiandrogen resistance, and lineage switch through upregulation of Sox2 [13,14], a reprogramming factor notably upregulated in enzalutamide-resistant prostate cancer cell lines [22].

Eleven percent of patients with localized disease had evidence of two or more TSG hits, suggesting that initiation of resistant disease can occur early in a subset of patients. We hypothesize that early compound loss of these TSGs results in aggressive disease marked by altered proliferative signaling and early genomic instability well before overt castration resistance.TMB and PGA, markers of global genomic instability, were significantly prognostic for relapse consistent with previous reports [23,24]. Multivariable analysis adjusting for genomic instability did not demonstrate a significant independent association of increasing gene hits and risk of relapse or castration resistance. The interpretation of this finding requires some consideration. Cumulative TSG loss may well be a representation of increased genome-wide mutational and copy number burden, the upstream mechanisms that remain unclearly defined. In turn, cumulative TSG loss may be a fundamental driver of genomic instability through aberrant genome maintenance and replication fidelity. Either association does not preclude a significant biological role of cooperative TSG loss in disease progression, and the sequence

and complex interplay of these drivers remain an area of active investigation.

The implications of early TSG aberrations may well be significant, as men with tumor TSG alterations who underwent definitive treatment were at an increased risk of recurrence, independent of clinical stage and ISUP grade. Genomic profiling for TSGs at the time of prostate biopsy may identify tumors harboring compound alterations and therefore identify men suitable for study of intensified systemic therapy (such as PI3K/Akt inhibitors for tumor *PTEN* loss, or chemotherapy). In contrast, we observed four (8%) TSG wild-type patients in the CRPC cohort who appeared to be prognostic outliers, with one death at 5.2 yr after CRPC diagnosis despite median OS of about 3 yr in modern trials of treatment-naive M1-CRPC patients [25,26]. It is notable that in the M1-CSPC cohort, there is no evidence of earlier TTCRPC with presence of TSG alterations. This may reflect imprecision in a hospital registry or indicate that response to primary ADT is not affected, as dominant clones are sensitive to castration, but resistant clones emerge with time (either present de novo or secondary) and impact the efficacy of CRPC therapies.

We included patients with monoallelic or biallelic TSG inactivation as "biomarker positive" in the primary analysis, based on preclinical and clinical evidence implicating TSG haploinsufficiency in prostate cancer progression [5,27]. This explains the higher frequency of TSG alterations observed in our cohort compared with other studies that have reported biallelic TSG inactivation in metastatic and primary prostate tumors [1,4]. Nevertheless, in the L-CSPC cohort, we demonstrated a significant association of any *TP53* and *PTEN* alteration and an increased risk of disease recurrence, with biomarker-positive alterations in these genes largely being due to monoallelic deletion, suggesting that hemizygous TSG loss is indeed prognostic. Encouragingly, adopting the same LOF definition in the validation cohort of L-CSPC yielded concordant results. Secondary analyses restricted to biallelic TSG inactivation again reproduced the direction of prognostic effect seen in the primary analysis in both localized and metastatic cohorts, although compound biallelic TSG loss was rare in hormone-sensitive disease.

This study has caveats and limitations of note. The cohorts identified were retrospective, and despite outcomes being comparable with prospective datasets, significant confounding factors cannot be excluded. We employed a pragmatic approach in the M1-CSPC cohort, including sequencing data from prostate biopsies taken at the diagnosis of localized disease and at the time of metastatic diagnosis, as sequenced metastatic biopsies of M1-CSPC patients at this time point were infrequent. Primary and metastatic tumors may have differed, and therefore there is a risk of underestimation of TSG alterations; however, a recent study comparing locoregional and metastatic CSPC tumors showed no statistically significant enrichment in *TP53*, *PTEN*, or *RB1* alterations in the latter disease stage [28]. Furthermore, recent data have highlighted the polyclonality of localized prostate cancer and the presence of subclonal alterations in TSGs such as *TP53* and *PTEN* [29,30]. As such, tumor profiling may have resulted in underdetection of clinically relevant, mutated subclones. The assignment of mutational significance and interpretation using targeted sequencing panels such as OncoPanel is also subject to variation, despite high confidence calling using variant filters and manual review. Furthermore, without paired germline data, the assay does not allow for definitive determination of variant origin (somatic vs germline). Last, the relatively nascent use of multigene tumor sequencing panels as part of clinical care at our institution (and other centers with similar programs) is a new enterprise with inherent limited follow-up in our CSPC cohorts, and therefore we expect outcome data to mature with longer follow-up.

5. Conclusions

In summary, we show that the presence of genomic alterations in *TP53*, *PTEN*, and *RB1* (and compound loss of these genes) in both early and advanced prostate cancer portends adverse prognosis. Detection of these alterations using efficient and increasingly affordable clinical tumor sequencing holds the potential to inform design of prospective therapeutic and biomarker trials for precision medicine as well as more accurate prognostication, by way of identifying high- and low-risk disease subgroups.

Author contributions: Anis A. Hamid had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.11.045.

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