Oncogenic Genomic Alterations, Clinical Phenotypes, and Outcomes in Metastatic Castration-Sensitive Prostate Cancer

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ABSTRACT

Purpose: The genomic underpinning of clinical phenotypes and outcomes in metastatic castration-sensitive prostate cancer is unclear.

Experimental Design: In patients with metastatic castration-sensitive prostate cancer at a tertiary referral center, clinical-grade targeted tumor sequencing was performed to quantify tumor DNA copy number alterations and alterations in predefined oncogenic signaling pathways. Disease volume was classified as high volume (≥4 bone metastases or visceral metastases) versus low volume.

Results: Among 424 patients (88% white), 213 (50%) had high-volume disease and 211 (50%) had low-volume disease, 275 (65%) had *de novo* metastatic disease, and 149 (35%) had metastatic recurrence of nonmetastatic disease. Rates of castration resistance [adjusted hazard ratio, 1.84; 95% confidence interval (CI), 1.40–2.41] and death (adjusted hazard ratio, 3.71; 95% CI, 2.28–6.02) were higher in high-volume disease. Tumors from high-volume disease had more copy number alterations. The NOTCH, cell cycle, and epigenetic modifier pathways were the highest-ranking pathways enriched in high-volume disease. *De novo* metastatic disease differed from metastatic recurrences in the prevalence of *CDK12* alterations but had similar prognosis. Rates of castration resistance differed 1.5-fold to 5-fold according to alterations in *AR, SPOP* (inverse), and *TP53*, and the cell cycle, WNT (inverse), and MYC pathways, adjusting for disease volume and other genomic pathways. Overall survival rates differed 2-fold to 4-fold according to *AR, SPOP* (inverse), WNT (inverse), and cell-cycle alterations. PI3K pathway alterations were not associated with prognosis once adjusted for other factors.

Conclusions: This study identified genomic features associated with prognosis in metastatic castration-sensitive disease that may aid in molecular classification and treatment selection.

Introduction

Treatment options for metastatic castration-sensitive prostate cancer are expanding (1). Both docetaxel and next-generation androgen receptor axis-directed therapies prolong overall survival (2–8). However, it is not clear which patients benefit most from intensified therapies, which increase toxicity and cost, or how clinical disease phenotypes can be used to find a drug class more likely to benefit a patient (1, 9). Some but not all trials show more favorable outcomes with docetaxel for patients with high-volume disease, characterized by the presence of visceral metastases or high-risk bone metastases (2, 10, 11). Similarly, it has been suggested that patients who initially presented with *de novo* metastases and those who developed metastatic recurrence after a diagnosis with localized disease may have differences in benefit from therapy (2, 12).

The genomic underpinning of clinical phenotypes in metastatic castration-sensitive prostate cancer has not been elucidated to date. It is also incompletely understood which tumor DNA alterations are drivers of aggressiveness in prostate cancer (13, 14), especially in castration-sensitive disease (1).

We set out to assess oncogenic alterations in tumor signaling pathways as the mechanistic basis of clinical phenotypes defined by disease volume and by timing of metastasis (*de novo* vs. metastatic recurrence). We further aimed to determine which genomic alterations are associated with clinical outcomes in metastatic castration-sensitive prostate cancer. We addressed these questions using clinical-grade–targeted tumor sequencing in a large cohort of patients with metastatic castration-sensitive prostate cancer.

Materials and Methods

Patient population and clinical characteristics

This study included patients with prostate cancer who underwent institutional review board-approved paired tumor–blood sequencing at a tertiary referral center after providing written informed consent. The study was conducted in accordance with the U.S. Common Rule. Patients had metastatic castration-sensitive disease at the time a

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Genomic and clinical data are available at https://www.cbioportal.org/study/summary?id.prad_mcspc_mskcc_2020.

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Translational Relevance

The genomic landscape of metastatic castration-sensitive prostate cancer is not well defined, and disease stratification for the purpose of initial treatment selection has primarily relied on clinical phenotypes, including volume of disease at the time of metastasis. Here, we describe tumor genomics in a large cohort of patients with metastatic castration-sensitive prostate cancer and show genomic features that are associated with clinical phenotypes, including with disease volume. We identify genomic alterations that are associated with prognosis in metastatic castration-sensitive disease (overall survival and time to castration resistance), demonstrating that alterations in *AR, TP53*, and the cell cycle and MYC pathways occur in tumors with worse prognosis, while alterations in *SPOP* and the WNT pathway occur in tumors with better prognosis. Our findings may aid in molecular classification of metastatic castration-sensitive prostate cancer, and pathways that are prognostically relevant could be targeted in studies of intensified upfront therapy.

specimen for genomic profiling was obtained. Disease extent had to be metastatic and before a potential future date of castration resistance. We first identified patients who had a tumor sample genomically profiled that had been confirmed as a primary or metastasis from prostate cancer by a pathologist. We set up a structured clinical research database for patient and sample characteristics, treatments, and clinical outcomes, including pilot testing for reliability and validity of team-based annotations. Data sources included pathology reports (primarily from rereview; if missing, external reports); patients' self-reported race, ethnicity, and smoking status; and the medical record, drawing from medical oncology, urology, or radiation subspecialty documentation from the initial presentation at the cancer center as well as visits before and after obtaining a sample for genomic profiling. Castration resistance and metastasis were determined by the primary treating physicians; in cases of discrepancies, the record was reviewed by more than one team member. Extent of disease, sites of disease, and bone disease volume were reviewed by the study radiologist (A.G. Wibmer) based primarily on bone scans and computed tomography. For high-volume disease, we used a modified definition, requiring presence of visceral metastases or at least four bone metastases, without requirements for extra-axial metastases unlike prior clinical trials (2, 6, 7); others were classified as low-volume disease. Additional imaging that had been obtained clinically was also reviewed for ambiguous cases.

Genomic profiling

For genomic profiling, both archival specimens or newly obtained specimens were formalin-fixed, and a peripheral blood sample was drawn as a source of germline DNA. Analysis of the germline sample was only performed in patients who signed a specific consent (15). The MSK-IMPACT panel, a hybridization capture-based clinical assay authorized by the FDA, was used to detect single-nucleotide variations, small insertions and deletions, copy number alterations, and structural rearrangements in 341 to 468 genes, depending on assay version (16). Clonality and allele-specific copy numbers were estimated using FACETS (17). Samples that had low tumor purity of 20% or less on histologic assessment or FACETS estimations, no detected somatic mutations, and no copy number alterations were excluded (6.7% overall; 4.1% of samples from metastatic castration-sensitive disease). If repeat samples during progression of metastatic castration-sensitive disease had been obtained (5% of patients), only the first sample was included.

As a measure of genomic instability, the burden of copy number alterations was inferred (fraction genome altered), also leveraging evenly distributed probes that tile common single nucleotide polymorphisms, and corrected for tumor purity (17). Tumor mutation burden was calculated as mutations per megabase. Potential actionability of tumor genomic alterations was annotated using OncoKB as of August 1, 2019 (18). Oncogenic alterations detected in the tumor and/or germline sample were grouped by major signaling pathways from pan-cancer analyses (19, 20), excluding the HIPPO and TGFβ pathways that were altered in <5% of samples within clinical subgroups. *TP53* was not grouped with DNA repair genes and considered separately, as were two individual genes relevant in prostate cancer (*AR, SPOP*).

Analysis

The primary prespecified analysis of clinical phenotype (low-volume disease vs. high-volume disease and *de novo* vs. metastatic recurrence) compared fraction genome altered and prevalence of oncogenic alterations in key signaling pathways. Prevalence differences and ratios were estimated using binomial regression, convergence-assisted by Poisson models (21). We adjusted for predefined factors, including disease volume (in analyses of timing of metastases) or timing of metastases (in analyses of disease volume), origin of sample (prostate in the setting of metastatic disease vs. metastasis), age at sampling, time from metastasis to sample, and continuous exposure to androgen deprivation therapy. Our goal was valid quantification of genomic differences between clinical phenotypes, not decision making; confidence intervals (CI) are not means for testing null hypotheses of no difference.

In a not hypothesis-driven exploratory analysis to probe for individual genes missed by pathway groupings, we compared prevalence of oncogenic gene alterations between phenotypes for genes that were altered in at least 5% of samples in any of the four clinical phenotype groups, using the Fisher exact test for binomial proportions with a Benjamini–Hochberg false-discovery rate for multiple-testing correction.

To assess associations of clinical phenotypes (22, 23) and pathway alterations with time to castration resistance as well as overall survival, we estimated hazard ratio (HR) using Cox proportional hazards regression and differences in three-year restricted mean survival times (24). Follow-up started when the genomically profiled sample was obtained via biopsy, and patients without event were censored at the last clinic visit (for castration resistance) or at last contact (for overall survival). In a sensitivity analysis of pathways and prognosis, the top two most frequently altered genes per pathway (if altered in >2.5% of samples) were assessed individually.

Results

Study population

From 424 patients (median age at sample collection, 66 years; interquartile range, 59–72), a sample obtained while the patient had metastatic castration-sensitive disease was sequenced between May 2015 and September 2018 (**Table 1**). The majority of patients had *de novo* metastatic disease ($n = 275$, 65%). Similar numbers of patients had low-volume disease and high-volume disease. As expected, patients with metastatic recurrences of initially nonmetastatic disease, compared to those with *de novo* metastatic disease, tended to be different in terms of age at sampling, prostate-specific antigen (PSA)

Table 1. Patient and sample characteristics, by disease volume at genomic profiling and by timing of metastases.

		By disease volume		By timing of metastases	
	All ***N* = 424**	**Low-volume disease** ***n* = 211**	**High-volume disease** ***n* = 213**	**Metastatic recurrence** ***n* = 149**	***De novo* metastatic** ***n* = 275**
Age at sampling (years)	65.6 (59.0–71.8)	65.3 (57.3–71.1)	65.8 (60.7–72.6)	68.3 (63.2–74.4)	64.2 (57.1–70.0)
Race					
Asian	12 (4%)	7 (4%)	5 (3%)	1 (1%)	11 (5%)
Black/African American	27 (8%)	13 (7%)	14 (8%)	13 (10%)	14 (6%)
White	312 (88%)	155 (88%)	157 (88%)	111 (88%)	201 (89%)
Other	2 (0%)	1 (0%)	1 (0%)	1 (1%)	1 (0%)
(Missing)	71	35	36	23	48
Timing of metastases					
Metastatic recurrence	149 (35%)	80 (38%)	69 (32%)	149 (100%)	0 (0%)
De novo metastatic	275 (65%)	131 (62%)	144 (68%)	0 (0%)	275 (100%)
Disease volume					
Low-volume disease	211 (50%)	211 (100%)	0 (0%)	80 (54%)	131 (48%)
High-volume disease	213 (50%)	0 (0%)	213 (100%)	69 (46%)	144 (52%)
Gleason grade at diagnosis					
<7	19 (5%)	11 (6%)	8 (4%)	19 (14%)	0 (0%)
3+4	30 (8%)	16 (8%)	14 (7%)	25 (18%)	5 (2%)
4+3	55 (14%)	33 (17%)	22 (12%)	42 (30%)	13 (5%)
8	88 (23%)	44 (23%)	44 (23%)	27 (19%)	61 (25%)
9–10	192 (50%)	90 (46%)	102 (54%)	27 (19%)	165 (68%)
(Missing)	40	17	23	9	31
PSA at diagnosis (ng/mL)	17.2 (6.6–88.8)	12.2 (6.0–42.2)	29.2 (6.9–257.7)	6.7 (4.5–11.0)	46.6 (12.7–245.8)
Sample tissue					
Bone	61 (14%)	38 (18%)	23 (11%)	35 (23%)	26 (9%)
Liver	15 (4%)	0 (0%)	15 (7%)	7 (5%)	8 (3%)
Lung	26 (6%)	0 (0%)	26 (12%)	21 (14%)	5 (2%)
Lymph node	93 (22%)	65 (31%)	28 (13%)	52 (35%)	41 (15%)
Other soft tissue	25 (6%)	3 (1%)	22 (10%)	21 (14%)	4 (1%)
Prostate	204 (48%)	105 (50%)	99 (46%)	13 (9%)	191 (69%)
On continuous ADT	91 (22%)	49 (23%)	42 (20%)	53 (36%)	38 (14%)

Note: Shown are counts (percentages across columns) or median (interquartile range).
Abbreviations: ADT, androgen deprivation therapy; PSA, prostate-specific antigen.

values at cancer diagnosis, disease volume, tissue sample, and continuous treatment with ADT (**Table 1**). Differences between patients with low-volume and high-volume disease were less pronounced, except for differences in profiled metastatic tissue (**Table 1**) due to the definition of high-volume disease. Median follow-up for development of castration resistance was 27.2 months and median follow-up for overall survival was 30.5 months.

Clinical phenotypes and prognosis

Of 213 patients with high-volume disease, 139 developed castration resistance, compared with 101 of the 211 patients with low-volume disease (adjusted HR, 1.84; 95% CI, 1.40–2.41; **Fig. 1A**). Disease volume was even more strongly associated with overall survival, with 77 deaths among patients with high-volume disease compared with 25 deaths among patients with low-volume disease (adjusted HR, 3.71; 95% CI, 2.28–6.02; **Fig. 1B**). Over 36 months (3 years) of follow up, patients with high-volume disease lived, on average, 4.8 months shorter (95% CI, 3.3–6.3) than patients with low-volume disease.

Associations between timing of metastases and prognosis were weaker. Of 275 patients with *de novo* metastatic disease, 175 developed castration resistance, compared with 65 of the 149 patients with metastatic recurrence. Timing of metastases was associated with development of castration resistance when adjusting for age at sampling, disease volume, and time from metastasis to sample (HR, 1.52; 95% CI, 1.11–2.10). However, timing of metastases was not prognostic once accounting for higher PSA at diagnosis and time since initial diagnosis (HR, 0.87; 95% CI, 0.53–1.43; **Fig. 1C**). There were 78 deaths among patients with *de novo* metastatic disease compared to 24 deaths among patients with metastatic recurrences (adjusted HR, 0.95; 95% CI, 0.41–2.21; **Fig. 1D**).

Genomics and clinical phenotypes

The burden of copy number alterations, a consequence of genomic instability and measured as fraction genome altered (median, 32%; interquartile range, 24–48), was higher in high-volume disease than in low-volume disease (adjusted mean difference in percentage points, 4.6; 95% CI, 1.5–7.7; **Fig. 2**). Fraction genome altered was similar between metastatic-recurrent and *de novo* metastatic disease (adjusted mean difference in percentage points, 1.1; 95% CI, -3.1 to 5.2; **Fig. 2**). As expected in noncastration-resistant prostate cancer, tumor mutation burden was mostly low (median, 2.6; interquartile range, 1.8–4.4) and similar across clinical phenotypes (Supplementary Table S1). Tumors had a median of three oncogenic alterations (interquartile range, 2–5; Supplementary Fig. S1). A total of 211 tumors (50%) had at least one potentially actionable alteration (Supplementary Fig. S2).

To assess oncogenic alterations in biologically grounded pathways as a potential basis of clinical phenotypes, 144 genes were grouped into 11 pathways (Supplementary Tables S2 and S3). In high-volume disease, for example, alterations in the NOTCH pathway were

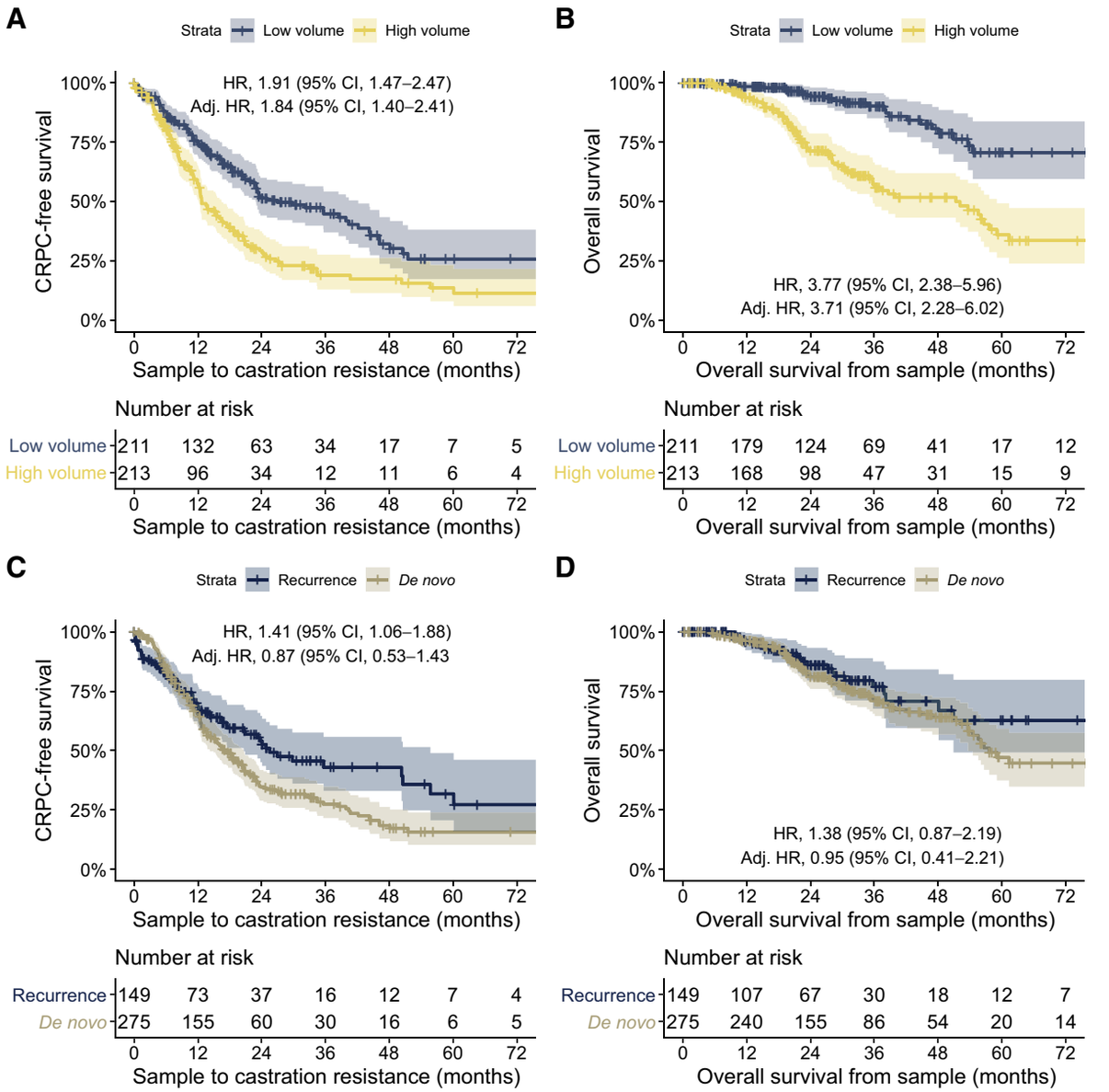

Figure 1.

Clinical phenotypes and outcomes. Kaplan–Meier curves by disease volume (low-volume vs. high-volume disease; **A** and **B**) and by timing of metastases (metastatic recurrence vs. *de novo* metastases; **C** and **D**) for time to castration-resistant prostate cancer (CRPC; **A** and **C**) and overall survival (**B** and **D**). Shaded areas denote 95% CIs for survival functions; hazard ratios are from Cox regression models. Adjusted models include timing of metastases (for models of disease volume) or disease volume (for models of timing of metastases) as well as age at sampling, time from diagnosis to sampling, time from metastasis to sampling, and prostate-specific antigen at cancer diagnosis.

4.2 percentage points more common (95% CI, 0.1–8.3; **Fig. 3A**); the next-ranked candidate pathways were the cell cycle and the epigenetic modifiers pathways. Results persisted when adjusting for modest differences in tumor purity or sequencing depth across clinical phenotypes, particularly by timing of metastases (Supplementary Table S1; Supplementary Figs. S3 and S4).

In comparisons by timing of metastatic disease (**Fig. 3B**), *AR* alterations were 8.6 percentage points (95% CI, 3.3–13.8) more frequent in metastatic recurrences than in *de novo* metastatic disease. However, this association was null once adjusted for continuous ADT treatment at the time of sampling. Alterations in SPOP and the cell-cycle and NOTCH pathways were less common in *de novo* metastatic disease, but results were imprecise.

There were no single genes differentially altered by volume of disease (Supplementary Fig. S5; Supplementary Table S4). *CDK12* alterations were 6.7 percentage points (95% CI, 3.0–10.4) more common in *de novo* metastatic disease compared with metastatic recurrence (false discovery rate, 0.037; Supplementary Table S5).

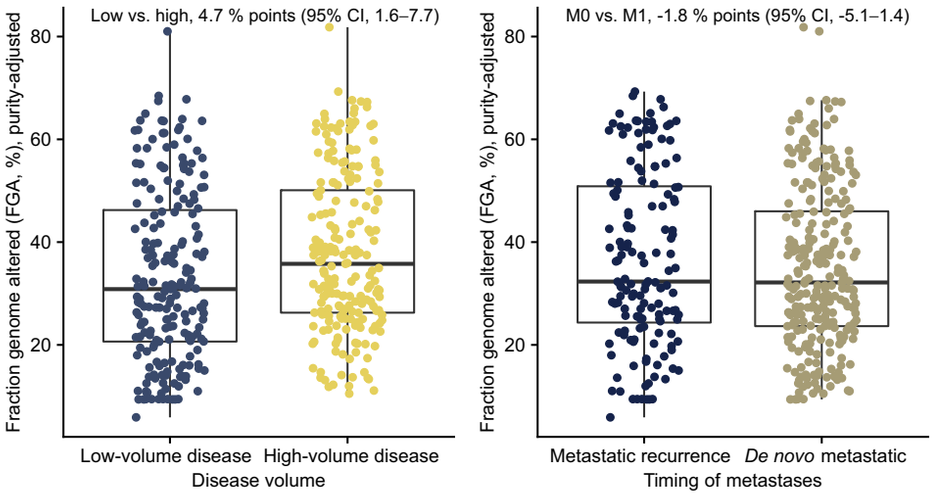

Figure 2.
Fraction genome altered, the proportion of genes with copy number alterations and a consequence of genomic instability, is compared by clinical phenotypes (with unadjusted mean group difference and 95% CI). Thick lines indicate medians, boxes span interquartile ranges.

Genomic alterations and prognosis

Rates of progression to castration resistance were 1.6-fold to 5-fold higher with alterations in *AR* and *TP53* as well as the cell-cycle and MYC pathways and approximately 1.5-fold lower with *SPOP* and WNT pathway alterations (**Fig. 4A**, right). Associations persisted in a multivariable model that also adjusted for alterations in all other pathways (**Fig. 4A**, right). Over 36 months of follow-up, on average, patients with pathway alterations had 6 to 11 months shorter (*AR*, cell cycle, MYC, *TP53*) and 2 to 5 months longer times to castration resistance (WNT, *SPOP*; **Fig. 4A**, left; Supplementary Table S6).

Overall survival rates (**Fig. 4B**, right) differed 2-fold to 4-fold according to alterations in *AR, SPOP* (inverse), and the cell cycle and WNT (inverse) pathways. Over 36 months of follow-up, differences in average survival were between 1 to 4 months (**Fig. 4B**, left).

A potential association between TP53 alterations and overall survival was attenuated in a multivariable model, as were associations of NOTCH pathway alterations with castration resistance; estimates for overall survival were imprecise. Notably, associations of alterations in DNA repair, PI3K, RAS/RAF/MAPK, and epigenetic modifiers pathways with castration resistance and overall survival were null, particularly in multivariable models (**Fig. 4**). In a *post hoc* sensitivity analysis, assessing the most commonly altered genes of each pathway (Supplementary Fig. S6), results were generally consistent. For *BRCA2* alterations, results were compatible with higher rates of castration resistance (adjusted HR, 1.60; 95% CI, 0.98–2.63); associations with overall survival could not be ruled out (adjusted HR, 1.08; 95% CI, 0.50–2.33). *PTEN* alterations were associated with a worse prognosis only if assessed in isolation; in multivariable models, there were no strong associations with castration resistance (adjusted HR, 1.27; 95% CI, 0.94–1.73) and overall survival (adjusted HR, 0.95; 95% CI, 0.58–1.56).

Discussion

In this large clinical-grade sequencing study of paired tumor–blood samples from patients with metastatic castration-sensitive prostate cancer, we identified genomic characteristics of high-volume disease and genomic characteristics of tumors with inferior prognosis. Tumors from high-volume disease had a higher fraction genome altered indicative of genomic instability, more oncogenic alterations in the NOTCH, cell cycle, and potentially epigenetic modifiers pathways, and an inferior prognosis compared with low-volume disease. We were unable to detect unequivocal differences between *de novo* metastatic disease and recurrent metastatic disease in both clinical outcomes and genomics, except for more frequent *CDK12* alterations in *de novo* metastatic disease. Importantly, we demonstrate that specific oncogenic signaling pathways are associated with two major clinical endpoints in metastatic castration-sensitive prostate cancer, castration resistance, and overall survival. Alterations in *AR, TP53*, and the cell-cycle and MYC pathways occurred in tumors with worse prognosis, while tumors with *SPOP* and WNT pathway alterations had better prognosis.

Genomic "landscape" studies of tumor DNA profiling in prostate cancer generally excluded metastatic castration-sensitive tumors, focusing on localized disease (25–30) or metastatic castration-resistant disease (13, 31–37). Exceptions are two smaller studies (14, 15) and a study that included archival castration-naïve tumors only from patients who developed castration-resistant disease (38). We connected genomics with clinical phenotypes and clinical outcomes in castration-sensitive disease. Differences between high-volume and low-volume disease in genomic instability, a poor prognostic factor at least in early prostate cancer (39, 40), may explain some of the differences in prognosis by disease volume, yet they are currently difficult to address therapeutically. Our focus on well-defined oncogenic signaling pathways (19) suggested some potentially better targets for the high-volume disease, a subgroup that has long been recognized to have inferior prognosis (22). Oncogenic alterations in single genes of the NOTCH pathway, such as *SPEN* (34), were individually uncommon, yet our analysis suggests this pathway as one potential driver of high-volume disease. NOTCH alterations have been suggested as a determinant of resistance to docetaxel (41), a therapy that some clinicians recommend just for patients with high-volume disease. Overall, it is critical that differences in pathway alteration prevalence be validated in future tumor sequencing studies. More precise estimates are particularly needed for additional candidates, such as the epigenetic modifiers pathway, *SPOP*, and *CDK12*, which emerged in a gene-level discovery effort. Future studies, which may need to be an order of magnitude larger, should also assess how genomic alterations differ by specific metastatic site, a distinction that a "volume" definition obscures.

De novo metastatic disease was associated with modestly inferior outcomes in unadjusted analyses compared with metastatic recurrences. Surprisingly however, once we accounted for systematic differences

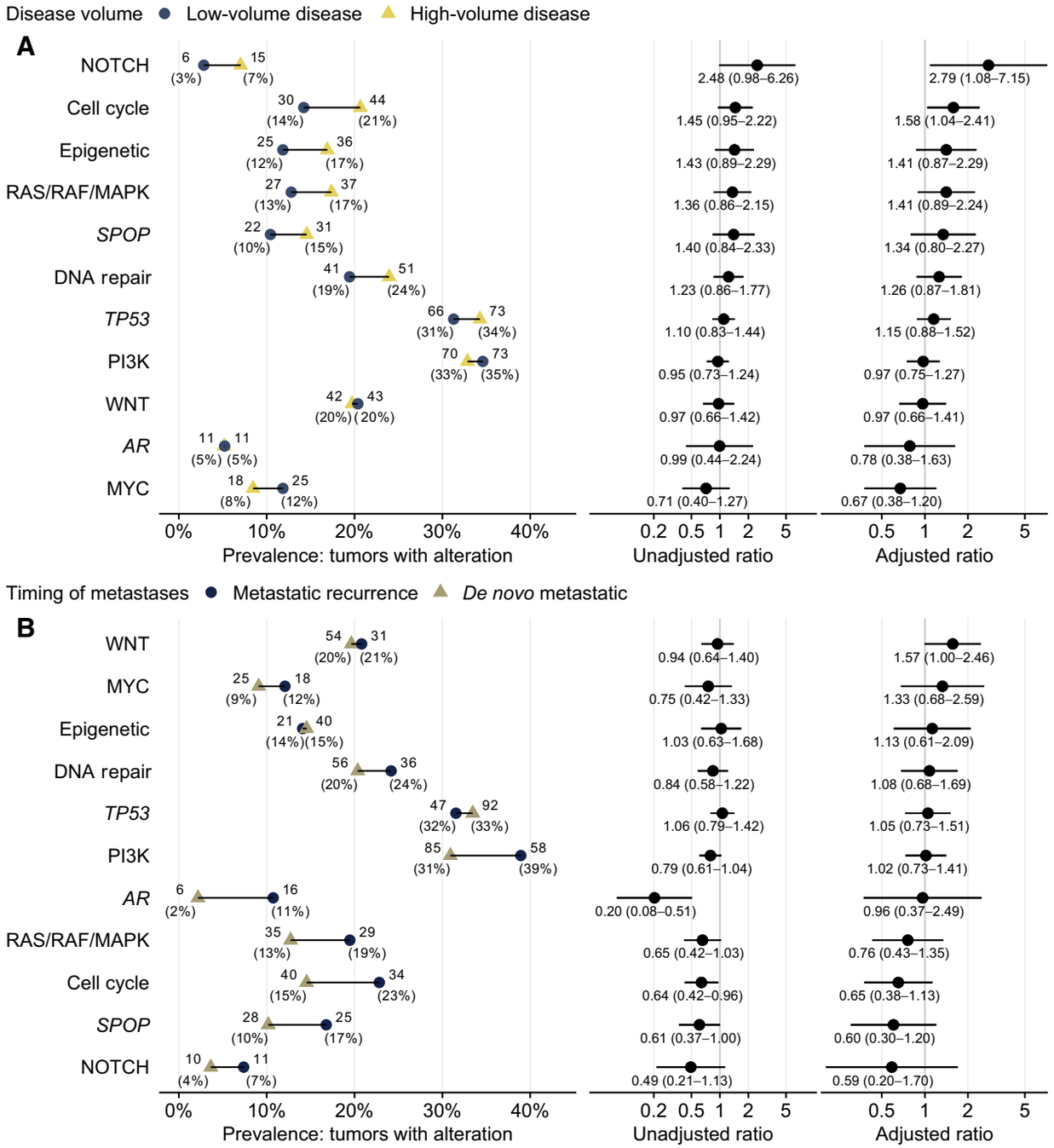

Figure 3.

Alterations in genomic pathways by clinical phenotype (**A**, disease volume; **B**, timing of metastases). The left panels show absolute prevalences of pathway alterations, including counts of samples with alterations (above symbols) and prevalence (below symbols). The right panels show prevalence ratios between clinical phenotypes (with 95% CIs). Higher ratios indicate that pathways had more frequent oncogenic alterations in high-volume disease (**A**) or in *de novo* metastatic disease (**B**). Pathways are ordered according to adjusted prevalence ratios. Adjusted models include timing of metastases (for models of disease volume) or disease volume (for models of timing of metastases), type of sample (prostate in the setting of metastatic disease vs. metastasis), age at sampling, time from metastasis to sample, whether the sample was obtained while on continuous androgen deprivation therapy, and sequencing depth.

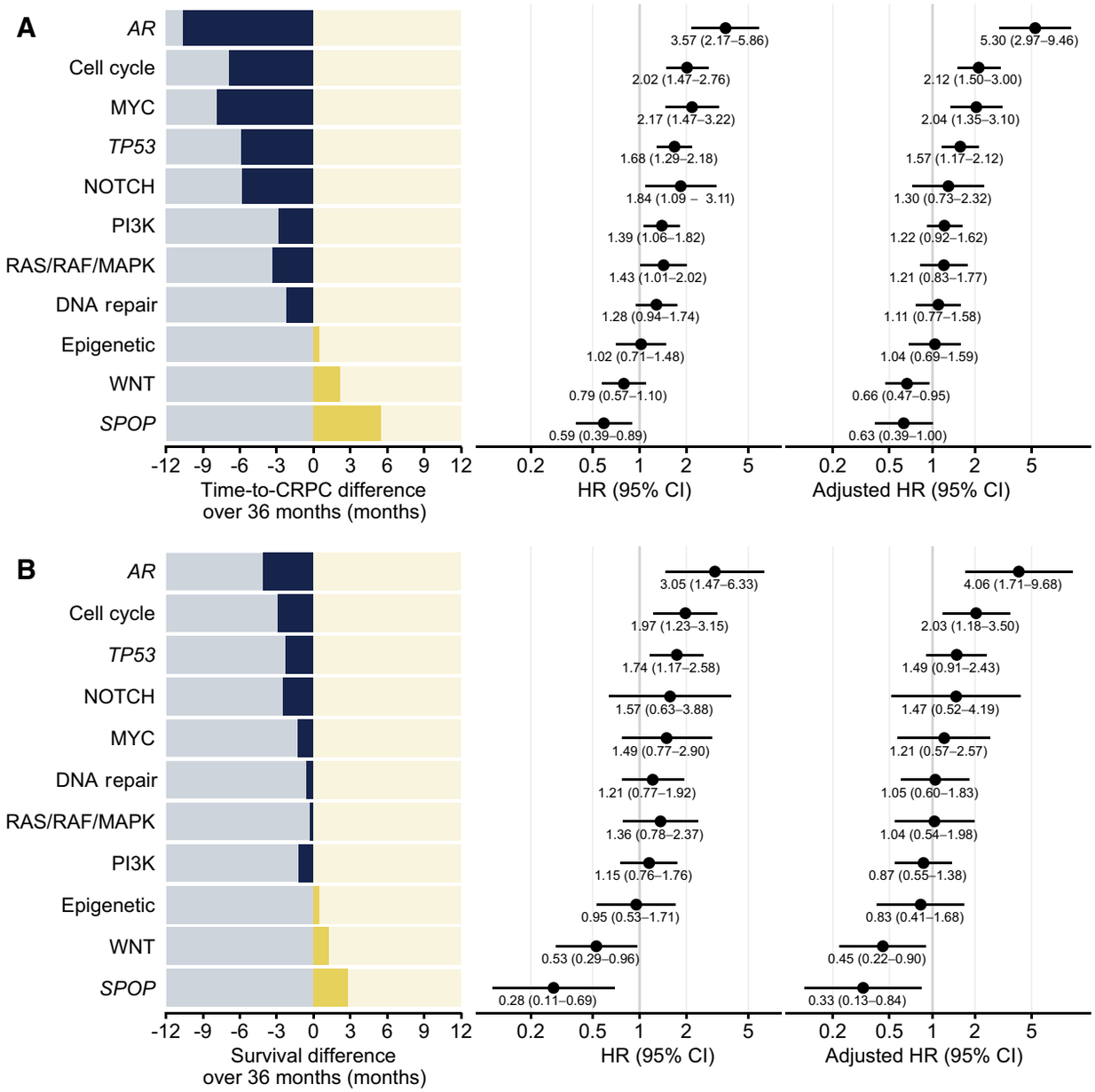

Figure 4.

Genomic pathways and time to castration resistance (**A**) and overall survival (**B**). The left panels show absolute differences in mean time to castration resistance or survival over a 36-month period associated with an oncogenic pathway alteration. The two right panels show hazard ratios (95% CI) from Cox proportional hazards models. Adjusted models include all genomic pathways mutually, disease volume, timing of metastases, age at sampling, PSA at cancer diagnosis, type of sample (prostate vs. metastasis), and fraction genome altered.

between patients who were diagnosed with metastatic prostate cancer *de novo* and those who experienced metastatic recurrence of initially nonmetastatic disease, we were unable to detect prognostic differences. The relatively small number of metastatic recurrences made results imprecise, and systematic differences between patient groups may have obscured true differences in genomics. Nonetheless, our data suggest that the timing of metastasis is likely not a strong prognostic factor in metastatic castration-sensitive disease, and differential treatment just based on this clinical feature may not be needed.

Genomic alterations that characterized tumors with poor prognosis did not appear to be created equal. *AR* alterations, while uncommon in the metastatic castration-sensitive setting, were the strongest predictor of development of castration resistance and death. Relatively strong associations between cell cycle and MYC pathways, as well as *SPOP* and *TP53* alterations, with prognosis in this disease state are concordant with observations in metastatic castration-resistant prostate cancer (13). However, WNT pathway alterations were associated with better prognosis, including longer overall survival when accounting for

clinical factors and other genomic alterations. This observation is in contrast to two smaller studies in metastatic castration-resistant prostate cancer (37, 42), where WNT pathway alterations, including *CTNNB1*, were associated with worse prognosis.

DNA repair and PI3K pathway alterations had weak to modest associations with development of castration resistance when assessed in isolation. Neither pathway remained associated with clinical outcomes once accounting for other genomic features and clinical factors. It is possible that specific alterations summarized in the DNA repair pathway could be drivers of poor prognosis, such as *BRCA2*. For the PI3K pathway and *PTEN* alterations, our data suggest no strong associations with disease progression and overall survival in this disease state, consistent with results in castration-resistant disease (13).

This study shares its main limitation with all other tumor sequencing studies published on prostate cancer to date (13–15, 25–38) in that it was hospital-based, relying on patients seen at an academic referral center. It is widely appreciated that such study populations are not representative of patients nationally or globally (affecting generalizability). Perhaps more importantly, such a restriction may also introduce selection bias (affecting validity) that is difficult to address analytically, particularly since only a subset of patients undergoes tumor biopsies. An important next step will be to nest studies of the tumor genome in ongoing population-based cohort studies with tumor biorepositories that can address these issues. With evolving sequencing technologies, future studies will also explore the cancer genome more broadly, complementary to our approach that concentrated on deep-coverage sequencing of known cancer susceptibility genes with curated functional annotation.

Taken together, our study takes the first step towards a genomic classification of metastatic castration-sensitive prostate cancer. In describing some of the genomic alterations that occurred more frequently in high-volume disease, we highlight how specific oncogenic signaling pathways may be a potentially targetable biologic underpinning of this poor-prognosis subtype. Our results on prognostically relevant genomic pathways highlight potential drivers of disease progression that may help prioritization in developing targeted therapies and that could be targeted in studies of intensified upfront therapy.

Disclosure of Potential Conflicts of Interest

H.I. Scher is an employee/paid consultant for Ambry Genetics Corp/Konica Minota, Inc., Pfizer, WCG Oncology, reports receiving commercial research grants to the institution from Epic Science, Illumina, Janssen, Menarini Silicon Biosystems, ThermoFisher, and is an advisory board member/unpaid consultant for Amgen, Bayer, ESSA Pharma, Janssen Research & Development, Janssen Biotech, Menarini Silicon Biosystems, and Sanofi Aventis. M.J. Morris is an advisory board member/unpaid consultant for Bayer, Blue Earth, Advanced Accelerator Applications, Tokai, ORIC, and Johnson and Johnson. D.B. Solit is an employee/paid consultant for Pfizer, Loxo Oncology, Lilly Oncology, QED Therapeutics, Vivideon Therapeutics, and Illumina. P.W. Kantoff reports paid consulting positions with Bavarian Nordic, SEER, DRGT, Progenity, and OncoCellMDX, ownership interest in Context, DRGT, Placon, and SEER, advisory board relationships with Merck, Roche, Context, and Tarveda, and other remuneration from Poling Law Firm. W. Abida is an employee/paid consultant for Clovis Oncology, Janssen Pharmaceutica, MORE Health, ORIC Pharmaceuticals, and reports receiving commercial research grants from AstraZeneca, Zenith Epigenetics, Clovis Oncology, and GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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References

1. VanderWeele DJ, Antonarakis ES, Carducci MA, Dreicer R, Fizazi K, Gillessen S, et al. Metastatic hormone-sensitive prostate cancer: clinical decision making in a rapidly evolving landscape of life-prolonging therapy. J Clin Oncol 2019;37:2961–7.
2. Kyriakopoulos CE, Chen YH, Carducci MA, Liu G, Jarrard DF, Hahn NM, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survival analysis of the randomized phase III E3805 CHAARTED trial. J Clin Oncol 2018;36:1080–7.
3. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017;377:352–60.
4. Gravis G, Boher JM, Joly F, Soulie M, Albiges L, Priou F, et al. Androgen deprivation therapy (ADT) plus docetaxel versus ADT alone in metastatic non castrate prostate cancer: impact of metastatic burden and long-term survival analysis of the randomized phase 3 GETUG-AFU15 trial. Eur Urol 2016;70:256–62.
5. James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med 2017;377:338–51.
6. Davis ID, Martin AJ, Stockler MR, Begbie S, Chi KN, Chowdhury S, et al. Enzalutamide with standard first-line therapy in metastatic prostate cancer. N Engl J Med 2019;381:121–31.
7. Chi KN, Agarwal N, Bjartell A, Chung BH, Pereira de Santana Gomes AJ, Given R, et al. Apalutamide for metastatic, castration-sensitive prostate cancer. N Engl J Med 2019;381:13–24.
8. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet 2016;387:1163–77.
9. Gillessen S, Attard G, Beer TM, Beltran H, Bossi A, Bristow R, et al. Management of patients with advanced prostate cancer: the report of the advanced prostate cancer consensus conference APCCC 2017. Eur Urol 2018;73:178–211.

10. Gravis G, Boher JM, Chen YH, Liu G, Fizazi K, Carducci MA, et al. Burden of metastatic castrate naive prostate cancer patients, to identify men more likely to benefit from early docetaxel: further analyses of CHAARTED and GETUG-AFU15 studies. Eur Urol 2018;73:847–55.
11. Clarke NW, Ali A, Ingleby FC, Hoyle A, Amos CL, Attard G, et al. Addition of docetaxel to hormonal therapy in low- and high-burden metastatic hormone sensitive prostate cancer: long-term survival results from the STAMPEDE trial. Ann Oncol 2019;30:1992–2003.
12. Finianos A, Gupta K, Clark B, Simmens SJ, Aragon-Ching JB. Characterization of differences between prostate cancer patients presenting with *de novo* versus primary progressive metastatic disease. Clin Genitourin Cancer 2018;16:85–9.
13. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci U S A 2019;116:11428–36.
14. Hamid AA, Gray KP, Shaw G, MacConaill LE, Evan C, Bernard B, et al. Compound genomic alterations of TP53, PTEN, and RB1 tumor suppressors in localized and metastatic prostate cancer. Eur Urol 2019;76:89–97.
15. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol 2017;2017.
16. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 2015;17:251–64.
17. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res 2016;44:e131.
18. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. JCO Precis Oncol 2017;2017.
19. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic signaling pathways in the cancer genome atlas. Cell 2018;173:321–37 e10.
20. Knijnenburg TA, Wang L, Zimmermann MT, Chambwe N, Gao GF, Cherniack AD, et al. Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. Cell Rep 2018;23:239–54 e6.
21. Spiegelman D, Hertzmark E.Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 2005;162:199–200.
22. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, et al. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med 1989;321:419–24.
23. Francini E, Gray KP, Xie W, Shaw GK, Valenca L, Bernard B, et al. Time of metastatic disease presentation and volume of disease are prognostic for metastatic hormone sensitive prostate cancer (mHSPC). Prostate 2018;78:889–95.
24. Trinquart L, Bill-Axelson A, Rider JR. Restricted mean survival times to improve communication of evidence from cancer randomized trials and observational studies. Eur Urol 2019;76:137–9.
25. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
26. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9.
27. Beltran H, Wyatt AW, Chedgy EC, Donoghue A, Annala M, Warner EW, et al. Impact of therapy on genomics and transcriptomics in high-risk prostate cancer treated with neoadjuvant docetaxel and androgen deprivation therapy. Clin Cancer Res 2017;23:6802–11.
28. Fraser M, Sabelnykova VY, Yamaguchi TN, Heisler LE, Livingstone J, Huang V, et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature 2017;541:359–64.
29. Wedge DC, Gundem G, Mitchell T, Woodcock DJ, Martincorena I, Ghori M, et al. Sequencing of prostate cancers identifies new cancer genes, routes of progression and drug targets. Nat Genet 2018;50:682–92.
30. Gerhauser C, Favero F, Risch T, Simon R, Feuerbach L, Assenov Y, et al. Molecular evolution of early-onset prostate cancer identifies molecular risk markers and clinical trajectories. Cancer Cell 2018;34:996–1011 e8.
31. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215–28.
32. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med 2016;22:369–78.
33. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–43.
34. Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, et al. The long tail of oncogenic drivers in prostate cancer. Nat Genet 2018;50:645–51.
35. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.
36. Quigley DA, Dang HX, Zhao SG, Lloyd P, Aggarwal R, Alumkal JJ, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. Cell 2018;174:758–69 e9.
37. Chen WS, Aggarwal R, Zhang L, Zhao SG, Thomas GV, Beer TM, et al. Genomic drivers of poor prognosis and enzalutamide resistance in metastatic castration-resistant prostate cancer. Eur Urol 2019;76:562–71.
38. Mateo J, Seed G, Bertan C, Rescigno P, Dolling D, Figueiredo I, et al. Genomics of lethal prostate cancer at diagnosis and castration resistance. J Clin Invest 2020;130:1743–51.
39. Hieronymus H, Murali R, Tin A, Yadav K, Abida W, Moller H, et al. Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death. Elife 2018;7.
40. Stopsack KH, Whittaker CA, Gerke TA, Loda M, Kantoff PW, Mucci LA, et al. Aneuploidy drives lethal progression in prostate cancer. Proc Natl Acad Sci U S A 2019;116:11390–5.
41. Domingo-Domenech J, Vidal SJ, Rodriguez-Bravo V, Castillo-Martin M, Quinn SA, Rodriguez-Barrueco R, et al. Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch- and hedgehog-dependent tumor-initiating cells. Cancer Cell 2012;22:373–88.
42. Wang L, Dehm SM, Hillman DW, Sicotte H, Tan W, Gormley M, et al. A prospective genome-wide study of prostate cancer metastases reveals association of wnt pathway activation and increased cell cycle proliferation with primary resistance to abiraterone acetate-prednisone. Ann Oncol 2018;29:352–60.