

TP53 Outperforms Other Androgen Receptor Biomarkers to Predict Abiraterone or Enzalutamide Outcome in Metastatic Castration-Resistant Prostate Cancer



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Abstract

Purpose: To infer the prognostic value of simultaneous androgen receptor (AR) and TP53 profiling in liquid biopsies from patients with metastatic castration-resistant prostate cancer (mCRPC) starting a new line of AR signaling inhibitors (ARSi).

Experimental Design: Between March 2014 and April 2017, we recruited patients with mCRPC ($n = 168$) prior to ARSi in a cohort study encompassing 10 European centers. Blood samples were collected for comprehensive profiling of CellSearch-enriched circulating tumor cells (CTC) and circulating tumor DNA (ctDNA). Targeted CTC RNA sequencing (RNA-seq) allowed the detection of eight AR splice variants (ARV). Low-pass whole-genome and targeted gene-body sequencing of AR and TP53 was applied to identify amplifications, loss of heterozygosity, mutations, and structural rearrangements in ctDNA. Clinical or radiologic progression-free survival (PFS) was estimated by Kaplan–Meier analysis, and independent associations were determined using multivariable Cox regression models.

Results: Overall, no single AR perturbation remained associated with adverse prognosis after multivariable analysis. Instead, tumor burden estimates (CTC counts, ctDNA fraction, and visceral metastases) were significantly associated with PFS. TP53 inactivation harbored independent prognostic value [HR 1.88; 95% confidence interval (CI), 1.18–3.00; $P = 0.008$], and outperformed ARV expression and detection of genomic AR alterations. Using Cox coefficient analysis of clinical parameters and TP53 status, we identified three prognostic groups with differing PFS estimates (median, 14.7 vs. 7.51 vs. 2.62 months; $P < 0.0001$), which was validated in an independent mCRPC cohort ($n = 202$) starting first-line ARSi (median, 14.3 vs. 6.39 vs. 2.23 months; $P < 0.0001$).

Conclusions: In an all-comer cohort, tumor burden estimates and TP53 outperform any AR perturbation to infer prognosis.

See related commentary by Rebello et al., p. 1699

Introduction

The androgen receptor (AR) remains the central target in the treatment of metastatic prostate cancer (mPC), which eventually

develops lethal castration resistance (mCRPC), for which current standard-of-care therapies lack prognostic biomarkers. Although second-generation AR signaling inhibitors (ARSi) are effective in

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Translational Relevance

Although single AR biomarkers and TP53 gene perturbations have been shown to be of prognostic value, no large-scale studies have simultaneously investigated multiple AR and TP53 biomarkers. Synchronous profiling of all outcome-associated somatic alterations in AR and TP53 in liquid biopsies of patients with metastatic castration-resistant prostate cancer (mCRPC; $n = 168$) prior to abiraterone and enzalutamide treatment demonstrates that TP53, but not AR, is an independently associated negative response biomarker. We present and validate a three-stratum risk stratification system using clinical variables and TP53 alterations to assist treatment decisions in mCRPC. Hence, efficient prognostication of patients with mCRPC, before starting abiraterone or enzalutamide treatment, is achievable by combining TP53 liquid biopsy profiling and clinical variables. Further comprehensive AR profiling studies are required to determine which patients have a relevant AR biomarker output.

both chemotherapy-naïve and -pretreated mCRPC, *a priori* resistance is observed in up to 40% of patients (1). Genomic analyses revealed pivotal roles for AR, PI3K, DNA repair, and cell-cycle pathways in mPC (2). AR alterations encompass copy number variants (CNV), mutations, and the expression of AR splice variants (ARV), which are associated with poor outcome on ARSi treatment (3–6). In addition, intra-AR genomic structural rearrangements (GSR) have been described in (pre)clinical mCRPC samples (7–9). DNA repair or PI3K pathway aberrations have been proposed as ARSi biomarkers, but the results are currently discordant (10–13). However, TP53 inactivation has consistently been associated with poor prognosis (11, 12, 14). To date, information on the simultaneous detection of multiple AR perturbations and other genomic events, and their association with outcome is lacking (9). Here, we investigated the prognostic value of a combined AR- and TP53-focused circulating tumor cell (CTC) and circulating tumor DNA (ctDNA) liquid biopsy to identify prognostic biomarkers for ARSi.

Materials and Methods

A detailed description of materials and methods is provided in Supplementary Materials and Methods. In brief, we recruited patients with mCRPC with histologically confirmed prostate adenocarcinoma, starting a new line of second-generation ARSi, that is, abiraterone or enzalutamide, for biochemically defined progressive disease (PD) according to European Association of Urology (EAU) guidelines (1). At baseline, 10–12 weeks follow-up of PD, a blood sample was collected for CellSearch CTC enumeration, CTC-ARV-targeted RNA sequencing (RNA-seq), and low-pass whole genome and targeted sequencing of plasma cell-free DNA (cfDNA) for AR and TP53 to infer amplifications, loss of heterozygosity, mutations, and structural rearrangements, as described previously (9). Treating physicians were blinded to the CTC/ctDNA results during clinical practice. Primary outcome measure was progression-free survival (PFS), according to Prostate Cancer Clinical Trials Working Group 3 criteria (15). Secondary outcomes encompassed PSA waterfall plots and confirmed $\geq 50\%$ PSA response rates at 10–12 weeks (16), and overall survival (OS). The association between somatic variations and

time-to-event outcomes were evaluated by Kaplan–Meier (KM) analysis with log-rank test and assessment of effect by uni- (UV-Cox) and multivariable Cox (MV-Cox) regression models, including the following covariates: PSA level, CTC count, and ctDNA fraction at baseline, prior chemotherapy, prior exposure to abiraterone or enzalutamide, and presence of visceral metastases (5, 17, 18). Cooccurrence was tested using χ^2 or Fisher exact tests. Correlations and comparisons by Pearson, Spearman, and Mann–Whitney tests, respectively. Statistical analysis was performed in R (v3.3.2), with two-sided $P < 0.05$ considered as statistically significant. This study was conducted in accordance with the Declaration of Helsinki, after a clinical protocol was reviewed, and ethical approval was acquired by ethical committees in Belgium (Antwerp University Hospital, Edegem, Belgium, registration number: B300201524217), The Netherlands (Erasmus Medical Centre Rotterdam, Rotterdam, the Netherlands, registration number: NL53474.078.15) and Sweden (Karolinska University Hospital, Solna, Sweden, registration number: 2016/101-32). All patients provided a written informed consent document.

Results

Patient cohort and sample collection

Between March 2014 and April 2017, 168 patients with mCRPC were recruited, starting ARSi (Supplementary Fig. S1A; Table 1). In total, 148 of 168 (88.1%) patients had not received prior ARSi for CRPC. We profiled 249 CTC and 252 ctDNA samples, with a baseline ARV and AR/TP53 gene profile in 131 and 145 evaluable samples, respectively, and matching datasets in 108 cases (Supplementary Fig. S1B). The median PFS in the studied cohort was 6.8 months [interquartile range (IQR): 3.4–13.2], with 129 of 168 (76.8%) patients progressed at the time of analysis. The median follow-up time was 12.4 months (IQR: 7–17.3), with 65 of 168 (38.7%) patients deceased at the time of analysis.

CTC and ctDNA profiling

CTC-ARV sequencing at baseline ($n = 131$), follow-up ($n = 61$) and PD ($n = 57$) demonstrated dominance of the full-length AR isoform, with ARV fractions ranging from $0.5\% \pm 1.6\%$, $0.06\% \pm 0.1\%$ to $1.6\% \pm 4.9\%$, respectively (Supplementary Fig. S2A). ARV expression demonstrated inter- and inpatient heterogeneity and was more prevalent in samples harvested at the time of PD. At baseline, ARVs were frequently coexpressed with AR-V3 (53/131, 40.4%) and AR-V7 (34/131, 25.9%) being the most prevalent constitutively active ARVs (Supplementary Table S1). AR45 and AR-V3 were most abundantly expressed (Supplementary Figs. S2B and S2C and S3).

Plasma AR sequencing revealed genomic alterations in 63 of 145 (43.4%), 14 of 45 (31.1%), and 33 of 62 (53.2%) patients at baseline, follow-up, and PD, respectively (Supplementary Fig. S4A). AR was amplified in 54 of 145 (37.2%), 9 of 45 (20%), and 26 of 62 (41.9%) patients at baseline, follow-up, and progression, respectively. Hotspot mutations were detected in 13 of 145 (8.9%), 3 of 45 (6.7%), and 7 of 62 (11.3%) patients at baseline, follow-up, and PD, respectively, with p.L702H and p.H875Y as most frequently detected. Tiled AR sequencing revealed GSRs in 26 of 145 (17.9%), 7 of 45 (15.6%), and 16 of 62 (25.8%) patients at baseline, follow-up, and PD, respectively. Excluding structural variants of unknown significance (SVUS) and focusing on rearrangements affecting coding or cryptic exon regions, an increased prevalence was observed at the time of PD compared

Table 1. Patient characteristics

| | All patients |
|--|--------------------|
| | <i>n</i> (%) |
| Patients | 168 (100%) |
| Age at registration, year, mean \pm SD | (76 \pm 7.7) |
| Tumor stage at diagnosis | |
| T1/2 | 45 (26.79%) |
| T3/4 | 41 (24.40%) |
| M1 | 45 (26.79%) |
| Node positive | 12 (7.14%) |
| Not specified | 25 (14.88%) |
| Gleason score at diagnosis | |
| ≤ 7 | 63 (37.50%) |
| 8–10 | 83 (49.40%) |
| Not specified | 22 (13.10%) |
| Primary treatment | |
| ADT (\pm RT) | 76 (45.24%) |
| Radical Px (\pm RT) | 61 (36.31%) |
| Radical Px + ADT | 5 (2.98%) |
| Other | 15 (8.93%) |
| Not specified | 11 (6.55%) |
| Previous chemotherapy | |
| Chemotherapy naïve | 100 (59.52%) |
| Chemotherapy pretreated | 68 (40.48%) |
| Previous ARSi for CRPC | |
| No | 148 (88.10%) |
| Yes | 20 (11.90%) |
| Initiating therapy | |
| Abiraterone acetate | 111 (66.07%) |
| Enzalutamide | 57 (33.93%) |
| Metastatic burden at start therapy | |
| LN only | 20 (11.90%) |
| Bone only | 73 (43.45%) |
| Bone and LN | 45 (26.79%) |
| Visceral and bone and/or LN | 26 (15.48%) |
| Not specified | 4 (2.38%) |
| Baseline blood chemistry | Median (IQR) |
| LDH, U/L (<i>n</i> = 119) | 335 (217–655.5) |
| AP, U/L (<i>n</i> = 123) | 102 (73–160.5) |
| PSA, μ g/L (<i>n</i> = 164) | 36.92 (13.5–144.9) |
| Baseline CTCs | Median (IQR) |
| CTC, #/7.5 mL (<i>n</i> = 164) | 2 (0–17.5) |

Abbreviations: ADT, androgen deprivation therapy; AP, alkaline phosphatase; IQR, interquartile range; LDH, lactate dehydrogenase; LN, lymph node; Px, prostatectomy; RT, radiotherapy.

with baseline [12/62 (19.4%) vs. 12/145 (8.3%) patients; χ^2 test, $P = 0.04$]. Also, the number of events in GSR-positive patients increased at progression (Mann–Whitney U test, $P = 0.014$), accompanied with more rearrangement complexity (Supplementary Fig. S4B and S4C). GSRs typically cooccurred with AR amplifications, with 43 of 49 (87.8%) GSR-positive samples having gained copy numbers (χ^2 test, $P < 0.0001$).

Plasma *TP53* sequencing revealed genomic alterations in 36 of 145 (24.8%), 12 of 45 (26.7%), and 27 of 62 (42.9%) patients at baseline, follow-up, and PD, respectively, with biallelic inactivation in 24 of 36 (66.7%), 6 of 12 (50.0%), and 17 of 26 (65.4%) of *TP53*-perturbed patients, respectively.

Integrating ARV data with genomic alterations in the AR gene

Comprehensive CTC and ctDNA profiles were available for 108, 31, and 49 patients at baseline, follow-up, and PD, respectively (Fig. 1). Of note, we observed that CTC-negative enumeration samples were occasionally positive for ctDNA and/or ARV expression in their temporally matched plasma and/or blood samples, respectively (Supplementary Fig. S5). For AR, when combining CNVs, GSRs, mutations and ARVs (excluding AR-

V1/2, which were expressed in nearly all patients), we detected perturbations in 77 of 108 (71.3%), 23 of 31 (74.2%), and 48 of 49 (97.9%) patients at baseline, follow-up, and PD, respectively. ARV expression (excluding AR-V1/2) occurred in patients with and without AR amplifications, which at baseline suggested a higher prevalence in AR-amplified disease (65.9% vs. 45.3%; χ^2 test, $P = 0.05$). However, ARV abundance was higher in AR-amplified ($P = 0.027$) or -rearranged ($P = 0.002$) samples obtained at PD. Interestingly, when focusing on exon1-deleting GSRs (i.e., ARv45), we observed increased expression levels of the exon 1b-2 junction, corresponding to the AR45 isoform (Mann–Whitney U test, $P = 0.002$; Supplementary Fig. S6).

CTC-ARV profiling and clinical outcome

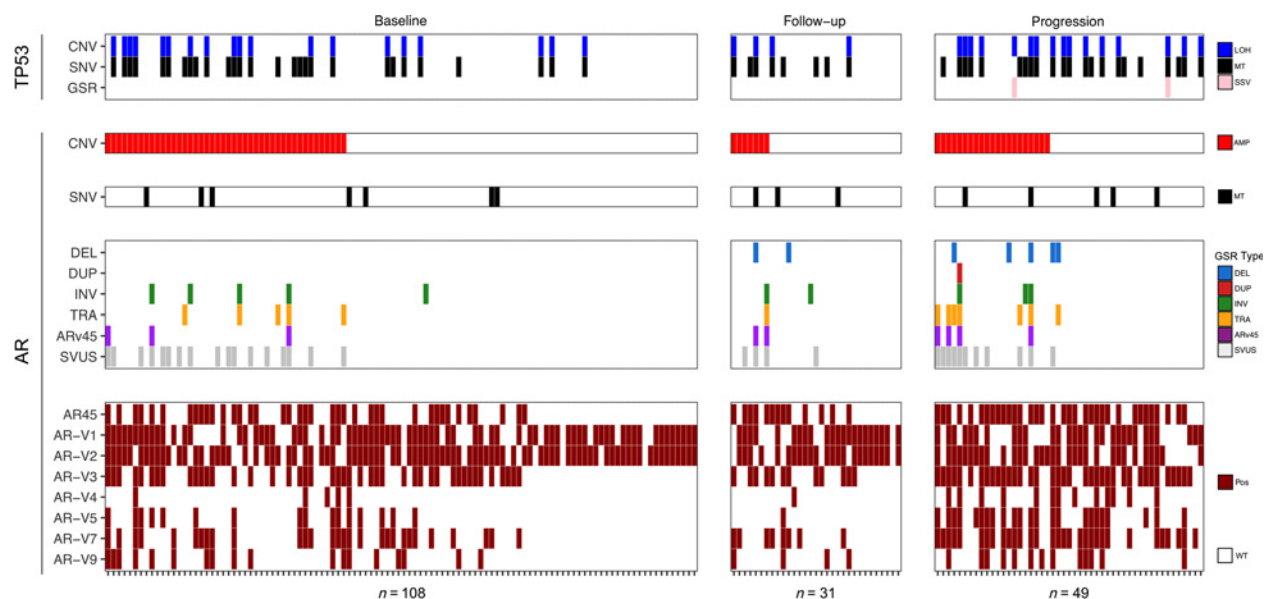
A shorter PFS was observed in patients expressing AR45, AR-V3, AR-V4, AR-V5, and AR-V7 at baseline (all $P < 0.05$; Supplementary Fig. S7). However, in MV-Cox analysis, the individual ARVs were not prognostic, whereas CTC count and prior chemotherapy exposure were independently associated with poor outcome (Supplementary Table S2). Log-rank testing identified a shorter overall survival in patients expressing AR45, AR-V3, AR-V5, AR-V7, and AR-V9 (all $P < 0.01$; Supplementary Fig. S7).

When combining PFS-associated ARVs from univariate analysis, we observed that 69 of 131 (52.6%) patients were expressing at least one of these ARVs, demonstrating a shorter PFS (median, 4.00 vs. 11.0 months, $P = 0.00014$; Fig. 2A). However, in MV-Cox analysis, combined ARV expression was not prognostic, and only CTC counts were independently associated with poor outcome [HR 1.33; 95% confidence interval (CI), 1.14–1.55; $P < 0.001$; Supplementary Table S2]. For 116 of 131 (88.5%) cases, PSA follow-up data at 10–12 weeks (or before in case of early PD) were available (Supplementary Fig. S8), which demonstrated fewer confirmed $\geq 50\%$ PSA responses in ARV-expressing patients (20% vs. 48%; χ^2 test, $P = 0.006$; Fig. 2A).

Plasma AR genomic profiling and clinical outcome

AR-amplified patients had a shorter PFS compared with patients who were copy number neutral (median, 3.9 vs. 9.5 months; $P < 0.0001$). Patients with intra-AR GSRs (with or without SVUS) had a shorter PFS compared with patients with a wild-type AR (median, 3.6 vs. 7.8 months, $P < 0.001$; Supplementary Fig. S9). No association between AR mutations and outcome was observed (Supplementary Table S2). For 132 of 145 (91%) cases PSA follow-up data were available, which demonstrated no association between genomic alterations and PSA response at 10–12 weeks (Supplementary Fig. S9). In MV-Cox analysis, AR amplification and GSRs lost significance, whereas the ctDNA fraction, baseline PSA level, and presence of visceral metastases were independently associated with poor outcome (Supplementary Table S2). Log-rank testing identified a shorter OS in AR-amplified (median, 11.2 vs. 29.0 months; $P < 0.0001$) and GSR-positive patients, regardless whether SVUS were included or excluded (median, 7.7 vs. 26.7 or 7.3 vs. 25.6 months; both $P < 0.001$; Supplementary Fig. S9). The 12 patients harboring GSRs within coding or cryptic exon regions [of whom 11/12 (91.7%) patients were AR-amplified] represented a unique subpopulation with worse PFS (median, 3.3 vs. 4.8 vs. 10.0 months; $P < 0.0001$) and overall survival (median, 7.3 vs. 11.2 vs. 29.7 months; $P < 0.0001$), compared with GSR-negative/AR-amplified and wild-type patients (Supplementary Fig. S10).

When combining PFS-associated genomic AR alterations from univariate analysis, we observed that 55 of 145 (37.9%) patients

**Figure 1.**

Comprehensive landscape of somatic *AR* and *TP53* perturbations in liquid biopsies from patients with mCRPC at baseline ($n = 108$), follow-up ($n = 31$), or progression ($n = 49$) on abiraterone or enzalutamide. Samples are grouped according to sample type. Top, *TP53* panel with copy number, mutation, and structural rearrangement status. Bottom, *AR* panel, encompassing a CNV panel, *AR* copy number stratified according to amplification status; SNV panel, hotspot mutations within the ligand-binding domain of *AR*; GSR panel, genomic structural rearrangements across the *AR* gene; ARV panel, presence or absence of *AR* splice variant expression. AMP, amplified; ARV, *AR* splice variants; ARV45, structural variant deletion *AR* exon 1, which may result in AR45 expression; CNV, copy number variation; DEL, deletion; DUP, duplication; INV, inversion; LOH, loss of heterozygosity; MT, mutant; Pos, positive; SNV, single nucleotide variation; SSV, significant structural variant; TRA, translocation; WT, wild type.

had a shorter PFS (median, 3.9 vs. 10.0 months; $P < 0.0001$; Fig. 2B). In MV-Cox analysis, the combined plasma-AR status lost significance, whereas ctDNA fraction (HR 1.02; 95% CI, 1.01–1.04; $P < 0.0001$), baseline PSA levels (HR 1.12; 95% CI, 1.00–1.26; $P = 0.047$), and presence of visceral metastases (HR 1.82; 95% CI, 1.11–3.00; $P = 0.02$) remained independently associated with poor outcome (Supplementary Table S2). No associations between the combined plasma-AR status and PSA response were observed (Fig. 2B).

Plasma *TP53* genomic profiling and clinical outcome

Patients with a *TP53* perturbation had a shorter PFS compared with patients who were wild type (median, 3.0 vs. 8.7 months; $P < 0.0001$; Fig. 2C). The poorest PFS was observed for patients harboring a biallelic inactivation, compared with patients with a monoallelic perturbation or wild-type genotype (median, 2.7 vs. 5.3 vs. 8.7 months; $P < 0.0001$). However, the PFS difference between mono- and biallelic inactivation was not significant ($P = 0.4$; Supplementary Fig. S11A). PSA follow-up data at 10–12 weeks demonstrated fewer confirmed $\geq 50\%$ PSA responses in *TP53*-perturbed patients (15.4% vs. 46.8%; χ^2 test, $P = 0.008$; Fig. 2C). In MV-Cox analysis, a perturbed *TP53* status was independently associated with poor outcome (HR 1.88; 95% CI, 1.18–3.00; $P = 0.008$), together with ctDNA fraction (HR 1.02; 95% CI, 1.01–1.03; $P = 0.0005$) and presence of visceral metastases (HR 1.72; 95% CI, 1.05–2.84; $P = 0.032$; Supplementary Table S2). Log-rank testing identified a shorter overall survival in *TP53*-perturbed disease (median, 7.8 vs. 26.7 months; $P < 0.0001$; Supplementary Fig. S11B).

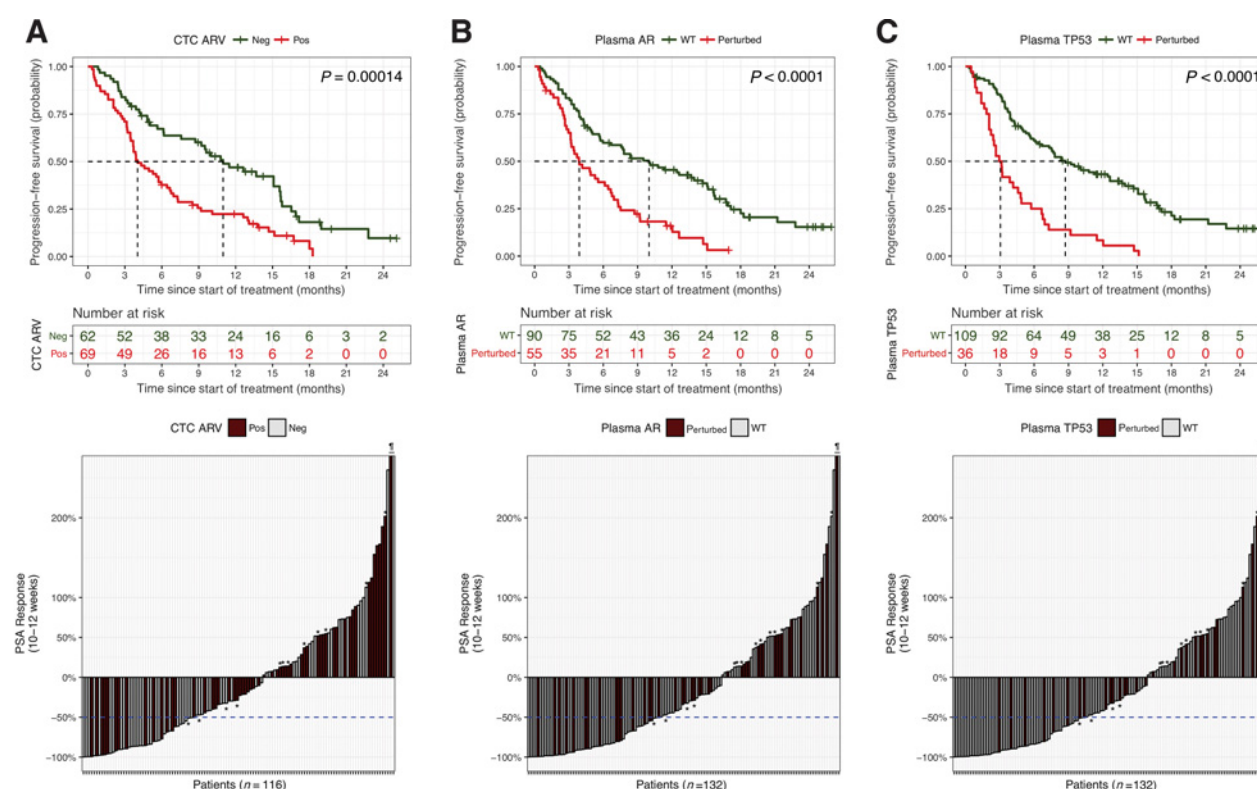
Benchmarking outcomes of ARV, genomic AR, and *TP53* profiling

In the light of previously published data (3, 5, 18, 19), we were surprised by our findings of lack of association between ARV expression, combined plasma AR status, and outcome in our MV-Cox analysis. Even considering different AR-V7 expression level thresholds for positivity failed to identify independent associations with outcome (Supplementary Fig. S12). We tested the associative power of *TP53* alterations against AR-derived biomarkers in a MV-Cox analysis, by including ARV, AR, and *TP53* genomic data (Supplementary Fig. S13A). Perturbed *TP53* status was the only molecular biomarker independently associated with poor outcome (HR 1.97; 95% CI, 1.14–3.40; $P = 0.015$), together with baseline PSA levels (HR 1.24; 95% CI, 1.07–1.44; $P = 0.005$) and presence of visceral metastases (HR 2.11; 95% CI, 1.21–3.66; $P = 0.008$). Even against the well-established AR amplification and AR-V7 biomarkers, *TP53* remained independently associated with poor outcome (HR 1.89; 95% CI, 1.08–3.32; $P = 0.026$; Supplementary Fig. S13B).

Inferring prognosis using clinical features and a *TP53*-driven liquid biopsy

To facilitate prognostication of patients initiating ARSi, we developed a scoring algorithm using the *TP53* MV-Cox regression coefficients (Supplementary Table S2; Fig. 3A). We generated a PFS score by summation of the individual variables multiplied by their corresponding Cox regression coefficient (Fig. 3B). Quartile index stratification of the PFS scores ($<Q1$, $Q1$ – $Q3$, and $\geq Q3$) identified three prognostic groups (good, intermediate, and poor) with different KM PFS estimates

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**Figure 2.**

AR splice variant expression in CTCs, detection of genomic *AR* and *TP53* perturbations in plasma cfDNA, PFS, and PSA response on abiraterone or enzalutamide. KM analysis of PFS (top) and waterfall (WF) plots of PSA responses after 10–12 weeks (or before in case of early disease progression) on therapy (bottom), stratified according to outcome-associated ARV expression in CTCs (A), genomic *AR* (B), or *TP53* (C) perturbations in plasma cfDNA at baseline. P value in KM plot is calculated via log-rank test. In WF plots, *, PFS < 10–12 weeks; †, PSA increase > 200%; dashed blue horizontal lines, 50% decrease in PSA. Neg, negative; Pos, positive; WT, wild type.

(median, 14.7 vs. 7.51 vs. 2.62 months; $P < 0.0001$). Next, we validated the developed classifier in an independent cohort of 201 patients with evaluable treatment-naïve mCRPC, initiating abiraterone or enzalutamide (14). Stratification on the basis of the PFS-score quartiles partitioned the independent cohort into three prognostic groups with 81 (40.3%), 89 (44.3%), and 31 (15.4%) patients with similar median PFS estimates of 14.3, 6.39, and 2.23 months, respectively (Fig. 3C and D).

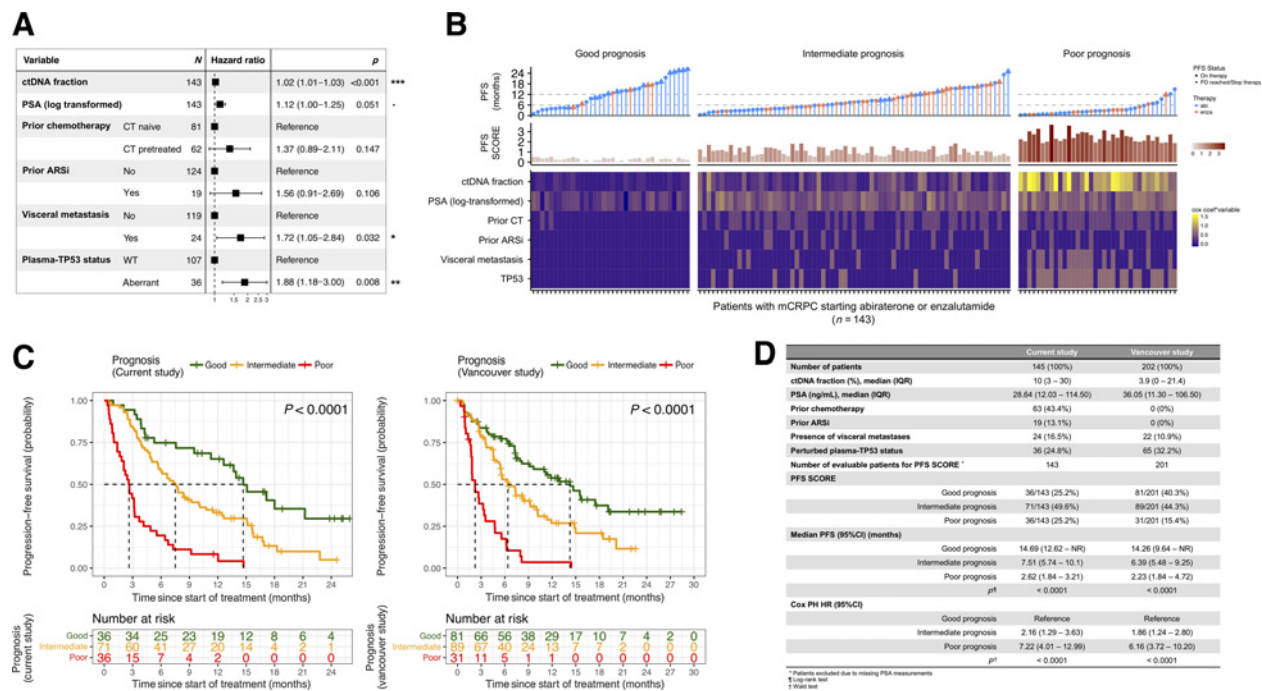
Discussion

This is the first large-scale prospective multicenter study to perform simultaneous profiling of CTC and ctDNA liquid biopsies from all-comer patients with mCRPC before, during, and at progression on ARSi. By accounting for both ARVs and *AR* genomic alterations, we observed that 71.3% of patients with mCRPC carry at least one relevant *AR* perturbation at baseline. Interestingly, other ARVs, besides AR-V7, are also associated with outcome in univariable analyses. In addition, 18% of patients with mCRPC demonstrate intra-*AR* rearrangements, which typically cooccur with *AR* amplifications, and have a poor prognosis. However, our key finding is that *TP53* inactivation outperforms any *AR*-derived biomarker as negative prognosticator for second-generation ARSi. Using a clinical feature and *TP53*-driven liquid biopsy-derived classifier, we observe that 50%–55% of patients

with mCRPC starting ARSi can be reliably stratified into good (median PFS ≥ 14.0 months) or poor (median PFS ≤ 2.5 months) prognosis groups.

This study demonstrates how *AR* perturbations, such as AR-V7 and *AR* amplifications, can be detected in the majority of patients with mCRPC; however, none of the *AR* biomarkers were independently associated with treatment outcomes in MV-Cox analyses. Although the initial discovery by Antonarakis and colleagues (20) suggested that AR-V7 could act as a negative response biomarker for ARSi, subsets of patients expressing AR-V7 still demonstrate clinical benefit (21). Hence the clinical utility of AR-V7 is currently controversial (22), and a recent consensus concluded that there is insufficient evidence to support the implementation of AR-V7 testing in clinical practice (23).

Intra-*AR* rearrangements have been described as a potential endocrine resistance mechanism, and could be detected in up to 50% of heavily pretreated patients with mCRPC using tumor tissue or plasma ctDNA (8, 9). Most recently, structural rearrangements were detected in 19 of 50 (38%) preselected patients with known high ctDNA fractions prior to ARSi and typically demonstrated inferior outcome (14). Here, we demonstrate for the first time how patients with intra-*AR* rearrangements encompass a unique subpopulation with poorest prognosis. However, in MV-Cox we observed that none of the *AR*-derived biomarkers were independently associated with outcome, thereby confirming the

**Figure 3.**

Development and validation of a three-stratum risk stratification system using clinical features and molecular profiling. **A**, Multivariable Cox regression analysis [HR (CI)] of PFS using baseline clinical characteristics, ctDNA fraction estimate, and *TP53* status in patients with mCRPC. CT, chemotherapy; WT, wild type. **B**, Multilevel landscape of Cox coefficient-adjusted variable values (bottom), calculated clinical progression (i.e., PFS) score (middle), and PFS (top). Patients are grouped according to the PFS score category (i.e., <Q1, Q1–Q3, and >Q3 level) and ordered according to increasing PFS. Horizontal dashed lines represent 12- and 6-month landmarks. CT, chemotherapy. **C**, KM analysis of PFS, stratified according to clinical progression score category at baseline for this study (i.e., training cohort, $n = 143$) and Vancouver Prostate Centre study (i.e., testing cohort, $n = 201$). P value is calculated via log-rank test. **D**, Performance characteristics of the three-stratum risk stratification system, comparing risk group prevalence, median PFS times, and Cox HRs.

recent report investigating the association between AR amplification and response to ARSi (14). Because both ctDNA fraction and CTC enumeration were independently associated with outcome in our MV-Cox analysis, our study exemplifies the importance adjusting for tumor burden estimates when performing biomarker discovery studies. Tumor burden may be correlated to the number of preexisting resistant cells harboring subclonal mutations before the start of therapy, which may prevent molecularly targeted trials to reach their primary endpoints (24, 25).

Despite not reaching statistical significance when associating with outcome, we believe that AR perturbations may still play a key role in the disease. However, there are inherent challenges with using AR as a baseline biomarker. AR biomarkers were detectable in the vast majority of patients at baseline and almost all at progression in our study. If at least one AR biomarker is detectable in the majority of men, comprehensive profiling needs to be undertaken to determine which patients express a relevant biomarker output from the AR locus in relation to the upcoming therapy. In addition, as the chemo-hormonal therapy landscape for mPC evolves (26–28), the somatic evolutionary trajectory of the AR-locus is likely to be altered and needs to be explored as guidelines are updated.

However, until the molecular heterogeneity of AR has been completely resolved, *TP53* profiling can be applied to identify poor prognosis patients. Beyond circulating and clinical disease burden estimates, *TP53* status remained significantly associated with outcome in our MV-Cox analysis. This emphasizes the

importance of looking into other pathways or transdifferentiation processes, which have been implicated in endocrine resistance and AR-independent tumor growth (2, 29, 30). Recent clinical studies have demonstrated an association between *TP53* inactivation and poor response to next-generation ARSi (11, 12, 14). Our study provides confirmatory evidence for the molecular characterization of *TP53*, reproducing its independent prognostic value, together with ctDNA fraction and presence of visceral metastasis, in an all-comer cohort of men with mCRPC.

In addition, we developed a robust and reliable three-stratum risk stratification system, using both clinical features and a *TP53*-driven liquid biopsy to identify patients with good and poor prognosis in the context of ARSi. Our PFS classifier was tested in a large mCRPC cohort ($n = 201$), recruited in a randomized clinical trial (RCT; ref. 14), and identified 31 of 201 (15.4%) patients in this independent cohort with poorest prognosis despite ARSi, who may be better served with other treatment modalities.

Limitations of this study include the absence or incomplete collection of data on patient performance status and routine clinical parameters. For example baseline alkaline phosphatase and lactate dehydrogenase concentrations were missing in approximately 30% of the studied cohort, and hence not included in MV-Cox analysis. In addition, the number of metastatic lesions was not collected. Formal performance status scores, which are associated with overall survival in patients with mCRPC starting first-line chemotherapy (31) but not with time to progression in

context of ARSi (14), are not collected as standard practice in the recruiting centers. We validated our prognostic classifier in an independent cohort of patients with *a priori* knowledge that *TP53*, ctDNA fraction, and visceral metastases were independently associated with outcome. However, and importantly, we demonstrate that our stratification method, which was generated on an all-comer cohort of men with mCRPC, gave similar PFS estimates and HR in a completely different cohort from an RCT. Although our study was prospectively designed to test the hypothesis that a combined ARV profiling strategy is prognostic in the context of ARSi, our exploratory plasma-derived biomarker analyses were undertaken retrospectively. Furthermore, our study of a heterogeneous cohort may be underpowered to identify PFS differences in specific subgroups of patients expressing ARVs.

Conclusions

This study is the first large-scaled prospective multicenter study to perform comprehensive AR and *TP53* profiling in CTCs and cfDNA in an all-comer cohort of men with mCRPC starting abiraterone or enzalutamide outside the context of a RCT. Besides emphasizing the importance of comprehensive AR profiling, a major strength of our study is the identification of a single molecular *TP53* biomarker and tumor burden-driven stratification system for all-comer patients commencing ARSi. The activity and efficacy of treatment selection driven by *TP53*, AR, and other molecular biomarkers will need to be tested in a future prospective interventional RCT.

Disclosure of Potential Conflicts of Interest

D. Schrijvers reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Janssen Pharmaceuticals, and reports receiving commercial research grants from Janssen Pharmaceutical Companies. M. Strijbos reports receiving speakers bureau honoraria from Ipsen, Janssen Pharmaceuticals, and Novartis, and is a consultant/advisory board member for Merck, Novartis, and Roche. No potential conflicts of interest were disclosed by the other authors.

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References

- Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, Gross T, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* 2017;71:630–42.
- Robinson D, Van Allen EM, Wu Y-M, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015;161:1215–28.
- Romanell A, Gasi Tandefelt D, Conteduca V, Jayaram A, Casiraghi N, Wetterskog D, et al. Plasma AR and abiraterone-resistant prostate cancer. *Sci Transl Med* 2015;7:312re10.
- Joseph JD, Lu N, Qian J, Sensintaffar J, Shao G, Brigham D, et al. A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. *Cancer Discov* 2013;3:1020–9.
- Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Zhu Y, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *J Clin Oncol* 2017;35:2149–56.
- Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, et al. Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. *JAMA Oncol* 2016;2:1441–49.
- Li Y, Hwang TH, Oseth LA, Hauge A, Vessella RL, Schmechel SC, et al. AR intragenic deletions linked to androgen receptor splice variant expression and activity in models of prostate cancer progression. *Oncogene* 2012;31:4759–67.
- Henzler C, Li Y, Yang R, McBride T, Ho Y, Sprenger C, et al. Truncation and constitutive activation of the androgen receptor by diverse genomic rearrangements in prostate cancer. *Nat Commun* 2016;7:13668.
- De Laere B, van Dam P-J, Whittington T, Mayrhofer M, Diaz EH, Van den Eynden G, et al. Comprehensive profiling of the androgen receptor in liquid biopsies from castration-resistant prostate cancer reveals novel intra-AR structural variation and splice variant expression patterns. *Eur Urol* 2017;72:192–200.
- Ferraldeschi R, Rodrigues DN, Riisnaes R, Miranda S, Figueiredo I, Rescigno P, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. *Eur Urol* 2015;67:795–802.
- Maughan BL, Guedes LB, Boucher K, Rajoria G, Liu Z, Klimek S, et al. p53 status in the primary tumor predicts efficacy of subsequent abiraterone and

- enzalutamide in castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis* 2018;41:884.
12. Hussain M, Daignault-Newton S, Twardowski PW, Albany C, Stein MN, Kunju LP, et al. Targeting androgen receptor and DNA repair in metastatic castration-resistant prostate cancer: results from NCI 9012. *J Clin Oncol* 2018;36:991–9.
 13. Mateo J, Cheng HH, Beltran H, Dolling D, Xu W, Pritchard CC, et al. Clinical outcome of prostate cancer patients with germline DNA repair mutations: retrospective analysis from an international study. *Eur Urol* 2018;73:687–93.
 14. Annala M, Vandekerckhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. *Cancer Disc* 2018;8:444–57.
 15. Scher HI, Morris MJ, Stadler WM, Higano C, Basch E, Fizazi K, et al. Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol* 2016;34:1402–18.
 16. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the prostate cancer clinical trials working group. *J Clin Oncol* 2008;26:1148–59.
 17. Scher HI, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D, et al. Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol* 2009;10:233–9.
 18. Conteduca V, Wetterskog D, Sharabiani MTA, Grande E, Fernandez-Perez MP, Jayaram A, et al. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study. *Ann Oncol* 2017;28:1508–16.
 19. Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, et al. Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. *JAMA Oncol* 2016;2:1441–9.
 20. Antonarakis ES, Lu C, Wang H, Lubner B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014;371:1028–38.
 21. Bernemann C, Schnoeller TJ, Luedeke M, Steinestel K, Boegemann M, Schrader AJ, et al. Expression of AR-V7 in circulating tumour cells does not preclude response to next generation androgen deprivation therapy in patients with castration resistant prostate cancer. *Eur Urol* 2017;71:1–3.
 22. Steinestel J, Bernemann C, Schrader AJ, Lennerz JK, Re: Antonarakis ES, Lu C, et al. Re: Emmanuel S. Antonarakis, Changxue Lu, Brandon Lubner, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *J Clin Oncol* 2017;35:2149–56: AR-V7 testing: what's in it for the patient? *Eur Urol* 2017;72:e168–9.
 23. Gillissen 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.
 24. Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, et al. Evolutionary dynamics of cancer in response to targeted combination therapy. *Elife* 2013;2:e00747.
 25. Le Tourneau C, Delord J-P, Gonçalves A, Gavoille C, Dubot C, Isambert N, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol* 2015;16:1324–34.
 26. Kyriakopoulos CE, Chen Y-H, 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;10:1080–7.
 27. 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.
 28. Smith MR, Saad F, Chowdhury S, Oudard S, Hadaschik BA, Graff JN, et al. Apalutamide treatment and metastasis-free survival in prostate cancer. *N Engl J Med* 2018;378:1408–18.
 29. Bluemn EG, Coleman IM, Lucas JM, Coleman RT, Hernandez-Lopez S, Tharakan R, et al. Androgen receptor pathway-independent prostate cancer is sustained through FGF signaling. *Cancer Cell* 2017;32:474–6.
 30. Zou M, Toivanen R, Mitrofanova A, Floch N, Hayati S, Sun Y, et al. Transdifferentiation as a mechanism of treatment resistance in a mouse model of castration-resistant prostate cancer. *Can Discov* 2017;7:736–49.
 31. Halabi S, Lin C-Y, Kelly WK, Fizazi KS, Moul JW, Kaplan EB, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. *J Clin Oncol* 2014;32:671–7.

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