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

**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 ctDNA (ctDNAseq) were 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 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...
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 clinical 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 (ctDNA) 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 ≥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 χ2 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.
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Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
</tr>
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<tbody>
<tr>
<td>Patients</td>
<td>168 (100%)</td>
</tr>
<tr>
<td>Age at registration, year, mean ± SD</td>
<td>76 ± 7.7</td>
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<tr>
<td>Tumor stage at diagnosis</td>
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<tr>
<td>T1/2</td>
<td>45 (26.79%)</td>
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<tr>
<td>T3/4</td>
<td>41 (24.40%)</td>
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<tr>
<td>M1</td>
<td>45 (26.79%)</td>
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<tr>
<td>Node positive</td>
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<td>25 (14.88%)</td>
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<tr>
<td>Gleason score at diagnosis</td>
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<tr>
<td>≤7</td>
<td>63 (37.50%)</td>
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<tr>
<td>8-10</td>
<td>83 (49.40%)</td>
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<tr>
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<td>22 (13.10%)</td>
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<td>Primary treatment</td>
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<tr>
<td>ADT (+RT)</td>
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<td>Radical Px (+RT)</td>
<td>61 (36.31%)</td>
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<tr>
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<td>Chemotherapy naive</td>
<td>100 (59.52%)</td>
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<tr>
<td>Chemotherapy pretreated</td>
<td>68 (40.48%)</td>
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<td>Previous ARSi for CRPC</td>
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<tr>
<td>No</td>
<td>148 (88.10%)</td>
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<td>Yes</td>
<td>20 (11.90%)</td>
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<tr>
<td>Atriplarone acetate</td>
<td>11 (66.07%)</td>
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<tr>
<td>Enzalutamide</td>
<td>57 (33.93%)</td>
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<tr>
<td>LN only</td>
<td>20 (19.0%)</td>
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<td>Bone only</td>
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<td>LDH, U/L (n = 119)</td>
<td>335 (217-655.5)</td>
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<td>AP, U/L (n = 123)</td>
<td>102 (73-160.5)</td>
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<td>CTC, #/7.5 mL (n = 164)</td>
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</table>

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: $\chi^2$ 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 ($\chi^2$ 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%; $\chi^2$ 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 exon1b-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 ≥50% PSA responses in ARV-expressing patients (20% vs. 48%; $\chi^2$ 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 was 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; $P < 0.001$), 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
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%; \( \chi^2 \) 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.
(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 \( \geq 14.0 \) months) or poor (median PFS \( \leq 2.5 \) 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
in our MV-Cox analysis, our study exemplifies 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 recommended 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.

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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-scale prospective multicenter study to perform comprehensive AR and TP53 profiling in CTCs and ctDNA 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 Ipsen, Janssen and Roche; M. Strijbos reports receiving speakers bureau honoraria from Ipsen, Janssen Pharmaceutical Companies, and Novartis, and is a consultant/advisory board member for Janssen Pharmaceuticals, and reports receiving commercial research grants from Janssen Pharmaceutical Companies. M. Strijbos also reports receiving an honorarium for a poster presentation (grant number K2010-70X-20430-04-3); and the Swedish Cancer Foundation (grant number 4-2689-2016); the Swedish Research Council (grant number 2018-00198/A1533); The Cancer Research Funds of Radiumhemmet, through the Radiumhemmet Cancer Research Foundation (grant number 4269-2016); the Swedish Research Council (grant number K2010-70X-20430-04-3); and the Swedish Cancer Foundation (grant number 09-0677).

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