Blood Biomarker Landscape in Patients with High-risk Nonmetastatic Castration-Resistant Prostate Cancer Treated with Apalutamide and Androgen-Deprivation Therapy as They Progress to Metastatic Disease

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ABSTRACT

Purpose: In the placebo-controlled SPARTAN study, apalutamide added to androgen-deprivation therapy (ADT) improved metastasis-free survival, second progression-free survival (PFS2), and overall survival (OS) in patients with nonmetastatic castration-resistant prostate cancer (nmCRPC). Mechanisms of resistance to apalutamide in nmCRPC require evaluation.

Patients and Methods: In a subset of patients from SPARTAN, aberrations were assessed at baseline and end of study treatment (EOST) using targeted next-generation sequencing or qRT-PCR. Circulating-tumor DNA (ctDNA) levels were assessed qualitatively. Select aberrations in androgen receptor (AR) and other common PC-driving genes were detected and summarized by the treatment group; genomic aberrations were summarized in ctDNA-positive samples. Association between detection of aberrations in all patients and outcomes was assessed using Cox proportional-hazards models and multivariate analysis.

Results: In 247 patients, the overall prevalence of ctDNA, AR aberrations, and *TP53* inactivation increased from baseline (40.6%, 13.6%, and 22.2%) to EOST (57.1%, 25.4%, and 35.0%) and was comparable between treatment groups at EOST. In patients who received subsequent androgen signaling inhibition after study treatment, detectable biomarkers at EOST were significantly associated with poor outcomes: ctDNA with PFS2 or OS (HR, 2.01 or 2.17, respectively; $P < 0.0001$ for both), any AR aberration with PFS2 (1.74; $P = 0.024$), and *TP53* or *BRCA2* inactivation with OS (2.06; $P = 0.003$; or 3.1; $P < 0.0001$).

Conclusions: Apalutamide plus ADT did not increase detectable AR/non-AR aberrations over ADT alone. Detectable ctDNA, AR aberrations, and *TP53/BRCA2* inactivation at EOST were associated with poor outcomes in patients treated with first subsequent androgen signaling inhibitor.

Introduction

A significant proportion of patients are treated for localized prostate cancer relapse, with sustained increase in prostate-specific antigen (PSA), which was traditionally treated with intermittent or continuous androgen-deprivation therapy (ADT; ref. 1). Recently, addition of androgen signaling inhibitors to ADT has shown a clear long-term benefit in delaying metastasis as well as extending survival (2–5).

In the past, several putative biomarkers were shown to be relevant in tumor progression and confer resistance to androgen receptor (AR)-targeted therapy in castration-resistant prostate cancer (CRPC). High circulating-tumor DNA (ctDNA) fraction, the proportion of tumor-derived DNA relative to total cell-free DNA (cfDNA) circulating in the blood, has been reported to be associated with clinical markers of tumor burden and poor response to treatment with abiraterone acetate plus prednisone or enzalutamide in metastatic CRPC (mCRPC; ref. 6). AR aberrations, such as splice variants, AR ligand-binding domain (LBD) mutations, and *AR* amplification, have been shown to confer resistance to enzalutamide and abiraterone acetate (7–10). Also, non-AR genomic aberrations, including *CDK12, TP53, RB1, BRCA1, BRCA2*, and *PTEN* inactivation and *MYC, MET*, and *PIK3CA* activation, have been reported to be increased in mCRPC, and some have been shown to be associated with poor prognosis and resistance to hormonal therapies (6, 10–12). *TP53* and *RB1* inactivation (loss) have also been shown to be prevalent in treatment-emergent small-cell neuroendocrine carcinoma (13, 14), an aggressive subtype of mCRPC.

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Translational Relevance

Patients with nonmetastatic castration-resistant prostate cancer (nmCRPC) with a rapidly rising prostate-specific antigen (PSA) are at high risk for developing metastases and for decreased survival. Apalutamide added to ongoing androgen-deprivation therapy (ADT) improves metastasis-free survival and overall survival over placebo plus ADT (5, 15). Androgen receptor (AR) activity is therefore an important driver of nmCRPC. Continuous ADT is associated with the development of AR aberrations, which confer resistance. We demonstrate that treatment of high-risk nmCRPC with apalutamide plus ADT, compared with ADT alone, does not increase the prevalence of select AR and non-AR aberrations, including expression of *AR* splice variants and mutations and copy-number changes in *AR, TP53, RB1, BRCA1/2, PTEN, PIK3CA, CDK12*, and *MYC*. Our findings provide a better understanding of nmCRPC biology and may help to guide AR-directed therapies in nmCRPC.

In the multicenter, randomized, double-blind, placebo-controlled, phase III SPARTAN study in patients with nonmetastatic CRPC (nmCRPC), the addition of apalutamide to ongoing ADT prolonged time to distant metastasis or death [metastasis-free survival (MFS); hazard ratio (HR), 0.28; 95% confidence interval (CI), 0.23–0.35; $P < 0.0001$] in the planned primary analysis (median follow-up, 20.3 months) compared with ADT alone (5, 16). In the final analysis (median follow-up, 52.0 months), apalutamide significantly improved overall survival (OS) compared with placebo (HR, 0.78; 95% CI, 0.64–0.96; $P = 0.0161$) despite the crossover of 19% of placebo-treated patients to apalutamide. Second progression-free survival (PFS2) was significantly longer in apalutamide- than placebo-treated patients in the primary analysis and continued to be improved in apalutamide-treated patients in the final analysis (HR, 0.55; 95% CI, 0.46–0.66; nominal $P < 0.0001$) over placebo (5, 15). Resistance mechanisms for apalutamide were first assessed in preclinical and phase II clinical studies in patients with mCRPC. The occasional acquisition of AR LBD mutations (8, 17) following apalutamide treatment has been shown. However, because of the small sample size and the lack of a control group in these studies, the true prevalence of AR LBD mutations when patients progress from nmCRPC to mCRPC is not well understood. Additional potential mechanisms of resistance to apalutamide also need to be evaluated.

In prostate cancer, identification of acquired resistance mechanisms is hampered by difficulties in obtaining metastatic tissue biopsies upon disease progression. Recently, liquid biopsies have been used successfully to study tumor clone dynamics (18), to identify predictive biomarkers associated with response to therapy (7, 10, 19–21), and to stratify patients by molecular subtype (22, 23). To understand the mechanisms of resistance in the nonmetastatic setting and at progression to metastatic disease, we used liquid biopsies at baseline and end of study treatment (EOST) in SPARTAN to profile a targeted set of biomarkers relevant in advanced prostate cancer.

In this analysis, we assessed aberrations in longitudinal samples from patients treated with apalutamide added to ADT compared with those treated with ADT alone. In addition, we assessed the association between biomarkers in the overall biomarker population and long-term outcomes.

Patients and Methods

The SPARTAN study design has been described previously (15, 16). Briefly, patients with nmCRPC were randomly assigned in a 2:1 ratio to receive 240 mg/d apalutamide or placebo added to ongoing ADT (5). Review boards at all participating institutions approved the study, which was conducted in accordance with current International Conference on Harmonisation guidelines for Good Clinical Practice and according to principles of the Declaration of Helsinki. All patients provided written informed consent. MFS, the primary endpoint, was defined as the time from randomization to the time of the first evidence of radiographically detectable bone or soft tissue distant metastasis assessed by blinded independent central review or death due to any cause, whichever occurred first. At the time of progression and development of mCRPC, patients were eligible to receive open-label abiraterone acetate and prednisone, provided by the sponsor, or any therapy deemed appropriate by their treating physician. PFS2 was defined as the time from randomization to investigator-assessed disease progression, including PSA progression, radiographically detected distant metastasis, symptomatic progression, or any combination thereof, after the first subsequent treatment for mCRPC or death from any cause, whichever occurred first. OS was defined as the time from randomization to death from any cause.

A subset of SPARTAN patients gave consent for optional biomarker sample collection and exploratory analysis following the amendment to the study protocol. Blood samples were collected at baseline and EOST, defined as the first MFS event or discontinuation of treatment. Patients who had at least one biomarker sample collected at EOST and analyzed before the first interim analysis (the clinical cutoff on May 19, 2017) were included in the biomarker population.

ctDNA and genomic AR and non-AR aberrations were assessed using next-generation sequencing of cfDNA isolated from plasma. Plasma was separated from whole blood that was collected into K2EDTA tubes and stored at −20°C or −70°C until testing. cfDNA isolation and amplification have been described previously (24). Targeted next-generation sequencing analysis was carried out using a proprietary target capture and analysis pipeline (Resolution Bioscience) described previously (25), with a set of probes covering 302 exons and over 1,500 single-nucleotide variant (SNV) loci across the genome. A gene panel assessed in this analysis is provided in Supplementary Methods; only genes altered with >5% prevalence are reported. Analytic validation studies of the Resolution Bioscience platform have found 95% limit of detection at 0.18% variant allele frequency for SNV and at a shift of 0.2 of raw copy number for CNV. Detectable levels of ctDNA were determined using a qualitative exploratory method assessing (i) a shift in the allele frequency of heterozygous SNPs, or (ii) a mutation detected with an allele frequency consistent with a somatic mutation, or (iii) a change in reading depth consistent with copy-number variation. Cell line titration studies using a genetically matched normal control found that the method detects tumor content down to 5%. AR LBD mutations evaluated in this study include L702H, W742C, H875Y, F877L, and T878A. Non-AR aberrations were summarized as inactivation, defined as either heterozygous or homozygous deletion or pathogenic inactivating (SNV/indel), and activation, defined as amplification or activating SNV. Inactivation was assessed in *CDK12, RB1, BRCA1, BRCA2*, and *TP53*, and activation was assessed in *MET, MYC*, and *PIK3CA*, the most frequently altered genes in metastatic prostate cancer (26). Only aberrations with known pathogenic consequences based on nonsense, frameshift, splice site mutations, or on ClinVar, the public archive of human genetic variants and interpretation of their significance to diseases (27), were assessed.

The assay was not designed to assess genomic fusions or allelic losses, and it was designed to detect deletions only in *TP53, CDK12, BRCA1*, and *BRCA2*. Genomic aberrations were summarized in patients with detectable levels of ctDNA.

ARv7 was assessed from whole blood collected into PAXGene blood RNA tubes (Qiagen) and stored at −20°C or −70°C until testing. Total whole-blood RNA was extracted and transcribed to complementary DNA using random hexamers. RT-PCR was used to detect ARv7 in four replicates using the Biomark's Fluidigm Dynamic Array microfluidic system (Expression Analysis). Means of replicates were summarized as positive (≤35-cycle threshold) or negative (>35-cycle threshold) detection. There are no markers/methods to determine tumor-specific RNA; therefore, the prevalence of ARv7 was evaluated relative to the number of patients for whom whole blood was collected and analyzed.

Prevalence of biomarkers was summarized in the total biomarker population and in the treatment groups and compared using the Fisher's exact test. AR aberrations were summarized individually or together to increase the sample size. Genomic aberrations were summarized in ctDNA-positive samples. Univariate associations between MFS, PFS2, and OS and biomarker status (detectable levels of ctDNA or presence of AR or non-AR aberrations) at baseline and between PFS2 and OS, and the biomarker status at EOST were assessed using Cox proportional hazards models to estimate HR and 95% CI. Association of biomarker status at baseline with outcomes was evaluated in the apalutamide and placebo groups. Association of biomarker status at EOST with outcomes was evaluated in pooled patients from both groups. Association between biomarker status at baseline or EOST and outcomes was assessed using either the first interim data cutoff (clinical cutoff on May 19, 2017) for MFS or the final data cutoff (clinical cutoff on February 1, 2020) for PFS2 and OS. PFS2 and OS were defined with delayed entry, that is, patients were not considered at risk of PFS2 and OS until they reached EOST (28) to account for a survival bias for patients who provided samples at EOST. Associations of genomic aberrations (i.e., activation or inactivation) with outcomes were adjusted to account for the prognostic effect of ctDNA using multivariate regression models. Multivariate associations of biomarker status were assessed in pooled patients from both treatment groups who had all biomarkers measured. Other comparisons were performed using the Wilcoxon test for continuous variables, the χ^2 test for categorical variables, and the log-rank test for time-to-event endpoints.

Results

Sample collection and analysis

The optional sample collection began following the amendment of the study protocol 6 months after the study had been initiated; therefore, only a proportion of SPARTAN patients provided samples for the exploratory biomarker analysis and not all patients who provided samples at EOST had baseline samples. Blood samples were collected from 675 patients at baseline and 297 patients at EOST (Supplementary Fig. S1A). The main focus of this analysis was to determine aberrations associated with progression from nonmetastatic to metastatic disease in apalutamide-treated patients. The second focus was to assess aberrations longitudinally from nonmetastatic disease at baseline to metastatic disease at progression. Therefore, we selected all available EOST samples collected by the time of the first interim analysis from 247 patients (biomarker population); among these patients, 149 also provided baseline samples (Supplementary Fig. S1A). Among 149 baseline blood samples, 133 plasma and 126 whole-blood samples were processed (Supplementary Fig. S1B). Among processed baseline samples, 110 plasma and whole-blood samples were from the same patients. Among 247 EOST samples, 240 plasma and 200 whole-blood samples were processed (Supplementary Fig. S1B). Of the processed EOST samples, 193 plasma and whole-blood samples were from the same patients. A total of 54.6% (131/240) of plasma samples and 63.0% (126/200) of whole-blood samples at EOST had matched baseline samples (Supplementary Fig. S1B).

SPARTAN study biomarker population

Of 247 patients in the biomarker population, 121 were treated with apalutamide plus ADT and 126 were treated with placebo plus ADT (Supplementary Fig. S1C). Baseline demographic and clinical characteristics were in general similar for apalutamide and placebo patients in the biomarker population and comparable with those in the overall intention-to-treat population (**Table 1**). Significant differences in baseline characteristics of the biomarker population compared with the overall population were observed in median PSA doubling time (PSADT), which was shorter, and in the proportion of patients with PSADT ≤6 months, which was larger. Median MFS, PFS2, and OS were also significantly shorter in the biomarker population compared with the overall population. The proportion of patients who progressed in the biomarker population was 51% ($n = 62$) in the apalutamide group and higher in the placebo group (74%; $n = 93$). In the overall biomarker population, 63% ($n = 155$) of patients progressed. In the total SPARTAN population randomized in the 2:1 ratio, the proportion of patients with distant metastasis or death had been observed in 22.8% (184/806) and 48.4% (194/401) in the apalutamide and placebo groups, respectively, and in 31.3% (378/1207) overall (5). Apalutamide significantly improved MFS in the biomarker population compared with placebo (HR, 0.35; 95% CI, 0.25–0.49; $P < 0.0001$). This favorable treatment effect of apalutamide was consistent with that in the overall study population (5, 15, 16). The majority of patients in the biomarker and overall populations (84% and 83%, respectively) received abiraterone acetate plus prednisone or enzalutamide as the first subsequent therapy after discontinuation of their primary study treatment (**Table 1**).

Genomic landscape at baseline and EOST in the SPARTAN biomarker population

ctDNA, a marker of tumor burden (6), was qualitatively detected from cfDNA in 40.6% of patients with nmCRPC at baseline (**Fig. 1**; Supplementary Table S1). Among ctDNA-positive samples at baseline, *AR* amplification was detected in 11.1%. AR LBD mutations were detected in only 1 patient (1.9%), and any AR genomic aberration was detected in 13.0% of patients.

Other non-AR genomic aberrations that may contribute to CRPC disease progression were assessed in the ctDNA-positive samples collected at baseline. These included inactivation of *CDK12, TP53, PTEN, RB1, BRCA1*, and *BRCA2* (6, 10, 11) and activation of *PIK3CA, MET*, and *MYC* (**Fig. 1**; Supplementary Table S1; ref. 10). Among patients with detectable ctDNA at baseline, *CDK12* inactivation, *BRCA2* inactivation, *PIK3CA* activation, *PTEN* inactivation, *RB1* inactivation, and *BRCA1* inactivation were detected in 5.6%, 5.6%, 1.9%, 1.9%, 1.9%, and 1.9% of patients, respectively (Supplementary Table S1). The most prevalent genomic aberration at baseline was *TP53* inactivation, seen in 22.2% of patients. No patients had activation of *MYC* or *MET* at baseline.

Samples collected at EOST from both treatment groups allowed for the evaluation of biomarkers in patients with prostate cancer that had

Table 1. Patient clinical and demographic characteristics at baseline and efficacy outcomes in SPARTAN.

	SPARTAN biomarker population			SPARTAN overall population		
	Apalutamide ($n = 121$)	Placebo ($n = 126$)	Total ($N = 247$)	Apalutamide ($n = 806$)	Placebo ($n = 401$)	Total ($N = 1,207$)
Median age (range; years)	76 (56–92)	72 (52–89)	74 (52–92)	74 (48–94)	74 (52–97)	74 (48–97)
Median PSADT (mo)	4.1	4.0	4.0[a]	4.4	4.5	4.4
PSADT, *n* (%)						
≤6 months	96 (79.3)	96 (76.2)	192 (77.7)[b]	576 (71.5)	284 (70.8)	860 (71.3)
>6 months	25 (20.7)	30 (23.8)	55 (22.3)	230 (28.5)	117 (29.2)	347 (28.7)
Bone-sparing agent use, *n* (%)	12 (9.9)	10 (7.9)	22 (8.9)	82 (10.2)	39 (9.7)	121 (10.0)
Nodal status at study entry, *n* (%)						
N0	103 (85.1)	97 (77.0)	200 (81.0)	673 (83.5)	336 (83.8)	1,009 (83.6)
N1	18 (14.9)	29 (23.0)	47 (19.0)	133 (16.5)	65 (16.2)	198 (16.4)
Prior prostate cancer therapy, *n* (%)						
Definitive local therapy	83 (68.6)	89 (70.6)	172 (69.6)	536 (66.5)	277 (69.1)	813 (67.4)
GnRH agonist	120 (99.2)	125 (99.2)	245 (99.2)	783 (97.1)	388 (96.8)	1,171 (97.0)
First-generation antiandrogen	98 (81.0)	102 (81.0)	200 (81.0)	593 (73.6)	290 (72.3)	883 (73.2)
Median MFS (95% CI; mo)	18.3 (14.8–21.9)	7.5 (7.2–10.9)	14.5[c] (11.0–14.7)	40.5 (NR–NR)	16.2 (14.6–18.5)	29.5 (27.8–NR)
Median PFS2 (95% CI; mo)	29.5 (25.8–35.4)	26.9 (24.2–29.7)	27.6[c] (25.8–30.8)	55.6 (53.0–61.2)	41.2 (37.8–46.2)	52.1 (49.8–54.2)
Median OS (95% CI; mo)	47.2 (40.9–51.4)	46.5 (41.1–57.7)	47.2[c] (42.3–51.4)	73.9 (61.2–NR)	59.9 (52.8–NR)	65.1 (60.5–NR)
First subsequent therapy, *n* (%)	$n = 97$	$n = 118$	$n = 215$	$n = 386$	$n = 285$	$n = 671$
AAP + enzalutamide	83 (85.5)	98 (83.1)	181 (84.2)	314 (81.3)	244 (85.6)	558 (83.2)
Chemotherapy	7 (7.2)	12 (10.2)	19 (8.8)	38 (9.8)	21 (7.4)	59 (8.8)
Other therapy	7 (7.2)	8 (6.8)	15 (7.0)	34 (8.8)	20 (7.0)	54 (8.0)

Abbreviations: AAP; abiraterone acetate plus prednisone; GnRH, gonadotropin-releasing hormone; NR, not reached.
[a]Biomarker versus overall populations, $P = 0.025$.
[b]Biomarker versus overall populations, $P = 0.046$.
[c]Biomarker versus overall populations, $P < 0.0001$.

progressed to metastatic disease detectable by conventional imaging or in those who discontinued study treatment. All assessed biomarkers were detectable with a higher prevalence at EOST over baseline (**Fig. 1**). Although 40.6% of patients had detectable ctDNA at baseline, this proportion significantly increased to 57.1% at EOST ($P = 0.003$; Supplementary Table S1). Among patients with ctDNA-positive samples, the proportion of those with *AR* amplification significantly increased from baseline to EOST (11.1%–28.5%; $P = 0.013$). Similarly, the ctDNA-adjusted prevalence of AR LBD mutations increased substantially from 1.9% at baseline to 8.8% at EOST, but the difference did not reach statistical significance (Supplementary Table S1). When both *AR* amplification and AR LBD mutations were taken into account, the proportion of patients with any AR genomic aberration significantly increased from 13.0% at baseline to 35.0% at EOST ($P = 0.002$; Supplementary Table S1). All assessed AR genomic aberrations were not statistically different in the apalutamide group over the placebo group at EOST (**Fig. 1**; Supplementary Table S1).

The prevalence of some genomic non-AR aberrations in the overall biomarker population was noticeably higher at EOST than at baseline (**Fig. 1**), but the difference of any type of non-AR aberrations did not reach statistical significance (Supplementary Table S1). In patients with detectable levels of ctDNA, aberrations in *CDK12, BRCA2, PIK3CA, PTEN*, and *TP53* increased from 5.6%, 5.6%, 1.9%, 1.9%, and 22.2%, respectively, at baseline to 8.8%, 15.3%, 9.5%, 5.1%, and 35.0%, respectively, at EOST (Supplementary Table S1). Aberrations in *MYC* increased from 0% to 7.3%; only 1 patient had *MET* aberrations at EOST (Supplementary Table S1). *RB1* and *BRCA1* aberrations were detected in 1 patient at baseline (1.9% for both) and in 2 (1.5%) and 5 (3.6%) patients, respectively, at EOST. There was no statistically significant increase in any of the assessed non-AR aberrations in the apalutamide group over the placebo group at EOST (**Fig. 1**; Supplementary Table S1). No patients in either the treatment group had both *TP53* and *RB1* inactivation at baseline or EOST (**Fig. 1**).

Overall, 43 of 69 (62.3%) apalutamide patients and 45 of 68 (68.2%) placebo patients who had detectable ctDNA harbored any genomic aberration at EOST (**Fig. 1**). The complete list of point mutations and aberrations at baseline and EOST is shown in Supplementary Table S2.

Landscape of transcriptional and genomic AR aberrations at baseline and EOST in the SPARTAN biomarker population

We next assessed an *AR* splice variant, ARv7, in plasma samples derived from whole-blood RNA. ARv7 was detected in 6.3% of patients at baseline and in 11.0% of patients at EOST, although the difference between EOST and baseline prevalence did not reach statistical significance. When we analyzed the prevalence of any AR aberration, transcriptional or genomic, among patients who had both cfDNA and whole-blood RNA evaluated at baseline ($n = 110$) and EOST ($n = 193$), 13.6% and 25.4% of patients had any genomic or transcriptional AR aberration at baseline and EOST, respectively ($P = 0.019$; Supplementary Table S1).

Notably, the prevalence of any AR aberration at EOST was also comparable between the two treatment groups (21.5% in apalutamide patients vs. 29.0% in placebo patients), consistent with findings for genomic AR aberrations. There was no difference in prevalence associated with either type of AR aberration between the apalutamide and placebo treatment groups at EOST (**Fig. 1**; Supplementary Table S1).

Association between biomarker status at baseline and efficacy outcomes

To evaluate the effect of biomarker status at baseline on MFS, PFS2, and OS in apalutamide and placebo-treated patients, we assessed the association of detected biomarkers with time to event (Supplementary

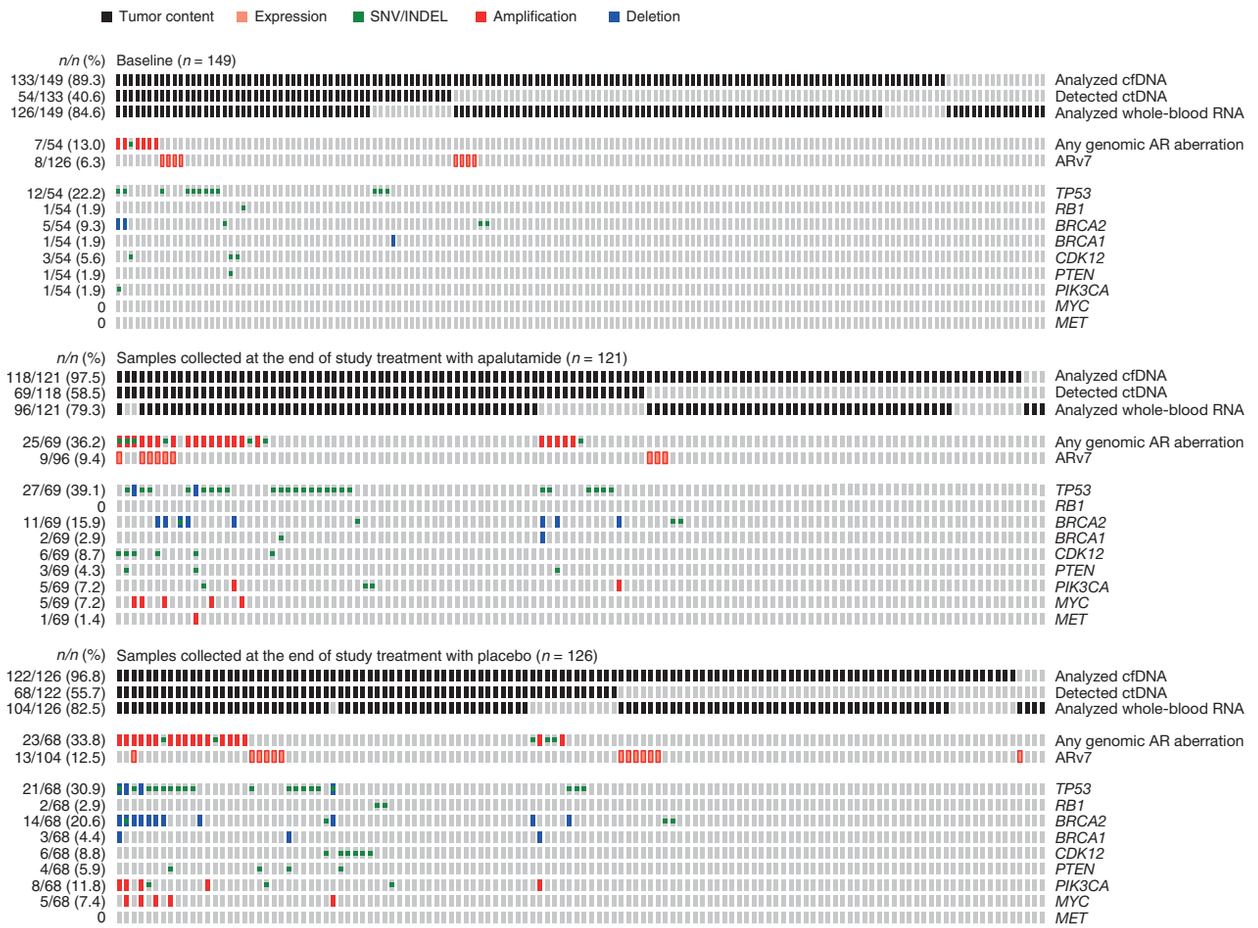

Figure 1.

Aberration landscape at baseline and EOST in the SPARTAN biomarker population. INDEL, insertion and deletion; SNV, single-nucleotide variation.

Table S3). We did not find any association or, in cases in which a statistically significant association was observed, the sample size was too small to allow for a meaningful interpretation. Given the low prevalence of biomarkers observed at baseline, it is difficult to determine whether the lack of association with outcome was due to low power or lack of signal.

Association between biomarker status at EOST and efficacy outcomes in the overall biomarker population

Because a substantial number of patients harbored at least one AR or non-AR aberration at EOST (**Fig. 1**), we sought to determine whether these aberrations are associated with poor prognosis in the overall biomarker population. We evaluated the association of biomarker status at EOST with PFS2 and OS in patients who received secondary treatment with abiraterone acetate plus prednisone or enzalutamide, the most common first subsequent therapy after the primary study treatment (**Table 2**). To account for a survival bias for patients who provided samples at EOST, we considered them at risk of PFS2 and OS after they reached EOST. We found that detectable levels of ctDNA were associated with shorter PFS2 and OS, and the presence of any AR aberration was associated with shorter PFS2. When individual types of AR aberrations were assessed, only the presence of *AR* amplification maintained the significant association with shorter PFS2. A similar trend for shorter OS was observed in patients with *AR* amplification, although it did not reach statistical significance. ARv7 expression did not appear to be associated with outcomes. No significant associations between AR LBD mutations with outcomes were found; however, this observation should be interpreted with caution because of the low prevalence of these mutations. The presence of *BRCA2* and *CDK12* inactivation at EOST was significantly associated with shorter PFS2 and OS, and *TP53* inactivation at EOST was significantly associated with shorter OS. Associations between *PTEN* inactivation, *BRCA1* inactivation, and *MYC* activation at EOST with outcomes should be interpreted with caution due to the small number of events. The presence of *PIK3CA* activation or *RB1* inactivation at EOST did not appear to be significantly associated with outcomes (Supplementary Table S4).

Multivariate analysis of biomarker status and efficacy in the overall biomarker population

We next sought to confirm whether biomarkers measured at EOST that were significantly associated with outcomes in univariate analyses maintained significant association with the outcomes in the multivariate analysis. Baseline PSA and PSADT, shown to be associated with improved MFS (29); Eastern Cooperative Oncology Group performance status (ECOG PS), one of the baseline stratification factors in

Table 2. Association of biomarker status at EOST with PFS2 and OS in patients treated with androgen signaling inhibitors as first subsequent treatment.

		PFS2				OS			
Biomarker		**Median (95% CI; mo)**	**Events/total, *n/n***	**HR (95% CI)**	***P***	**Median (95% CI; mo)**	**Events/total, *n/n***	**HR (95% CI)**	***P***
ctDNA	Detected	18.3 (14.0–22.8)	91/137	2.01 (1.45–2.81)	<**0.0001**	34.1 (26.5–40.7)	90/137	2.17 (1.51–3.12)	<**0.0001**
	Undetected	30.7 (26.1–39.5)	58/100			57.1 (49.8–NR)	43/103		
ARv7	Positive	22.8 (7.4–29.3)	7/14	0.97 (0.45–2.11)	0.946	24.8 (22.2–NR)	4/7	1.27 (0.45–3.62)	0.652
	Negative	26.0 (23.0–28.8)	88/130			47.2 (37.1–55.8)	35/57		
AR amplification	Positive	13.8 (7.8–18.4)	18/24	2.91 (1.62–5.25)	**0.0004**	23.9 (8.4–33.6)	9/11	1.75 (0.78–3.92)	0.171
	Negative	27.6 (25.1–31.1)	77/120			51.3 (42.3–62.0)	30/53		
AR LBD mutations	Positive	10.3 (7.4–26.2)	4/4	2.06 (0.74–5.75)	0.168	18.1 (18.1–NR)	1/3	0.35 (0.05–2.58)	0.301
	Negative	25.8 (22.8–28.8)	91/140			46.5 (35.9–54.3)	38/61		
Any AR aberration	Positive	15.5 (11.4–23.0)	25/36	1.74 (1.08–2.80)	**0.024**	23.9 (8.4–49.4)	10/15	1.14 (0.54–2.40)	0.727
	Negative	27.6 (24.2–32.8)	70/108			51.3 (42.3–58.6)	29/49		
TP53 inactivation	Positive	16.1 (10.6–24.9)	25/36	1.13 (0.70–1.84)	0.620	30.2 (23.2–38.4)	31/36	2.06 (1.28–3.32)	**0.003**
	Negative	25.4 (19.6–28.5)	93/141			51.4 (45.3–61.7)	71/141		
BRCA2 inactivation	Positive	14.1 (7.8–18.0)	15/19	2.30 (1.31–4.02)	**0.004**	28.9 (18.1–33.5)	17/19	3.13 (1.83–5.36)	<**0.0001**
	Negative	25.5 (22.8–28.8)	103/158			50.0 (44.1–57.1)	85/158		
PTEN inactivation	Positive	12.3 (10.6–16.1)	6/6	3.30 (1.42–7.70)	**0.006**	27.4 (15.5–32.5)	5/6	1.90 (0.76–4.74)	0.168
	Negative	24.9 (22.2–27.5)	112/171			46.5 (42.0–52.7)	97/171		
CDK12 inactivation	Positive	7.5 (7.4–15.5)	6/8	2.56 (1.08–6.09)	**0.034**	27.9 (19.3–37.0)	7/8	2.23 (1.00–4.98)	**0.050**
	Negative	25.1 (22.2–27.6)	112/169			47.2 (42.1–54.0)	95/169		
BRCA1 inactivation	Positive	11.3 (10.6–16.5)	4/4	4.75 (1.67–13.5)	**0.004**	22.1 (13.1–NR)	4/4	4.80 (1.70–13.6)	**0.003**
	Negative	24.9 (19.7–27.5)	114/173			46.5 (42.0–54.0)	98/173		
MYC activation	Positive	9.9 (8.4–NR)	5/7	3.48 (1.36–8.91)	**0.009**	22.2 (8.4–31.0)	6/7	1.91 (0.82–4.42)	0.132
	Negative	24.9 (19.7–27.5)	113/170			46.5 (42.0–54.0)	96/170		

Note: Bold represents significant values.

Table 3. Multivariate analyses of associations of patient and disease characteristics at baseline and biomarker status at EOST with PSF2 and OS in patients treated with androgen signaling inhibitors as first subsequent treatment.

	PFS2		OS	
	Median (95% CI), 25.5 (22.3–28.1; mo) Events/total, *n/n*: 95/144		Median (95% CI): 46.5 (42.0–52.7; mo) Events/total, *n/n*: 82/144	
Variable	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Baseline characteristic				
ECOG PS	1.26 (0.76–2.08)	0.363	1.14 (0.66–1.98)	0.637
PSA	1.08 (0.94–1.25)	0.278	1.17 (1.00–1.37)	0.052
PSADT	0.72 (0.41–1.27)	0.257	0.65 (0.36–1.20)	0.172
Aberration at EOST				
ctDNA	1.66 (1.03–2.69)	**0.039**	1.51 (0.86–2.65)	0.149
AR aberrations	1.49 (0.83–2.64)	0.179	0.77 (0.42–1.41)	0.398
TP53 inactivation	0.97 (0.52–1.82)	0.930	2.64 (1.47–4.73)	**0.001**
PTEN inactivation	1.98 (0.55–7.12)	0.294	1.49 (0.34–6.64)	0.598
BRCA2 inactivation	1.75 (0.85–3.60)	0.127	3.04 (1.45–6.40)	**0.003**
BRCA1 inactivation	5.49 (0.94–31.92)	0.058	1.79 (0.28–11.64)	0.541
CDK12 inactivation	2.89 (0.98–8.51)	0.055	2.36 (0.90–6.16)	0.081
MYC activation	2.59 (0.90–7.47)	0.079	1.04 (0.35–3.06)	0.944

Note: Bold represents significant values.

SPARTAN (5, 15); and the presence of ctDNA were also taken into account (**Table 3**). In the overall biomarker population of patients treated with androgen signaling inhibitors as first subsequent treatment, independent associations of biomarkers at EOST with outcomes were found only between detectable ctDNA and PFS2 (HR, 1.66; 95% CI, 1.03–2.69; $P = 0.039$), *TP53* inactivation and OS (2.64; 95% CI, 1.47–4.73; $P = 0.001$), and *BRCA2* inactivation and OS (3.04; 95% CI, 1.45–6.40; $P = 0.003$).

Discussion

In the SPARTAN study, patients with nmCRPC with rapidly rising PSA experienced improved MFS, PFS2, and OS with the addition of apalutamide to ongoing ADT treatment (5, 15, 16) compared with ADT alone. Despite the delay in disease progression seen with apalutamide treatment, a substantial proportion of patients eventually progressed and received subsequent therapy for mCRPC. We investigated the status of detectable ctDNA and AR and non-AR aberrations, which have been shown previously to be associated with disease burden, poor prognosis, and resistance to AR-targeted therapy, at baseline and/or EOST, in patients with nmCRPC treated with apalutamide or placebo plus ADT. We also studied the association of these biomarkers with clinical outcomes.

The SPARTAN biomarker population is a subset of the overall patient population. Some of the baseline disease characteristics indicate more aggressive disease in the biomarker population. Thus, the proportion of patients who progressed was higher, and baseline PSADT and median time to outcomes were shorter than in the overall population. These observations may be explained by the enrichment of the biomarker population with patients who reached EOST and, therefore, with those who progressed on therapy or discontinued treatment by the time of study unblinding. This population may be also enriched with patients who developed primary resistance to AR inhibition and who may have poorer outcomes, as evidenced by the shorter time to MFS across treatment groups in the biomarker population versus the overall population. Nevertheless, despite the greater number of early progressors in the biomarker population, apalutamide treatment prolonged MFS over placebo, consistent with the overall study results (5, 16), suggesting that observations in the biomarker population can be applicable in the overall SPARTAN population.

The evolution of prostate cancer from nonmetastatic to metastatic disease is accompanied by an increase in tumor burden. Our observation that detectable ctDNA, a surrogate marker of tumor burden (6), was less prevalent at baseline when patients were nonmetastatic, but increased by EOST when a large proportion of patients progressed to metastatic disease, support this. The prevalence of ctDNA detected in SPARTAN at EOST was similar to that previously reported in mCRPC, although the detection methods differed (6). Furthermore, similar to prior findings (6), the presence of ctDNA was an independent prognostic factor of shorter PFS2 for patients who received androgen signaling inhibitors after EOST.

As tumor burden grows, tumor heterogeneity, manifested by the acquisition of genomic and transcriptional aberrations, also increases, consistent with our findings. Thus, we observed that the prevalence of *AR* amplification, a common AR aberration linked to disease progression (6), significantly increased from baseline to EOST in the SPARTAN biomarker population to the prevalence similar to that observed in mCRPC (10). Similarly, AR LBD mutations, which have been reported to modulate AR signaling through ligand and cofactor affinity and potentially result in a gain of function for AR signaling in the presence of other steroids (30), also increased from baseline to EOST. The reported prevalence of AR LBD mutations in mCRPC varied between 12% and 26% in different studies (Supplementary Table S5; refs. 6, 10, 12, 31). We observed lower AR LBD mutation prevalence at EOST in SPARTAN (9%). AR genomic aberrations increased even when they were normalized by the presence of detectable ctDNA. A similar increase from baseline to EOST was observed for the ARv7 transcript, which has been characterized previously as one of the most important constitutively active *AR* splice variants associated with resistance to enzalutamide and abiraterone acetate (7). Detection of ARv7 expression in 11% of patients from the SPARTAN biomarker population at EOST was lower than the approximately 30% previously reported in mCRPC (7). The prevalence of any AR aberration, either genomic or transcriptional, was also increased from baseline to EOST.

Several major deleterious non-AR genomic aberrations associated with poor prognosis and resistance to hormonal therapy in prostate cancer also increased by EOST in our analysis, consistent with our finding for AR aberrations. Thus, we observed that inactivation of *CDK12*, a regulator of transcription and genomic stability (11), *BRCA2*, homologous recombination repair gene (6, 11), and tumor suppressors *TP53* and *RB1* (6, 10, 11), was detected in an increased proportion of patients from baseline to EOST. We also observed a substantial increase from baseline to EOST in detection of oncogene *PIK3CA* (11) and proto-oncogene *MYC* (10) activation. The observed prevalence of non-AR aberrations at EOST was in general lower than that reported in mCRPC, although it varied between studies (Supplementary Table S5; refs. 6, 10, 31). Our findings suggest that although SPARTAN patients at EOST had developed mCRPC, their disease burden was likely lower than that seen in cross-sectional observational studies of mCRPC. This may occur because SPARTAN patients, who entered the study with no demonstrable metastases on conventional imaging, were followed with imaging at relatively short intervals (16 weeks). Our assay was not designed to assess large deletions, explaining low levels of *PTEN* aberration in our analysis. Nevertheless, we observed the increased prevalence of aberrations from baseline to EOST, which is consistent with increased tumor heterogeneity, despite the fact that only 63% of patients in the biomarker population progressed. Increasing detection of aberrations described in EOST specimens from SPARTAN underscores the genomic complexity in patients who progress to mCRPC.

We did not observe a higher prevalence of ctDNA in apalutamide versus placebo-treated patients at EOST. The prevalence of genomic AR and non-AR aberrations in ctDNA-positive samples and *AR* splice variant seen in apalutamide- and placebo-treated patients at EOST was also comparable despite a longer median treatment exposure with apalutamide plus ADT (18.3 months) versus placebo plus ADT (7.5 months). These findings suggest that apalutamide does not induce the acquisition of common mechanisms of AR resistance to a greater degree than observed with ADT alone. Our findings may provide confidence in clinical adoption of early treatment with apalutamide in combination with ADT; however, we cannot ignore the fact that the lower proportion of patients who progressed in the apalutamide group versus the placebo group may affect our ability to detect the difference.

Our findings may have implications for treating patients with nmCRPC after progression. We observed the association between the presence of any AR aberration and shorter PFS2 in the univariate analysis. This association was likely driven by the presence of *AR* amplification, supporting previous findings in mCRPC (6). The association between AR aberrations and poor outcome did not maintain significance in the multivariate analysis, also consistent with previous findings (6). Similar to other findings (6, 32), inactivation of *TP53* or *BRCA2* was associated with shorter OS in both univariate and multivariate analyses in our study, suggesting their potential as independent prognostic biomarkers of poor survival for patients receiving AR inhibitors after progression.

The SPARTAN biomarker population is a subset of the SPARTAN study population that includes those who progressed on therapy or discontinued treatment by the time of study unblinding. Therefore, the biomarker population may be enriched for patients with primary resistance to AR inhibition and may have poorer outcomes, as evidenced by the shorter time to MFS across treatment groups in the biomarker population versus the overall population. Nevertheless, despite the greater number of early progressors in the biomarker population, apalutamide treatment prolonged MFS, PFS2, and OS over placebo, consistent with the overall study results (4, 5).

The design of our analysis has a number of limitations. As our analysis assessed aberrations present at the time of progression, patients whose disease became resistant to therapy, that is, worse players, are prevalent in this analysis. However, we believe that the biomarker population represents the overall SPARTAN population because we observed consistent treatment benefit in MFS with apalutamide. The benefit was diminished in PFS2 and OS because patients were considered at risk at the time of progression. Another limitation is that only a subset of SPARTAN patients was tested for biomarkers at baseline and EOST, and only 55% of plasma samples and 63% of whole-blood samples collected at EOST had matched samples at baseline. Our findings should be further examined in the context of prospective studies to assess their generalizability. A limited number of assessed samples and low detectable levels of ctDNA and aberrations at baseline did not allow a thorough assessment of association between biomarker detection and clinical outcomes to evaluate a relative impact of biomarkers on apalutamide- versus placebo-treated patients. This assessment should be examined separately with the larger sample set, which was beyond the scope of this analysis. The limited number of prostate-related genes and types of aberrations were assessed in this study.

In our hypothesis-generating analysis of a subset of patients with nmCRPC and rapidly rising PSA receiving continuous ADT before study entry, we observed a low prevalence of aberrations at baseline that increased by EOST in both the apalutamide and placebo treatment groups, consistent with growing tumor burden and evolving heterogeneity. However, despite substantially longer treatment exposure to apalutamide and presumed stronger AR signaling inhibition with apalutamide plus ADT compared with placebo plus ADT, the prevalence of AR aberrations remained comparable at the end of treatment with apalutamide and placebo.

Authors' Disclosures

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