Published in final edited form as:

Prostate. 2021 May ; 81(6): 326–338. doi:10.1002/pros.24110.

Nivolumab plus Ipilimumab, with or without Enzalutamide, in AR-V7-expressing Metastatic Castration-Resistant Prostate Cancer: A Phase-2 Nonrandomized Clinical Trial

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Abstract

Background: AR-V7-positive metastatic prostate cancer is a lethal phenotype with few treatment options and poor survival.

Methods: The two-cohort nonrandomized phase 2 study of combined immune checkpoint blockade for AR-V7–expressing metastatic castration-resistant prostate cancer (STARVE-PC) evaluated nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg), without (cohort 1) or with (cohort 2) the anti-androgen enzalutamide. Co-primary endpoints were safety and PSA response rate.

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Contributions: Eugene Shenderov: Clinical investigation, coordination of the investigation, clinical trial statistical analysis, drafting of the manuscript, design of the clinical trial, review and revision of the manuscript, and approval of final manuscript. **Rana Sullivan, Alice Jordan, Donna Dowling, Rana Harb**: Clinical investigation, review and revision of the manuscript, and approval of final manuscript. **Hao Wang and Wei Fu**: Clinical trial statistical analysis, review and revision of the manuscript, and approval of final manuscript**. Joseph Schonhoft and Adam Jendrisak**: EPIC CTC assays, review and revision of the manuscript, and approval of final manuscript. **Michael A. Carducci and Mario A. Eisenberger**: Clinical investigation, review and revision of the manuscript, and approval of final manuscript. **James R. Eshleman, Jun Luo and Drew M. Pardoll**: Translational research, review and revision of the manuscript, and approval of final manuscript. **Karim Boudadi and Charles G. Drake**: Design of the clinical trial, review and revision of the manuscript, and approval of final manuscript. **Emmanuel S. Antonarakis**: Clinical investigation, design of the clinical trial, drafting of the manuscript, review and revision of the manuscript, and approval of final manuscript.

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Secondary endpoints included time-to-PSA-progression (PSA-PFS), time-to-clinical/radiographic-progression (PFS), objective response rate (ORR), PFS lasting >24 weeks, and overall survival (OS).

Results: Thirty patients were treated with ipilimumab plus nivolumab (N=15, cohort 1, previously reported), or ipilimumab plus nivolumab and enzalutamide (N=15, cohort 2) in patients previously progressing on enzalutamide monotherapy. PSA response rate was 2/15 (13%) in cohort 1 and 0/15 in cohort 2, ORR was 2/8 (25%) in cohort 1 and 0/9 in cohort 2 in those with measureable disease, median PSA-PFS was 3.0 (95%CI 2.1-NR) in cohort 1 and 2.7 (95%CI 2.1–5.9) months in cohort 2, and median PFS was 3.7 (95%CI 2.8–7.5) in cohort 1 and 2.9 (95%CI 1.3–5.8) months in cohort 2. Three of 15 patients in cohort 1 (20%, 95%CI 7.1–45.2%) and 4/15 patients (26.7%, 95%CI 10.5–52.4%) in cohort 2 achieved a durable PFS lasting >24 weeks. Median OS was 8.2 (95%CI 5.5–10.4) in cohort 1 and 14.2 (95%CI 8.5–NA) months in cohort 2. Efficacy results were not statistically different between cohorts. Grade-3/4 adverse events occurred in 7/15 cohort 1 patients (46%) and 8/15 cohort 2 patients (53%). Combined cohort (n=30) baseline alkaline phosphatase and cytokine analysis suggested improved OS for patients with lower alkaline phosphatase (HR 0.30, 95% CI 0.11–0.82), lower circulating IL-7 (HR 0.24, 95% Cl 0.06–0.93) and IL-6 (HR 0.13, 95% Cl 0.03–0.52) levels, and higher circulating IL-17 (HR 4.53, 95% CI 1.47–13.93) levels. There was a trend towards improved outcomes in men with low sPD-L1 serum levels.

Conclusions: Nivolumab plus ipilimumab demonstrated only modest activity in patients with AR-V7-expressing prostate cancer, and was not sufficient to justify further exploration in unselected patients. Stratification by baseline alkaline phosphatase and cytokines (IL-6, −7, and −17) may be prognostic for outcomes to immunotherapy.

Keywords

immunotherapy; prostatic cancer; ipilimumab; nivolumab; prognostic biomarker; enzalutamide

INTRODUCTION

AR-V7 is a truncated molecule capable of mediating constitutively active androgen receptor signaling[1]. Detection of AR-V7 in advanced prostate cancer (PCa) is associated with poor prognosis and aggressive course[2,3]. Circulating tumor cell (CTC)-based AR-V7-positive prostate cancers are generally resistant to novel hormonal therapies including abiraterone and enzalutamide[2,4]. Additionally, although AR-V7-positive prostate cancers may preferentially benefit from taxane chemotherapies including docetaxel and cabazitaxel, the prognosis remains poor[5,6]. Consequently, AR-V7-positive PCa patients usually have a median progression-free survival (PFS) of 3–4 months and a median overall survival (OS) of only 7–9 months, with infrequent longer term responders[4,7]. Therefore, proper patient treatment stratification and development of effective therapies for AR-V7-expressing advanced prostate cancer represents an urgent unmet clinical need.

Immune-checkpoint blockade may represent a treatment paradigm able to confer therapeutic benefit to this patient subset, as inhibition of cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and/or the programmed death 1 (PD-1) receptor has resulted in meaningful

antitumor responses in multiple aggressive cancer types[8,9]. At the expense of additional side-effects, blockade of both PD-1 and CTLA-4 has generally proven more efficacious than inhibition of either pathway alone[10]. Indeed, monotherapy with anti-CTLA-4 therapy or anti-PD-1/PD-L1 therapies has shown limited clinical benefit in prostate cancer to date[11,12]. This is, in part, thought to be attributed to prostate cancer being a low–mutation-burden tumor[13] that is immunologically "cold" with few tumor-infiltrating T cells[14]. However, some subtypes of prostate cancer, like AR-V7-expressing prostate cancers, may be associated with a greater rate of DNA-repair gene mutations making them more likely to respond to immunotherapy[15] as hypermutated tumors or those harboring DNA repair defects have shown greater sensitivity to immune-checkpoint inhibition[16,17]. Recently, Sharma and colleagues showed that metastatic castration-resistant prostate cancer (mCRPC) patients treated with anti-CTLA-4 (ipilimumab) plus anti-PD-1 (nivolumab) demonstrated responses of 10 to 25% depending on chemotherapy treatment status, especially when stratified by tumor mutational burden (TMB). We have previously reported the safety and efficacy of the combination of nivolumab plus ipilimumab in 15 AR-V7-positive mCRPC patients who had progressed on (and stopped) the second-generation androgen receptor antagonist enzalutamide on the first cohort of the Study of Combined Immune Checkpoint Blockade for AR-V7–Expressing Metastatic Castration-Resistant Prostate Cancer (STARVE-PC) Phase II trial[18]. In that report, two patients achieved a partial response, the median OS was 8.2 months, and seven patients (46%) reported grade 3–4 treatment-related adverse events (TRAEs). Notably, the trend in outcomes appeared to be better in patients with DNA-repair mutations, but was limited by small sample size with only 6/15 men having such mutations. Prior studies have shown that PD-L1, the ligand for PD-1, is upregulated on dendritic cells in men with mCRPC either progressing on or refractory to enzalutamide[19]. Consequently, a second cohort of the STARVE-PC trial was added which required patients to have enzalutamide as their most recent therapy, and enzalutamide was continued for study duration with the concurrent use of ipilimumab and nivolumab.

We hypothesized that AR-V7-positive mCRPC patients on enzalutamide would have increased PD-L1 expression making them more susceptible to treatment with combined immune-checkpoint blockade, and that this approach would be safe and tolerable. Herein, we present the clinical findings of the second cohort of the phase-2 STARVE-PC trial, as well as overall updated results for all 30 patients, and we report exploratory immunological studies identifying potential biomarkers of response.

MATERIALS AND METHODS

Study Design and Patient Eligibility

This trial was a single-institution two-cohort open-label phase 2 study conducted at The Johns Hopkins Hospital. Patients received treatment by intravenous infusion consisting of 3 mg/kilogram of nivolumab plus 1 mg/kilogram of ipilimumab every 3 weeks for 4 doses, followed by a maintenance regimen of 3 mg/kilogram of nivolumab every 2 weeks thereafter. The two cohorts were open to enrollment sequentially, and there was no randomization. Treatment continued until radiographic progression, unequivocal clinical progression, development of unacceptable toxicity, or withdrawal of consent.

Eligible patients had histologically confirmed, progressive, metastatic castration-resistant prostate cancer (mCRPC) with detectable AR-V7 transcripts using the Johns Hopkins CTC-based clinical-grade AR-V7 assay[20,21]. Cohort 1 subjects had to progress through initial hormonal therapy, either by orchiectomy or by using a GnRH agonist in combination with an anti-androgen. Cohort 2 subjects had to have enzalutamide as their most recent therapy and enzalutamide was continued for study duration despite prior progressive disease. Additional eligibility criteria included: an ECOG performance-status of 0–1, at least 18 years of age, serum testosterone <50 ng/dL with ongoing androgen-deprivation therapy, adequate organ (liver, kidney, bone marrow) function, and availability of new or archival tumor tissue for biomarker analysis. Key exclusion criteria included a second active malignancy within 5 years, prior immune-checkpoint inhibitor therapy, active brain or meningeal metastases, history of autoimmune disease, or requirement for systemic corticosteroids.

The primary endpoint was the PSA response rate, defined as a ≥50% decline in PSA from baseline maintained for ≥4 weeks. Secondary endpoints included time-to-PSA-progression (PSA-PFS), time-to-clinical/radiographic-progression (PFS), objective response rate (ORR) according to RECIST 1.1 criteria[22] in patients with measurable disease, PFS lasting >24 weeks (termed “durable PFS”), and overall survival (OS). PSA-progression was defined as a ≥25% increase in PSA from baseline or nadir, requiring confirmation ≥4 weeks later (PCWG2 criteria[23]). Clinical/radiologic-progression was defined as unequivocal symptomatic progression (worsening disease-related symptoms or new cancer-related complications), or radiographic progression (CT scan showing ≥20% enlargement in sum diameter of soft-tissue target lesions [RECIST1.1]; bone scan showing ≥2 new osseous lesions not related to bone flare) or death, whichever occurred first. Safety and adverse effects were also tabulated.

Study assessments were prospectively defined. PSA measurements were obtained at baseline and every 4 weeks on study. Radiographic evaluations (CT of chest/abdomen/pelvis and technetium-99 bone scans) were performed at baseline and every 12 weeks. Physical examination, toxicity assessments, and laboratory studies (complete blood count, comprehensive metabolic panel, thyroid function) were performed every 4 weeks. Safety was assessed by collecting and grading adverse events according to CTCAE v4.0 criteria.

This was an investigator-initiated trial (NCT02601014) designed by the principal investigators (E.S.A. and E.S.) and funded by Bristol Myers-Squibb who also provided both study drugs free of cost. The study was approved by the Johns Hopkins University IRB, and was overseen by an independent scientific review committee and a data and safety monitoring committee. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients provided written informed consent before participation.

DNA sequencing and DNA-repair gene analysis

All 15 patients in cohort 1 underwent prospective tumor DNA sequencing as previously described[18]. Twelve of 15 men in cohort 2 underwent germline (Color Genomics) and somatic (Foundation Medicine, or Personal Genome Diagnostics) genomic testing for

clinical purposes at different stages of treatment utilizing various commercial next-generation DNA sequencing assays. In addition to examining sequence alterations and microsatellite instability, we generated estimates of mutation burden for each tumor (Personal Genome Diagnostics [PGDx], Baltimore, MD, and Foundation Medicine, Boston, MA). We subsequently focused on sequence alterations in the DNA-repair genes. Only protein-truncating alterations or ClinVar[24] designated pathogenic alterations were classified as pathogenic or likely pathogenic in the current analysis. Patients were considered to be DNA repair-deficient (DRD-positive [DRD+]) if they had at least one pathogenic mutation in a gene involved in DNA-damage repair[25]; otherwise they were classified as DRD-negative (DRD–).

CTC detection, and phenotypic heterogeneity (Shannon index)

Circulating tumor cell (CTC) detection was performed as described previously[11,13,26]. Briefly, blood from each subject was collected in Streck tubes, shipped to Epic Sciences (San Diego, CA) and underwent automated immunofluorescent staining to identify CTCs by a combination of morphological features and malignant biomarkers (CK and AR N-terminal) in the absence of CD45 expression. CTC phenotypic heterogeneity was assessed using the Shannon index as previously described using the Epic Sciences platform[11]. As previously reported[18], a high Shannon index was one with a score of ≥1.5 and a low Shannon index was one with a score of <1.5.

Cytokine, chemokine and soluble factor ELISA analysis

Patient baseline sera was collected prior to treatment and custom Luminex ELISA was performed as per manufacturer's instructions (R&D Systems, a Bio-Techne brand, Minneapolis, MN). The analytes included in these studies were soluble B7-H3, soluble PD-L1, soluble Galectin-3, soluble 4–1BB, IFN-γ, IL-2, Il-1b, IL-5, IL-7, IL-13, IL-17, IL-36b, IL-33, IL-6, IL-8, IL-15, IL-23, and TNF-a. Associations with clinical outcomes were analyzed using the lower tertile group (<33rd percentile) compared against the middle and upper tertile group (>33rd percentile).

Statistical analyses

The primary clinical trial endpoint was PSA 50% response, and a response rate above 5% was considered clinically meaningful. The trial was powered for each cohort separately. Accordingly, a sample size of 15 patients with 3 observed PSA responses would produce a 90% confidence interval of 6–44% for the PSA response rate, which would be above the null hypothesis of 5% response rate. The null hypothesis would therefore be rejected if ≥3 of 15 patients achieved a PSA response.

Analyses of response endpoints (e.g. PSA response, ORR) were expressed as proportions with 2-sided Agresti-Coull binomial 95% confidence intervals. Time-to-event endpoints (e.g. PSA-PFS, PFS, OS) were analyzed using the Kaplan-Meier method and 95% confidence intervals were generated using the generalized Brookmeyer-Crowley method after log-transformation. Clinical outcomes were compared among patients who were DRD+ and DRD– (primary biomarker analysis), as well as according to other biomarker categories (CTC heterogeneity), and baseline cytokine/chemokine profiles). To examine associations

between clinical outcomes and biomarker status, response endpoints were compared using Fisher's exact test, and time-to-event endpoints were compared using the log-rank test with Cox proportional-hazards models to estimate hazard ratios. All tests were two-sided, and P values ≤0.05 were considered significant. Analyses of biomarkers were primarily hypothesis generating without adjustment for multiple testing. Statistical analyses were performed using R (version 3.4.3) and GraphPad Prism version 8.4.3.

RESULTS

Clinical Trial Outcomes

Patients (N=30; both cohorts) were enrolled from March 2016 through March 2020. In total, 52 patients were assessed for eligibility on the basis of a preliminary positive AR-V7 test that was repeated prior to enrollment, 22 were excluded, and 30 patients were treated with at least 1 dose of the study drugs (Fig. 1). Fifteen patients on Cohort 1 received Nivolumab plus Ipilimumab without next-generation anti-androgen as previously reported[18], and 15 patients on Cohort 2 (who were previously progressing on Enzalutamide monotherapy) received Nivolumab plus Ipilimumab together with continuation of Enzalutamide as described henceforth.

The baseline characteristics for all patients, and by trial Cohort, are summarized in Table 1. Median age for Cohort 2 participants was 70.5 years, 33% had ECOG performance-status of 1, median baseline PSA was 151 ng/mL, 40% had visceral metastases (liver 20%, lung 6.7%, adrenal gland 6.7%, and peritoneum 6.7%), and 40% had received ≥4 prior regimens for metastatic castration-resistant prostate cancer (mCRPC). At the time of data cutoff (August 15, 2020), median follow-up was 9.9 (range, 2.3–17.3) months, and 8 patients remained alive. Baseline characteristics were generally balanced between cohorts, even though the two cohorts were not randomly assigned. Treatment exposure and patient disposition are shown in Table S1. Cohort 1 median (range) treatment duration was 2.4 (0.9–8.6) with patients receiving a median of 3 combination doses, and cohort 2 was 2.8 (0.7–14) with a median of 4 combination doses received. Primary causes for discontinuation were study drug toxicity (6 cohort 1 patients [40%] and 3 cohort 2 patients [20%]) and disease progression (9 cohort 1 patients [60%] and 11 [73.3%] cohort 2 patients).

All patients in Cohort 2 were evaluable for efficacy (summarized in Table 2). Overall, none of 15 men achieved a PSA response. Among the 9 patients with measurable soft-tissue disease, the objective response rate (ORR) was 0%. Median PSA-PFS was 2.7 (95%CI 2.1–5.9) months (Fig. 2A), and median PFS was 2.9 (95%CI 1.3–5.8) months (Fig. 2B). Four of 15 patients (26.7%, 95%CI 10.5–52.4%) achieved a "durable PFS >24 weeks". Median OS was 14.2 (95%CI 8.5–NA) months (Fig. 2C). Efficacy results between cohorts were not statistically different, as summarized in Table 2. Overall, combining data from cohorts 1 and 2, median PFS-PSA was 2.8 months, median PFS was 3.0 months, and median OS was 9.5 months (Supp. Fig. 1). Patient time to progression for each patient by cohort, DRD, and TMB is shown in supplementary Figure 2.

Safety and Adverse Events

Sixteen grade 3–4 adverse events occurred in 8 of 15 patients in cohort 2 (53%, Table 3). Two cases of fatigue, two cases of diarrhea/colitis, two cases of elevated lipase, two cases of adrenal insufficiency, and one case each of pain, syncope, hyponatremia, anemia, hypothyroidism, hypertension, hypophysitis, and decreased leukocyte count were observed in total. Immune-related adverse events were of particular interest, and there were seven events (affecting 47% of patients) possibly or probably related to autoimmune phenomena: two episodes of colitis, two episodes of adrenal insufficiency, one episode of hypothyroidism, one episode of hypophysitis, and an episode of decreased leukocyte count; pneumonitis was not observed. Treatment discontinuation was required in 3 of 15 cohort 2 patients. Overall, the most common toxicities of any grade that developed during or after treatment were GI, dermatitis/pruritus, pain, and fatigue. There were no treatment-related deaths. No notable adverse event differences were observed between cohorts (Table 3).

DNA-based biomarker outcomes

Microsatellite instability was not noted in any patient. Mean tumor mutational load was estimated at 2.9 (range, 1–6) mutations/Mb in cohort 2 compared to 2.7 (range, 1–10) mutations/Mb in cohort 1; median TMB for the overall patient population was cohort 2.0 mutations/Mb. DNA-repair deficient (DRD+) status in cohort 2 was noted for 2 of 15 patients (13%) who harbored potentially deleterious somatic and/or germline mutations in a least one DNA-repair gene (Table 4). Patient 6 had a somatic *CDK12* mutation with frameshift mutation and loss of heterozygosity as a basis for biallelic inactivation without durable response noted, and patient 8 had a somatic mutation in *ATM* (durable response noted); there were no observed *BRCA1/2* mutations. No germline mutations were observed in cohort 2.

As biological data emerging for enzalutamide during the course of the trial has not shown compelling data for enzalutamide sensitizing for immunotherapy[27], biomarker hypothesis-generating analyses were performed for the overall AR-V7 positive nivolumab plus ipilimumab treated cohort (N=30).

Clinical outcomes of the DRD+ and DRD– patients are summarized in Figure 3 and Supplementary Figure 3. PSA-PFS, PFS, and OS response measures did not differ statistically between DRD+ and DRD- cases, but were numerically greater among DRD+ patients. We next examined clinical outcomes according to TMB status greater than or equal to 5 (one standard deviation above mean, mean 2.8, standard deviation 2.1). Again, there were no observed PSA-PFS, PFS, or OS differences (Supp. Fig. 4), although there was a trend towards greater PFS in the TMB-high group. As the conventional definition of high TMB is ≥10 muts/Mb, it is possible that no TMB effect was noted due to the fact that there were no truly TMB-high patients in this study. To examine the prognostic impact of CTC phenotypic heterogeneity, we compared outcomes in patients with a high (≥1.5) versus low (<1.5) Shannon index (Supp. Fig. 5). No statistical associations were observed for PSA-PFS, PFS, or OS; although trends for improved outcomes were suggested in the Shannon-high group.

Cytokine, chemokine and soluble factor analysis

PD-L1, the primary ligand of PD-1, is known to have both a cell membrane presence (mPD-L1) and can also be secreted as a soluble molecule (sPD-L1) into the plasma. High sPD-L1 has been correlated with a poor prognosis in various cancers, but has not been studied in prostate cancer. We hypothesized that sPD-L1 levels may represent an indirect measure of mPD-L1, will be available for most samples unlike difficult to obtain metastatic biopsies, and that baseline serum levels might serve as a biomarker of clinical response especially in conjunction with co-measured immune cytokines. Extreme analysis looking at the lowest tertile versus the middle and upper tertiles of sPD-L1 levels was utilized to enrich for possible responders versus non-responders (Fig. 4). Accordingly, PSA-PFS was 4.2 versus 2.7 months in the lowest tertile versus the 2 upper tertiles (HR 0.72, 95%CI 0.32–1.63), PFS was 3.6 versus 2.9 months (HR 0.40, 95%CI 0.31–1.60), and OS was 10.7 versus 8.2 months (HR 0.53, 95%CI 0.22–1.29). Adjustment for patient age and concurrent enzalutamide treatment status suggested a trend toward improved OS for patients in the lowest tertile of sPD-L1 levels (HR 0.35, 95% CI 0.11–1.11, Fig. S8)

Supplementary figures 6–8 show forest plots summarizing PSA-PFS, PFS, and OS outcomes according to various additional cytokines, chemokines and soluble factors (collected at baseline, before ipilimumab/nivolumab administration). For each analyte, outcomes were compared for patients in the lowest tertile versus the middle and upper tertiles with median survival summarized in panels A, unadjusted hazard ratios in panels B, and adjusted hazard ratio (for patient age and enzalutamide treatment status) in panels C. Adjustment for patient age and enzalutamide status suggested improved OS for patients with lower circulating concentrations of IL-7 (HR 0.24, 95% Cl 0.06–0.93) and IL-6 (HR 0.13, 95% Cl 0.03–0.52), and higher circulating concentrations of IL-17 (HR 4.53, 95% CI 1.47–13.93). This held true for IL-6 across PSA-PFS (HR 0.28, 95% CI 0.09–0.87) and PFS (HR 0.19, 95% CI 0.05–0.67) as well.

We also explored the relationship between some clinical factors and outcomes. Analysis of baseline alkaline phosphatase, but not hemoglobin, showed improved median OS for patients with alkaline phosphatase below 1.5x upper limit of normal both pre (HR 0.4, 95% Cl 0.16–0.99) and post (HR 0.3, 95% Cl 0.11–0.82) adjustment for patient age and enzalutamide treatment status (Fig. S9).

DISCUSSION

Patients with metastatic prostate cancer have a median survival of 3–5 years; however, AR-V7-expressing PCa is a lethal phenotype with significantly shortened survival times and inadequate treatment options[28]. Herein, we present clinical outcomes from cohort 2, as well as a combined analysis of cohorts 1 and 2, of the STARVE-PC phase II trial specifically targeting AR-V7-positive mCRPC with ipilimumab 1 mg/k plus nivolumab 3 mg/kg immunotherapy. This study of 30 total patients represents the largest report of immunotherapy-treated AR-V7 PCa to-date, building on our first-in-field report of cohort 1 results[18]. The trial did not meet the primary endpoint of PSA response in either cohort. However, a subset of patients demonstrated modest clinical activity to combined immune-checkpoint blockade and could be identified by low alkaline phosphatase level (<1.5x ULN),

as suggested in previous studies of CTLA-4 blockade with ipilimumab[29], as well as circulating cytokine status prior to treatment. No additional exposure data including prior lines of therapy, clinical features, or laboratory tests were noted to be associated with response. These findings support further evaluation of immunotherapy in a biomarker-defined manner in mCRPC patients.

In a subset of patients with metastatic disease of multiple cancer types including NSCLC, melanoma, RCC, and bladder cancer, immune-checkpoint inhibitors have been shown to provide durable, and in some cases complete, responses. Prostate cancer has been shown to be poorly T-cell infiltrated, i.e. immunologically "cold," and this likely explains why immune-checkpoint blockade using single-agent CTLA-4 or PD-1/PD-L1 antibodies has yielded few objective responses in PCa[12,30], although notable exceptional responses have been observed in some PCa patients with mismatch repair deficient (MMRd)[16] or homologous-recombination deficient (HRD) tumors[31]. Nivolumab plus ipilimumab for mCRPC in the recently reported Checkmate-650 trial showed an ORR of 25% in pre-chemotherapy patients, and an ORR of 10% in post-chemotherapy mCRPC patients, but at the expense of 4 treatment-related deaths in 90 patients (4.4%) and 43 grade 3–4 TRAEs in ~42–53% of patients. Comparatively, our cohort had no treatment-related deaths, though immune-related adverse events were frequently observed. A possible explanation is the different treatment regiments: Checkmate-650 utilized ipilimumab 3 mg/kg and nivolumab 1 mg/kg, while this study used ipilimumab 1 mg/kg and nivolumab 3 mg/kg. The KEYNOTE-029 and CheckMate-511 clinical trials have previously shown increased toxicity from 3 mg/kg versus 1 mg/kg ipilimumab consistent with the present differences observed[32,33].

None of the patients in the STARVE-PC trial had MMRd cancers, and the initial encouraging activity noted in a subset of patients harboring germline and/or somatic mutations in DNA-repair genes in the preliminary cohort 1 dataset were not reproduced in cohort 2, or in the combined analysis. It is possible that our results were influenced by prioritization of PCa patients with DNA-repair genes into PARP-inhibitor trials that were enrolling concurrently to cohort 2 at our institution resulting in no *BRCA1/2* mutations in cohort 2. Different DRD mutations have been shown to differentially impact the tumor microenvironment and the likely response to immune checkpoint therapy as in this tria[34]. Furthermore, recent clinical trial data on homologous repair-deficient PCa responsiveness to immunotherapy is mixed overall[30,35]. Also, while patients with high CTC heterogeneity had favorable responses in Cohort 1, this trend was not statistically significant in Cohort 2. TMB has also been reported as a potential biomarker of response in ipilimumab plus nivolumab-treated lung cancer[17], and recently by Sharma and colleagues in ipilimumab plus nivolumab-treated mCRPC[35]. In our analysis, TMB status did not correlate with therapeutic response, although a trend was seen for greater PFS (but not OS) in the TMB-high group. This may have been due to the fact that we were underpowered to show a significant association due to the small overall sample size (n=30).

We also hypothesized that patients treated with ipilimumab plus nivolumab and concurrent enzalutamide, in cohort 2, would have increased clinical responses, but we did not observe this outcome. The hypothesis that enzalutamide increases PD-L1 expression in mCRPC[19,36]

was further not born out in the recently published trial by Graff and colleagues where the authors could not find increased PD-L1 expression in enzalutamide-treated mCRPC[30]. Unfortunately, reliable PD-L1 expression analysis is complicated by multiple confounding issues including tumor cell versus tumor-infiltrating-lymphocyte expression, the cutoffs utilized, and obtaining biopsies for tissue staining for all patients leading to patient subsets being studied. Interestingly, the primary ligand of PD-1, i.e. PD-L1, is known to have both a cell membrane presence (mPD-L1) and can be secreted as a soluble molecule (sPD-L1) into the plasma. Our secondary hypothesis that sPD-L1 levels may serve as a biomarker of clinical response was not statistically proven, but a favorable OS trend for those with lower sPD-L1 expression was noted in the performed post-hoc analysis. These results suggest that in prostate cancer, high sPD-L1 expression may correlate with poor prognosis, as observed in various other cancers including lung cancer[37], gastric cancer[38–40], rectal cancer[41], lymphoma[37,42], renal cell carcinoma, hepatocellular carcinoma[43], esophageal cancer[44,45], and soft tissue sarcoma[46]. However, this is the first time that sPD-L1 is studied in prostate cancer. Interestingly, lower levels of IL-6 and −7 were both associated with improved outcomes, as was higher levels of IL-17. This is consistent with prior literature showing elevated IL-6 in patients with prostate cancer being strongly associated with higher disease stage, presence of metastases, and poor prognosis[47–49]. Compared to patients with benign prostate hyperplasia patients with PCa are more likely to express IL-7[50]. The role of IL-17 in tumorigenesis is still being explored with both pro- and anti-tumor immunity roles described[51].

Our study has several limitations. Primarily, AR-V7 is an uncommon phenotype leading to a small sample size (n=30, overall). Our ability to interrogate the impact of concurrent administration of enzalutamide was even more limited, due to only 15 patients being enrolled per cohort. Our biomarker data is hypothesis-generating, without type I error control for multiple comparisons. It will require future validation studies with larger patient populations. The lack of a placebo group, or single ipilimumab or nivolumab treatment groups, limits the ability to understand if sPD-L1 would predict response to either agent alone or if it defines better prognosis tumors regardless of immunotherapeutic intervention.

In conclusion, our collective data from the STARVE-PC trial suggest that the combination of nivolumab 3 mg/kg plus ipilimumab 1 mg/kg demonstrates acceptable safety but only modest activity in a patients with AR-V7-expressing prostate cancer, and was not sufficient to justify further exploration in unselected patients. The intriguing association between immunotherapy efficacy and alkaline phosphatase, sPD-L1 as well as circulating cytokine levels of IL-6, −7, and −17 requires additional confirmation in prostate cancer. Optimization of other immune checkpoint targets, patient selection with further evaluation of biomarkers, and dosing optimization is still required to yield successful clinical results in treatment resistant AR-V7-positive prostate cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

We thank all patients, families, and caregivers who participated in this study. This trial is registered at ClinicalTrials.gov (NCT02601014).

Funding: This investigator initiated research was sponsored by Bristol-Myers Squibb (BMS, Princeton, NJ) who also provided both study drugs free of cost. This work was also partially supported by National Institutes of Health Cancer Center Support Grant P30 CA006973 (ES, ESA), Prostate Cancer Foundation Young Investigator Award (ES), Department of Defense grant W81XWH-16-PCRP-CCRSA (ESA) and grant W81XWH-19-1-0511 (ES), and the Bloomberg-Kimmel Institute for Cancer Immunotherapy (ES, DMP, ESA).

Conflicts of Interest: Emmanuel S. Antonarakis is a paid consultant/advisor to and or received institutional research funding from Janssen, Astellas, Sanofi, Dendreon, Genentech, Pfizer, Amgen, Lilly, Bayer, Tokai, Clovis, AstraZeneca, Novartis, BMS; he is the co-inventor with lead inventor Jun Luo of an AR-V7 test that has been licensed to Qiagen. Joseph Schonhoft and Adam Jendrisak are employees of Epic Sciences and receive stock options. The other authors made no disclosures.

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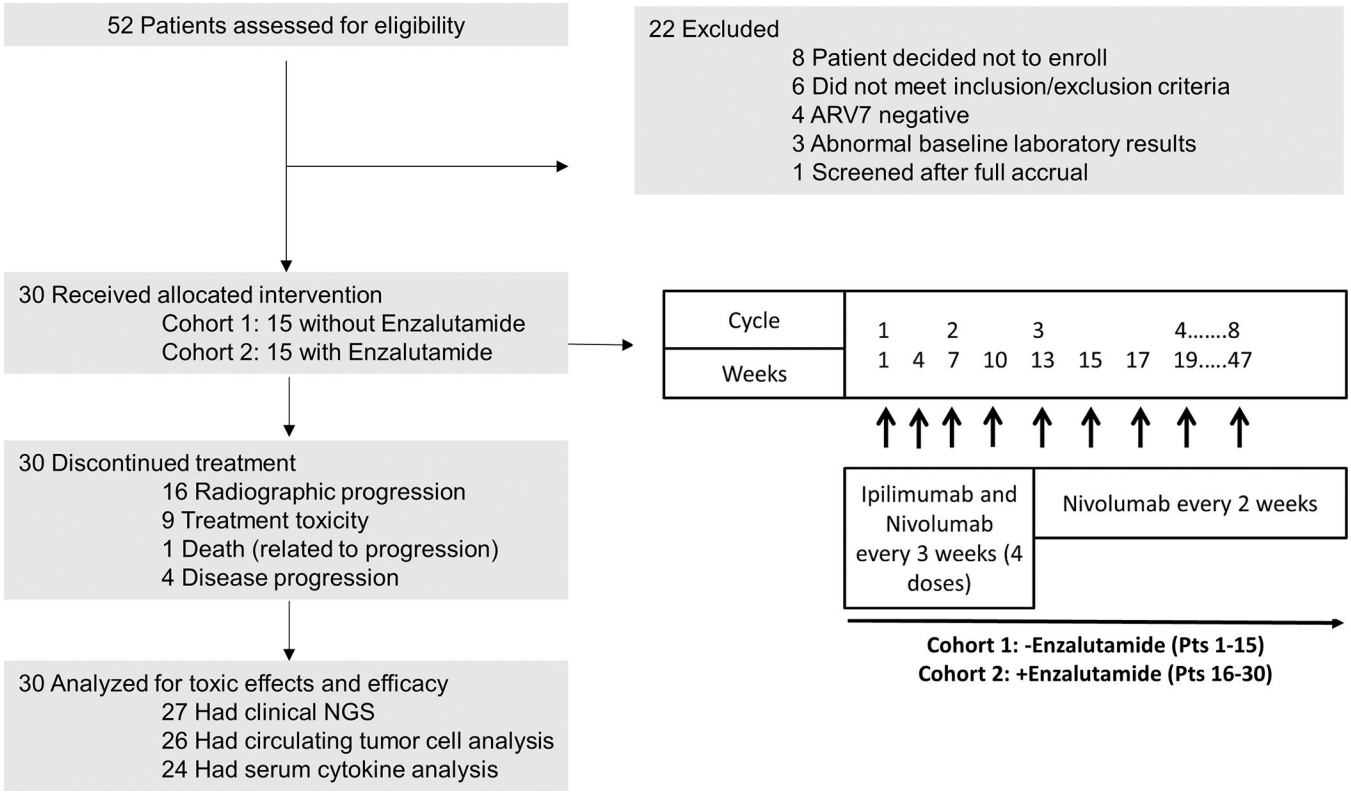

Figure 1.
Study Flowchart

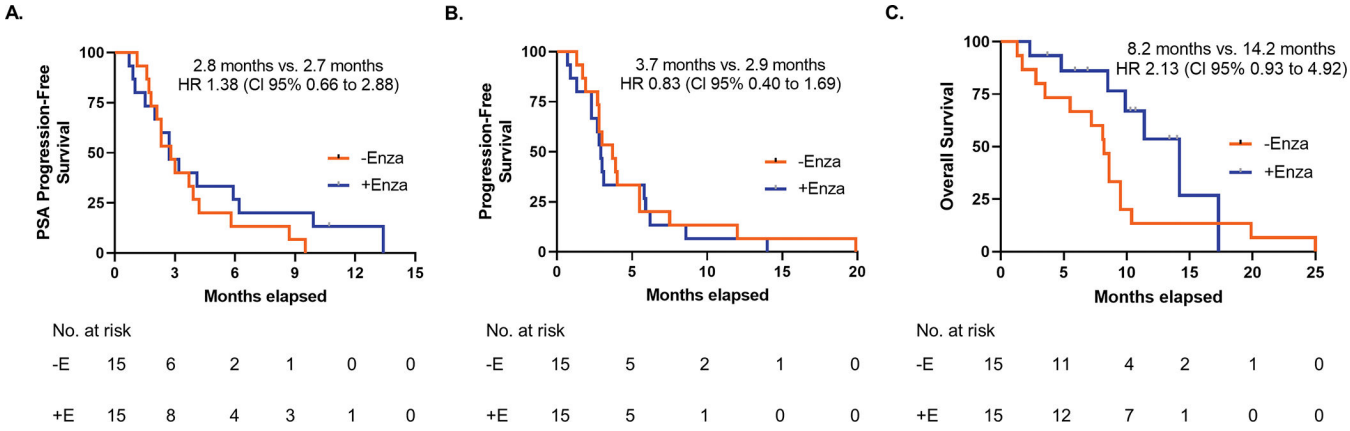

Figure 2.

Time to event outcomes according to Enzalutamide treatment (cohort 1 vs 2). **(A)** PSA-PFS according to Enzalutamide treatment cohort. **(B)** PFS according to Enzalutamide treatment cohort. **(C)** OS according to Enzalutamide treatment cohort. Orange line without Enzalutamide and blue line with Enzalutamide treatment.

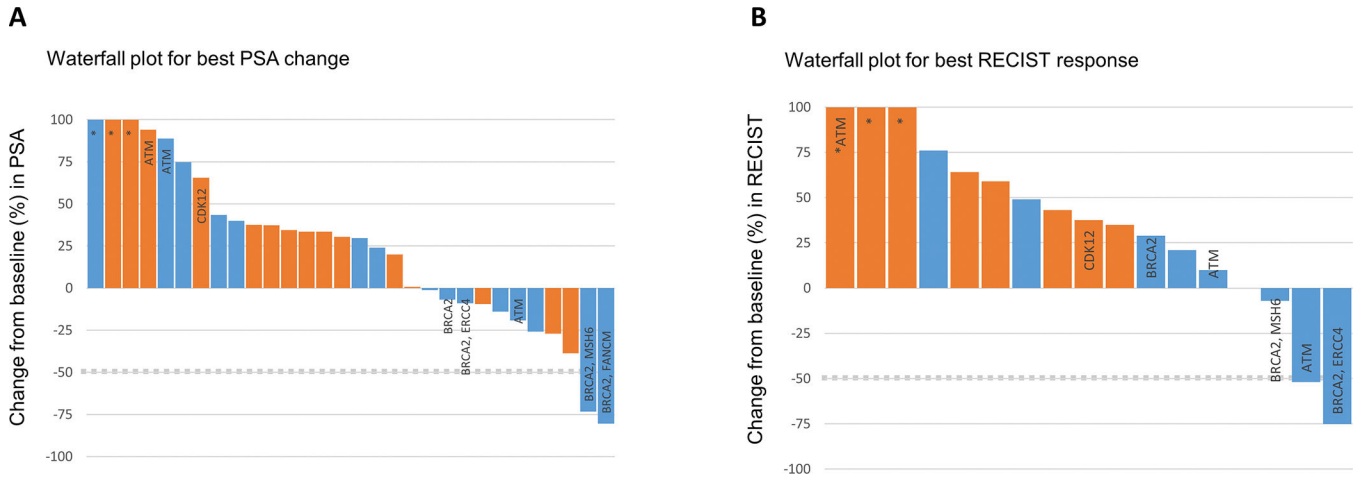

Figure 3.

PSA responses and radiographic responses according to Enzalutamide treatment and superimposed patient specific DRD mutations. **(A)** PSA response according to Enzalutamide treatment. **(B)** RECIST 1.1 Response according to Enzalutamide treatment. Dashed lines indicates PSA50 response. Orange bars indicate with Enzalutamide and blue bars without Enzalutamide treatment. *Indicates values truncated at 100%.

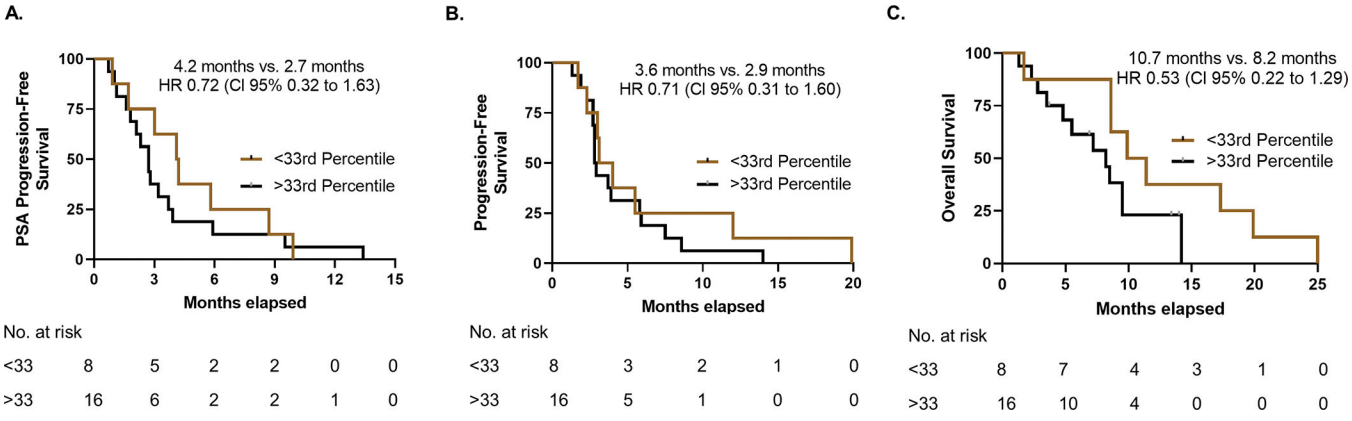

Figure 4.

Time to event outcomes according to soluble PD-L1 level (lower vs. mid+upper tertile). **(A)** PSA-PFS according to soluble PD-L1 <33rd percentile or >33rd percentile. **(B)** PFS according to soluble PD-L1 <33rd percentile or >33rd percentile. **(C)** OS according to soluble PD-L1 <33rd percentile or >33rd percentile. Brown line soluble PD-L1 below 33rd percentile and black line soluble PD-L1 above 33rd percentile.

Table 1.

Baseline characteristics of patients in Cohort 1, Cohort 2, and the combined population

Baseline Characteristic	Ipi / Nivo (N=15)	Enzalutamide + Ipi / Nivo (N=15)	All Patients (N=30)
Median age at study entry, N (range) - yrs	65 (52–76)	70.5 (54–77)	67 (52–77)
Race, N (%)			
white	13 (86.7%)	13 (86.7%)	26 (86.7%)
black	1 (6.7%%)	2 (13.3%)	3 (10%)
other	1 (6.7%%)	0 (0%)	1 (3.3%)
ECOG performance score, N (%)			
0	8 (53.3%)	10 (66.7%)	18 (60%)
1	7 (46.7%)	5 (33.3%)	12 (40%)
Gleason sum at diagnosis, N (%)			
≤7	4 (26.7%)	4 (26.7%)	8 (26.7%)
≥8	11 (73.3%)	11 (73.3%)	22 (73.3%)
PSA (ng/mL) median (range)	115 (31–7576)	151.4 (4.4–1316.9)	135.7 (4.4–7576)
Hemoglobin, N (%)			
<11 g/dL	7 (46.7%)	5 (33.3%)	12 (40%)
≥11 g/dL	8 (53.3%)	10 (66.7%)	18 (60%)
Alkaline phosphatase, N (%)			
<1.5 × upper limit of normal	7 (46.7%)	6 (40%)	13 (43.3%)
≥1.5 × upper limit of normal	8 (53.3%)	9 (60%)	17 (56.7%)
Number of prior regimens for CRPC, N (%)			
1	0 (0%)	3 (20%)	3 (10%)
2	4 (27%)	0 (0%)	4 (13.3%)
3	2 (13%)	6 (40%)	8 (26.7%)
≥4	9 (60%)	6 (40%)	15 (50%)
Prior treatments, N (%)			
Abiraterone	12 (80%)	13 (86.7%)	25 (83.3%)
Enzalutamide	12 (80%)	15 (100%)	27 (90%)
Abiraterone + Enzalutamide	9 (60%)	13 (86.7%)	22 (73.3%)
Docetaxel	13 (85%)	5 (33.3%)	18 (60%)
Cabazitaxel	5 (33%)	0 (0%)	5 (16.7%)
Sipuleucel-T	7 (47%)	3 (20%)	10 (33.3%)
Radium-223	4 (27%)	4 (26.7%)	8 (26.7%)
Presence of bone metastases, N (%)			
yes	15 (100%)	15 (100%)	30 (100%)
no	0 (0%)	0 (0%)	0 (0%)

Baseline Characteristic	Ipi / Nivo (N=15)	Enzalutamide + Ipi / Nivo (N=15)	All Patients (N=30)
Presence of visceral metastases, N (%)			
Yes, any	10 (66.7%)	6 (40%)	16 (53.3%)
Liver	6 (40%)	3 (20%)	9 (30%)
Lung	3 (20%)	1 (6.7%)	4 (13.3%)
Adrenal gland	0 (0 %)	1 (6.7%)	1 (3.3%)
Peritoneum	1 (6.7%)	1 (6.7%)	2 (6.7%)
No	5 (33.3%)	9 (60%)	14 (46.7%)
Presence of nodal metastases, N (%)			
Yes	7 (47.0%)	6 (40%)	13 (43.3%)
No	8 (53.0%)	9 (60%)	17 (56.7%)

Table 2.

Overall outcomes for Cohort 2, Cohort 1, and the combined population

	Overall (N=30)	Enzalutamide + Ipi / Nivo (N=15)	Ipi / Nivo (N=15)	HR (95%CI)
PSA_{50} , N (%) (95% CI)	2/30 (6.7%) (0.8%, 22.4%)	0/15 (0%) (0, 23.9%)	2/15 (13.3%) (2.5%, 39.1%)	-
ORR, N (%) (95% CI)	2/17 (11.8%) (2.0%, 35.6%)	0/9 (0%) (0, 34.5%)	2/8 (25%) (6.3%, 59.9%)	-
PFS at 24 wks (95% CI)	7/30 (23.3%) (11.5%, 41.2%)	4/15 (26.7%) (10.5%, 52.4%)	3/15 (20%) (6.3%, 46.0%)	-
PSA-PFS (mo), (95% CI)	2.7 (2.1, 4.1)	2.7 (1.0, 5.9)	2.8 (1.7, 3.9)	1.43 (0.66 – 3.08)
PFS (mo), (95% CI)	3.0 (2.7, 5.5)	2.9 (1.3, 5.8)	3.7 (1.9, 5.5)	0.82 (0.40 – 1.71)
OS (mo), (95% CI)	9.5 (8.1, 11.4)	14.2 (8.5, NA)	8.2 (2.8, 9.5)	2.13 (0.93 – 4.92)

Table 3.

Summary of treatment related adverse events (AEs) in Cohort 2, and summary of grade 3–4 AEs and immune related adverse events (irAEs) by Cohort. All grade 3–4 and grade 1–2 occurring >1 time are shown.

Adverse Event	All Grade (total) AEs, N (%)	Grade 1–2 AEs, N (%)	Grade 3–4 AEs, N (%)
Grade 3–4 AEs by Cohort (patients (%))	**Overall: 15/30 (50%)**	**Cohort 1: 7/15 (46%)**	**Cohort 2: 8/15 (53%)**
Grade 3–4 irAEs by Cohort (patients (%))	**Overall: 12/30 (40%)**	**Cohort 1: 5/15 (33%)**	**Cohort 2: 7/15 (47%)**
Grade 3–4 AEs leading to treatment discontinuation by Cohort (patients (%))	**Overall: 9/30 (30%)**	**Cohort 1: 6/15 (40%)**	**Cohort 2: 3/15 (20%)**
GI (Constipation/Anorexia/Nausea/WT loss)	14 (93 %)	14 (93 %)	0 (0 %)
Dermatitis/Pruritis	14 (93 %)	14 (93 %)	0 (0 %)
Pain	12 (80 %)	12 (80 %)	1 (7 %)
Fatigue	9 (60 %)	7 (47 %)	2 (13 %)
Neurological	7 (47 %)	6 (40 %)	1 (7 %)
Electrolyte Change (Hypocalcemia, Hyponatremia)	6 (40 %)	5 (33 %)	1 (7 %)
Anemia	5 (33 %)	4 (27 %)	1 (7 %)
Flu-like symptoms	5 (33 %)	5 (33 %)	0 (0 %)
Diarrhea/Colitis	5 (33 %)	3 (20 %)	2 (20 %)
Amylase/Lipase increased	4 (27 %)	2 (13 %)	2 (13 %)
Hypothyroidism	4 (27 %)	3 (20 %)	1 (7 %)
Dysgeusia	3 (20 %)	3 (20 %)	0 (0 %)
Adrenal insufficiency	2 (13 %)	0 (0 %)	2 (13 %)
AST/ALT increased	2 (13 %)	2 (13 %)	0 (0 %)
Dyspnea	2 (13 %)	2 (13 %)	0 (0 %)
Muscle weakness	2 (13 %)	2 (13 %)	0 (0 %)
Hematuria	2 (13 %)	2 (13 %)	0 (0 %)
Hot flashes	2 (13 %)	2 (13 %)	0 (0 %)
Thrombocytopenia	2 (13 %)	2 (13 %)	0 (0 %)
Decreased WBC	1 (7 %)	0 (0 %)	1 (7 %)
HTN	1 (7 %)	0 (0 %)	1 (7 %)
Hypophysitis	1 (7 %)	0 (0 %)	1 (7 %)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 4.

Summary of Cohort 2 clinical response by DNA repair deficiency (DRD) mutations and tumor mutational load (TMB)

Patient No.	Prior Systemic Treatment	Basline PSA	PSA50 achieved	RECIST Response Noted	Durable PFS (≥24 wks)	DRD status	DNA repair gene	MSI	TMB (Muts/Mb)	Source of tumor cells	Platform
1	Abi, Enza, Rad223, BETi	465.4	No	No	No	-	None	MSS	2.0	Prostate	PGDxCS
2	Abi, Enza, Rad223, TGF-βRI	14.4	No	No	No	-	None	MSS	1.0	Prostate	PGDxCS
3	Abi, Enza	8.2	No	No	No	-	None	MSS	4.0	Prostate	FOne
4	Abi, Enza, Doce, Sip-T, Rad223, PI3Ki	195.9	No	No	YES	-	None	MSS	2.0	Plasma	PGDxPS
5	Abi, Enza, BAT	4.4	No	No	No	-	None	MSS	2.0	Prostate	PGDxCS
6	Abi, Enza, BAT	151.4	No	No	No	+	CDK12 [K523fs*87+ LOH)]	MSS	1.0	Lymph Node	FOneCDx
7	Abi, Enza, Doce, Doxo, Lut177-PSMA	8.3	No	No	YES	-	None	MSS	6.0	Prostate	FOne
8	Abi, Enza, BAT	859.7	No	No	YES	+	ATM [Loss exons 30–34]	MSS	4.0	Prostate	FOne
9	Enza	444.2	No	No	No	ND	-	-	-	-	-
10	Abi, Enza, Doce, Sip-T, Rad223, BAT	238.6	No	No	No	-	None	MSS	3.0	Plasma	PGDxPS
11	Abi, Enza, BAT	25.9	No	No	No	ND	-	-	-	-	-
12	Abi, Enza, Doce	224	No	No	YES	ND	-	-	-	-	-
13	Enza	1316.9	No	No	No	-	None	MSS	5.0	Prostate	PGDxCS
14	Abi, Enza, Doce	8.7	No	No	No	-	None	MSS	4.0	Prostate	FOne
15	Abi, Enza, Sip-T, mTORi	120	No	No	No	-	None	MSS	1.0	Prostate	FOneCDx

Abbreviations: Abi, Abiraterone; Enza, Enzalutamide; Rad223, Radium-223; BETi, BET inhibitor; TGF-βRI, TGF-beta receptor inhibitor; Doce, Docetaxel; Sip-T, Sipuleucel-T; PI3Ki, PI3K inhibitor, BAT, Bipolar androgen therapy; Doxo, Doxorubicin; Lut177-PSMA, Lutetium177-PSMA; mTORi, mTOR inhibitor, PGDxCS, PGDx CancerSELECT 125; FOne, Foundation One; FOneCDx, FoundationOne CDx; PGDxPS, PGDx PlasmaSELECT 64.