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PD-1/PD-L1 Pathway Inhibitors in Advanced Prostate Cancer

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Abstract

Introduction: Pharmacological inhibition of immune checkpoint receptors or their ligands represents a transformative breakthrough in the management of multiple cancers. However, immune checkpoint inhibitors have yet to be FDA-approved for the management of metastatic prostate cancer (PCa), the commonest non-cutaneous malignancy in men.

Areas covered: We review our current understanding of the PD-1/PD-L1 pathway in cancer, the use of anti-PD-1/PD-L1 therapeutics in PCa, and potential subgroups of PCa patients who may derive the greatest benefit from these agents (such as men with tumors that have expression of PD-L1 and/or high mutational load). We also review the prior and current clinical trials evaluating the blockade of PD-1/PD-L1 in PCa, highlighting some of the key ongoing studies of greatest relevance to the field.

Expert commentary: Clinical trials investigating PD-1/PD-L1 inhibitors should be encouraged in patients with PCa. While it is unlikely that immune checkpoint monotherapies will produce long-lasting responses in a substantial proportion of patients, there is early evidence of activity in some patient subsets. These subgroups may include those with high PD-L1 expression, those with hypermutated or microsatellite-unstable tumors, and those enriched for germline and/or somatic DNA-repair gene mutations (e.g. intraductal/ductal histology, primary Gleason pattern 5, and perhaps AR-V7-positive tumors).

Keywords

cancer; prostate; PD-1; PD-L1; immunotherapy

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Declaration of Interest

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1) Introduction:

Prostate Cancer (PCa) is the most frequent noncutaneous cancer in men worldwide, accounting for 19% of new cases of cancer in men in the United States ¹. In 2017, it was estimated that there were 164,690 new cases and 29,430 deaths from PCa ¹. The treatment approaches for localized PCa include active surveillance, radiotherapy or surgery, and the 5-year survival rate approaches 100% ². In metastatic disease, androgen deprivation therapy (ADT) alone or in combination with chemotherapy ^{3–4} or abiraterone ^{5–6} is the backbone of the initial treatment.

Unfortunately, many patients with metastatic disease develop castration-resistant prostate cancer (mCRPC), defined as disease progression despite low serum testosterone levels. Even after several advances in the last decade, with the approval of numerous medications which demonstrated overall survival (OS) improvements (docetaxel ⁷, sipuleucel-T ⁸, cabazitaxel ⁹ abiraterone ¹⁰, enzalutamide ¹¹ and radium-223 ¹²), median OS still averages 2 to 3 years after the tumor becomes resistant to ADT ^{13–14}.

The unprecedented success of the immune checkpoint inhibitors (Table 1) which act on the PD-1/PD-L1 pathway has provided evidence that the patient's immune system can be modulated to combat advanced and metastatic cancers. Therefore, since PCa is the most common malignancy in men, and many of the current treatments for advanced disease produce a high frequency of side effects negatively impacting quality of life, the use of these promising immune therapies has become very attractive. In this review, we summarize and discuss the rationale, translational background and clinical trials evaluating blockade of the PD-1/PD-L1 pathway in PCa.

2) Immune checkpoints and the PD-1/PD-L1 pathway in cancer

The molecular mechanisms of T cell antigen recognition, regulation and function ¹⁵ began to be better elucidated in the 1980s. By the mid-1990s, it was clear that T cell activation was even more elaborate than previously thought. For example, during the process of proliferation and functional differentiation, T cell activation also stimulates some inhibitory pathways that can attenuate and even abolish T cell responses ¹⁶. It is now recognized that tumors have mechanisms of suppressing the antitumor immune response, including production of cytokines, recruitment of immunosuppressive cells, and upregulation of co-inhibitory receptors known as immune checkpoints ¹⁷.

The immune checkpoint pathway CTLA-4/B7 (cytotoxic T-lymphocyte associated protein-4, also known as CD152 and its ligand, respectively), downregulates T-cell activation, diminishing antitumor immunity, and allowing cancer cells to survive ¹⁸. Ipilimumab, a fully human monoclonal antibody targeting CTLA-4, blocks the CTLA-4/B7 pathway, permitting the immune system to recognize and kill cancer cells. Ipilimumab was approved by the FDA in 2011 as the first drug which showed overall survival (OS) benefit in metastatic melanoma ¹⁹.

The PD-1 pathway includes the programmed death protein-1 (PD-1) and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). This pathway has emerged as a mechanism for immune

tolerance whereby tumor cells can suppress an antitumor immune response by blocking effector functions and reducing T cell killing capacity²⁰. PD1 and PD-L1 inhibitors such as nivolumab and pembrolizumab (targeting PD1), and atezolizumab, avelumab and durvalumab (targeting PD-L1), have changed the landscape of cancer therapy in the past 2 years. These drugs have received FDA approval based on their unprecedented benefit in many cancer types including melanoma^{21,22}, non-small cell lung cancer (NSCLC)^{23,24}, urothelial cancer²⁵, renal cell carcinoma (RCC)²⁶ and head and neck cancer²⁷. Therefore, taking into account the high prevalence of PCa, its invariable lethal outcome when metastatic castration-resistant disease develops, and the success of PD-1/PD-L1 blockade in other cancers, the use of checkpoint inhibitors in PCa appears to be a rational approach.

3) Rationale for the use of PD-1 and PD-L1 inhibitors in advanced prostate cancer

The role of the immune system in the advanced PCa was established clinically after the success of sipuleucel-T, an autologous active cellular immunotherapy. A phase III trial using this therapy showed a significant improvement in OS in men with asymptomatic or minimally symptomatic mCRPC⁸. Patients who received sipuleucel-T had a relative reduction of 22% (HR 0.78, 95% CI 0.61–0.98) in the risk of death compared with the placebo group, translating into an improvement of 4.1 months in the median OS⁸. Thereby, it was suggested that correct modulation of the immune system could benefit patients and improve outcomes in mCRPC.

Although the checkpoint inhibitors have proven their efficacy in many different cancers, their activity is limited to a certain percentage of patients, varying from 10%²⁷ to 40%²² depending on the tumor type. Therefore, there is a need to identify biomarkers of response to checkpoint inhibitors, in order to determine which patients will benefit from the right therapy. This is especially important in tumors with low objective response rates to immunotherapy, such as PCa. To date, there are several biomarkers under development that can help to predict the benefit of CTLA-4 and PD-1/PD-L1 inhibition in different types of cancer, such as tumor cell or immune cell PD-L1 expression and the somatic mutational load of the cancer.

a) PD-L1 expression in prostate cancer

Tumor cell PD-L1 expression has been demonstrated to be a valuable prognostic²⁸ and predictive^{29,30} biomarker for PD-1 inhibitor sensitivity in some cancers. However, the relationship between this biomarker and its clinical significance is not perfect, and varies among the different types of solid tumors. Also, the different methods of evaluation of PD-L1 expression, and the different cut-offs to consider positivity/negativity of this biomarker are not yet standardized (TABLE 2).

Translational studies investigating the prognostic impact of PD-L1 expression in human prostate cancer are sparse. The first study which tested a PD-1 inhibitor in PCa (17 PCa patients from the phase 1 study of nivolumab) was able to evaluate tumor tissue in 2 of these patients, but neither of them expressed PD-L1 in tumor or immune cells³¹. Some

subsequent studies showed low or no PD-L1 expression^{32,33}, however, recent data using standardized PD-L1 evaluation, showed higher expression levels (TABLE 1). PD-L1 expression in PCa, as well as in other cancers, depends on the assay cut-off, technique and the antibody clone used in the evaluation. It is important to note that most PCa studies have evaluated primary prostate specimens only, and that there is little data about PD-L1 expression on metastatic lesions.

The clinical significance of PD-L1 expression and its impact on the prognosis of PCa was also evaluated in a few studies. One of them, which assessed 209 patients in two independent cohorts of primary PCa patients following radical prostatectomy, showed an association between high PD-L1 expression and significantly shorter biochemical recurrence-free survival in both cohorts ($P = 0.022$ and $P = 0.009$)³⁴. In this same study, PD-L1 (moderate to high expression) was also associated with higher proliferation rates (Ki-67, $P < 0.001$), higher Gleason score ($P = 0.004$), and androgen receptor (AR) expression ($P < 0.001$)³⁴. These findings suggested one mechanism of evasion from immune surveillance which could be responsible for the higher risk of recurrence in patients with high PD-L1 expression.

One important hypothesis that has been raised is that the PD-L1 expression might be a dynamic biomarker in PCa. One study including patients who had progressed on the anti-androgen enzalutamide, evaluated PD-L1/PD-L2 expression in dendritic cells (DCs) in blood, and compared it with patients who had responded or were naïve to enzalutamide³⁵. That study showed, for the first time, that mCRPC patients progressing on enzalutamide have an increased prevalence of PD-L1/PD-L2 positivity³⁵. Men who initially responded well to enzalutamide (PSA decline $> 50\%$) had lower circulating PD-L1/2 positive DCs than those who had no PSA decline. In progressing patients, more PD-L1/2 positive DCs were associated with poorer response to enzalutamide and shorter treatment duration ($p < 0.05$). This suggests that the increase in expression of PD-L1 could be a mechanism of immune evasion and resistance to enzalutamide. It also raises the hypothesis that, as a dynamic biomarker, PD-L1 expression could perhaps be modulated, making it a reasonable new strategy to potentially increase the activity of PD-1/PD-L1 inhibitors in PCa. This rationale was reinforced after a pilot trial which demonstrated that pembrolizumab was active in patients who had progressed on enzalutamide (and who continued on enzalutamide after pembrolizumab was added), showing an impressive response rate of 20% (4/20)³⁶. This trial is discussed in more detail below.

b) Tumor mutational load

Tumors that have a high somatic mutational load tend to have a greater and more sustainable benefit from immune checkpoint inhibitors across many cancer types. This can be explained based on the fact that tumors with an elevated number of mutations, especially nonsynonymous alterations, create more mutation-associated neoantigens (MANAs) that might be recognized by the immune system, releasing T cells to attack the cancer cells³⁷. At least four important studies consolidate this hypothesis, both in the context of CTLA-4³⁸ and PD-1/PD-L1^{39,40,41} pathway inhibition. Not coincidentally, two tumor types with the

highest average number of mutations, melanoma¹⁹²² and NSCLC²³²⁴, were the first two cancers in which PD-1 inhibitors proved successful.

The prevalence of somatic mutations is highly variable between and within cancer types, ranging from about 0.1 mutations per megabase (Mb) to >100 mutations per Mb⁴². This translates into a total number of nonsynonymous mutations ranging from about 5 to >5000 tumor mutations per cancer exome⁴³. On average, PCa has between 50–100 nonsynonymous DNA alterations per cancer exome (*i.e.* 1–2 mutations per Mb), and is not generally considered to be a cancer with a high mutational load⁴³. However, within the same type of cancer, the number of nonsynonymous mutations and consequently the mutational load can be extremely diverse. For example, colorectal tumors without microsatellite instability (MSI) have approximately 70 nonsynonymous mutations/tumor exome while MSI-high colorectal cancers can have >1,500 mutations per exome⁴³. This enormous disparity is partially explained because of inherited or acquired DNA repair defects (DRD), which can cause accumulation of DNA errors, increasing the number of nonsynonymous mutations and the mutational burden. There are multiple different types of DNA repair defects, each causing a unique spectrum and signature of mutations across the tumor genomic landscape, but a discussion of these diverse DRD mechanisms is beyond the scope of this review.

Recently, it was uncovered that a significant percentage of mCRPC harbors either germline or somatic mutations in different types of DNA repair genes, especially in those of homologous recombination (HR) repair, with germline HR mutations being present in 8–12%^{44,45} and somatic HR mutations being present in 20–25%⁴⁴ of advanced prostate cancers. Similar to the MSI-high tumors, which have a high mutational load and remarkable responses to checkpoint inhibitors (discussed below), defects in HR pathway genes may also be associated with increases in neoantigen load⁴⁶ and could also potentially be predictive of response to immunotherapy. The notion that HR mutations (*i.e.* not just MMR mutations) may be associated with high expression of PD-L1 and increased tumor-infiltrating lymphocytes⁴⁶, also a factor that can potentially impact checkpoint inhibitor response, makes clinical trials with HR-deficient PCa patients an attractive option.

The Cancer Genome Atlas Research (TCGA) network reported that up to 40% of endometrial cancers and up to 20% of colon cancers have high numbers of mutations, being considered hypermutated tumors⁴⁷. Recently it was described that some subtypes of advanced PCa, perhaps up to 5–12%, may also be hypermutated owing to underlying mismatch repair (MMR) gene mutations and MSI-high phenotypes (from *MSH2* and *MSH6* structural rearrangements)⁴⁸. Furthermore, an association has been observed between ductal adenocarcinoma of the prostate, a rare and aggressive histopathologic variant⁴⁹, and MMR deficient tumors⁵⁰. That study included 10 consecutive patients with ductal prostate cancer and showed that 40% had an underlying MMR gene alteration (2 patients in *MSH2*, 1 in *MSH6* and 1 in *MLH1*); three of these patients were also hypermutated. One patient with a hypermutated (MSI-high) tumor had an impressive response to the anti-PD-1 therapy, pembrolizumab, even though he had been progressed on many prior systemic therapies (abiraterone, enzalutamide, docetaxel, carboplatin and cabazitaxel). The patient had a

baseline PSA of 177.35 ng/mL and after 3 cycles of pembrolizumab his PSA was less 100 ng/mL ⁵⁰.

The presence of intraductal/ductal histology and lymphovascular invasion may also be associated with an increased prevalence of germline DRD mutations. In a recent study that evaluated 150 unselected patients with recurrent or metastatic PCa, it was shown that patients with germline mutations were more likely than germline-negative men to harbor intraductal/ductal histology (48% vs 12%, $P<0.01$), and were also more likely to have lymphovascular invasion (52% vs 14%, $P<0.01$). Finally, very high Gleason grades, specifically primary Gleason pattern 5 (*i.e.* 5+4=9 and 5+5=10), may associated with MSH2 loss in 8–10% of patients compared to 1–2% in other Gleason grades ⁵¹. In that study, loss of MSH2 protein was also associated with hypermutation and tumor infiltrating lymphocyte density; factors which can increase the efficacy of immune checkpoint blockade therapies.

The hypothesis that hypermutated tumors, especially those that have MMR gene mutations, are extremely sensitive to the immunotherapy approaches was confirmed in an important prospective clinical trial ⁴⁰. This study enrolled patients with 12 different advanced cancers, including colorectal cancer, endometrial, gastroesophageal, pancreas and prostate, and treated them with pembrolizumab, an anti-PD-1 antibody ⁴⁰. Despite the fact that all patients had progressed on at least one previous systemic therapy, an impressive overall response rate (ORR) of 53% (95% CI 42–64%) was seen and 21% of patients achieved complete radiographic responses ⁴⁰. One PCa patient was enrolled in this trial and had a complete and durable tumor response (a response that lasted for 24 weeks or more). This study demonstrated that with a reliable biomarker, a subgroup of patients (including PCa patients) can benefit from PD-1 blockade. Based on these data, the FDA granted accelerated approval to pembrolizumab in May 2017, for adult and pediatric patients with unresectable or metastatic solid tumors (of any histology), with MSI-high or deficient MMR genes after progression on a prior systemic treatment ⁵². This was the first cancer type-agnostic FDA approval in oncology, and marked the beginning of a genomically-defined characterization of cancer drug approvals.

4) Immune Checkpoint Inhibitors in Prostate Cancer

After the success of sipuleucel-T in mCRPC, ipilimumab has been evaluated in different doses, schedules and combinations in PCa patients. The first phase I study evaluating ipilimumab in mCRPC was published more than one decade ago ⁵³. This trial treated 14 patients, 12 with a single dose of ipilimumab and 2 were re-treated with two doses after PSA progression on the first dose. Of 14 patients treated, 2 (14%) had a PSA decline higher than 50%, showing the potential activity of this drug in PCa ⁵³. The phase II trial using Ipilimumab in combination with androgen deprivation therapy (ADT) versus ADT alone evaluated 108 patients with advanced PCa, showing undetectable PSA levels at 3 months in 55% versus 38% of patients, respectively ⁵⁴. Also, the combination of radiation therapy and ipilimumab demonstrated manageable toxicity and antitumor activity ⁵⁵. These three trials encouraged further evaluation of the blockade of CTLA-4 pathway alone or in combination with radiotherapy or ADT in larger confirmatory trials (Table 3).

Subsequently, a phase III trial evaluated a single dose of bone-directed radiotherapy (8 Gy) followed by either ipilimumab or placebo (every 3 weeks for up to four cycles) in mCRPC patients after progression on docetaxel chemotherapy. Despite showing a superior PFS (4.0 vs 3.1 mo; HR 0.70 95% CI. P<0.0001) and PSA response rate (13.1% vs 5.2%) favoring ipilimumab, the primary endpoint of OS was not met⁵⁶. Data from prespecified and *post hoc* subgroup analysis suggested that ipilimumab might possibly improve the OS (modified OS 22.7 versus 15.8 mo; HR 0.62, P=0.038) for patients with a better prognostic profile, particularly those without visceral (liver) metastases⁵⁶. Despite these data supporting further evaluation of ipilimumab in patients with lower disease burden and absence of visceral disease, the phase III trial which evaluated ipilimumab in asymptomatic or minimally symptomatic chemotherapy-naïve mCRPC patients also did not reach its primary endpoint (OS 28.7 mo for ipilimumab vs 29.7 mo for placebo, 95% CI 0.88–1.39, p=0.366)⁵⁷. However, this trial also showed a PFS improvement in the ipilimumab arm (5.6 mo vs 3.8 mo, HR 0.67; 95% CI, 0.55–0.81), with an early separation of the Kaplan-Meier curves sustained over time⁵⁷. Taken together, these two phase III trials showed a consistent and plausible PFS benefit, supporting the hypothesis that there may be a subgroup of patients who benefit from the checkpoint inhibition^{56,57}. Therefore, it would be desirable to identify this subgroup and to develop a biomarker to predict benefit and response to this and others immune checkpoint inhibitors.

5) Completed Clinical Trials with PD-1/PD-L1 inhibitors in Prostate Cancer

a) PD1-Inhibitors:

To date, there are a few completed and published clinical trials evaluating the use of PD-1 or PD-L1 inhibitors in PCa (TABLE 3). The first trial which assessed the safety and activity of a PD-1 inhibitor in PCa was published in 2012³¹. In this phase 1 trial, 17 patients with mCRPC received nivolumab, a monoclonal antibody against PD-1. Despite objective response rates of 28% in patients with melanoma, 27% in patients with RCC and 18% in patients with NSCLC, no objective response rate was seen in the 6 response-evaluable PCa patients (although one patient achieved a 29% objective tumor response and a 70% PSA response)³¹.

The first evidence of meaningful activity of PD-1 blockade in men with mCRPC was demonstrated in a phase II trial which evaluated pembrolizumab in chemotherapy-naïve patients progressing (but continuing) on enzalutamide⁵⁸. All patients had initially responded to enzalutamide (*i.e.* excluding those with primary refractory disease to enzalutamide), and upon subsequent PSA progression pembrolizumab was added. Ten patients were enrolled in the initial pilot phase, and 30% (3/10) had a PSA 50% response, most with remarkable and durable responses. Of the three patients who achieved a response, all had a PSA nadir of <0.1 ng/mL (from 2502.8, 70.7 and 46.1 ng/mL down to <0.01, 0.08 and 0.02 ng/mL, respectively). Also, at a median follow up of 30 weeks (range 16–55), these three patients remained free of progression at 30, 55, and 16 weeks of follow-up respectively. Importantly, somatic genetic analysis of one of the three pembrolizumab responders showed an MSI-high tumor⁵⁸, corroborating the rationale that cancers with high mutational loads may respond favorably to PD-1 inhibitors⁵⁹. This study recently reported an updated analysis now

incorporating 20 patients³⁶. Of the second set of 10 patients, one had a PSA reduction of 50%, totaling 4 responses out of 20 patients (20%). These data suggest that PD-1 inhibitors either can restore the sensitivity of antiandrogen therapy or can be effective despite enzalutamide failure. Perhaps innovative studies evaluating these two possibilities could be feasible and relevant to understand the mechanisms of sensitivity and resistance to both PD-1 inhibitors and enzalutamide³⁶.

Pembrolizumab was also tested as a monotherapy (without enzalutamide) in patients with advanced PCa in the KEYNOTE-028 study. This phase Ib basket trial which assessed the efficacy and safety of pembrolizumab enrolled 477 patients with several cancer types, including mCRPC⁶⁰. The prostate cancer cohort comprised 23 mCRPC patients who were required to have measurable disease as well as tissue-based PD-L1 expression 1% (in tumor or immune cells). Among these 23 patients, the objective response rate was 13% (3/23), and 39% had stable disease lasting >12 weeks. In patients who achieved an objective response, the median duration of response was 59 weeks (range 28–62 weeks). Median OS was 8 months, a reasonable OS given that 74% of patients had received two or more prior systemic therapies for metastatic CRPC disease⁶⁰. These encouraging results have led to a large study, the KEYNOTE-199 trial (NCT02787005), which is evaluating pembrolizumab in five different cohorts, three as monotherapy and two in combination with enzalutamide. Pembrolizumab alone will be tested in men with PD-L1 positive measurable disease, PD-L1 negative measurable disease, and in patients who have bone-predominant non-measurable disease. The other two cohorts are evaluating the addition of pembrolizumab in patients who are progressing on enzalutamide, in both measurable-disease and in bone-predominant disease cohorts.

The combination of PD-1 inhibitors with other drugs has also been tested in prostate cancer (TABLE 3). The addition of ipilimumab to nivolumab (*i.e.* combined immune checkpoint blockade) was evaluated in androgen receptor splice variant-7 (AR-V7)–positive mCRPC patients⁶¹, a subgroup of patients who are resistant to antiandrogen therapy⁶² and have a worse prognosis⁶³. A secondary rationale was that AR-V7–positive prostate cancers are believed to harbor a greater number of DNA repair gene mutations, which may render these tumors more responsive to immunotherapy. This phase II study enrolled 15 mCRPC patients with detectable AR-V7 in the blood, and patients received nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for 4 doses and then maintenance of nivolumab 3 mg/kg every 2 weeks until disease progression or unacceptable toxicity. The primary endpoint was PSA >50% response rate, and the secondary objectives were ORR, PFS, durable PFS (lack of clinical or radiographic progression for 24 weeks), and OS. Targeted sequencing for DRD mutations was also performed on each patient. Overall, 40% (6/15) of patients had some pathogenic DRD mutation (*BRCA2*, *ATM*, *ERCC4*). Of the 8 patients with measurable disease, 2 had a radiographic response (ORR=25%), and both of them were patients with DRD mutations. Furthermore, PFS and durable PFS were statistically superior in DRD-positive compared to DRD-negative patients: 7.5 vs 2.9 months ($p=0.027$), and 50% vs 0% ($p=0.044$), respectively; and there was a trend towards benefit in DRD-positive patients for OS (9.5 vs 7.2 months, $p=0.421$) and PSA 50% responses (17% vs 0%, $p=0.400$).⁶¹ It is important to note that although 46% of men experienced grade-3/4 adverse events (including several immune-related toxicities), there were no treatment-related deaths

in this study, suggesting that even in patients with a tendency to be older and frail, such as PCa patients, the combination of two checkpoint inhibitors seems to be reasonably safe and feasible. Also, this study showed that DNA repair mutations may be enriched in AR-V7 disease, and that the combination of a PD-1 inhibitor and a CTLA-4 inhibitor has antitumor activity in mCRPC patients who harbor DNA repair abnormalities, even if they are AR-V7-positive⁶¹. An ongoing trial (NCT03061539) is also evaluating the same combination, nivolumab plus ipilimumab, in prostate cancers with different immunogenic signatures. Almost two hundred metastatic castration-resistant patients with MMR deficiency, DRD mutations or a high CD8 T cell infiltrate will be enrolled in that trial.

Another trial which tested immunotherapy combinations is a phase I trial which evaluated the addition of cabozantinib to nivolumab, with or without ipilimumab, in different genitourinary cancers including mCRPC⁶⁴. This trial showed that both combinations (nivolumab plus cabozantinib, and nivolumab plus cabozantinib and ipilimumab) were safe and well-tolerated. Overall, the combinations were active in genitourinary tumors (ORR=37%), particularly in urothelial carcinoma (N=16, ORR=44%). Of the 9 mCRPC patients included in this trial, 1 patient had a partial response (ORR=11%), and 6 patients had stable disease (SD=66%)⁶⁴.

b) PD-L1 Inhibitors:

To date, three PD-L1 inhibitors have been approved by the FDA for use in the US. Atezolizumab, avelumab, and durvalumab were approved for urothelial carcinoma⁶⁵ and NSCLC^{66,67}, urothelial and merkel cell carcinoma⁶⁸, and urothelial carcinoma⁶⁹, respectively. The first clinical trial which used a PD-L1 inhibitor in PCa patients included 18 mCRPC patients who received avelumab⁷⁰. This phase I trial accepted patients who had progressed on a previous treatment and involved avelumab 10 mg/kg every 2 weeks. Patients who had progressed on an androgen receptor antagonist could continue it and avelumab was added. Both avelumab alone and in combination with enzalutamide were well tolerated. However, neither avelumab alone nor in combination with enzalutamide showed any favorable responses. Of the 5 patients who received the combination, 4 (80%) had stable disease as the best objective response and 1 had progressive disease. Of the 12 patients who received avelumab alone, 11 were evaluable for radiographic response but none were observed (3 PD, 8 SD)⁷⁰. The PSA doubling time of 3 patients was prolonged, hypothesizing that despite no ORR, some patients could benefit from this therapy by prolonging the time to disease progression.

A different PD-L1 inhibitor, durvalumab, was evaluated in combination with olaparib, a PARP inhibitor, and the results were presented recently⁷¹. This phase II trial included 19 patients with mCRPC who had received previous treatment with either abiraterone or enzalutamide and had tumor tissue available for genetic DNA sequencing. Patients tolerated the combination therapy relatively well, with only one grade-4 toxicity being observed. Genetic sequencing (both germline and somatic) in 17 patients with available DNA showed that 35% (6/17) harbored a DRD mutation (all were *BRCA2* lesions: 4 were somatic-only, 2 were germline mutations). The overall PSA 50% response rate was 44% (7/16) in unselected patients and 83% (5/6) among the patients who harbored DRD (*BRCA2*) mutations. The

single patient who harbored a DRD lesion and who did not achieve a PSA 50 response had a decrease in PSA of 43%. The radiologic response rates seen were CR/PR=29% (5/17), SD=47% (8/17), PD=12% (2/17) and unevaluable=12% (2/17). The 6-month and 9-month PFS were 87% (95% CI: 56–96%) and 58% (95% CI: 8–88%) respectively. This trial is being expanded to enroll a total of 50 mCRPC patients, so that the activity and safety of the durvalumab/olaparib combination can be further clarified in a larger sample set.

6) EXPERT COMMENTARY:

The advent of PD-1/PD-L1 inhibitors may be the biggest advance in cancer care in the last decades. Despite that, it is now clear that these drugs do not appear to have a great efficacy in unselected PCa patients. However, results from trials using CTLA-4 inhibitors in PCa, showing a PFS advantage and some PSA responses, indicate that the notion that there is a subgroup of patients who benefit from this checkpoint inhibition is reasonable.⁵⁶⁵⁷

Well-known biomarkers of response to checkpoint inhibitors such as PD-L1 expression²⁹³⁰ and high mutational burden⁴¹ could be present in some subgroups of PCa patients. Examples of subgroups of PCa patients who could be enriched for PD-L1 expression are aggressive tumors³⁴, cancers which have progressed on antiandrogen therapy (especially enzalutamide)³⁵³⁶, and tumors that harbor germline or somatic DRD mutations⁷². In other cancers, different factors could change the expression of PD-L1 across time and could have an impact in PCa, such as radiation exposure⁷³, tyrosine kinase inhibitors (TKI) therapy⁷⁴ and chemotherapy⁷⁵. All these variables might be evaluated in future clinical trials with checkpoint immunotherapy for PCa.

High mutational burden could be seen in patients who harbor HRD⁷² or MMR⁴⁸ mutations, and some pathological findings can enrich these two findings, such as ductal/intraductal histology and Gleason pattern 5. All these subgroups together can represent more than 1/3 of mCRPC patients, making the use of PD-1/PD-L1 inhibitors a potentially attractive strategy for clinical trials in these patients. The other promising subgroup which showed benefit with PD-1 plus CTLA-4 inhibition was the AR-V7 positive subset⁶¹. Preliminary data suggested that these patients may be enriched for DRD mutations, and maybe because of that they have a better response than unselected patients.

New mechanisms of resistance to PD-1 blockade may explain the low success of the PD-1/PD-L1 inhibitors in unselected PCa patients. Epigenetic alterations are involved in the regulation of gene expression in key biological processes, and some of these epigenetic changes can cause T cell exhaustion and consequently anti-PD-1 resistance⁷⁶⁷⁷. Perhaps the combination of PD-1/PD-L1 inhibitor and drugs that act in specific epigenetic pathways, such as the bromodomain/BET inhibitors⁷⁸, which also play a role in androgen receptor signaling, could potentially overcome mechanisms of resistance to PD-1/PD-L1 inhibitors, in addition to causing cell damage by a different mechanism of action. In the same context, PARP inhibitors, Wnt pathway inhibitors, AR-targeted therapies and AKT/PI3K pathway inhibitors also might be reasonable combinations with PD-1/PD-L1 blockade. Finally, some epigenetic alterations also can provide prognostic information. Two studies demonstrated that the methylation of the PD-1 promoter is associated with biochemical recurrence in PCa,

and perhaps strategies that decrease methylation of this checkpoint inhibitor might improve the outcomes of these patients⁷⁹⁸⁰.

Also, in patients with PCa with a tendency to be older and frailer, PD-1/PD-L1 inhibitors (when used as monotherapies) tend to be generally well tolerated. In previous phase 3 trials using CTLA-4 inhibitors, an immunotherapy that is more toxic than the PD-1/PD-L1 inhibitors, no treatment-related death were seen⁵⁵⁵⁶, making these therapies an attractive approach for these patients.

In conclusion, the use of PD-1/PD-L1 inhibitors for advanced prostate cancer should be encouraged in the setting of clinical trials (Table 5). It will also be necessary to identify the patient subgroups which can benefit from these therapies and to try to develop a biomarker panel to predict benefit and response of these and others checkpoint inhibitors. For now, treatment of MMR-deficient or MSI-high advanced prostate cancers with pembrolizumab is supported by the FDA label, but outside of this setting the use of immune checkpoint inhibitors should be considered investigational in prostate cancer.

7) Five Year View:

What will the prostate cancer immunotherapy landscape look like 5 years from now? We believe that PD-1/PD-L1 inhibitors will be approved to treat some proportion of advanced PCa patients, perhaps those with germline and/or somatic HRD mutations (e.g. *BRCA2*, *ATM*, *CDK12*). We also anticipate that there will be stronger data to support the use of PD-1/PD-L1 inhibitors in men with MMR-deficient prostate cancer, resulting from dedicated trials focusing specifically on prostate cancer populations. These molecular subgroups are expected to be enriched for PD-L1 expression and high mutational burden, factors that increase the efficacy of immunotherapy in other cancer types, such as melanoma and non-small cell lung cancer. Some clinical and pathologic factors could potentially help to identify these subgroups, such as patients with high-grade tumors (associated with PD-L1 expression) and tumors with primary Gleason pattern 5 disease, ductal/intraductal histology and perhaps AR-V7 positive cancers (all of which may be associated with pathogenic DNA-repair gene mutations). Owing to the modest efficacy of immune checkpoint inhibitors in unselected PCa populations, we believe that future studies should be designed to pre-select patients with a high chance of deriving benefit from PD-1/PD-L1 blockade, such as the different subsets of patients discussed above.

Another strategy that could potentially increase the efficacy of PD-1/PD-L1 blockade in PCa would be to evaluate these checkpoint inhibitors in conjunction with other systemic therapies, in particular with other agents that may act synergistically with these antibodies. Some examples may include combinations of different classes of immune checkpoint inhibitors with each other, such as PD-1/PD-L1 inhibitors given together with CTLA-4 inhibitors. This combined immune checkpoint strategy has already shown impressive results in melanoma and renal cell carcinoma, and preliminary results suggest that it may be effective in some AR-V7–positive tumors, especially those who also harbor pathogenic HRD mutations. Additionally, the combination of poly (ADP-ribose) polymerase (PARP) inhibitors, a class of drug which blocks another important DNA-repair pathway (i.e. base

excision repair), and which has already shown encouraging results in mCRPC, could also act synergistically together with PD-1/PD-L1 inhibitors in PCa patients, perhaps even in those without underlying HRD mutations.

Finally, we believe that over the next 5 years (after understanding which subsets of patients may benefit from PD-1/PD-L1 blockade, and what therapies are better to combine with these checkpoint inhibitors), it will also be imperative to elucidate both primary and acquired resistance mechanisms to checkpoint inhibitors in PCa. Understanding these resistance mechanisms will further aid in designing rational combination strategies to overcome or reverse these escape pathways. In the case of primary PD-1/PD-L1 resistance, immune-adjuvant approaches (e.g. vaccine strategies) may be required to simultaneously induce tumoral T cell infiltration with concurrent removal of the immunological breaks. In closing, while it is impossible to predict exactly what the 5-year future may hold, we are confident that PD-1/PD-L1 approaches will be an important part of advanced prostate cancer therapeutics, perhaps not as monotherapy strategies in unselected patients, but with a focus on pathologic or molecular subsets of patients, or perhaps in a more expanded population in the context of the right combination approach. The initial challenges with PD-1/PD-L1 inhibitors in this disease should not discourage us or prompt us to give up on this class of therapy for prostate cancer patients; rather, it should motivate us further to dig deeper and uncover biomarkers of sensitivity as well as appropriate combination approaches. We are just beginning to scratch the surface of discovery.

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8.

Key Issues:

- The success of PD-1 and PD-L1 inhibitors across multiple different cancer histologies has provided evidence that the antitumor immune system can be modulated to combat advanced and metastatic cancers.
- Because prostate cancer (PCa) is the most common noncutaneous malignancy in men, the use of these immune checkpoint-targeting therapies in this disease has become an attractive notion.
- Although PD-1/PD-L1 inhibitors may have modest efficacy in unselected PCa patients, accumulating data suggest that there may be a subgroup of men who benefit more from immune checkpoint inhibition.
- Some potential biomarkers of sensitivity to immune checkpoint inhibitors may include PD-L1 expression (in tumor cells, in immune cells) and high mutational burden (e.g. in patients harboring mismatch repair [MMR] or homologous repair deficiency [HRD] mutations).
- In addition, specific histologic subtypes may enrich for MMR or HRD alterations, including the presence of ductal/intraductal morphology, primary Gleason pattern 5 (5+5 or 5+4) disease, and possibly the presence of the AR-V7 splice variant.

Table 1:

Immune checkpoint-blocking antibodies approved by the Food and Drug Administration (FDA) and their corresponding indications:

Drug	Target	Indication
Ipilimumab	CTLA-4	Melanoma
Nivolumab	PD-1	Melanoma, non-small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma, Hodgkin's lymphoma, squamous-cell carcinoma of the head and neck, urothelial carcinoma, colorectal cancer with high microsatellite instability (MSI-high) or mismatch repair deficiency (dMMR)
Pembrolizumab	PD-1	Melanoma, non-small cell lung cancer, Hodgkin's lymphoma, squamous-cell carcinoma of the head and neck, urothelial carcinoma, gastric cancer, all solid tumors with high microsatellite instability (MSI-high) or mismatch repair deficiency (dMMR)
Atezolizumab	PD-L1	Non-small cell lung cancer, urothelial carcinoma
Avelumab	PD-L1	Merkel-cell carcinoma, urothelial carcinoma
Durvalumab	PD-L1	Urothelial carcinoma

Table 2:

Completed clinical trials using CTLA-4 Inhibitors in Prostate cancer

Drug	Phase	Number of patients	Setting	Results	Reference
Ipilimumab	I	14	mCRPC	PSA50: 14% (2/14)	53
Ipilimumab + ADT	II	108	Locally advanced PCa	Undetectable PSA at 3 months: ADT + Single dose of Ipilimumab: 55% ADT alone: 38%	54
Ipilimumab + radiation therapy	I/II	75	mCRPC after progression on an anti-androgen therapy	PSA decline in evaluable patients: 3 mg/kg: 27% (4/15) 5 mg/kg: 17% (1/6) 10 mg/kg: 16% (8/50)	55
Ipilimumab after radiation therapy	III	799	mCRPC after progression on docetaxel	OS 11.2 m for ipilimumab vs 10.0 m for placebo (HR 0.85, 0.72 – 1.00, p=0.053) 1 y OS 46.8% for ipilimumab vs 40.4% for placebo 2 y OS 26.2% for ipilimumab vs 15.0% for placebo PFS 4.0 m for ipilimumab vs 3.1 m for placebo (HR 0.70, 0.61 – 0.82, p< 0.0001) PSA50 response 13.1% for ipilimumab vs 5.2% for placebo	56
Ipilimumab	III	400	Asymptomatic or minimally symptomatic mCRPC chemotherapy naïve	OS 28.7 m for ipilimumab vs 29.7 for placebo (HR 1.1, 0.88 – 1.39, p=0.3667). PFS 5.6 m for ipilimumab vs 3.8 m for placebo (HR 0.67, 0.55 – 0.81) PSA50 response 23% vs 8%	57

Table 3:

Studies examining PD-L1 expression in Prostate Cancer

Setting	Number of patients	PD-L1 detection Antibody/clone	Tumor surface expression cutoff for positivity	Percentage of PD-L1 positivity	Reference
primary prostate cancer specimens	2	5H1 (Yale University, New Haven, CT)	5%	0%	31
primary prostate cancer specimens	20	5H1 (Yale University, New Haven, CT)	5%	15%	32
primary prostate cancer specimens	209	EPR1161 (Abcam, Cambridge, MA)	No cut-off	moderate to high PD-L1 expression was detected in 61.7% of cases (377/611).	34
primary prostate cancer specimens	16	015 (Sino Biological, PA)	A three-tier score was defined for PD-L1 staining intensity: 0 (no signal), 1+ (light signal) and 2+ (high signal) in 50% neoplastic cells.	Eight of 16 cases expressed PD-L1 (50 %), with 19 % scored as strong 2+	81
radical prostatectomy (RP) from intermediate- to high-risk prostate cancer who underwent RP after Neo-AAPL treatment.	88 (44 abiraterone treated and 44 control) 130 hormone naïve pts	SP263 (Ventana Medical Systems, Tucson, AZ)	0 (negative or < 1%), 1 (1%–4%), 2 (5%–24%), 3 (25%–49%), 4 (50%).	Abiraterone treated pts: Neoadjuvant treatment: 7% No neoadjuvant treatment: 21% Hormone naïve prostate cancers: 1% 14%	82
primary prostate cancer specimens	402	E1L3N (Cell Signaling, Danvers, MA)	no staining = 0, weak staining = 1, moderate staining = 2, and strong staining = 3. PD-L1+ stromal cells and PD-L1+ lymphocytes were scored as number of positive stained cells per 0.6 mm diameter core as follows: 0 = 0–3, 1 = 4–10, 2 = 11–15, and 3 = > 15	PD-L1 staining in tumor epithelial (TE) cells was positive in 371/402 (92%) cases, and 236/402 (59%) cases had a high PD-L1 intensity score. In addition, 267/402 (66%) of patients had PD-L1+ stromal cells.	83
primary prostate cancer specimens	25	22C3 (Agilent, Santa Clara, CA)	Stained slides were counterstained with hematoxylin and cover slipped for review and scoring by a pathologist on a semiquantitative 0 to 5 scale. A score of 3 to 5 on the semiquantitative 0 to 5 score was deemed “high” expression whereas a score of 0 to 2 was deemed “low” expression	Low: 92% (23/25) High: 8% (2/25)	33

Table 4:

Completed clinical trials evaluating PD-1 or PD-L1 inhibitors in Prostate Cancer

Drug	Phase	Number of patients	Setting	Results	Reference
Nivolumab	I	17	Dose escalating trial. Multiple cancers.	PSA response (6%) 1/17. No radiologic response was seen.	31
Pembrolizumab + enzalutamide	II	20	Enzalutamide resistant PCa	PSA Response: 20%	5836
Pembrolizumab	Ib	23	mCRPC	ORR=13%	60
Pembrolizumab	II	1	Mismatch repair deficient tumor	ORR=100%	40
Nivolumab + Ipilimumab	II	15	mCRPC AR-V7 positive	PSA50 Response=13% (2/15) ORR=25%	61
Nivolumab + Cabozantinib, Nivolumab + Ipilimumab + Cabozantinib	I	9	mCRPC	SD=66% (6/9) PR=11% (1/9)	64
Avelumab + Enzalutamide	I	18	mCRPC	Best Response Rate: PD 23.5% (4/17) SD 70.5% (12/17) N/A 6% (1/17) NA	70
Durvalumab + Olaparib	II	19	mCRPC	PSA50 Response: 44% 6-month PFS: 86.7% Median PFS: not reached	71

Table 5:

Ongoing trials evaluating PD-1 or PD-L1 inhibitors alone or in combinations in Prostate Cancer

Drug	Combination	Setting	Phase	Number of patients	Primary Outcome (s)	Trial ID
Pembrolizumab	Cohort A: Olaparib Cohort B: Docetaxel Cohort C: Enzalutamide	mCRPC	Ib/II	210	Safety and PSA response rate (decline of 50% from baseline twice 3 weeks apart).	NCT02861573
Pembrolizumab	DNA vaccine	mCRPC	I/II	32	Safety, 6-month PFS, median time to radiographic progression, ORR, PSA Response	NCT02499835
Pembrolizumab	Radium-223	mCRPC	II	45	The extent of immune cell infiltration	NCT03093428
Pembrolizumab	Monotherapy (cohorts 1–3), or with ongoing Enzalutamide (cohorts 4,5)	mCRPC	II	370	ORR	NCT02787005
Pembrolizumab	ADXS31–142	mCRPC	I/II	51	Safety	NCT02325557
Pembrolizumab	Enzalutamide	mCRPC	II	58	PSA Response	NCT02312557
Nivolumab	PROSTVAC-F	Neoadjuvant cohort, and mCRPC cohort	I/II	29	Safety Changes in T-cell infiltration in the tumor after neoadjuvant treatment	NCT02933255
Nivolumab	Ipilimumab	mCRPC	II	90	ORR, rPFS	NCT02985957
Nivolumab	Ipilimumab, plus ongoing Enzalutamide	mCRPC in Arv7 positive patients	II	15	PSA Response and/or ORR	NCT02601014
Nivolumab	Monotherapy	mCRPC with DNA repair defects	II	45	PSA Response	NCT03040791
Nivolumab	Ipilimumab	mCRPC with specific immunogenic signatures	II	175	Radiologic response, PSA response, CTC (circulating tumor cells) conversion rate	NCT03061539
Atezolizumab	Radium-223	mCRPC	I	45	Safety, ORR	NCT02814669
Atezolizumab	Sipuleucel-T	mCRPC	I	34	Safety	NCT03024216
Atezolizumab	Enzalutamide + atezolizumab vs Enzalutamide alone	mCRPC	III	730	OS	NCT03016312
Atezolizumab	CPI-444	mCRPC, and other solid tumors	I/Ib	534	Safety	NCT02655822
Avelumab	Talazoparib	mCRPC, and other solid tumors	Ib	296	Safety, ORR	NCT03330405
Avelumab	Monotherapy	mCRPC, and other solid tumors	I	1753	Safety, ORR	NCT01772004
Avelumab	Monotherapy	Neuroendocrine phenotype	II	18	ORR, rPFS, OS	NCT03179410
Durvalumab	Tremelimumab	Chemotherapy-naïve PCa	II	27	Safety, PSA-PFS at 12 months	NCT03204812
Durvalumab	With/without Tremelimumab	mCRPC	II	74	ORR	NCT02788773