JBUON 2018; 23(6): 1796-1802
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

The expression of PD-L1 in patients with castrate prostate cancer treated with enzalutamide

Igor Richter[1,2], Tomas Jirasek[3], Ivana Havlickova[3], Radmila Curcikova[3], Vladimír Samal[4], Josef Dvorak[2], Jirí Bartos[1]

[1]Department of Oncology, Regional Hospital Liberec, Liberec, Czech Republic; [2]Department of Oncology, Thomayer Hospital Prague and First Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Department of Pathology, Regional Hospital Liberec, Liberec, Czech Republic; [4]Department of Urology, Regional Hospital Liberec, Liberec, Czech Republic

Summary

Purpose: *The purpose of our retrospectively study was to evaluate the PD-L1 expression in patients with metastatic castration-resistant prostate cancer (mCRPC) treated with enzalutamide.*

Methods: *A total of 33 patients with mCRPC were treated with enzalutamide. All patients were previously treated by one or two lines of chemotherapy. Enzalutamide was administered in the standard dose (160 mg orally once daily as four 40 mg capsules). No corticosteroids were concomitantly administered. PD-L1 expression was determined semiquantitavely by immunohistochemistry.*

Results: *Enzalutamide was well tolerated with predominantly G1-2 toxicity. G3-4 anaemia was found in 6 patients and G3-4 thrombocytopenia in 2 patients. One patient had cerebral hemorrhage. The median progression free survival (PFS) was 7.0 months (95% CI 6.1-7.9). The median overall survival (OS) was 8.4 months (95% CI: 5.1-11.7). The shorter OS was noted in the subgroup of patients with decreasing hemoglobin levels during enzalutamide treatment with hazard ratio (HR) 0.155 (95% CI 0.053-0.449) and in patients with Gleason score 8-10 with HR 0.334 (95% CI 0.12-0.927) according to the regression analysis. All tissue samples were scored as negative in the detection of PD-L1.*

Conclusions: *The expression of PD-L1 in prostate cancer cells as potential new predictive biomarker was not confirmed. Further studies are needed to clarify this topic.*

Key words: *castration-resistant, enzalutamide, metastatic prostate cancer, PD-L1*

Introduction

Prostate cancer is the most common malignant neoplasm in men worldwide and second cause of cancer related death. Initially, prostate cancer is sensitive to androgen deprivation therapy (ADT), but later progresses to castration-resistant prostate cancer (CRPC). Since its approval in 2004, docetaxel was the only agent until 2010 that had proven survival benefit in metastatic CRPC (mCRPC) [1,2]. Some studies demonstrated that CRPC had persistent androgen receptor (AR) signaling despite castrate levels of serum androgens, indicating that many tumors in this state remain sensitive to further targeting this pathway [3-5]. Enzalutamide (MDV3100) is a second-generation antiandrogen with a significant increase in affinity for AR. Enzalutamid prevents nuclear translocation and coactivator recruitment of the ligand-receptor complex [6,7]. Enzalutamide belongs to androgen receptor targeting agents (ARTA). The AFFIRM study was an international randomized double-blind phase III trial that evaluated enzalutamide compared with to placebo in patients with mCRPC in the post-docetaxel setting. The primary endpoint was overall survival (OS). The median OS was 18.4 months

Correspondence to: Igor Richter, MD, PhD. Department of Oncology, Regional Hospital Liberec, Husova 10, 460 63, Liberec, Czech Republic.
Tel: +420 777 599 847, +420 485312267, Fax: +420 485312027, E-mail: igor.richter@seznam.cz
Received: 15/03/2018; Accepted: 20/04/2018

(95% CI 17.3 to not yet reached) in the enzalutamide group versus 13.6 months (95% CI 11.3-15.8) in the placebo group (HR 0.63, 95% CI 0.53-0.75, p<0.001) [8].

Strategies to improve the clinical outcomes that should be evaluated in future clinical trials include incorporation of more active agents. A very examined topic in oncology is immunotherapy, which is used in various types of tumors such as melanoma, non-small cell lung cancer (NSCLC), renal cell cancer and others [9-11]. The immune checkpoint inhibitors are becoming mainstream in systemic cancer treatment. Programmed cell death-1 (PD-1) and its ligand (PD-L1) belong to one type of immune-inhibitory checkpoint molecules that suppress T cell-mediated immune response, leading to the development of tumors [12]. PD-1 (CD279) is a cell surface receptor that belongs to the immunoglobulin superfamily and also a member of the extended CD28/CTLA-4 family. PD-1 is mainly expressed by activated T cells [13,14]. PD-L1 (B7-H1, CD274) has been identified as cell-surface glycoprotein belonging to the B7 family [15]. PD-L1 is mainly expressed on the surface of tumor cells and antigen-presenting cells in various solid malignancies [16-20]. PD-1/PD-L1 pathway plays a prominent role in immune regulation by delivering inhibitory signals to maintain the balance in T-cell activation, tolerance and immune-mediated tissue damage. The PD-L1 expression has been associated with improved clinical outcome in some types of tumors. Clinical trials demonstrated that monoclonal antibodies which target PD-1 or PD-L1 enhance T cell functions, leading to impressive outcomes in patients with melanoma, renal cell carcinoma, NSCLC and bladder cancer [21-23]. Phase I data from men with mCRPC suggest that PD-1 blockade is less effective in prostate cancer than in other tumor types [24].

The expression of PD-L1 in prostate cancer cells and its prognostic or predictive significance is unclear. The aim of our study was to evaluate our first clinical experience in patients with mCRPC treated by enzalutamide and to detect the PD-L1 expression in prostate cancer cells.

Methods

Patients

Between November 2015 and June 2017 a total of 33 patients with mCRPC have been treated with enzalutamide in our hospital. Their median age was 71 years (range 55-83). All patients had ECOG performance status 0-2. The patient demographic data are shown in Table 1. All the patients were previously treated with one or two lines of chemotherapy, receiving docetaxel in the first line of mCRPC. Enzalutamide was administered in the standard dose (160 mg orally once daily in four 40 mg capsules). No corticosteroids were concomitantly administered. The median pretreatment hemoglobin concentration was 128.5 g/l (range 91-160), the median pretreatment leukocytes concentration was 6.5×10^9/l (range 3.7-20.5), the median pretreatment platelet concentration was 202×10^9/l (range 67-575). The median of PSA concentration before starting enzalutamide therapy was 105 μg/l (range 2.2-above 5000). The objective tumor response was defined by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.

Immunohistochemical assessment of PD-L1 expression

Archival tissue blocks of 24 patients with primary prostate carcinoma were selected for further studies. Nine patients were not evaluated because of specimen unavailability. Neoplastic tissue was obtained by needle punction in 22 patients and in 3 of them tissue blocks obtained by radical prostatectomy were also available. In the remaining 2 patients punction blocks were not present and specimens were obtained by radical prostatectomy (one patient) and transurethral resection (one patient). Paraffin-embedded tissue samples were obtained from the archive of the departments of Pathology in the Regional Hospital Liberec, Hospital Česká Lípa, Hospital Mladá Boleslav, Plzen Hospital Laboratory. Tissue of human tonsill palatina and squamous cell carcinoma of head & neck region were used as positive controls. All bioptic specimens were fixed in buffered formalin and embedded in paraffin. Five μm thin sections were stained with haematoxylin and eosin. Histologic grading was assessed from available specimens independently by three

Table 1. Characteristics of patients treated with enzalutamide

Characteristics	*Patients, n=33 n (%)*
Age	
Median (range), years	71 (55-83)
≥75	8 (24.2)
Disease location	
Bone	29 (87.9)
Lymph nodes	7 (21.2)
Visceral	4 (12.1)
No. of previous cytotoxic chemotherapy	
1	33 (100)
2	10 (30.3)
ECOG performance status	
0 or 1	30 (90.9)
2	3 (9.1)
PSA	
Median (range), ng/ml	116 (4.5-5000)
Gleason score	
≤7	21 (63.6)
8-10	12 (36.4)

experienced pathologists (T.J., I.H. and R.C.). For immunohistochemical purposes the sections were placed on poly-D-lysine-coated glass slides. Rabbit monoclonal antibody recognizing PD-L1, clone 28-8 (Abcam, Cambridge, UK) was diluted 1:400. A standard immunohistochemical procedure was applied to all specimens using Ventana BenchMark XT autostainer following antibody data-sheet recommended procedure. Pre-treatment with cell conditioning solution (CC1) and OptiView detection system with 3,3-diaminobenzidine purchased from Roche, Prague, Czech Republic) were used to visualize the immunohistochemical reactions. Immunostaining results were semiquantitatively evaluated by three of the authors (T.J., I.H. and R.C.) in the whole tissue sections in specimens as follows: 0: no positive staining; 1: up to 1% positive cells; 2: 1-10% positive cells; 3: 10-50% positive cells. The evaluation was performed using multihead microscope (Olympus, Prague, Czech Republic); consensus on Gleason score grading was achieved viewing slides in the same multiheaded microscope in few controversial cases as well.

Statistics

The statistical evaluation was performed by using the Number Cruncher Statistical Systems 9 NCSS program (Kaysville, Utah, USA). Overall survival (OS) = time from starting enzalutamide till death or the date of the last patient visit (censored data). Progression-free survival (PFS) = time from starting enzalutamide to disease progression or the last control of a patient without progression (censored data). The OS and DFS were assessed using Kaplan-Meier analysis with log rank test. Multivariate analysis was performed using the Cox regression method. Statistical significance was set at $p<0.05$.

Results

The enzalutamide therapy was well tolerated. One patient had grade IV cerebral hemorrhage. The most common type of grade I-II non-hematological toxicity was fatigue, nausea and epistaxis. Grade I-II anaemia was found in 3 patients and grade III-IV in 6 patients. Grade I-II leukopenia was found in 3 cases. Grade III-IV thrombocytopenia was noted in 2 patients (Table 2). The median of hemoglobin nadir was 121.5g/l (range 67-160), of leukocytes nadir was 5.6×10^9/l (range 3.2-17.7), and of platelet nadir was 184×10^9/l (range 32-371).

At the time of assessment (30 Nov 2017), 24 patients had disease progression. The median of PFS was 7.0 months (95% CI 6.1-7.9) (Figure 1). A total of 18 patients died. The median of OS was 8.4 months (95 % CI: 5.1-11.7) (Figure 2). The proportion of patients with decline of PSA >50% was 63.6%.

The shorter OS was described in a subgroup of patients with decrease of hemoglobin concentration during the enzalutamide treatment with HR 0.155 (95% CI 0.053-0.449) and in patients with Gleason score 8-10 with HR 0.334 (95% CI 0.12-0.927)

Table 2. Toxicity of enzalutamide treatment

Events	*Grade I-II n (%)*	*Grade III-IV n (%)*
Anemia	3 (9.1)	6 (18.2)
Thrombocytopenia	0 (0)	2 (6.1)
Leukocytopenia	3 (9.1)	0 (0)
Fatigue	6 (18.2)	0 (0)
Nausea	3 (9.1)	0 (0)
Liver function tests abnormalities	1 (3)	0 (0)
Cerebral hemorrhage	0 (0)	1 (3)
Epistaxis	3 (9.1)	0 (0)
Diarrhea	1 (3)	0 (0)
Constipation	1 (3)	0 (0)
Anorexia	2 (6.1)	0 (0)
Spastic muscle contraction	0 (0)	0 (0)

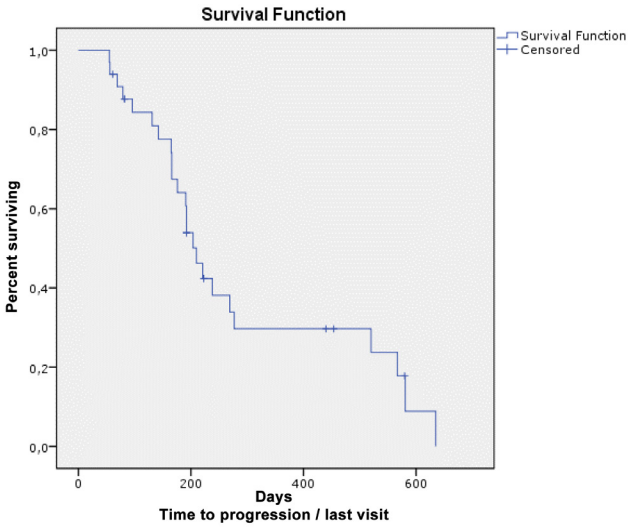

Figure 1. Progression-free survival (PFS): The median of PFS was 7.0 months (95% CI 6.1-7.9).

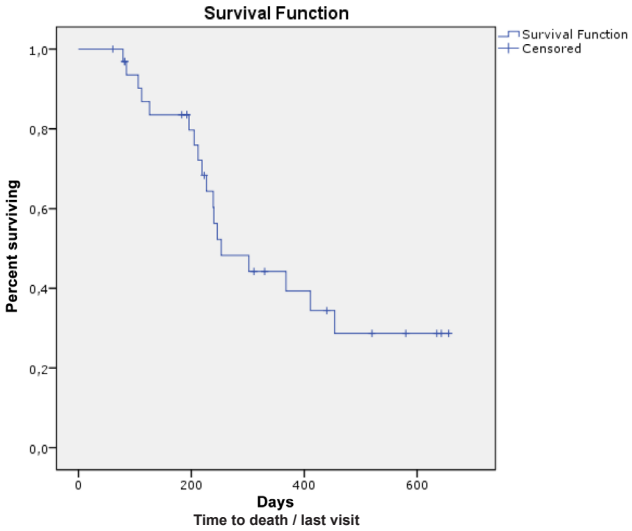

Figure 2. Overall survival (OS): The median of OS was 8.4 months (95 % CI: 5.1-11.7).

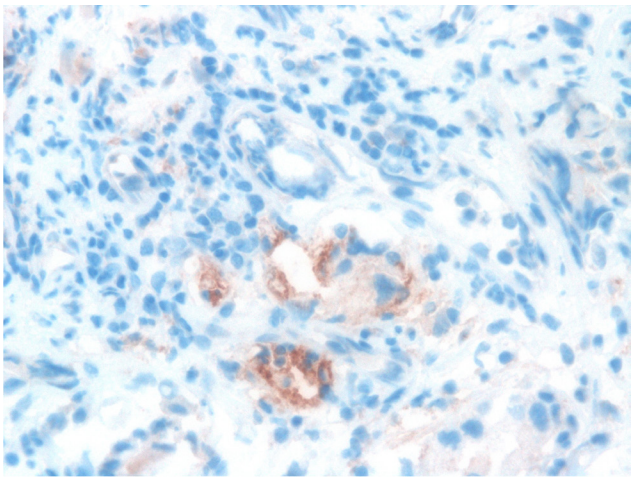

Figure 3. Focal strong staining was observed in <1% of neoplastic cells (negative results). Original magnification 200×.

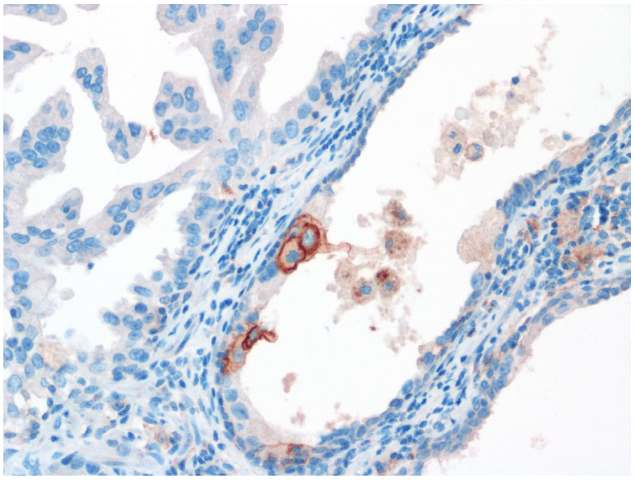

Figure 4. Focal positive staining for PD-L1 in <1% of neoplastic cells (negative result) in concomitantly present prostatic intraepithelial neoplasia. Original magnification 200×.

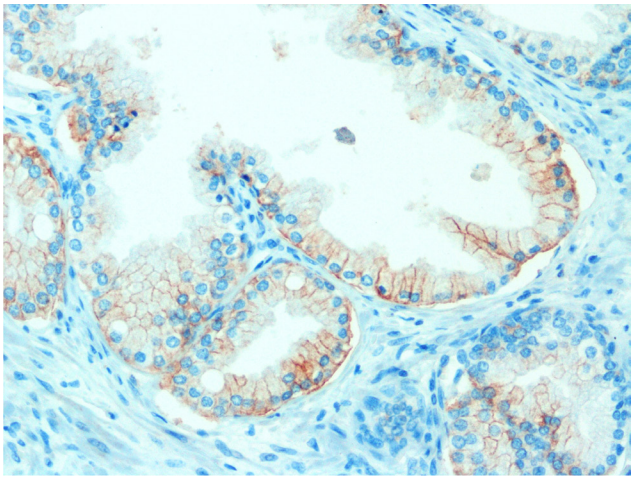

Figure 5. Focal PD-L1 expression in non-neoplastic prostatic cells (<1%), while neoplastic tissue was completely negative in this case. Original magnification 200×.

according to multivariate regression analysis. Age, pretreatment concentration of PSA, duration of previously hormonal treatment, and the number of chemotherapy lines were not demonstrated to have significant prognostic influence on OS (Table 3).

All tissue samples were scored as negative in immunodetection of PD-L1. Focal strong staining was observed in <1% of neoplastic cells (negative result) in one patient with transurethral resection (Figure 3). Focal positive staining for PD-L1 was also present in <1% of neoplastic cells (negative result) in prostatic intraepithelial neoplasia (PIN, Figure 4) tissue in the resection specimen in one patient. Invasive neoplasia was however completely PD-L1-negative. Focal PD-L1 staining was also present in non-neoplastic prostatic glands (<1%) in the patient with resection specimen only, while neoplastic tissue was completely negative in this case (Figure 5).

Discussion

Our retrospective study demonstrated our first experience with enzalutamide in the post-docetaxel setting in patients with mCRPC. Enzalutamide was very well tolerated with only one case of grade III-IV non-hematological toxicity. One patient with cardiac comorbidities had a cerebral hemorrhage. It is difficult to determine the direct influence of enzalutamide to this adverse event. The enzalutamide treatment is associated with the possibility of the seizures. In the AFFIRM study seizures were described in 5 of the 800 patients (0.96%) treated with enzalutamide while no seizures were reported in the placebo group [8]. The PREVAIL trial, that evaluated enzalutamide in patients with mCRPC before previous chemotherapy, seizures were described in one case [25]. We did not demonstrated seizures in our study. The most common type of non-hematological toxicity was fatigue, which could be also related with progression of disease. The median OS was 8.4 months which is less than in the AFFIRM study. The first reason could be the shorter time of observation in our retrospective study (7.6 months). The second reason for the lower median OS in our study could be that fewer patients participated in the following systemic treatment after enzalutamide (21.7%) compared with 42% in the AFFIRM study. The time to PSA progression in the AFFIRM study was 8.3 months, and the median radiographic PFS was 8.3 months. In our study the median PFS was 7.0 months. In Cox regression analysis we demonstrated the negative prognostic influence on OS in subgroups of patients with decreasing hemoglobin concentra-

Table 3. Brief presentation of regression analysis of factors influencing overall survival

Factors	*HR (95% CI)*	*p value*
Hemoglobin nadir (≤128.5 vs. >128.5 g/l)	0.155 (0.053 - 0.449)	0.021
Gleason score (≤7 vs. 8 - 10)	0.334 (0.120 - 0.927)	0.037
Pretreatment PSA (≤ 105vs. >106 ng/ml)	0.449 (0.128 - 1.568)	0.798
Duration of hormonal treatment (≤12 vs >12 months)	0.721 (0.271 - 1.547)	0.825
No. of chemotherapy lines (1 vs. 2 lines)	0.682 (0.197 - 2.363)	0.813
Age (≤75 vs. >75 years)	0.683 (0.189 - 2.469)	0.819

tion during the enzalutamide treatment and with Gleason score 8-10.

Currently we do not have any predictive factors for ARTA therapy in clinical practice. In their study, Antonakis et al. demonstrated that detection of androgen-receptor splice variant 7 messenger RNA (AR-V7) in circulating tumor cells from men with advanced prostate cancer was associated with resistance to ARTA therapy. The patients with detectable AR-V7 had lower PSA response, shorter PSA progression-free survival, shorter radiographic and clinical progression-free survival and shorter OS [26]. On the other hand, a recently published study demonstrated that the expression of AR-V7 in circulating tumor cells does not preclude response to ARTA therapy in patients with CRPC [27]. In the Czech Republic the AR-V7 detection in prostate cancer cells is not recommended in clinical practice [28].

The new potential predictive factor in patients with mCRPC which could be studied is the expression of PD-1 or PD-L1 in prostate cancer cells. The expression of PD-1/PD-L1 in prostate cancer is unclear [24,29]. Martin et al. reported that human prostate cancer cell lines PC3 and DU145 constitutively express PD-L1. In 20 whole-mount human primary prostate cancer samples, 3 were found positive in PD-L1 staining, which represents 15% of the cases. The PD-L1 positivity was defined as 5% of cells with membrane staining [30]. In our study we did not demonstrate the expression of PD-L1 in primary prostate cancer cells. We chose the cutoff PD-L1 expression according to the KEYNOTE-012 trial with positivity of at least 1% of tumor cells [31]. We discussed the possibility of laboratory error in the determination of PD-L1 expression and we performed the assessment of PD-L1 expression in squamous cell carcinoma of head and neck, where the expression of PD-L1 was described approximately in 50% of cases [32]. In these cells, strong (3+) expression of PD-L1 was demonstrated (Figure 6). We determined the PD-L1 expression using immunohistochemical methods. This type of detection of PD-L1 expression has a limitation because of its subjectivity and unclear definition of positive tumor PD-L1 staining [33,34]. Immunostaining results in our study were evaluated independently by three experienced histopathologists who were not familiar with the treatment results of patients. The possible problem of the examination of PD-L1 in biopsies is the focal analysis of the tumor, while the major parts of tumor cells could remain without examination. PD-L1 expression has two patterns, focal expression and diffuse expression. Even from the same sample, biopsy may result in a bias due to the focal nature of PD-L1 expression in many tumors [35].

The other unknown question is whether patients with enzalutamide-resistant CRPC may have highly expressed PD-L1 in the prostate cancer cells. Bishop et al. in their study demonstrated for the first time that enzalutamide resistance is associated with high frequency of PD-L1 expression, not only in the tumor, but in circulating immune cells as well [36]. The problem is the fact, that not all patients who respond to PD-1 or PD-L1 therapies exhibit tumor expression of PD-L1, and that only a very small sample of CRPC tumors have been assessed for PD-L1 expression as we mentioned above [29]. The result is that the use of PD-1/L1 blockade is even more difficult for CRPC patients. Preliminary findings from a phase 1b study with pembrolizumab in heavily pretreated PD-L1 positive advanced prostate cancer patients reported an ORR of 13%, with a median duration response of 59 weeks and stable disease in 39% of the enrolled patients [37]. A phase II study which evaluated pembrolizumab in combination with enzalutamide in mCRPC patients upon progression on enzalutamide alone showed PSA decline of ≥ 50% in 20% of the patients and some of them remained progression-free for up to 60 weeks [38]. Patients with progression after enzalutamide were treated with pembrolizumab, which was added to standard dose of enzalutamide. Three of the first 10 patients enrolled in this phase II trial experienced rapid PSA reductions to ≤ 0.2 ng/ml. Two of these 3 patients had baseline tumor biopsies. Immunohistochemistry from these biopsies showed PD-L1 expression [39].

Conclusions

This restrospective study presented our first experience with enzalutamide in patients with mCRPC in the postdocetaxel setting. The treatment was effective and very well tolerated. We did not detect the expression of PD-L1 in prostate cancer cells as potential new predictive biomarker. Further studies are needed to clarify this topic.

Conflict of interests

The authors declare no conflict of interests.

References

1. Tannock IF, de Wit R, Berry WR et al. Docetaxel plus prednisone or mitoxantron plus prednisone for advanced prostate cancer. N Engl J Med 2004;351:1502-12.
2. Petrylak DP, Tangen CM, Hussain MH et al. Docetaxel and estramustine compared with mitoxantrone plus prednisone for advanced refractory prostatae cancer. N Engl J Med 2004;351:1513-20.
3. Sartor AO. Progression of metastatic castrate-resistant prostate cancer: Impact of therapeutic intervention in the post-docetaxel space. J Hematol Oncol 2011;4:18.
4. Chen CD, Welsbie DS, Tran C et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2003;10:33-9.
5. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer directed therapies targeting the androgen-receptor signaling axil. J Clin Oncol 2005;23:8253-61.
6. Tran C, Ouk S, Clegg NJ et al. Development of a second-generation antiandrogen for treatment af advanced prostate cancer. Science 2009;324:787-90.
7. Jung ME, Ouk S, Yoo D et al. Structure-activity relationship for thiohydantoin androgen receptor antagonists for castration-resistant prostate cancer (CRPC). J Med Chem 2010;53:2779-96.
8. Scher HI, Fizazi K, Saad F et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187-97.
9. Hodi FS, O´Day SJ, McDermott DF et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
10. Brahmer J, Reckamp KL, Baas P et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373:123-35.
11. Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803-13.
12. Ichikawa M, Chen L. Role of B7-H1 and B7-H4 molecules in downregulating effector phase of T-cell immunity: novel cancer escaping mechanism. Front Biosci 2005;10:2856-60.
13. Fanoni D, Tavecchio S, Recalcati S et al. New monoclonal antibodies against B-cell antigens: possible new strategies for diagnosis of primary cutaneous B-cell lymphomas. Imunol Lett 2011;134:157-60.
14. Terme M, Ullrich E, Aymeric L et al. Il-18 induces PD-1-dependent immunosuppression in cancer. Cancer Res 2011;71:5393-9.
15. Freeman GJ, Long AJ, Iwai Y et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:1027-34.
16. Katsuya Y, Fujita Y, Horinouchi H et al. Immunohistochemical status of PD-L1 in thymoma and thymic carcinoma. Lung Cancer 2015;88:154-9.
17. Nakanishi J, Wada Y, Matsumoto K et al. Overexpression of B7-H1 (PD-L1) significantly associated with tumor grade and postoperative prognosis in human urothelial cancers. Cancer Immunol Immunother 2007;56:1173-82.
18. Nomi T, Sho M, Akahori T et al. Significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 2007;13:2151-7.
19. Fay AP, Sgnoretti S, Callea M et al. Programmed death ligand-1 expression in adrenocortical carcinoma: an exploratory biomarker study. J Immunother Cancer 2015;3:3.
20. Richter I, Jirásek T, Dvořák J et al. The prognostic effect of neoadjuvant chemoradiotherapy on change of PD-L1 expression in patients with locally advanced rectal adenocarcinoma. JBUON 2017;22:875-81.
21. Ohaegbulam KC, Assal A, Lazar-Molnar E et al. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med 2015;21:24-33.
22. Alexandrov LB, Nik-Zainal S, Wedge DC et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
23. Allison JP. Immune checkpoint blockade in cancer therapy: the 2015 Lasker-DeBakey Clinical Medical Research Award. JAMA 2015;314:1113-4.
24. Brahmer JR, Drake CG, Wollner I et al. Phase I study of single-agent antiprogrammed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010;28:3167-75.
25. Beer TM, Amstrong AJ, Rathkof DE et al. Enzalutamide in metastatic prostatae cancer before chemotherapy. N Engl J Med 2014;371:424-33.
26. Antonarakis ES, Lu C, Wang H et al. AR-V7 and resistance to enzalutamid and abirateron in prostate cancer. N Eng J Med 2014;371:1028-38.

27. Bernemann C, Schnoeller TJ, Luedeke M et al. Expression of AR-V7 in circulating tumour cells does not preclude response to next generation androgen deprivation therapy in patiens with castration resistant prostate cancer. Eur Urol 2017;71:1-3.
28. Büchler T, Bobek V, Kološtová K et al. AR-V7 androgen receptor variant as predictor of response to androgen-receptor targeting agents used to treat castration-refractory metastatic prostate cancer. Klin Onkol 2018;31:9-14.
29. Taube JM, Klein AP, Brahmer JR et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD.1 therapy. Clin Cancer Res 2014;20:5064-74.
30. Martin AM, Nirschl TR, Nirschl CJ et al. Paucity of PD-L1 expression in prostatae cancer: Innate and adaptive immune resistance. Prostate Cancer Prostatic Dis 2015;18:325-32.
31. Muro K, Chung HC, Shankaran V et al. Pembrolizumab for patients with PD-L1 positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol 2016;17:717-26.
32. Wang X, Teng F, Kong L. PD-L1 expression in human cancer and its association with clinical outcomes. Onco Targets Ther 2016;9:5023-39.
33. Gandhi L, Balmanoukian A, Hui R et al. Abstract CT105: MK-3475 (anti-PD1 monoclonal antibody) for non-small cell lung cancer (NSCLC): antitumor activity and association with tumor PD-L1 expression. Cancer Res 2014;74:CT105.
34. Marti AM, Martinez P, Navaro A et al. Concordance of PD-L1 expression by different immunohistochemistry (IHC) definitions and in situ hybridization (ISH) in squamous cell carcinoma (SCC) of the lung. J Clin Oncol 2014;32: (Suppl 25, abstr 7569).
35. Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogenity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883-92.
36. Bishop JL, Sio A, Angeles A et al. PD-L1 is highly expressed in enzalutamide resistant prostate cancer. Oncotarget 2014;6:234-42.
37. Hansen A, Massard C, Ort PA et al. Pembrolizumab for patients with advanced prostate adenocarcinoma: preliminary results from the KEYNOTE-028 study. Ann Oncol 2016;27(Suppl 6, abstract 725PD).
38. Graff JN, Alumkal JJ, Drake CG et al. First evidence of significant clinical activity of PD-1 inhibitors in metastatic castration resistant prostate cancer (mCRPC). Ann Oncol 2016;27(Suppl 6, abstract 7190).
39. Graff JN, Alumkal JJ, Drake CG et al. Early evidence of anti-PD-1 activity in enzalutamide - resistant prostate cancer. Oncotarget 2016;7:52810-7.