Original paper

Programmed cell death protein 1 and programmed death ligand 1 expression in neuroendocrine carcinomas of the urinary bladder

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Neuroendocrine carcinomas (NECs) of the urinary bladder are rare and aggressive, without an effective treatment approach. Immune checkpoint inhibitor agents targeting the interaction of programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) have been approved for urothelial carcinoma, but their use for small-cell carcinoma (SmCC) of the urinary bladder is unclear. Thus, we analyzed PD-1 and PD-L1 expression in NECs, particularly in SmCC of the urinary bladder. We used PD-1 antibody and two different biomarkers for PD-L1 antibodies, Ventana SP263 (Ventana Medical Systems, Inc.) and AM26531AF-N (Acris Antibodies GmbH). Among the 12 SmCC cases, 16.7% stained positive on immune cells for PD-1 and 33.0% for PD-L1. PD-L1 positivity on tumor cells was 25.0% of SmCC cases. The overall survival was shorter in PD-L1-positive patients compared with PD-L1-negative patients (15.4 months vs. 27.6 months). Large cell carcinoma (n = 1) was strongly and diffusely stained with PD-L1, and this case had the longest survival (68 months) in the group. Immune checkpoint inhibitors may be an alternative treatment option in SmCC of the urinary bladder. Furthermore, the absence of PD-L1 may be a good prognostic parameter.

Key words: immune checkpoint, PD-1, PD-L1, small-cell carcinoma, urinary bladder.

Introduction

Neuroendocrine tumors of the urinary bladder are rare, comprising < 1% of all urinary bladder malignancies [1]. The 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs classifies these tumors into four types, which are, in order of frequency, small-cell carcinoma (SmCC), large-cell neuroendocrine carcinoma (LCNEC), well-differentiated neuroendocrine tumors, and paraganglioma [2]. Although clinical symptoms, such as hematuria, are similar to other urothelial carcinomas (UCs), neuroendocrine carcinomas (NECs) are more aggressive than UCs. Despite therapy, approximately 80% of patients die within 5 years of diagnosis [3]. The patients are predominantly male (male : female, 5 : 1) and > 60 years old [4]. NEC diagnosis is based on histomorphological and immunohistochemical evaluation of transurethral resection (TUR) specimens.

SmCC pathogenesis remains controversial, and there are several hypotheses regarding its origin. The most persuasive of these is origination from a multipotential common stem cell, which has the ability to differentiate into various cell types. Other theories include origination from malignant transformation of urinary bladder neuroendocrine cells or from urothelial metaplastic changes [4].

SmCC patients are usually at an advanced stage (stage III or IV) when diagnosed. In reports of large case series, almost all cases had invaded into the muscle layer or further, and the most frequent sites of metastasis were pelvic and retroperitoneal lymph nodes, liver, bone, brain, and lung [5, 6, 7, 8]. Nearly 40% of SmCC cases comprise mixed tumor components of small cell and UCs [5]. Due to the rarity of these tumors, there is no standard treatment. In surgically resectable disease, the preferred treatment choice is neoadjuvant chemotherapy (CT), followed by radical resection, or adjuvant CT with or without radiotherapy (RT) in operated patients [4]. In advanced disease, CT based on platinum is preferred [4]. The prognosis of SmCC patients is poor, and 5-year survival rates of 63.6%, 15.4%, and 10.5% for stage II, III, and IV, respectively, were reported in a large patient series [5]. Additionally, the prognosis of cases of pure small-cell histology was worse than that of mixed cases. LCNEC, which is rarer than SmCC, has close similarities with SmCC in terms of its theory of origin, disease behavior, patient survival, and treatment approaches [9].

Besides surgery and CT and RT modalities, immune checkpoint inhibitors are a new and promising choice of treatment in various cancer types, including UC. The efficacy of these drugs in NECs of the urinary bladder remains uncertain. There are a few reports of the interaction between SmCC cells and immune cells and the usefulness of immune checkpoint inhibitors in these patients [3, 10, 11]. The first described case of a successful clinical and radiological response to pembrolizumab (a programmed cell death protein 1 [PD-1] inhibitor) treatment has led to more studies of the immune blockade in SmCC [12].

The present study investigates PD-1 and programmed death ligand 1 (PD-L1) expression in NECs of the urinary bladder in a single-institution cohort.

Material and methods

We performed a retrospective single-institution study of 13 cases of NECs of the urinary bladder diagnosed at our institute between 2009 and 2019. We obtained clinical, treatment, and follow-up patient data from the hospital database system.

We used an automated slide stainer (BenchMark XT; Ventana Medical Systems, Inc., Oro Valley, AZ, USA) to prepare formalin-fixed paraffin-embedded tissues for immunohistochemical staining with monoclonal antibody MRQ-22 (Ventana Medical Systems, Inc.) to detect PD-1 and two different antibodies, AM26531AF-N (Acris Antibodies GmbH, Herford Germany) and Ventana SP263, for detecting PD-L1, according to the manufacturer's instructions. The PD-1 and PD-L1 results were separately evaluated in both tumor cells and immune cells in the tumor microenvironment for each tumor component. Programmed cell death protein 1 was scored as positive when ≥ 5% of the population was stained. PD-L1 was scored as positive when membranous staining was present in ≥ 5% of the population.

Ethical committee approval was not an obligation institutionally; hence the research was not performed directly on human subjects, but on human tissue samples preserved in the archive of the department.

Results

Among the 13 patients with NECs of the urinary bladder in our study cohort, 12 were male and one was female. The mean age was 61.9 years (range, 31-79 years). There were four cases (30.8%) of pure SmCC, one case (7.7%) of pure LCNEC, and eight cases (61.5%) of mixed SmCC, of which seven comprised UC components, and one was urachal mucinous adenocarcinoma.

All cases were muscle-invasive (TNM stage ≥ T2). According to the TNM staging system, 6 cases (46.2%) were stage II, and 7 cases (53.8%) were at an advanced stage (5 cases, stage IV; two cases, stage III). The sites of metastasis included iliac and retroperitoneal lymph nodes (n = 3), lung and liver (n = 2), and bone (n = 2).

Only 3 patients (23.1%) were still alive (mean overall survival [OS], 40 months; range 23-68 months). The mean OS for the 10 deceased cases was 21.5 months (range: 2-60 months). We were able to obtain the treatment data in nine cases, which were as follows: CT alone (n = 2), CT combined with RT (n = 3); and radical cystectomy followed by CT and RT (n = 2). Two patients did not receive any treatment following TUR, and were deceased in 2 and 6 months, respectively. The patient data are presented in Table I.

Programmed cell death protein 1 and programmed death ligand 1 results

We did not detect PD-1 positivity in any tumor cells. Lymphocytes in the tumor microenvironment revealed positivity in 6 cases, of which three cases were in the UC component, 2 cases were SmCC, and 1 case was LCNEC.

Programmed death ligand 1 positivity in tumor cells differed according to the antibody used (Figs. 1, 2). We detected more positive cases by Ventana SP263 compared with the AM26531AF-N antibody (Table II). The percentages of the positive tumor cells are also given in Table I. Of note, a small number of tumor cells (< 5%) were positive in urothelial component in case 6. The LCNEC case was diffusely positive with both PD-L1 antibodies (Figs. 3, 4). PD-L1-positive immune cells were detected with AM26531AF-N antibody in two UC cases. In contrast,

Table I. The clinicopathologic and immunohistochemical data of the cases

	Age/ Sex	Diagnose	Metastasis	Stage	Treatment	Follow-up (months)	Status	PD1		PDL-1 (Ventana /SP263)	
								Tumor cells	Immune cells	Tumor cells	Immune cells
1	51/M	SmCC	None	II	CT	23	Alive	–	–	–	–
2	62/M	SmCC	None	II	CT	20	Deceased	–	–	+ (30%)	–
3	51/M	SmCC	None	II	CT+RT	9	Deceased	–	+	+ (40%)	+
4	78/F	SmCC	Vertebra met	IV	None	2	Deceased	–	–	–	+
5	65/M	UC	None	II	N/A	29	Alive	–	+	+ (10%)	–
		SmCC						–	–	–	–
6	72/M	UC	Lung, liver met, bilateral iliac lymph node met	IV	N/A	5	Deceased	–	–	–	–
		SmCC						–	–	–	–
7	79/M	UC	Sacrum, vertebra met (2 years)	IV	CT+RT	48	Deceased	–	–	–	–
		SmCC						–	–	–	–
8	52/M	UC	Perivesical invasion	III	N/A	60	Deceased	–	+	–	–
		SmCC						–	–	–	–
9	69/M	UC	Prostate invasion	III	N/A	21	Deceased	–	–	+ (70%)	+
		SmCC						–	–	+ (60%)	+
10	49/M	UC	Iliac and retroperitoneal lymph node met	IV	CT+RT	19	Deceased	–	+	–	+
		SmCC						–	–	–	–
11	75/M	UC	None	II	None	6	Deceased	–	–	+ (10%)	–
		SmCC						–	–	–	–
12	31/M	Mucinous adenoca	Lung, liver met, bilateral iliac lymph node met	IV	Radical surgery+ CT+RT	25	Deceased	–	–	–	–
		SmCC						–	+	–	+
13	71/M	LCNEC	None	II	Radical surgery+ CT+RT	68	Alive	–	+	+ (70%)	–

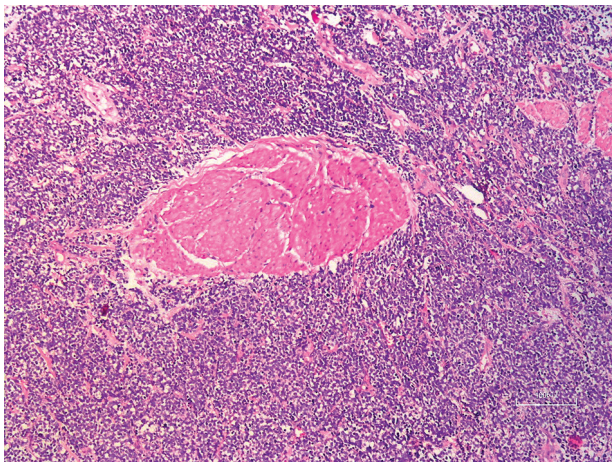

Fig. 1. Small cell carcinoma of the urinary bladder invading the muscle layer (HE, magnification 100×)

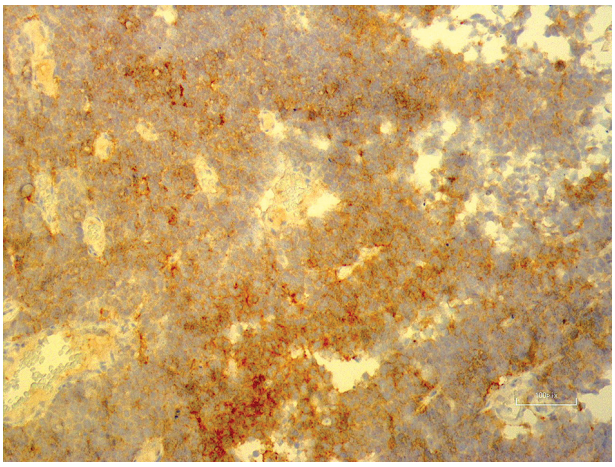

Fig. 2. PD-L1 positivity in tumor cells of small cell carcinoma with Ventana SP263 antibody (IHC, magnification 200×)

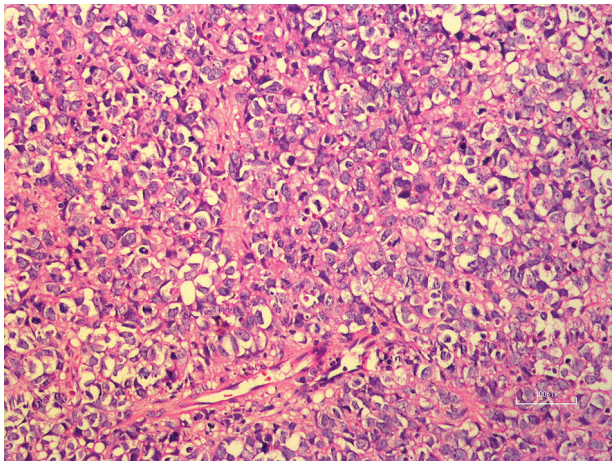

Fig. 3. Large cell neuroendocrine carcinoma (LCNEC) of the urinary bladder (HE, magnification 200×)

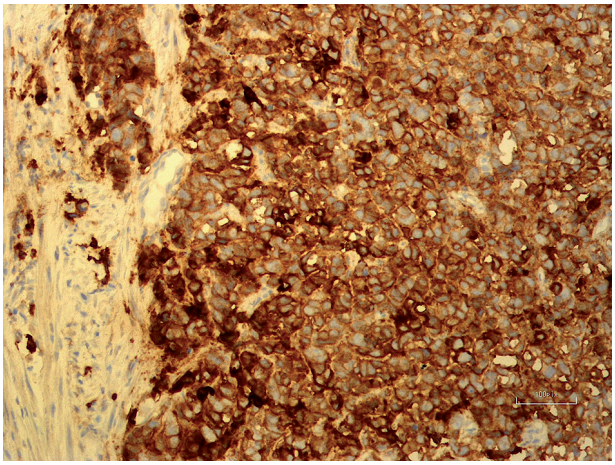

Fig. 4. LCNEC case with diffuse and strong positivity by PD-L1(IHC, magnification 400×)

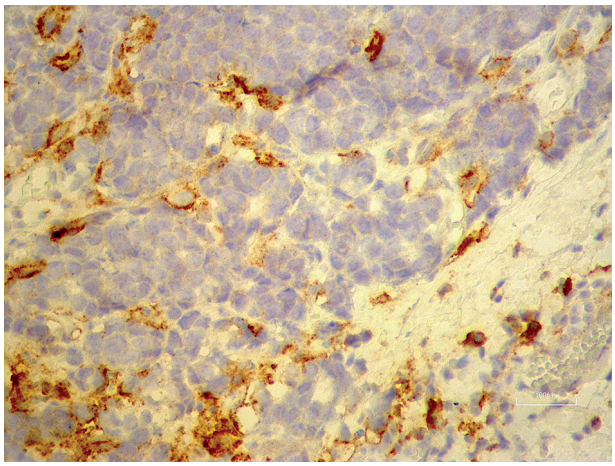

Fig. 5. PD-L1 positivity on immune cells in small cell carcinoma (IHC, magnification 400×)

we detected six cases of PD-L1 positive immune cells, including four SmCC cases and two UC cases, with Ventana SP263 (Figure 5). Table II summarizes the PD-1-positive and PD-L1-positive case distribution according to the histopathologic subtypes. Due to the presence of more positivity in tumor cells using Ventana SP263, we used these results when comparing our cohort data with those of the literature regarding the approved treatment choice.

All cases of PD-L1-positive tumors were non-metastasized (n = 6). Of them, four patients were deceased. Cases of two alive patients were LCNEC (n = 1) and mixed carcinoma with PD-L1 positivity in the UC component (n = 1). The histological subtypes of the deceased patients' tumors included two pure SmCCs and two mixed carcinomas, of which one was PD-L1 positive in both tumor components, and the other was PD-L1 positive in the UC component only (Table I). Programmed death ligand 1 positivity was present in 5/12 of the SmCC cases, regardless

Table II. Distribution of PD-1 and PD-L1 (two different antibodies) results based on histopathological subtypes

	PD-1		PD-L1 (Ventana/SP263)		PD-L1 (AM26531AF-N)	
	Tumor cells	Immune cells	Tumor cells	Immune cells	Tumor cells	Immune cells
Small cell carcinoma (n = 12)	0	2	3	4	2	0
Large cell NEC (n = 1)	0	1	1	0	1	0
Urothelial carcinoma (n = 7)	0	3	3	2	1	2
Mucinous adenocarcinoma (n = 1)	0	0	0	0	0	0
Total number	0	6	7	6	4	2

of the cell type. These five patients were deceased (mean OS, 15.4 months). Of the seven PD-L1-negative patients, two were alive after a mean of 26 months, whereas the other 5 PD-L1-negative patients were deceased (mean OS, 27.6 months; range, 5-60 months). The OS for PD-L1-positive cases was lower than that of PD-L1-negative cases, although this was not statistically significant due to the limited number of cases (U = 12, Z = 27, p = 0.216; Mann-Whitney U test).

Discussion

Programmed cell death protein 1 is a transmembrane protein expressed on various immune cells, including T cells, B cells, and natural killer cells [13]. Programmed cell death protein 1 plays an inhibitory role by binding its ligand (PD-L1), which is found on antigen-presenting cells and tumor cells. This PD-1 and PD-L1 interaction results in inhibition of tumor cell apoptosis by T cells, so the immune response against the tumor becomes blocked [14]. Thus, immune checkpoint inhibitor agents targeting both PD-1 and PD-L1 could reactivate cytotoxic T cells to work against cancer cells [15]. Various immune checkpoint inhibitor anticancer drugs targeting the PD-1/PD-L1 checkpoint have been approved for clinical use in some cancers, including metastatic melanoma, non-small-cell lung carcinoma (NSCLC), renal cell carcinoma, and UC. Additionally, there are ongoing clinical trials in other solid tumors and hematological cancers [15, 16, 17].

The accumulating data over the past few years indicate that the determination of PD-1 and PD-L1 plays a vital role in choosing candidate carcinomas likely to benefit from the treatment and as predictive biomarkers. Immunohistochemistry (IHC) is widely used to detect the presence of these proteins. However, heterogeneity of tumor expression, the lack of a consistent scoring system, and the presence of different companion biomarkers on different IHC assay platforms, such as Ventana SP263, Ventana SP142, Dako 22C3 (Dako, Carpinteria, CA, USA), and Dako 28-8 (Dako), cause controversial results in the literature [10, 18, 19]. Thus, data comparisons between different assays, especially in NSCLC, show significant variability [20, 21, 22].

Programmed death ligand 1 expression has been reported in 20-30% of UCs [18]. Previous studies have shown that PD-L1 positivity correlates with the TNM T-stage as well as worse survival in UC patients with a high PD-L1 positivity compared with patients with a low PD-L1 positivity [23]. However, Davick *et al.* did not identify a significant difference in OS between patients with PD-L1-positive and those with PD-L1-negative tumors in their cohort [10]. Additionally, although not statistically significant, patients with PD-L1-positive tumors had slightly better OS compared to PD-L1-negative ones in their study [10]. Similar findings were also reported, particularly in viral-related cancers (vulvar and cervical squamous cell carcinoma [SCC], oropharyngeal SCC, and Merkel cell carcinoma) and in NSCLC and melanomas. This is thought to be related to the elicitation of the immune response by these tumors [10].

Due to the rarity of NECs of the urinary bladder, the literature is limited to a few studies in terms of PD-1 and PD-L1 expression of these tumors. In a larger study with 165 cases of different types of urinary bladder cancer, including only three SmCC cases, PD-L1 positivity was only reported for one SmCC case [10]. Furthermore, in a recently reported study, all of the 12 pure SmCC cases contained PD-1-positive lymphocytes, which we only found in 2/12 (16.7%) of the SmCC tumors in our study [3]. Such a difference between two studies may be related to the use of different antibody clones and to the heterogeneity of these tumors.

Programmed death ligand 1 results for SmCCs of the urinary bladder are limited to only a few reports [3, 10, 11]. We were unable to compare our data directly with these reports because of the variation in companion biomarkers. Sanguino et al. used Ventana SP142 for PD-L1 staining and reported focal (< 5%) staining in tumor cells in 2/19 (10.5%) SmCCs and strong PD-L1 staining in tumor stromal

mononuclear cells in 4/19 cases (21.1%) [11]. Similarly, PD-L1 positivity in tumor cells was reported in only 1/12 (8.3%) SmCC cases (E1L3N monoclonal antibody; Cell Signaling Technology, Danvers, MA, USA) and 1/3 (33.3%) SmCC cases (antibody not mentioned) in other studies and was 3/12 (25.0%) in our study [3, 10]. The higher value in our study appears to be related to the biomarker that we used (Ventana SP263).

In contrast to Sanguino *et al*., who reported 3/4 (75.0%) PD-L1-positive cases to be alive, in our cohort, all PD-L1-positive SmCC cases (n = 5), either in tumor cells or in immune cells, alone or together, were deceased (mean OS 15.4 months). Furthermore, the OS for PD-L1-positive cases was lower than that for PD-L1-negative cases (15.4 months vs. 27.6 months), which was also in contrast with the latter study (41 months vs. 14 months) [11]. Our results revealed that PD-L1 positive cases of SmCCs of the urinary bladder had poorer OS. Of note, PD-L1 expression status appeared unrelated to metastasis risk in our study.

We were unable to find any data in the literature regarding immune checkpoint status in LCNEC of the urinary bladder. Although we had only one case in our cohort, it is noteworthy that this case was one of the three alive patients in the group and had the longest follow-up period in our cohort (68 months). Additionally, unlike other SmCC cases, PD-L1 positivity with strongly membranous staining was detected in 70% of tumor cells. Thus, we speculate that PD-L1 positivity may be correlated with better survival in LCNEC of the urinary bladder, and this requires further investigation.

In the literature, urachal mucinous adenocarcinoma has not been reported as a component in mixed neuroendocrine carcinomas of the bladder, previously. The single case in our cohort (case 12) was the youngest patient with an advanced stage. Programmed cell death protein 1 and PD-L1 was null both in tumor cells and the microenvironment in the urachal adenocarcinoma component. We think that this extremely rare tumor will contribute to the literature.

In conclusion, PD-1 and PD-L1 interaction play a vital role in tumor progression, survival, and treatment approach. Because of their rarity, the predictive and prognostic role of PD-L1 in NECs of the urinary bladder still remains controversial and undetermined compared with UCs. Our results show that SmCCs and LCNEC of the urinary bladder may benefit from approved immune checkpoint inhibitor treatment. More data are necessary to understand the prognostic role of PD-1/PD-L1 interaction in these tumors.

The authors declare no conflict of interest.

The authors would like to thank Enago (www.enago.com) for the English language review.

References

1. Kouba E, Cheng L. Neuroendocrine tumors of the urinary bladder according to the 2016 World Health Organization classification: Molecular and clinical characteristics. Endocr Pathol 2016; 27: 188-199.
2. Moch H, Humphrey PA, Ulbright TM, et al. WHO Classification of Tumours of the Urinary System and Male Genital Organs. 4th ed. IARC Press, Lyon 2016.
3. Mandelkow T, Blessin NC, Lueerss E, et al. Immune Exclusion Is Frequent in Small-Cell Carcinoma of the Bladder. Dis Markers 2019; 2019: 2532518.
4. Ismaili N. A rare bladder cancer – small cell carcinoma: review and update. Orphanet J Rare Dis 2011; 6: 75.
5. Choong NW, Quevedo JF, Kaur JS. Small cell carcinoma of the urinary bladder. The Mayo Clinic experience. Cancer 2005; 103: 1172-1178.
6. Cheng L, Pan CX, Yang XJ, et al. Small cell carcinoma of the urinary bladder: a clinicopathologic analysis of 64 patients. Cancer 2004; 101: 957-962.
7. Iczkowski KA, Shanks JH, Allsbrook WC, et al. Small cell carcinoma of urinary bladder is differentiated from urothelial carcinoma by chromogranin expression, absence of CD44 variant 6 expression, a unique pattern of cytokeratin expression, and more intense gamma-enolase expression. Histopathology 1999; 35: 150-156.
8. Siefker-Radtke AO, Dinney CP, Abrahams NA, et al. Evidence supporting preoperative chemotherapy for small cell carcinoma of the bladder: a retrospective review of the M. D. Anderson cancer experience. J Urol 2004; 172: 481-484.
9. Akdeniz E, Bakirtas M, Bolat MS, et al. Pure large cell neuroendocrine carcinoma of the bladder without urological symptoms. Pan Afr Med J 2018; 30: 134.
10. Davick JJ, Frierson HF, Smolkin M, et al. PD-L1 expression in tumor cells and the immunologic milieu of bladder carcinomas: a pathologic review of 165 cases. Hum Pathol 2018; 81: 184-191.
11. Sanguino A, Samiei A, Pasricha G, et al. Programmed death-ligand 1 (PD-L1) expression in small cell carcinoma of bladder. J Clin Oncol 2019; 37 (15 Suppl): e16019-e16019.
12. Wilde L, Ali SM, Solomides CC, et al. Response to pembrolizumab in a patient with chemotherapy refractory bladder cancer with small cell variant histology: a case report and review of the literature. Clin Genitourin Cancer 2017; 15: e521-e524.
13. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677-704.
14. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. Cell Death Dis 2015; 6: e1792.
15. Alsaab HO, Sau S, Alzhrani R, et al. PD-1 and PD-L1 Checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol 2017; 8: 561.
16. Cserni G, Serfozo O, Ambrózay É, et al. Spontaneous pathological complete regression of high-grade triple-negative breast cancer with axillary metastasis. Pol J Pathol 2019; 70: 139-143.
17. Cybulska-Stopa B, Ługowska I, Jagodzińska-Mucha P, et al. Immune checkpoint inhibitors therapy in older patients (≥ 70 years) with metastatic melanoma: a multicentre study. Postepy Dermatol Alergol 2019; 36: 566-571.
18. Aggen DH, Drake CG. Biomarkers for immunotherapy in bladder cancer: a moving target. J Immunother Cancer 2017; 5: 94.

19. Patel SP, Kurzrock R. PD-L1 Expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther 2015; 14: 847-856.
20. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 Immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. J Thorac Oncol 2017; 12: 208-222.
21. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016; 387: 1540-1550.
22. Sacher AG, Gandhi L. Biomarkers for the clinical use of PD-1/PD-L1 inhibitors in non-small-cell lung cancer: a review. JAMA Oncol 2016; 2: 1217-1222.
23. Nakanishi J, Wada Y, Matsumoto K, et al. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. Cancer Immunol Immunother 2007; 56: 1173-1182.

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