ejpmr, 2019,6(2), 01-09.

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

PD-1/PD-L1 BLOCKADE IN METASTATIC BLADDER CANCER TREATMENT: WHAT CAN WE DO NOW FOR THE PATIENTS?

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Article Received on 22/11/2018 **Article Revised on 12/12/2018** **Article Accepted on 03/01/2019**

ABSTRACT

The incidence of bladder cancer (BC) is increasing and this malignant tumor is common urological malignancy in human beings. The conventional general treatment to decrease or control the tumor cells are not satisfactory, so the treatment of BC has changed over time to revolve round not only impacted by chemotherapy and surgery, but also impacted by the use of immunotherapy. Tumor immunotherapy is a general term for enhancing the antitumor immune response by mobilizing the host's immune defense mechanism or by giving certain biologically active substances. Blocking checkpoints is durable clinical responses across multiple tumor types, including BC. However, for some special patients, it is fail to control tumor growth by the blockade of PD-1/PD-L1. Now, we perform a literature review about the clinical trials of PD-1/PD-L1 immune checkpoint blockades especially for BC patients, introducing comprehensive assessment about biomarkers and investigators for the aim of precise treatment for those patients and what we can do to improve the effects of immunotherapy for BC.

KEYWORDS: PD-1/PD-L1,bladder cancer.

INTRODUCTION

As one of the common malignancies over the world, 5-year survival of muscle invasive bladder cancer (MIBC) patients is short and with poor outcomes.[1-3] Bladder cancer (BC) is one type of immunogenic tumor in the world[4], so bacille Calmette-Guerin (BCG) was used as an immunotherapy for patients with non-muscle invasive bladder cancer (NMIBC)[5], and BCG was the first immune drug approved by the USA Food and Drug Administration (FDA) for BC.[6] What is different from NMIBC, the immunotherapy for MIBC is to block the checkpoint of Programmed death-1 (PD-1) or programmed death ligand 1 (PD-L1). PD-1 and PD-L1have been found to be expressed by BC patients.[7] PD-L1 participates in the process that cancer cells escape the attack of activated immune cells. The molecule PD-L1 combines with the matched molecule PD-1, a special receptor different from B7-1 and B7-2. The discovery that cancer cells avoid being identified by the immune system through expressing PD-L1 on tumor cells membrane, this rational mechanism provides ideal to develop antibody of PD-1 and PD-L1 to prevent cancer cell escaping. Erlmeier at el. found that UC patients whose tumor cells over expressed PD-L1were insensitivity to single chemotherapy[8] and on the other hand, immunotherapy can slow the progression of tumors and prolong the life of tumor patients. However, there are many challenges to make a personal immunotherapy for BC patients.

PD-1 and PD-L1: One member of the CD28/CTLA-4 Ig subfamily is PD-1, all of those members have similar functions.[9] The region of PD-1 out of cell contains a single Ig V-like domain.[10,11] The tail of PD-1 does not contain any SH2- or SH3-binding motifs, which is different from CD28 and CTLA-4. PD-1 differs from CTLA-4 and CD28 in 21–33% sequence of a single N-terminal Ig V- like. PD-L1 (B7-H1) and PD-L2 (B7-DC) can combine with PD-1; they have recently been identified as two new members of the B7 family.[12] There is about 40% identify between PD-L1 and PD-L2 amino acid, while PD-Ls and B7s is about 20% similar. PD-L1 and PD-L2 have faultless ability to combine with PD-1. Not only immune cells, including T cells, B cells can express PD-L1[13], but also non-hematopoietic cells can express this immunoglobulin.[12,14-18] On the other cells, such as macrophages can express PD-L2.[12,13,19-21]

In normal conditions, the immune system doesn't attack self-antigen providing checkpoints by the regulator. However, while the cancer cells express those checkpoint, they will also not be recognized by immunity systems, the worse result is that overproducing checkpoints will exhaust the antigen-specific effective T-cell with the result of unlimited amplification of tumor cells in this special environment. On the contrary, while blocking their receptors on immune effector cells will lead to reduce tumor escape and eliminate cancer in

principle.[22-25] The naive T cells activated rely on antigen usually need through two major stages.[26] First, while interacting with the antigen presented on the major histocompatibility complex (MHC) which is on the surface of an antigen-presenting cell (APC), the T cell will be activated, this progress is essential for inflammatory cells including T lymphocytes, microglia, and macrophages.[27] Then CD28, which is one of co-stimulatory molecules on the T-cell, binds CD80 (B7-1) or CD86 (B7-2) on the APC.[28] These complex two steps to activate the naive T cells are rigorous to regulate immune responses (Figure 1A). The progress of activating optimal T-cells needs B7 molecules on APCs to costimulate the ligation of the co-receptor CD28 on T-cells.[29] If the immune response is activated, activated T cells will express factors to attenuate the immune response with proliferating T cell and producing cytokine.[30] But while PD-L1 on the surface of tumor cells binds with PD-1 receptor on cytotoxic T lymphocytes, tumor cells can avoid being destructed (Figure 1B).PD-1 is expressed on T cells, which cause tumor cells not to be identified by immunity system.[31,32,33] While PD-1 interacts with its receptor expressed by tumor cells, such as PD-L1 and PD-L2, leading to fewer TCR-mediated proliferate and cytokine product. This new ideal to cure BC patients by using PD-L1 monoclonal antibodys (mAbs) is originated from preventing PD-L1combining with PD-1. PD-L1 and PD-L2 competed for PD-1 binding, while blocking PD-L1 and PD-L2, will lead to largely prolifer T cells and product more immunity factors to prevent the progress of tumor (Figure 1C).[34]

PD-1/PD-L1Targeting Immunotherapy in the Clinical Treatment: In 2005, Hiroyuki N et al. found that mice knocked out of PD-1 gene would suffer from autoimmune diseases, and then the hypothesis that PD-L1 plays an important role in managing peripheral tolerance was extracted.[35] The tumor cells are not sensitive to conventional chemotherapy because of the PD-1/PD-L1 axis, however, if PD-L1 or PD-1 are blockaded or the PD-L1 gene is silenced, the status of patients are obvious been improved.[36] In 2014, with the satisfying tumor responses in clinical trials in melanoma, the antibodies interacting with PD-1/PD-L1 immune checkpoint were quickly allowed to be used, such as nivolumab and pembrolizumab.[23,37,38] In 2015, FDA approval nivolumab to be an antibody for the metastatic squamous non-small-cell lung carcinoma (NSCLC) patients.[39] Then, a phase I clinical trial reveals that this monoclonal antibody can obvious improve the patients' status who suffering from melanoma, NSCLC and certain other solid tumors.[40] Another anti-PD-L1 monoclonal antibody, MPDL3280A, bring inspiring news for patients with melanoma, NSCLC and urinary cancers after treated with this antibody, their conditions are under the control.[41] In the phase I trial, with the great results, FDA approved this monoclonal antibody to be used for urinary cancers patients.[7] There are also further studies of anti-PD-L1 antibodies being done currently.[39]

Treatments for Bladder Cancer Patients: The incidence rate of BC is the fourth in the United States of men[42] and BC is the second most common urological malignancy in humans.[43] According to the TNM staging standard, BC is classified into 2 groups, NMIBC and MIBC.There are 70% of BC patients presenting with NMIBC, which tend to recur and the standard treatment for those is transurethral resection of bladder tumor(TURBt). The preservation of 30% BC patients presenting as MIBC are treated with radical cystectomy, radiation, and chemotherapy.[44] The first one of the two risks of BC is the gender and the second is smoking, a 10-fold variation was found in men and about 2-fold to 6-fold was found among smokers, smoking man has a higher risk than nonsmoking woman.[45] The standard to treat advanced metastatic BC is based on cisplatinum-based chemotherapy, the result is that those patients have a median overall survival (OS) about 60 weeks with this treatment.[46,47] However, not all patients are appropriate for cisplatinum-based treatment, 30%–50% of these patients cannot benefit from this project.[48,49] Tumor samples express more PD-1 than normal bladder tissue[50] and that induce the extra cellular PD-L1 to interact with PD-1leading tumor cells to escape from the attacks of immune cells. Maybe the immune-based treatments can be used in UC. Immunotherapy has been used only in advanced cancer forms. However, the novel inhibitors of PD-1and PD-L1 exhibit specific and unique mechanisms of action, the fact is that the benefit is found in the use of BCG for NMIBC in the last 30 years.[51,52] To prevent the progress of disease and prolong the life of MIBC patient , immunotherapy is an effective choice. A surprising phenomenon is that even if the patients are at the same BC stage and treated with the same immunotherapy, the ultima outcome can be diversity which indicates that something may be different in those people, personalized medicine were proposed by investigator, biomarker can make this ideal come true for different patients to be treated with personal scheme.[53]

PD-1 Blockade in Bladder Cancer: Nivolumab (BMS-936558) , within the power to be used in locally advanced urothelial carcinoma (aUC) or metastatic urothelial carcinoma(mUC) by the FDA, is one of the human PD-1 monoclonal antibodies. The patients with aUC or mUC being treated with nivolumab had an exciting result. In the phase I/II(Clinical Trials Number, NCT01928394) (Table 1), 86 patients with mUC were enrolled and all of them are older than 18 years old, the expression of PD-L1 was assessed. Treated those patients with 3 mg/kg every 2 weeks, and median OS was 41.7weeks (95% confidence interval (CI) 31.3-69.4) , median PFS was 12.0 weeks (95% CI6.4–25.3) in the all population, however, while the patients with PD-L1 expression was more than 1%, the median PFS was 23.6 weeks (95% CI 6.0 – 48.0) but that one of the others was 12.0 weeks (95% CI 6.0–27.9).[54]

Pembrolizumab (MK-3475) combines with PD-1 directly, in several recent clinical trials, BC patients were treated with pembrolizumab, the results were satisfactory in the terms of safety, response and survival of those people. A phase Ib trail(Clinical Trials Number, NCT01848834) (Table 1) was done in 115 patients over 18 with recurrent or mUC or having progressed cancers after treated with cisplatin therapy, but only 33 patients met the standards that the tumor cells must expressed at least 1% PD-L1. According to the evaluation after those population were treated with at the dose of 10mg/kg every 2 weeks, median PFS was 8.6 weeks (95% CI 8.6-17.1), and the Median OS was 55.7weeks (95% CI 21.4-85.7).[55] In phase II study (Clinical Trials Number, NCT02335424) (Table 1), the enrolled 374 patients with disease progressive while based on cisplatin chemotherapy. Those population treated at the dose of 200 mg every 3 weeks discontinuously.[56] After that, 542 patients were examined in another phase III trial (Clinical Trials Number, NCT02256436) (Table 1), the inclusion criteria of those patients was that someone had recurred aUC or the state of him was not controlled after platinum-based chemotherapy. Patients having the percentage of cells expressed PD-L1 at lest10% relative to total cells were deemed PD-L1 positive. The median OS in the patients treated with pembrolizumab was 44 weeks(95% CI, 34.3 to 50.6) and OS in those treated with chemotherapy was 32 weeks (95% CI, 26.1 to 35.6). The median OS among patients positive for PD-L1in the pembrolizumab group was 34.3 weeks (95% CI,21.4 to 52.7) compared to 22.3weeks (95% CI,17.1 to 31.7) in the chemotherapy group.[57]

PD-L1 Blockade in Bladder Cancer: Durvalumab (MEDI4736) is anti-PD- L1 immune checkpoint inhibitor, which is a safety and efficacy immunotherapy for patients with MIBC, especially in the PD-L1-positive subgroup.[58] The early studies reported using durvalumab is safety and effective in patients with refractory squamous NSCLC, which is also the same in patients with MIBC.[59] In a phase I/II(Clinical Trials Number, NCT01693562) (Table 1), 191 patients(over 18 years) with advanced UC or mUC were registered and using durvalumab at a dose of 10 mg/kg every 2 weeks by intravenous infusion for them until disease were still progression. The result of median PFS was 6.4 weeks (95%CI, 6-8.1weeks) and the OS was 78.0 weeks (95%CI, 34.7 weeks to not estimable).[60] In 2016, FDA approved durvalumab for MBC patients if the status progressed on platinum-based treatment. Massard et al. treated patients with UC whose status couldn't be improved or were cisplatin-ineligible as the object of observation[58], and they reported the results from the UBC expansion cohort using.

Avelumab, a monoclonal antibody, binds to PD-L1. A dose-escalation(1 mg every kilogram, 3 mg every kilogram, 10 mg every kilogram and 20 mg every kilogram) phase 1a trial (Clinical Trials Number, NCT01772004) that examined the characters of avelumab, such as safety, tolerability and pharmacokinetics, this trail indicated that avelumab intravenous injected every 2 weeks at a dose of 10mg/kg was the best.[61] After that, Manish R Patel et al. made another phase 1trail (Clinical Trials Number, NCT01772004) (Table1), they chose the 329 patients over 18 years old with advanced UC or mUC, 249 patients of those enrolled population accepted a dose of 10 mg/kg every 2 weeks. The median PFS of patients taking avelumab was 6.6 weeks(95% CI 6.1–11.4), while the median OS of them was 27.9 weeks (95% CI 20.6–40.7) longer than median PFS. The ORR was fifty percent in the PD-L1-positive patients group while it was four point three percent in the group that the patients were with PD-L1-negative tumors. After 6 months, the PFS was 58.3% in the PD-L1-positive group comparing with 16.6% in the group of opposite states of PD-L1 expression.[62]

Atezolizumab（MPDL3280A）is the first PD-L1 inhibitor to be approved by FDA for UC. There are some factors influencing the effect for different patients, for example, years and renal impairment are the two factors leading patients with UBC to tolerate MPDL3280A, which largely contribute to the lack of renal toxicity. In this trail I (Clinical Trials Number, NCT01375842) (Table 1), 205 patients were evaluated by immunohistochemistry (IHC) expressed on tumor-infiltrating immune cell (TIIC) and at a dose of 15 mg/kg every 3 weeks. The result is that an ORR is 43% for a PD-L1 TIIC IHC score of 2/3, and 11% for a score of 0/1. These indicate that MPDL3280A may make a great contribution in treating MBC[7]. In a phase II trial (Clinical Trials Number,NCT02108652）(Table 1), 310 advanced UC or mUC patients over18 are studied and their disease have not been controlled after treated with platinum-based chemotherapy, the most important criteria to choose patients whose status from 0 to 1 according to Eastern Cooperative Oncology Group performance. In this trail, the dose at 1200 mg of Atezolizumab are intravenous injected every 3 weeks. The results is that the ORR is 15%(95% CI 11–19) in the all patients, if the percentage of PD-L1-positive immune cells more than 5%, the ORR is 27% (95% CI 19–37) , in the other patients, the ORR is 18% (95% CI 13–24) [63]. And then FDA approved atezolizumab to be used for mUC patients who progressed after treated with cisplatin [64] and in June 2014.

Tumor Microenvironment of Bladder Cancer

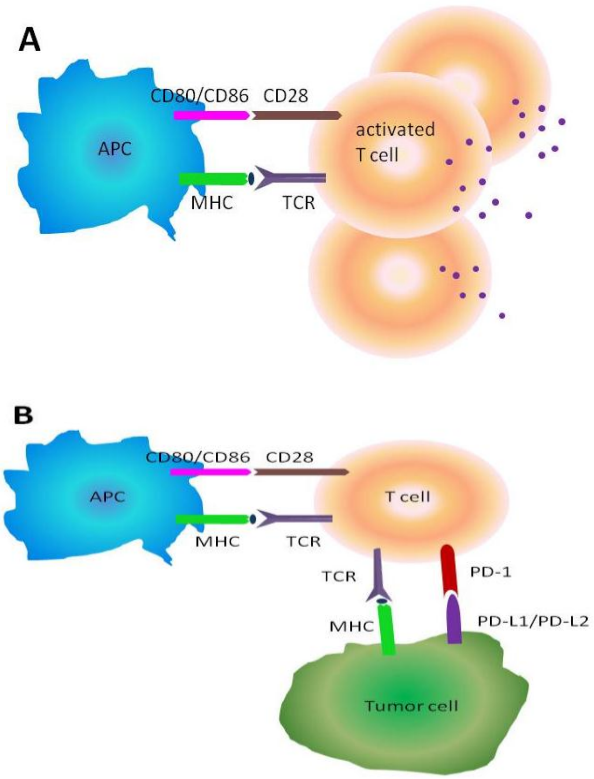
The immune reaction can be influenced by many factors in the tumor microenvironment (TME), from the view point of tumor, the factors include the cancer antigens and major histocompatibility complex on cancer cells; standing in the angle of immune cells, the elements contain the action of T cell and the infiltration of T cells into tumors. Boorjian et al. observed that the patients with UC will express more PD-L1 than people with early stage cancers, this independently factor predict all-cause mortality.[65] At 2017, Dr. Takuro Noguchi at el. found

that tumor escape is not only correlative to overepression PD-L1 on the tumor cells, but also relative to those in the host cell, specially the tumor associated macrophages.[66] The next year, Tang H at el. published a paper on the view that the PD-L1 on the host immune cell limits the transport of T cells reducing the efficacy of the PD-1/PD-L1 blockage treatment.[67] The expression of PD-L1 belonging to change the former and the PD-1 belonging to the latter in the TME can change the status of BC patients presenting as inflamed immune deserts.[68] Dense CD8+ T-cell infiltrates in this inflamed tumor, in general, CD8+ T cells recognize cancer-associated antigens and producing IFN- gamma which stimulate tumor-infiltrating immune cells or tumor cells to express PD-L1.[69-70] Therefore, the first phenomenon in this inflamed tumors is a preexisting CD8+ T-cell response to them, and establishing anticancer immunity by intratumoral PD-L1 expression. By checking the number of CD8+ T-cell and its activity may provide some information to evaluate the progression and prognosis of this disease.[72]

Hugo W et al. revealed that enrichment of BRCA2 mutation in melanoma patients are responsed to PD-1 immune checkpoint blockade and they also found a transcriptional signature which may indicate resistant to PD-1 immune checkpoint blockade.[73] Miao D et al found that losing PBRM1 in ccRCC may influence response to anti-PD-1 immunotherapy for ccRCC.[74] Progress of tumor formation related to the accumulation of somatic mutations, Chen found that somatic mutation influence the immunogenicity.[75] By studying the mutation of inflamed tumors, such as melanoma, epithelial cancer and clear cell renal cell carcinoma (ccRCC), reveal that nonsynonymous mutations on tumors maybe generate T cells specific for neo-epitopes.[76-82] PD-L1/PD-1 pathway maybe related to nonsynonymous mutations of many tumors.[41,83-85] A high frequency of somatic mutations in BC was found by Lawrence et al. through analysis the exome sequences.[86] Mutated cellular transcripts lead to produce tumor neoantigens presented on the surface of APCs, and then those neoantigens would enhance host immune recognition which is a critical first step in generating a robust antitumor response. In short, the features, such as significantly higher proportion of immune cell infiltrates, the personal expression of CD8+T-cells or CD4+ T-cells, biomarker of tumor cells, genomic and transcriptomic features of BC, maybe explain the mechanism why the effectiveness of the blockade of PD-1/PD-L1 for BC patients are different and give guidance for doctor about what check they should do for personal treatment.

New ways of treatment to improve the effectiveness of PD-1/PD-L1 blockade: About the treatment for those population, published studies revealed that PD-1 pathway activated in MIBC and the same sample over expressed PD-1 and cytotoxic T-lymphocyte antigen-4 (CTLA-4), those two pathway were costimulated in MIBC indicate that combine anti-CTLA4 and anti-PD-1/PD-L1 therapies might be better than single therapy.[50] A previous clinical study(Clinical Trials Number, NCT01844505) revealed that anti-PD-1 blockade plus anti- CTLA-4 is more effective in melanoma than any one alone[87], a years later, that conclusion was confirmed in NSCLC[59] (Clinical Trials Number, NCT02000947). But there is no clinical trial to certify that in MIBC, maybe combining those two different immunologic checkpoint inhibitor will improve median OS of BC patients.

The future of immunotherapy in Metastatic Bladder Cancer patients: The immunotherapy of inhibiting the PD-1/PD-L1 for the BC patients was efficient and improved their survival rates, however, this was not successful for the other patient. What is the reason and what can the scientist do to find the heterogeneity in those two groups of patients? There are several researches did to find the mechanism about cancers, those studies provide us with new ideas to find the personal treatment for BC. For example, before the treatment, a check about expression of PD-L1 of the patients inefficient with chemotherapy, unable to accept surgery or no response to radiotherapy should be done. More clinical research should be done, such as the comprehensive immune profiling of BC patients who are sensitive to checkpoint treatment of PD-1/PD-L1, the special mutation of BC patients. To summarize, there is still no uniform standard of immunotherapy for the patients, with the approval of FDA on the drugs of immunologic checkpoint inhibitor for BC. But while the mechanism is more clear, more and more patients will benefit from the improvement of the immune escape mechanism.



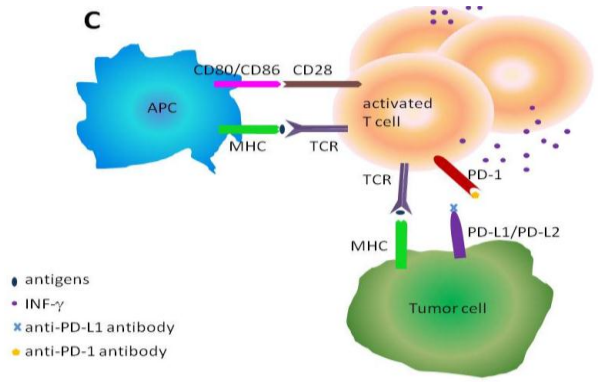


Figure. 1.

Legend:The progress of T-cell activated and anticancer immune responseof PD-1/PD-L1inhibited.
(A)APCsexpress a specific antigen that is presented to T cells in a peptide MHC.T cells recognize this presented antigen with their TCR and, together with binding of CD28; withresults of decreasing T-cell proliferation and cytokine production.
(B)PD-1 engagement with PD-L1 leads to inhibitcytokine production, such asINF-γ, as well as inhibition of T-cell proliferation.
(C)That theanti-PD-L1 antibody or anti-PD-1 antibody combine with PD-L1 or PD-1 leads to T-cell proliferation and cytokine production, such as INF-γ.
PD-1 = programmed cell death 1; PD-L1 = programmed cell death receptor ligand 1;PD-L2= Programmed death-ligand 2; TCR=T-cell receptor;APC = Antigen presenting cell; INF-γ = Interferon-γ.

PD-1/PD-L1 blockade in Metastatic Bladder Cancer treatment: what can we do now for the patients？

Table. 1: Summary of checkpoint inhibitors in bladder cancers.

Target	Antibody	Number of Patients	Criteria of selecting patients	Dose	Phase	Times	Results	NCT Trail Number
Blockade PD-1	Nivolumab (BMS-936558)	86	age ≥18 years	3 mg/kg	I/II	every 2 weeks	Median OS: 41.7weeks (95% CI, 31.3 to 69.4) median PFS: 12.0 weeks (95% CI, 6.4 to 25.3) median PFS: 23.6 weeks (95% CI, 6.0 to 48.0) PD-L1expression>=1% median PFS : 12.0 weeks (95% CI, 6.0 to 27.9) PD-L1expression < 1%	NCT01928394
	Pembrolizumab (MK-3475)	115	age ≥18 years Tumor cells expressed more than 1% PD-L1	10 mg/kg	Ib	every 2 weeks	PFS: 8.6 weeks (95% CI, 8.6 to 17.1) median OS: 55.7weeks (95% CI, 21.4 to 85.7)	NCT01848834
		374	disease progressive while based on cisplatin chemotherapy	200mg	II	every 3 weeks	NA	NCT02335424
		542	had recurred advanced urothelial or the state of him was not controlled after platinum-based chemotherapy	200 mg	III	every 2 weeks	OS: 44 weeks (95% CI, 34.3 to 50.6) median OS: 32 weeks (95% CI, 26.1 to 35.6)	NCT02256436
Blockade PD-L1	Durvalumab (MEDI4736)	191	age ≥18 years with advanced or metastatic UC	10 mg/kg	I/II	every 2 weeks	median PFS: 6.4 weeks (95%CI, 6.0 to 8.1) OS: 78.0 weeks (95%CI, 34.7 to not estimable)	NCT01693562
	Avelumab (MSB0010718C)	329	age ≥18 years with advanced or metastatic UC	10 mg/kg	I	every 2 weeks	median PFS: 6.6 weeks (95% CI, 6.1 to 11.4) median OS: 27.9 weeks (95%CI, 20.6 to 40.7)	NCT01772004
	Atezolizumab （MPDL3280A）	205	UBC PD-L1 positive	15mg/kg	I	every 3 weeks	ORR:43% (a PD-L1 TIIC IHC score of 2/3) ORR:11% (a score of 0/1)	NCT01375842
		310	age ≥18 years Eastern Cooperative Oncology Group performance status: 0 to 1	1200mg	II	every 3 weeks	ORR:15% (95% CI, 11.0 to 19.0) ORR: 27% (95% CI, 19.0 to 37.0) the percentage of PD-L1-positive immune cells >= 15% ORR:18% (95% CI, 13.0 to 24.0) the percentage of PD-L1-positive immune cells < 15%	NCT02108652

PD-1, programmed cell death 1; median OS, median overall survival; CI, confidence interval, median PFS, median progression free survival; PFS, progression free survival; PD-L1, programmed cell death receptor ligand 1; ORR, overall response rate.

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