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Perspective

Programmed death ligand-1/programmed death-1 inhibition therapy and programmed death ligand-1 expression in urothelial bladder carcinoma

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Abstract

After two decades of unchanged paradigms, the treatment strategies for advanced urothelial bladder cancer have been revolutionized by emerging programmed death ligand-1 (PD-L1)/programmed death-1 (PD1) inhibition therapy. Increased evidence is demonstrating the efficacy of PD-L1/PD1 inhibition therapy in both second-line and first-line settings. However, the percentage of patients who benefit from anti-PD-L1/anti-PD1 therapy is still low. Many questions have been raised in the development of biomarker-driven approaches for disease classification and patient selection. In this perspective, we discuss PD-L1/PD1 expression in urothelial bladder carcinoma, review approved anti-PD-L1/anti-PD1 agents for bladder cancer treatment and current ongoing studies investigating combination treatment strategies, and explore PD-L1 expression status for the evaluation of bladder cancer immunotherapy.

Keywords: Immune checkpoint therapy; Urothelial carcinoma; Bladder cancer; Programmed death-ligand 1; Programmed death-1

Introduction

With an estimated 430,000 new diagnoses and 165,000 deaths globally per year, urothelial bladder cancer has been the ninth most common cancer in the world.[1] In general, urothelial cancers are divided into two general categories: low grade and high grade, which have distinctive clinical and pathological presentation and develop through different molecular pathways. In this perspective, we will focus our discussion only on the high grade urothelial cancers.[2] Clinically, 70% of bladder cancer patients are diagnosed with non-muscle invasive high-grade disease and usually treated with transurethral tumor resection. Adjuvant therapies, including instillation of chemotherapy and Bacille Calmette-Guerin (BCG) vaccine therapy into the bladder, often lead to reduction in recurrences and prevent progression.[3] For patients with muscle invasive disease, the major goal of treatment is to determine if the bladder lesion can be managed independently or additional systemic approaches are

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required to improve the control of advanced diseases. Currently, cisplatin-based combination chemotherapy is the first-line treatment for locally advanced or metastatic disease. Although some new chemotherapy regimens and combination approaches have been developed over the years, the response rate of those patients is still less than 50%−70%.[4] Patient 5-year survival rate is only 15% and median overall survival is around 12 months.[5]

High grade urothelial bladder cancers usually display a high burden of somatic mutations. High variability in driver mutations and heterogeneity within tumors bring challenges for the development of targeted therapies. In the past 30 years, the mainstay of treatment of combination chemotherapy remained unchanged. On the other hand, high somatic mutation frequencies carry a high antigenic expression, which in turn results in an optimal target for immunotherapy. In 2016, the first anti-programmed death ligand-1 (PD-L1) agent atezolizumab was approved for the second-line treatment of urothelial carcinoma,[6] leading to the seismic progress in bladder cancer treatment. Since then, another four immune checkpoint inhibitors have been approved by U.S. Food and Drug Administration (FDA) for the treatment of patients with locally advanced or metastatic urothelial carcinoma. In National Comprehensive Cancer Network 2019 Guideline, atezolizumab and pembrolizumab have been recommended as the first-line systemic therapy for patients with locally advanced or metastatic urothelial cell carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 expression. Despite encouraging outcomes obtained from a variety of clinical trials, it is noted that not all patients can benefit from immune checkpoint inhibition therapy; some patients who demonstrate encouraging initial responses can acquire resistance over time. Therefore, developing predictive factors and identifying target patient are critical for the improvement of immunotherapy strategies. Here, we summarize the approved PD-L1/programmed death-1 (PD-1) inhibition therapies, and review the current studies of the assessment of PD-L1 expression for prognosis prediction of urothelial bladder cancer.

PD-L1 expression in bladder cancer

PD-L1, also known as B7 homolog 1 (B7−H1), is a protein that in human is encoded by the cluster of differentiation (CD) 274 gene.[7] As a ligand of PD-1, PD-L1 binding with PD-1 on lymphocytes delivers a signal that inhibits T-cell receptor (TCR)-mediated activation of interleukin-2 (IL-2) production and T cell proliferation. PD-L1/PD-1 interaction is essential in the development of peripheral immune tolerance. This mechanism is exploited by cancer cells to develop the evasion of antitumor immunity.[8] Other than the regulation of immune response, a recent study also found tumor PD-L1 promoted cell-intrinsic growth through the inhibition of autophagy and mammalian target of rapamycin (mTOR) activation in the absence of PD-1.[9] Thus, it is generally accepted that PD-L1 tumor expression correlates with increased progression, and this could be an indicator of cancer prognosis and molecular subtyping.[10]

However, in the study of PD-L1 status in bladder cancer, controversial results have been reported. A retrospective study tested the messenger RNA (mRNA) expression of PD-1, PD-L1 and CD3 in tumors of patients with stage pT1 non-muscle-invasive bladder cancer (NMIBC). The results indicated that patients with high PD-L1 mRNA expression gained an improved survival, which might be related to a higher immune competence.[11] Previously, Bellmunt et al[12] examined PD-L1 expression in formalin-fixed paraffin-embedded urothelial tumor samples through immunohistochemistry (IHC). In this study, PD-L1 expression in tumor cells and expression in tumor-infiltrating mononuclear cells were differentially analyzed. The authors concluded that PD-L1 expression in tumor cells was not predictive of patient survival. However, positive PD-L1 status in tumor-infiltrating mononuclear cells was significantly associated with longer survival in patients with metastases. In contrast, another early study based on IHC and flow cytometry demonstrated that tumor specimens from patients with higher grade showed significantly higher percentages of tumor-associated B7−H1. Tumor-associated B7−H1 expression was significantly associated with a high frequency of postoperative recurrence and low survival rate.[13] A recent study showed that PD-L1 was widely expressed on tumor immune cell infiltrates but not on tumor cells in high grade T1 bladder tumors. There was no correlation between PD-L1 positivity and outcomes.[14] The variation among these studies might be attributed to different PD-L1 antibodies, quantification methods, tumor characteristics, and cell types (tumor cells versus tumor infiltrating lymphocytes, etc.) analyzed. Thus, the role of PD-L1 expression as a prognostic marker for bladder cancer is still unclear.

Anti-PD-L1/anti-PD1 agents for bladder cancer treatment

Historically, bladder cancer has been treated successfully with immune therapy. Intravesical instillation of BCG was the first immunotherapy approved for non-muscle invasive bladder cancer by the FDA in 1990,[15] and has been an important part of the treatment to prevent disease progression and recurrence in patients with bladder cancer.[16,17] Today, PD-L1/PD1 immune checkpoint inhibition therapy provides a new treatment option for advanced or metastatic bladder cancer. Five anti-PD-L1/anti-PD1 agents have been approved for urothelial carcinoma treatment by U.S. FDA since 2016, including three PD-L1 inhibitors, atezolizumab, durvalumab, and avelumab, and two PD-1 inhibitors, pembrolizumab and nivolumab (Table 1).

The first immune checkpoint agent, atezolizumab, was approved by U.S. FDA in May 2016 for the treatment of patients with locally advanced or metastatic urothelial carcinoma whose disease has worsened during or following platinum-containing chemotherapy. Atezolizumab is an engineered humanized PD-L1 immunoglobulin G (IgG)1 antibody to block PD-1 binding. The phase I trial demonstrated atezolizumab had durable activity in metastatic urothelial carcinoma patients, especially in patients with high expression of PD-L1 in tumor-infiltrating immune cells.[18,19] The subsequent multi-center single-arm phase II study reported the clinical efficacy of atezolizumab in the treatment of metastatic urothelial carcinoma patients after unsuccessful platinum-based chemotherapy.[20] In this study, 315 patients with inoperable locally advanced or metastatic urothelial carcinoma with progressed disease after platinum-based chemotherapy were enrolled to accept 1200 mg intravenous atezolizumab administration. The objective response rate was 15% in all patients. The median overall survival was 7.9 months in all patients. Atezolizumab also demonstrated survival benefit as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma in a recent phase II trial.[21] In April 2017, accelerated approval to atezolizumab for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin chemotherapy has been granted by U.S. FDA. However, in a recent Phase III study aimed to assess the safety and efficacy of atezolizumab versus chemotherapy in patients with locally advanced or metastatic urothelial carcinoma after progression with platinum-based chemotherapy, atezolizumab did not show significantly longer overall survival benefit than chemotherapy in these patients with PD-L1 overexpressing tumors.[22] More studies are currently underway to evaluate the long-term survival benefit of atezolizumab combination treatment.

Pembrolizumab is a humanized monoclonal IgG4 PD-1 antibody, which was approved for patients with locally advanced or metastatic urothelial cell carcinoma who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1 or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 expression by U.S. FDA. In a multi-center single-arm phase II study, 370 cisplatin-ineligible patients with advanced urothelial cancer who had not been previously treated with systemic chemotherapy were recruited to receive at least one dose of pembrolizumab treatment. 24% of patients had a centrally assessed objective response.[23] In another open-label randomized phase III trial, 542 patients with advanced urothelial cancer that recurred or progressed after platinum-based chemotherapy were assigned to receive pembrolizumab treatment or the investigator's choice of chemotherapy with paclitaxel,

Table 1
Five anti-PD-L1/anti-PD-1 agents approved for urothelial carcinoma by U.S. FDA.

Drugs	Category	U.S. FDA first approval date	U.S. FDA approval for UC	Clinical trials
Atezolizumab	PD-L1 IgG1 antibody	May 18, 2016	May 18, 2016 April 18, 2017	GO 27831 (Phase I) IMvigor210 (Phase II) IMvigor211 (Phase III)
Pembrolizumab	PD1 IgG4 antibody	September 4, 2014	May 18, 2017	Keynote 052 (Phase II) Keynote 012 (Phase I) Keynote 045 (Phase III)
Nivolumab	PD1 IgG4 antibody	December 22, 2014	February 2, 2017	Checkmate 032 (Phase I) Checkmate 275 (Phase II)
Avelumab	PD-L1 IgG1 antibody	March 23, 2017	May 9, 2017	JAVELIN (Phase I)
Durvalumab	PD-L1 IgG1 antibody	May 1, 2017	May 1, 2017	MEDI4736 (Phase I/II)

PD-L1: programmed death ligand-1; PD-1: programmed death-1; FDA: Food and Drug Administration; UC: urothelial carcinoma; IgG: immunoglobulin G.

docetaxel, or vinflunine. The median overall survival in the total population was 10.3 months in the pembrolizumab group, as compared with 7.4 months in the chemotherapy group ($P = 0.002$).[24]

Nivolumab is an engineered humanized PD-1 IgG4 antibody approved by U.S. FDA in February 2017 for second-line therapy in previously platinum treated metastatic urothelial carcinoma.[25,26] The multi-center, single-arm phase II study of nivolumab enrolled 270 patients with metastatic or surgically unresectable locally advanced urothelial carcinoma. These patients accepted the treatment of nivolumab 3 mg/kg intravenously every 2 weeks. Confirmed objective response was 19.6% in all patients.[25] Durvalumab is a selective, human IgG1 monoclonal antibody that blocks PD-L1 binding to PD-1 and CD80, and received accelerated approval by U.S. FDA for the second-line treatment of metastatic urothelial carcinoma in May 2017. A phase 1/2 dose-escalation and dose-expansion study evaluating the safety and antitumor activity of durvalumab reported that the initial overall response rate for all patients was 31.0%.[27,28] Avelumab, another anti-PD-L1 IgG1 antibody, also received the accelerated approval by U.S. FDA in May 2017. The phase Ib multicenter study evaluated the safety and efficacy of avelumab treatment in patients with urothelial carcinoma progressing after platinum-based chemotherapy and unselected for PD-L1 expression.[29] The confirmed objective response rate was 18.2%. Further pooled analysis which included 161 post-platinum patients with advanced metastatic urothelial carcinoma demonstrated a 17% best overall or partial response rate.[30] Anti-PD-L1/anti-PD-1 combination treatment strategies have been increasingly investigated.[31–34] By mechanism, current ongoing studies in this field can be generally divided into two categories: immune checkpoint inhibitor combined with traditional chemotherapy and radiotherapy, and using cytotoxic T-lymphocyte-associated protein (CTLA) antibodies and other immunotherapies to increase the sensitivity of immune checkpoint inhibitor. Selected ongoing clinical trials are summarized in Table 2 and Table 3.

PD-L1 expression status for the evaluation of bladder cancer immunotherapy

Notably, the percentage of patients who benefited from anti-PD-L1/anti-PD1 therapy is generally less than 20%–30%. Demonstrating the mechanism of treatment resistance and elucidating the determinants of treatment response are fundamentally important for the development of effective treatment strategies. Among all biomarkers for the selection of immune checkpoint treatment, PD-L1 expression status has been mostly explored. In lung cancer and melanoma, PD-L1 status evaluation has been established as an important step for anti-PD-L1/anti-PD-1 therapy.[35] However, in urothelial carcinoma, the clinical significance of PD-L1 expression still remains controversial. PD-L1 status in tumor was found no association with response rate in previous atezolizumab, pembrolizumab, and nivolumab trials.[21,25,26]

In these studies, PD-L1 status is mostly evaluated by IHC staining. Distinct assay and scoring method could significantly influence the evaluation results. Currently, FDA has approved PD-L1 IHC 22C3 pharmDx assay, PD-L1 IHC 28-8 pharmDx, and ventana PD-L1 (SP142) assay for the evaluation of non-small-cell lung cancer.[36] Because of different techniques, targeted epitopes, and positive levels, the same tissue specimen can generate distinct results.[37] Another major question is cell subtype selection for the analysis. More evidence is needed to reveal the association of PD-L1 expression among different cell subtypes in immune cells, cancer cells and stroma. Thus, analysis of PD-L1 using multiplex system will be tremendously valuable. In addition, urothelial bladder cancer demonstrates highly heterogeneity within tumor, which also increases the possibility of false negative through analysis of one tissue biopsy sample. This heterogeneity of PD-L1 expression was quantitatively accessed in non-small-cell lung cancer tissue samples.[38] In this study, assessment of 588 serial section fields of view from whole tissue showed discordant expression at a frequency of 25%, raising the challenges of using PD-L1 expression alone to predict immune checkpoint treatment response.[39] Other than PD-L1 status, tumor mutational load and analyses of the Cancer Genome Atlas subtypes were found to be associated with atezolizumab treatment response.[20] In this study, tumor mutation load was examined before the treatment in 150 patients by sequencing 315 cancer-related genes. Significant increases of median mutation load in atezolizumab responders were observed compared to non-responders ($P < 0.0001$). Recent new data further demonstrated the prediction value of tumor phenotype with regard to $CD8^+$ T effector cells, tumor mutation burden, and transforming growth factor-β (TGF-β) signaling in fibroblasts for treatment outcome of atezolizumab.[40] Tumor mutation burden and $CD8^+$ T-effector cell phenotype were positively associated with atezolizumab response. Interestingly, TGF-β signaling

Table 2
Selected ongoing studies investigating combination treatment strategy: immune checkpoint inhibitor plus chemotherapy/radiotherapy.

Study ID	Study title	Population	Treatment	Primary outcome	Phase
NCT02621151	Pembrolizumab (MK3475), Gemcitabine, and Concurrent Hypofractionated Radiation Therapy for Muscle-Invasive Urothelial Cancer of the Bladder	Patients with muscle-invasive urothelial cancer who are not candidates for or decline radical cystectomy	Pembrolizumab + Concurrent radiation and gemcitabine	Two-year bladder-intact disease-free survival rate	Phase 2
NCT03617913	Avelumab in Combination With Fluorouracil and Mitomycin or Cisplatin and Radiation Therapy in Treating Participants With Muscle-Invasive Bladder Cancer	Patients with muscle-invasive bladder cancer	Avelumab + Fluorouracil and mitomycin or cisplatin and radiation therapy	Complete response rate	Phase 2
NCT03775265	Chemoradiotherapy With or Without Atezolizumab in Treating Patients With Localized Muscle Invasive Bladder Cancer	Patients with localized muscle invasive bladder cancer	Atezolizumab + Cisplatin/fluorouracil/ gemcitabine, mitomycin and radiation therapy	Bladder-intact event-free survival (BI-EFS)	Phase 3
NCT03472274	DUrvalumab (MEDI4736) and TREmelimumab in NEOadjuvant Bladder Cancer Patients (DUTRENEO)	Patients with muscle-invasive bladder cancer	Durvalumab + Tremelimumab + Cisplatin-based neoadjuvant chemotherapy	Evidence of residual disease based on pathological review of the surgical specimen	Phase 2
NCT03529890	Radio-Immunotherapy Before Cystectomy in Locally Advanced Urothelial Carcinoma of the Bladder (RACE IT)	Patients with locally advanced bladder cancer	Nivolumab + Radiation therapy of the pelvis	Rate of patients with completed treatment	Phase 2
NCT03601455	Radiation Therapy and Durvalumab With or Without Tremelimumab in Treating Participants With Unresectable, Locally Advanced, or Metastatic Bladder Cancer	Patients with unresectable, locally advanced, or metastatic bladder cancer	Radiation therapy and durvalumab + Tremelimumab	Progression-free survival (PFS) and incidence of adverse events	Phase 2
NCT02662062	Pembrolizumab With Chemoradiotherapy as Treatment for Muscle Invasive Bladder Cancer (PCR-MIB)	Patients with maximally resected via transurethral resection (TURBT) non-metastatic muscle invasive bladder cancer	Pembrolizumab + Cisplatin + Radiotherapy	Number of patients with grade 3 or 4 acute toxicities	Phase 2
NCT03288545	A Study of Enfortumab Vedotin Plus Pembrolizumab and/or Chemotherapy for Patients With Urothelial Bladder Cancer (EV-103)	Patients with locally advanced or metastatic urothelial cancer	Enfortumab vedotin + Pembrolizumab + Cisplatin/carboplatin/gemcitabine	Type, incidence, severity, seriousness, and relatedness of adverse events; type, incidence, and severity of laboratory abnormalities	Phase 1
NCT03747419	Avelumab and Radiation in Muscle-Invasive Bladder Cancer	Patients with muscle-invasive bladder cancer	Avelumab + Radiation therapy	Complete response rate	Phase 2

Table 3
Selected ongoing studies investigating combination treatment strategy: increasing sensitivity of immunotherapy.

Study ID	Study title	Population	Treatment	Primary outcome	Phase
NCT03980041	Study to Evaluate the Efficacy/Safety of IPI-549 in Combination With Nivolumab in Patients With Advanced Urothelial Carcinoma (MARIO-275)	Advanced urothelial cancer patients who have progressed or recurred following treatment with platinum-based chemotherapy	Nivolumab + IPI 549	Objective response rate (ORR)	Phase 2
NCT03773666	A Feasibility Study of Durvalumab ± Oleclumab as Neoadjuvant Therapy for Muscle-invasive Bladder Cancer (BLASST-2)	Patients with muscle-invasive bladder cancer before surgery	Durvalumab + Oleclumab	Number of participants without dose-limiting toxicity (DLT)	Phase 1
NCT02845323	Neoadjuvant Nivolumab With and Without Urelumab in Patients With Cisplatin-Ineligible Muscle-Invasive Urothelial Carcinoma of the Bladder	Patients with cisplatin-ineligible muscle-invasive urothelial carcinoma of the bladder	Nivolumab + Urelumab	Immune response to treatment with nivolumab and urelumab compared to nivolumab monotherapy measured by tumor infiltrating cluster of differentiation (CD) 8^+ T cell density at cystectomy	Phase 2
NCT03258593	Durvalumab and Vicinium in Subjects With High-Grade Non-Muscle-Invasive Bladder Cancer Previously Treated With Bacillus Calmette-Guerin (BCG)	Patients who have bladder cancer that has not spread to the muscle in the bladder and was treated unsuccessfully with Bacillus Calmette-Guerin	Durvalumab + Vicinium	Safety and tolerability	Phase 1
NCT03138889	A Study of a CD122-Biased Cytokine (NKTR-214) in Combination With Anti-PD-1 (Pembrolizumab) and of NKTR-214 in Combination With Anti-PD-L1 (Atezolizumab) in Patients With Select Advanced or Metastatic Solid Tumors (PROPEL)	Patients with stage III or stage IV melanoma, locally advanced or metastatic urothelial carcinoma, or stage IV non-small-cell lung cancer (NSCLC)	NKTR-214 + Pembrolizumab/ atezolizumab	Incidence of treatment-emergent adverse events; recommended phase 2 dose (RP2D) of NKTR-214 in combination with pembrolizumab or atezolizumab	Phase 1
NCT03123055	A Study of B-701 in Combination With Pembrolizumab in Treatment of Locally Advanced or Metastatic Urothelial Cell Carcinoma (FIERCE-22)	Patients with locally advanced or metastatic urothelial cell carcinoma	B-701 + Pembrolizumab	Initial safety and determination of RP2D according to dose-limiting toxicity; safety and tolerability of B-701 (vofatamab) plus pembrolizumab; efficacy of B-701 (vofatamab) plus pembrolizumab measured by ORR	Phase 1/2

in fibroblasts, which was associated with lack of response, may restrict T cell penetration into the center of the tumor. Mouse model study indicated that co-inhibition of TGF-β and PD-L1 converted tumor from an excluded to an inflamed phenotype for immune therapy.[40]

Conclusion

To date, PD-L1/PD1 inhibition therapy proved its efficacy in patients with advanced urothelial bladder cancer. New evidences in treatment agents and combination strategies are constantly emerging. However, there is an unmet need in the development of biomarker-driven approach for disease classification and patient selection. PD-L1 expression standalone as an evaluation criterion has many limitations, and more refined technologies such as multiplex analytic platform or surrogate markers (TMB) are needed. With more planned studies that tailor the treatment in a patient-specific condition, future precision immune therapy can be expected and yield the improved benefits for bladder cancer patients.

Conflict of interest

None.

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