Urothelial carcinoma (UC), originating in the bladder or upper urinary tract, is the most common histological type of cancer. Currently, platinum-based cytotoxic chemotherapy is the standard treatment for metastatic UC (mUC) and the preferred treatment option in the perioperative (neoadjuvant and/or adjuvant) setting of muscle invasive bladder cancer (MIBC). In addition, intravesical bacillus Calmette-Guerin immunotherapy or chemotherapy is applied as the adjuvant therapeutic option in non-muscle invasive bladder cancer (NMIBC) after transurethral resection, to prevent recurrence and progression. In recent years, with an increased understanding of cancer immunobiology, systemic immunotherapies targeting immune checkpoint inhibition has been explored and clinically used in the area of UC. The programmed cell death 1 receptor (PD-1) and its ligand (PD-L1) are important negative regulators of immune activity, preventing the destruction of normal tissues and autoimmunity. To date, five immune checkpoint inhibitors blocking PD-1 (pembrolizumab, nivolumab) or PD-L1 (atezolizumab, durvalumab, and avelumab) have been approved by the United States Food and Drug Administration (US-FDA) for first- or second-line use in mUC, based on durable therapeutic response and manageable safety profiles observed in relevant clinical trials. In addition, the clinical use of several immune checkpoint inhibitors is currently being tested for MIBC and NMIBC. In this article, we review the current and ongoing clinical trials, regarding immune checkpoint inhibitors, being conducted in various clinical settings of UC, including mUC, MIBC, and NMIBC.

Keywords: Immunotherapy; PD-1 inhibitor; PD-L1 inhibitor; Urinary bladder neoplasms

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50% of patients with metastatic UC (mUC) do not qualify for cisplatin-based chemotherapy, owing to poor performance status and impaired renal function. Thus, these patients were primarily treated with carboplatin-based regimens, presenting an ORR of 30%–40% for 9–10 months [5,6]. Patients with recurrence after first-line treatment, or who show progress while receiving first-line treatment, have a particularly poor prognosis. Unfortunately, second-line chemotherapies, including paclitaxel, pemetrexed, docetaxel, and vinflunine, have shown only modest efficacy with an ORR of 12% and a median OS of 5–7 months [5,7,8].

To date, several immunotherapeutic agents that block immune checkpoints, such as programmed cell death 1 receptor (PD1) (nivolumab/pembrolizumab), PD-1 ligand-1 (PD-L1) (durvalumab/avelumab), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (ipilimumab/tremelimumab), have been investigated and/or clinically used in various types of cancers, including UC. Among these, five immune checkpoint inhibitors (atezolizumab, pembrolizumab, nivolumab, durvalumab, and avelumab) have already received approval from the US Food and Drug Administration (US-FDA). Also, atezolizumab and pembrolizumab were approved by Korea-FDA for the treatment of mUC after cisplatin failure; these two drugs were also approved as the first-line treatment in patients with cisplatin-ineligible mUC. In this article, we aimed to review the current and ongoing clinical trials being performed in various clinical settings of UC, including mUC, muscle invasive bladder cancer (MIBC), and non-muscle invasive bladder cancer (NMIBC).

Rationale for ImmuneCheckpoint Inhibitors in Bladder Cancer

T cell–mediated immunity consists of several sequential phases: clonal selection of antigen-presenting cells and the activation, proliferation, transition, and implementation of direct effector function. These phases are controlled by equilibrium between inhibitory and stimulatory signals [9]. In a non-tumor environment, immune checkpoint proteins control the immune system and prevent autoimmunity. Immune checkpoint proteins follow inhibitory pathways that physiologically counterbalance the co-stimulatory pathways to appropriately adjust the immune responses [10].

Generally, cancer cells evade antitumor immunity by adopting active immune escape strategies as follows: (1) diminishing MHC-I expression, and hence CD8+ T cell activity; (2) defective antigen processing and presentation, thereby causing reduced recognition by T cells; and (3) increasing the expression of co-inhibitory (i.e., immune checkpoint) molecules [11]. Since most cancers select the immune checkpoints to evade the immune system attack by blocking the effector T-cell functions, antitumor immunity may be recovered by antibodies that inhibit the receptor-ligand interaction and deactivate the immune checkpoints [12]. Currently, the most investigated and clinically related immune checkpoint molecules are PD-1, PD-L1, and CTLA-4.

The wide mutational spectrum of UC might be advantageous in establishing efficient immunotherapies for this disease, since mutations might induce more neo-antigens that are recognized as ‘non-self’ by the circulating T cells, thereby inducing an immune response [13]. A recent study demonstrated that high mutational burden is observed in UC, melanoma, and non-small cell lung cancer [14]. In addition, the clinical efficacy of PD-1-L1 blockade was identified to be proportional to the high tumor mutational burden, especially in melanoma and non-small cell lung cancer [14,15]. Consequently, based on this cancer immunologic data, several monoclonal antibodies, that block the ligand-receptor interaction for immune checkpoints and/or its functional consequences, have been developed and clinically applied for the management of UC, including bladder and upper urinary tract cancers [16-19].

ImmuneCheckpoint Inhibitors After Platinum-Based Chemotherapy in Metabolic UC

To date, the clinical trials regarding immune checkpoint inhibitors in UC have mainly focused on platinum-refractory mUC. An open-label, multi-center, single-arm, phase II IMvigor210 trial, cohort 2 (NCT02108652) assessed the clinical efficacy and safety of atezolizumab in patients with locally advanced or mUC whose disease had progressed during or following previous treatment with platinum-based chemotherapy or who had disease progression within 12 months of treatment with a platinum-based neoadjuvant or adjuvant chemotherapy [20,21]. In that trial, a total of 310 patients received atezolizumab, fixed dose of intravenous 1,200 mg every three weeks. The observed ORR for all patients by independent review was 14.5%. The immune cell (IC) 2/3 subgroup (assessed by immunohistochemistry, correlating to ≥5.0% of ICs) had a clinically meaningful ORR of 26.0% versus 9.0% in the IC 0/1. The median OS was 7.9 months for the entire cohort and 11.4 months for patients with IC2/3 PD-L1 expression with a median follow-up duration of 14.4 months. Grade 3 to 4 treatment-related adverse events (TRAEs) were reported in 16.0% of the patients. Although this study was limited by phase II trial,
atezolizumab received US-FDA approval for platinum-refractory mUC in May 2016 [20-22]. In a recent multi-center, randomized phase III IMvigor211 clinical trial (NCT02302807), the clinical efficacy and safety of atezolizumab (1,200 mg intravenously, every three weeks) compared to chemotherapy (investigator’s choice of vinflunine, paclitaxel, or docetaxel) were reported in 901 patients with mUC, whose disease had progressed despite platinum-based chemotherapy [23]. As a primary endpoint of the trial with atezolizumab, no significant improvement of OS was observed in the subset of 234 patients with ≥5% expression of PD-L1 on tumor-infiltrating ICs (median, 11.1 months vs. 10.6 months; hazard ratio [HR], 0.87; 95% confidence interval [CI], 0.63–1.21); ORR was similar (23% vs. 22%). Although a higher response rate was found with atezolizumab in patients with increased PD-L1 expression compared to that in patients with lower levels of PD-L1 expression, this patient subset also had a higher response rate to chemotherapy. Safety analysis of the intention-to-treat population found that the incidence of grade 3 or 4 TRAEs was lower with atezolizumab than with chemotherapy (20% vs. 43%), as was the incidence of treatment discontinuation (7% vs. 18%). However, the duration of response was longer in the atezolizumab group than in the chemotherapy group (159 months vs. 83 months). This long durability of atezolizumab was also identified in the IMvigor210 trial [24].

A randomized, phase III clinical trial related to pembrolizumab (200 mg every three weeks for 24 months) was performed in patients with mUC, in whom the disease had progressed during or after platinum-based chemotherapy (KEYNOTE-045 or NCT02256436) [25]. Patients were included irrespective of PD-L1 expression level. The pembrolizumab group showed significantly longer OS (103 months vs. 7.4 months; HR, 0.73; 95% CI, 0.59–0.91) and a higher ORR (21.1% vs. 11.4%) compared to that in the chemotherapy (paclitaxel, docetaxel, or vinflunine) group. The 12-month OS rates for the pembrolizumab and chemotherapy groups were 43.9% and 30.7%, and the 18-month OS rates were 36.1% and 20.5%, respectively. No statistically significant difference was found in progression-free survival (median, 21 months vs. 33 months; HR, 0.98%; 95% CI, 0.79–1.16). Serious TRAEs were less frequent with pembrolizumab than with chemotherapy (15.0% vs. 43.9%) [25].

A multi-center, phase II single-arm study assessing nivolumab (3 mg/kg every two weeks) in patients with platinum-refractory mUC has also been reported (NCT02887996 or CheckMate-275) [26]. The confirmed ORR was 19.6% in the entire study cohort, with a median OS of 8.7 months; this ORR was also observed (28.4%) in the PD-L1 high expression group (defined as ≥5%). Grade 3–4 TRAEs occurred in approximately 15.0% vs. 43.9% of patients [26]. Nivolumab (240 mg, intravenously, every two weeks) was approved by the FDA in February 2017. Subsequently, an alternative schedule of administering 480 mg nivolumab every four weeks was approved, based on clinical pharmacology analyses and safety assessments.

Durvalumab (10 mg/kg, intravenously, every two weeks) was injected to patients with advanced UC that had progressed during or after the previous platinum-based chemotherapy, either for metastatic disease or for progressive disease after less than 2 months of adjuvant or neoadjuvant chemotherapy (NCT01693562). In this phase I/II study, ORR in the entire cohort was 17.8%, including 9 (37%) with complete response (CR), and the median OS of 18.2 months. The ORR was 27.8% and 51% in the PD-L1 high and PD-L1 low or negative expression group, respectively, when applying a 25% cut-off value. Grade 3 to 4 TRAEs were observed in 68% of patients [27].

In a phase Ib trial, avelumab showed clinical efficacy in patients with platinum-refractory mUC (JAVELIN Solid Tumor trial, NCT01772004) [28]. Confirmed ORR was 17%, including 6% CR, 11% partial response (PR), and median OS 65 months. This ORR was observed (24%) in the high PD-L1 expression group (defined in this study as ≥5% staining of tumor cells only) [28]. Safety profiles showed a low rate of grade 3 to 4 TRAEs (8%) and no treatment-related deaths. The outcomes of the clinical trials regarding anti-PD-1 or L1 inhibitors are summarized in Table 1.

### IMMUNE CHECKPOINT INHIBITORS IN PLATINUM-INELIGIBLE METABOLIC UC

Up to 50% of patients with advanced UC do not qualify for cisplatin-based chemotherapy due to age or comorbidity (ie, impaired renal function, neuropathy, and heart failure). Although comparative phase III data are not available for a first-line setting, a phase II study supports the use of immune checkpoint inhibitors.

In a multicenter, single-arm phase II study, atezolizumab (total dose 1,200 mg, every three weeks) was used as first-line therapy in 119 patients with advanced or mUC, who were not eligible for treatment with a cisplatin-based regimen [29]. At a median follow-up of 17 months, ORR was observed in 27 patients (23%), including 11 (9%) with CR. Median duration of response was not reached, and 19 out of 27 patients continued to respond at the time of analysis. Median OS for the entire cohort was 16 months; TRAEs mirrored those used after cisplatin-based chemotherapy or
In another single-arm phase II trial, the first-line use of pembrolizumab was investigated in patients with mUC who were not eligible for cisplatin-based chemotherapy (NCT02335424 and KEYNOTE-052) [30]. The ORR was 24% in all treated patients, including 5% CR and 19% PR. In particular, the high PD-L1 expression group (positive score of 10% or more) showed a high ORR (38%). Grade 3 to 4 TRAEs were observed in 15.0% of all treated patients. These results for the first-line use of an immune checkpoint in mUC are comparable to those of the existing carboplatin-based combination chemotherapy, including gemcitabine/carboplatin and methotrexate/carboplatin/vinblastine regimens [6], in terms of efficacy and safety. On the basis of the outcomes of these phase II trials, atezolizumab and pembrolizumab have gained accelerated US-FDA approval for first-line use in cases with cisplatin-ineligible mUC. The outcomes of these trials are also summarized in Table 1.

**IMMUNE CHECKPOINT INHIBITOR COMBINATIONS**

Immune checkpoint inhibitors showed clinical efficacy and manageable adverse effects in advanced UC. To enhance the treatment response of immune checkpoint inhibitors and provide more effective pathway blockade in UC, several trials have been reported and various combined approaches are ongoing.

Several PD-1/PD-L1 inhibitors in conjunction with chemotherapeutic agents are being investigated for treating mUC. Theoretically, cytotoxic chemotherapy can cause tumor cell lysis, potentially enhancing immunogenicity by promoting tumor antigen production [9]. Trials have combined pembrolizumab with gemcitabine or docetaxel (NCT02437370) [31] and ipilimumab, a CTLA-4 checkpoint inhibitor, with gemcitabine/cisplatin chemotherapy as a first-line treatment in patients with mUC (NCT01524991) [32]. Three phase III studies are currently ongoing to assess the first-line use of combined chemo-immunotherapy. The IMvigor130 trial compares atezolizumab plus gemcitabine/carboplatin vs. gemcitabine/carboplatin alone in patients with mUC who are ineligible for cisplatin-based chemotherapy (NCT02807636) [33]. The KEYNOTE-361 is a three-arm phase III study comparing pembrolizumab alone, pembrolizumab plus gemcitabine/cisplatin or carboplatin, and chemotherapy alone in patients with mUC who had not been treated with prior systemic chemotherapy (NCT02853305) [34]. The CheckMate-901 is also a multi-arm phase III trial that compares nivolumab and ipilimumab
combination, nivolumab plus gemcitabine/cisplatin or carboplatin, and chemotherapy alone in previously untreated patients with mUC (NCT03036098) [35]. Patients are currently being enrolled in these trials and the forthcoming results of these trials are awaited.

Effective targeted therapies, specific to a portion of tumors with certain driver mutations, might induce release of neo-antigens by triggering target-directed tumor cell killing that generates a durable antitumor response and enhances sensitivity to checkpoint inhibition [13]. In particular, the drugs of interest are inhibitors that target the fibroblast growth factor receptor (FGFR) and vascular endothelial growth factor (VEGF) pathways, which are usually highly expressed in UC [36,37].

Phase I trials that combine durvalumab with the FGFR inhibitor AZD4547 (NCT02546661) [38] and pembrolizumab with the anti-VEGFR2 monoclonal antibody ramucirumab (NCT02443324) [39] have been performed as a second-line and beyond therapy in platinum-refractory mUC. Table 2 summarizes the ongoing clinical trials combining immune checkpoint inhibition with other therapeutic modalities.

Another promising approach to enhance the effectiveness of treatment is the use of a dual checkpoint blockade, which involves the combination of PD1/PD-L1 and CTLA-4 inhibitors. CheckMate-032, an open-label, multicenter, phase 1/2 studies that combine nivolumab and ipilimumab have provided the first indication of the clinical utility of this combination in the treatment of mUC (NCT01928394) [40]. Patients were received with either of two combination schedules, nivolumab 1 mg/kg+ipilimumab 3 mg/kg (N1I3) or nivolumab 3 mg/kg+ipilimumab 1 mg/kg (N3I1) every 3 weeks for four cycles, followed by nivolumab 3 mg/kg every 2 weeks, or nivolumab monotherapy 3 mg/kg (N3) every 2 weeks. ORR was 38.5%, 25.7%, and 25.6% in N1I3, N3I1 and N3 groups, respectively after a minimum follow-up of 39 months in N1I3 group (n=26), 130 months in the N3I1 group (n=104) and 145 months in N3 group (n=78). The frequency of drug-related grade 3–4 adverse events were similar in both combination groups (30.8% in N1I3 vs. 31.7% in N3I1), and higher than in the N3 group (23.1%). TRAEs caused discontinuation in 7.7% (N1I3), 12.5% (N3I1), and 3.9% (N3) of patients. One death was reported in the N3I1 group (pneumonitis) and two were reported in the N3 group (pneumonitis and thrombocytopenia) [40]. A randomized, open-label, multi-center phase III study (DANUBE NCT02516241) is currently underway to investigate the efficacy and safety of durvalumab, with or without tremelimumab, compared to that of standard-of-care chemotherapy, as first-line treatment for patients with unresectable and/or mUC [41].

Lymphocyte activation gene 3 (LAG3) is an immune checkpoint molecule that is extremely expressed on the surface of activated ICs, including T cells, B cells, and NK cells. T cell immunoglobulin and mucin-3 (TIM-3) are other checkpoint proteins expressed on several ICs, such as T cells, NK cells, and monocytes. OX40, is a protein binding affinity to the tumor necrosis factor receptor family, transduces a co-stimulatory signal during T-cell activation. Patients with mUC are being actively enrolled for several phase I trials that study TIM-3 antagonists (NCT02608268) [42], LAG3 inhibitors (NCT01968109) [43], and OX40 agonists (NCT02528357) [44], with and without PD-1/PD-L1 inhibitors. Currently, an anti-LAG3 mAb (BMS-986016) is being studied in conjunction with nivolumab in a phase 1 trial (NCT01968109) [43]. Similarly, an OX40 agonist (GSK3174998) is being investigated in conjunction with pembrolizumab in a phase I trial (NCT02528357) [44]. Another interesting approach for promoting the immune response further targets the T-cell microenvironment. Indoleamine 2,3-dioxygenase (IDO), which is an intracellular enzyme made by tumor cells, and plays an important role in the pathways that generate immune-suppressive metabolites [45]. Likewise, considering that high intracellular adenosine concentrations have a inhibitory effect on cytotoxic T-cell function, the adenosine A2A receptor (A2AR) is a potential target for cancer treatment [46,47]. Currently, patients with mUC are being enrolled in several clinical trials (NCT02178722 [48], NCT02318277 [49], and NCT02655822 [50]) with regard to IDO or A2AR inhibitors, combined with PD-1 or L1 checkpoint inhibitors. The ongoing trials using dual checkpoint blockade are listed in Table 2.

### IMMUNE CHECKPOINT INHIBITORS IN NON-METASTATIC MIBC OR NMIBC

Although surgical therapies, including radical cystectomy and transurethral resection of bladder tumor (TURBT), are primarily applied as the primary standard therapeutic modalities for MIBC and NMIBC, adjuvant or neoadjuvant therapy using various anti-tumor drugs is frequently used to improve oncologic outcome. According to current guidelines, neoadjuvant chemotherapy using cisplatin-based combination regimen is recommended in patients with cT2-4aN0M0 MIBC, and the use of adjuvant cisplatin-based combination chemotherapy is selectively recommended for patients with locally advanced (pT3/4) and/or lymph node-positive disease [4,5]. In the case of NMIBC, the use of intravesical treatment with bacillus Calmette-Guerin or chemotherapeutic agents after TURBT has
| Clinical trial number | Drug(s) investigated | Clinical setting | Study design | Phase | Primary endpoint |
|-----------------------|----------------------|-----------------|-------------|-------|------------------|
| NCT02437370 [31]      | Pembrolizumab+docetaxel or gemcitabine | Second- or third-line | Non-randomized, double arm  
Arm A: pembrolizumab+docetaxel  
Arm B: pembrolizumab+gemcitabine | I | MTD |
| NCT01524991 [32]      | Ipilimumab+gemcitabine+cisplatin | Fist-line | Single-arm UC cohort | II | One-year OS |
| NCT02807636 (IMvigor130) [33] | Atezolizumab±gemcitabine/carboplatin or cisplatin | First-line | Randomized, double-blind, placebo-controlled, three-arm  
Arm 1: atezolizumab+gemcitabine/carboplatin or cisplatin  
Arm 2: placebo+gemcitabine/carboplatin or cisplatin  
Arm 3: atezolizumab alone | III | PFS, OS, AEs |
| NCT02853305 (KEYNOTE-361) [34] | Pembrolizumab±gemcitabine/carboplatin or cisplatin | First-line | Randomized, controlled, three arm  
Arm 1: pembrolizumab  
Arm 2: pembrolizumab+gemcitabine/carboplatin or cisplatin  
Arm 3: chemotherapy (gemcitabine/carboplatin or cisplatin) | III | PFS, OS |
| NCT03036098 (CheckMate-901) [35] | Nivolumab+ipilimumab or gemcitabine/cisplatin or carboplatin | First-line | Randomized  
Experimental group: nivolumab+ipilimumab or nivolumab+gemcitabine/cisplatin or carboplatin  
Comparator group: chemotherapy (gemcitabine/cisplatin or carboplatin alone) | III | PFS, OS |
| NCT02546661 [38]      | Durvalumab±AZD4547 (FGFR tyrosine kinase inhibitor) | Second- or third-line | Randomized to durvalumab alone or the combination | I | AEs |
| NCT02443324 [39]      | Pembrolizumab+ramucirumab (anti-VEGFR2 antibody) | Second-line and beyond | Non-randomized, single-arm for multiple tumors including UC | I | DLTs |
| NCT01928394 (CheckMate-032) [40] | Nivolumab±ipilimumab | Any line | Randomized, single arm for multiple tumors including UC | I/II | ORR |
| NCT02516241 (DANUBE) [41] | Durvalumab±tremelimumab | First-line | Three-arm randomization to durvalumab alone, durvalumab+tremelimumab, or chemotherapy (gemcitabine/carboplatin) | III | OS |
| NCT02608268 [42]      | MBG453 (TIM-3 antagonist)+PDR001 (anti-PD-1 antibody) | Second-line and beyond | Non-randomized to MBG453 alone or MBG453 in combination PDR001 for multiple tumors including UC | I/II | AEs, ORR, DLTs |
| NCT01968109 [43]      | Nivolumab±BMS-980616 (anti-LAG3 antibody) | Any line | Randomized, multiple tumor types including UC  
Arm 1: BMS-980616 alone  
Arm 2: BMS-980616+nivolumab | I/II | AEs, ORR, DCR, DOR |
| NCT02528357 [44]      | Pembrolizumab±GSK3174998 (OX40 agonist) | Any line | Non-randomized, multiple tumor types including UC  
Part 1: GSK3174998 alone  
Part 2: GSK3174998+pembrolizumab | I | AEs, DLTs |
| NCT02178722 [48]      | Pembrolizumab+epacadostat (indoleamine 2,3-dioxygenase inhibitor) | Second-line and beyond | Single-arm, multiple tumor types including UC | I/II | DLTs, ORR |
| NCT02318277 [49]      | Durvalumab+epacadostat | Second-line and beyond | Single-arm, multiple tumor types including UC | I/II | DLTs, AEs, ORR |
| NCT02655822 [50]      | Atezolizumab±CPI-444 (adenosine-A2A receptor antagonist) | Any line | Randomized, multiple tumor types including UC, dose selection study of CPI-444, 1 arm combining with atezolizumab | I | DLTs, ORR, AEs, MDL |

UC, urothelial carcinoma; PD-1, programmed cell death 1 receptor; MTD, maximum tolerated dose; OS, overall survival; PFS, progression-free survival; AE, adverse event; DLT, dose-limiting toxicity; ORR, objective response rate; DCR, disease control rate; DOR, duration of response; MDL, maximum dose level.
been recommended, based on risk group stratification for recurrence and progression [51]. Based on the accumulating evidence of immune checkpoint inhibitor in mUC, several immune checkpoint inhibitors are also being examined in MIBC and NMIBC settings. Three phase III trials are currently being implemented to identify the efficacy of atezolizumab (NCT02450331 or IMvigor010) [52], nivolumab (NCT02632409 or CheckMate-274) [53], and pembrolizumab (NCT03244384) [54] compared to that of placebo in the adjuvant setting of MIBC. Immune checkpoint inhibitors are currently being tested in a neoadjuvant setting as a phase I or II trial for nivolumab plus ipilimumab (NCT03887761) [55], nivolumab plus urelumab vs. nivolumab alone (NCT02845323) [56], pembrolizumab (NCT02736266) [57], pembrolizumab in conjunction with gencitabine, with or without cisplatin (NCT02365766) [58], and atezolizumab (NCT02362309) [59]. In the case of NMIBC, several PD-1 and PL-L1 inhibitors are currently being tested for the use of adjuvants (intravenous or intravesical administration) in a phase I or II study design for pembrolizumab (NCT03167151) [60], NCT02808143 [61], NCT02625961 [62]), atezolizumab (NCT02844816 [63], NCT02792192 [64]), and durvalumab (NCT02901548) [65]. Patients are currently being enrolled in trials for MIBC and NMIBC, and the results are thus not yet available. The details of these trials are summarized in Table 3.

### PREDICTIVE BIOMARKERS FOR IMMUNE CHECKPOINT INHIBITION IN UC

Although no commercialized biomarker is available, to predict the response to immune checkpoint inhibitors, several biomarker candidates including PD-L1 expression, tumor mutational (neo-antigen) burden, The Cancer Genome Atlas (TCGA) subtype, and interferon γ (IFN-γ) expression signature, are currently being explored in relation to UC; however, further validation is recommended.

#### 1. PD-L1 expression

The expression of PD-L1 on tumor and/or ICs has been rigorously examined as a potential biomarker, but the data remain unconcluded; they range from a strong association with overall responses, using a composite biomarker required for patient selection in KEYNOTE-052 (pembrolizumab), IMvigor210 cohort 2 (atezolizumab), CheckMate-275 (nivolumab) [26], and durvalumab study [27,66] to no association as identified in IMvigor210 cohort 1 (atezolizumab) [20] and KEYNOTE-045 (pembrolizumab) [25]. These disparities could be attributed to several factors, such as selection of PD-L1 expression either tumor cell or IC, diverse PD-L1 cut-off values, application of 4 different methods for PD-L1 IHC scoring, intra-tumoral heterogeneity of PD-L1 expression, mismatch of PD-L1 expression between primary tumor and metastases, and the possibility of continuous changes in PD-L1 expression due to the animated nature of the tumor microenvironment [67,68]. Most importantly, up to 10% of patients with PD-L1-negative tumors are known to respond to anti-PD-L1 therapy, suggesting that the negative predictive role of PD-L1 as a biomarker is inadequate to rule out patients from potentially life-prolonging therapy.

#### 2. Tumor mutational (neo-antigen) burden

The high mutational burden shows an obvious predictive implication for the response to the checkpoint inhibitor, since the adaptive cancer immunity is dependent on the recognition of neo-antigens encoded in tumor mutations [13,14]. In a recently published trial on atezolizumab in UC, the median mutational load of responders was higher than that of non-responders (12.4 mut/Mb vs. 6.4 mut/Mb) [20]. However, whether mutational density alone can predict patient response still needs to be clarified. A wide variation is found regarding the upper range in non-responders of over 50 mut/Mb, with many responders having fewer than 10 mut/Mb even tumors with relatively fewer neo-antigens, such as in renal cancer, respond to immunotherapy [14,69].

#### 3. TCGA subtype

The genomic analyses of UC have found several molecular clusters, including the luminal and basal subtypes or TCGA clusters I to IV [70,71]. Integrated analytical results suggested that the TCGA UC subtype correlates with favorable response to PD-1/PD-L1-targeted immunotherapy [71]. Atezolizumab is likely to be the most effective drug in the luminal cluster II subtype in the IMvigor210 study, whereas nivolumab was the most efficacious drug in the basal type I in the CheckMate-275 study [20,26]. The reasons for these discrepancies in the mUC subtype might be associated with tissue source. Both cohorts of IMvigor210 and CheckMate-275 allowed biopsy specimens from primary tumor, lymph nodes, or metastatic lesions for TCGA subtyping, which may have caused incorrect tumor classification. The criteria for molecular subtyping varied in each study, posing a challenge in standardizing TCGA classification. Nevertheless, the luminal I subtype, which is related to the upregulation of the FGFR pathway and high concentration of FGFR3 mutations and/or translocations, was insufficient of an immune signature, had low expression
| Clinical trial number | Drug(s) investigated | Clinical setting | Study design | Phase | Primary endpoint |
|-----------------------|----------------------|------------------|--------------|-------|------------------|
| NCT02450331 (IMvigor010) [52] | Atezolizumab | Adjuvant in muscle invasive UC | Randomized, placebo-controlled | III | DFS |
| NCT02632409 (CheckMate-274) [53] | Nivolumab | Adjuvant in muscle invasive UC | Randomized, double-blind, placebo-controlled | III | DFS |
| NCT03244384 [54] | Pembrolizumab | Adjuvant in MIBC | Randomized, placebo-controlled | III | OS, DFS |
| NCT0387761 [55] | Nivolumab+ipilimumab | Neoadjuvant in muscle invasive UC | Single-arm trial | I | Number of patients that underwent surgical resection <12 weeks after the study started |
| NCT02845323 [56] | Nivolumab+urelumab (IgG4 monoclonal antibody to CD137) | Neoadjuvant in MIBC | Randomized to nivolumab alone or the combination | II | Immune response measured by tumor infiltrating CD8+ T cell density at cystectomy |
| NCT02736266 [57] | Pembrolizumab | Neoadjuvant in MIBC | Single-arm trial | II | pCR at the time of radical cystectomy |
| NCT02365766 [58] | Pembrolizumab+gemcitabine ±cisplatin | Neoadjuvant in muscle invasive UC | Non-randomized | I/II | Phase 1b: number of patients with AEs; Phase II: rate of pathologic muscle invasive response at radical cystectomy |
| NCT02662309 [59] | Atezolizumab | Neoadjuvant in MIBC | Single-arm trial | II | pCR at cystectomy, pre-and post-treatment of CD8 and/or CD3 change |
| NCT03167151 [60] | Pembrolizumab | In intermediate risk of recurrent NMIBC | Randomized | I/II | Safety, tolerability, and toxicity |
| NCT02808143 [61] | Pembrolizumab+BCG solution | In high-risk of BCG refractory NMIBC | Single-arm trial, intravesical use | I | MTD |
| NCT02625961 (KEYNOTE-057) [62] | Pembrolizumab | In high-risk of BCG unresponsive NMIBC | Single-arm trial, intravesical use | II | CR, DFS |
| NCT02844816 [63] | Atezolizumab | In high-risk of BCG unresponsive NMIBC | Single-arm trial, intravesical use | II | CR |
| NCT02792192 [64] | Atezolizumab (intravenous use)±intravesical BCG | In high-risk of BCG naïve or relapsing/unresponsive NMIBC | Non-randomized | I/II | AEs, DLTs, MTD, CR |
| NCT02901548 [65] | Durvalumab | In BCG refractory carcinoma in the bladder | Single-arm trial, intravenous use | II | CR |

UC, urothelial carcinoma; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle invasive bladder cancer; BCG, bacillus Calmette-Guérin; DFS, disease-free survival; OS, overall survival; pCR, pathologic complete response; AE, adverse event; MTD, maximum tolerated dose; CR, complete response rate; DLT, dose-limiting toxicity.
of PD-L1 in tumor cell, and suggested low responses to atezolizumab and nivolumab.

4. Immune gene expression profiling (IFN-γ expression signature)

Immune gene expression profiling can be a more useful biomarker for predicting the response to immune checkpoint inhibition than PD-L1 expression alone, since it can be quantified from multiple cell types within a cancer specimen, which is probably a better representative of the tumor microenvironment [72,73]. In UC, the expression of IFN-γ inducible, CXCL9, CXCL10, and T-helper-1-type chemokines showed a close linkage with response to atezolizumab [20]. In the CheckMate-275 trial with nivolumab, a high expression of 25-gene IFN-γ signature was closely associated with almost 25-fold response to nivolumab than that without [28]. However, the negative predictive value of this gene panel remains a concern, since some responses were also identified in patients with a non-inflamed cytokine signature.

CONCLUSIONS

Immune checkpoint inhibitors have shown long-term durable response and tolerable safety profiles in several clinical trials. However, approximately 70% to 80% of patients may remain unresponsive to immune checkpoint inhibition. Therefore, further research on combining immune checkpoint inhibitor therapy with other therapeutic modalities, such as cytotoxic chemotherapy or different therapeutic targets, is needed to reinforce the effects of immunotherapies. Additional clinical trials with longer clinical follow-up for the PD-1/PD-L1 inhibitors are required to define their role in treating locally advanced and mUC in the first-line setting, and to define their potential for neoadjuvant and/or adjuvant therapy. Finally, the unavailability of specific biomarkers for predicting treatment responses remains a major limitation on the use of immune checkpoint inhibitors in real clinical practice. The existence of predictive biomarkers will enable selective use of immune checkpoint inhibition in the expected responders. Therefore, identification of these biomarkers should be consistently investigated and validated through additional supportive basic and clinical research.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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REFERENCES

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374-403.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:6-30.
3. Jung KW, Won YJ, Kong HJ, Lee ES. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2015. Cancer Res Treat 2018;50:303-16.
4. Clark PE, Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, et al. NCCN guidelines insights: bladder cancer, version 2.2016. J Natl Compr Canc Netw 2016;14:1213-24.
5. Alfred Witjes J, Lebret T, Compérat EM, Cowan NC, De Santis M, Bruins HM, et al. Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol 2017;71:462-75.
6. De Santis M, Bellmunt J, Mead G, Kerst JM, Leahy M, Maroto P, et al. Randomized phase II/III trial assessing gemcitabine/carboplatin and methotrexate/carboplatin/vinblastine in patients with advanced urothelial cancer who are unfit for cisplatin-based chemotherapy: EORTC study 30986. J Clin Oncol 2012;30:191-9.
7. Galsky MD, Pal SK, Lin SW, Ogale S, Zivkovic M, Simpson J, et al. Real-world effectiveness of chemotherapy in elderly patients with metastatic bladder cancer in the United States. Bladder Cancer 2018;4:227-38.
8. Niegisch G, Gerullis H, Lin SW, Pavlova J, Gondos A, Rudolph A, et al. A real-world data study to evaluate treatment patterns, clinical characteristics and survival outcomes for first- and second-line treatment in locally advanced and metastatic urothelial cancer patients in Germany. J Cancer 2018;9:1337-48.
9. Lichtenegger FS, Rothe M, Schnorfeil FM, Deiser K, Krupka C, Augsberger C, et al. Targeting LAG-3 and PD-1 to enhance T cell activation by antigen-presenting cells. Front Immunol 2018;9:385.
10. Baksh K, Weber J. Immune checkpoint protein inhibition for cancer: preclinical justification for CTLA-4 and PD-1 blockade and new combinations. Semin Oncol 2015;42:363-77.
11. Marin-Acevedo JA, Soyano AE, Dholaria B, Knutson KL, Lou Y. Cancer immunotherapy beyond immune checkpoint inhibitors. J Hematol Oncol 2018;11:8.
12. Sharma P, Allison JP. Immune checkpoint targeting in cancer
therapy: toward combination strategies with curative potential. Cell 2015;161:205-14.
13. Efremova M, Finotello F, Rieder D, Trajanoski Z. Neoantigens generated by individual mutations and their role in cancer immunity and immunotherapy. Front Immunol 2017;8:1679.
14. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
15. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124-8.
16. Kates M, Sopko NA, Matsui H, Drake CG, Hahn NM, Bivalacqua TJ. Immune checkpoint inhibitors: a new frontier in bladder cancer. World J Urol 2016;34:49-55.
17. Kim J. Immune checkpoint blockage therapy for bladder cancer treatment. Invest Clin Urol 2016;57 Suppl 1:S98-105.
18. Chism DD. Urothelial carcinoma of the bladder and the rise of immunotherapy: J Natl Compr Canc Netw 2017;15:1277-84.
19. Donin NM, Lenis AT, Holden S, Drakaki A, Pantuck A, Beldegrun A, et al. Immunotherapy for the treatment of urothelial carcinoma. J Urol 2017;197:14-22.
20. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909-20.
21. Perez-Gracia JL, Loriot Y, Rosenberg JE, Powles T, Necchi A, Hussain SA, et al. Atezolizumab in platinum-treated locally advanced or metastatic urothelial carcinoma: outcomes by prior number of regimens. Eur Urol 2018;73:462-8.
22. Inman BA, Longo TA, Ramalingam S, Harrison MR. Atezolizumab: a PD-L1-blocking antibody for bladder cancer. Clin Cancer Res 2017;23:1886-90.
23. Powles T, Durán I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMVigor211): a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2018;391:748-57.
24. Necchi A, Joseph RW, Loriot Y, Hoffman-Censits J, Perez-Gracia JL, Petrylak DP, et al. Atezolizumab in platinum-treated locally advanced or metastatic urothelial carcinoma: post-progression outcomes from the phase II IMVigor210 study. Ann Oncol 2017;28:3044-50.
25. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017;376:1015-26.
26. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2017;18:312-22.
27. Powles T, O'Donnell PH, Massard C, Arkenau HT, Friedlander TW, Hoimes CJ, et al. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. JAMA Oncol 2017;3:e172411.
28. Patel MR, Ellerton J, Infante JR, Agrawal M, Gordon M, Aljumaily R, et al. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. Lancet Oncol 2018;19:51-64.
29. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet 2017;389:67-76.
30. Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol 2017;18:1483-92.
31. Lara P, Beckett L, Li Y, Parikh M, Robles D, Aujla P, et al. Combination checkpoint immunotherapy and cytotoxic chemotherapy: pembrolizumab (pembrolizumab) plus either docetaxel or gemcitabine in patients with advanced or metastatic urothelial cancer. J Clin Oncol 2017;35:398.
32. Galsky MD, Wang H, Hahn NM, Twardowski P, Pal SK, Albany C, et al. Phase 2 trial of gemcitabine, cisplatin, plus ipilimumab in patients with metastatic urothelial cancer and impact of DNA Damage response gene mutations on outcomes. Eur Urol 2018;73:751-9.
33. US National Library of Medicine. Study of atezolizumab as monotherapy and in combination with platinum-based chemotherapy in participants with untreated locally advanced or metastatic urothelial carcinoma (IMVigor130) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02807636.
34. Powles T, Gschwend JE, Loriot Y, Bellmunt J, Gecgi Z, Vulestke C, et al. Phase 3 KEYNOTE-361 trial: pembrolizumab (pembro) with or without chemotherapy versus chemotherapy alone in advanced urothelial cancer. J Clin Oncol 2017;35:TPS4590-TPS.
35. Galsky MD, Powles T, Li S, Hennicken D, Sonpavde G. A phase 3, open-label, randomized study of nivolumab plus ipilimumab or standard of care (SOC) versus SOC alone in patients (pts) with previously untreated unresectable or metastatic urothelial carcinoma (mUC; CheckMate 901). J Clin Oncol
36. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, et al. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 1993;143:1255-62.

37. Lamy A, Gobet F, Laurent M, Blanchard F, Varin C, Moulin C, et al. Molecular profiling of bladder tumors based on the detection of FGFR3 and TP53 mutations. J Urol 2006;176:2686-9.

38. US National Library of Medicine. Open-label, randomised, multi-drug, biomarker-directed, phase 1b study in pts w/ muscle invasive bladder cancer (BISCOY) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02546661.

39. US National Library of Medicine. A study of ramucirumab plus pembrolizumab in participants with gastric or GEJ adenocarcinoma, NSCLC, transitional cell carcinoma of the urothelium, or biliary tract cancer [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02443324.

40. US National Library of Medicine. A study of nivolumab by itself or nivolumab combined with ipilimumab in patients with advanced or metastatic solid tumors [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT01928394.

41. Powles T, Galsky MD, Castellano D, Heijden MSVD, Petrylak DP, Armstrong J, et al. A phase 3 study of first-line durvalumab (MEDI4736) ± tremelimumab versus standard of care (SoC) chemotherapy (CT) in patients (pts) with unresectable Stage IV urothelial bladder cancer (UCB): DANUBE. J Clin Oncol 2016;34:TPS4574-TPS.

42. US National Library of Medicine. Safety and efficacy of MBG453 as single agent and in combination with PDR001 in patients with advanced malignancies [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02608268.

43. Ascietro PA, Bono P, Bhatia S, Melero I, Nyakas MS, Svane IM, et al. LBA18Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior anti-PD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched populations. Ann Oncol 2017;28:mdx440.011-mdx440.011.

44. Infante J, Ahlers CM, Hodi FS, Postel-Vinay S, Schellens JH, Heymach JV, et al. Abstract CT027: A phase I, open-label study of GSK3174998 administered alone and in combination with pembrolizumab in patients (pts) with selected advanced solid tumors (ENGAGE-1). Cancer Res 2016;76:CT027-CT.

45. Pedersen AW, Kopp KL, Andersen MH, Zocca MB. Immuno-regulatory antigens-novel targets for cancer immunotherapy. Chin Clin Oncol 2018;7:19.

46. Cekic C, Day YJ, Sag D, Linden J. Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. Cancer Res 2014;74:7250-9.

47. McCaffery I, Laport G, Hotson A, Willingham S, Patnaik A, Beeram M, et al. Biomarker and clinical activity of CPI-444, a novel small molecule inhibitor of A2A receptor (A2AR), in a Ph1b study in advanced cancers. Ann Oncol 2016;27(Suppl 6):vi114-35.

48. Smith DC, Gajewski T, Hamid O, Wasser JS, Olszanski AJ, Patel SP, et al. Epacadostat plus pembrolizumab in patients with advanced urothelial carcinoma: preliminary phase I/II results of ECHO-202/KEYNOTE-037. J Clin Oncol 2017;35:4503.

49. US National Library of Medicine. A study of epacadostat (INCB024360) in combination with durvalumab (MEDI4736) in subjects with selected advanced solid tumors (ECHO-203) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02318277.

50. US National Library of Medicine. Phase 1/1b study to evaluate the safety and tolerability of CPI-444 alone and in combination with atezolizumab in advanced cancers [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02655822.

51. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 2017;71:447-61.

52. US National Library of Medicine. A study of atezolizumab versus observation as adjuvant therapy in participants with high-risk muscle-invasive urothelial carcinoma (UC) after surgical resection (IMvigor010) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02450331.

53. US National Library of Medicine. An investigational immunotherapy study of nivolumab, compared to placebo, in patients with bladder or upper urinary tract cancer, following surgery to remove the cancer (CheckMate 274) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02632409.

54. US National Library of Medicine. Pembrolizumab in treating patients with locally advanced bladder cancer [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT03244384.

55. US National Library of Medicine. Neo-adjuvant bladder urothelial carcinoma combination-immunotherapy (NABUCCO) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT03387761.

56. US National Library of Medicine. Neoadjuvant nivolumab with and without urelumab in patients with cisplatin-ineligible...
57. US National Library of Medicine. Neoadjuvant pembrolizumab for muscle-invasive urothelial bladder carcinoma [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02365766.

58. US National Library of Medicine. Neoadjuvant pembrolizumab in combination with gemcitabine therapy in cis-eligible/ ineligible UC subjects [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02662309.

59. US National Library of Medicine. Preoperative MPDL3280A in transitional cell carcinoma of the bladder (ABACUS) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02625961.

60. US National Library of Medicine. Pembrolizumab in intermediate risk recurrent non-muscle invasive bladder cancer (NMIBC) (PemBla) [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02081433.

61. US National Library of Medicine. Pembrolizumab and BCG solution in treating patients with recurrent non-muscle-invasive bladder cancer [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02844816.

62. US National Library of Medicine. Safety and pharmacology study of atezolizumab alone and in combination with bacille calmette-guérin (BCG) in high-risk non-muscle-invasive bladder cancer (NMIBC) participants [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02792192.

63. US National Library of Medicine. Phase 2 durvalumab (Medi4736) for bacillus calmette-guérin (BCG) refractory urothelial carcinoma in situ of the bladder [Internet]. [cited 2018 Jun 7]. Available from: https://clinicaltrials.gov/ct2/show/NCT02901548.

64. Massard C, Gordon MS, Sharma S, Rafii S, Wainberg ZA, Luke J, et al. Safety and efficacy of durvalumab (Medi4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer. J Clin Oncol 2016;34:3119-25.

65. Aggen DH, Drake CG. Biomarkers for immunotherapy in bladder cancer: a moving target. J Immunother Cancer 2017;5:94.

66. Drake CG, Bivalacqua TJ, Hahn NM. Programmed cell death ligand-1 blockade in urothelial bladder cancer: to select or not to select. J Clin Oncol 2016;34:3115-6.

67. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373:1803-13.

68. Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152-65.

69. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014;507:315-22.

70. Gajewski TF. The next hurdle in cancer immunotherapy: overcoming the non-T-cell-inflamed tumor microenvironment. Semin Oncol 2015;42:663-71.