Effects and Mechanisms of Checkpoint Inhibitors (CTLA-4, PD-1 and PD-L1 Inhibitors) as New Immunotherapeutic Agents for Bladder Cancer

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Abstract

Since intravesical Bacillus Calmette-Guerin (BCG) began to be used for bladder cancer, our understanding of the importance of immune mechanisms in bladder cancer has steadily grown. With developments in immunotherapy in recent years, the use of new immunotherapeutic agents for bladder cancer, especially chemotherapy-resistant invasive and metastatic cancers, has opened the way for research in this area. Of these new therapeutic agents, this article reviews studies published on PubMed or listed on the ClinicalTrials.gov website as of December 2017 regarding the effects and mechanisms of action of checkpoint inhibitors [cytotoxic t-lymphocyte associated protein-4, programmed cell death 1 receptor (PD-1) and PD-1 ligand inhibitors] on bladder cancer. Because checkpoint inhibitors were first used for chemotherapy-resistant bladder cancer after identification of positive expression in tumor cells and especially in tumor-infiltrating mononuclear cells, significant objective response rates and survival advantages have been reported. Research continues regarding the use of these agents as first- and second-line treatment for metastatic disease in combination with chemotherapy; their efficacy in neoadjuvant, adjuvant, and bladder-preserving approaches to muscle-invasive bladder cancer (MIBC) disease, and their use in non-muscle-invasize bladder cancer (NMIBC), especially BCG-refractory disease. Depending on the results of these ongoing studies, immunotherapy may direct the treatment of bladder cancer in the future.

Keywords: Bladder cancer, immunotherapy, PD-1, PD-L1, CTLA-4

Introduction

Bladder cancer ranks fifth among the most common types of cancer (1). Seventy-five percent of cases are identified as non-muscle-invasive bladder cancers (NMIBC) [(pathologic stage Ta, T1 and carcinoma *in situ* (CIS)] following transurethral resection. European Organization for Research and Treatment of Cancer risk groups have been defined to predict the recurrence and progression of NMIBC, and follow-up and treatment protocols are recommended based on this classification (2). Intravesical Bacillus Calmette-Guerin (BCG) therapy is the only agent that reduces recurrence and progression to muscle-invasive bladder cancer (MIBC), and is especially recommended for high-risk patients (2,3). The search for alternative treatments is ongoing due to the risk of toxicity or unresponsiveness to BCG therapy. Immunotherapy is the most important and newest of these research areas, yet is also the oldest (because it forms the basis of the BCG mechanism of action).

There are several reasons that immunotherapy is a favorable treatment modality for bladder cancer. Firstly, bladder cancer has one of the highest mutation rates among all cancers. Therefore, it has high antigenic potential (4). Secondly, because the tumor is surrounded by a large surface in the intravesical area, it is easily accessible and suitable for local treatment. Thirdly, follow-up is easy because response to treatment can be observed visually. Despite these advantages, however, treatment success in bladder cancer is not at the desired level. For this reason, it is also an important target in research on new immunotherapeutic agents (5).

The aim of this review is to discuss bladder cancer immunology and the role of new immunotherapeutic agents (inhibitors) in the treatment of bladder cancer in light of the current literature. The contents are presented within the following subheadings: Immunotherapy in bladder cancer, BCG and bladder cancer, and checkpoint inhibition and inhibitors. Substantial attention

is given to the most studied group of compounds, checkpoint inhibitors, in the treatment of locally invasive and metastatic bladder cancers. This is followed by sections concerning the role of checkpoint inhibitors in the neoadjuvant and bladder-preserving approach to MIBC and their role in the treatment of NMIBC. Finally, we present checkpoint expressions in urothelial tumors.

The Basis of Immunotherapy in Bladder Cancer

While the relationship between the immune system and the foundations of neoplasia has been known since 1891, BCG was shown to be an effective agent in the treatment of bladder cancer by Morales et al. (6) in 1976. BCG has been used in the treatment of bladder cancer since that time and is still, over 40 years later, recommended for the treatment of high-risk NMIBC.

Bacillus Calmette-Guerin and Bladder Cancer

Calmette et al. (7) developed BCG from *Mycobacterium bovis* as an antituberculosis vaccine. However, after the link between malignancy and the immune system was established, BCG began to be used in the treatment of cancer. After showing efficacy against implanted tumors in mice, BCG was used in leukemia, melanoma, and head and neck cancers; it was first applied in the bladder as endoscopic intralesional injection for melanoma metastasis (8,9). Later, Morales et al. (6) demonstrated the efficacy of intravesical BCG in patients with NMIBC. In their study, which was the first to describe the use of BCG in bladder cancer, patients who had frequent recurrence and could not undergo total resection were treated with 120 mg intravesical BCG and intradermal BCG injections for 6 weeks (6). Since the first studies (6,10), BCG has becommen a standard therapeutic agent in use from the 1990s to the present, especially for patients with high-risk NMIBC.

The mechanism of action of BCG is based on immune system activation and the immune response. The immune response begins with the macropinocytosis of BCG into the urothelial cells, followed by the upregulation of major histocompatibility complex class 2 molecules and cytokine release. This results in migration of Th1 lymphocytes to the area around the tumor and the formation of a cytotoxic immune response mediated by CD8+ lymphocytes, natural killer (NK) cells, and granulocytes (11).

Checkpoint Inhibition and Inhibitors

Immune checkpoint inhibition is at the forefront of current cancer research. It was approved by the United States Food and Drug Administration (FDA) following positive results from phase 3 trials on checkpoint inhibition in melanoma, non-small cell lung cancer, and renal cell carcinoma. However, the focus of research is inverse to severity in urinary system malignancies, especially urothelial carcinoma. Most of the research and FDA approvals related to checkpoint inhibition pertain to locally invasive and metastatic patient groups. Therefore, we divided studies investigating checkpoint inhibitors in urothelial carcinoma into those focusing on locally invasive and metastatic bladder cancer in Table 1, MIBC in Table 2, and NMIBC in Table 3.

The mechanism of checkpoint inhibition targets T cell regulation, increasing T cell and antitumor activity by suppressing inhibitor signals. This shows that, in addition to the previously known T cell receptor (TCR) activation, there are many co-stimulatory and inhibitory molecules on the surface of T cells and that these influence T cell behavior (12,13). The most important of these are cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and programmed cell death 1 receptor (PD-1) and its ligands (PD-L1 and PD-L2) (Figure 1), which will be discussed in detail in the next section.

Cytotoxic T-Lymphocyte Associated Protein-4 and Ipilimumab (CTLA-4 Inhibitor)

CTLA-4 expressed on the surface of T cells is known to be among the molecules involved in T cell activation. CTLA-4 competes with CD28, an immunostimulant receptor, for B7 ligands (B7-1 and B7-2) found on the surface of antigen-presenting cells (APCs). However, the B7/CTLA-4 complex inhibits T cell activation in the lymphoid tissue instead of enhancing it as the CD28 complex does. This shows that CTLA-4 inhibition can promote an immune response. Therefore, when the B7/CTLA-4 interaction is blocked by ipilimumab, a monoclonal anti-CTLA-4 antibody, the T cell balance is shifted towards activation, increasing the antitumor effects. Ipilimumab first received FDA approval for metastatic melanoma (14). In one large trial, 799 patients with metastatic castration-resistant prostate cancer that progressed after docetaxel chemotherapy for urologic malignancies were given a placebo or ipilimumab following external radiotherapy applied to the bone (15). While no difference in overall survival was observed between the groups, ipilimumab yielded more favorable outcomes in terms of prostate-specific antigen reduction and progression-free
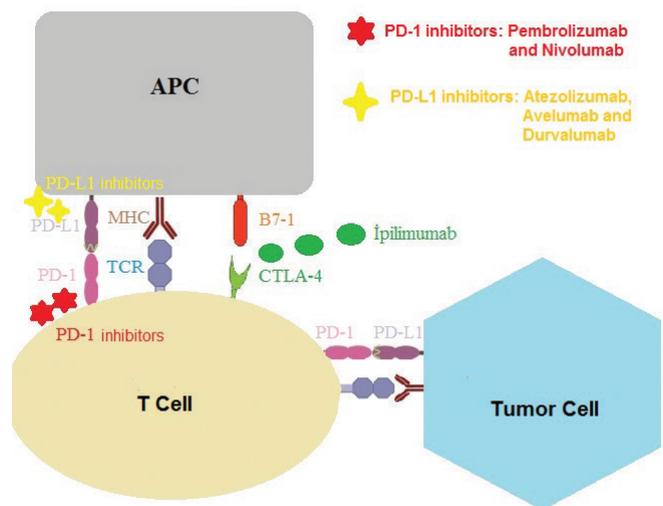

Figure 1. The receptor-ligand relationship in checkpoint inhibition, and the cells and checkpoints affected by inhibitors: cytotoxic T-lymphocyte associated protein-4, programmed cell death 1 receptor, and programmed cell death 1 ligand

CTLA-4: Cytotoxic T-lymphocyte associated protein-4, PD-L1: Programmed cell death 1 ligand, PD-1: Programmed cell death 1 receptor, APC: Antigen-presenting cell, TCR: T cell receptor

survival (4 months vs 3.1 months). In another study, 12 patients whose clinical stage was T1-T2N0M0 were given 2 doses of ipilimumab prior to cystectomy and side effects resulted in delayed cystectomy in 2 patients (16). Phase 2 trials evaluating the combination of ipilimumab with gemcitabine and cisplatin in patients with advanced disease are ongoing. Side effects of ipilimumab include vitiligo, rashes, pruritus, anorexia, fatigue, diarrhea, and in a small number of patients, immune-related effects requiring steroid treatment (14). Trials of ipilimumab conducted in the locally invasive and metastatic patient group are presented in Table 1.

Programmed Cell Death 1 Receptor and Its Ligands (PD-L1 and PD-L2)

PD-1 receptor (CD279) and its two ligands PD-1 ligand 1 (PD-L1, B7-H1, and CD274) and PD-1 ligand 2 (PD-L2, B7-DC, and CD273) are cell surface glycoproteins from the B7 family of coinhibitory molecules. PD-L1 is found on the surfaces of APCs, T cells, NK cells, stem cells, and various non-hematopoietic cells in humans (17). PD-L2 has been shown in a few studies to be expressed in a small number of cells. PD-L1 and PD-L2 bind to the PD-1 receptor expressed by T cells, and these ligands are also found in APCs such as macrophages, dendritic cells, and B cells. This receptor and its ligands are important molecules involved in T cell immunomodulation. The PD-1 receptor inhibits TCR-mediated T cell function, as does CTLA-4. However, unlike CTLA-4, they are believed to exert this effect in the tumor microenvironment (18). Upregulation of PD-L1 in tumor cells is considered a mechanism of PD-1 pathway activation and immune escape (19). Indeed, immunohistochemical studies have shown that increased PD-L1 expression is associated with advanced stage bladder cancer and high-grade tumors (20). Therefore, the following sections include a detailed discussion of studies investigating the effect of inhibitory drugs that target PD-1 and PD-L1 in bladder cancer.

Atezolizumab (PD-L1 Inhibitor)

Atezolizumab, a monoclonal immunoglobulin G1 antibody that binds PD-L1, came into use following FDA approval in the treatment of advanced stage bladder cancer that progresses despite platinum-based chemotherapy (12,21). This approval was obtained by examining data obtained from phase 1 and 2 trials and based on the presence of PD-L1 in tissue samples taken from advanced stage patients prior to treatment. In a phase 1 trial, response was observed in 25% of the patients, with 2 patients showing complete response. However, this response was found to rely not on the immunohistochemical scores of tumor cells, but rather on the scores of tumor infiltrating mononuclear cells (TIMCs). In a phase 1 trial involving 68 patients with metastatic urothelial carcinoma who had received prior treatment (93% had previously undergone cisplatin-based chemotherapy; systemic therapy failed in 72%), the objective response rate at 6-week follow-up was 50% among those with a high PD-L1 expression in the TIMCs, compared to only 8.3% among those who were PD-L1 negative. Overall, 57% of patients experienced a side effect such as anorexia, fatigue, nausea, weakness, and shivering (12,13). This was followed by

the results of the phase 2 IMvigor210 (NCT02108652) study (21). In that trial, 310 inoperable and metastatic patients with an Eastern Cooperative Oncology Group performance score of 0 or 1 were evaluated. PD-L1 expression in TIMCs was determined using SP142 assay. Tumors were grouped according to expression rate: <1%; ≥1% to <5%; and ≥5%. The overall response rate was 15% among the 310 patients, and response was found to be associated with expression rates (higher response rate with higher TIMC PD-L1 expression: 26% response rate at with ≥5% expression, 18% with ≥1% to <5% expression, and 15% overall response rate). Median survival time was 11.7 months, and median progression-free survival time was found to be 2.1 months regardless of PD-L1 expression status. In the 2nd cohort of this study (NCT02108652), atezolizumab treatment resulted in an objective response rate of 16% in all patients and a 28% objective response rate in patients with ≥5% PD-L1 expression in TIMCs, after a median follow-up of 1.5 years (21,22). The 12-month overall survival rate of patients with ≥5% PD-L1 expression in TIMCs was 50%, compared to 37% in the entire patient population (21,22).

In terms of the adverse effect profile of atezolizumab in the IMvigor 211 trial (NCT02302807), 69% of patients overall experienced a side effect. The most common adverse effects were fatigue (31%), nausea (14%), anorexia, pruritus, fever, diarrhea, rashes, and arthralgia. Pneumonia and dyspnea were serious side effects (21,22). Based on these results, atezolizumab was approved by the FDA in May 2016 for the treatment of patients with locally advanced or metastatic urothelial carcinoma with disease progression during or after platinum-based chemotherapy. A study expected to be completed in the spring of 2018 (NCT02807636) is investigating the effect of atezolizumab ± gemcitabine/carboplatin or chemotherapy with cisplatin alone on patients with locally advanced or metastatic urothelial carcinoma. Studies investigating the effects of atezolizumab in MIBC and NMIBC are presented in Tables 2 and 3.

Pembrolizumab (PD-1 Inhibitor)

In February 2017, the FDA approved the evaluation of pembrolizumab as a first-line treatment in patients with urothelial carcinoma who are not eligible for cisplatin-based chemotherapy and as a second-line treatment in patients with urothelial carcinoma that progresses during or after platinum-based chemotherapy. In the phase 1b trial investigating anti-PD-1 pembrolizumab, 33 patients with recurrent or metastatic urothelial cancer were examined and 41% PD-L1 expression was noted in the tumor cells (23). In the median follow-up period of 11 months, overall response and complete response rates of 24% and 10% were obtained. In the KEYNOTE-012 (NCT01848834) phase 1b trial, the objective response rate was 25% and 12-month progression-free survival was 19% in the total population, while the objective response rate was 38% for tumors with positive PD-L1 expression (>1% in tumor nests) (24). In KEYNOTE-012, side effects were observed in 61% of patients, and were most commonly reported as fatigue (18%), peripheral edema (12%), and nausea (9%) (25). In KEYNOTE-052 (NCT02335424), a phase 2 study in which pembrolizumab was given as first-line therapy to patients with

Table 1. Studies of checkpoint inhibitors in locally invasive and metastatic urothelial carcinoma

Study ID	Drugs	Cohort	Design	Phase	N	Primary outcome measure	Schedule
NCT01524991	Ipilimumab, Gemcitabine, Cisplatin	Urothelial carcinoma, 1 arm	Single arm	2	36	One-year overall survival	January 2012-March 2018
NCT02108652 (Cohort 2) (IMvigor 210)	Atezolizumab	Bladder locally invasive/metastatic, 1 arm	Single arm	2	310	Objective response rate	May 2014-May 2018
NCT02302807 (IMvigor211)	Atezolizumab/Vinflunine, Paclitaxel, Docetaxel	Bladder locally invasive/metastatic, 2 arms	Randomized	3	932	Overall survival	January 2015-November 2017
NCT02807636 (IMvigor130)	Atezolizumab/ Carboplatin, Gemcitabine, Placebo, Cisplatin	Urothelial carcinoma, locally invasive/metastatic, 2 arms	Randomized	3	1200	Overall and progression-free survival	June 2016-July 2020
NCT01848834 (KEYNOTE-012)	Pembrolizumab	Cancer, solid tumors including urothelial carcinoma 1 arm	Single arm	1	297	Safety and side effects	May 2013-January 2018
NCT02256436 (KEYNOTE-045)	Pembrolizumab/Vinflunine, Paclitaxel, Docetaxel	Urothelial carcinoma advanced stage, 2 arms	Randomized	3	542	Overall and progression-free survival	November 2014-January 2019
NCT02335424 (KEYNOTE-052)	Pembrolizumab	Urothelial carcinoma, locally invasive/inoperable/metastatic, 1 arm	Single arm	2	350	Objective response rate	February 2015-June 2018
NCT02437370	Pembrolizumab/Docetaxel, Gemcitabine	All urothelial carcinomas	Non-randomized	1	38	Safety and side effects	August 2015-December 2019
NCT01928394	Nivolumab, Ipilimumab, Cobimetinib	Advanced stage, metastatic solid tumors, including bladder	Randomized	1/2	1150	Objective response rate	October 2013-December 2018
NCT02387996 (CheckMate 275)	Nivolumab	Bladder, inoperable/metastatic, 1 arm	Single arm	2	386	Objective response rate	March 2015-October 2018
NCT02632409 (CheckMate 274)	Nivolumab/Placebo	Bladder, upper urinary tract urothelial carcinoma, 2 arms	Randomized	3	640	Disease-free survival	February 2016-May 2020
NCT01693562	Durvalumab	Advanced stage solid tumors, 1 arm	Single arm	1/2	1022	Safety and side effects	August 2012-July 2018
NCT01772004 (JAVELIN solid tumors)	Avelumab	Locally invasive/metastatic solid tumors, including urothelial carcinoma, 1 arm	Single arm	1	1753	Toxicity	February 2013-May 2018
NCT02603432 (JAVELIN bladder 100)	Avelumab	Bladder, locally invasive/metastatic, 2 arms	Randomized	3	668	Progression-free survival, objective response	April 2016-July 2020
NCT02496208	Cabozantinib S-Malate, Ipilimumab, Nivolumab	Metastatic genitourinary tumors	Non-randomized	1	135	Side effects	July 2015-December 2017
NCT02553642	Nivolumab/Nivolumab + Ipilimumab	Bladder, melanoma, metastatic, 1 arm	Single arm	2	70	Response rate	September 2015-September 2018
NCT02516241 (DANUBE)	Durvalumab/Durvalumab + Tremelimuma/Cisplatin, Carboplatin, Gemcitabine	Urothelial carcinoma, stage 4, 3 arms	Randomized	3	1005	Efficacy	November 2015-July 2019

advanced stage inoperable and metastatic urothelial carcinoma, the objective response rate was 24% in the first 100 patient analysis and 36.7% in patients whose PD-L1 expression rates in tumor and immune cells were >10% (26). In the KEYNOTE-045 (NCT02256436) phase 3 trial, the overall survival in the chemotherapy and pembrolizumab randomization in patients with previously treated metastatic urothelial cancer was 10.3 months in the pembrolizumab arm and 7.4 months in the chemotherapy arm (27). Combinations of pembrolizumab with docetaxel or gemcitabine (NCT02437370) and gemcitabine or cisplatin (NCT02690558) are currently being investigated in ongoing studies. Combinations of pembrolizumab with chemotherapy and radiotherapy are also being investigated in studies currently in progress (NCT02662062 and NCT02621151). Studies investigating the effects of pembrolizumab in MIBC and NMIBC are presented in Tables 2 and 3.

Nivolumab (PD-1 Inhibitor)

Nivolumab is a monoclonal antibody against PD-1. Following its use in other types of cancer, nivolumab was approved by the FDA for use in the treatment of renal cell carcinoma in November 2015 and for use in patients with locally advanced or metastatic urothelial carcinoma progressing for one year after platinum-based chemotherapy in February 2017. In a trial including patients with metastatic urothelial cancer (NCT01928394), the objective response rates for patients with ≥1% and <1% PD-L1 expression in tumor cells were 24% for 26%, respectively, and overall survival for the entire population was 9.7 months (28). Approximately 21.8% of patients experienced grade 3-4 side effects, the most common of which were lipase elevation (5.1%), amylase elevation (3.8%), and fatigue (28). In the CheckMate 275 study (NCT02387996), an objective response rate of 19.6% was achieved with nivolumab in patients with metastatic urothelial cancer. The objective response rate was 16.1% for those with a low or negative PD-L1 expression in the tumor (<1%), while this rate was 28.4% for those with ≥5% PD-L1 expression (29). CheckMate 274 (NCT02632409) is an ongoing phase 3 trial in which nivolumab is evaluated versus a placebo after surgery in patients with bladder or upper urinary tract cancer.

Durvalumab (PD-L1 Inhibitor)

Durvalumab, a monoclonal antibody against PD-L1, was evaluated by the FDA in February 2016 for patients with inoperable or metastatic urothelial bladder cancer that progressed during or after standard platinum-based chemotherapy.
In a phase 1/2 durvalumab trial involving patients with inoperable or metastatic urothelial bladder cancer (NCT01693562), the objective response rate was 31%, while this rate was 0% in the low/negative PD-L1 subgroup (<25%) and 46% in the high PD-L1 subgroup (≥25%) (30). The most common side effects observed in the study were fatigue (13%), diarrhea (10%), and decreased appetite (8%).

Avelumab (PD-L1 Inhibitor)

This anti-PD-L1 monoclonal antibody is in the early stages of research for more than 15 types of cancer, including bladder cancer. Avelumab has a different mechanism than other PD-L1

Table 2. Studies of checkpoint inhibitors in patients with muscle-invasive bladder cancer

Study ID	Drugs	Cohort	Design	Phase	N	Primary outcome measure	Schedule
NCT02662309 (ABACUS)	Atezolizumab followed by cystectomy	Bladder, MIBC, neoadjuvant effect, 1 arm	Single arm	2	85	Efficacy, pathologic response	February 2016-March 2019
NCT02736266	Pembrolizumab followed by cystectomy	Bladder, MIBC, neoadjuvant effect, 1 arm	Single arm	2	90	Efficacy, pathologic response	February 2017-June 2019
NCT02662062 (PCR-MIB)	Pembrolizumab, Cisplatin, Radiotherapy	Bladder, MIBC, bladder-preserving approach, 1 arm	Single arm	2	30	Safety and toxicity	August 2016-January 2024
NCT02690558	Pembrolizumab, Gemcitabine, Cisplatin	Bladder, MIBC, neoadjuvant combination, 1 arm	Single arm	2	39	Efficacy	May 2016-May 2024
NCT02621151	Pembrolizumab, TUR-BT, Gemcitabine, Radiotherapy	Bladder, MIBC, 1 arm	Single arm	2	54	Two-year bladder limited disease rate	October 2014-May 2024
NCT02662062	Pembrolizumab/ Cisplatin, Radiotherapy	Bladder, MIBC, 1 arm	Single arm	2	30	Safety	August 2016-January 2024
NCT02560636 (PLUMMB)	Pembrolizumab and radiotherapy	Bladder, inoperable MIBC, 1 arm	Single arm	1	34	Safety and toxicity	June 2016-June 2019

MIBC: Muscle-invasive bladder cancer, TUR-BT: Transurethral resection of bladder tumour

Table 3. Studies of checkpoint inhibitors in patients with non-muscle-invasive bladder cancer

Study ID	Drugs	Cohort	Design	Phase	N	Primary outcome meausre	Schedule
NCT02451423	Atezolizumab	Bladder, NMIBC, refractory to BCG, 1 arm	Single arm	2	42	Complete response rate	April 2016-December 2019
NCT02625961 (KEYNOTE-057)	Pembrolizumab	Bladder, high-risk NMIBC, refractory to BCG, 1 arm	Single arm	2	260	Complete response rate, Disease-free survival	February 2016-December 2021
NCT02324582 (MARC)	Pembrolizumab and BCG	Bladder, high-risk NMIBC, refractory to BCG, 1 arm	Single arm	1	15	Safety	June 2015-November 2020

BCG: Bacillus Calmette-Guérin, NMIBC: Non-muscle-invasive bladder cancer

Table 4. Programmed cell death 1 ligand expression in urothelial tumor tissue

Author	N	Tissue	Tissue preservation method	PD-1/PD-L1 antibody	PD-L1 positivity limit (%)	PD-L1 expression rate in tumor cells (%)
Faraj et al. (37)	56	Cystectomy, bladder	Formalin-fixed paraffin block	5H1 PD-L1 mouse monoclonal	≥5	20
Bellmunt et al. (38)	160	Cystectomy and TUR-BT, bladder	Formalin-fixed paraffin block	405.9a11 PD-L1 mouse monoclonal	≥5	20
Boorjian et al. (35)	314	Cystectomy, bladder	Formalin-fixed paraffin block	5H1 PD-L1 mouse monoclonal	≥5	12
Nakanishi et al. (39)	65	Bladder, ureter, renal pelvis	Frozen section pathology	M1H1 PD-L1 mouse monoclonal	>12.2	
Inman et al. (20)	280	Bladder	Formalin-fixed paraffin block	5H1 PD-L1 mouse monoclonal	≥1	28
Wang et al. (40)	50	Bladder	Formalin-fixed paraffin block	Pdcd-1L1 (H-130) rabbit polyclonal	>10	72

TUR-BT: Transurethral resection of bladder tumour, PD-L1: Programmed cell death 1 ligand, PD-1: Programmed cell death 1 receptor

inhibitors. It exhibits antibody-dependent cellular cytotoxic activity in addition to PD-L1 inhibition. This causes the direct destruction of tumor cells, but this activity can also cause lysis of other cells expressing PD-L1 and lead to specific toxicities (31). In the JAVELIN solid tumor phase 1b study (NCT01772004), the objective response rate was 16.5% among patients whose cancer progressed after platinum-based chemotherapy or who were ineligible for platinum (32). Infusion-related reactions (22.5%) and fatigue (14.7%) were the most common side effects (32). The phase 3 JAVELIN bladder 100 study (NCT02603432) is currently ongoing.

PD-1/PD-L1 and CTLA-4 Inhibitor Combination

PD-1 is mainly involved in the effector phase of T-cell activation, and the PD-1/PD-L1 interaction primarily occurs during presentation of antigens to memory T cells in peripheral tissues (33). CTLA-4 is expressed by regulatory T cells and memory CD4 cells and functions during the early activation of T cells found in lymphatic tissues (33). Therefore, the combination of treatments aimed at the inhibition of these two targets is logical.

The combination of nivolumab and ipilimumab is being investigated as part of the CheckMate 032 trial. Cohort A (n=26) received a combination of nivolumab (1 mg/kg) and ipilimumab (3 mg/kg), cohort B (n=104) received a combination of nivolumab (3 mg/kg) and ipilimumab (1 mg/kg) (34). A higher response rate was observed in the cohort with the higher dose ipilimumab (39%) compared to the cohort with the lower dose (26%) (34). However, overall survival time was similar in both groups (7.3 months versus 10.2 months) (34). Another ongoing study (NCT02553642) investigates PD-L1 expression and rates of response to nivolumab/ipilimumab combination therapy in patients with locally advanced/inoperable or metastatic urothelial carcinoma. Other studies evaluating combinations of nivolumab and ipilimumab (NCT01928394, NCT02496208) are summarized in Table 1.

The combination of durvalumab and tremelimumab, a CTLA-4 inhibitor, versus standard chemotherapy in patients with stage 4 urothelial bladder cancer is currently being assessed in the DANUBE trial (NCT02516241), expected to be completed in 2019.

In addition to these agents, T cell surface receptors such as B7-H3 and OX40, which regulate cell activation and efficacy, have also been discovered as potential therapeutic targets. Boorjian et al. (35) have suggested that high expression of the glycoprotein B7-H3 in urothelial tumors may be associated with upregulation of PD-1.

The Role of Checkpoint Inhibitors in the Treatment of Muscle-Invasive Bladder Cancer

Neoadjuvant MVAC (methotrexate, vincristine, adriamycin, cisplatin) or gemcitabine and cisplatin combinations are recommended regimens for MIBC. However, a large proportion of patients relapse after radical cystectomy (36). Furthermore,

standard chemotherapy agents are not available for the patient group that is ineligible for platinum-based chemotherapy. Relevant studies involving checkpoint inhibitors and their combinations with chemotherapy and radiotherapy are in the research phase. Some of these studies are presented in Table 2. Besides these, the option of immunotherapy for MIBC patients who are ineligible for cystectomy or want bladder-preserving treatment is one of the current topics being discussed, and there are ongoing studies involving this patient group. Some of these studies are also shown in Table 2.

The Role of Checkpoint Inhibitors in the Treatment of Non-Muscle-Invasive Bladder Cancer

Research continues regarding the use of checkpoint inhibitors in the treatment of locally invasive and metastatic urothelial carcinoma, especially bladder cancer, and the role of these drugs in treatment. Their combinations with both neoadjuvant and adjuvant chemotherapy and chemo-radiotherapy, and the role of these combinations as first- and second-line therapies constitute a broad research area. A clearer picture is expected to develop in the 2020s. The next step for these treatments, which have already been investigated for NMIBC, involves studies targeting the 40% of the patient population that develops recurrence and progression into MIBC despite intravesical BCG therapy. Some relevant studies that are in progress, especially those involving BCG-refractory patient group, are listed in Table 3.

PD-L1 Expression in Urothelial Tumor Tissue

Studies examining PD-L1 expression levels in urothelial carcinoma have yielded differing results. These studies are briefly summarized in Table 4. In one of those studies, pathologic specimens of 56 patients who underwent radical cystectomy due to bladder cancer were examined and ≥5% PD-L1 expression was observed in 20% of them. However, it was shown that PD-L1 expression and cytotoxic CD8+ T cell density were not associated with the clinicopathologic data (37). Bellmunt et al. (38) reviewed the pathology specimens of 160 patients who underwent transurethral resection of bladder tumour (TUR-BT) or radical cystectomy and defined a threshold of ≥5% for PD-L1 positivity on tumor cells. Positive PD-L1 expression was detected in 40% of TIMCs and was associated with longer survival in metastatic disease (38). In a study of 314 cystectomy specimens, ≥5% PD-L1 expression was observed in urothelial tumor cells and the expression of PD-1 in TIMCs was markedly increased (35). In a study of 65 patients, >12.2% PD-L1 expression was associated with high tumor grade and low recurrence-free survival (39). Another study demonstrated that increasing tumor stage was associated with higher PD-L1 expression positivity rate (≥1%) (7%, 16%, 23%, 30%, and 45% in Ta, T1, T2, T3/4, and CIS tumors, respectively) (20). Yet another report stated that >10% PD-L1 expression was associated with high-grade, muscle invasion, recurrence, and shorter survival (40). However, there are certain factors that make it difficult to direct compare these studies evaluating PD-L1 expression. These include differences in the organ sampled and collection method (TUR-BT, cystectomy, nephroureterectomy), differences in immunohistochemical analysis (formalin-fixed paraffin block vs. frozen tissue), different PD-L1 antibodies (5H1, M1H1, and Pdcd-1L1) and differences in expression positivity rates (ranging from 1% to 12.2%) (20,35,39).

Conclusion

Although BCG is an important step in the treatment of NMIBC, additional treatments are needed in patients with treatment failure, as in locally invasive or metastatic bladder cancer. Of the immunotherapeutic agents investigated for this purpose, checkpoint inhibitors (CTLA-4, PD-1, and PD-L1 inhibitors) have provided favorable objective response rates and longer survival in locally advanced, inoperable, and metastatic bladder cancer, especially depending on expression levels in TIMCs and tumor cells. Although research priorities are inverse to disease severity, we look forward to the outcomes of ongoing studies in order to use these inhibitors in neoadjuvant, adjuvant, and bladder-preserving approaches to MIBC and in patients with BCG-refractory NMIBC.

Questions

1. What are the role and mechanism of action of Bacillus Calmette-Guerin in bladder cancer immunotherapy?
2. What is the role of checkpoints in the immune response and what are the effects of their inhibition?
3. What is the role of checkpoint inhibitors in the current treatment of bladder cancer?
4. What are the expectations regarding checkpoint inhibitors in the treatment of non-muscle invasive bladder cancer?

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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