

Basic Concepts in Bladder Cancer Immunotherapy

J. Ryan Mark, MD; Jean Hoffman-Censits, MD; and Leonard G. Gomella, MD

Abstract

The landscape of available treatment for metastatic urothelial cancer is rapidly changing due to the emergence of new monoclonal antibodies that target the PD-1/PD-L1 interaction known as checkpoint inhibitors. These new agents utilize a principle established long ago with the use of *Bacillus Calmette-Guérin* by urologists for non-muscle-invasive bladder cancer that the immune system can be modulated to act against bladder cancer. The success of checkpoint inhibitors in early clinical trials has led to rapid FDA approval and interest in immunotherapy by the public and clinicians alike. It is important for all to be aware of this changing landscape; thus, we review the basic concepts behind the development of these newly available drugs.

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of bladder cancer. The role of immune-mediated anti-cancer effects was suspected when granulomatous inflammation was found on biopsy rather than urothelial carcinoma.² Morales' results were confirmed by larger SWOG and European Organisation for Research and Treatment of Cancer randomized clinical trials, leading to FDA approval of intravesical BCG immunotherapy for localized bladder cancer in 1990. It has remained the most effective treatment option for superficial bladder cancer for the last 30 years.³

The Role of the Immune System in Bladder Cancer

Immune suppression has been implicated as a risk factor for developing cancer, as increased rates of malignancy are seen in both transplant and elderly patients. Specifically, with immune suppression, there is a 3-fold increased risk of developing bladder cancer.⁴ Still, the majority of malignancies occur in individuals with competent immune systems, which implies a failure in immune surveillance in these individuals. As such, the ability to avoid detection and destruction by the immune system is now recognized as a defining characteristic of cancer.⁵

The process that explains the immune system's interaction in cancer development, proposed by Chen et al, is termed the "cancer-immunity cycle." Rapid proliferation of cancer cells, and ultimately necrosis, releases antigens—termed neoantigens—that are unique to the cancer cell. These neoantigens are processed by antigen-presenting cells (APCs) of the innate immune system and presented to T cells on major histocompatibility complex (MHC) I and II within lymph nodes.⁶ Co-stimulatory factors influence the production of effector T cells that should recognize these antigens as foreign and recruit an immune response, leading to cancer-cell death. Alternatively, inhibitory signals will produce T regulatory cells that recognize these antigens as self; in such a case, the immune system is suppressed, allowing cancer-cell survival. Newly activated cytotoxic T lymphocytes (CTLs) enter the circulatory system and infiltrate tumor tissue, where they bind to tumor antigens presented on MHC molecules. If the appropriate co-stimulatory molecules are present, the immune system is activated and tumor cell death is promoted, releasing more antigens and contributing to the cycle of tumor destruction.

Introduction

On May 18, 2016, atezolizumab became the first anti-PD-L1 inhibitor to gain FDA approval for use in patients with advanced urothelial carcinoma. Approval of an additional 4 anti-PD-1/PD-L1 inhibitors would follow.¹ These exciting new treatments take advantage of interactions between cancer cells and their host's immune system, thereby offering patients an alternative to cytotoxic therapies. Widespread use of these novel agents requires that clinicians gain familiarity with the growing field of immunotherapy. In this review, we discuss basic concepts for understanding the immune system's interactions with malignant cells, as well as report on the currently available immunotherapies in the treatment of bladder cancer.

The susceptibility of bladder cancer to immune-mediated destruction is not a new concept, as demonstrated by the use, decades ago, of *Bacillus Calmette-Guérin* (BCG) by urologists for non-muscle-invasive bladder cancer. Based on an observation that tuberculosis patients had fewer incidental findings of cancer at autopsy, studies were performed throughout the 1950s demonstrating tumor resistance in animals after BCG inoculation. This ultimately led Morales et al in 1976 to the discovery that intravesical BCG reduced the recurrence rate

However, if inhibitory signals are present on the tumor cell surface, then the CTLs recognize the cancer as self and allow the tumor's survival.⁶

The process above contributes to the constant surveillance of mutations and subsequent cancer control known as the “immune-editing hypothesis.”⁷ Three phases—elimination, equilibrium, and escape—either control cancer or support its progression. Elimination involves the effective response by T cells when mutated cells are destroyed. Local development of a tumor occurs in the equilibrium phase, during which incomplete control by the immune system allows malignant cells to persist, grow, and develop more mutations. Inhibitory cosignaling or T-cell energy leads to the escape phase, resulting in local progression and metastasis.⁷ Ultimately, the goal of successful immunotherapy is to push the tumor into the elimination phase by manipulating the cancer immunity cycle such that tolerance of the developing tumor is prevented.

Encouraging an anticancer immune response relies first on the ability of the immune system to identify malignant cells. The greatest success in achieving immune activation has been in tumors that carry a high degree of somatic mutations.⁸ The mutational load leads to increased neoantigen production and subsequent immune detection. Urothelial cancer is highly mutated, with only melanoma and some lung cancers bearing higher mutational loads.⁹ The activity of BCG immunotherapy is based upon this concept, as nonspecific activation of the immune system leads to bladder cancer recognition and apoptosis. While the precise mechanism is still unclear, installation of BCG exposes urothelial cells to an attenuated mycobacterium; this results in a local inflammatory response and the release of cytokines—interferon (IFN) and interleukin—to recruit the innate immune system.¹⁰ As a result of this inflammation, APCs engulf and present both BCG and, more importantly, local tumor antigens via MHC complexes to T lymphocytes. This activates the adaptive immune system against the bladder tumor.

The addition of cytokines to BCG to improve response has been investigated. Intravesical BCG plus interferon alpha (IFN α) has failed to show improved responses over BCG alone in BCG-naïve patients.^{11,12} It is thought that the poor response to IFN α may be due to the limited contact time with the bladder. Currently, a phase III multicenter clinical trial sponsored by the Society of Urologic Oncology Clinical Trials Consortium is underway to investigate if a recombinant adenovirus delivering the IFN α gene to the urothelium in a unique formulation can improve responses after BCG failure (NCT01687244). Results from the phase II clinical trial in 40 patients after BCG failure demonstrated a 35% 1-year recurrence-free survival.¹³

Another notable investigational virus-based immunotherapy for the treatment of non-muscle-invasive bladder cancer is the oncolytic virus CG0070. Intravesical delivery of the

virus is thought to cause damage to retinoblastoma-deficient cells, leading to selective destruction of urothelial cancers. The agent also carries the granulocyte-macrophage colony-stimulating factor cytokine gene, which promotes enhanced local inflammatory reaction. Recently presented interim results from a phase II trial of 36 patients receiving intravesical CG0070 after 2 failed induction courses of BCG showed a 44% objective response rate (ORR) at 6 months. In patients with bladder carcinoma in situ, 52% of patients were disease-free at 6 months, with long-term results pending (NCT02365818).¹⁴

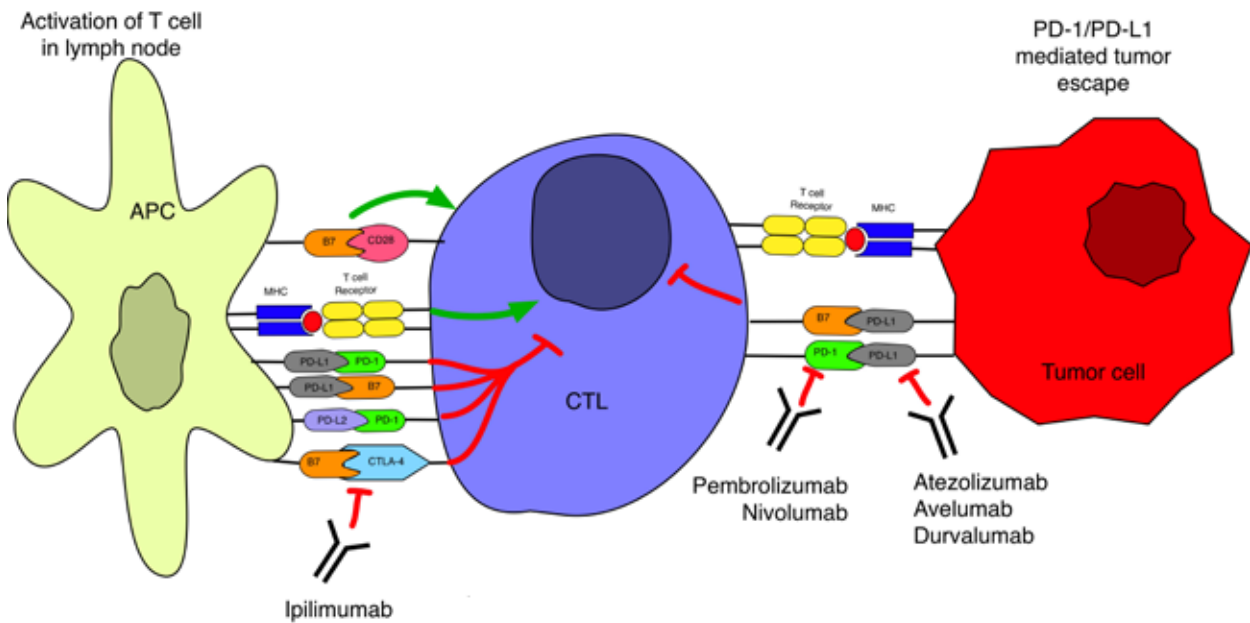
Checkpoint Inhibition in Bladder Cancer

Among the most promising novel immunotherapies in urothelial cancer are immune checkpoint inhibitors. When MHC-bound tumor antigens are presented to the T-cell receptor (TCR), simultaneous co-stimulatory binding of the CD28 and B7 ligands are necessary for CTL-mediated tumor destruction.¹⁵ Conversely, co-inhibitory signals produced by the binding of PD-1 on the T cell to its ligand, PD-L1, on the tumor, and to APC blunt the immune response; this allows the tumor to evade immune-mediated destruction. This pathway has been extensively studied in bladder cancer, as urothelial carcinoma cells can have rich PD-L1 expression. The presence of PD-L1 is both common in advanced disease and predictive of all-cause mortality after cystectomy.¹⁶ New monoclonal antibodies to both PD-1 and PD-L1 disrupt the interaction of these 2 cellular proteins, allowing for the stimulation of infiltrating CTLs and subsequent tumor destruction (**Figure**).

The FDA-approved anti-PD-1/PD-L1 agents available for systemic use in advanced bladder cancer in the United States are shown in the **Table**. Atezolizumab was the first anti-PD-L1 antibody to gain FDA approval. Breakthrough therapy status was granted after phase I data from 67 patients—all with metastatic urothelial cancer who had failed prior chemotherapy—showed that they had a 43% ORR if their tumors had a high level of PD-L1 expression on tumor-infiltrating lymphocytes, and an 11% ORR if they did not.¹⁷ Subsequently, cohort 2 of the phase II trial IMvigor210 confirmed the efficacy of atezolizumab. In that cohort's 310 patients, all with metastatic urothelial carcinoma and whose tumors had progressed on or post platinum, treatment with atezolizumab yielded an ORR of 15%, higher than the historic 10% response rate seen with single-agent chemotherapy, which was the rate the researchers had expected. Complete response (CR), a phenomenon nearly unheard-of in prior postplatinum studies, was demonstrated in 6% of patients. Patients with the highest levels of PD-L1 expression had a CR rate of 11% and an ORR of 26%.¹⁸ Atezolizumab was well tolerated in this heavily pretreated population, many of whom had renal insufficiency or impaired functional status. Furthermore, responses tended to be durable. These data led to accelerated FDA approval of atezolizumab in the second line and beyond in May 2016.

Cohort 1 of the IMvigor210 trial included 123 cisplatin-inel-

FIGURE. Checkpoint Blockade With PD-1/PD-L1 Inhibitors



Interaction of PD-1/PD-L1/PD-L2 inhibits T-cell-mediated tumor killing. PD-1/PD-L1/PD-L2 interaction is usually associated with suppression of the immune-mediated antitumor response. Blocking the binding of PD-1 to the PD-L1 ligand allows for a more robust antitumor response.

APC indicates antigen-presenting cell; MHC, major histocompatibility complex.

igible, treatment-naïve patients with locally advanced or metastatic urothelial cancer. The ORR with 17 months of follow-up was 28%, with 6% of patients achieving a CR. Patients with low PD-L1 expression still achieved an ORR of 21%.¹⁹ The biologic rationale for differential responses based on PD-L1 expression between cohorts 2 and 1 in IMvigor 210 has not been explained. These data led to accelerated FDA approval in January 2017 for first-line atezolizumab in platinum-ineligible patients with locally advanced or metastatic urothelial cancer.

Despite these encouraging results, the phase III IMvigor 211 trial of second-line chemotherapy versus atezolizumab failed to meet the primary endpoint of improved overall survival (OS).²⁰

However, in the phase III KEYNOTE-045 trial, the PD-1 inhibitor pembrolizumab did show improved OS compared with second-line chemotherapy. A total of 542 patients who had tumor progression on or post platinum based chemotherapy in the perioperative or advanced setting were assigned to pembrolizumab once every 3 weeks or single-agent paclitaxel, docetaxel, or vinflunine. The pembrolizumab arm had improved OS (10.3 vs 7.4 months) compared with chemotherapy, with fewer grade 3 or greater adverse events (15% vs 49.4%).²¹ Pembrolizumab has since been approved for use in metastatic urothelial cancer in the second line as of May 2017. Based on

the 28.6% ORR in the KEYNOTE-052 trial, which enrolled cisplatin-ineligible treatment-naïve patients, pembrolizumab is also approved in the first-line setting. Pembrolizumab differs from atezolizumab in that it is a monoclonal antibody to PD-1 instead of PD-L1, so it acts on the surface of the T cell; atezolizumab acts on PD-L1 expressed both on the tumor surface and on APCs. Additionally, PD-1 binds to the PD-L2 receptor (Figure). By blocking PD-1, both PD-1/PD-L1 and PD-1/PD-L2 interactions are disrupted. Conversely, anti-PD-L1 has no effect on PD-1/PD-L2 binding. The relevance of this is not yet understood and whether or not this has contributed to the differing results of IMvigor211 and KEYNOTE-045 regarding OS has yet to be seen.²²

Nivolumab is another anti-PD-1 monoclonal antibody that gained accelerated FDA approval for second-line use following platinum-based chemotherapy. Approval came in February 2017 after the phase II trial CheckMate 275 demonstrated a 19.6% ORR, with a median response duration of 10.3 months.²³ The anti-PD-L1 inhibitors durvalumab and avelumab were also given accelerated FDA approval. The phase I trial of durvalumab included 61 patients, 40 of whom demonstrated >25% PD-L1 positivity in tumor or immune cells. The ORR was 31%; however, none of the PD-L1-nega-

TABLE. Checkpoint Inhibitors Approved for Systemic Use in Advanced Bladder Cancer, as of October 2017

Drug	Manufacturer	Target	Companion Biomarker	Approved Use in Urothelial Carcinoma	Date(s) of Approval for Bladder Cancer	Approval in Other Malignancies
Atezolizumab	Genentech	PD-L1	Ventana PD-L1 (SP142) ^a	1, 2, 3	5/18/2016 ^{1,2} 4/17/2017 ³	Non-small-cell lung
Avelumab	Pfizer	PD-L1	Dako 73-10	1, 2	5/9/2017	Merkel cell
Durvalumab	AstraZeneca	PD-L1	Ventana PD-L1 (SP263) ^a	1, 2	5/1/2017	—
Nivolumab	Bristol-Myers Squibb	PD-1	Dako 28-8 ^b	1, 2	2/2/2017	Melanoma, non-small-cell lung, classical Hodgkin lymphoma, colorectal, head and neck squamous cell, renal cell carcinoma, hepatocellular cancer, gastric cancer
Pembrolizumab	Merck	PD-1	Dako 22C3 ^b	1, 2, 3	5/18/2017	Melanoma, non-small-cell lung, head and neck squamous cell, classical Hodgkin lymphoma, microsatellite instability-high (various forms), hepatocellular cancer, gastric cancer

1. Locally advanced or metastatic urothelial carcinoma with progression during or after first-line treatment with platinum-based chemotherapy.
2. Locally advanced or metastatic urothelial carcinoma progressing within 12 months of neoadjuvant / adjuvant platinum-based chemotherapy.
3. Locally advanced or metastatic urothelial carcinoma in those who are cisplatin-ineligible.

^aFDA approved companion biomarker.

^bFDA approved biomarker in other malignancies.

tive patients had a response. For PD-L1–positive patients, the ORR was 46.4%.²⁴ Avelumab was tested in the JAVELIN phase I trial, which included 44 patients with metastatic urothelial carcinoma; they had had at least 1 prior chemotherapy or were platinum-ineligible. The ORR in this study was 18.2%, with an ORR of 50% in PD-L1–positive patients versus 4.3% in PD-L1–negative ones.²⁵ Avelumab is unique in that it causes antibody-dependent, cell-mediated cytotoxicity, which directly lyses cells independent of PD-1/PD-L1 checkpoint blockade. However it remains to be seen if, compared with the other anti-PD-L1 monoclonal antibodies, this results in improved efficacy or a differing safety profile.²⁶

PD-L1 tissue biomarker testing is not mandated by the FDA in routine clinical practice. Key differences in biomarker testing technique—including on tumor cells, on tumor-infiltrating cells, or both, and varying cutoffs for positivity of PD-1/PD-L1—prohibit direct comparison of biomarker data in these studies. Phase I and II trials of atezolizumab evaluated expression of PD-L1 on infiltrating-immune cells using immunohistochemistry (IHC) staining with the Ventana SP142 assay and a cut-off point of 5% for determining high and low expression.^{17,18} In the KEYNOTE studies of pembrolizumab, IHC staining for PD-L1 on tumor cells was determined using the 22C3 antibody and a cutoff of 1% or more to select for PD-L1 positivity.²¹ The CheckMate 275 study of nivolumab used both 1% and ≥5% staining on tumor cells as a positive biomarker, but used yet another proprietary IHC stain, Dako PD-L1.²³ Durvalumab

was studied with the Ventana SP263 assay on both tumor and infiltrating T cells with a cutoff point of 25%, while in the avelumab trial, biomarker positivity was defined as ≥5% PD-L1 tumor staining using a Dako assay.^{24,25} Further understanding of the relevance of PD-1/PD-L1 IHC staining as a biomarker to predict response is complicated by the variable and changing expression of PD-L1 in tumors, making clinical use of these assays a source of continuing investigation.

It has been well described in melanoma and other malignancies that patients can have variable responses to immune checkpoint inhibitor therapy, including initial RECIST progression of the disease, and—unlike comparable chemotherapy outcomes—still achieve a long-term clinical benefit.²⁷ In the IMvigor210 trial, atezolizumab treatment was continued post progression in 134 patients. In 19% of these individuals, there was a 30% or greater decrease in their target lesion on subsequent follow-up.²⁸ This observation has been consistent with use of checkpoint inhibitors in other genitourinary malignancies; CheckMate 025, for instance, led to the approval of nivolumab in metastatic renal cell carcinoma. In this study, 140 patients were treated after progression and 14% demonstrated a >30% reduction in tumor volume.²⁹ Modified RECIST criteria are used in many immunotherapy studies to account for the phenomenon of initial progression followed by response.

Patients with urothelial cancers tend to be older, with comorbidities that include cardiovascular disease, renal insufficiency, impaired function status, hearing issues, and

peripheral neuropathy. These problems can be exacerbated by, or may preclude, cisplatin-based chemotherapy. The PD-1/PD-L1 checkpoint inhibitors are generally more tolerable than chemotherapy. Unlike the toxicity of chemotherapy, which tends to be progressive and cumulative, the development of toxicity on checkpoint blockade therapy can be immune-related, with a variable time course. Treatment delay or discontinuation is the mainstay of treatment, with steroids or immune-modulating therapy reserved for patients with serious or life-threatening toxicity. These agents have an unclear impact on the long-term efficacy of the immunotherapy.³⁰

Future Directions

Excitement surrounding checkpoint blockade has spread as other molecular interactions involved in the immune evasions of cancers are investigated. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibition by ipilimumab has been extensively studied in melanoma. Functionally, the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a CD28 type receptor found on the surface molecule of regulatory T cells. Interaction with the B7 ligand functions to dampen activation of CTLs by inhibiting the stimulatory effect of CD28/B7 coupling (Figure). By adding a monoclonal antibody against CTLA-4, APCs are better able to stimulate a T-cell response to a neoantigen.³¹ Results of ongoing studies will indicate the effectiveness of CTLA-4 inhibition in bladder cancer; however, early data about neoadjuvant ipilimumab administered before cystectomy suggest that CTLA-4 inhibition results in an increase of tumor-infiltrating CD4 T lymphocytes, which has corresponded to clinical benefit in melanoma patients.³²

Ipilimumab has also been shown to be active in combination with nivolumab. Ten patients with metastatic urothelial cancer that had progressed on nivolumab monotherapy received combination therapy. Of the 10 patients, 1 had a partial response and 4 had stabilization of their disease after the addition of ipilimumab to their immunotherapy regimen.³³

Conclusion

The successful treatment of bladder cancer with BCG has hinted at the promise immunotherapy holds for the treatment of this disease. As our understanding of immune-mediated pathways increases, a familiarity with the immune system and the therapies used to manipulate it will be necessary for all clinicians. The many targetable molecules in immune-mediated pathways, as well as a drug combination that is outside the scope of this discussion, are currently in development. These recently discovered and not-yet-known interactions between the immune system and cancer cells will hopefully bring us closer to curing urothelial malignancies.

Author affiliations: J. Ryan Mark, MD, and Leonard G. Gomella, MD, are with Department of Urology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA. Jean Hoffman-Censits is with the Department of Medical Oncology,

Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA.

Address correspondence to: J. Ryan Mark, MD, Department of Urology, Sidney Kimmel Cancer Center, Thomas Jefferson University, 1025 Walnut St, Suite 1100, Philadelphia, PA 19107. E-mail: James.R.Mark@jefferson.edu.

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