Immunotherapy: The Wave of the Future in Bladder Cancer?

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Abstract

Urothelial cell carcinoma (UC) is one of the most common cancers and one of the most deadly. Metastatic UC is particularly hard to treat, because it is typically diagnosed when patients are elderly and have medical comorbidities. Many patients with metastatic UC are unable to receive cisplatin-based chemotherapy, due to older age at diagnosis and comorbidities, and even when platinum chemotherapy can be administered, it has limited success in prolonging survival. Recently, improved understanding of molecular targets and immunologic characteristics of urothelial tumor cells has resulted in new therapeutic approaches that may help optimize first- and second-line therapy. The most exciting of these approaches is inhibition of cytotoxic T-lymphocyte–associated antigen 4 or programmed cell death protein 1. These so-called "immune checkpoint" proteins are negative regulators of T-cell immune function, and inhibiting these proteins results in increased activation of the immune response to tumors. Two checkpoint inhibitors, atezolizumab and nivolumab, are now approved by the Food and Drug Administration as second-line therapy for advanced UC, and a wealth of clinical trials of these and other agents are ongoing. This review shows how oncology clinicians can incorporate checkpoint inhibitors into the management of patients with locally advanced or metastatic UC. It also introduces other forms of immunotherapy that are being investigated in bladder cancer: antibody-drug conjugates, vaccines, adoptive immunotherapy, and recombinant Bacillus Calmette–Guérin.

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Introduction

Bladder cancer is the sixth most common form of cancer in the United States and ranks fourth among men.[1] In 2017, it is expected that there will be 79,030 new cases of bladder cancer and 16,870 deaths due to the disease.[1] There is a male:female ratio of about 3:1 for new cases and about 2:1 for deaths.[1] Multiple factors may account for this, including the greater likelihood of men being smokers and their greater risk of occupational exposure to chemicals. Approximately 2.4% of men and women in the United States will be diagnosed with bladder cancer at some point during their lifetime, and an estimated 587,426 people are living with the disease.[2]

Worldwide, 90% of cancers that form in the bladder, the renal pelvises (the lower part of the kidneys), the ureters, and the proximal urethra derive from urothelium, a specialized mucous membrane.[3] The cancers are, therefore, called urothelial cell carcinoma (UC). Because the urothelium is also termed the transitional epithelium, UC is sometimes referred to as transitional cell carcinoma.

Urothelial carcinoma is divided into 3 categories, each differing in prognosis and management. Most new cases (70%-80%) are diagnosed as non–muscle-invasive UC (NMIUC), which is associated with a 15-year survival rate of 62% to 95%.[4] For these tumors, transurethral resection of bladder tumor is the standard of care, with adjuvant intravesical bacillus Calmette–Guérin (BCG) or intravesical chemotherapy added when the NMIUC is high-risk.

Twenty to 30% of cases are diagnosed at a later stage, when one or more tumors have invaded the muscularis mucosa, which forms a layer on the bladder wall.[4] This muscle-invasive disease (MIUC) can progress rapidly to metastatic disease, so it is treated much more aggressively, with partial or radical cystectomy, and with neoadjuvant or adjuvant chemotherapy.

The third category of disease, metastatic UC (mUC), is very challenging to treat, partly because bladder cancer is most frequently diagnosed between the ages of 75 to 84 years (median age at diagnosis 73 years), with only 2% of patients diagnosed before age 45.[2] Up to half of patients with mUC are unable to receive cisplatin-based chemotherapy, the standard of care for first-line treatment, due to poor performance status or medical comorbidities such as renal

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impairment, ischemic heart disease, or peripheral neuropathy.[5] For previously untreated patients who receive platinum chemotherapy, overall survival (OS) is about 9 to 15 months,[6] and the response rates with second-line chemotherapies are generally 20% or less, with median survival of 6 to 9 months.[7] Altogether, the 5-year survival rate for mUC is dismal, 5%.[2]

Thus, more effective and less toxic options for systemic treatment of advanced bladder cancer are urgently needed. As in certain other solid tumors, immune checkpoint inhibition is an exciting development in UC therapy. Atezolizumab and nivolumab are approved by the Food and Drug Administration (FDA) for second-line therapy, and a number of other checkpoint inhibitors are in clinical trials. This supplement reviews how oncology clinicians can incorporate checkpoint inhibitors into the management of patients with locally advanced or metastatic UC, and it introduces other forms of immunotherapy that are being investigated in bladder cancer.

The First Immunotherapy: BCG

Bacillus Calmette–Guérin, a weakened form of the bovine tuberculosis bacterium *Mycobacterium bovis,* was initially developed as a tuberculosis vaccine. In 1976, a Canadian oncologist, Alvaro Morales, described tumor responses in 4 of 10 patients who received intravesical BCG for NMIUC.[8] Subsequent randomized trials confirmed reduced rates of recurrence and progression, with positive effects on mortality. In 1990, the FDA approved intravesical BCG as the first cancer immunotherapy, and it has since become the gold standard for treating high-risk NMIUC. Multiple BCG substrains are used globally for UC immunotherapy, and they have different product characteristics and strengths, which lead to different dosing levels and schedules. However, all strains are effective and are considered clearly superior to intravesical chemotherapy.[9,10] The American Urological Association recommends 3 years of maintenance therapy for patients who can tolerate it.[11] The optimal schedule is unknown, but a schedule evaluated by the Southwestern Oncology Group in a randomized trial was associated with significantly increased recurrence-free survival compared with induction BCG alone.[12] In that study, BCG was instilled weekly for 3 weeks at months 3, 6, 12, 18, 24, 30, and 36 from the start of 6 weeks of induction therapy.

The most frequent adverse events (AEs) associated with BCG are urinary frequency, irritative bladder symptoms, hematuria, low-grade fever, and flu-like symptoms. These are generally tolerable with supportive care. However, severe AEs can occur, typically due to local or systemic infection with live BCG. These uncommon events include BCG sepsis, granulomatous prostatitis, granulomatous epididymo-orchitis, allergic reaction, contracted bladder, hepatitis, and pneumonitis. They require discontinuation of BCG and, usually, rapid initiation of antituberculous therapy.[13]

A number of other potential problems make BCG a suboptimal therapy. Some 30% to 45% of patients do not respond completely, and of those who do, up to 50% will have a recurrence with substantial risk of progression to MIUC.[14] Furthermore, up to 20% of patients cannot tolerate BCG because of AEs.[14] Healthcare workers are also at risk because of the use of live bacterium. Finally, issues associated with the BCG manufacturing process have complicated matters by creating a worldwide shortage.

While the mechanisms of BCG are not fully understood, it is known that BCG is internalized by urothelial cells and bladder cancer cells, some of which can be killed by direct BCG-related toxicity. In addition, internalized BCG induces production of proinflammatory cytokines that recruit immune effector cells, including CD4+ T cells, CD8+ T cells, and natural killer cells. These cells are a major source of Th1 cytokines that induce cytotoxic killing of cancer cells. Thus, response to BCG requires an intact immune system.[13,15,16]

Immune Checkpoint Inhibitor Therapy

Background

The mechanism of checkpoint inhibitors is much different from that of BCG and warrants a brief review of cancer immunology. To distinguish self from nonself, the immune system relies on T-cell receptors recognizing and binding to antigens presented by a protein complex called the major histocompatibility complex (MHC) on the surface of antigen-presenting cells (APC) (Figure 1).[17] However, the MHC presents self-antigens as well as foreign antigens, and T cells need to distinguish those they should attack. When the CD28 protein on the T cell binds to the B7 protein on the APC, the T cell is stimulated.

Once activated, T cells upregulate expression of cell-surface proteins called "immune checkpoints" that help to control immune response. When engaged with their ligands, checkpoint proteins modulate T-cell activation, Th1 cytokine production, and cell-mediated cytotoxicity. This physiologic "braking" of the immune system is important because it prevents unchecked inflammation and autoimmunity. In a cancer patient, however, it can allow tumor cells, which would normally be recognized by T cells, to evade the immune system.

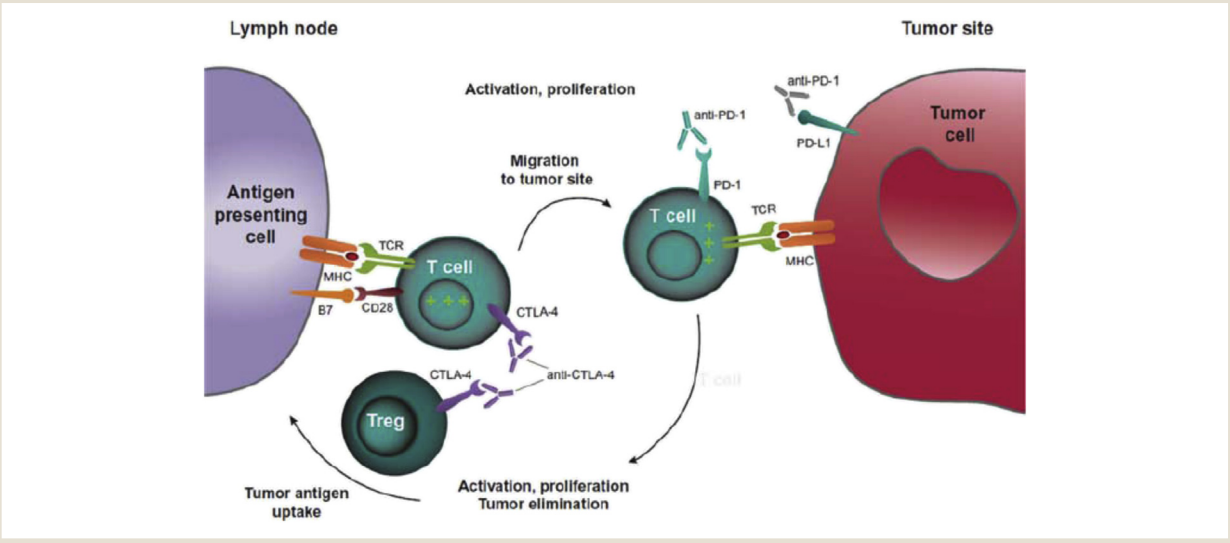
A large number of checkpoint proteins have been identified, and to date, drugs that inhibit 2 of them, cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), have demonstrated significant clinical activity. These proteins are thought to operate at different stages of an immune response (Figure 1). CTLA-4 is considered the lead checkpoint, because it stops initial T-cell activation, typically in lymph nodes. The PD-1 pathway is located in the tumor microenvironment, where it dampens ongoing immune responses after T cells have been activated. Unlike CTLA-4, which is expressed only on T cells, PD-1 is expressed on a broad range of cells, including activated and "exhausted" (nonfunctional) T cells, tumor-infiltrating T cells, and APCs, such as B cells, dendritic cells, and macrophages.

Mechanisms of Checkpoint Inhibition

CTLA-4 Blockade. As explained above, for a T cell to become fully activated, the B7 molecule on the APC must bind to the CD28 receptor on the partially activated T cell (Figure 1). Expression of CTLA-4 on a T cell interferes with this process, in that CTLA-4 outcompetes CD28 and binds with B7, which results in downregulation of T-cell activity. Monoclonal antibodies against CTLA-4 block the interaction between CTLA-4 and its B7 ligands, allowing activation of more T cells.

PD-1 and PD-L1 Blockade. The PD-1 pathway consists of the PD-1 receptor and its 2 ligands, PD-L1 and PD-L2. Both PD-1 and

Figure 1 Schematic of an Immune Response to a Tumor Cell, Showing 2 Immune Checkpoint Pathways (CTLA-4 and PD-1) and Opportunities for Blocking Them



Abbreviations: CTLA-4 = cytotoxic T-lymphocyte–associated antigen 4; MHC = major histocompatability complex; PD-1 = programmed cell death protein 1; PD-L1 = PD ligand 1; TCR = tumor cell receptor.[17]

PD-L1 become highly expressed on many types of tumors, including UC, and the PD-1/PD-L1 pathway is probably dominant for allowing tumors to escape from the immune system. The PD-2 ligand is expressed by a more limited cell population and has not been well studied.

Binding of PD-1 with PD-L1 inhibits T-cell proliferation, production of Th1 cytokines, and cytolytic activity, which leads to the "exhaustion" of T cells. Monoclonal antibodies against PD-1 or PD-L1 allow T cells to remain activated, restore the activity of those that have become nonfunctional, and reduce the immunosuppression produced by T-regulatory cells.

Humanized monoclonal antibodies that block CTLA-4 (ipilimumab, tremelimumab), PD-1 (nivolumab, pembrolizumab), or PD-L1 (atezolizumab, durvalumab, avelumab) have all demonstrated antitumor activity in patients with UC. Clinical data on each of these agents are presented below.

Mutational Load and Checkpoint Inhibition

A strong rationale for using checkpoint inhibitors in UC is that this tumor type carries the third highest mutation rate of all studied cancers.[6] Mutant proteins result in the production of abnormal antigens, called neoantigens, that the immune system can recognize as foreign because of their novelty. In patients with melanoma or lung cancer, high mutational load has been correlated with better response rates to immune checkpoint blockade.[18,19]

Issues in Using Checkpoint Inhibitors

Pseudoprogression. Because checkpoint inhibitors work by "restarting" an antitumor immune response, patients' response to them is affected by the kinetics and efficacy of each patient's immune system and its interplay with tumors and metastases.[17] In some patients, response patterns to checkpoint inhibitors differ from those seen with chemotherapy or targeted agents. Fast activation of an antitumor immune response can lead to edema and an influx of immune cells into the tumor site, which can masquerade as tumor progression until the tumor shrinks.[20] Similarly, tumor shrinkage in some patients is delayed until after the appearance of new lesions.

These response patterns are termed "pseudoprogression" because by the conventional criteria for judging tumor response (ie, Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1),[21] they would be classified as progressive disease. This is a serious concern, because oncologists normally use RECIST to judge whether a patient is failing drug therapy and needs alternative treatment.

The immune-related response criteria (irRC) were developed to capture patterns of response beyond those seen with cytotoxic agents.[22] Table 1 compares RECIST with the irRC.[23] One of the important differences between the 2 sets of response criteria is the concept of total tumor burden. The irRC call for evaluation of all lesions to define the response pattern; the appearance of new lesions is not considered progressive disease per se.

In clinical trials of ipilimumab, an anti–CTLA-4 agent, in advanced melanoma, nearly 10% of 227 patients were initially characterized as having progressive disease but later had evidence of activity that was consistent with response.[22] A review of trials of PD-1 and PD-L1 inhibitors showed that about 4% of 1126 patients had immune-related patterns of response that did not meet RECIST criteria.[20] In bladder cancer, the rate was 1.5%. Not all patients in these trials were evaluated using the irRC, so the rates are probably underestimates.

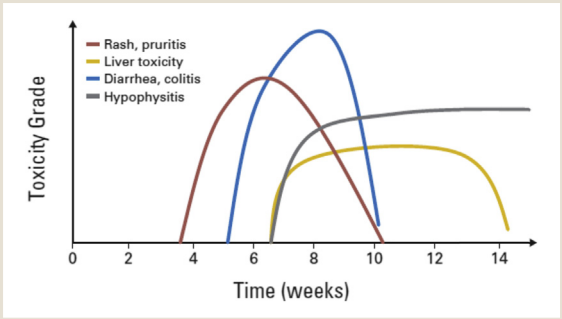
As more patients with UC receive checkpoint inhibitors, clinicians should keep in mind these important principles: (a) the

Table 1 Comparison of Key Differences Between RECIST v1.1 and Immune-Related Response Criteria[23]

Category	RECIST v1.1	irRC
Measurement of tumor burden	Unidimensional	Bidimensional
Target lesions	Maximum, 5[a]	Maximum, 15 index lesions
New lesion	Results in progressive disease at first appearance	Up to 10 new visceral lesions and 5 cutaneous lesions may be added to the sum of the products of the two largest perpendicular diameters of all index lesions at any time point
Complete response	• Disappearance of all target and nontarget lesions • Nodes must regress to <10 mm short axis • No new lesions • Confirmation required	
Partial response	• ≥30% decrease in tumor burden compared with baseline • Confirmation required	• ≥50% decrease in tumor burden compared with baseline[b] • Confirmation required
Progressive disease	• ≥20% + 5-mm absolute increase in tumor burden compared with nadir • Appearance of new lesions or progression of nontarget lesions	• ≥25% increase in tumor burden compared with baseline, nadir, or reset baseline[b] • New lesions added to tumor burden • Confirmation required
Stable disease	Neither partial response nor progressive disease	

Abbreviations: irRC = immune-related response criteria; RECIST v1.1 = Response Evaluation Criteria in Solid Tumors, version 1.1.
[a]For the present analyses, the maximum number of target lesions was 10.
[b]If an increase in tumor burden is observed at the first scheduled assessment, the baseline is reset to the value observed at the first assessment.

Figure 2 Kinetics of Immune-related Adverse Events With Ipilimumab[26]



appearance of measurable antitumor activity may take longer than for cytotoxic therapies; (b) responses may occur after conventional progressive disease is noted; (c) discontinuation of therapy may be inappropriate in some cases unless progressive disease is confirmed; (d) allowance should be made for "clinically insignificant" progressive disease (eg, small new lesions in the presence of other responsive lesions); and (e) durable stable disease may represent antitumor activity.[22,24]

Immune-related Adverse Events. Checkpoint inhibitor therapy is often associated with immune-related AEs (irAEs) that differ from those associated with cytotoxic chemotherapy. They can cause inflammation in nearly every organ system, since the immune system is being "revved up" systemically, and they occur in characteristic patterns, sometimes quite delayed or even arising after treatment has ended.[25] Figure 2 gives an example, showing the major categories of irAEs associated with ipilimumab and the characteristic patterns in the timing of their occurrence.[26] Across tumor types, common irAEs include dermatologic effects, constitutional symptoms, endocrine effects, hepatotoxicity, and pulmonary effects.[27] In this review, the section on clinical trials of checkpoint inhibitors describes the types and rates of irAEs that are being noted in UC.

PD-1 and PD-L1 blockers seem to be associated with lower rates of irAEs compared with anti–CTLA-4 agents.[28,29] A potential explanation is that CTLA-4 is highly expressed on regulatory T cells, which are abundant in multiple tissue types including the skin, gut, and liver.[30] In contrast, expression of PD-L1 is limited to tumors and areas of active inflammation.

Whichever checkpoint inhibitor is administered, patients should be carefully selected for treatment and be closely monitored, both during treatment and for 1 year after therapy has ended.[25,31] It should be noted, though, that most trials have excluded patients with preexisting autoimmune conditions, so there are no data about how to use checkpoint inhibitors in that setting. Detailed recommendations for managing irAEs are given below.

Use of PD-L1 as a Biomarker. Results from retrospective biopsy studies in UC disagree about whether baseline PD-L1 expression on tumor cells is associated with survival and tumor aggressiveness. Some research found PD-L1 to be a biomarker for prognosis,[32-34] some showed no relationship between PD-L1 expression and survival,[35] and some had mixed results.[36,37] This discordance is attributable at least in part to the different methodologies for measuring PD-L1 status, which include differences in the antibody clones used, the positivity cutoffs, and the type of cells analyzed (tumor cell or tumor-infiltrating immune cell).[30]

As reflected in the section on clinical trial data below, it still has not been determined whether PD-L1 status will be useful as a predictive biomarker. Notably, a proportion of patients in UC trials who have no measurable PD-L1 at baseline have gone on to respond to PD-1 or PD-L1 therapy. This raises concerns, since excluding PD-L1–negative patients may exclude potential responders to therapy.

Clinical Trials of Checkpoint Inhibitors

CTLA-4 Inhibitors. Ipilimumab was the first checkpoint inhibitor to be approved by the FDA. It binds to CTLA-4, blocking its interaction with its B7 ligands. Ipilimumab was approved by the FDA based on significantly improved OS in metastatic melanoma, a highly immunogenic cancer.[38]

However, an early phase 2 study of ipilimumab in advanced UC demonstrated an objective response rate (ORR) of only 5%.[39] This agent has shown more promise when combined with chemotherapy. Data have been presented from a phase 2 trial in which 36 patients

with mUC received 2 cycles of gemcitabine plus cisplatin (GC), followed by 4 cycles of GC plus ipilimumab.[40] After median follow-up of 10.4 months, the ORR was 64% and median progression-free survival (PFS) was 8 months. However, the trial did not demonstrate superiority of ipilimumab on the primary endpoint, 1-year OS, which was 14.6 months.

A published phase 2 trial of ipilimumab plus gemcitabine in mUC suggests efficacy and manageable toxicity, but the study was closed early due to enrollment delays and loss of funding.[41]

Tremelimumab, another CTLA-4 blocker, has FDA orphan drug status for treatment of malignant mesothelioma. A phase 2 study in advanced solid tumors, including UC, is ongoing (ClinicalTrials.gov identifier NCT02527434).

Atezolizumab. Atezolizumab binds to PD-L1 and blocks its interactions with both the PD-1 and B7.1 (also known as CD80) receptors. In May 2016, it was approved by the FDA as second-line treatment for locally advanced and metastatic UC, following platinum-containing chemotherapy, and is now a standard of care in this setting. At the same time, a complementary diagnostic test was approved to help guide decision-making: the SP142 assay (Ventana, Tucson, AZ, USA), which uses immunohistochemistry (IHC) to evaluate PD-L1 status on tumor-infiltrating immune cells. Subsequently, atezolizumab was approved as second-line treatment of metastatic non—small-cell lung cancer (NSCLC). It has also demonstrated clinical activity in melanoma, breast cancer, and renal cancer.[42]

A phase 1 trial of second-line atezolizumab in mUC showed that a checkpoint inhibitor can produce clinically significant responses in bladder cancer.[43] The trial had such striking results that the FDA granted the drug breakthrough therapy status, and later gave it priority review for advanced UC, before granting approval.

Responses to atezolizumab in this study were associated with the PD-L1 status of tumor-infiltrating immune cells, measured using IHC. After at least 6 weeks of follow-up, the ORR was 43% for the 30 patients categorized as IHC 2/3 (having at least 5% of cells PD-L1 positive). In comparison, the ORR was 11% for the 35 patients categorized as IHC 0/1 (having <5% of cells PD-L1 positive); in fact, 8% of PD-LD negative patients responded. The IHC 2/3 group had a 7% complete response (CR) rate.[43]

Many patients in this study had poor prognostic factors at baseline: 75% had visceral metastases, 19% had hemoglobin levels <10 g/dL, 33% had creatinine clearance <60 mL/min, 59% had an Eastern Cooperative Oncology Group performance score (ECOG PS) of 1, and 42% were started on atezolizumab within 3 months of previous chemotherapy. Even so, only 4% of patients had a grade 3 treatment-related AE, and none had a grade 4/5 treatment-related AE. The most common toxicities, thought to be related to immune system activation, were decreased appetite and fatigue, always grade 1 or 2.[43]

An updated analysis of this study reported on 94 efficacy-evaluable patients who had at least 12 weeks of follow-up.[44] The ORR was 27%, including a 10% CR rate, and the median duration of response was 22.1 months. At a median follow-up of 24 months, median OS was 10.6 months, the 1-year OS rate was 47%, and the 2-year OS rate was 29%. Grade 3/4 treatment-related AEs were recorded for 8% of the 95 safety-evaluable patients, and no treatment-related deaths were reported.

Approval of second-line atezolizumab by the FDA was based on IMvigor210, a multicenter phase 2 trial.[6] The 310 participants had inoperable locally advanced or metastatic UC that had progressed after platinum-based chemotherapy. PD-L1 expression was assessed by IHC using the SP142 assay and was categorized as IHC0 (<1% PD-L1-positive immune cells), IHC1 (≥1% but <5%), and IHC2/3 (≥5%). The patients were evenly distributed among these groups.

To assess whether RECIST v1.1 adequately captures response to atezolizumab, the investigators designated 2 co-primary endpoints: ORR according to RECIST v1.1 and ORR according to irRC. The study was designed to detect an improvement in ORR by RECIST compared with a historical 10% response rate.[6]

The primary analysis showed that, according to RECIST, treatment with atezolizumab (1200 mg every 3 weeks) resulted in a significantly improved ORR for each IHC group compared with the historical response rate. After a median follow-up of 11.7 months, the ORR was 15% (15 CR) by RECIST and 19% (16 CR) by irRC. Of PD-L1 negative patients, 8% to 13% responded (depending on the criteria used), including 2 who had CR. The median duration of response had not yet been reached in any IHC group. At the time of data cutoff, ongoing responses were reported in 38 (84%) of the 45 responding patients and the median time to response was 2.1 months.[6]

Median PFS according to RECIST was 2.1 months in all patients and was similar across IHC groups. However, when the irRC were used, median PFS increased in all groups. The effect was most pronounced in the IHC2/3 group: median PFS was 4.0 months in that group, versus 2.9 months in the IHC1/2/3 group and 2.7 months overall. Median OS was 11.4 months in the IHC2/3 group, 8.8 months in the IHC1/2/3 group, and 7.9 months overall (Figure 3).[6]

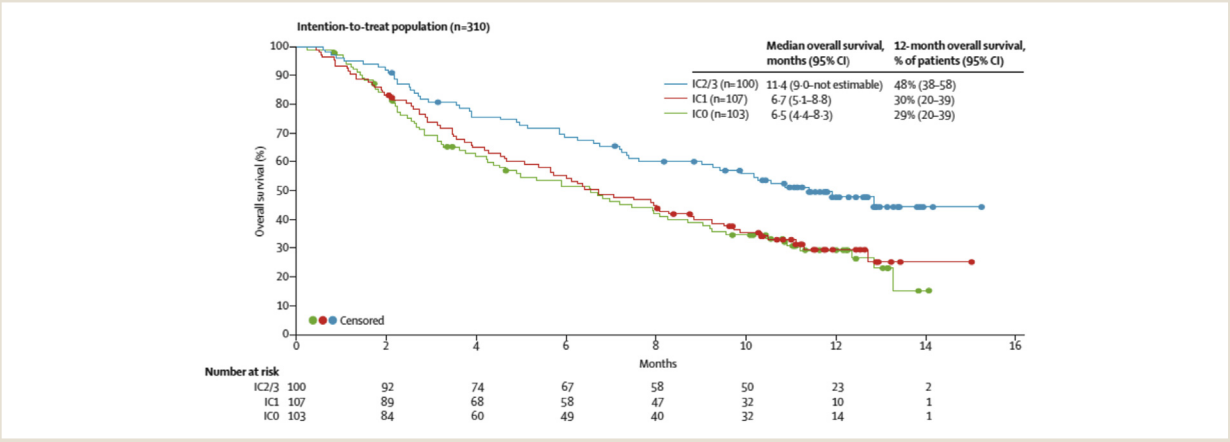
To account for the possibility of pseudoprogression, 121 patients were treated beyond progression for a median of 7.8 weeks (range, 0-51 weeks). Of these, 17% subsequently experienced target lesion reduction of at least 30% from their baseline scans.[6]

Atezolizumab was well tolerated. Treatment-related AEs of any grade were reported for 69% of the 310 patients, most of them mild or moderate in severity. Sixteen percent of patients had a grade 3/4 treatment-related AE. Most of these AEs were mild to moderate in severity. Immune-related AEs of any grade affected 7% of patients, with pneumonitis, increased aspartate aminotransferase (AST), increased alanine aminotransferase (ALT), rash, and dyspnea being the most common, each occurring in 1% of patients. Grade 3/4 immune-related AEs were reported for 5% of patients. Treatment-related serious AEs occurred in 11% of patients. The withdrawal rate due to AEs was 4%.[6]

With longer follow-up of this cohort (median 14.4 months), the investigators detected evolution of responses, including additional CR, in both IHC groups.[45] Overall the ORR was 15% (17 CRs) by RECIST and 19% (18 CRs) by irRC. Median OS was 7.9 months and the 1-year OS rate was 37%.

First-line atezolizumab was studied in a separate cohort in IMvigor210.[46] The 119 patients had mUC and were ineligible for cisplatin due to renal impairment, hearing loss, peripheral neuropathy, or ECOG PS2. After median follow-up of 17.2 months, the ORR was 23%, which included a 9% CR rate. In contrast to the

Figure 3 Overall Survival of Platinum-refractory Subjects in the IMvigor210 Study of Atezolizumab[6]



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other cohort in IMvigor210, responses were approximately equivalent across IHC groups. This difference might reflect biologic differences in the tumors (platinum-refractory vs. platinum-ineligible) or the fact that most platinum-refractory tumors were heavily pretreated.[47]

Median duration of response was not reached, median PFS was 2.7 months, and median OS was 15.9 months.[46] Thus, OS in IMvigor210 was similar to that with first-line cisplatin/gemcitabine in advanced UC[48] and better than that with first-line carboplatin/gemcitabine.[49] Treatment-related AEs experienced by at least 10% of patients were fatigue (30%), diarrhea (12%), and pruritus (11%).[46] Immune-related AEs occurred in 12% of patients, including 7% that were grade 3/4. The discontinuation rate due to AEs was 8%. There was 1 death due to sepsis, considered treatment-related.

A randomized, multicenter phase 3 trial, IMvigor211, is under way to compare atezolizumab with chemotherapy in advanced UC (NCT02302807).

Nivolumab. Nivolumab binds to the PD-1 receptor and blocks its interactions with both PD-L1 and PD-L2. In February 2017, it was FDA-approved as second-line therapy for locally advanced and metastatic UC, following platinum-containing chemotherapy. It is also approved for metastatic melanoma, NSCLC, renal cell carcinoma, and head and neck squamous cell carcinoma.

The approval of nivolumab in UC was based on CheckMate 275, a multicenter, single-arm, phase 2 trial in patients with metastatic or advanced UC who had disease progression or recurrence despite at least 1 platinum-based chemotherapy regimen.[50] About half of the 265 patients evaluable for efficacy (46%) had PD-L1 expression of ≥1%, and 30% had PD-L1 expression of ≥5%. The ORR, the primary endpoint, was 19.6%, and the CR rate was 2.0%. Higher response rates were observed among patients with PD-L1 expression ≥1% (23.8%) and ≥5% (28.4%). Responses were also observed in patients with PD-L1 <1% (16.1%).

Median duration of response was not reached, and response was ongoing among 77% of the 52 responders at the time of the analysis. After median follow-up of 7 months, the median PFS was 2.0 months. The median OS was 8.74 months in all treated patients, 5.95 months in patients with PD-L1 <1%, and 11.3 months in patients with PD-L1 expression ≥1%.[50]

Among all 270 patients who received nivolumab, 18% experienced a grade 3/4 treatment-related AE. The most frequent were fatigue (2%), diarrhea (2%), asthenia (1%), and rash (1%). Three deaths were judged to be treatment-related, all in patients with metastatic disease: pneumonitis 1 day after the second nivolumab infusion; acute respiratory failure 15 days after the fourth infusion, in a patient with lung lymph node metastases; and cardiovascular failure 16 days after the first infusion.[50]

Interim results have been published from CheckMate 032, a single-arm, multicenter, phase 1/2 trial involving 78 previously treated patients with mUC who were enrolled regardless of PD-L1 status.[51] They were treated with nivolumab 3 mg/kg every 2 weeks. The primary endpoint, ORR by RECIST, was 24.4% after minimum follow-up of 9 months (median, 15.2 months). The ORR did not differ substantially according to whether PD-L1 expression was <1% or ≥1%. The median duration of response was 9.4 months. Median PFS was 2.8 months and median OS was 9.7 months.

Grade 3/4 treatment-related AEs occurred in 22% of the 78 patients, most commonly elevated lipase (5%), elevated amylase (4%), and fatigue, maculopapular rash, dyspnea, decreased lymphocyte count, and decreased neutrophil count (3% each). Immune-related AEs of any grade were dermatologic (42%), gastrointestinal (10%), renal (9%), hepatic (5%), and pulmonary (3%). Eight patients (10%) had a serious AE that was considered treatment-related: colitis, diarrhea, mouth ulceration, nausea, oral pain, thrombocytopenia, fatigue, hyponatremia, acute kidney injury, or pneumonitis. The cases of thrombocytopenia and pneumonitis were grade 4 and required nivolumab discontinuation, and the patients subsequently died.[51]

Pembrolizumab. Pembrolizumab binds to the PD-1 receptor and blocks its interactions with both PD-L1 and PD-L2. It is FDA-approved for metastatic melanoma, NSCLC, and head and neck cancer. Data have been published from KEYNOTE-012, a phase 1b trial in recurrent or metastatic UC that selected for patients with PD-L1–positive tumor cells.[52] Pembrolizumab 10 mg/kg was given every 2 weeks for up to 24 months. Of 27 patients who had measurable disease at baseline, the ORR was 26% over median follow-up of 13 months, with an 11% CR rate and 15% PR rate per RECIST. The median duration of response was 10 months. Median PFS was 2 months, the 12-month PFS rate was 15%, median OS was 13 months, and the 12-month OS rate was 50%.

The most common treatment-related AEs in the KEYNOTE-012 safety population (n = 33) were fatigue (18%) and peripheral edema (12%). Grade 3 treatment-related AEs occurred in 5 patients (15%), of whom 2 discontinued treatment (due to myositis/rhabdomyolysis or hypercalcemia). Four patients had single irAEs (grade 2 myositis, grade 2 uveitis, grade 3 colitis, grade 3 pruritus) and 2 patients had 2 irAEs (grade 3 maculopapular rash and grade 3 stasis dermatitis; grade 3 myositis and grade 3 rhabdomyolysis). There were no treatment-related deaths.[52]

An international, randomized phase 3 trial of pembrolizumab, KEYNOTE-045, recently demonstrated a survival benefit of checkpoint inhibition over an active comparator in previously treated advanced UC.[53] Pembrolizumab 200 mg 3 times weekly was compared with paclitaxel, docetaxel, or vinflunine in 524 patients. Treatment was scheduled to be given for up to 2 years, but the Independent Data Monitoring Committee recommended that the trial be stopped early based on the results of a prespecified interim analysis. After median follow-up of 9 months, pembrolizumab was associated with significantly improved OS compared with chemotherapy (HR, 0.73; $P = .002$; median 10.3 vs. 7.4 months). There was no significant difference between treatment arms with regard to PFS, the other primary endpoint. The ORR was significantly better with pembrolizumab, 21.1%, than with chemotherapy, 11.4%. Compared with chemotherapy, pembrolizumab was associated with fewer any-grade treatment-related AEs (60.9% vs. 90.2%) and fewer grade 3 to 5 treatment-related AEs (15.0% vs. 49.4%). Four patients in each arm died due to treatment-related AEs.

First-line pembrolizumab is being studied in KEYNOTE-052, a multicenter phase 2 trial that involves patients with advanced UC who were ineligible for cisplatin.[54] Patients are receiving pembrolizumab 200 mg every 3 weeks for up to 24 months. An interim analysis has been presented on the first 100 patients after median follow up of 8 months. The ORR, the primary endpoint, was 24% by RECIST, which included a CR rate of 6%. The median duration of response had not been reached. The most common treatment-related AE was fatigue (14%), and 16% of patients experienced a grade 3/4 treatment-related AE.

Investigational PD-L1 Inhibitors. Two additional PD-L1 inhibitors, durvalumab and avelumab, have shown promising activity in multiple cancers, including UC. For durvalumab, interim results have been presented on patients with inoperable or metastatic UC, heavily pretreated with platinum-based regimens, who are participating in a phase 1/2 dose-escalation, dose-expansion study.[55] PD-L1 expression on tumor cells and tumor-infiltrating immune cells was assessed with the Ventana SP263 assay. Altogether, 61 patients were given durvalumab 10 mg/kg every 2 weeks for a median of 4 doses.

Treatment-related AEs occurred in 64% of patients, most frequently fatigue (13%) or diarrhea (10%).[55] There were 3 cases of grade 3/4 treatment-related AEs: infusion-related reaction, tumor flare, and acute kidney injury (biopsy-proven nephritis). Of the 42 patients who have been evaluated for efficacy, with follow-up of at least 12 months, 16 (38%) had an objective response by RECIST. Nearly all of the responders, 15/16, had PD-L1–positive tumor cells or tumor-infiltrating immune cells. The FDA has granted durvalumab the breakthrough therapy designation for patients with PD-L1–positive UC.

Avelumab is being studied as second-line therapy for mUC in JAVELIN, a large phase 1b trial. Data have been reported on 44 patients, unselected for PD-L1 status, who received 10 mg/kg every 2 weeks and were followed for a median of 11 months.[56] The most common treatment-related AEs were infusion-related reaction (20.5%), fatigue (20.5%), asthenia (11.4%), and nausea (11.4%). Grade 3/4 treatment-related AEs were asthenia, myositis, decreased appetite, and elevated creatine phosphokinase or AST (each 1 event). The ORR by RECIST was 18.2%, including 2 complete responses and 6 partial responses. Among the 35 patients who could be evaluated for PD-L1 expression, the ORR was substantially higher in those who were PD-L1–positive than in those who were not (50.0% vs. 4.3%). Likewise, the 24-week PFS rates were 58.3% and 16.6%, respectively. Overall, the 12-month OS rate was 50.9%.

In light of these encouraging results, an additional 129 patients with mUC were enrolled in JAVELIN, including 9 who were platinum-ineligible.[57] With a median treatment duration of 10.4 weeks, the rate of grade 3/4 treatment-related AEs was 7%, and only fatigue (reported by 2 patients) occurred in more than 1 patient. There was 1 treatment-related death due to pneumonitis. Among 109 patients with at least 4 months of follow-up, the ORR was 16.5% with 3 complete responses and 15 partial responses. Median PFS was 6.1 weeks and the 12-week PFS rate was 35.6%.

A randomized, open-label, phase 3 trial, JAVELIN Bladder 100, is comparing avelumab plus best supportive care as maintenance treatment versus best supportive care alone.[58] The study population is patients with locally advanced or metastatic UC whose disease did not progress after first-line platinum-containing chemotherapy.

Dual Checkpoint Inhibition

As explained above, there are differences in the timing and location of the checkpoint pathways. Consequently, the combination of an anti–CTLA-4 agent with an anti–PD-1/PD-L1 therapy might have an additive or even synergistic effect, resulting in greater and longer-lasting antitumor immune response.[17]

Several studies in metastatic melanoma have demonstrated that dual checkpoint inhibition with ipilimumab plus nivolumab significantly increases ORR and PFS.[59,60] Unfortunately, this improved efficacy came at the cost of substantially increased toxicity. Grade 3/4 treatment-related AEs occurred in 54% to 55% of patients on combination therapy, versus 24% to 27% of patients on ipilimumab monotherapy.[59,60] Sequential treatment may prove to be more tolerable than combination treatment, because prior CTLA-4

inhibition does not seem to predispose patients to develop irAEs with PD-1 inhibitors.[17]

For now, several studies are investigating whether combined CTLA-4 and PD-1/PD-L1 blockade is a feasible approach for UC. One of these, DANUBE, is a randomized, multicenter, open-label phase 3 study that is comparing durvalumab with or without tremelimumab against standard-of-care chemotherapy as first-line treatment for unresectable and/or metastatic UC.[61] In a phase 1/2 study, the combination of nivolumab and ipilimumab will be compared with nivolumab alone in advanced UC and other solid tumors (NCT01928394).

Data presented from a phase 1 study show that the combination of nivolumab and cabozantinib, an oral drug that inhibits MET/VEGF, was well tolerated and had clinical activity in mUC and other genitourinary tumors.[62] In an expansion of that study, cabozantinib will be administered with nivolumab or with nivolumab plus ipilimumab (NCT02496208).

Early-Stage Checkpoint Inhibition

Because mUC responds to checkpoint inhibitors, it stands to reason that a histologically similar primary tumor should respond as well.[63] Researchers are investigating whether the use of checkpoint inhibitors in MIUC may serve as a better-tolerated neoadjuvant or adjuvant treatment than traditional chemotherapy for selected patients. For example, in a phase 2 trial, 12 patients with MIUC received 1 or 2 doses of ipilimumab before radical cystectomy.[64] In this neoadjuvant setting, the safety profile was acceptable and measurable immunologic effects were noted in all patients. Trials that are planned or under way include IMvigor010, a randomized, multicenter, phase 3 trial of adjuvant atezolizumab (NCT02450331); the phase 2 ABACUS trial of neoadjuvant atezolizumab (NCT02662309); and two phase 2 trials of neoadjuvant pembrolizumab.[65,66]

Checkpoint inhibitors are also being studied in NMIUC. A phase 2 study of pembrolizumab, KEYNOTE-057, is under way for patients with BCG-refractory, high-risk NMIUC.[67] Another phase 2 trial will evaluate neoadjuvant atezolizumab in patients who have BCG-refractory NMIUC and patients with MIUC who are candidates for cystectomy but are ineligible for neoadjuvant chemotherapy (NCT02451423).

BCG might someday be combined with checkpoints inhibition in patients with NMIUC, to prevent tumor progression. Even with aggressive endoscopy, intravesical BCG fails after 5 years, on average.[68] This appears to happen at least partly because the initial immunosuppression with BCG is overcome when tumors upregulate their expression of PD-L1 to "escape" the immune system.[68] One study showed that 11 of 12 patients with NMIUC who failed BCG had extremely abundant levels of PD-L1 expression in tumor cells.[69]

Intravesical delivery of checkpoint inhibitors is another area of exploration. This approach might minimize the irAEs associated with high systemic concentrations while maintaining high local concentrations at the tumor site.[4]

Minimizing and Managing irAEs

Most patients with UC are over 70 years old, and an estimated 40% have some degree of renal impairment.[68] It is important to take every precaution so that checkpoint inhibitor therapy is well tolerated and does not interfere with quality of life.

Prior to Treatment. In addition to the typical elements of a physical examination, a baseline electrocardiogram and chest x-ray should be obtained prior to initiating checkpoint inhibitor therapy. A thoracic computed tomography scan should be performed with thin sections with and without injection, for use as a baseline reference in case pulmonary toxicity arises. All preexisting symptoms should be carefully considered, especially those related to intestinal transit, dyspnea and coughing, rash, nausea, and headaches, as well as indications of motor or sensory neuropathy and arthralgia.[25]

The patient history is also extremely important, because patients with a personal or family history of autoimmunity are at increased risk of irAEs. In addition, a history of previous infections and risk of viral infections such as HIV or viral hepatitis may indicate an increased risk of irAEs.[25]

Laboratory tests should include[25]:

- Complete blood count
- Serum electrolytes: sodium, potassium, alkaline reserve, calcium, phosphorus, uric acid, urea, creatinine with estimated glomerular filtration rate
- Glycemia
- Total bilirubin, AST, ALT, gamma-glutamyl transferase, prealbumin
- Albuminemia, C-reactive protein
- Thyroid-stimulating hormone (TSH), T4
- Cortisol and adrenocorticotropic hormone (ACTH) first thing in the morning
- Luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone
- Proteinuria: morning sample, fasting if possible—better than a urine dipstick to detect low levels of proteinuria and tubular proteinuria
- Urinary sediment
- QuantiFERON tuberculosis or tuberculin skin test in case of anterior exposure
- Virology: HIV, HCV and HBV serology
- Antinuclear antibodies, thyroid peroxidase antibody, thyroglobulin antibody

When feasible, it is desirable to obtain plasma/serum biobanking before the beginning of immunotherapy.[25]

Patient Education About irAEs. Early identification of irAEs is crucial, both to maximize the tolerability and duration of treatment, and to prevent irreversible damage. Patients should be closely monitored during treatment, but even more important is to encourage patients to self-monitor for early signs of emerging irAEs (Table 2). Patients should maintain regular contact with nurses or caseworkers, and it is important to clearly establish what communication channels patients can use to report new or worsening symptoms promptly. Also, patients should be made aware of which side effects are associated with the specific therapy they are receiving. Diarrhea, colitis, hypophysitis, and pruritus are more common with anti−CDLA-4 therapy, whereas thyroid dysfunction, arthralgias and myalgias, vitiligo, and rash are more common with PD-1 and PD-L1 blockers.[25,28]

Table 2 Symptoms That Patients Receiving Immune Checkpoint Inhibitors Should Report[25]

Symptom	Possible irAE (Non-inclusive)
Febrile headache	• Dysimmune meningitis
Non-febrile headache	• Dysimmune hypothyroidism • Progressive: hypophysitis • Acute/subacute: stroke due to vasculitis
Acute confusion	• Febrile confusion: dysimmune meningoencephalitis • Afebrile confusion: encephalitis, hypophysitis • Acute/subacute: stroke due to vasculitis • Hyperosmolar coma linked to dysimmune diabetes
Chest pain or arrhythmia	• Dysimmune pericarditis • Dysimmune myocarditis • Dysimmune pleurisy • Dysimmune gastritis
Dyspnea	• Dysimmune interstitial lung disease • Hydrops, pleurisy autoimmune • Dysimmune pericarditis • Dysimmune myocarditis
Asthenia	• Endocrine dysfunction • Renal failure on dysimmune nephropathy • Neurological and muscular dysfunction • Dysimmune hemolytic anemia • Induced connective tissue disease
Peripheral edema	• Dysimmune nephropathy with glomerulonephritis • Dysimmune pericarditis, dysimmune myocarditis • Dysimmune hypothyroidism • Dysimmune vasculitis, APLS with thrombosis • Dysimmune neuropathy
Weight loss	• Dysimmune gastritis • Dysimmune enterocolitis • Celiac disease • Dysimmune hyperthyroidism • Dysimmune hypophysitis • Dysimmune adrenal insufficiency
Influenza syndrome, fever	• ILI reaction to immunotherapy • Dysimmune colitis • Hyperthyroidism • Thrombosis • Vasculitis
Neurologic symptoms including sensory loss or motor deficits	• Dysimmune mononeuritis • Dysimmune polyradiculoneuritis/ Guillain–Barré • Encephalitis • Myelitis • Vasculitis • Myasthenia
Rash or itching	• Dysimmune hypo/hyperthyroidism • Immune-related hives, eczema
Diarrhea or abdominal pain	• Dysimmune enterocolitis • Celiac disease • Dysimmune hyperthyroidism
Nausea, vomiting	• Dysimmune enterocolitis • Ketoacidosis due to dysimmune diabetes • Dysimmune adrenal insufficiency • Dysimmune nephropathy • Dysimmune pancreatitis • Dysimmune hepatitis
Red or painful eye, visual impairment	• Dysimmune uveitis • Dysimmune retinitis • Dysimmune optic neuritis • Dysimmune encephalitis

Management of Specific irAEs. Table 3 gives the typical management of irAEs by grade.[25] Generally, the factors to be considered are whether inpatient care is required, whether symptomatic treatment alone will be sufficient, whether immunotherapy should be suspended or terminated, and whether corticosteroid therapy or an additional immunosuppressive drug should be considered. In most cases of moderate to severe side effects, collaboration with an organ specialist is recommended, except for side effects that are easily managed.

Constitutional Effects. When a patient presents with mild diarrhea, clinicians should consider other etiologies that may be responsible, such as *Clostridium difficile* infection or other bacterial/viral pathogens. It is also important to determine whether the patient is experiencing diarrhea (increase in frequency of stool) or colitis (abdominal pain, and radiographic or endoscopic findings of colonic inflammation).[27,31]

Clinicians should emphasize to patients the need to maintain hydration and consider recommending the American Dietary Association's colitis diet. In addition, antimotility agents (oral diphenoxylate hydrochloride and atropine sulfate 4 times a day) may be used. In cases in which no infectious cause has been identified and symptoms continue for more than 3 days, or increase in severity, patients should receive an oral or IV corticosteroid.[27,70]

In severe cases or if patients do not experience relief with an oral corticosteroid, hospitalization for IV corticosteroid therapy, hydration, and electrolyte management will be needed. Infliximab should be considered if IV steroid treatment does not result in symptom resolution.[27,70]

Dermatologic Effects. Like many irAEs, dermatologic effects can occur at any time, but they typically arise early in the course of treatment.[27,28] Grade 1 and 2 rashes can usually be treated with topical corticosteroid cream, but consider an oral steroid for grade 2 rash on a case-by-case basis, and definitely for grade 3/4 rash.[28,70] For pruritus, oral hydroxyzine or diphenhydramine may be helpful. Consultation with a dermatologist is recommended for grade 4 skin toxicities, such as Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full-thickness dermal ulceration, necrosis, or hemorrhage.[31] In these severe cases, permanent discontinuation of checkpoint blockade should be considered.[25,27]

Hepatotoxicity. In patients with increased AST or ALT levels, viral hepatitis and other causes of hepatitis should be excluded. In cases of mild AST/ALT elevation, checkpoint blockade can be continued, although the patient should be cautioned to avoid excessive alcohol consumption. In cases of moderate elevation (3 to 5 times the upper limit of normal [ULN]), withhold checkpoint inhibitor therapy and initiate oral or IV corticosteroid treatment. In patients with

Table 3 Typical Management of Immune-related Adverse Events[25]

Severity— CTCAE Grade	Ambulatory Versus Inpatient Care	Corticosteroids	Other Immunosuppressive Drugs	Immunotherapy
1	Ambulatory	Not recommended	Not recommended	Continue
2	Ambulatory	Topical steroids or Systemic steroids oral 0.5-1 mg/kg/day	Not recommended	Suspend temporarily[a]
3	Hospitalization	• Systemic steroids • Oral or i.v. • 1-2 mg/kg/day for 3 days then reduce to 1 mg/kg/day	• To be considered for patients with unresolved symptoms after 3-5 days of steroid course • Organ specialist referral advised	Suspend and discuss resumption based on risk/benefit ratio with patient
4	Hospitalization consider intensive care unit	• Systemic steroids i.v. methylprednisolone • 1-2 mg/kg/day for 3 days then reduce to 1 mg/kg/day	• To be considered for patients with unresolved symptoms after 3-5 days of steroid course • Organ specialist referral advised	Discontinue permanently

Some dysimmune toxicities may follow a specific management: this has to be discussed with the organ specialist.
[a]Outside skin or endocrine disorders where immunotherapy can be maintained.

elevations 5 to 20 times ULN, in addition to steroid treatment, a CTLA-4 blocker should be permanently discontinued, and permanent discontinuation of anti-PD-1/PD-L1 drugs should be considered. If elevations are >20 times ULN, any checkpoint inhibitor therapy should definitely be permanently discontinued.[27,28]

Hepatitis may continue for an extended period of time and may call for extended or repeated corticosteroid tapers (minimum of 3 weeks suggested) and/or additional immunosuppression. In rare cases, elevations in AST and ALT can be steroid-refractory.[27]

Endocrine Effects. The most common endocrine-related irAEs are hypophysitis (pituitary inflammation) and hypothyroidism. Because these AEs may present with nonspecific symptoms such as fatigue and headache, making the diagnosis can be clinically challenging. Laboratory studies of the pituitary axis should be obtained in patients who present with symptoms suggestive of endocrinopathy.[27,28,70]

Radiographic studies to look for enhancement and enlargement of the pituitary and laboratory measurements of ACTH and TSH can aid in the diagnosis. Laboratory findings can help distinguish among the likely endocrinopathies: hypophysitis is associated with low ACTH and TSH, whereas primary adrenal insufficiency is associated with low cortisol/abnormal cortisol stimulation test and high ACTH. Primary hypothyroidism is characterized by low free T4 and high TSH. It is important to distinguish primary hypothyroidism from hypophysitis, which can result in secondary hypothyroidism (low free T4 and low TSH). Management of hypothyroidism involves replacement with thyroid hormone (levothyroxine).[27]

Irreversible primary adrenocortical insufficiency can occur in patients on checkpoint inhibitor therapy. In addition, adrenal crisis associated with dehydration, hypotension, and electrolyte imbalances such as hyperkalemia and hyponatremia can arise quickly and require immediate hospitalization for IV corticosteroid therapy. This situation calls for advice from an endocrinologist, aggressive hydration, and evaluation for sepsis.[27,70]

Pulmonary Effects. Imaging studies should be obtained in any patient presenting with a new pulmonary symptom, such as an upper respiratory infection, cough, or shortness of breath. Pulmonary irAEs include pneumonitis and sarcoidosis. In patients who present with moderate to severe respiratory symptoms, a bronchoscopy should be obtained to exclude infectious causes. In severe cases, high-dose corticosteroid therapy will be needed, and concomitant immunosuppression with infliximab, mycophenolate mofetil, or cyclophosphamide can be considered.[27]

Less Common irAEs. Elevations in lipase levels are usually asymptomatic and can be monitored without treatment. When pancreatitis is suspected clinically, amylase and lipase should be checked.[27,70] An oral steroid and/or hospitalization may be required in severe cases.[28]

Renal abnormalities are usually asymptomatic and resolve with steroid treatment. When mild elevations occur, advise the patient about adequate hydration and discontinue any medications that may cause renal injury. Moderate to severe elevations require steroid treatment and may require discontinuation of therapy.[28,70]

Steroid treatment can be helpful. In consultation with a neurologist, plasmapheresis and IV immunoglobulin may be considered.[27,70]

Patients with mild symptoms may respond to analgesia with nonsteroidal anti-inflammatory drugs. In patients with moderately severe symptoms, consider prednisolone at a low dose (10-20 mg/day). Patients with severe symptoms should be treated with a higher steroid dose and promptly referred to a rheumatologist.[28]

Consultation with an ophthalmologist is recommended. Treatment with a topical intraocular corticosteroid such as 1% prednisolone acetate suspension may alleviate symptoms. An oral steroid can be used for grade 3/4 or refractory cases.[27,70]

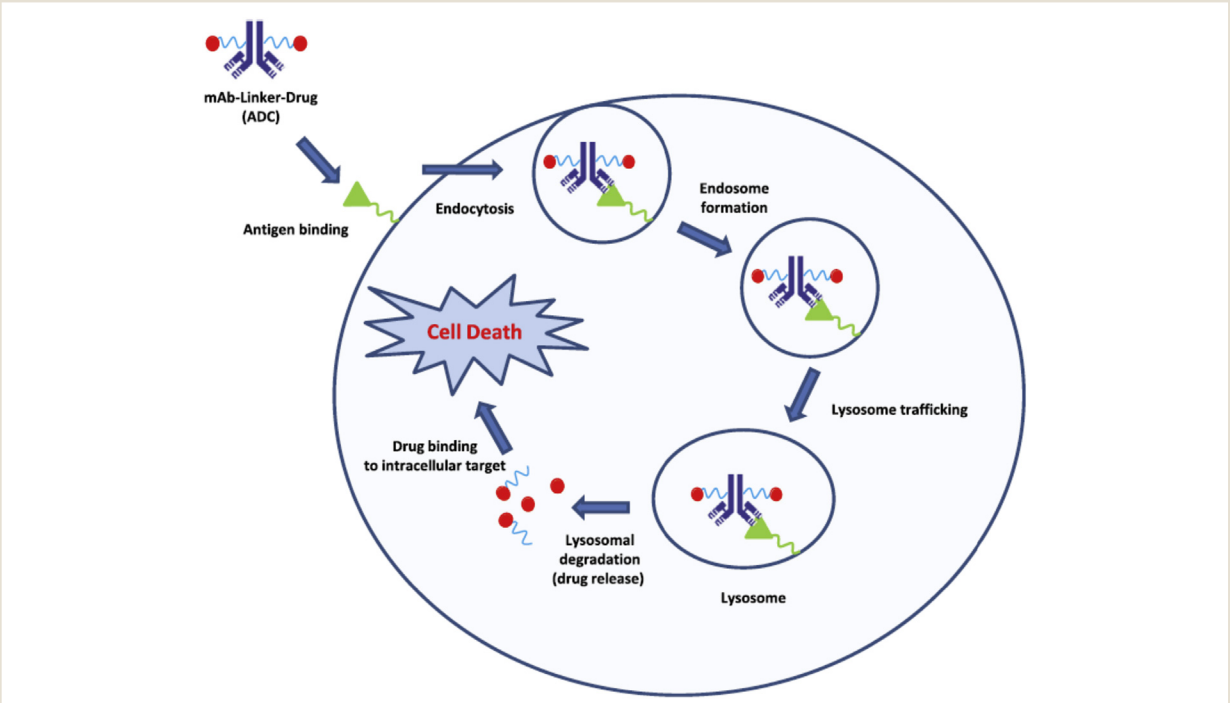
If abnormalities reach grade 2, laboratory monitoring is needed every 2 days. If symptoms persist or worsen, begin corticosteroid therapy.[31]

Other Forms of Immunotherapy

Antibody-Drug Conjugates

An antibody-drug conjugate (ADC) combines the targeting capability of a monoclonal antibody with the antitumor activity of a highly potent cytotoxic drug. The cytotoxic drug is usually a small

Figure 4 Schematic Representation of the Mechanism of Action of Antibody–Drug Conjugates[71]



molecule that has high systemic toxicity. It is chemically linked (conjugated) to a monoclonal antibody that has been engineered to bind to a specific tumor-associated antigen. This makes the drug combination very specific to tumor cells, which limits systemic toxicity. A familiar example of an ADC is trastuzumab emtansine, which is FDA-approved for HER2-positive metastatic breast cancer. This drug combines trastuzumab with the cytotoxic agent DM1.

Patients receive an ADC by infusion. The chemical link between the monoclonal antibody and the cytotoxic agent is stable in the bloodstream, and the ADC circulates until it binds to receptors on the exterior of the target tumor cells (Figure 4).[71] The ADC enters a tumor cell through a process known as endocytosis, and once inside the cell, the cytotoxic agent is released from the antibody and kills the tumor cell.

Sacituzumab govitecan is an ADC that combines hRS7, a humanized monoclonal antibody that targets the Trop-2 receptor (expressed by many solid tumors), and SN-38.9, the active metabolite of irinotecan. The FDA has designated it a breakthrough therapy for metastatic triple-negative breast cancer and is giving it fast-track review in lung cancer. Interim results presented from a phase 1/2 study show that sacituzumab govitecan also has significant clinical activity in platinum-resistant mUC.[72] Among 13 patients evaluable for efficacy, the ORR was 46% (all partial responses) and the clinical benefit ratio (partial response plus stable disease for >4 months) was 57%. Median PFS was 8.1 months and median OS was 10.8 months, with 79% of patients still alive. Among 15 patients evaluable for safety, the grade 3+ treatment-related AEs that occurred in more than 5% of patients were neutropenia (9%), diarrhea (9%), and staphylococcal bacteremia (9%). No patient developed antibodies to the antibody or the cytotoxic drug.

Two other investigational ADCs, enfortumab vedotin and ASG-15ME, are both designed to deliver a cytotoxic agent called monomethyl auristatin E (MMAE). The monoclonal antibodies in these ADCs target Nectin-4 and SLITRK6, respectively, 2 proteins that are highly expressed in UC.

Data have been presented from phase 1 trials of these compounds in patients with previously treated mUC.[73,74] In the study of ASG-15ME, a total of 42 patients were evaluable at doses considered active, of whom 1 had a complete response and 13 had partial responses (ORR = 33%).[73] That included 5/12 patients (42%) who had failed checkpoint inhibitor therapy. Median PFS was 16 weeks and median duration of response was 15 weeks. After median duration of treatment of 13 weeks, the most common treatment-related AE was fatigue (44%), and 20% of patients had grade 3/4 treatment-related AEs. Ten patients had reversible ocular AEs, of which 1 was grade 3.

For 33 response-evaluable patients on enfortumab vedotin, the ORR was 30%, including 3/12 patients (25%) who had failed checkpoint inhibitor therapy.[74] At the dose selected for phase 2 studies, the ORR was 57%. Median PFS and duration of response were both 16 weeks. Among the 42 safety-evaluable patients, the median duration of treatment was 12 weeks. The most common treatment-related AE was fatigue (38%), and 24% of patients had grade 3/4 treatment-related AEs. Grade 1/2 ocular symptoms developed in 21% of patients.

Vaccines

The purpose of a therapeutic cancer vaccine is to activate APCs that express a specific antigen. These cells then drive the proliferation of specific T cells that can destroy tumor cells. Vaccines have advantages over monoclonal antibodies. They can prevent tumor growth, so that continual antibody treatment is unnecessary, and they are associated with less risk of tumor escape. Two main categories of vaccines are being studied in UC: those that target oncoproteins and those that target tumor-associated antigens.

Targeting the Oncoprotein HER2. As most oncology clinicians know, HER2 is a growth factor receptor whose overexpression is associated with a number of malignancies, notably breast cancer. According to a meta-analysis of 9 studies, HER2 expression in patients with UC is associated with tumor grade, lymph node metastasis, and disease-free survival.[75]

DN24-02 (formerly known as lapuleucel-T) is a vaccine prepared by isolating peripheral blood monocytes from an individual patient, then culturing those cells with a proprietary fusion protein that combines recombinant (genetically engineered) HER2 with granulocyte macrophage colony-stimulating factor. The process matures the mononuclear cells into APCs.[30]

The NeuACT randomized, phase 2 trial compared adjuvant DN2402 or standard of care surveillance in patients with HER2+ UC who were at high risk of relapse after surgery.[76] The 142 patients had a median of 13.2 months of follow up. Rates of OS and disease-free survival did not differ significantly between the treatment groups, but DN2402 increased APC activation, and cytokine patterns showed a boost in immunologic effect. The researchers concluded that APC activation and immune responses persisting for >1 year suggest T-cell activation and immune memory generation.

Targeting Tumor-Associated Antigens. Cancer testis antigens are a large group of strongly immunogenic tumor-associated antigens that are expressed in a variety of tumors but not in normal tissue, except testis and placenta. In a study of tumor samples from 95 patients with high-grade UC, most tumors (77%) expressed at least one cancer testis antigen, and 61% of samples expressed more than one.[77] The most commonly expressed antigens were proteins known as MAGE-A4 and MAGE-A3. In similar studies, 43% to 46% of bladder tumors expressed MAGE-3.[78,79]

Recombinant MAGE-A3 protein, administered as an intramuscular vaccine with an immunostimulant known as AS15, is being studied in 3 clinical trials in UC. A recently completed phase 1 trial evaluated the vaccine in patients with NMIUC, but no results have been reported (NCT01498172). Another phase 1 trial, recently completed with no data reported, assessed whether intravesical BCG would enhance T-cell responses following immunization with the vaccine (NCT01498172). An ongoing phase 2 trial, MAGNOLIA, is evaluating adjuvant use of the vaccine in patients with MIUC (NCT01435356).

Two other tumor-associated antigens, mucin-1 (MUC-1) and carcinoembryonic antigen (CEA), are highly expressed or altered in most cancers, including UC. The PANVAC vaccine, which has demonstrated efficacy against a range of tumor types, targets both of those antigens. It consists of a primary vaccination with a recombinant vaccinia vector followed by multiple boosts with a recombinant fowlpox vector. Besides containing antigens against MUC-1 and CEA, the vectors contain transgenes for 3 human T-cell costimulatory molecules that enhance immune response.

An ongoing phase 2, randomized study is evaluating whether patients who fail initial BCG will respond better to PANVAC plus BCG than to BCG alone as the second course of treatment.[80] Currently, among patients who have recurrence of NMIUC after an initial induction course of BCG, only 35% experience 12-month disease-free survival following their second BCG cycle.[80]

Vesigenurtacel-L, also known as HS-410, is a whole cell—based vaccine that is specifically designed to treat high-grade NMIUC. It utilizes heat shock proteins from a UC cell line to "chaperone" UC-specific antigens and present them to the patient's immune system. A whole-cell vaccine presents a full range of tumor-associated antigens simultaneously, which has the theoretical advantage of stopping tumor cells from escaping the immune system. Based on the safety results from a phase 1/2 trial in UC (NCT02010203), the vaccine has received fast track designation from the FDA. The phase 2, multicenter, randomized, double-blind portion of the trial will evaluate patients who receive BCG plus vesigenurtacel-L or BCG plus placebo.

Combining Vaccines With Checkpoint Inhibitors. In the past, vaccines have been limited by a state called "T-cell exhaustion," in which T cells become dysfunctional and checkpoint molecules become upregulated on T-cell surfaces. The PD-1 and PD-L1 checkpoint inhibitors can reverse T-cell exhaustion, so it is possible that vaccines will prove to be most effective when combined with a checkpoint inhibitor. Vaccines are being combined with nivolumab or pembrolizumab in ongoing UC trials (NCT02897765 and NCT02432963, respectively).

Adoptive T-cell Therapy

Adoptive T-cell therapy is a collective term for several types of passive immunotherapy in which T cells are removed, manipulated in some way outside the body, and reinfused into the patient. This provides more rapid immunity that relying on the immune system to generate tumor-specific T cells. Before the T cells are reinfused, patients require nonmyeloablative leukoreductive therapy with radiation and chemotherapy.

One approach to adoptive T-cell therapy is to extract tumor-infiltrating lymphocytes (TILs) from tumors, enhance and expand them outside the body, and then reinfuse them into the patient. The only study to date in mUC showed that reinfusion was technically feasible in half of 12 subjects, and there were no major AEs.[81]

An adaptation of this approach is to perform genetic sequencing of the tumor, to identify mutations, and subsequently probe the TILs, once they are removed, to see if they recognize any of the mutations. Then TILs can be reinfused that contain mutation-specific T cells. Because UC has the third highest rate of mutations among major malignancies,[6] patients with UC may benefit from this type of technology, which is being investigated by the National Cancer Institute.

Either type of adoptive immunotherapy with TILs is challenging, because TILs have to be extracted through an invasive procedure and grown outside the body. To overcome these difficulties, some researchers are isolating T-cell receptors (TCRs) from peripheral blood, genetically modifying them to make them specific to a tumor antigen, and reinfusing them. A preliminary study of this approach showed its effectiveness in melanoma and synovial cell carcinoma.[82] As explained in the section on vaccines, cancer testis antigens are highly expressed in UC, and they may be good candidates for this type of therapy.

One of the challenges in treating cancer, including UC, is that tumors can escape the immune system by removing their surface expression of MHC molecules. A recent approach to adoptive immunotherapy is to create chimeric antigen receptors (CARs), which have innate antitumor activity and do not need to use the MHC to recognize targets. CARs are genetically engineered receptors that combine the antigen-binding site of a monoclonal antibody with the signal-activating machinery of a T cell, so that they bind directly to specific tumor antigens. Exciting responses to CAR-modified T-cell therapy have been reported in B-cell malignancies,[83] but as with most other forms of adoptive immunotherapy, no studies in UC have been reported.

Coming Full Circle: Recombinant BCG

Because of the limitations of BCG, numerous efforts are being made to genetically engineer improved products, using new knowledge of molecular biology and mycobacterial genetics. The 2 main classifications of recombinant BCG (rBCG) being investigated are products that secrete Th1 cytokines and those that have a non-live BCG subcomponent.

As explained above, BCG requires a Th1 immunologic response to produce an antitumor effect. One of the most popular approaches to rBCG is to genetically manipulate BCG strains so that they generate different mouse and human Th1 cytokines such as IL-2, IL-12, IL-18, IFN-α, or IFN-γ. These strains induce a long-lasting immunostimulatory effect, so theoretically they could be used at a lower dose. However, there is still potential for side effects from the live BCG, as well as from the cytokines. Therefore, Th1 cytokine—secreting rBCG may prove to be best suited to patients who do not respond to BCG induction and those who relapse following BCG therapy.

Non-live BCG subcomponents are just as immunologically active as live BCG, but their use should avoid the serious side effects that can accompany live BCG infection of cells. Subcomponents being evaluated in laboratory studies include mycobacterium cell wall extract (MCWE) and various BCG subunit proteins and antigens. Of 61 patients with carcinoma in situ of the bladder who were treated with MCWE, 41% had negative cystoscopies and biopsies 60 weeks after therapy, and toxicity was minimal.[84] However, randomized trials are still needed to compare BCG subcomponent—based rBCG with BCG itself and provide a definite answer about efficacy.

Conclusions

Cancer immunotherapy, now a popular strategy for targeting numerous tumor types, was pioneered in bladder cancer with BCG. However, mUC does not benefit from BCG, and even localized disease is characterized by high recurrence and progression rates. Immune checkpoint inhibition, especially with PD-1 and PD-L1 blockers, is the most exciting development in the systemic therapy of UC since platinum-based chemotherapy. Two agents are FDA-approved for advanced UC, and a number of others are in clinical trials, including in the adjuvant and neoadjuvant settings.

Because checkpoint inhibitors have been so successful in other cancers, they have largely skipped preclinical studies in UC, which will be needed in order to understand how to bolster antitumor immune responses. Moreover, additional clinical trials are needed to determine whether biomarkers can be identified for patients who are most likely to benefit from checkpoint inhibitors, to evaluate potential treatment combinations, and to establish how to sequence immunotherapy with chemotherapy.

Antibody-drug conjugates, vaccines, adoptive immunotherapy, and rBCG are other promising strategies for immunotherapy in UC. They may someday be combined with established treatments such as surgery, radiation, and chemotherapy. With further research, immunotherapy is expected to come to the forefront of the treatment of UC.

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