Immunotherapy in Urothelial Cancer, Part 1: T-Cell Checkpoint Inhibition in Advanced or Metastatic Disease

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Abstract: Cancer of the urothelium is the sixth most common cancer in the United States and is seen predominantly in men. Most cases of this disease present as non–muscle-invasive bladder cancer (NMIBC), with cancer recurrence or progression to muscle-invasive cancer in more than 50% of patients after initial therapy. NMIBC is an immune-responsive disease, as indicated by the use of intravesical bacillus Calmette-Guérin as treatment for more than 3 decades. More recently, immunotherapy has seen much progress in a variety of cancers, including advanced and metastatic bladder cancer, in which historical 5-year survival rates are approximately 15%. The advent of T-cell checkpoint inhibitors, especially those directed at programmed death 1 (PD-1) and its ligand (PD-L1), has had a significant effect on the therapy of advanced urothelial cancer. This had led to accelerated approval by the US Food and Drug Administration for atezolizumab and nivolumab in advanced urothelial cancer previously treated with platinum-based chemotherapy. In addition, level 1 evidence supports the use of pembrolizumab over single-agent tubulin-directed chemotherapy in the same setting. Several other treatments with immune-mediating mechanisms of action are in development and hold great promise, including monoclonal antibodies directed at other checkpoint molecules, oncolytic virus therapy, adoptive T-cell therapy, combination immunotherapy, and antibody-drug conjugates. This review focuses on the recent development of T-cell checkpoint inhibitors in advanced and metastatic urothelial cancer and addresses their potential use in combination. It also discusses a spectrum of novel immunotherapies with potential use in urothelial cancer.

Introduction

Cancer of the urothelium is the sixth most common cancer in the United States. It affects men approximately 4 times more often than women, primarily whites.[1] In approximately 10% to 20% of patients, non–muscle-invasive bladder cancer (NMIBC) progresses to muscle-invasive bladder cancer, and the disease recurs in 10% to

30% of cases.[2] Stage is the most important independent prognostic variable for assessing the probability of progression and survival. The 5-year survival rate is approximately 77% for all stages of bladder cancer, compared with less than 15% for metastatic bladder cancer.[3,4] Surgery with chemotherapy is a standard of care, but effective options for patients who do not have chemosensitive disease or cannot receive cisplatin-based chemotherapy have been limited. Novel therapies are being explored to provide additional options for patients who otherwise would have poor outcomes.

Immunotherapy has been developed in recent years for use in a variety of cancers, including bladder cancer. Immunotherapy harnesses the immune system to recognize and destroy cancer cells. Intravesical bacillus Calmette-Guérin (BCG), a type of attenuated mycobacterium, was the first type of immunotherapy used to help trigger an immune response, activating immune cells in the bladder as therapy for NMIBC; it was approved by the US Food and Drug Administration (FDA) in 1990. In 1995, high-dose interleukin 2 (IL-2) therapy was approved for metastatic renal cell cancer and melanoma based on durable complete responses (CRs). In 2010, the FDA approved sipuleucel-T (Provenge, Dendreon) immunotherapy for the treatment of early castration-resistant prostate cancer.[5] Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), a protein receptor that downregulates the immune system, was the first immune checkpoint receptor to be targeted clinically; the result was a survival advantage in patients with advanced melanoma.[6,7] Ipilimumab (Yervoy, Bristol-Myers Squibb), a monoclonal antibody that activates the immune system by targeting CTLA-4, was successful in improving overall survival (OS) in two phase 3 studies in advanced melanoma.[8-11] These data led to FDA approval of ipilimumab in 2011. More recently, research has been conducted on programmed death 1 (PD-1) and its ligand PD-L1, which are part of the family of checkpoint inhibitors. CTLA-4 and PD-1 are receptors that inhibit T-cell activation by distinct mechanisms. CTLA-4 and PD-1 both negatively regulate T-cell activation, but CTLA-4 inhibits AKT phosphorylation by using protein phosphatase 2A (PP2A) to mediate the suppression of T-cell activation. In contrast, PD-1 signaling inhibits AKT phosphorylation by preventing the CD28-mediated activation of phosphoinositide 3-kinase (PI3K).[12,13]

PD-L1 is expressed on some tumor cells and many immune cells and binds to PD-1 on immune cells. The binding of these checkpoint proteins suppresses the immune response. By blocking this interaction, checkpoint inhibitor monoclonal antibodies "release the brakes" on the immune system, allowing immune cells to attack tumors. The complex formed by the T-cell receptor, major histocompatibility complex, and antigen interacts with the first T-cell activation signal. A second, costimulatory signal from antigen-presenting cells (APCs) is necessary for the completion of successful T-cell activation (eg, B7 from APC binding CD28 on the T cell). Without a costimulatory signal, T cells become anergic. After T-cell activation, CTLA-4 on the T cell is upregulated, placing a damper on the T-cell response. On the APC side, B7-1 and B7-2 are upregulated in inflammatory settings. B7 can be either costimulatory or coinhibitory.[14-16] The net effect is to prevent runaway T-cell activation. PD-1 is also expressed on activated T cells, and expression is induced by inflammatory cytokines at a site of inflammation. PD-1 interacts with PD-L1 on APCs, with the net effect of preventing excessive tissue damage and autoimmunity at a site of infection.[17] By inhibiting PD-L1, the signals that prevent the body's immune system from attacking the cancer are lifted.

Monoclonal antibodies specific for PD-1 and PD-L1 induce tumor regression in patients who have advanced melanoma, renal cancer, lung cancer, or head and neck squamous cell cancer, with relatively low rates of toxicity. Pembrolizumab (Keytruda, Merck) and nivolumab (Opdivo, Bristol-Myers Squibb) were the first checkpoint inhibitors in the anti–PD-1 pathway family to be approved by the FDA for the treatment of refractory melanoma, and atezolizumab (Tecentriq, Genentech) was the first anti–PD-L1 antibody approved for the treatment of metastatic bladder cancer after failure of chemotherapy. PD-1 is a receptor normally involved in downregulating immune responses and promoting peripheral self-tolerance. PD-L1 and PD-L2, which are the 2 main ligands of PD-1, are variably expressed. Many tumors have made use of the PD-1/PD-L1 pathway as a mechanism to evade immune surveillance and destruction.[8,18-23] In murine models, expression of PD-L1 on the mastocytoma cell line increased apoptosis in active tumor-reactive T cells, suggesting a possible target for cancer immunotherapy.[14,23,24] The hypothesis was that exploitation of the PD-1/PD-L1 pathway in various tumors was a mechanism to evade immune surveillance and destruction. Drugs targeting the PD-1/PD-L1 pathway have led to more durable responses in selected patients compared with other therapies, such as targeted agents and cytotoxic chemotherapy. Here, we review the development of immunotherapy in bladder cancer and discuss its evolving role in the landscape of bladder cancer.

Systemic Therapy for Advanced or Metastatic Bladder Cancer

In the 1990s, the combination of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) was shown to

be superior to cyclophosphamide, doxorubicin, and cisplatin (CISCA), with a median OS of 12.5 months vs 8.2 months in patients with metastatic bladder cancer. MVAC also had a higher overall response rate (ORR) in these patients.[25,26] Gemcitabine and cisplatin had an efficacy similar to that of MVAC with less toxicity, including less neutropenia, neutropenic sepsis, and mucositis.[4] However, 5-year survival rates were higher and tolerability was much better with dose-dense MVAC (ddMVAC) given with granulocyte colony–stimulating factor (G-CSF) than with standard MVAC, and this regimen is considered a standard of care in patients who have good performance status and renal function.[27]

Recurrent or metastatic bladder cancer is treated with combination chemotherapy regimens. Even in the era of immune checkpoint inhibitors, these regimens remain an important treatment for patients with metastatic disease owing to the high response rate and the potential for long-term survival. In patients who are not eligible for cisplatin treatment because of comorbidities, gemcitabine and carboplatin can be used. This combination was found to achieve more favorable cancer control and have a better toxicity profile compared with methotrexate, carboplatin, and vinblastine (M-CAVI), a modification of the MVAC regimen that incorporates carboplatin.[20,28] Additional regimens without cisplatin include paclitaxel/gemcitabine and docetaxel/gemcitabine, which are associated with ORRs in the range of 33.3% to 51.6% and a median progression-free survival (PFS) of 5.8 to 9.5 months.[29-31] Single agents typically are not preferred owing to a short response duration and a lack of known OS benefit.

Second-line single-agent options derived from phase 2 studies show modest activity and include pemetrexed (Alimta, Lilly), docetaxel, ifosfamide, and nab-paclitaxel (Abraxane, Celgene), all of which produce ORRs of 28% or less.[32-38] Vinflunine is approved in Europe for the second-line treatment of urothelial cancer on the basis of a randomized phase 3 trial that showed an improvement in OS compared with best supportive care.[38] Vinflunine did not gain approval in the United States, however, likely because the trial demonstrated a low ORR (9%), a relatively small OS benefit (2.3 months) with statistical significance only on multivariate analysis, and substantial toxicity.[38] Ramucirumab (Cyramza, Lilly), a vascular endothelial growth factor (VEGF)–targeted antibody, has shown promise in the second-line setting; the combination of ramucirumab and docetaxel doubled PFS in comparison with docetaxel as a single agent in a randomized phase 2 study.[39] Phase 3 trials, including RANGE, which completed accrual in 2017,[40] will seek to define further the role of this combination for advanced bladder cancer.

Checkpoint Inhibitors in Advanced or Recurrent Urothelial Cancer

Atezolizumab

Atezolizumab is a humanized monoclonal immunoglobulin G1 (IgG1) antibody against PD-L1. The drug blocks signaling of the ligand through interaction with B7-1 and PD-1 receptors, but it does not inhibit signaling between PD-L2 and the PD-1 receptor.[41] The FDA granted accelerated approval for atezolizumab in patients with metastatic urothelial cancer after failure of a platinum regimen on the basis of results in cohort 2 of the phase 2 IMvigor 210 study, which included 310 patients treated every 3 weeks (Table 1).[42] The primary endpoint was the ORR by 2 measures: the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 response (on central review) and the investigator-determined immune-modified RECIST response. The ORR was 15% (95% CI, 11%-20%), with a higher ORR of 27% (95% CI, 19%-37%) in the patients whose tissue exhibited higher levels of PD-L1 immunohistochemistry staining (IHC 2+/3+) as measured with the Ventana PD-L1 (SP142) Assay from Roche. Responses and some CRs occurred even in the absence of staining showing expression of PD-1 or PD-L1 on tumor tissue. CRs occurred in 15 patients (5%). The median duration of response had not yet been reached at a median follow-up of 11.7 months; ongoing responses were noted in 38 of 45 responding patients (84%). Overall responses were also observed even in the absence of expression of PD-L1 on tumor-infiltrating immune cells. Atezolizumab was approved by the FDA in May 2016 without a specific requirement for tissue staining for PD-L1 to select patients for treatment, although a companion diagnostic was included in the approval.[43,44]

Toxicities included fatigue, nausea, anorexia, pruritus, fever, diarrhea, rash, and arthralgia. There were 23 incidents (7%) of immune-related adverse events (AEs), including pneumonitis, elevated liver enzymes, and rash, and 15 of these (5%) were of grade 3 or 4 intensity; the authors also note that 22% of the patients had an AE that required corticosteroid treatment.[43,44]

Atezolizumab was also evaluated in the first-line setting in patients with metastatic urothelial cancer who were cisplatin-ineligible in cohort 1 of the IMvigor 210 study. Of 119 patients treated, 83 (70%) had baseline renal impairment and 24 (20%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 2 as the primary reason for ineligibility for cisplatin treatment. With a median follow-up of 17.2 months, the ORR was 23% (95% CI, 16%-31%), the CR rate was 9%, and 19 of 27 responses were ongoing. The median response duration was not reached. The PD-L1 score in tumor-infiltrating lymphocytes was not associated with and did not

predict response in this cohort. Responses occurred across all PD-L1 and poor prognostic factor subgroups. The median PFS was 2.7 months. The median OS was 15.9 months. Treatment-related AEs that occurred at a rate exceeding 10% included fatigue, diarrhea, and pruritus. There was 1 treatment-related death due to sepsis, and 9 patients (8%) had AEs that led to treatment discontinuation. Immune-related AEs occurred in 12% of patients.[42] Atezolizumab demonstrated a better OS than prior standard chemotherapy, such as carboplatin and gemcitabine, despite a lower response rate and shorter PFS. It was also less toxic.[28] These data provided the basis for accelerated approval in April 2017 for atezolizumab in patients with metastatic urothelial cancer who are ineligible for cisplatin treatment. The IMvigor 211 phase 3 trial comparing atezolizumab with chemotherapy in 932 patients who have urothelial cancer and progression on or after platinum has completed accrual, and data are awaited.[45] Other ongoing trials of atezolizumab are investigating its role in the neoadjuvant and adjuvant settings.[46,47]

Nivolumab

Nivolumab, a fully human IgG4 monoclonal antibody to PD-1, received FDA breakthrough therapy designation in June 2016 for unresectable, locally advanced, or metastatic urothelial carcinoma progressing on or after a platinum-containing regimen, primarily on the basis of findings from a phase 2 trial.[48] Data for nivolumab were reported from the CheckMate 032 trial, in which patients with platinum-pretreated urothelial cancer received nivolumab every 2 weeks. The primary endpoint was investigator-evaluated ORR by RECIST 1.1. The PD-L1 IHC 28-8 pharmDx assay from Dako was used, with a cutoff of less than 1% staining vs at least 1% staining for PD-L1 expression. The ORR was 24.4% (95% CI, 15.3%-35.4%), with 5 CRs; responses were seen in 26.2% of those with less than 1% staining and in 24% of those with at least 1% staining. Although immune-related AEs were not specifically reported, 10% of patients experienced gastrointestinal AEs, 5% hepatic AEs, 3% pulmonary AEs, and 42% skin AEs, with 1% to 3% of these reaching grade 3 or 4 intensity. There were 2 treatment-related deaths, with 1 death caused by thrombocytopenia and 1 death caused by pneumonitis.[48]

CheckMate 275 was a large follow-up phase 2 trial of 270 patients with metastatic or surgically unresectable urothelial carcinoma whose disease had progressed despite platinum-based chemotherapy.[49] The primary endpoint was ORR. With a median follow-up of 7.0 months (range, 0.1-13.4), the confirmed ORR was 19.6% (95% CI, 15.0%-24.9%). Responses were independent of tumor PD-L1 expression as determined with the Dako PD-L1 IHC 28-8 pharmDx assay at cutoffs of 1% and 5%. The median duration of response was not reached. The median OS was 8.74 months (95% CI, 6.05-not estimable). Grade 3/4 treatment-related AEs occurred in 17.8% of patients; these were most commonly fatigue and diarrhea. Based on data from the two phase 2 studies, nivolumab received accelerated approval in February 2017 for urothelial cancer previously treated with platinum agents.

Pembrolizumab

Pembrolizumab is a humanized IgG4 antibody against PD-1. It blocks the signaling of both ligands (PD-L1 and PD-L2) with no cytotoxic activity (antibody-dependent cellular cytotoxicity [ADCC] or complement-dependent cytotoxicity [CDC]). KEYNOTE-012 included a phase 1b expansion cohort of patients with metastatic urothelial cancer who had at least 1% of tumor cells staining for PD-L1 with the Dako PD-L1 IHC 22C3 pharmDx assay. In this study, 24% of patients had not received any prior systemic therapy. The ORR was 27.6% (95% CI, 12.7%-47.2%), including 3 CRs. Immune-related toxicities included 1 episode of uveitis, and grade 3 events included myositis/rhabdomyolysis, rash, and colitis.[50]

KEYNOTE-045 was a randomized phase 3 trial comparing pembrolizumab 200 mg every 3 weeks with paclitaxel, docetaxel, or vinflunine for previously treated metastatic urothelial cancer.[51] The coprimary endpoints were PFS and OS in all patients and in the patients who had a combined positive score of at least 10% for PD-L1 as determined with the 22C3 antibody. The trial accrued 542 patients, 270 of whom received pembrolizumab and 272 chemotherapy. Pembrolizumab significantly prolonged OS in both the entire group of patients who received it (hazard ratio [HR], 0.73; 95% CI, 0.59-0.91; *P*=.0022) and the subgroup of patients with 10% or more PD-L1–expressing tumor who received it (HR, 0.57; 95% CI, 0.37-0.88; *P*=.0048). At a median follow-up of 14.1 months, the median OS in the total population treated with pembrolizumab was 10.3 months (95% CI, 8.0-11.8) vs 7.4 months (95% CI, 6.1-8.3) in the those treated with chemotherapy. The median OS in the 10% PD-L1–positive subgroup treated with pembrolizumab was 8.0 months (95% CI, 5.0-12.3) and was 5.2 months (95% CI, 4.0-7.4) in the 10% PD-L1–positive subgroup treated with chemotherapy. There was no difference between PFS with pembrolizumab and PFS with chemotherapy in the all-patient groups or in the 10% PD-L1–positive subgroups. Pembrolizumab showed an OS benefit in all subgroups examined, including patients with liver metastases and those with a PD-L1 combined positive score of less than 1%. Interestingly, the benefit of pembrolizumab was greater in current or former smokers than in those who had never smoked. When all patients in

each group were considered, the ORR for pembrolizumab was 21.1% compared with 11.4% for chemotherapy (*P*=.0011). The estimated proportion of responders with a response duration of at least 12 months was 68% in the pembrolizumab group and 35% in the chemotherapy group. Treatment-related AEs occurred in 69.1% of the pembrolizumab-treated patients and in 90.2% of the chemotherapy-treated patients. Fewer grade 3 or higher treatment-related AEs were reported with pembrolizumab than with chemotherapy (15.0% vs 49.4%). The most common treatment-related AEs were pruritus (19.5%), fatigue (13.9%), and nausea (10.9%) with pembrolizumab; they were alopecia (37.6%), fatigue (27.8%), and anemia (24.3%) with chemotherapy. Treatment was discontinued in 5.6% of the patients on pembrolizumab and in 11% of those on chemotherapy. KEYNOTE-045 provides the first level 1 evidence for the benefit of T-cell checkpoint inhibitors over chemotherapy in advanced urothelial cancer, in this case with pembrolizumab in patients previously treated with platinum-containing chemotherapy. Approval by the FDA and other regulatory agency is anticipated.

KEYNOTE-052 is a phase 2 study of pembrolizumab in patients with advanced or metastatic urothelial cancer who are ineligible for cisplatin treatment.[52] Results have been reported from the initial 100 patients of a 350-patient accrual. Of the 100 patients treated, 45% had baseline renal impairment and 43% had an ECOG performance status of 2 as the primary reason for the designation of ineligibility for cisplatin treatment. With 8 months of median follow-up, the ORR was 24%, the CR rate was 6%, and 20 of 24 responses were ongoing. The median response duration was not reached. A higher combined positive score for PD-L1 with the 22C3 antibody in tumor cells and/or tumor-infiltrating lymphocytes was associated with a higher response rate. Responses occurred across all combined positive score subgroups. Median OS was not reached at the time of reporting. Treatment-related AEs occurring at a rate exceeding 10% included fatigue, diarrhea, and pruritus. There was 1 treatment-related death due to sepsis, and there were 9 patients (9%) with AEs leading to treatment discontinuation. Immune-related AEs occurred in 12% of patients. Further follow-up and accrual are needed, but pembrolizumab has shown promising activity in the setting of patients ineligible for cisplatin treatment.

Durvalumab

Durvalumab (Imfinzi, AstraZeneca) is an engineered human IgG1 antibody against PD-L1, with mutations in the Fc domain to reduce ADCC and CDC.[24] In a phase 1/2 study with an expansion cohort for pretreated urothelial cancer, 61 patients were given durvalumab every 2 weeks for 1 year. After the first 20 patients had been enrolled, accrual was restricted to patients with PD-L1 staining on more than 5% of tumor cells with the Ventana PD-L1 (SP263) Assay.[21] The ORR was 31% (95% CI, 18%-47%), and response was strongly associated with tissue staining for PD-L1. The ORR was 46% in the group with high PD-L1 expression (defined as ≥25% staining in tumor cells or immune cells) compared with 0 in the 14 patients with a low rate of PD-L1 positivity (<25% staining). Although 10% of patients experienced diarrhea, the investigators reported no cases of colitis and no pneumonitis events. Grade 3 toxicities occurred in 5% of patients and included acute kidney injury and infusion-related reaction. Tumor flare was noted in 2% of patients. Durvalumab received accelerated approval in May 2017 for use in patients with metastatic urothelial bladder cancer that progressed during or after 1 standard platinum-based regimen.

Avelumab

Avelumab (Bavencio, EMD Serono/Pfizer) is a fully human IgG1 antibody that inhibits PD-L1. As part of a large phase 1b trial, 129 patients with urothelial cancer were treated with avelumab. Among 109 patients with more than 4 months of follow-up, the confirmed ORR was 16.5% (95% CI, 10.1%-24.8%), with 3 CRs and 15 partial responses (PRs); 17 of the 18 were ongoing (94.4%) at the time of reporting. A total of 78 patients (60.5%) had a treatment-related AE; the most common (≥10%) AEs were infusion-related reaction (22.5%) and fatigue (14.7%). There were 9 patients (7.0%) who had grade 3/4 treatment-related AEs. There was 1 treatment-related death, from pneumonitis.[53] A follow-up phase 3 trial is planned. The response rates and toxicity profiles for immune checkpoint inhibitors are summarized in Table 1.

Incorporating Immune Checkpoint Inhibition Into Treatment for Urothelial Cancer

Many questions remain unanswered with regard to the optimal incorporation of immune checkpoint inhibitors into the treatment paradigm for metastatic urothelial cancer. A major question is how survival with these agents compares directly with survival with cytotoxic chemotherapy; currently, the treatment paradigm still offers cisplatin-based combination chemotherapy first. Phase 3 randomized studies comparing immune checkpoint inhibitors with salvage chemotherapy, primarily taxane agents, have yielded our first level 1 evidence for immune checkpoint inhibitor superiority: KEYNOTE-045 for pembrolizumab,[51] with data from IMvigor 211 for atezolizumab to follow soon.[45] A second major question is whether the immune checkpoint A inhibitors should

Table 1. Summary of Response and Toxicity Profiles for Immune Checkpoint Inhibitors Tested in Patients With Metastatic Urothelial Cancer

Agent, Study	N	ORR, %	Time to Response, mo	Median OS, mo	Rate of Treatment-Related AEs ≥Grade 3, %
Ineligible for first-line cisplatin					
Atezolizumab, IMvigor 210 cohort 1 (NCT02951767)	119	23.5	NR	15.9	15
Pembrolizumab, KEYNOTE-052 (NCT02335424)	100	24	2.0	NR	16
Previously treated					
Atezolizumab, IMvigor 210 cohort 2 (NCT02108652)	310	15	2.1	7.9	16
Durvalumab (NCT01693562)	61	31	NR	NR	5
Nivolumab, CheckMate 032	78	24.4	1.48	9.7	23
Nivolumab, CheckMate 275	270	19.6	1.87	8.74	17.8
Pembrolizumab, KEYNOTE-012 (NCT01848834)	33	27.6	2.1	NR	15
Pembrolizumab, KEYNOTE-045 (NCT02256436)	270	21.1	2.1	10.3	15
Avelumab, JAVELIN Solid Tumor (NCT01772004)	129	16.5	1.5	NR	7

AE, adverse event; N, number of patients; NR, not reported; ORR, overall response rate; OS, overall survival.

be incorporated into first-line therapy. KEYNOTE-361[54] will compare chemotherapy (gemcitabine/cisplatin or gemcitabine/carboplatin) alone vs pembrolizumab alone vs chemotherapy plus pembrolizumab as first-line therapy. IMvigor 130[55] has a design similar to that of KEYNOTE-361 but uses atezolizumab. JAVELIN Bladder 100[56] is a maintenance study comparing avelumab plus best supportive care with best supportive care alone after platinum-based chemotherapy. These randomized studies will be critical in better defining the value of immune checkpoint inhibitors and their optimal use.

Given the substantial toxicity of standard chemotherapy, immunotherapy has the additional appeal of providing an alternative to standard chemotherapy, although most trials to date of first-line immunotherapy for recurrent, advanced bladder cancer are limited to patients ineligible for platinum. The FDA gave accelerated approval to atezolizumab in 2016 for patients who have progression on platinum-based chemotherapy or who have metastatic or progressive disease less than 12 months after adjuvant or neoadjuvant chemotherapy. The FDA is considering data on a range of PD1/PD-L1–directed therapies (atezolizumab, pembrolizumab, nivolumab, durvalumab, and avelumab) in patients who have platinum-refractory disease or are ineligible for platinum treatment. Finally, there is interest in combining immunotherapy with chemotherapy in the first-line setting or as maintenance, in the hope of increasing the rate of long-term cancer-free survival (Table 2).

Combination Immunotherapy in Bladder and Urothelial Cancer

One of the areas of active investigation in immunotherapy research is combining immunotherapy with other immune checkpoint inhibitors and determining the sequence in

which they should be given. These studies of dual checkpoint blockage and sequencing initially investigated melanoma and lung cancer. The first major study to combine CTLA-4 inhibition with PD-1/PD-L1 inhibition was in melanoma.[57] In the 3-arm phase 3 CheckMate 067 trial, 945 patients with untreated unresectable or metastatic melanoma were randomly assigned to receive nivolumab (n=316), ipilimumab (n=315), or nivolumab plus ipilimumab followed by nivolumab alone (n=314). The median PFS was 11.5 months for the combination, 6.9 months for nivolumab monotherapy, and 2.9 months for single-agent ipilimumab. Single-agent nivolumab reduced the risk for progression by 45% vs ipilimumab (HR, 0.55; 00.5% CI, 0.42-0.73; *P* <.0001). The ORR was 58% with the combination vs 19% with ipilimumab. The median duration of response with the combination was not met. In the study, ORRs were higher for the combination vs nivolumab monotherapy across levels of tumor PD-L1 expression.[57] Pembrolizumab also has been combined with ipilimumab in melanoma. Early results showed an ORR of 57%, with 5 CRs (5%) and 56 PRs (52%).[58]

In a study of first-line nivolumab monotherapy vs nivolumab-based combinations in advanced non–small cell lung cancer (NSCLC), nivolumab/ipilimumab resulted in a response rate of 39% (n=38) and a disease control rate of 74% (95% CI, 57%-87%). The frequency of treatment-related grade 3/4 AEs leading to discontinuation was very low in the lower ipilimumab dose cohort, again indicating that low-dose, less frequent anti–CTLA-4 therapy combined with anti–PD-1 therapy may produce optimal clinical results, even in the first-line setting. The ORR for patients with at least 1% PD-L1 expression in both nivolumab/ipilimumab combination regimen cohorts was 57%, which was double that seen in previously reported monotherapy arms. Efficacy was enhanced in the combination regimen cohorts as PD-L1 expression increased, with a response rate of up to a 92% in those patients with at least 50% PD-L1 expression.[59] Because of the success of combination therapies in melanoma and NSCLC, similar studies are currently being conducted in bladder cancer, including a study of nivolumab combined with ipilimumab.[60]

The difference between PD-L1– and PD-1–directed therapy is also being investigated. In theory, PD-1 inhibitors should block interaction with both PD-L1 and PD-L2, resulting in broader coverage and greater efficacy but increased toxicity. PD-L1 inhibitors do not provide PD-L2 coverage, potentially resulting in a decreased antitumor response but also less autoimmune toxicity. In practice, the relative efficacy of anti–PD-1 and anti–PD-L1 drugs is still to be determined. Therefore, studies alternating between PD-L1 and PD-1 combinations have been conducted because the advantages and disadvantages of each remain unclear.

In addition to dual checkpoint blockade, ongoing studies are being conducted on checkpoint blockage with costimulatory receptor agonists, chemotherapy, innate immune cell stimulators, small molecules, indoleamine 2,3-dioxygenase inhibition, and adoptive cell therapy in bladder and solid tumors (Table 2).

Options After Checkpoint Inhibitors, Including Novel Immunotherapies

Current options after progression on checkpoint inhibitor therapy are limited, given both our lack of experience in this space and the lack of efficacy of standard single-agent chemotherapy after progression on platinum-based therapy. There are, however, several novel immunotherapy options in development.

Oncolytic virus therapy is being investigated in many tumors, including urothelial cancer. Oncolytic viruses alter the environment in the tumor cell, allowing the viruses to replicate in and lyse tumor cells only. This approach is designed to destroy tumor cells without harming normal cells by causing immune cells to attack the cancer cells.[61-63] In vitro and in vivo studies of CG0070, an altered oncolytic adenovirus, have demonstrated the selective replication, cytotoxicity, granulocyte-macrophage colony–stimulating factor (GM-CSF) production, and antitumor efficacy of CG0070 in several preclinical models of bladder transitional cell carcinoma.[64] In a phase 1 study of CG0070 in 35 patients with NMIBC, high urine levels of GM-CSF were detected in all patients. The CR rate across all cohorts was 48.6%, with a median duration of 10.4 months.[65] A phase 3 study is testing CG0070 in patients with NMIBC whose disease has failed to respond to BCG therapy and who refused cystectomy.[66] Enadenotucirev is an oncolytic virus that uses actin-resistant DNAse I expression to reduce tumor growth.[67] A phase 1 trial currently under way in patients with metastatic or advanced epithelial cancers, including bladder cancer, that is not responding to standard therapy is looking at enadenotucirev in combination with pembrolizumab.[68] Finally, preclinical studies of intralesional therapy with Coxsackievirus A21 have shown benefit in melanoma, with the possibility of durable responses.[69] The immunostimulatory features of Coxsackievirus are thought to be based on synergy of its immunogenic effects and its direct oncolytic activities to promote tumor regression. This has led to a phase 1 trial of Coxsackievirus A21 administration via intravesical instillation and in sequential combination with low-dose mitomycin C in patients with NMIBC.[70]

Another new method of immunotherapy is adoptive T-cell therapy. T cells are removed from a patient with cancer and genetically modified or treated. Then, tumor-specific cytotoxic T cells are infused back into the

Table 2. Immunotherapy Combination Trials in Bladder Cancer

Study	Arms	Line of Therapy	N	Phase
HCRN GU10-148 (NCT01524991)	Gemcitabine + cisplatin + ipilimumab	First-line	36	2
DANUBE (NCT02516241)	Durvalumab + tremelimumab vs durvalumab vs standard-of-care chemotherapy	First-line	1005	3
IMvigor 130 (NCT02807636)	Atezolizumab vs atezolizumab + platinum-based chemotherapy vs platinum-based chemotherapy	First-line	1200	3
KEYNOTE-361 (NCT02853305)	Pembrolizumab +/- platinum-based combination chemotherapy vs chemotherapy	First-line	990	3
JAVELIN Bladder 100 (NCT02603432)	Avelumab + BSC vs BSC alone after first-line platinum-based chemotherapy	Maintenance	668	3
D4884C00001 (NCT02527434)	Durvalumab +/-tremelimumab vs tremelimumab alone, sequenced or in combination, for patients with advanced tumors	Second-line	66	2
BISCAY (NCT02546661)	Durvalumab +/- targeted agent matched to tumor profile: FGFR, PARP, WEE1, or PI3K inhibitor	First-line, second-line, or third-line	140	1b/2
Ludwig Institute for Cancer Research-2014-11 (NCT02643303)	Durvalumab, tremelimumab, and poly-ICLC (a TLR3 agonist) in patients with advanced cancers	Second-line	102	1/2
MSKCC 15-126 (NCT02553642)	Nivolumab +/- ipilimumab	Second-line	120	2
BMS CA209-032 (NCT01928394)	Nivolumab +/- ipilimumab	Second-line	1150	1/2
NCI Center for Cancer Research (NCT02496208)	Nivolumab + cabozantinib (Cabometyx, Exelixis) +/- ipilimumab	Second-line	66	1/2
BMS CA224-020 (NCT01968109)	Anti-LAG3 +/- nivolumab	Second-line	360	1
Celldex CDX1127-06 (NCT02543645)	Varlilumab (CDX1127) + atezolizumab	Second-line	55	1
Corvus CPI-444-001 (NCT02655822)	CPI-444 +/- atezolizumab	Second-line	534	1
Plexxikon (NCT02452424)	CSF1R, KIT, or FLT3 inhibitor (PLX3397) + pembrolizumab	Second-line	400	1/2
PsiOxus Therapeutics (NCT02636036)	Enadenotucirev (oncolytic virus) + nivolumab	Second-line	30	1
UC Davis (NCT02437370)	Pembrolizumab (MK3475) + docetaxel or gemcitabine in platinum-pretreated urothelial cancer	Second-line	38	1
Yale (NCT02443324)	Ramucirumab (VEGFR2 inhibitor) + pembrolizumab	Second-line	155	1
USC (NCT02717156)	Pembrolizumab + sEphB4-HSA	Second-line or later	60	2
California Cancer Consortium / NCI CTEP	Atezolizumab +/- eribulin (Halaven, Eisai)	Platinum-exposed or platinum-ineligible patients	66	2, randomized

BSC, best supportive care; CSF1R, colony-stimulating factor 1 receptor; CTEP, Cancer Therapy Evaluation Program; FGFR, fibroblast growth factor receptor; LAG3, lymphocyte activation gene 3; NCI, National Cancer Institute; PARP, poly(adenosine diphosphate-ribose) polymerase; PI3K, phosphoinositide 3-kinase; TLR3, Toll-like receptor 3; VEGFR2, vascular endothelial growth factor receptor 2.

patient with the goal of having them recognize, target, and destroy tumor cells. The earliest breakthrough was in metastatic melanoma, in which there were a large number of CRs and durable responses beyond 5 years. Adoptive immunotherapy was first studied by using tumor-infiltrating lymphocytes to treat patients with advanced melanoma, with more than 50% of patients responding to treatment.[71,72] Although patients with melanoma and those with hematologic malignancies (with the use of CD19-targeted chimeric antigen receptor T cells) have benefited from adoptive cell therapies, the same results have not been seen in other cancers.[69,73] Phase 1 and 2 trials with engineered T cells in head and neck cancer are now being completed.[73,74] In addition, phase 1 studies are examining the use of T cells engineered to recognize the NY-ESO-1, MAGEA4, PRAME, survivin, and SSX markers in patients with other solid tumors, including bladder cancer.[75]

Monoclonal antibodies in combination with cytokines are also being studied. Cytokines are involved in cell signaling; they are immunomodulating agents produced by broad range of cells to help control immune activity. High-dose IL-2, which functions to enhance the activity of the immune system against tumors, has been used in metastatic melanoma and renal cell carcinoma with significant durable responses.[76,77] Cytokines such as IL-2 are able to enhance antitumor immunity by stimulating T cells, B cells, monocytes, macrophages, lymphokine-activated killer cells, and natural killer cells. ALT-801 is a biologic compound in which IL-2 is genetically fused to a humanized soluble T-cell receptor directed against the p53-derived peptides expressed on tumor cells. ALT-801 functions as a tumor-targeted IL-2 immunotherapeutic combined with an anti–T-cell receptor antibody, which then promotes targeting of immunostimulatory activity to the site of p53-overexpressing tumor cells in the tumor microenvironment. Currently, 2 studies are underway: a trial of ALT-801 in combination with cisplatin and gemcitabine in muscle-invasive or metastatic bladder cancer, and a phase 1/2 trial of ALT-801 in combination with gemcitabine in patients with NMIBC who have failed BCG therapy.[78,79] Studies of other immunotherapy options are listed in Table 3.

Building on the success of brentuximab vedotin (Adcetris, Seattle Genetics) in lymphoma and of adotrastuzumab emtansine (T-DM1; Kadcyla, Genentech) in human epidermal growth factor receptor 2 (HER2)–positive breast cancer, antibody-drug conjugates are an active area of investigation in clinical trials in urothelial cancer. Antibody-drug conjugates are constructed so that an anticancer drug is coupled to an antibody that specifically targets an extracellular tumor marker, with the aim of selectively delivering the anticancer agent to the tumor. The goals are to increase cancer cell kill and diminish side effects.[80] In urothelial cancer, several agents look promising, including AGS15E (also called ASG-15ME). This agent targets SLITRK6, which is commonly expressed on urothelial cancer epithelial cells, and delivers the microtubule-disrupting agent monomethyl auristatin E.[81,82] In a phase 1 dose-escalation study of 42 evaluable patients with urothelial cancer, 1 patient had a CR and 13 patients had PRs (ORR, 33%), including 4 of 11 patients (36%) with liver metastases and 5 of 12 patients (42%) whose disease failed to respond to checkpoint inhibitor therapy. The most common treatment-related AE was fatigue (44%). A total of 23 patients (50%) had grade 3/4 AEs, 9 (20%) of which were considered related to treatment. There were 10 patients with reversible ocular AEs, of which 1 was a grade 3 toxicity. This trial will be expanded into a phase 2 trial of AGS15E in urothelial cancer at the maximum tolerated dose. In addition, enfortumab vedotin (also called AGS-22E) is an antibody-drug conjugate directed at Nectin-4, which is commonly expressed on epithelial cells in bladder and other cancers.[82] In a phase 1 trial of 33 patients with urothelial cancer who had an evaluable response, 10 had a PR (ORR, 30%), including 4 of 10 patients (40%) with liver metastases and 3 of 12 (25%) whose disease failed to respond to checkpoint inhibitor therapy. A total of 91% of patients had AEs. The most common treatment-related AE was fatigue (38%). There were 23 patients (70%) with grade 3/4 AEs, which were considered treatment-related in 10 patients (24%). There were 9 patients (21%) with ocular AEs, which were grade 1 or 2. This study will be further expanded in patients with urothelial cancer.[83]

Limitations of Immunotherapy

The current limitations of PD-1/PD-L1 therapy in urothelial cancer include the following: (1) efficacy limited to a relatively small subset of patients, (2) poor biomarker delineation of patients who will or will not benefit from therapy, and (3) major toxicity in a small subset of patients.

PD-1/PD-L1 checkpoint inhibitors are generally much better tolerated than some prior approved agents, such as high-dose IL-2 and ipilimumab. Common side effects of PD-1 and PD-L1 inhibitors include dermatologic toxicities such as rash and pruritus, which occur in approximately 50% of patients treated with ipilimumab; diarrhea or colitis; hepatotoxicity; and endocrinopathies. The most common endocrinopathies reported with ism, although type 1 diabetes also has been reported. AEs occurring less frequently are pneumonitis, asymptomatic elevation of lipase and amylase, renal insufficiency, and ophthalmologic disorders such as episcleritis and conjunctivitis. Most of these toxicities resolve with drug

Table 3. Immunotherapy Options After Checkpoint Inhibitors

Study	Arms	Line of Therapy	N	Phase
BOND2 (NCT02365818)	Oncolytic virus CG0070 in high-grade NMIBC after BCG failure	Second-line	122	3
SPICE (NCT02636036)	Enadenotucirev + PD-1 inhibitor	Second-line	30	1
CANON (NCT02316171)	Coxsackievirus A21 +/- mitomycin C in NMIBC	First-line	15	1
TACTASOM (NCT02239861)	TAA-specific CTLs (adoptive T-cell therapy)	Second-line	18	1
RANGE (NCT02426125)	Docetaxel +/- ramucirumab	Second-line	524	3
CA-ALT-801-01-10 (NCT01326871)	ALT-801 + cisplatin + gemcitabine (phase 1b and phase 2) ALT-801 + gemcitabine (phase 2 only)	First-line	90	1/2
CA-ALT-801-01-12 (NCT01625260)	Gemcitabine + ALT-801 in NMIBC after BCG	Second-line	52	1/2
B-701-U21 (NCT02401542)	Arm 1: B-701, an anti-FGFR3 antibody, in locally advanced or metastatic bladder cancer Arm 2: Docetaxel +/- B-701	Second-line	211	1/2
MK-6018-001 (NCT02346955)	CM-24 (MK-6018) +/- pembrolizumab in selected advanced or recurrent malignancies	Second-line	196	1
GEN702 (NCT02552121)	Tisotumab vedotin (HuMax-TF-ADC)	Second-line in tumors expressing tissue factor	44	1/2
AGS15E-13-1 (NCT01963052)	AGS15E monotherapy in metastatic urothelial cancer	Second-line or later	45+	1/2
ASG-22CE-13-2 (NCT02091999)	ASG-22CE (enfortumab vedotin) in metastatic urothelial cancer and other solid tumors expressing Nectin-4	Second-line or later	200	2

BCG, bacillus Calmette-Guérin; CTL, cytotoxic T lymphocyte; FGFR3, fibroblast growth factor receptor 3; NMIBC, non–muscle-invasive bladder cancer; PD-1, programmed death 1; TAA, tumor-associated antigen.

withdrawal or therapy with corticosteroids or tumor necrosis factor-α blockade.[84-88] The endocrinopathies are the exception to this reversibility; they tend to be permanent, and patients require ongoing monitoring and thyroid and/or corticosteroid hormone replacement therapy. Combining immunotherapy with other agents, with the goal of improved efficacy, will most certainly result in incremental side effects. It is important to determine whether these side effects will limit the use of combination treatment.

Conclusion

The recent advent of PD-1/PD-L1–directed immunotherapy has changed urothelial cancer therapy, particularly for patients whose cancers have progressed on platinum-based therapy or who are not eligible for cisplatin treatment in the first-line metastatic setting. Continued clinical trials are needed to establish the place of these and other immunotherapy agents in the treatment of bladder cancer. Given the therapeutic desert that existed previously, these new agents are welcome, but they must be well managed for optimal efficacy and for the early recognition and management of AEs.

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