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REVIEW ARTICLE

Role of immunotherapy in bladder cancer: past, present and future

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Abstract
As research focus in oncology has recently shifted from oral targeted therapy to immunomodulation, the era of successful drug development in bladder cancer has just begun. This has led to unprecedented approval of five immunotherapeutic agents by regulatory agencies for metastatic bladder cancer within a span of 12 months. With an initial triumph of anti-programmed cell death-1 (anti-PD-1) and anti-programmed cell death ligand-1 (anti-PDL-1) drugs, ongoing efforts are aimed at identification and validation of new druggable immune targets to consolidate the initial gains. In this paper, we review the role of immunotherapy in the treatment of bladder cancer as well as the various emerging immunotherapeutic agents and their possible use in bladder cancer.

Keywords Bladder cancer · Immunotherapy

Introduction

Bladder cancer is the most common malignancy involving the urinary system. Around 430,000 people are diagnosed with bladder cancer every year globally, while 165,000 patients die of it [1]. About 75% of new cases and deaths are in men. The overall 5- and 10-year survival rates for muscle-invasive bladder cancer (MIBC) with treatment are about 50 and 36%, respectively [2]. This has remained largely unchanged for the last 18 years. Since the development of cisplatin and gemcitabine combination chemotherapy for advanced bladder cancer [3], no major improvements in therapeutic spectrum have been achieved until recently (Fig. 1). Disappointingly, the 5-year survival rate for metastatic bladder cancer is only 15%. Patients with metastatic bladder cancer are treated with platinum-based combination chemotherapy in the first-line setting yielding a median overall survival (OS) of around 15 months [3]. Second-line chemotherapies, such as paclitaxel, pemetrexed, docetaxel, and vinflunine, have limited efficacy, with a median survival of approximately 7 months [4, 5].

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Immunotherapy has rapidly shifted the treatment paradigm for many cancers in the recent past including melanoma, renal cancer (RCC), and lung cancer. Preclinical data suggest that bladder cancer is immunogenic [6]. PDL-1 expressed on the tumor cell surface or on host immune cells in the tumor microenvironment engages the PD-1 receptor on activated cytotoxic T-cells leading to downregulation of the host immune response against tumor cells. PDL-1 inhibitors block off this negative feedback loop and hence accentuate immune response against tumor cells [7]. In this paper, we will review the role of current immunotherapeutic agents available for the treatment of bladder cancer both as monotherapy and in combination with either chemotherapy or other immunotherapeutic drugs. We will also discuss various novel immunotherapy agents that are currently in development phase and their possible role in the future treatment paradigm.

Bacillus Calmette–Guerin (BCG) immunotherapy

The oncological use of BCG, a live attenuated strain of Mycobacterium *bovis*, was first reported in a case series of nine patients in 1976 [8]. It has been the standard of care treatment for non-muscle-invasive bladder cancer (NMIBC) for some decades. Its mechanism of action in the treatment of bladder cancer is still not fully understood. It is believed that exposure to BCG suppresses tumor cell growth in a dose-dependent manner by a local immune response as

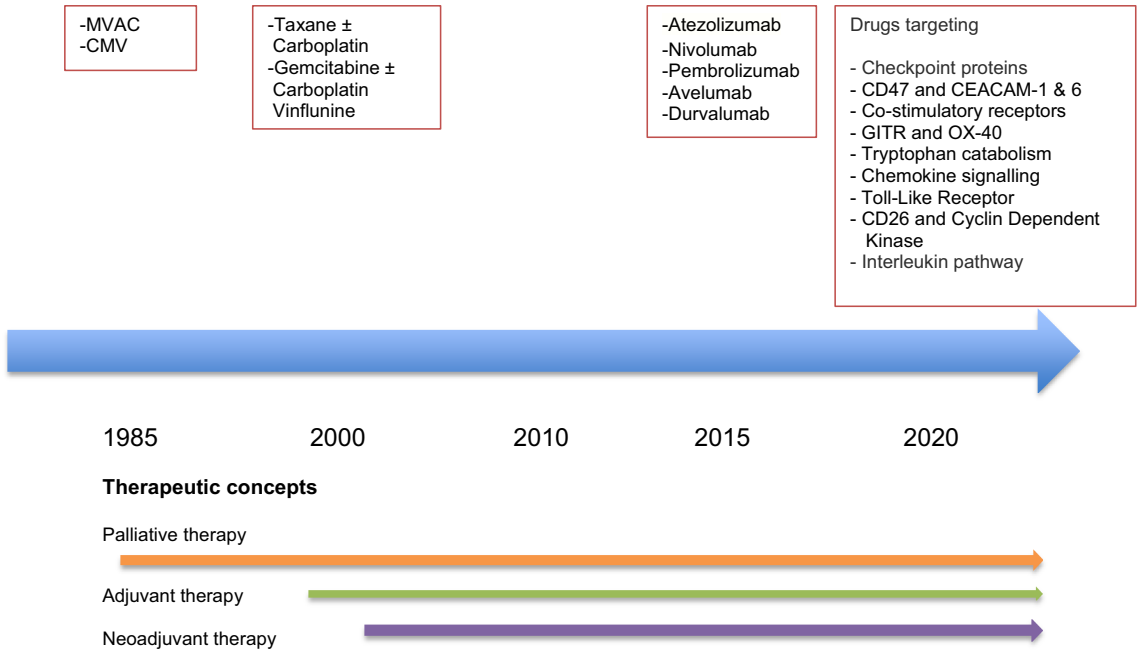

Fig. 1 Overview of drug development in bladder cancer. *MVAC* methotrexate, vinblastine, doxorubicin, and cisplatin, *CMV* cisplatin, methotrexate, and vinblastine, *GITR* glucocorticoid-induced tumor necrosis factor receptor

evidenced by infiltration of bladder with inflammatory cells and a sharp rise in urine levels of cytokines [9, 10]. The current guidelines recommend use of intravesical induction BCG immunotherapy in patients with an intermediate-risk or high-risk NMIBC (T1, Tis, and high-grade Ta) [11, 12]. Several randomized studies have suggested BCG immunotherapy being superior to various intravesical chemotherapy agents in reducing recurrences and delaying disease progression [13]. A meta-analysis of nine randomized trials reported a significantly lower rate of local tumor relapse in patients who received intravesical BCG after undergoing transurethral tumor resection as compared to patients treated with transurethral resection alone or transurethral resection followed by intravesical chemotherapy [14]. There are recent data to support the use of maintenance intravesical BCG therapy in selected patients with NMIBC. A randomized trial showed that in high-risk NMIBC, recurrence-free survival was improved when maintenance BCG was administered for 3 years; however, for intermediate-risk NMIBC, 1 year of maintenance treatment was sufficient [15].

Unfortunately, nearly 40% of patients with NMIBC fail BCG therapy [16]. Many causes have been postulated including insufficient treatment, occult invasive or metastatic disease, inadequate immune response, gradual wanning of immune response, or natural resistance-associated macrophage protein-1 (NRAMP-1) gene polymorphism [17].

In recent years, BCG shortage has been a challenge for oncologists around the world. With a definitive cessation of production of the main strain (i.e., Immucyst®) by the end of 2018, alternative strategies are being sought including using different strains [18, 19]. However, this is a major problem calling for alternative strategies.

In future, BCG-cell wall and BCG-cell wall skeleton (BCG-CWS) has been proposed to replace live BCG to induce the same immunological response but without a risk of systemic infection. BCG-CWS nanoparticle administered intravesically in rodent models has shown to inhibit tumor growth [20]. Furthermore, activating the anti-tumor immune response in bladder cancer with viruses instead of BCG is an experimental concept that has been tested using an adenovirus-mediated p53 gene-transfer technique and replication-competent granulocyte macrophage colony-stimulating factor-armed adenovirus with encouraging results [21, 22].

Another future prospect is to combine BCG therapy with PD-1 inhibitors such as pembrolizumab for high-risk NMIBC either intravesically (NCT02808143) or intravenously (NCT02324582). The findings from these trials may result in a significant change in the current practice for NMIBC.

Checkpoint inhibitors

Ipilimumab

Ipilimumab is a monoclonal antibody directed against cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) and currently approved for the treatment of metastatic melanoma. Over the last few years, clinical trials have demonstrated

promising activity both as monotherapy and in combination in various tumor types. Its potential activity in bladder cancer was first observed in 2010 in a small translational study when used as neoadjuvant therapy in surgically resectable, localized bladder cancer [23]. Two doses of ipilimumab (either at 3 or 10 mg/kg) were administered to 12 patients prior to undergoing radical cystectomy. There was pathological evidence of tumor downstaging in two-third specimens, whilst one-third of patients changed to negative urine cytology (or fluorescent in-situ hybridization) for malignant cells.

Recent data suggest that some chemotherapy drugs (e.g., standard dose gemcitabine) mediate their anti-tumor effect by inducing immunogenic cell death [24]. Other drugs such as cisplatin are also known to modulate tumor immunity. Combinational therapies including CTLA-4 antagonist, immune checkpoint antagonist, and chemotherapy have been under investigation in bladder cancer lately. A phase-2 trial investigated ipilimumab in combination with cisplatin and gemcitabine chemotherapy in metastatic bladder carcinoma [25]. This trial involved 36 treatment-naive patients (majority with visceral metastases) who were treated with six cycles of above combination. The objective response rate (ORR) was 23%, median progression-free survival (PFS) of 8 months, median overall survival rate (OS) of 14.6 months, and 1-year survival rate of 59%. The most common grade-3/4 adverse events (AEs) were neutropenia (36%), hyponatremia (31%), and anemia (25%), while the most common grade-3/4 immune-related AEs were colitis (6%) and hypophysitis (3%). Although this study did not meet the primary end-point of 1-year survival rate of 80%, however, it set a new milepost for future research and reaffirmed the notion of bladder cancer being immunogenic. This trial at least showed that this combination is feasible and active, but more work needs to be carried out to identify the cohort of patients who would most benefit from combined chemotherapy and CTLA-4 blockade.

Significant efforts are currently underway to enhance immune activation by combining anti-CTLA-4 and anti-PD-1 agents. Checkmate032, a phase-1/2 open-label trial evaluated the safety and efficacy of nivolumab combined with ipilimumab in metastatic solid tumors. The bladder cancer cohort studied two different combinations; ipilimumab 3 mg/kg plus nivolumab 1 mg/kg or ipilimumab 1 mg/kg plus nivolumab 3 mg/kg given every 3 weeks for 4 cycles followed by nivolumab monotherapy given every 2 weeks in patients with previously treated metastatic bladder cancer [26]. A promising ORR of 38.5% (ipilimumab 3 mg/kg dose) as compared to 26% (ipilimumab 1 mg/kg dose) was observed. The median PFS was 4.3 months as compared to 2.6 months, while the median OS was 10.2 months as compared to 7.3 months [27]. These rates were independent of PDL-1 expression status. The most common grade-3/4 AEs in the former combination were diarrhoea (7.7%) and pneumonitis (3.8%); while elevated liver enzymes (5.8%) and diarrhoea (4.8%) were observed in the latter treatment group. Further assessment of this combination in large long-term trials to validate above findings is suggested.

Atezolizumab

Atezolizumab is a high-affinity engineered humanized immunoglobulin G1 monoclonal antibody that inhibits interaction between PDL-1 and PD-1. Preclinical as well as clinical data advocate that atezolizumab can reinstate anti-tumor activity of T-cells that is most relevant in patients with suppressed immunity [28]. Atezolizumab has broad spectrum activity against a variety of tumors. In the initial dose-escalation phase-1 safety and tolerability study using adaptive design, atezolizumab treatment was well tolerated up to the maximum administered dose of 20 mg/kg. Treatment-related grade-3/4 AEs were seen in 13% patients (immune-related grade-3/4 AEs in 1% only). Significant anti-tumor activity with rapid and durable responses was observed in all tumor-specific cohorts. In addition, important pharmacodynamic studies were performed to delineate the mechanism associated with treatment response and relationship between PDL-1 and outcomes. Following this study, research efforts were launched to further evaluate potential role of atezolizumab in metastatic bladder cancer to meet this unmet need.

IMvigor210 was a two-cohort, multicentre, international, single-arm, phase-2 trial with primary end point of ORR. Cohort-1 of this study enrolled 310 patients with inoperable locally advanced or metastatic bladder carcinoma whose disease had progressed after the previous platinum-based chemotherapy [29]. These participants were treated with intravenous atezolizumab (1200 mg, given every 3 weeks). PDL-1 expression on tumor-infiltrating immune cells (ICs) was assessed using Ventana PDL-1 (SP-142) assay and defined by the percentage of PDL-1-positive immune cells; IC0 ($<1\%$), IC1 ($\geq 1\%$ but $<5\%$), and IC2/3 ($\geq 5\%$). ORR was 9% in IC0 group, 26% in the IC2/3 group, 18% in the IC1/2/3 group, and 15% in overall cohort. The responses were durable and ongoing in 84% of responders. Median OS was 11.4 months in patients in the IC2/3 group, 8.8 months in the IC1/2/3 group, and 7.9 months for the entire cohort of patients. Biomarker work has shown that higher levels of PDL-1 expression on immune cells were associated with higher response rates and longer survival. Atezolizumab seemed to be safe and generally well tolerated in this heavily pre-treated and highly co-morbid population. Only 16% of patients had a grade-3/4 treatment-related AE that is similar to data from the initial study.

Cohort-2 of IMvigor210 enrolled 119 treatment naïve patients with metastatic bladder cancer who were deemed ineligible to receive cisplatin (by treating physician) and

received atezolizumab as above [30]. ORR in this cohort was 24% including a complete response rate (CRR) of 9%, whilst 70% responses were ongoing. Median PFS was 2·7 months, while median OS was 15·9 months. It is interesting to note that patients aged 80 years or above (21%) had median survival duration of 14·8 months.

Exploratory immune and genetic analysis of archival tumor specimens from IMvigor210 study suggested encouraging response of tumors based on their intrinsic subtypes; luminal type-2 tumors had the best response, even though basal tumors had immune-infiltrate predominance [31]. Both PDL-1 levels and intrinsic subtype were reported to be of independent prognostic value. However, further research is needed to confirm its validity and routine use.

Another interesting phenomenon reported later in 2017 was the better overall survival if the patients were continued on atezolizumab at the time of progression as compared to patients where atezolizumab was discontinued [32]. Median OS for those continuing atezolizumab beyond progressive disease was 12.8 months, compared to 3.6 months for those not treated with atezolizumab beyond progressive disease. However, further studies need to be done to ascertain its replicability with other checkpoint inhibitors. After receiving a breakthrough therapy designation, atezolizumab was initially approved by the United States Food and Drug Administration (US FDA) based on data from IMvigor210 in 2016 for the treatment of metastatic bladder carcinoma following progression on platinum-containing therapy. Subsequently, it has been approved in 2017 for the treatment of patients with advanced bladder carcinoma who are not eligible to receive platinum-based therapy as the first-line therapy.

IMvigor211, a phase-3 trial, compared atezolizumab with the second-line chemotherapy and disappointedly failed to show its superiority in terms of OS [33]. 931 patients with locally advanced or metastatic bladder cancer after failure of platinum-containing chemotherapy were randomized to either atezolizumab or chemotherapy (of investigator's choice). The randomization was stratified according to PDL-1 expression in the same way as IMvigor 210 (i.e., IC0, IC1, and IC2/3). The primary end-point was OS; however, it was analyzed with a hierarchial-fixed sequence in pre-specified populations: IC2/3 first, followed by IC1/2/3, and then intention-to-treat population thereafter, only if statistical significance was achieved at each step. Median OS was found to be not statistically significant between atezolizumab arm and chemotherapy arm (11.1 versus 10.6 months) in IC2/3 cohort. This precluded further formal statistical analyses. These findings were in contrast to results of Keynote045 and raised further doubts about PDL-1 status being a reliable biomarker. Interestingly, in exploratory analyses, median OS in ITT population was numerically better in atezolizumab arm (8.6 versus 8.0 months). Furthermore, atezolizumab arm performed better when compared against taxanes (8.3 versus 7.5 months) than it did against vinflunine chemotherapy (8.3 versus 9.2 months) and could be a reason why chemotherapy arm performed better as a whole. Median duration of response was found to be 21.7 months for atezolizumab compared with 7.4 months for chemotherapy. The study also explored tumor-mutation-burden as a surrogate marker for treatment response in IC2/3 cohort. Patients with high tumor-mutation-burden had a median OS of 17.8 months with atezolizumab versus 10.6 months with chemotherapy. Further studies are needed to confirm its reliability as a marker for treatment response. Atezolizumab was again better tolerated as compared to chemotherapy with fewer grade-3/4 AEs (20 vs 43%) and fewer treatment discontinuations (7 vs 18%) in ITT cohort.

Atezolizumab is looking to further expand its use in bladder cancer. A phase-3 trial (IMvigor130) is looking at atezolizumab as monotherapy or in combination with platinum-based chemotherapy in patients with untreated locally advanced or metastatic bladder carcinoma. Other trials are looking into its use as neoadjuvant treatment in MIBC (NCT02662309), in combination with BCG in high-risk NMIBC (NCT02792192) and monotherapy use in recurrent BCG-unresponsive NMIBC (NCT02844816).

Nivolumab

Nivolumab is a fully human monoclonal antibody against PD-1 with clinical activity reported in multiple tumor types. In the phase-1 trial of nivolumab in 17 patients with various solid tumors, maximum tolerated dose (MTD) was not reached as no dose-limiting toxicity was observed at any dose up to 20 mg/kg [34]. The pharmacokinetic analysis showed area under concentration–time curve (AUC) to be linear. Preliminary anti-tumor activity was seen in three patients who had a partial response.

Further development of nivolumab continued in melanoma, non-small cell lung cancer, renal cancer, bladder cancer, and various other malignancies. Subsequently, a phase-1/2 Checkmate032 study of nivolumab was conducted in 78 (previously) heavily treated metastatic bladder cancer patients with study primary-end-point being ORR by investigator assessment. These participants had multiple lines of chemotherapy including platinum-doublet in the past. They received nivolumab 3 mg/kg intravenously every 2 weeks until disease progression or unacceptable toxicity. This cohort had similar characteristics as the cohort in IMvigor210 study except for only 13% patients over the age of 75 years was recruited. The investigators reported an ORR of 24.4% including five patients with a complete response regardless of PDL-1 status [26]. The median duration of response was 9·4 months, while encouragingly, of 19 responders, 12 had an ongoing response with a median OS of 9·7 months. Of note,

grade-3/4 treatment-related AEs occurred in 22% patients with skin (42%), gastrointestinal (10%), renal (9%), and hepatic (5%) being most common.

Checkmate275 study by the same investigators was designed to further delineate anti-tumor activity of nivolumab in 265 advanced bladder cancer patients with similar characteristics as in Checkmate032. This time, however, the response was also assessed by tumor PDL-1 expression (≥ 5 and $\geq 1\%$) that was determined at the time of screening using Dako 28-8 assay. Demographic and baseline clinical characteristics, although generally well balanced across PD-L1 subgroups (< 1 vs. $\geq 1\%$ expression and < 5 vs. $\geq 5\%$ expression); they differed in terms of performance status and PDL-1 expression (with higher number of patients with PDL-1 expression of < 5 and $< 1\%$). The study reported ORR of 20% in the entire study population; noticeably higher in patients with PDL-1 $> 5\%$ (28.4%), while lower for patients with PDL-1 expression $> 1\%$ and less than $< 1\%$ (23.8 and 16.1%, respectively) [35]. This is somewhat different to IMvigor210 results where PDL-1 $< 1\%$ was associated with only 9% ORR, although PDL-1 expression was measured on immune cells rather than tumor cells. Thus, Checkmate275 study hinted at a treatment response even in patients with low PDL-1 expression raising a concern if PDL-1 expression is a reliable predictive marker for treatment response in bladder cancer. Again, a majority of the patients with objective response had ongoing treatment response. The median OS was 8.7 months in the overall population, 11.3 months in the PDL-1 $> 1\%$ subgroup, and 5.9 months in the PDL-1 $< 1\%$ subgroup. The safety profile of nivolumab was similar to the previous experience from Checkmate032 study. However, the lack of control arm due to it being phase-2 study currently limits our ability to compare above results with the second-line chemotherapy. In an exploratory analysis [36], similar to IMvigor 211, tumor-mutation-burden was found to have strong association with better ORR, PFS, and OS. This again raises a possibility of tumor-mutation-burden to be a possible prognostic/predictive treatment-response marker for bladder cancer in future. In keeping with the theme of faster access to cancer drugs, nivolumab was approved by the US FDA based on above findings for the treatment of patients with locally advanced or metastatic bladder cancer after failure of a platinum-containing regimen.

Nivolumab is being tested as adjuvant treatment after radical cystectomy ± neoadjuvant chemotherapy prior in Checkmate274 (NCT02632409), while a neoadjuvant trial in combination with urelumab (an anti-4-1BB agonist antibody) is about to open (NCT02845323). Another study is looking into nivolumab in combination with cabazitaxel followed by ipilimumab in the metastatic setting (NCT02496208).

Pembrolizumab

Pembrolizumab is a highly selective humanized monoclonal antibody that blocks interaction between PD-1 and PD-L1/PDL-2. There are three monotherapy trials of pembrolizumab in bladder cancer that have been reported in the recent years. The efficacy of pembrolizumab was first reported in a small phase-1b trial that included 33 heavily pre-treated patients with advanced bladder cancer [37]. Only patients with at least 1% PDL-1 expression detected on the tumor cells or in tumor stroma were included in this trial. They received pembrolizumab 10 mg/kg every 2 weeks for a period of 24 months and until complete response, progression, or unacceptable toxicity. ORR was seen in 26% patients of whom 11% had a complete response. Subsequently, phase-2 Keynote052 trial evaluated the activity and safety of 200 mg pembrolizumab administered on a 3-weekly basis (for 24 months) in treatment-naive cisplatin-ineligible patients with metastatic or locally advanced bladder cancer [38]. The investigators reported an ORR of 24% that was comparable to findings of IMvigor210 atezolizumab study. It is important to note that Dako 22C3 assay was used to assess the response in PDL-1 positive population by combined positive score (tumor and immune cell PDL-1 expression) of > 1 and $> 10\%$ which was 25.4 and 38%, respectively. 83% responses were ongoing. 6-month survival of 67% was reported. Tolerability was acceptable with most common grade-3 AEs of fatigue (2%), raised alkaline phosphatase (1%) and colitis (1%).

The pivotal phase-3 Keynote045 trial was designed to evaluate pembrolizumab at a dose of 200 mg administered on a 3-weekly basis against investigator's choice of chemotherapy (paclitaxel, docetaxel, or vinflunine) in 542 patients with metastatic bladder cancer who had progressed after platinum-based chemotherapy [39]. PDL-1 expression was categorized in a similar fashion to Keynote052, but only response to PDL-1 levels of $\geq 10\%$ was assessed which made up around 30% of the total cohort. The ORR in the pembrolizumab group was around 20% (as expected) as compared to 11% in the chemotherapy arm; ongoing response was noted in 72% of the patients. The median OS was reported to be 10.3 versus 7.4 months, respectively, in ITT population. One-year survival was 43.9% in pembrolizumab arm as compared to 30.7% in chemotherapy arm. Interestingly, however, when PDL-1 levels of 10% or above were taken into account, median OS was 8 months in the pembrolizumab group, while 5.2 months in the chemotherapy group. This raised doubts about PDL-1 being a reliable marker in urothelial cancers. Median PFS was 2.1 months in the pembrolizumab group, while 3.3 months in the chemotherapy arm. Moreover, there was no statistically significant difference between in PFS of patients with PDL-1 of $\geq 10\%$

than ≤ 1%. Pembrolizumab was deemed better tolerable than chemotherapy with pruritis and fatigue being the most common adverse events. Unfortunately, quality of life was not measured across patient groups in this study. Based on Keynote045 findings, the US FDA in May 2017 approved pembrolizumab for the treatment of patients with metastatic bladder cancer who have disease progression during or after platinum-based therapy or within 1 year of neoadjuvant/adjuvant treatment with platinum-based chemotherapy.

Pembrolizumab has also been studied in combination with a variety of cytotoxic agents. In a trial of 12 patients with metastatic bladder cancer, pembrolizumab in combination with chemotherapy (docetaxel or gemcitabine) showed good efficacy with an ORR of 33%, while commonest grade-3 AEs (likely chemotherapy-related) being anemia (38%), fatigue (31%), and neutropenia (31%) [40]. This study provides the initial evidence that a combination of pembrolizumab and chemotherapy may be beneficial in bladder cancer; however, mechanism of immunogenic cell death by interplay of immunotherapy and chemotherapy as well as detailed safety profile needs to be further elucidated.

Keynote361 is an ongoing phase-3 study with pembrolizumab with or without platinum-based combination chemotherapy versus chemotherapy alone in patients with advanced bladder carcinoma as the first-line treatment. This trial aims to study if immunotherapy in combination with chemotherapy is superior to chemotherapy alone with regard to PFS and OS in treatment-naive patients. Other trials are exploring various new applications of pembrolizumab including monotherapy use in patients with high-risk NMIBC (Keynote057), in combination with BCG (MARC) in high-risk NMIBC, in combination with BCG in patients with recurrent NMIBC (NCT02808143), and in combination with chemoradiotherapy for MIBC (PCR-MIB).

Pembrolizumab is also currently being investigated in neoadjuvant setting as monotherapy (NCT02736266) as well as in combination with platinum-doublet chemotherapy (NCT02690558) or with gemcitabine in platinum-eligible/ineligible population (NCT02365766). Another trial is investigating pembrolizumab as maintenance therapy after the initial chemotherapy in the metastatic disease (NCT02500121). Pembrolizumab is being utilized in combination with radiation in an ongoing phase-1 trial in MIBC or metastatic bladder cancer (PLUMMB) to improve outcomes. Pembrolizumab is planned to be continued after conclusion of radiation therapy for a year or until disease progression (NCT02560636). Some other ongoing trials are evaluating combination of pembrolizumab with various novel agents in the early phase trials including; B-701, a fibroblast growth-factor receptor antagonist (NCT02925533) and Vorinostat (NCT02619253).

Avelumab

Avelumab (a fully humanized antibody against PDL-1) with its dual mechanism of action assists in harnessing body's immune response against malignancy. In the first phase-1 study involving 44 patients with platinum-refractory bladder cancer, avelumab administered at 10 mg/kg biweekly demonstrated ORR of 17.3% [41]. Ongoing responses were observed in 79% of the patients. The median PFS was 1.6 months; the median OS was 8.2 months with a 12-month OS rate of 41.9%. Using a Dako proprietary assay, PDL-1 staining on the tumor cells and the tumor-associated immune cells was grouped in > 5% as positive and < 5% as negative. ORR in PDL-1-positive patients was 25.6%, while 13.7% in PDL-1-negative tumors. Grade-3/4 AEs were present in only 10.4% patients. Of note 20% patients had infusion-related reaction that has not been observed with any other PD-1/PDL-1 inhibitor before. A planned pooled analysis of 242 metastatic bladder cancer patients from two cohorts of Javelin trial reported similar infusion-related reaction rate but well tolerated otherwise with promising clinical activity, regardless of tumor PDL-1 status [42]. Lack of randomized treatment assignment and a relatively small sample size are the major limitations of above studies.

Based on Javelin trial findings, the US FDA granted approval to avelumab in May 2017 for the treatment of patients with metastatic bladder cancer who have disease progression during or after platinum-based therapy or within 1 year of neoadjuvant/adjuvant treatment with platinum-based chemotherapy.

A randomized phase-3 trial of avelumab plus best supportive care versus best supportive care alone as maintenance therapy in patients with metastatic bladder carcinoma who are not progressing after the first-line platinum-based therapy is underway (NCT02603432).

Durvalumab

Durvalumab is a selective, engineered human antibody that blocks PDL-1 binding to PD-1 and CD80. A recent phase-1/2 trial of durvalumab reported significant anti-tumor activity in 191 previously treated metastatic bladder cancer patients [43]. In this single-arm, open-label, non-randomized study, durvalumab was administered at 10 mg/kg on a biweekly basis for 12 months. Treatment with durvalumab was associated with ORR of 17.8% in all evaluable patients although as high as 46% in patients with positive PDL-1 (cutoff 25% using Ventana SP-263). In addition, in the PDL-1 positive patients, 68% experienced a ≥ 30% target lesion reduction from baseline as compared to 9% in the PDL-1 negative arm. The investigators more recently presented an updated analysis with ORR of 17.8% once 191 metastatic bladder cancer patients have been treated

[44]. As expected ORR was 27.6% in patients with a high PDL-1 expression as compared to 5.1% in patients with a low expression. Median PFS was 1.5 months, while median OS was 18.2 months in ITT population. The safety profile of durvalumab was consistent with other immunotherapeutic agents including fatigue, musculoskeletal pain, constipation, decreased appetite, nausea, and peripheral edema (treatment-related grade-3/4 AEs 6.8%) needing only 1.6% patients to discontinue treatment. In light of these results, the US FDA granted accelerated approval to durvalumab this year for the treatment of patients with metastatic bladder carcinoma who have disease progression during or after platinum-based therapy or within 1 year of neoadjuvant/adjuvant treatment with platinum-based chemotherapy.

Durvalumab is currently being studied in BCG-refractory NMIBC (NCT02901548). A phase-3 trial (NCT02516241) is evaluating the combination of durvalumab and tremelimumab in previously untreated bladder cancer (NCT02516241), while another study is looking at the combination therapy as neoadjuvant treatment for patients ineligible for cisplatin-based chemotherapy (NCT02812420). Another study with durvalumab and radiotherapy followed by adjuvant durvalumab in MIBC (NCT02891161) is in recruitment phase. Several ongoing clinical trials are also exploring its efficacy in other solid tumors.

A comparison of the results of the studies along with the most common treatment-related AEs is listed in Tables 1 and 2.

The future of immune oncology in bladder cancer

Currently, over 50 different immuno-oncology drugs directed against several distinct steps of a well-recognized immunologic cascade that is rendered dysfunctional by a growing tumor are being investigated in various solid organ malignancies. Moving the way forward, these agents, if demonstrate a good response, may attain an important role as solitary or adjunctive treatment (either with chemotherapy or other immunotherapeutic agents) in the near future in various cancers including bladder cancer. The aim of course would be to improve upon the durable responses achieved with recent advances in the field of bladder cancer by checkpoint inhibitors.

New targets and upcoming novel immunotherapeutic agents in the treatment of bladder cancer

Drugs targeting checkpoint proteins

Lymphocyte activation Gene-3 (LAG-3)

LAG-3 is expressed on cell surface of lymphocytes and has been recently recognized as an important new target in cancer immunology [45]. Anti-LAG-3 drugs have exhibited enhanced activation of antigen-specific T-cells at the tumor site and resulting in reduced tumor growth. Preclinical studies have revealed widespread co-expression of PD-1 and LAG-3 on tumor-infiltrating T-cells in several cancers and dual (Anti-LAG-3/anti-PD-1) blockade has shown good synergistic results in animal tumor models [46]. Two such drugs BMS986016 and LAG-525 are currently under evaluation in solid organ malignancies. Further clinical evaluation of these agents (as monotherapy or in combination) in bladder cancer is awaited.

Killer-cell Ig-like receptors (KIR)

KIRs are expressed on mature natural-killer (NK) cells whose ligands are HLA molecules. Binding of HLA molecules to KIR results in inhibitory signalling that decreases NK cell-mediated tumor destruction. Lirilumab (anti-KIR) was tested in a dose-escalation study and was deemed well tolerated [47]. It is being evaluated in solid organ malignancies in combination with nivolumab and ipilimumab. A phase-1/2 trial recently reported significant clinical activity of lirilumab (in combination with nivolumab) in patients with advanced platinum-refractory squamous cell cancer of head and neck [48]. Further data from other expansion cohorts of this trial will provide important information on future development of these agents in other malignancies.

Drugs targeting CD47 and CEACAM-1 and 6

CD47

CD47 is an inhibitory signal protein present on tumor cells to avoid phagocytosis [49]. Preliminary data also suggest that CD47 is upregulated in various cancers. Thus, this protein has all the characteristics for a good molecular target. Monoclonal antibodies are being developed to target this cell-surface antigen. Two molecules CC-90002

Table 1 Summary results of studies of approved PD-1/PDL-1 inhibitors in patients with bladder cancer

Agent	Trial name	Study type	Population	ORR	PDL-1 assay	Median OS (months)
Atezolizumab	IMvigor210	Phase 2	Cohort-1 = inoperable locally advanced or metastatic urothelial carcinoma pre-treated with platinum-based chemotherapy Cohort-2 = cisplatin-ineligible metastatic urothelial cancer	15% overall 26% in IC2/3 population 24% in ITT population	Ventana (SP-142)	7.9 in ITT population 11.4 in IC2/3 population 14.8 in ITT population
	IMvigor211	Phase 3	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	14% in IC1/2/3 population		11.6 in IC2/3 population
Nivolumab	Checkmate 032	Phase 1/2	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	24.4% in ITT population	Dako 28 − 8	9.7 in ITT population
	Checkmate 275	Phase 2	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	20% in ITT population 28.4% in population with > 5% PDL-1 expression		8.7 in ITT population 11.3 in population with > 1% PDL-1
Pembrolizumab	Keynote 052	Phase 2	Treatment naïve, cisplatin-ineligible, locally advanced or metastatic urothelial cancer	24% in ITT population 38% in population with > 10% PDL-1	Dako 22C3	6-month survival 67% in ITT population
	Keynote 045	Phase 3	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	20% in population with > 10% PDL1 expression		10.3 in ITT population 8 in population with > 10% PDL1 expression
Avelumab	Javelin for Solid tumors	Phase 1b	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	17.3% in ITT population 25.6% in population with > 5% PDL1 expression	Dako proprietary assay	8.2 in ITT population
Durvalumab		Phase 1/2	Advanced or recurrent urothelial carcinoma pre-treated with platinum-based chemotherapy	17.8% in ITT population 27.6% in population with > 25% PDL-1 expression	Ventana SP-263	18.2 in ITT population

(NCT02367196) and Hu5F9-G4 (NCT02216409) are being evaluated in solid and hematological malignancies

Table 2 Summary of treatment-related adverse events in studies of approved PD-1/PDL-1 inhibitors in patients with bladder cancer

Treatment-related event	Atezolizumab [29]	Pembrolizumab [35]	Avelumab [38]	Durvalumab [40]
Fatigue	30 (3)	13 (1)	20.5 (0)	13 (0)
Diarrhoea	12 (2)	9 (1)	9 (0)	9.8 (0)
Pruritus	11 (1)	19 (0)	6.8 (0)	3.3 (0)
Anorexia	9 (1)	8.6 (0)	4.5 (2.3)	8.2 (0)
Rash	4 (1)	0.8 (0.4)	9 (0)	NR
Hypothyroidism	7 (0)	6.4 (0)	6.8 (0)	NR
Liver derangement	4 (3)	NR	4.5 (2.3)	NR
Autoimmune colitis	1 (1)	2.3 (1)	NR	NR
Infusion-related reaction	4 (3)	0.8 (0)	20.5 (0)	3.3 (1.6)
Pyrexia	5 (0)	NR	NR	6.6 (0)
Dyspnoea	3 (0)	4 (2.3)	2.3 (0)	NR
Thrombocytopenia	3(0)	NR	NR	NR
Renal failure	2 (2)	0.8 (0.8)	NR	1.6 (1.6)
Arthralgia/Arthritis	4 (0)	NR	2.3 (0)	6.6 (0)
Asthenia	3 (0)	5.6 (0.4)	11.4 (2.3)	6.6 (0)
Adrenal insufficiency	NR	0.4 (0.4)	NR	NR
Sensory neuropathy	NR	0.8 (0)	NR	NR

Data presented as overall percentage and grade 3–4 percentage

NR not reported

at present. As bladder cancer is considered very immunogenic, future efforts should focus on stimulating phagocytosis in bladder cancer.

CEACAM-1 and CEACAM-6

CEACAM are the members of the carcinoembryonic antigen (CEA) family of immunoglobulin glycoprotein cell adhesion molecules (CAM) and being increasingly recognized as playing a key role in modulation of melanoma, lung, bladder, and other malignancies [50].

CEACAM1 is postulated to have a role in cancer progression, invasion, and tumor angiogenesis. CEACAM-1 is present both on tumor cells and T-cells and once activated undergoes trans-oligomerization and prevents immune activation preventing tumor cell destruction by T-cells and NK cells [51]. Blocking CEACAM-1 with a drug CM-24 is expected to cause more cancer-specific activation rather than general immune activation and it is being evaluated in a phase-1 study (NCT02346955).

CEACAM-6 is an adhesion molecule that binds cytotoxic T-cells inhibiting their activation and resulting in tumor-sparing [51]. L-DOS47 targets CEACAM-6 and is been evaluated (with pembrolizumab) in lung cancer (NCT02340208).

Drugs targeting co-stimulatory receptors

CD-137 (4-1BB)

CD-137 is a co-stimulatory receptor present on cytotoxic and regulatory T-cells (T_{regs}) as well as NK cells. Its functions include activation of cytotoxic T-cells and inhibiting suppressive functions of T_{regs}. This is a potentially suitable therapeutic target in bladder cancer.

A phase-1 dose finding study of urelumab, a monoclonal antibody agonist of CD-137, showed transaminitis as dose-limited toxicity and determined 0.1 mg/kg (three weekly) recommended phase-2 dose [52]. A phase-1/2 study combining urelumab with nivolumab showed ORR of 50% in melanoma (regardless of PDL-1 status), and lung, head, and neck cancer patients [53]. This combination was well tolerated with common treatment-related AEs being fatigue, transaminitis, and anemia.

Another similar drug PF-05082566 was evaluated in a phase-1 study in combination with rituximab in patients with relapsed or refractory non-Hodgkin lymphoma with good response rates [54]. It is being evaluated in solid organ malignancies (NCT01307267). These agents need further exploration in bladder malignancy in view of preliminary immunological activity demonstrated in above studies.

CD27

CD27 is a co-stimulatory receptor that belongs to the tumor necrosis factor receptor superfamily and is expressed both on T-cells, B-cells, and NK cells. Varlilumab, a CD27-agonist antibody, has shown promising anti-tumor activity in a phase-1 trial in patients with hematological malignancies [55]. A study of varlilumab and atezolizumab combination in patients with advanced bladder cancer is underway (NCT02543645). Varlilumab is also being investigated in various combinations in several other malignancies (NCT02413827, NCT02335918, and NCT02270372).

CD40

CD40 is largely expressed on antigen presenting cells (APCs) and is associated with APC maturation and immune enhancement, resulting in tumor-specific T-cell activation. CP-870,893, an anti-CD40, in two separate phase-1 studies in patients with advanced solid tumors showed encouraging activity with grade-1/2 cytokine release-syndrome being the most common AE [56, 57]. Lucatumumab (HCD122), ADC-1013, SEA-CD40, and APX005M are other anti-CD40 agents that are currently being investigated.

Glucocorticoid-induced tumor necrosis factor receptor (GITR) agonists and OX-40 agonists

GITR is expressed on T_{regs} and induce activation of CD4+ and CD8+ cells. Preclinical studies of GITR-agonistic antibodies (including in combination with checkpoint inhibitors) showed preliminary signal of activity [58]. Phase-1 studies of TRX518 (NCT01239134) and MK-4166 (NCT02132754) in solid tumors are currently underway.

OX-40 is expressed on CD4+, CD8+, and NK cells, and potentiates T-cell receptor signalling on the surface of T-lymphocytes, leading to their activation and enhancement of T_{regs} activity. In a phase-1 trial of OX40 agonist, 9B12/MEDI0562 showed limited anti-tumor activity with acceptable safety profile in patients with metastatic solid malignancies refractory to the conventional therapy [59]. Although no patient achieved a partial response of > 30% overall tumor shrinkage, however, at least one tumor nodule regressed in 12 patients and no change in the measurement of target lesions was observed in six additional individuals. Transient lymphopenia, fatigue, rash, and flu-like symptoms with fever and chills were the most common AEs. A humanized version of the same drug (MEDI0562) is being tested in patients with solid organ malignancies in a phase-1 study (NCT02318394). Another trial using RG7888/MOXR0916 in combination with atezolizumab with or without bevacizumab is recruiting patients with metastatic carcinomas (NCT02410512).

Drug targeting tryptophan catabolism

Indoleamine 2,3-dioxygenase-1 (IDO1) is a tryptophan-catabolizing enzyme expressed in many cancers that induces immune tolerance by suppressing T-cell activity. IDO1 has been linked to the progression of bladder cancer with some prognostic significance [60]. Hence, it could be a future target for the treatment of bladder cancer.

Epacadostat is an effective inhibitor of IDO1 and currently in the early phase of clinical development [61]. It is being investigated both as monotherapy and in combination in several malignancies. It was evaluated in combination with pembrolizumab in a phase-1/2 study involving 54 patients with metastatic cancer [62]. An interim analysis was encouraging as in seven evaluable melanoma patients, ORR was 57%, and disease control rate (DCR) was 86%. In five evaluable renal cell cancer patients, ORR was 40%. In another study, epacadostat administered with ipilimumab in patients with metastatic melanoma yielded an ORR of 30% [63]. Combinational studies of epacadostat with nivolumab are currently in progress (NCT02327078).

A phase-1 study of single-agent indoximod involving 48 advanced cancer patients concluded this agent to be safe up to 2000 mg taken twice daily [64]. Although there were no partial or complete responses, durable stable disease (> 6 months) was observed in five patients.

The postulate that IDO inhibitors may potentiate the anti-tumor activity by improving response to the conventional chemotherapy has led to combinational studies. Indoximod has been evaluated in combination with docetaxel in a dose-escalation study in 27 patients with metastatic cancer [65]. Investigators reported a partial response rate of 18%, stable disease lasting less than 6 months in 36%, and stable disease lasting over 6 months in 4% of patients. Common grade-3/4 AEs included neutropenia and febrile neutropenia (both 13%). In a phase-1b study, indoximod was given with ipilimumab in metastatic melanoma with good tolerability [66]. This study has progressed into phase-2 where indoximod is planned to be given with clinician choice of either ipilimumab, pembrolizumab, or nivolumab. In case of disease progression, therapy can be switched from one checkpoint-inhibitor (anti-CTLA-4 or anti-PD-1) to another while continuing indoximod (NCT02073123).

GDC 0919 is yet another experimental agent employing the same pathway. In a phase-1a study of 19 patients with recurrent/advanced solid tumors, MTD was not reached; however, 800 mg twice a day on a 21-/28-day cycle was well tolerated [67]. 44% had stable disease at the time of interim analysis with acceptable toxicity with an exception of one grade-4 lower gastrointestinal hemorrhage. A phase-1b study is currently recruiting patients with locally advanced or metastatic solid tumors for GDC-0919 and atezolizumab combination (NCT02471846).

Drug targeting chemokine signalling

The presence of immune cells in tumor microenvironment largely depends on chemokine ligands on these cells and their receptors on tumor cells [68]. Chemokines are structurally divided into four subgroups, namely, CXC, CC, CX3C, and C. Targeting chemokine pathway could provide us an important breakthrough in cancer treatment.

CXCR1/2

CXCR1/2-CXCL8 axis activates multiple intracellular signalling pathways that regulate proliferation and differentiation of immune cells. This axis also mediates progression of multiple cancers and hence is associated with early relapse and poor prognosis. Preclinical data suggest that CXCR1/2 axis is involved in bladder cancer progression and the development of metastasis, necessitating the need for further investigation into the role of inhibitors of CXCR1/2 [69].

Reparixin, an inhibitor of CXCR1/2, has already shown activity in combination with paclitaxel both in hormone receptor positive and triple receptor negative breast cancer [70]. However, its impact on the bladder cancer immune microenvironment remains to be studied.

AZD5069, a CXCR2 inhibitor, is being evaluated in combination with durvalumab for solid cancers including bladder cancer in an early phase study [71]. Interim results have suggested clinical benefit with manageable safety profile. The finals results of this study are expected next year (NCT02499328).

CXCR4

Activation of CXCR4-CXCL12 axis activates intracellular pathways that are associated with cancer progression and development of metastasis. Recent evidence suggests that bladder cancer cells express CXCR4 and its upregulated in metastatic disease. Taken together, multiple therapies are under development targeting this pathway to modulate immune response. BL8040, LY2510924, and PTX9908 are currently undergoing evaluation in various solid and hematological malignancies. Clinical trials using these agents both as monotherapy and in combination with immune checkpoint inhibitors with strong pharmacodynamic end-points are needed in advanced bladder cancer.

CSF1R

Preclinical studies have reported that granulocyte colony-stimulating factor receptors (G-CSFRs) are expressed on the surface of bladder cancer cells. Colony-stimulating factor-1 expressed in tumor surfaces is responsible for activating tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC) resulting in an imbalance between T-cell population in their microenvironment which results in chemotherapy resistance [72]. The precise role of G-CSFRs in bladder cancer physiology is not yet understood. PLX3397, JNJ-40346527, FPA008, AC-708, and IMC-CS4 are all CSF1R inhibitors in various phases of clinical development.

PLX3397 was evaluated in combination with paclitaxel in a phase-1b study in patients with advanced solid tumors [73]. Of 23 patients that were evaluable for treatment response, four had a partial response (including one with bladder cancer), while another 10 had stable disease. PLX3397/paclitaxel combination will be further evaluated in the I-SPY-2 neoadjuvant breast cancer trial (NCT01525602).

Toll-like receptor (TLR) agonists

TLRs enhance immunity through recognition of microbial pathogen-associated molecular patterns (PAMPs) and endogenous danger signals (DAMPs) released from dying cells. It has been reported that TLR expression is reduced in bladder cancers. TLR agonists have been able to activate immune response against bladder cancer in preclinical studies [74].

Disappointingly, TLR agonists as a single agent have shown poor efficacy in earlier trials, thus, necessitating to be further evaluated in combination with other agents to enhance their immunostimulatory effects. VTX-2337 is a TLR8 agonist that in combination with cetuximab in patients with head and neck cancer in a phase-1b clinical trial showed good tolerability and treatment response [75]. Another trial of VTX-2337 in combination with pegylated doxorubicin involving patients with metastatic ovarian cancer is currently underway (NCT02431559).

In a double-blinded phase-2 trial, MGN1703 (another TLR9 agonist) has shown promising activity (against placebo) as a maintenance therapy in 59 patients with metastatic colorectal cancer who had normalized CEA after the first-line induction therapy [76]. In a subgroup of patients with high activated NKT cell counts at baseline, there was a significant improvement in PFS. These results are encouraging but need further validation due to small study sample and immature survival data. These agents may potentially have broader utility and should also be explored in bladder as well as other immunogenic cancers.

Drugs targeting interleukin pathway

ALT-801

Recombinant human interleukin-2 (IL-2) is known to be able to induce durable complete responses in a small number of patients with metastatic melanoma and kidney cancer;

however, it is associated with significant toxicities such as hypotension, capillary leak syndrome, and oliguria. ALT-801 is an innovative immunotherapeutic fusion protein consisting of IL-2, linked to a single-chain T-cell receptor domain that recognises a peptide epitope (aa264-272) of the human p53 antigen displayed on cancer cells in the context of HLA-A*0201 (p53+/HLA-A*0201). It is more immune stimulatory than IL-2 alone and potent against solid/hematological malignancies in patients with tumors that are positive for p53 (aa 264–272)/HLA-A*0201 [77]. A phase-1/2 study in advanced/metastatic bladder cancer in 62 patients of whom majority were chemotherapy-refractory, reported efficacy and safety of ALT-801 in combination with gemcitabine and cisplatin [78]. ORR was observed in 35% (including complete responses), while grade-3/4 toxicities mostly hematological were seen in 50% patients.

A phase-1 trial evaluated two cycles of induction with intravenous ALT-801 (4 doses, days 3, 5, 8, 15) and gemcitabine (2 doses, days 1, 8) 13 days apart in BCG-resistant high-risk NMIBC [79]. Patients who had a biopsy-proven complete response (CR) received one maintenance cycle and underwent response assessment. Of the six patients who received induction and maintenance treatment, CR was observed in three, which was durable in two patients lasting ≥ 18 months.

There may be a therapeutic potential for combining ALT-801 with chemotherapy or other immunotherapeutic agents in future.

ALT-803

Interleukin-15 (IL-15) is a key factor for the development, proliferation, and activation of natural-killer cells and CD8+ memory T-cells. ALT-803 is a novel IL-15 agonist (N72D) with enhanced IL-15 biological activity and has so far been studied in animal models only. It has demonstrated durable anti-tumor activity in breast and colon murine models [80]. Intravesical ALT-803 along with BCG treatment has shown to reduce tumor burden in bladder cancer in rat models [81]. Further clinical studies in combination with other immunotherapeutic agents in muscle-invasive and metastatic bladder cancer are warranted.

Drugs targeting CD26 and cyclin-dependent kinase (CDK)

CD26 expression on T-helper cells correlates well with anti-tumor activity in vivo [82]. YS110 is a monoclonal antibody with high affinity to the CD26 antigen. The first-in-human study evaluated YS110 in 33 patients with advanced cancers. There was no complete or partial response, but prolonged stable disease was observed in half the of study population [83]. Common AEs reported were low-grade asthenia and pyrexia. It is important to note that most of study patients had treatment-refractory mesothelioma with at least three prior lines of therapies.

Flavopiridol, a pan-CDK inhibitor, for intravesical use has shown promising activity in bladder cancer in preclinical models and is expected to be evaluated soon in clinical trials [84]. Another CDK4-6 inhibitor, palbociclib, is being evaluated in treatment-refractory metastatic bladder cancer (NCT02334527).

Vaccines

Although there are ever increasing number of immune targets and emerging therapeutic agents in bladder oncology, cancer vaccines that had previously been an aspiration only have now become an expanding area of immunotherapeutics. NEO-PV-01 is a unique vaccine employing the concept of neoantigens. Tumor cell-surface neoantigens are the "unique to cancer DNA sequences", that once identified, are synthesized in the lab and mixed with an adjuvant immune enhancer. Typically, around 5% of the mutated genes are potential neoantigens. It is being tested in a phase-1 study along with nivolumab in bladder cancer (NCT02897765).

Vesigenurtacel-L (HS-410) consists of an allogeneic cell line, selected for high expression from a series of bladder tumor antigens, transported by cell-secreted gp96-Ig to APCs thus, activating CD8+ cytotoxic T-cells. A phase-2 trial of 78 patients who were either BCG-naïve or recurrent, with intermediate or high-risk NMIBC were randomized against placebo in combination with 6 weeks of induction BCG, followed by 6 weeks of HS-410 and further followed by 3-weekly treatments at 3, 6, and 12 months [85]. The combination was well tolerated. The immunologic activity was measured by tumor-infiltrating lymphocytes (TIL) being 60% negative pre-treatment as compared to 15% negative post-treatment.

A meta-analysis reviewing expression of human epidermal growth-factor receptor-2 (HER-2) in bladder cancer confirmed the presence in 27.8–85.2% of all bladder cancers and its presence was related with higher tumor grade, lymph node metastasis, and poor disease-specific survival [86]. DN24-02 is an autologous cellular immunotherapy vaccine that targets HER-2 receptor and is currently being investigated in a randomized phase-2 study in patients with high-risk HER-2 positive bladder cancer (NCT01353222).

Modified vaccinia virus Ankara vaccine expressing p53 (p53MVA) made from a gene-modified virus may help the body build a strong immune response to kill tumor cells. A phase-1 trial in combination with pembrolizumab is in progress (NCT02432963).

Drug conjugates

Antibody-drug conjugate (ADC) is a novel way of utilizing immune system to work synergistically with chemotherapy to improve outcomes. Sacituzumab govitecan (IMMU-132) is an ADC consisting of a humanized anti-Trop-2 monoclonal antibody (hRS7) conjugated with the active metabolite of irinotecan, SN-38. This makes use of Trop-2, a receptor widely expressed in different tumors conferring targeted delivery of chemotherapy to cancer cells. In a small study of 32 platinum-pre-treated patients with metastatic bladder cancer, sacituzumab monotherapy was well tolerated and was associated with an ORR of 36% and a median PFS of 7.2 months [87]. Because of intriguing data in several other tumor types, as well, the US FDA has recently granted breakthrough therapy designation to sacituzumab. Future large clinical studies could provide a confirmation of the value of this agent in bladder cancer.

Modified T-cells with innate anti-tumor activity using chimeric antigen receptors (CARs)

The concept of development of genetically modified T-cells with innate anti-tumor activity using chimeric antigen receptors (CARs) is still experimental in epithelial malignancies but has been successfully trialed in patients with relapsed acute lymphoblastic leukaemia resulting in a high remission rate [88]. This opens up a new immunotherapeutic possibility in bladder cancer research models.

Oncolytic viruses

Coxsackie virus A21 (CVA21) is a bio-selected oncolytic and immunotherapeutic strain of Coxsackie family given intratumorally to provoke an immune response. It is being tested alone as well as in combination with pembrolizumab in a phase-1 trial (NCT02043665).

Another phase-1b clinical trial combining oncolytic virus, Ad11/Ad3 chimeric group-B adenovirus with nivolumab is underway in metastatic cancers (NCT02636036).

Photoimmunotherapy

Monoclonal antibody-photo-absorber conjugate is a novel selective method of delivering light therapy enticing good immune response in bladder cancer. T_{regs} are known to suppress immune response against cancers and result in tumor immune escape. Photoimmunotherapy can generate good immune response by systemic depletion of T_{regs} in the tumor microenvironment. A direct cytotoxic effect of photoimmunotherapy results from the release of free radicals. This effect requires three components; a photosensitive molecule, light of a specific wavelength, and oxygen. A study in 2013 reported the efficacy and safety of Radachlorin, a photosensitive drug, in patients with BCG-refractory high-grade NMIBC [89]. Recurrence-free rates of 91 and 64% at 12 and 24 months were reported, respectively.

It has been tested successfully [90] in experimental models in EGFR-positive NMIBC using panitumumab as a photo-absorber. Further studies could prove its benefit in bladder cancer.

Conclusion

The role of immunotherapy in treatment paradigm for various cancers is rapidly expanding. Bladder cancer being immunogenic is another perfect target for further evaluation of these checkpoint inhibitors. These immunotherapeutic agents have already demonstrated promising activity and will likely play a dominant role in changing the future landscape of bladder cancer treatment. As discussed above, several studies are ongoing to find the best tolerated dose of newer agents alone or in combination with other chemotherapeutic and established immunotherapeutic agents. Of note, the conditional regulatory approval of immunotherapeutic agents in bladder cancer has been based on ORR only and survival data are currently lacking. The future utility of these agents would depend on survival results from ongoing post-approval confirmatory trials.

The future successful development of immunotherapy in bladder cancer would involve overcoming many obstacles including better understanding of tumor heterogeneity, establishing mechanism of primary and secondary treatment resistance, developing effective synergistic combinations (and regimens) without increased level of treatment related toxicities, and tackling a high cost of new agents in the era of constrained resources. In the battle against these unique challenges, the incorporation of unparalleled genomic information and new biomarkers in clinical trials may lead the way forward. The cancer drug development community should resist the temptation to use uncontrolled small studies and relying solely on surrogate end-points. Future clinical trials in bladder oncology should be geared towards new innovative designs that are enriched with patients who would most likely gain an improvement in survival.

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