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Anti-Tumour Treatment

A review on the evolution of PD-1/PD-L1 immunotherapy for bladder cancer: The future is now

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ABSTRACT

The treatment of bladder cancer has evolved over time to encompass not only the traditional modalities of chemotherapy and surgery, but has been particularly impacted by the use of immunotherapy. The first immunotherapy was the live, attenuated bacterial Bacillus Calmette–Guérin vaccine, which has been the standard of care non-muscle-invasive bladder cancer since 1990. Modern immunotherapy has focused on inhibitors of checkpoint proteins, which are molecules that impede immune function, thereby allowing tumor cells to grow and proliferate unregulated. Several checkpoint targets (programmed death ligand-1 [PD-L1] programmed cell death protien-1 [PD-1], and cytotoxic T-lymphocyte associated protein 4 [CTLA4]) have received the most attention in the treatment of bladder cancer, and have inhibitor agents either approved or in late-stage development. This review describes the most recent data on agents that inhibit PD-L1, found on the surface of tumor cells, and PD-1 found on activated T and B cells and macrophages. Atezolizumab is the only member of this class currently approved for the treatment of bladder cancer, but nivolumab, pembrolizumab, durvalumab, and avelumab all have positive results for this indication, and approvals are anticipated in the near future. The checkpoint inhibitors offer an effective alternative for patients for whom previously there were few options for durable responses, including those who are ineligible for cisplatin-based regimens or who are at risk of significant toxicity. Research is ongoing to further categorize responses, define ideal patient populations, and investigate combinations of checkpoint inhibitors to address multiple pathways in immune system functioning.

Introduction

Bladder cancer is the fifth most common cancer in the United States (US), and as of 2012, is the ninth most common cancer diagnosed worldwide, affecting 430,000 people and resulting in 165,000 deaths annually [1,2]. The greatest risk factor for bladder cancer is tobacco smoking and worldwide incidence rates correspond with smoking prevalence [2]. Although significant time, effort, and spend has been dedicated to bladder cancer research, overall incidence and mortality rates have changed little over the past 20 years [1–3]. Worldwide incidence rates have remained relatively stable for two primary reasons: (1) although smoking rates have declined, population growth has resulted in a greater number of smokers [4]; and (2) the current prevalence of bladder cancer reflects smoking behaviors from 20 to 30 years ago when cigarette smoking was more widespread, at least in developed countries [5]. Furthermore, even in high-income countries where standards of care are high, outcomes are relatively unchanged from 20 years ago, contributing to the stagnant mortality rates. For example, in Norway, 5-year relative survival among men diagnosed with bladder cancer was 73% during the period of 1994–1998 and 76% during the period of 2009–2013 [6]. New treatments are clearly needed.

Advances in treatment

Treatment for an individual patient is designed around tumor stage, size, and grade, as well as the overall health and preferences of the patient. Thus, accurate classification of bladder tumors is critical. The World Health Organization (WHO) introduced the first international, systematic approach to the grading of urothelial cancers in 1973 [7,8]. These guidelines represented a significant

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advancement in the management of bladder neoplasia but lacked clearly defined criteria for each grade, resulting in misclassification of tumors [8]. In 2004, the WHO grading system was revised to eliminate the ambiguities of the original system, and a minimally amended update of the 2004 revision was published in 2016 [9,10]. The utility of the 2004 guidelines was validated in a study of 1515 patients who underwent transurethral resection of primary non-muscle invasive bladder cancer (NMIBC) that demonstrated distinct differences in progression rates and mortality for the revised categories, and this system is, therefore, widely used by pathologists today [11,12].

Modern therapy for bladder cancer encompasses surgical, chemotherapeutic, radiologic, and immunotherapeutic modalities that have their origins primarily in the 19th century when scientific institutions, journal publications, and medical congresses were established, facilitating dialog among researchers and clinicians and spurring advances across medical fields [13]. Although bladder cancer as a malady was probably recognized since ancient times, tumors affecting the bladder were first mentioned by Lacuna in 1551 and the first surgeries specifically targeting bladder tumors were performed in the 16th and 17th centuries, as cited in Herr 2006 [13]. In 1910, Edwin Beer was the first to use electroresection to treat papillary bladder tumors, and since then, TURBT, first introduced in the 1930s, has become the primary avenue for obtaining histologic samples for diagnosis/staging and also for curing patients by removing the tumor tissue [13,14].

Cancer chemotherapy is a product of the 20th century and the accidental discovery during World War I that exposure to mustard gas depleted bone marrow cells and lymph nodes [15]. This discovery sparked an explosion of research into anticancer agents, and in 1978 the U.S. Food and Drug Administration (FDA) approved cisplatin for testicular and ovarian cancers, which was expanded in 1993 to bladder cancer, and was the first chemotherapy drug approved for bladder cancer.

Although typically thought of as a modern invention, as cited in Decker 2009, immunotherapy has its origins in the late 19th century, when William B. Coley noted that patients with cancer went into remission after developing erysipelas [16]. He experimented by injecting mixtures of live and inactivated *Streptococcus pyogenes* and *Serratia marcecsens* directly into tumors, achieving remission in patients with a variety of malignancies, including sarcoma, lymphoma, and testicular cancer [16]. However, because a mechanistic explanation for his results was at the time, elusive, and research into surgery and radiotherapy was becoming predominant, immunotherapy was not further pursued. Meanwhile, in 1908, Albert Calmette and Camille Guérin began the development of a vaccine against tuberculosis (TB), as cited in Herr 2008 [17]. They found success in 1921 when they administered the vaccine to a baby whose mother had died of TB and whose grandmother was close to death from TB. The baby was protected from TB and the live, attenuated bacterial vaccine, labeled Bacillus Calmette–Guérin, or BCG, after its creators, was put into widespread use. In the meantime, it was recognized that TB had antitumor effects, and a series of experiments beginning in the 1950s showed that mice injected with BCG demonstrated resistance to challenge with transplanted tumors [17,18]. In 1969, investigations in humans began when BCG (administered by scarification or percutaneous inoculation) was tried in acute lymphoblastic leukemia, and via the intratumoral route for intradermal and in-transit metastases of malignant melanoma. Further clinical trials were conducted using BCG against lung, prostate, colon, and kidney cancers, but the results were disappointing. However, animal experiments showed that *topical* BCG was highly effective against bladder tumors, with the first human study of intravesical BCG conducted

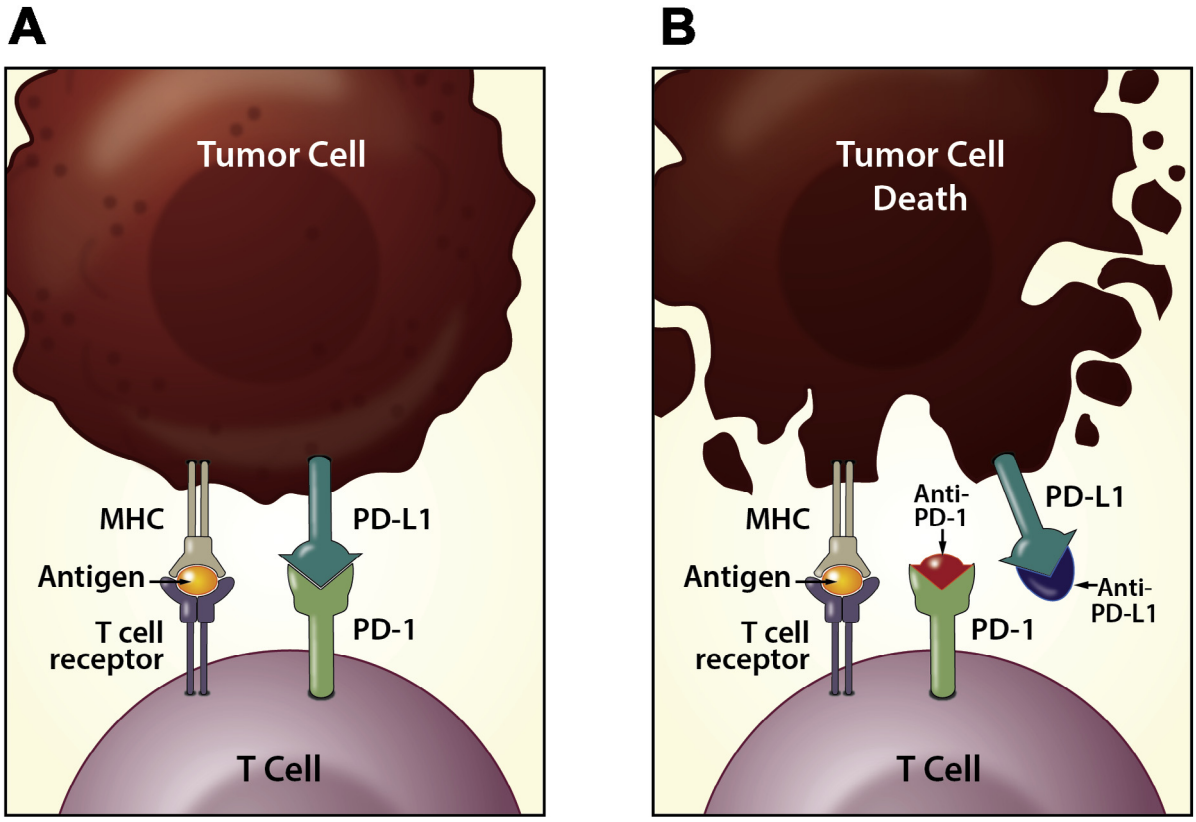

Fig. 1. Mechanism of action of PD-1 and PD-L1 inhibitors. A. PD-L1 binds to PD-1 and inhibits T-cell killing of tumor cells. B. Blocking PD-L1 or PD-1 allows T-cell killing of tumor cells. MHC = major histocompatibility complex; PD-1 = programmed cell death protein-1; PD-L1 = programmed death ligand-1.

in 1976 [19]. In 1990, the FDA approved the use of intra-vesical BCG for patients with superficial bladder cancer, and it is still the recommended standard of care for high-grade noninvasive bladder cancer [17,20].

The modern era of immunotherapy

The premise of immunotherapy is for the body to heal itself. Immunotherapy, such as BCG, functions by allowing or stimulating the immune system to do what it has evolved to do, namely, protect the body from foreign invaders and other threats. Although malignant cells exhibit differences in antigenicity from healthy cells that prime them as targets for the immune system, they have evolved a number of mechanisms that allow them to evade immune recognition [21]. Cancer cells can downregulate the expression of tumor antigens (molecules that are unique to tumor cells) on the cell surface so that they are no longer detected as foreign [22]. They can express other proteins on the cell surface, that induce immune cell deactivation [23], and they can induce cells in the tumor microenvironment to release cytokines, such as transforming growth factor beta (TGF-β), that suppress immune responses while promoting tumor cell proliferation and survival [24].

The mechanism of action of BCG is not fully elucidated but is thought to elicit an immune response in much the same way as native TB. It has been found that BCG shows a predilection for entering bladder cancer cells where it is broken down and the resulting antigenic fragments combine with the histocompatibility complex on the tumor cell surface to induce cytokines and direct cytotoxicity responses [25].

The potential of immunotherapy has energized research in this area, with a broad array of therapeutic modalities under investigation, including allogeneic stem cell transplants, antineoplastic vaccines, proinflammatory cytokines, chimeric antigen receptors, and adoptive T-cell transfer, among others. One of the most promising has been the use of monoclonal antibodies to block the negative co-signaling molecules that prevent an effective immune response. The result of these ‘‘checkpoint” inhibitors is the reinvigoration of T-cell–mediated antitumor activity [26].

Focus on checkpoint inhibitors

The primary role of T cells is to distinguish healthy cells from pathogens or malignant cells through the activation or deactivation of various receptors on the T-cell surface. As mentioned previously, malignant cells can escape detection through cell surface molecules that interact with the receptors on T cells to, in essence, mimic the signals released by healthy cells. The result is an immune system that remains inactive against malignant cells, allowing their unregulated growth and proliferation. Because these molecules and their associated receptors on T cells keep the immune system ‘‘in check,” by impeding immune functioning, they are collectively called checkpoint proteins. Checkpoint inhibitors interfere with the effects of these checkpoint proteins, which serves to ‘‘release the brakes” on the immune system.

Three checkpoint targets (programmed death ligand-1 [PD-L1] programmed cell death protein-1 [PD-1], and cytotoxic T-lymphocyte associated protein 4 [CTLA-4]) have been the primary focus of investigation for the treatment of bladder cancer, and have inhibitor agents either approved or in late-stage development. In the late 1990s/early 2000s, the binding of PD-L1, found on the surface of tumor cells, to PD-1 found on activated T and B cells and macrophages, was shown to result in a net immunosuppressive effect (See Fig. 1) [27–29]. In addition to bladder cancer cells, PD-L1 is expressed on the cell surface of numerous cancers, including melanoma, renal cell carcinoma, lung cancer, head and neck cancers, ovarian cancer, and hematologic malignancies, and is found in normal tissues, such as heart, lung, and placenta [30,31]. Agents that inhibit PD-L1 include atezolizumab, durvalumab, avelumab, and BMS-936559 (MDX-1105); and inhibitors of PD-1 include nivolumab and pembrolizumab. Another checkpoint molecule that has been extensively studied is CTLA-4, which is expressed exclusively on T cells. CTLA-4 has been implicated in the regulation of immune system functioning since the mid-1990s, when it was shown that loss of CTLA-4 led to lymphoproliferation and fatal multi-organ tissue destruction in mice [32,33]. Ipilimumab and tremelimumab are two agents that block the activities of CTLA-4. An overview of the status of planned and ongoing clinical trials of these agents in bladder cancer is provided in Table 1.

Rationale for PD-L1/PD-1 axis inhibition

Levels of PD-L1 expression have been shown to correlate with bladder cancer severity and outcome. It has been found that tumors that express higher levels of PD-L1 (on tumor cells) are more likely to be considered high-grade, and patients experience higher frequencies of postoperative recurrence and poorer survival in organ-confined disease [34–36]. In addition, PD-L1 tumor cell expression is associated with increased resistance to BCG therapy, which is thought to be related to the associated suppression of the immune system, since a fully functioning immune system is required for BCG efficacy [35]. In contrast, a recent study found that PD-L1 expression did not differ between NMIBC and muscle-invasive bladder cancer (MIBC) and that the PD-L1 expression in tumor-infiltrating mononuclear cells (in immune cells) was predictive of longer overall survival (OS) times in patients who developed metastases and received subsequent chemotherapy. It is suspected to be due to the specificity of these cells against the tumors. Overall, cancers with the highest mutational burden, such as bladder cancer, seem to benefit the most from checkpoint blockade because of the greater T-cell-mediated antitumor immune response elicited by these cancers [37]. Agents in this class have been found to be active in other malignancies since 2012, but the first report of the clinical activity of checkpoint inhibitors in MIBC was published in 2014 [38]. Since then there has been a veritable explosion of data, much of which has yet to be fully published. The most recent clinical trial results (both published and in congress abstracts) for bladder cancer are presented in Table 2.

Atezolizumab

Atezolizumab was the first PD-L1 inhibitor found active in bladder cancer [38], and is currently the only PD-L1 inhibitor specifically approved for patients with locally advanced or metastatic urothelial carcinoma, who progressed on or after platinum-based chemotherapy [39]. This monoclonal antibody was granted accelerated approval by the FDA in May 2016, and is pending approval in Europe. The initial studies of atezolizumab from 2014 were in non-small-cell lung cancer (NSCLC), with approval for this indication granted in October 2016. It is still under investigation for renal cell carcinoma, melanoma, triple-negative breast cancer, and others.

Cohort 2 of the phase 2 IMvigor 210 trial (NCT02108652) in patients ($N = 310$) with inoperable, platinum-treated, locally advanced or metastatic urothelial carcinoma was the basis for FDA approval. This analysis showed that atezolizumab (1200 mg IV q3w) resulted in an objective response rate (ORR) of 16% for all patients and a 28% ORR in those with $\geqslant 5\%$ of PD-L1 expressing tumor-infiltrating immune cells (IC) after 1.5 years median follow-up [40,41]. Of those patients who experienced a response, these responses tended to be durable, with the median duration of response not reached after a medium of 17.5 months of

Table 1
Overview of planned and ongoing clinical trials of PD-1/PD-L1 inhibitors for bladder cancer with or without other malignancies.

Clinicaltrials.gov number (name)	Phase	Treatments	Population	Planned patients (N)	Date started	Planned completion date
NCT02108652 (IMvigor 210)	2	Atezolizumab	Inoperable, platinum-treated, locally advanced/ metastatic urothelial carcinoma	439/119 separate cohort of cisplatin-ineligible patients	May 2014	Preliminary results reported
NCT02302807 (IMvigor 211)	3	Atezolizumab vs Paclitaxel + docetaxel + vinflunine	Locally advanced/metastatic urothelial carcinoma after failure with platinum-containing chemotherapy	932	January 2015	November 2017
NCT02807636 (IMvigor 130)	3	Atezolizumab + chemotherapy vs Chemotherapy	Locally advanced/metastatic urothelial carcinoma	1400	June 2016	June 2018
NCT02792192	1b/2	Atezolizumab + BCG vs BCG	High-risk NMIBC	70	June 2016	November 2020
NCT02655822	1/1b	Atezolizumab + CPI-444 vs CPI-444	Non-small cell lung cancer, malignant melanoma, renal cell cancer, triple-negative breast cancer, head and neck cancer, colorectal cancer, bladder cancer	534	January 2016	December 2018
NCT02844816	2	Atezolizumab	Recurrent BCG-unresponsive NMIBC	143	February 2017	February 2019
NCT02662309 (ABACUS)	2	Preoperative atezolizumab	Transitional cell carcinoma of the bladder	85	February 2016	March 2019
NCT02450331 (IMvigor 010)	3	Adjuvant atezolizumab vs observation	PD-L1-positive, high-risk MIBC after cystectomy	700	October 2015	April 2022
NCT02451423	2	Atezolizumab	BCG-refractory NMIBC or muscle-invasive TCC appropriate for cystectomy and refusing or ineligible for neoadjuvant chemotherapy	42	April 2016	December 2019
NCT01928394 (CheckMate 032)	1/2	Nivolumab + ipilimumab vs nivolumab	Triple-negative breast cancer, gastric cancer, pancreatic adenocarcinoma, and small cell lung cancer, bladder cancer, and ovarian cancer	1100	October 2013	December 2018
NCT02553642 (CA209-260)	2	Nivolumab + ipilimumab vs nivolumab	Advanced melanoma or bladder cancer	120	September 2015	September 2017
NCT02845323	2	Nivolumab + urelumab vs nivolumab	MIBC ineligible for cisplatin-based chemotherapy	44	September 2016	January 2019
NCT02496208	1	Nivolumab + cabozantinib-s-malate + ipilimumab vs Nivolumab + cabozantinib-s-malate	Advanced/metastatic urothelial carcinoma and other genitourinary tumors	66	July 2015	December 2017
NCT02423343	1b/2	Nivolumab + galunisertib	Advanced refractory solid tumors	100	October 2015	March 2019
NCT01714739	1	Nivolumab + lirilumab	Select advanced solid tumors	162	October 2012	July 2019
NCT02387996 (CheckMate 275)	2	Nivolumab	Metastatic or unresectable urothelial cancer w/ progression/recurrence following platinum-based chemotherapy	242	March 2015	October 2017
NCT02632409 (CheckMate 274)	3	Nivolumab vs placebo	Bladder or upper urinary tract cancer following surgery	640	February 2016	October 2020
NCT01848834 (KEYNOTE-012)	1b	Pembrolizumab	Advanced triple-negative breast cancer, advanced head and neck cancer, advanced urothelial cancer, advanced gastric cancer	297	May 2013	November 2016
NCT02256436 (KEYNOTE-045)	3	Pembrolizumab vs paclitaxel + docetaxel or vinflunine	Recurrent or progressive metastatic urothelial cancer after failure with platinum-containing chemotherapy	470	October 2014	May 2017
NCT02560636 (PLUMMB)	1	Pembrolizumab vs Radiotherapy	Locally advanced/metastatic bladder cancer	34	June 2016	June 2019
NCT02621151	2	Pembrolizumab + gemcitabine + radiotherapy	MIBC who are ineligible for or decline cystectomy	54	October 2014	May 2024
NCT02324582 (MARC)	1	Pembrolizumab + BCG	High-risk NMIBC postsurgery	15	June 2015	May 2017
NCT02808143	1	Pembrolizumab + BCG	High-risk, BCG-refractory NMIBC	27	July 2016	January 2019
NCT02662062 (ANZUP PCR-MIB)	2	Pembrolizumab + cisplatin + radiotherapy	Non-metastatic MIBC who are ineligible for or decline cystectomy	30	August 2016	January 2024
NCT02736266 (PURE01)	2	Neoadjuvant Pembrolizumab + chemo-radiotherapy	MIBC prior to cystectomy	90	May 2016	November 2017
NCT02335424 (KEYNOTE-052)	2	Pembrolizumab	Advanced/unresectable or metastatic urothelial cancer who are ineligible for cisplatin	350	February 2015	June 2018
NCT02625961 (KEYNOTE-057)	2	Pembrolizumab	High-risk NMIBC unresponsive to BCG	260	February 2016	May 2020
NCT02710396	2	Pembrolizumab	NSCLC, head and neck cancer, bladder cancer, esophageal squamous cell carcinoma, transitional cell carcinoma	120	March 2016	March 2023

(*continued on next page*)

Table 1 (*continued*)

Clinicaltrials.gov number (name)	Phase	Treatments	Population	Planned patients (N)	Date started	Planned completion date
NCT02043665 (STORM/ KEYNOTE-200	1	Pembrolizumab + Coxsackie virus A21	NSCLC, castration-resistant prostate cancer, melanoma, bladder cancer	90	January 2014	August 2019
NCT02690558	2	Pembrolizumab + gemcitabine + cisplatin	MIBC prior to cystectomy	39	May 2016	April 2020
NCT02365766	1b/2	Neoadjuvant Pembrolizumab + gemcitabine + cisplatin (Cohort 1) or Pembrolizumab + gemcitabine (Cohort 2)	MIBC or urothelial cancer with/without cisplatin-eligible disease	81	May 2015	March 2018
NCT02500121	2	Pembrolizumab vs placebo	Metastatic urothelial cancer	200	November 2015	November 2019
NCT02346955 (MK-6018–001)	1	Pembrolizumab + CM-24 (MK-6018) vs CM-24	NSCLC, melanoma, bladder cancer, colorectal cancer, gastric cancer, ovarian cancer	196	February 2015	May 2019
NCT02452424	1/2a	Pembrolizumab + PLX3397	Melanoma, NSCLC, ovarian cancer, triple-negative breast cancer, squamous cell carcinoma of the head and neck, bladder cancer, pancreatic ductal adenocarcinoma, gastric cancer	400	June 2015	July 2019
NCT02178722 (KEYNOTE-137/ECHO-202)	1/2	Pembrolizumab + epacadostat	Malignant solid tumors, lymphoma, diffuse large B-cell carcinoma, NSCLC, transitional cell carcinoma of urinary tract, triple-negative breast cancer, squamous cell of head and neck, ovarian neoplasms, adenocarcinoma of the endometrium, renal cell carcinoma, microsatellite-instability high colorectal cancer	403	June 2014	November 2017
NCT02437370	1	Pembrolizumab + docetaxel vs pembrolizumab + gemcitabine	Platinum pre-treated urothelial cancer	38	August 2015	December 2019
NCT02432963	1	Pembrolizumab + modified vaccinia virus Ankara vaccine-expressing p53	Bladder cancer, colon cancer, estrogen-receptor-negative cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, HER2/Neu-negative cancer, melanoma, NSCLC, pancreatic cancer, progesterone-receptor-negative cancer, rectal carcinoma, renal cell carcinoma, soft tissue sarcoma, triple-negative breast cancer	12	November 2015	April 2017
NCT02619253	1/1b	Pembrolizumab + vorinostat	Advanced renal or urothelial cell carcinoma	42	January 2016	May 2018
NCT02636036 (SPICE)	1	Pembrolizumab + enadenotucirev	Metastatic or advanced epithelial tumors	30	January 2016	June 2019
NCT02501096	1b/2	Pembrolizumab + lenvatinib	Non-small cell lung cancer, renal cell carcinoma, endometrial cancer, urothelial cancer, squamous cell carcinoma of the head and neck, or melanoma	150	July 2015	October 2017
NCT02717156	2	Pembrolizumab + Recombinant EphB4-HSA Fusion Protein	Metastatic urothelial cancer refractory to platinum	60	June 2016	June 2020
NCT01693562	1/2	Durvalumab	Histologically or cytologically confirmed advanced solid tumor who are ineligible or progressed on first-line therapy	1173	August 2012	July 2018
NCT02546661 (BISCAY)	1b	Durvalumab + AZD4547 vs durvalumab + olaparib vs durvalumab + AZD1775 vs durvalumab vs AZD4547	MIBC who progressed on prior treatment	110	August 2016	June 2018
NCT02516241 (DANUBE)	3	Durvalumab + tremelimumab vs chemotherapy	Stage IV urothelial bladder cancer	1005	November 2015	September 2019
NCT02812420	Pilot	Durvalumab + tremelimumab	MIBC with high-risk urothelial carcinoma who are ineligible for cisplatin-based neoadjuvant chemotherapy	15	September 2016	September 2018
NCT02643303	1/2	Durvalumab + tremelimumab + tumor microenvironment modulator, polyICLC	Head and neck squamous cell carcinoma, breast cancer, sarcoma, Merkel cell carcinoma, cutaneous T-cell lymphoma, melanoma, renal cancer, bladder cancer, prostate cancer	102	October 2016	August 2022
NCT02527434	2	Durvalumab + tremelimumab vs durvalumab vs tremelimumab	Urothelial bladder cancer, triple-negative breast cancer, pancreatic ductal adenocarcinoma	76	November 2015	April 2018
NCT02118337	1/2	Durvalumab + MEDI0680 vs MEDI0680	Select advanced malignancies	150	May 2014	January 2019
NCT02603432 (JAVELIN Bladder 100)	3	Avelumab	Locally advanced or metastatic urothelial cancer that did not worsen during or following completion of first-line chemotherapy	668	April 2016	July 2019
NCT01772004 (JAVELIN	1	Avelumab	Non-small cell lung cancer (first line or post-platinum), metastatic breast cancer, colorectal	1700	January 2013	May 2018

Table 1 (*continued*)

Clinicaltrials.gov number (name)	Phase	Treatments	Population	Planned patients (N)	Date started	Planned completion date
Solid Tumor)			cancer, urothelial carcinoma (secondary), mesothelioma, gastric/GEJ cancer (first line switch maintenance and second line), ovarian cancer (secondary and platinum refractory + liposomal doxorubicin), renal cell carcinoma (second line), melanoma and head, neck squamous cell carcinoma, castration-resistant prostate cancer, adrenocortical carcinoma, urothelial carcinoma (efficacy), and gastric/GEJ cancer (third line)			
NCT01943461 (JAVELIN Solid Tumor JPN)	1	Avelumab	Japanese subjects with metastatic or locally advanced solid tumors for which no standard therapy exists or standard therapy has failed	57	September 2013	May 2017

BCG = Bacillus Calmette–Guérin; GEJ = gastroesophageal junction; MIBC = muscle-invasive bladder cancer; NMIBC = non-muscle invasive bladder cancer; NSCLC = non-small cell lung cancer; PD-L1 = programmed death ligand 1.

Table 2
Summary of the most recent reported results of studies of PD-1/PD-L1 inhibitors in patients with bladder cancer.

Agent	Trial name	Phase	Dose	Population	Patients (N)	ORR (%)	Most common reported AEs	Reference
Atezolizumab	IMvigor 210	2	1200 mg IV q3 w	Inoperable, platinum-treated, locally advanced/metastatic urothelial carcinoma	310/119 separate cohort of cisplatin-ineligible patients	16% overall 28% in those with ⩾5% of PD-L1 IC 24% in cisplatin-ineligible patients	Fatigue = 31% Nausea = 14%	[40–42]
Nivolumab	CheckMate 032	1/2	3 mg/kg IV q2 w	Metastatic urothelial cancer after ⩾1 prior line of platinum-based therapy	78	24% in those with PD-L1 ⩾ 1% expression 26% in those with PD-L1 < 1% expression	Grade 3/4: Increased lipase = 5.1% Increased amylase = 3.8% Fatigue = 2.6% Decreased neutrophils = 2.6% Dyspnea = 2.6%	[44]
Pembrolizumab	KEYNOTE-045	3	200 mg q3 w	Previously treated metastatic urothelial cancer	542	21% overall	Pruritus = 20% Fatigue = 14% Nausea = 11% Diarrhea = 9% Decreased appetite = 9%	[53]
Durvalumab		1/2	10 mg/kg IV q2 w	Inoperable/metastatic urothelial bladder cancer	61	31% overall 46% in those with PD-L1 expression 0% in those without PD-L1 expression	Fatigue = 13% Diarrhea = 10% Decreased appetite = 8%	[54]
Avelumab	JAVELIN Solid Tumor	1b	10 mg/kg IV q2 w	Metastatic urothelial carcinoma	129	17% overall 50% in those with PD-L1 expression	Infusion-related reactions = 23% Fatigue = 15%	[58,59]

AEs = adverse events; CPS = combined positive score; IC = immune cells; IV = intravenous.

follow-up [40,41]. The 12-month OS in patients with ⩾5% of PD-L1-expressing tumor-infiltrating IC was 50% compared with 37% for the overall population [40,41]. Classifying patients based on The Cancer Genome Atlas (TCGA) subtype found that immune cell PD-L1 prevalence was highly enriched in the basal subtype versus the luminal subtype, while tumor PD-1 expression was seen almost exclusively in the basal subtype [41]. Although response to atezolizumab occurred in all TCGA subtypes, it was significantly higher in the luminal cluster II subtype than the others, suggesting that subtypes differ in other immune parameters besides PD-L1 [41]. In Cohort 1 of patients who were chemotherapy-naïve in the metastatic setting and ineligible for cisplatin ($N = 119$), atezolizumab resulted in an ORR of 24% and median OS of 14.8 months across IC categories, and responses were durable, with the median duration of response not reached at 14.4 months of follow-up [42].

In Cohort 2 of this phase 2 trial, the most common adverse reactions with atezolizumab in the overall population were fatigue (31%) and nausea (14%) [40,41]. The rate of grade 3/4 adverse events was 16%, and the rate of discontinuation from the study due to adverse events was low [40,41]. A randomized phase 3 study of atezolizumab versus chemotherapy in patients with

locally advanced or metastatic urothelial cancer that had progressed after platinum-based chemotherapy has completed accrual (IMvigor 211; NCT02302807).

A study of atezolizumab plus or minus gemcitabine/carboplatin or cisplatin versus chemotherapy alone is currently recruiting patients with treatment-naïve locally advanced or metastatic urothelial carcinoma, and is due to be completed in the spring of 2018 (NCT02807636).

Nivolumab

Nivolumab is a monoclonal antibody directed against PD-1, and was the first PD-1 inhibitor approved anywhere in the world when it received marketing approval for unresectable melanoma from Japan in July 2014. It was originally granted accelerated approval by the FDA, and is currently approved in the United States for use in metastatic melanoma (December 2014), NSCLC (March 2015), renal cell carcinoma (November 2015), Hodgkin lymphoma (May 2016), squamous cell cancer of the head and neck (November 2016), and recently for the treatment of patients with locally advanced or metastatic urothelial carcinoma whose disease has progressed during a period of up to 1 year after first-line platinum-containing chemotherapy (February 2017). Nivolumab was approved for advanced melanoma and NSCLC in Europe in 2015, and for renal cell carcinoma in April 2016. The first clinical trial results released in 2012 were in patients ($N = 296$) with advanced solid malignancies, including melanoma, NSCLC, prostate cancer, renal cell cancer, and colorectal cancer [43]. Response rates were in the order of 18% to 28%, and 65% of responses lasted for a year or more in patients with $\geqslant 1$ year of follow-up [43].

Recent results from the nonrandomized, phase 1/2 CheckMate 032 study (NCT01928394) of nivolumab (3 mg/kg IV q2w) in patients ($N = 78$) with metastatic urothelial cancer showed an ORR of 24% for those with PD-L1 expression $\geqslant 1\%$ on tumor cells (TC) versus 26% for those with PD-L1 expression <1%, and overall survival was 9.7 months for the entire population [44]. About 21.8% of patients experienced grade 3/4 adverse events, with increased lipase (5.1%), increased amylase (3.8%), and fatigue, decreased neutrophils, and dyspnea (2.6% each) as the most common; grade 5 pneumonitis and thrombocytopenia occurred in 1 patient each (2.6%) [44].

The single-arm, open-label CheckMate 275 study (NCT02387996) of nivolumab (3 mg/kg IV q2w) in patients with metastatic urothelial cancer who have received prior therapy ($N = 265$) demonstrated an ORR of 19.6% for the total population, 16.1% in those with low or no PD-L1 tumor expression (<1%), and 28.4% in those with PD-L1 tumor expression $\geqslant 5\%$ after a median 7-months follow-up [45]. Median PFS was 2.0 months and the median OS was 8.7 months [45]. A total of 18% of patients experienced grade 3/4 adverse events (fatigue and diarrhea; 2% each) and 1% of patients experienced a grade 5 event [45]. A phase 3 study (CheckMate 274; NCT02632409) of nivolumab versus placebo after surgery in patients with bladder or upper urinary tract cancer is ongoing.

A number of studies are investigating nivolumab plus ipilimumab in different cancers. However, a study of ipilimumab alone added to chemotherapy showed little additional effect over chemotherapy alone in patients with metastatic urothelial cancer [46]. Nevertheless, this is a logical combination since they have complimentary mechanisms of action. PD-1 acts primarily during the effector phase of T-cell activation and the PD-1/PD-L1 interaction occurs primarily in peripheral tissues and organs upon representation of antigens to memory T-cells [47]. CTLA-4 is expressed by regulatory T cells and memory CD-4 cells and is functional during early activation of T cells in lymphatic tissues [47]. As part of the CheckMate 032 study, the combination of nivolumab plus ipilimumab is being investigated: Cohort A ($n = 26$) nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg), and Cohort B ($n = 104$) nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) [48]. The cohort investigating the higher dose of ipilimumab had a numerically greater response rate of 39% (95% confidence interval [CI], 20.2–59.4) versus 26% (95% CI, 17.9–35.5) for the lower dose [48]. Overall survival was similar in both groups: Cohort A = 10.2 months (95% CI, 4.5-NR); Cohort B = 7.3 (95% CI, 5.6–11.4 months) [48]. Median progression-free survival remained less than 5 months in both groups. Adverse events were in line with those previously seen with these drugs in other tumors. Overall these data suggest the combination with the higher dose of ipilimumab may be preferable compared with the lower dose for future development. Comparative studies need to be conducted with these combinations and front-line chemotherapy. Two additional studies in bladder cancer of nivolumab and ipilimumab are currently ongoing. One phase 1 study (NCT02496208) of patients with metastatic genitourinary tumors combines nivolumab, ipilimumab, and cabozantinib, a small molecule inhibitor of c-Met and vascular endothelial growth factor receptor 2. The second study (NCT02553642) is examining the relationship between PD-L1 expression and response to nivolumab/ipilimumab combination therapy in patients with locally advanced/unresectable or metastatic urothelial carcinoma. These studies are expected to be completed at the end of 2017.

Pembrolizumab

Pembrolizumab is a monoclonal antibody that targets the PD-1 receptor, and was approved by the FDA in September 2014 and in Europe in July 2015 for the treatment of advanced melanoma. In October 2015 and October 2016, it was approved by the FDA for the treatment in NSCLC metastatic and first-line settings, respectively; in August 2016, received accelerated approval for head and neck cancer, and in February 2017, was granted priority review for urothelial carcinoma as first-line treatment of patients who are ineligible for cisplatin-containing therapy and as second-line treatment for patients whose disease progressed on or after platinum-containing chemotherapy. The approval for NSCLC in Europe was granted in August 2016. The first positive report of pembrolizumab, published in 2013, was of 135 patients with advanced melanoma who demonstrated durable tumor responses after a median follow-up of 11 months [49].

The KEYNOTE-012 (NCT01848834) phase 1b study showed that in second-line therapy for patients ($N = 28$) with advanced urothelial cancer, the ORR was 25% and the 12-month PFS rate was 19% for the overall population with pembrolizumab (10 mg/kg every 2 weeks); for patients with tumors positive for PD-L1 expression (defined as >1% in tumor nests or a PD-L1-positive band in stroma by a prototype immunohistochemistry assay), the ORR was 38% [50]. The safety analysis of KEYNOTE-012 ($N = 33$) showed that fatigue was the most common adverse event (18%), followed by peripheral edema (12%), and nausea (9%); 15% had grade 3–5 adverse events and 1 patient discontinued due to grade 3 rhabdomyolysis [51].

A planned interim analysis of the first 100 patients in KEYNOTE-052 (NCT02335424) a phase 2 study of pembrolizumab (200 mg q3w) as first-line therapy in patients with advanced/unresectable or metastatic urothelial cancer has reported an ORR of 24.0% unselected subjects and 36.7% in those with $\geqslant 10\%$ combined positive score (CPS; tumor and immune cell PD-L1 expression) after median 8-month follow-up [52]. Moreover, complete responses were seen in 6.0% of all-comers and 13.3% of those with high CPS [52]. Adverse events were common (67%), comprising mainly of fatigue (14%), and 16% experienced a grade 3/4 adverse event [52].

KEYNOTE-045 (NCT02256436), a randomized phase 3 trial of pembrolizumab (200 mg q3w) versus chemotherapy in patients with previously treated metastatic urothelial cancer, showed an OS of 10.3 months with pembrolizumab versus 7.4 months with

chemotherapy for a hazard ratio of 0.73 (95% CI, 0.59–0.91). The survival benefit was observed regardless of PD-L1 expression [53]. These results showed for the first time, there is an agent that improves survival in the second-line setting. The ORR was also significantly improved with pembrolizumab (21.1% vs 11.4%; $P = 0.0011$). Although the incidence of most adverse events was lower in the pembrolizumab arm, the incidence of pruritus was higher with pembrolizumab (20%) than chemotherapy (3%), as were other immune-mediated AEs, including thyroid abnormalities (9% vs 2%), pneumonitis (4% vs 0.4%), and colitis (2% vs 0.4%) [53].

Pembrolizumab is also being investigated in combination with docetaxel or gemcitabine (NCT02437370), and with gemcitabine and cisplatin (NCT02690558). A phase 2 trial of pembrolizumab added to concurrent gemcitabine and radiation in patients with MIBC is underway (NCT02621151), as is a study of pembrolizumab plus cisplatin and radiotherapy (NCT02662062).

Durvalumab

Durvalumab, a monoclonal antibody against PD-L1, was granted breakthrough therapy designation by the FDA in February 2016 for patients with PD-L1 inoperable or metastatic urothelial bladder cancer whose tumor has progressed during or after a standard platinum-based regimen. It is also currently under investigation for the treatment of NSCLC, head and neck cancer, gastric cancer, pancreatic cancer, hepatocellular carcinoma, mesothelioma, and hematologic cancers.

Breakthrough Therapy designation was based on the phase 1/2 study (NCT01693562) of durvalumab (10 mg/kg IV q2w) in patients ($N = 61$) with inoperable or metastatic urothelial bladder cancer [54]. The ORR was 31% in the overall population and 46% in the PD-L1 high (defined as TC or IC $\geqslant$25%) subgroup versus 0% in the PD-L1 low/neg subgroup (defined as TC and IC <25%) [54]. The median duration of response has not yet been reached (range: 4–49 weeks), and responses were ongoing in 12 of 13 patients at the time of publication [54]. The most common adverse events were fatigue (13%), diarrhea (10%), and decreased appetite (8%), and grade 3 adverse events occurred in 5% of patients; there were no grade 4 or 5 events [54]. This trial is currently ongoing with a larger cohort of patients with urothelial bladder cancer, with results expected early 2017.

The combination of durvalumab plus the CTLA-4 inhibitor, tremelimumab, which is currently being examined (DANUBE; NCT02516241) versus standard-of-care chemotherapy in patients with stage IV urothelial bladder cancer, is expected to be completed in 2019. This 3-arm trial ($N = 1004$) compares standard chemotherapy with single agent durvalumab and the combination of durvalumab and tremilimumab, with overall survival as the primary endpoint. Another study (estimated enrollment $N = 15$) is planned to begin recruiting at the end of 2016 to evaluate durvalumab plus tremelimumab in urothelial carcinoma patients who are ineligible for neoadjuvant chemotherapy (NCT02812420). Preliminary results of a phase 1 trial (NCT02118337) of durvalumab in combination with the anti-PD-1 monoclonal antibody, MEDI0680 (AMP-514), in patients with select advanced solid malignancies ($N = 30$) showed a 15% ORR and a 35% disease control rate, and the most common adverse events were pruritus (17%), diarrhea and fatigue (both 13%), and flushing, peripheral edema, and pyrexia (each 10%) [55].

Avelumab

This anti-PD-L1 monoclonal antibody is in the initial stages of development for more than 15 types of cancers, including bladder. Avelumab differs from the other PD-L1 inhibitors in that in addition to inhibiting PD-L1, it possesses antibody-dependent, cell-mediated cytotoxicity, which results in direct lysis of tumor cells, but may also be potentially involved in specific toxicities involving the lysis of non-tumor cells with PD-L1 expression [56]. Although autoimmune adverse events were rare in the Javelin Solid Tumor Phase 1 trial (NCT01772004), of the 168 total patients with a variety of solid tumors, 3 (1.8%) patients experienced autoimmune hepatitis, with 1 case resulting in death in a patient with liver metastasis [57]. Avelumab received breakthrough therapy designation by the FDA for Merkel cell carcinoma in November 2015, but has not yet been approved for this indication.

Results from the ongoing JAVELIN Solid Tumor phase 1b trial (NCT01772004) presented at the 2016 European Society for Medical Oncology annual meeting showed that the overall ORR was 16.5%, the median PFS was 6.1 weeks, and the PFS rate at 12 weeks was 35.6% for avelumab (10 mg/kg IV q2w) in patients with metastatic urothelial carcinoma who progressed after platinum-based chemotherapy or were platinum-ineligible ($N = 129$) [58]. In an earlier analysis of patients with PD-L1–positive tumors ($N = 12$) presented at the 2016 Genitourinary Cancers Symposium, the ORR was 50% and the PFS rate at 12 weeks was 58% [59]. The most common adverse events included infusion-related reactions (22.5%) and fatigue (14.7%); 1 patient died due to treatment-related pneumonitis [58]. The phase 3 JAVELIN Bladder 100 study (NCT02603432) as first-line treatment in the maintenance setting is currently ongoing. This maintenance design is distinct from other studies in the era of immune-oncology therapy.

Testing for PD-L1

Note that the anti-PD-1/PD-L1 agents approved for use in the United States for a variety of cancers do not specify PD-L1 expression as a prerequisite for use, with the exception of pembrolizumab, which has a testing requirement for use in NSCLC (Tumor Proportion Score [TPS] $\geqslant$ 50% for first-line and TPS > 1% for second-line) but not in melanoma [39,60,61]. Although the respective clinical trials for all of the agents in this class against a variety of tumor types have shown that efficacy correlates with PD-L1 expression, responses have also been seen in patients whose tumors tested negative for PD-L1 [62]. The recent randomized phase 3 data with pembrolizumab showed the CPS PD-L1 biomarker incorporating both tumor cell membrane and immune cell PD-L1 staining was both predictive and prognostic in platinum-refractory disease [53]. In the phase 1/2 study of durvalumab that utilized an assay that measured PD-L1 expression for both TC and IC, defining PD-L1 status on the basis of expression on TC or IC (high defined as TC or IC $\geqslant$25% and low defined as TC and IC <25%), separately, did not result in a clear distinction between responders and nonresponders, but was predictive when looking at TC or IC expression as a combined measure [54]. A recent investigation of tumor samples from 160 patients with urothelial carcinoma using mouse monoclonal anti-PD-L1 antibodies, showed that PD-L1 expression in tumor cells (defined as $\geqslant$5%) was not predictive of OS, while PD-L1 expression in tumor-infiltrating mononuclear cells (score of 2–4 considered positive) significantly correlated with longer survival in patients who developed metastases [63]. Although not definitive, these studies raise the question of whether testing for PD-L1 should be conducted on both TC and IC. While the presence of PD-L1 can be predictive of response, the lack of PD-L1 expression should not preclude use of these agents since some patients with negative results upon testing have exhibited responses.

Additionally, there is lack of criteria defining what constitutes positive versus negative expression, and PD-L1 expression appears to be a continuum, which makes it difficult to define distinct categories of positive and negative. For example, in NSCLC trials of nivolumab the cutoff was 1% to 5% positive cells at biopsy, but for pembrolizumab it was 1% (positive) to 50% (strongly positive),

and for atezolizumab it was 1% to 50% TC and 1% to 10% IC [62,64–66]. Furthermore, testing for PD-L1 is not standardized, and it is unknown whether the different tests can be interchanged with different treatments or across indications. Currently, 4 PD-L1 assays have been analytically validated and used in clinical trials but each has been developed in conjunction with a specific inhibitor, and therefore, may not be able to be used across the class. Also, different reagents from different commercial sources may have different sensitivities [67,68]. The Blueprint PD-L1 IHC Assay Comparison Project was undertaken to provide information on the analytical and clinical comparability of the 4 PD-L1 IHC assays used in clinical trials (22C3, 28-8, SP263, and SP142), using NSCLC as the tumor type [69]. This study showed that 3 (22C3, 28-8, SP263) of the 4 assays tested were closely aligned with respect to TC staining, but greater variability across all of the assays was due to lack of training in IC scoring [69]. The investigators concluded that interchanging assays and cut-off values could lead to "misclassification" of PD-L1 status for some patients [69]. Finally, PD-L1 expression is heterogeneous within tumors, between the primary tumor and metastases, and may appear and disappear over time [68,70]. Therefore, since the data are still evolving with respect to PD-L1 as a prognostic/predictive biomarker, testing patients with bladder cancer before initiating therapy with these agents is currently reserved for clinical trials. However, the PD-L1 status of a patient's tumor may facilitate discussion on the benefits and risks of treatment options, and more importantly, help set therapeutic expectations during the physician and patient dialogue.

Conclusions

It is an exciting time to be involved in the treatment of bladder cancer. A new era in immunotherapy is dawning and is the culmination of the century of research that preceded, beginning with the discovery of BCG in 1908. The introduction of the checkpoint inhibitors in this century offers real hope for patients for whom previously there were few options for durable responses, including those who are ineligible for cisplatin-based regimens on the basis of age, comorbidities, or patient acceptance. Cisplatin is known for its potential for nephrotoxicity, ototoxicity, and emesis [71], and the PD-L1 inhibitors seem to be relatively well tolerated, without the propensity for renal damage; and therefore, may be a viable alternative for many patients. The improved tolerability of immunotherapy over chemotherapy and radiation directly correlates with its targeted mechanism of action. Moreover, research is ongoing to further categorize responses and define ideal patient populations, including contribution of compromised immunogenic capability, influence of prior therapy, and efficacy and tolerability with combination use. Specifically, research is engaged in combinations of checkpoint inhibitors even beyond PD-1/PD-L1 plus CTLA-4, such as indoleamine 2,3-dioxygenase (IDO) inhibitors, lymphocyte activation gene 3 (LAG-3), 4-1BB (CD137), T-cell immunoglobulin and mucin-domain-containing-3 (TIM-3), colony-stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily, member 4 (OX40), and others, to address multiple pathways in immune system functioning. However, there is no doubt that immunotherapy will change the standard of care of bladder cancer long into the future.

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