Novel immunotherapeutic approaches to the treatment of urothelial carcinoma

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***Abstract*:** Immunotherapy has long played a role in urothelial cancers with the use of bacille Calmette Guérin (BCG) being a mainstay in the treatment of nonmuscle invasive bladder cancer. Novel therapeutic approaches have not significantly impacted mortality in this population and so a renaissance in immunotherapy has resulted. This includes recombinant BCG, oncolytic viruses, monoclonal antibodies, vaccines, and adoptive T-cell therapy. Herein, we provide a review of the current state of the art and future therapies regarding immunotherapeutic strategies for urothelial carcinoma.

Keywords: immunotherapy, NMIBC, urothelial carcinoma

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Introduction

Urothelial cancer remains a challenge to treat, as evidenced by slowly declining yearly incidence rates, yet stable death rates for the last 15 years. Urothelial cancer is the fourth most common cause of cancer in men, with 74,000 new cases reported and 16,000 deaths in 2015 [Siegel *et al.* 2015]. In recent years, we have seen an escalation in the utilization of immune-based strategies for the treatment of all cancers, including urothelial cancer. Immunotherapy in fact has a substantial and relatively successful history in the treatment of urothelial cancer, with bacille Calmette–Guérin (BCG) currently accepted as the mainstay of treatment for nonmuscle-invasive bladder cancer (NMIBC) after transurethral resection. However, BCG has limited efficacy and the potential for side effects; high-risk NMIBC remains difficult to treat with high long-term rates of recurrence and disease progression. Therefore, there has been a concerted effort to develop new and innovative immunotherapy strategies. In this review, we provide a comprehensive assessment of current and potential future immune-based therapies for the treatment of urothelial cancer (Table 1).

BCG

BCG is a live, attenuated strain of *Mycobacterium bovis* initially developed by Calmette and Guérin for use as a vaccine to prevent tuberculosis. It has now become the foundation for the treatment of high-grade NMIBC with numerous studies demonstrating that administration of intravesical BCG to patients with superficial bladder tumors and carcinoma *in situ* (CIS) reduces recurrence rates and progression to muscle-invasive disease [Herr *et al.* 1988; Sylvester *et al.* 2005]. BCG acts as a nonspecific stimulant to the reticuloendothelial system and induces a local inflammatory response with subsequent infiltration of granulocytes, macrophages, natural killer cells, dendritic cells, and lymphocytes. This influx of immune cells leads to the local secretion of a wide array of cytokines, including interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, granulocyte macrophage colony stimulating factor (GM-CSF), and soluble intercellular adhesion molecule 1 [Jackson *et al.* 1995]. T-cell-mediated immunity predominates and is associated with increased tumor destruction. Importantly, an anti-BCG-specific immune response that is induced *via* antigen presentation by dendritic cells to T cells amplifies the response elicited by BCG. Ratliff and colleagues demonstrated in well designed experiments that the absence of either CD4 or CD8 T-cell subsets eradicated the antitumor activity of intravesical BCG immunotherapy for bladder cancer [Ratliff *et al.* 1993].

Despite a defined benefit, 20–50% of patients will develop disease recurrence within 15 years of a successful induction cycle [Cookson *et al.* 1997], while only 25–45% of these patients will benefit

Table 1. Novel targeted therapies for urothelial cancer.

Treatment modality	Targets	Stage of development
rBCG		
Th1 cytokine based	IL-2, IL-12, IL-18, IFN-α, IFN-γ, S1PT	Preclinical/animal models
BCG subcomponent based	Cell wall, cell wall skeleton, subunit protein antigens	Phase II/III
Oncolytic viruses		
rAd-IFN/Syn3	IFN-α2b	Phase II
CG0070	GM CSF	Phase II/III
Monoclonal antibodies		
Tumor-associated antigens (TAAs)	hCG-β	Phase I
Checkpoint blockade inhibitors	CTLA-4, PD-1, PD-L1	Phase I
Vaccines		
DN24-02, AdHER dendritic cell vaccine	HER2	Phase II
Cancer testis antigens (CTAs)	NY-ESO-1, MAGE-A3	Phase II
PANVAC	MUC-1, CEA	Phase II
Adoptive T-cell therapy	CTAs (e.g. NY-ES0-1), TAAs	Preclinical/feasibility study

CEA, carcinoembryonic antigen; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; GM-CSF, granulocyte macrophage colony stimulating factor; hCG-β, human chorionic gonadotropin β chain; HER2, human epidermal growth factor receptor 2; IFN, interferon; IL, interleukin; MUC-1, mucin-1; PD, programmed death; rAd, recombinant adenovirus; rBCG, recombinant bacille Calmette–Guérin; S1PT, pertussis toxin subunit S1.

from a second induction course [Haaff *et al.* 1986]. Lastly, availability of BCG has become a relevant issue as we are now experiencing unprecedented national shortages.

Recombinant BCG

Recent advances have focused on genetically engineered recombinant BCG (rBCG) strains that provide a novel tactic for modification of BCG to overcome some of the limitations of conventional BCG therapy. The two major rBCG strategies employed at this time are T helper 1 (Th1) cytokine-based rBCG and BCG-subcomponent-based rBCG.

Th1 cytokine-based rBCG

Since evidence has shown a Th1 immune response to be critical for successful intravesical BCG immunotherapy, genetically engineered rBCG constructs that allow for the ability to secrete Th1-stimulating cytokines have been developed. Common Th1 cytokine-based rBCG strategies include secretion of IL-2, IL-12, IL-18, IFN-α, and IFN-γ. IL-2 has been shown to enhance the proliferation of cytotoxic lymphocytes *in vitro* as well as augment the cytotoxic activity of natural killer (NK) cells and monocytes [Henney *et al.* 1981; Malkovsky *et al.* 1987]. Therefore, increased levels of IL-2 in the urine of patients shortly after treatment with BCG are indicative of a BCG-specific response [De Jong *et al.* 1990]. Studies looking at IL-2-based rBCG strains have shown that relative to BCG alone, IL-2 secreting rBCG strains can improve antigen-specific proliferation, induce a more favorable IFN-γ:IL-4 ratio, elicit higher levels of Th1 cytokines, and enhance antitumor cytotoxicity [Murray *et al.* 1996; Slobbe *et al.* 1999; Young *et al.* 2002]. As a result, IL-2 secreting rBCG may provide clinical benefit in the treatment of NMIBC and additional clinical trials are warranted.

IL-12 has a synergistic effect with IL-2 in producing a cytotoxic T-cell response, and has been shown to increase IFN-γ production, specifically enhance the Th1 immune response, augment NK-cell activity, and induce tumor regression in animal models of cancer [Brunda *et al.* 1993; Nastala *et al.* 1994; O'Donnell *et al.* 2004]. IL-12 is instrumental in the BCG immune response, as BCG therapy was found to be ineffective for treatment of murine bladder cancer in IL-12 knockout mice [Riemensberger *et al.* 2002]. Unfortunately, a phase I study of the intravesical administration of human recombinant IL-12

alone in patients with recurrent superficial transitional cell carcinoma of the bladder proved to be ineffective as it showed no clinically relevant evidence of antitumor or immunologic effects [Weiss *et al.* 2003]. IL-12-based rBCG strains have been constructed for treatment of *Mycobacterium* tuberculosis infection and initial results in animal models are positive with strong evoked immunogenicity and Th1-cell responses [Deng *et al.* 2011; Lin *et al.* 2012]. Therefore, IL-12-based rBCG constructs may have the potential of enhancing intravesical BCG for treatment of NMIBC; further clinical trials in humans are necessary.

IL-18 is primarily secreted by activated macrophages and, along with IL-2 and IL-12, is thought to play a synergistic role in the induction of a Th1 immune response. Luo and colleagues developed an rBCG strain that functionally secretes mouse IL-18 [Luo *et al.* 2004]. This IL-18-secreting rBCG stain was shown to increase production of IFN-γ and GM-CSF, decrease production of IL-10, increase cellular proliferation, and stimulate higher differentiation of IFN-γ-secreting cells in mice splenocytes. Furthermore, IL-18 rBCG also enhanced BCG-induced macrophage cytotoxicity against murine bladder tumor cells (MBT-2) in a dose-dependent manner. Therefore, IL-18-based rBCG strains show promise as potential agents for NMIBC immunotherapy.

IFN-α, unlike the cytokines mentioned previously, has been extensively utilized in conjunction with BCG for the treatment of patients with superficial bladder cancers. IFN-α has been shown to have a wide array of antitumor properties, including enhancement of the Th1 immune response, triggering of apoptosis *via* increased TNF-related apoptosis-inducing ligand expression, increased antigen detection, and reduced angiogenesis in bladder tumors [Slaton *et al.* 1999; Papageorgiou *et al.* 2007]. A national multicenter phase II trial of combination BCG plus IFN-α-2B for treatment of recurring superficial bladder cancer displayed strong efficacy and minimal side effects [Joudi *et al.* 2006]. However, in one study in 236 patients with frequently recurring Ta/T1 grade 1–2 NMIBC, mitomycin C instillations followed by monthly BCG significantly reduced long-term disease recurrence in relation to alternating instillations of BCG and IFN-α2b [Jarvinen *et al.* 2015]. Therefore, it is unclear at this point in time if combination therapy of BCG with IFN-α offers any appreciable clinical benefit compared with BCG alone. IFN-α-secreting rBCG strains have been generated, and studies have shown these strains to be more effective than wild-type BCG in inducing IFN-γ production from peripheral blood mononuclear cells (PBMCs) and inducing PBMC cytotoxicity against bladder cancer cell lines *in vitro* [Luo *et al.* 2001; Liu *et al.* 2009; Ding *et al.* 2012].

IFN-γ is a critical cytokine in the BCG-mediated antitumor response and has been shown to effectively inhibit growth in bladder cancer cell lines [Hawkyard *et al.* 1992]. In one study, a murine IFN-γ-secreting rBCG strain was shown to upregulate major histocompatibility complex (MHC) class 1 expression on murine bladder cancer cell lines relative to rBCG control strain [Arnold *et al.* 2004]. In addition, intravesical instillation of IFN-γ-secreting rBCG led to enhanced recruitment of CD4+ T cells into the bladder as well as increased local expression of IL-2 and IL-4. Furthermore, in a murine orthotopic bladder cancer model, IFN-γ-secreting rBCG significantly improved survival relative to a control group that received wild-type control BCG.

Pertussis toxin-based rBCG has also been constructed. One study in mice revealed that rBCG expressing pertussis toxin subunit S1 (rBCG S1PT) can stimulate a strong antigen-specific Th1-dominant cellular response characterized by increased IFN-γ production and decreased IL-4 production [Nascimento *et al.* 2000]. A recent study conducted in orthotopic bladder cancer animal models demonstrated that rBCG-S1PT immunotherapy leads to bladder weight reduction as well as increased survival times relative to BCG treatment [Andrade *et al.* 2010].

BCG subcomponent-based rBCG

Another avenue of research has focused on non-live BCG subcomponents, including BCG cell wall and various BCG proteins and antigens to induce the same immune response as live BCG in the treatment of NMIBC while possibly avoiding serious side effects associated with live BCG infection. Zlotta and colleagues purified several BCG subcomponents, including the cell wall, various polysaccharides, and purified native proteins and found these subcomponents could enhance a Th1 immune response as well as increase lymphocyte-mediated cytotoxicity against bladder tumors *in vitro* [Zlotta *et al.* 2000]. The BCG cell wall has been found to be the most potent Th1 response inducer compared with the other subcomponents of BCG. In a 2009

multicenter study, a total of 55 patients with CIS of the bladder, 82% of whom had disease that failed to respond to previous BCG therapy, received induction plus maintenance therapy with intravesically administered mycobacterial cell wall-DNA complex (MCC) at two different doses (4 and 8 mg) [Morales *et al.* 2009]. The complete response rate at 26 weeks was 27.3% in the 4 mg group and 46.4% in the 8 mg group. In addition, the MCC was well tolerated by both dose groups with 90% of all adverse events being mild to moderate in nature. A phase II/III clinical trial [ClinicalTrials.gov identifier: NCT00406068] assessing MCC in the treatment of patients with NMIBC who were BCG refractory has been completed. Unfortunately, a phase III trial [ClinicalTrials.gov identifier: NCT01200992] evaluating MCC *versus* mitomycin C for the treatment of BCG refractory NMIBC closed early due to poor accrual.

Apart from MCC, the BCG cell wall skeleton (BCG CWS) is a potential substitute for live BCG. However, clinical use has been difficult due to unfavorable physiochemical characteristics preventing effective penetration of the urothelium, an essential step in the induction of the immune cascade. Delivery systems such as liposomal vectors and lipid nanoparticle packaging have been studied to improve delivery of BCG CWS and show promise for tumor growth inhibition in animal models of bladder cancer [Miyazaki *et al.* 2011; Nakamura *et al.* 2014].

Lastly, rBCG strategies utilizing various other BCG subunit proteins and antigens such as PstS1, MPT-64, and Ag85B have been evaluated and show efficacy in enhancing cytotoxicity on superficial bladder cancers in both *in vitro* and orthotopic murine bladder models [Sanger *et al.* 2004; Yu *et al.* 2007; Begnini *et al.* 2013]. Interestingly, poly-rBCG DNA vaccines that specify multiple BCG antigens such as Ag85A, Ag85B, Mpt64, and PstS3 have been developed and shown to elicit Th1-predominant immune responses, inhibit tumor growth, and prolong the survival of bladder tumor bearing mice [Lee *et al.* 2004].

Oncolytic virus therapy

Oncolytic virus therapy takes advantage of an altered environment within a tumor cell, such that intravesically administered viruses replicate only in tumor cells and not in normal cells. Oncolytic viruses act *via* direct destruction of tumor cells and through stimulation of host antitumor immune responses. Several different viruses, such as adenovirus, herpes simplex virus, reovirus, and oncolytic vaccina virus, have been studied for oncolytic properties in the preclinical setting, with a few moving into early phase clinical trials [Potts *et al.* 2012]. Here, we focus on the following two recombinant adenovirus-based therapies: rAd-IFNα/Syn3 and CG0070. RAd-IFNα/Syn3 is a nonreplicating recombinant adenovirus vector encoding IFN-α2b. Syn3 is a clinical surfactant excipient which enhances the adenoviral mediated transduction (process by which foreign DNA is introduced into the tumor cells *via* the viral vector) of urothelial cancer cells [Dinney *et al.* 2013]. In a phase I trial in 17 patients with recurrent NMIBC, intravesical rAd-IFNα/Syn3 was well tolerated and 6 of 14 patients (43%) with detectable urine IFNα had a complete response at 3 months, which lasted an average of 31 months [Dinney *et al.* 2013]. There is currently an ongoing phase II study examining intravesical administration of rAd-IFN/Syn3 in patients with BCG-refractory or relapsed bladder cancer [ClinicalTrials.gov identifier: NCT01687244].

CG0070 is a selectively replicating oncolytic adenovirus that replicates in cancer cells with defective retinoblastoma tumor suppressor protein (Rb), which is commonly mutated in many bladder cancers. Furthermore, CG0070 is designed to encode human GM CSF [Ramesh *et al.* 2006]. In a phase 1 study of CG0070 in 35 patients with NMIBC, high urine GM CSF levels were detected in all patients and the complete response rate across all cohorts was 48.6% with a median duration of 10.4 months [Burke *et al.* 2012]. A phase II/III study examining the efficacy of CG0070 in patients with NMIBC whose condition has failed to respond to prior BCG is currently ongoing [ClinicalTrials.gov identifier: NCT01438112].

Monoclonal antibodies

An exciting area of research in the field of immunotherapy for urothelial cancer focuses on monoclonal antibodies directed against tumor-associated antigens. One such example is human chorionic gonadotropin-β chain (hCG-β), which is found in 30–40% of bladder cancers and whose expression correlates with more aggressive cancers, more advanced disease, higher recurrence rates, and decreased survival rates [Iles, 2007]. CDX-1307 is a novel monoclonal antibody under study for the treatment of bladder cancer [Morse *et al.* 2011a]. CDX-1307 consists of a fusion between B11, a human monoclonal antibody specific for the mannose

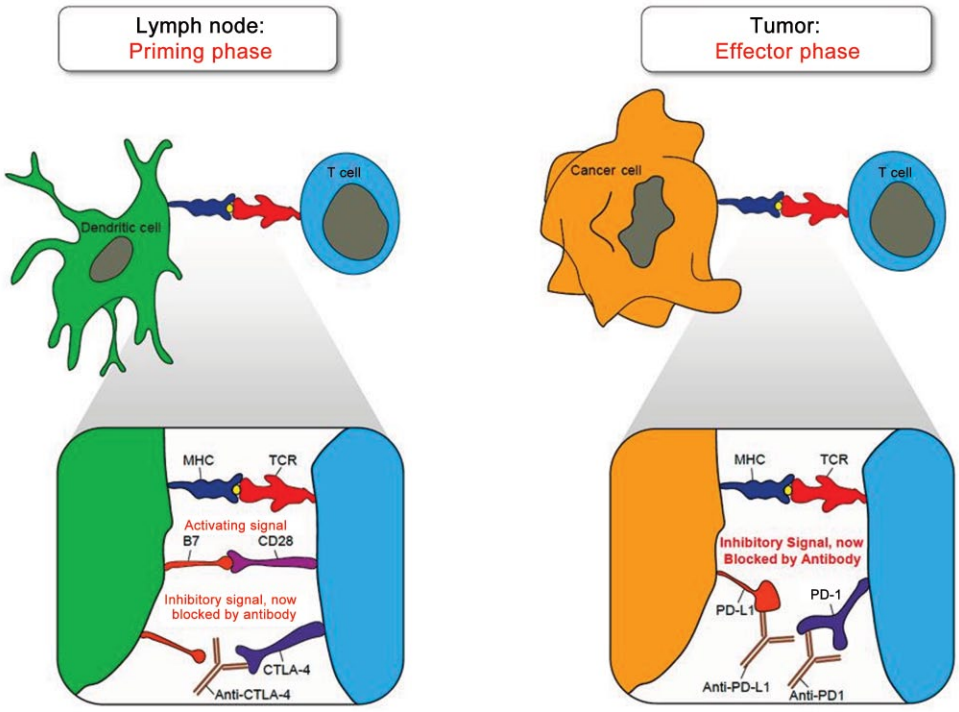

Figure 1. Mechanism of action for checkpoint blockade inhibitors. CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; MHC, major histocompatibility complex; PD, programmed death; TCR, T-cell receptor (Courtesy of *Wkly* 2015; 145: w14066).

receptor on antigen-presenting cells (APCs), and hCG-β. This structure allows CDX-1307 to attach to APCs and become internalized, with subsequent presentation of hCG-β to T cells and antigen-specific activation. A phase I study in patients with advanced epithelial malignancies revealed that CDX-1307 was well tolerated and induced hCG-β-specific humoral and T-cell responses when coadministered with toll-like receptor (TLR) agonists [Morse *et al.* 2011b]. Unfortunately, a study of CDX-1307 vaccine for treatment of patients with newly diagnosed muscle-invasive bladder cancer [ClinicalTrials.gov identifier: NCT01094496] was terminated early due to slow enrollment.

Checkpoint inhibitors

Checkpoint blockade inhibitors offer another potential strategy for the treatment of urothelial cancers. Immune checkpoints are inhibitory pathways that physiologically counterbalance costimulatory signals to modulate the immune response and keep T-cell proliferation in check. These inhibitory mechanisms are critical for maintaining self-tolerance, controlling the overall duration and strength of the immune response, and minimizing damage to healthy tissues. Cancer cells can potentially take advantage of this mechanism by overexpressing immune checkpoint molecules, which effectively blunts the immune response in the immediate tumor microenvironment. Therefore, monoclonal antibodies capable of disrupting the inhibitory receptor–ligand interactions involved in immune checkpoint mechanisms have been developed as potential strategies to treat cancers (Figure 1).

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is an immune checkpoint protein receptor that antagonizes the interaction between CD28 on T cells and B7 on APCs, and therefore inhibits the secondary costimulatory signal that APCs provide to T cells. This process is a normal physiologic mechanism that works to modulate the immune response as appropriate and inhibit excessive T-cell proliferation. However, in certain cancers, T-regulatory cells in the tumor microenvironment can constitutively express CTLA-4, which suppresses an effective antitumor immune response [Rothschild *et al.* 2015]. Stemming from this concept, CTLA-4 blocking antibodies have been produced as immunotherapy for cancer. The antibody ipilimumab, an anti-CTLA-4 agent, demonstrated survival benefit in a landmark phase III randomized clinical trial

conducted in patients with previously treated metastatic melanoma [Hodi *et al.* 2010]. Patients receiving ipilimumab plus glycoprotein 100 (gp100) had a median overall survival of 10 months, as compared with 6.4 months for patients receiving gp100 alone. However, grade 3 or 4 immune-related adverse events occurred in 10–15% of patients treated with ipilimumab in this study. There are currently several ongoing trials assessing ipilimumab in urologic malignancies, mainly for prostate cancer and kidney cancer. Liakou and colleagues conducted a presurgical clinical trial with anti-CTLA-4 antibody in six patients with localized bladder cancer demonstrating increased expression of inducible costimulator (ICOS) in CD4 T cells from peripheral blood and tumor tissue [Liakou *et al.* 2008]. Increase in these CD4 (+) ICOS (hi) cells led to an increase in the effector: regulatory T-cell ratio and increased production of IFN-γ. Subsequently, a phase I trial assessing ipilimumab treatment in 12 patients with localized urothelial carcinoma of the bladder demonstrated a tolerable safety profile with most drug-related adverse events consisting of grade 1/2 toxicities [Carthon *et al.* 2010]. Furthermore, all 12 patients had increased presence of CD4 (+) ICOS (hi) T cells in both tumor tissue and systemic circulation. Further trials are warranted to assess the efficacy of anti-CTLA-4 agents for the treatment of urothelial cancers.

Another critical immune checkpoint mechanism involves interactions between programmed death 1 (PD-1) receptor found mainly on T cells and its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-1 is a 288 amino acid type 1 transmembrane protein that is expressed on activated T cells and acts *via* its downstream pathways to limit the proliferation and activity of T cells to suppress autoimmunity and tissue damage during an inflammatory response [Keir *et al.* 2008]. Both type 1 and type II IFNs upregulate PD-L1 and PD-L2 expression as part of the inflammatory response in peripheral tissues [Eppihimer *et al.* 2002]. Several different cancer types, including urothelial cancers, take advantage of this checkpoint blockade inhibition by overexpressing PD-L1 in order to evade immune-mediated destruction [Zou and Chen, 2008]. In urothelial cancers, PD-L1 expression by tumor cells is correlated with higher levels of BCG unresponsiveness, advanced stage, higher postoperative recurrence, and poor survival [Inman *et al.* 2007; Nakanishi *et al.* 2007]. Stemming from these results, various forms of PD-L1 manipulation have been developed as potential immunotherapy for aggressive muscle-invasive cancers. A recent phase I clinical trial assessed the anti-PD-L1 antibody MPDL3280A, which inhibits the interaction of PD-L1 with PD-1 for the treatment of metastatic urothelial bladder cancer [Powles *et al.* 2014]. Of the 67 patients assessed for efficacy of treatment, 50 patients (75%) had visceral metastases at baseline and 48 patients (72%) had already received and failed to respond to two or more previous systemic treatments. For patients with a minimum of 6 weeks of follow up, the objective response rate (ORR) in patients with strong PD-L1 expression by immunohistochemistry (IHC) staining of tumor-infiltrating immune cells (⩾5% PD-L1 positive) was relatively high at 43%, whereas the ORR in patients with weak PD-L1 expression (⩽5% PD-L1 positive) was 11%. Furthermore, treatment with MPDL3280A had a favorable toxicity profile with most adverse events being grade 1 or 2 and transient in nature. Overall, decreased appetite and fatigue were the most commonly reported toxicities. Preliminary findings from the phase Ib KEYNOTE-012 [ClinicalTrials.gov identifier: NCT01848834] study assessing the PD-1 inhibitor pembrolizumab also revealed promising results with an ORR of 25% in 33 patients with advanced urothelial cancer (51.5% failing at least two previous systemic therapies) [Gupta *et al.* 2015]. There are currently several ongoing clinical trials assessing checkpoint blockade inhibitors, mainly anti-CTLA-4 agents and PD/PD-L1 inhibitors, in various combinations as well as in conjunction with other cytotoxic therapies [Wu *et al.* 2015]. These trials are now proposed in NMIBC given the encouraging preliminary results in metastatic urothelial cancer.

In addition, there are various other immune checkpoint proteins that are currently being studied in basic science laboratories, and are on the verge of clinical evaluation in urothelial cancer. They include HLA-G, B7-H3, B7-H4, LAG3, and TIM3, among others [Carosella *et al.* 2015]. Development of monoclonal antibodies to these immune checkpoints is an active field and may demonstrate a future role in the treatment of urothelial cancers.

Vaccines

An additional avenue of immunotherapy research for urothelial cancer has focused on vaccine development. Potential targets include oncoproteins such as human epidermal growth factor receptor 2 (HER2), cancer testis antigens (CTAs), and tumor-associated antigens such as

carcinoembryonic antigen (CEA) and Mucin-1 (MUC-1). A meta-analysis that looked into the prognostic role of HER2 in bladder cancer revealed that HER2 is expressed in 27.8–85.2% of bladder cancers and that HER2 expression is correlated with higher tumor grade, lymph node metastasis, and poor disease-specific survival [Zhao *et al.* 2015]. DN24-02 is an autologous cellular immunotherapy vaccine designed to target the HER2 receptor that is currently being investigated in a randomized phase II study in patients with high-risk HER2+ urothelial carcinoma [ClinicalTrials.gov identifier: NCT01353222]. It consists of autologous PBMCs and APCs that are activated *ex vivo* with a recombinant fusion protein and then subsequently infused back into the patient. Preliminary results revealed that in 30 patients who had completed three infusions, DN24-02 had the ability to activate APCs, increase HER2-specific antibody responses, and increase expression of T-cell cytokines consistent with immunological prime-boost response. In addition, most adverse events (92.3%) were grade 1–2 in severity [Bajorin *et al.* 2014]. Another HER2 vaccine, AdHER2/neu dendritic cell vaccine, is currently being investigated at the National Institutes of Health Clinical Center as part of a phase I trial [ClinicalTrials.gov identifier: NCT01730118] in patients with HER2+ metastatic solid tumors and bladder cancer. In a mouse model of breast cancer, vaccination with an adenoviral ErbB-2/neu vaccine demonstrated the ability to cure large established subcutaneous HER2-expressing breast cancers as well as lung metastatic disease [Park *et al.* 2008]. Furthermore, it was shown that the antitumor activity involved antibody-mediated blockade of HER2 function.

Cancer testis antigens

CTAs are a group of tumor-associated antigens that have little to no expression in normal adult tissues, variably increased expression in many different cancers, and the ability to induce a strong immune response. In a study examining the expression patterns of nine CTAs in a panel of high-grade urothelial carcinomas of the bladder, Sharma and colleagues found that at least one CTA was expressed in 77% of the tumor samples and that 61% of these samples expressed more than one CTA [Sharma *et al.* 2006]. Two CTAs studied in relation to urothelial cancer include NY-ESO-1 and MAGE-A3. Coadministration of a recombinant NY-ESO-1 protein vaccine with BCG and GM CSF in six patients with localized bladder cancer post cystectomy resulted in NY-ESO-1-specific antibody responses in five of six patients [Sharma *et al.* 2008]. A phase I study assessing NY-ESO-1 vaccine with or without sirolimus in the treatment of patients with NY-ESO-1-expressing solid tumors is currently underway and has completed recruitment [ClinicalTrials.gov identifier: NCT01522820]. Another phase I study [ClinicalTrials.gov identifier: NCT01498172] examining the coadministration of BCG with recMAGE-A3 + adjuvant AS15 (recMAGE-A3 + AS15 ASCI) for the treatment of NMIBC has been completed and the results are pending. A phase II trial evaluating the efficacy of recMAGE-A3 + AS-15 in patients with muscle-invasive bladder cancer after cystectomy is ongoing and has completed recruitment [ClinicalTrials.gov identifier: NCT01435356].

Pox-viral-based vaccine therapy

PANVAC is a cancer vaccine therapy that contains transgenes for the tumor-associated antigens MUC-1 and CEA as well as three human T-cell costimulatory molecules (B7.1, intracellular adhesion molecule 1, and leukocyte function-associated antigen 3). These costimulatory molecules can augment an immune response in the setting of weakly immunogenic cancer antigens such as MUC-1 and CEA [Madan *et al.* 2007]. In various malignancies including bladder cancer, cell surface expression of MUC-1 can be significantly increased, and increased MUC-1 expression patterns have been shown to correlate with higher stage and grade of bladder cancer [Ahmad *et al.* 2015]. CEA levels are also increased in certain bladder cancers and are found in up to 76% of high-grade bladder tumors [Cardillo *et al.* 2000]. A phase II study assessing the use of PANVAC + BCG therapy *versus* BCG alone in patients with high-grade NMIBC whose condition has failed to respond to prior BCG therapy is currently under accrual [ClinicalTrials.gov identifier: NCT02015104].

Adoptive T-cell therapy

Adoptive T-cell therapy is an innovative and highly personalized cancer therapy that involves extraction of human T cells, expansion and manipulation of these cells *ex vivo*, and subsequent reinfusion of these proliferated immune cells back into the patient. This therapeutic avenue began with the discovery of tumor-infiltrating lymphocytes (TILs). It was found that the presence of immune

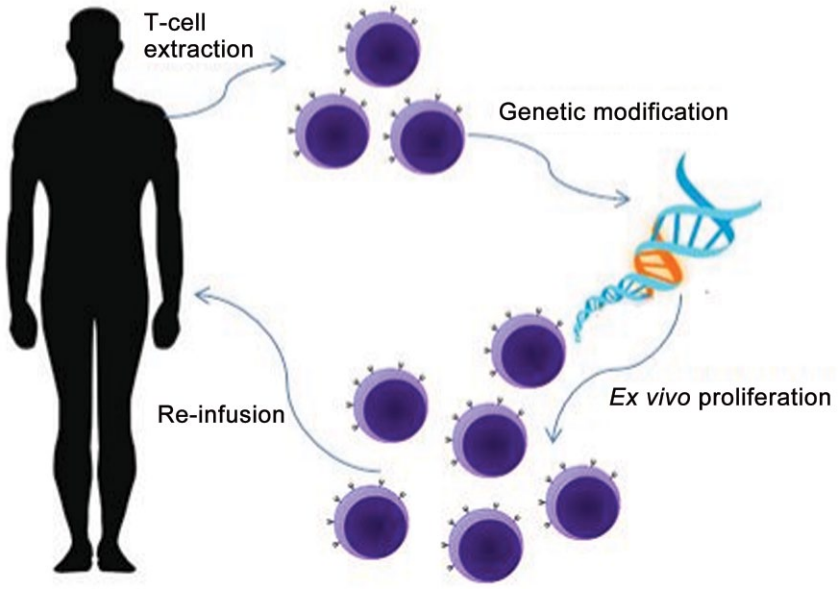

Figure 2. Adoptive T-cell therapy.

infiltrate in tumors correlated with better prognosis following therapy [Duong *et al.* 2015]. As a potential therapeutic strategy, researchers have isolated tumor-infiltrating cells from various cancers and reinfused these cells back into patients after *ex vivo* proliferation. Further studies demonstrated that better clinical responses are observed when patients received a lymphodepleting preparative regimen before cell reinfusion [Rosenberg and Restifo, 2015]. Recently, this strategy was tested as a feasibility study in patients with advanced urothelial urinary bladder cancer [Sherif *et al.* 2010]. Tumor reactive lymphocytes were extracted from sentinel nodes draining human bladder cancer, expanded, and then subsequently reinfused back into patients. In 6 of 12 patients, it was feasible to administer the treatment without any major adverse effects, while technical failures were encountered in the other six patients.

Despite initial success, TIL extraction with subsequent reinfusion is not without its challenges. TILs are difficult to isolate from cancers other than melanoma. In addition, TIL therapy requires invasive procedures for extraction as well as the ability to grow *ex vivo*. In an attempt to broaden the scope of adoptive T-cell therapy to other cancers, techniques have been developed to modify host T cells with genetically engineered antitumor T-cell receptors (TCRs). This process involves isolation of T cells from peripheral blood and viral transduction with vectors in these cells to express a recombinant TCR specific for a chosen tumor antigen. These cells are then reinfused back into the patient (Figure 2). CTAs, as described earlier, are appropriate targets for this therapy because they have low expression in normal tissue and high expression in certain cancers. Genetically engineered TCRs directed against CTAs seem to be a viable option for urothelial cancers, given the high expression of CTAs in many urothelial tumors.

Therapy with genetically modified TCRs requires TCR binding to antigen processed by APCs and presented on MHC receptors. Therefore, certain tumors can potentially escape detection by immune cells by downregulating molecules such as MHC class 1. To overcome this limitation, recombinant receptors called chimeric antigen receptors (CARs) have been developed [Barrett *et al.* 2015]. CARs consist of Ab-binding domains fused to T-cell signaling domains, allowing the direct binding of T cells to antigen on cancer cell surfaces that is non-MHC restricted. This, in effect, arms T cells with antitumor activity capable of targeting specific tumor-associated antigens regardless of MHC class 1 expression in the tumor cells [Maude *et al.* 2014]. The future application of adoptive T-cell therapies to urothelial cancers is very promising.

Conclusion

The field of immunotherapy offers exciting and potentially effective strategies for the treatment of urothelial cancers. Limited progress in survival outcomes for patients compels us to search for novel therapeutic options. BCG therapy has a storied and successful history in the treatment of superficial urothelial cancers and will continue to play an integral role. Other avenues such as rBCG, oncolytic viruses, monoclonal antibodies, vaccines, and adoptive T-cell therapy show promise as treatment for cancers of the urothelial tract. Combination therapies involving immunotherapy in addition to other therapeutic modalities such as cytotoxic therapy, radiation, ablation, or surgery may be the future for treatment of many different types of urothelial cancers. Additional clinical trials are warranted in this area. Future directions should focus on finding neoantigens specific for urothelial cancer, utilizing next-generation sequencing to predict responsiveness to immunotherapy, and formulating personalized therapeutic plans using adoptive T-cell therapy.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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