REVIEW

Nonmuscle invasive bladder cancer: a primer on immunotherapy

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ABSTRACT   Intravesical Bacillus Calmette-Guérin (BCG) has long been the gold standard treatment of nonmuscle invasive bladder cancer. Recently, there has been an emergence of novel immunotherapeutic agents, which have shown promise in the treatment of urothelial cell carcinoma. These agents aim to augment, modify, or enhance the immune response. Such strategies include recombinant BCG, monoclonal antibodies, vaccines, gene therapy, and adoptive T-cell therapy. Here, we review the emerging immunotherapeutics in the treatment of nonmuscle invasive bladder cancer.

KEYWORDS   Urothelial cell carcinoma; Bacillus Calmette-Guérin (BCG); immunotherapy; bladder cancer

Introduction

Bladder cancer is the 9th most common malignancy in the world. Urothelial cell carcinoma is the most common histological subtype accounting for 90% of all cases[1]. There was an estimated 74,000 newly diagnosed cases in the United States last year and 16,000 deaths attributed to bladder cancer[2]. Intravesical Bacillus Calmette-Guérin (BCG) represents one of the largest success stories in immunotherapy. It has been shown to decrease recurrence rates and progression to muscle invasive disease in patients with carcinoma *in situ* (CIS) and superficial tumors[3,4]. Treatment with a six-week course of intravesical BCG following transurethral resection remains the gold standard for managing patients with high-grade nonmuscle invasive bladder cancer (NMIBC). This review encompasses the most recent advances in immunotherapeutic strategies for NMIBC (**Table 1**).

BCG

BCG is a live attenuated strain of Mycobacterium bovis, originally developed in 1921 as a tuberculosis vaccine. The exact mechanism by which intravesical BCG exerts its

therapeutic effect is not well understood. It is known to induce a local inflammation within the bladder mucosa following instillation, characterized by an influx of inflammatory cells and production of inflammatory cytokines. This cascade is initiated by the internalization of BCG into the urothelial cells via attachment of its fibronectin attachment protein to fibronectin, an extracellular matrix protein (**Figure 1**)[5]. This leads to the influx of granulocytes, macrophages, natural killer (NK) cells, dendritic cells and lymphocytes[6,7]. Cytokines such as IL-1, IL-2, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) have been identified in the urine following repeated BCG instillations[8]. In addition to this non-specific inflammatory response, a BCG-specific immune response also plays a role in its antitumor effect. Antigen presentation to T cells is thought to be essential to the BCG response, as studies have shown that CD4+ or CD8+ T cell depleted mice have impaired antitumor activity[9].

Although the generation of a Th1 mediated immune response is needed for BCG to exert its antitumor effect, there is growing evidence that the innate immune response may also contribute to the antitumor activity of BCG therapy. NK cells, display cytotoxic activity independent of MHC antigen presentation. To determine the antitumor property of NK cells, NK-deficient beige mice with were orthotopically implanted with MB49 bladder tumors with increasing doses of tumor burden and compared to wildtype mice with corresponding implanted tumor volumes. At the same tumor dose, the beige mice demonstrated reduced survival compared to their wildtype counterparts[10]. The

Table 1 Summary of immunotherapeutic options and their respective targets

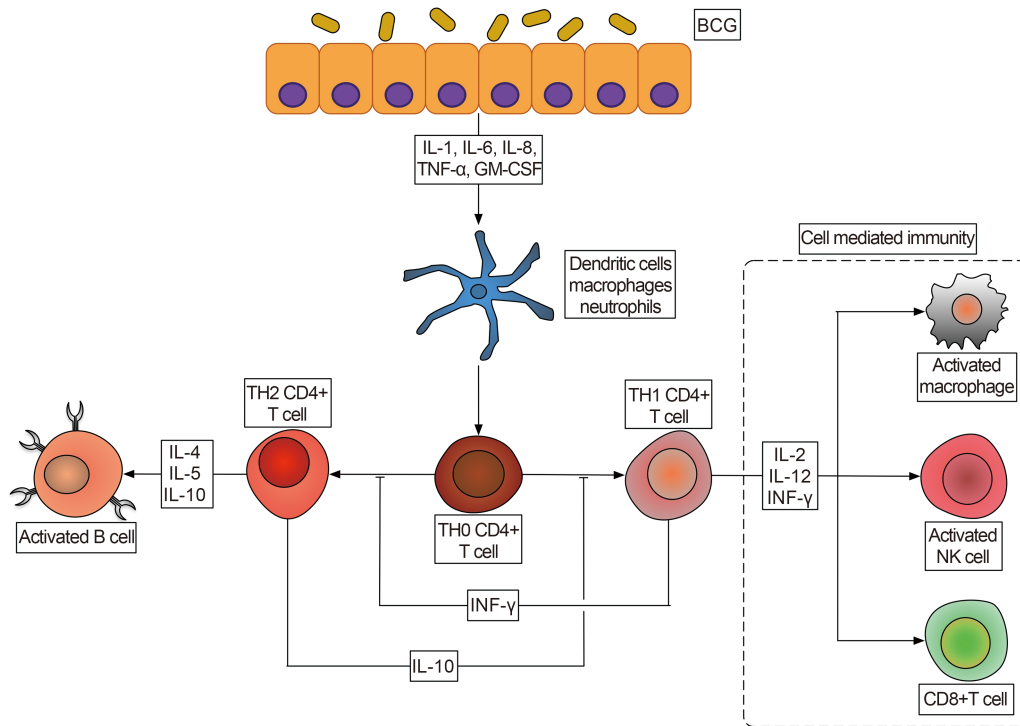| Immunotherapeutic agent	Target
Recombinant BCG (rBCG)	
Th1 cytokine secreting	IL-2, IL-12, IL-18, INF-α, INF-γ
BCG subcomponent	Mycobacterium cell wall extract
Non-BCG subcomponent	Pertussus S1P1
Monoclonal antibodies	
Tumor associated antigens (TAAs)	β-hCG
Checkpoint inhibitors	CTLA-4, PD-L1
Vaccines	
AdHER2/neu dendritic cell vaccine	HER2/neu
Cancer testis antigens (CTAs)	NY-ESO-1, recMAGE-A3
PANVAC	MUC-1, CEA, T cell costimulatory molecules
Adoptive T cell therapy	
Tumor infiltrating lymphocytes (TILs)	CTAs (NY-ESO-1)
Chimeric antigen receptor (CAR)	TAAs

Figure 1 Immune cascade as a proposed mechanism of action following intravesical BCG therapy. Adapted from Askland et al.[5] Adv. Urol. 2012.

cytotoxic effect of NK cells results from the release of direct cytotoxic agents as the expression of secreted or membrane bound cytokines, including TNF-related apoptosis-inducing ligand (TRAIL)[11]. In response to IFN-α stimulation, T cells, NK cells, dendritic cells and macrophages express TRAIL, inducing downstream caspase activity in tumor cells by

binding to a family of death receptors, DR4 and DR5, ultimately resulting in apoptosis[12]. Neutrophils also express TRAIL in response to direct recognition of BCG cell wall components on toll-like-receptor 2 (TLR2), and amplify TRAIL expression in response to IFN-α[13]. Neutrophil recruitment is an early response to BCG, preceding T cell involvement, and occurs due to the release of chemokines such as IL-8 by the bladder epithelium. Inflammatory cytokine and TRAIL expression are necessary for the antitumor property of BCG therapy.

Despite the efficacy of intravesical BCG for NMIBC, 20%-50% of patients will recur following one induction course[14,15]. Of these patients, only 25%-45% will respond to a second treatment course[16]. Additionally, the use of BCG may be limited in some patients due to severe local side effects or systemic toxicity.

Recombinant BCG (rBCG)

To decrease toxicity and improve the efficacy of intravesical BCG, rBCG has been utilized as an alternate immunotherapy strategy. The advent of genetic engineering has allowed for recombinant BCG strains to express immunomodulatory exogenous genes. Alternatively, BCG subcomponents have been engineered to decrease the local toxicity of BCG while still producing a comparable immunogenic response. The two major classifications of rBCG strategies are Th1 cytokine-secreting rBCG and non-live BCG subcomponent rBCG.

Th1 cytokine-secreting rBCG

A Th1 immunologic response is essential for BCG to produce its antitumor effect[17]. This has led to genetically manipulating BCG strains to secrete Th1 cytokines as a strategy to enhance effective therapy. Such strategies include IL-2, IL-12, IL-18, IFN-α, and IFN-γ secreting rBCG. *In vitro* immune assays have shown that IL-2 augments the cytotoxicity of NK cells as well as monocytes[18,19]. IL-2 is also essential for generating an immune response *in vivo*. In patients treated with intravesical BCG, studies have shown IL-2 levels peaks in the urine within 2-6 hours following treatment[20,21]. IL-2 rBCG profoundly potentiates the immune response[22]. In deer immunized with either BCG or IL-2 rBCG, IL-2 secreting rBCG induces a strong IFN-γ: IL-4 ratio, which may skew the immune response towards a Th1 response[23,24].

IL-12 has demonstrated antitumor activity in murine adenocarcinoma and sarcoma models[25]. This cytokine induces production of high levels of IFN-γ and TNF-α. IL-12

also enhances NK activity, in addition to the alloreactive lymphocyte and cytotoxic T-cell responses. The role of IL-12 is not only to generate a Th-1 response, but also to inhibit the progression of a Th-2 immune response[26]. A study by O'Donnell et al.[27] showed that the use of IL-12 in an orthotopic murine bladder cancer model resulted in a dose dependent response of tumor regression and long term survival. Unfortunately recombinant IL-12 monotherapy has failed to show significant antitumor activity in phase 1 clinical trials[28].

IL-18 acts synergistically with BCG to induce a Th1 immune response. Luo et al.[29] showed that IL-18 secreting rBCG results in increased IFN-γ production and augmented macrophage cytotoxicity in bladder cancer cells. The increased levels of IFN-γ may allow use of rBCG IL-18 alone or in combination with lower and safer doses of BCG[30].

INF-α has been studied in bladder cancer cell lines *in vitro* and has demonstrated ability to induce apoptosis via TRAIL expression[31]. In addition, IFN-α has also demonstrated *in vivo* inhibition of tumor growth and tumor vascularization when studied in murine bladders implanted with human bladder cancer cells[32]. A large phase 2 trial studied the efficacy of IFN-α in combination with intravesical BCG administration, showing a 59% and 45% recurrence free survival at 24 months in BCG naïve and BCG failed patients, respectively. It is unclear if this treatment option is superior to intravesical BCG monotherapy, and a phase 3 trial would be necessary to investigate this further[33]. Although no trials to date have studied the efficacy of IFN-α as a single agent in humans, *in vitro* studies have shown rBCG-IFN-α to enhance the Th1 IFN-γ immune response of human peripheral blood mononuclear cells (PBMC). Compared to BCG alone, rBCG-IFN-α showed superior PBMC cytotoxicity against human bladder cell lines[34–36].

As previously discussed, IFN-γ is essential for cell-mediated immunity and its role in BCG therapy has been well described, showing an inhibitory effect on bladder cancer cells[37]. *In vitro* rBCG-IFN-γ upregulates major histocompatibility complex (MHC) -1 in murine bladder cell lines, leading to incremental therapeutic efficacy in orthotopic mice[38].

A subunit from the bacteria Bordetella pertussis, S1PT, has also been genetically engineered for expression in rBCG. Spleen cells from rBCG-S1PT-vaccinated mice exhibited increased levels of IFN-γ and decreased levels of IL-4, leading to a dominant cellular immune response and a decreased humoral immune response[39,40]. In a mouse orthotopic tumor model, rBCG-S1PT therapy resulted in bladder tumor weight reduction and increased survival time compared to the control group[40].

BCG subcomponent based

Using non-live immunologically active BCG subcomponents is an attractive alternative to using live attenuated BCG as a method for decreasing the toxicity associated with BCG[41]. In one study, 61 patients with carcinoma *in situ* were treated with intravesical mycobacterium cell wall extract (MCWE). A negative biopsy was seen in 62.5%, 49.3%, and 41.1% of patients at 12, 24, and 60 weeks following treatment respectively[42]. Though these results are promising, to date there has not been a study with results that compare BCG cell wall components to local chemotherapy. A phase 3 study investigating the intravesical EN3348, a mycobacterial cell wall-DNA complex, compared to mytomycin C, was terminated early due to lack of accrual (NCT01200992). One limitation of MCWE is related to stability in solution making it challenging to use in the clinical. To overcome the unfavorable biophysical properties, Miyazaki et al. [43] used octaarginine liposomes (R8 liposomes) as a vector for transporting BCG cell wall skeleton into the cytoplasm of murine bladder tumor cells. This led to inhibition of bladder carcinogenesis making it a prospective substitute for BCG for immunotherapy against NMIBC[43].

Mycobacterium phlei cell wall nucleic acid complex (MCNA) has been postulated to exert antineoplastic activity by exhibiting immunotherapeutic effect as well as direct chemotherapeutic effect, and has such has been used in a trial as intravesical therapy to treat high grade bladder cancer[44]. Although the study concluded that MCNA therapy lowered the risk of progression and cystectomies, the FDA advisory panel recently voted against its Biologics Application License, citing the lack of study power and lack of control group[45]. PstS1 is a phosphate binding subunit of the Mycobacterium permease protein found on the cell membrane[46,47]. This protein subunit is a remarkably immunogenic antigen[48]. *In vitro* data shows PstS1 may be exploited as a potent immunostimulatory antigen, resulting in increased cytotoxicity, IFN-γ release, and proliferation of PBMCs. When applied to *in vivo* experiments, therapeutic effects were observed in mice treated with intravesical recombinant PstS1 for orthotopic bladder tumors, as mice treated with intravesical PstS1 showed significantly prolonged survival compared to PBS control[49]. Other protein subcomponents such as MPT-64, and Ag85B have also demonstrated antitumor potential[50,51].

Monoclonal antibodies

Monoclonal antibodies targeting tumor associated antigens (TAAs) are another novel immunotherapy strategy that may yield auspicious results. One example of such a TAA is β-hCG, which is elevated in the serum and urine of 30%-40% of bladder cancer patients. Elevated β-hCG may be associated with advanced disease and increased mortality[52]. CDX-1307 is a monoclonal antibody that targets β-hCG, which may have utility in the treatment of bladder cancer. The antibody consists of B11, a monoclonal antibody against the mannose receptors of antigen presenting cells (APCs), fused to β-hCG. Internalization of CDX-1307 and subsequent presentation of the β-hCG on APCs leads to both cellular and humoral responses via antigen presentation to CD4+ and CD8+ T cells. This suggests that CDX-1307 may provide therapeutic benefit in the subset of patients with β-hCG expressing bladder cancer[53].

Checkpoint inhibitors are a novel approach to overcoming the cancer induced immune dysfunction that would otherwise eliminate cancer cells before they are clinically evident. Tumors maintain immune tolerance through a variety of mechanisms including maintaining protective cell types in the tumor microenvironment, producing soluble molecular factors, and expressing co-inhibitory receptors. These co-inhibitory receptors are commonly referred to as immune checkpoints, and lead to a reticence of tumor specific effector cells such as T cells. Thus, strategies to interrupt the immune check points have recently been developed to overcome tumor immune resistance.

Activation of T cells requires two major stimulatory signals. The first is provided by the recognition of MHCs on APCs by the T cell receptor (TCR). The second signal is provided by B7 stimulatory molecules on APCs and recognized by CD28 on the T cell. Expansion and activation of the T cell follows the recognition of both the stimulatory signals. Activated T cells express cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) to compete with CD28 costimulatory receptor for the secondary B7 activation signal provided by the APC, thus preventing excessive T cell proliferation. T regulatory cells within the tumor microenvironment can constitutively express CTLA-4 attenuate tumor specific T cell response[54]. Ipilimumab is a monoclonal antibody against CTLA-4, and a phase 3 study showed increased survival in patients with metastatic melanoma who were administered the antibody. Patients randomized to the control arm received glycoprotein 100 (gp100) and demonstrated overall survival of 6.4 months. Patients in the experimental arm received ipilimumab, with or without gp100, and demonstrated an overall survival of 10.0 months, a significant improvement compared to gp100 alone. Though these studies show exciting results for patients

with metastatic disease, a study by Liakou et al. [55] studied the use of ipilimumab in presurgical patients with bladder cancer. Six patients with localized bladder cancer were treated with ipilimumab and were found to have expression of inducible costimulatory (ICOS), a T cell specific surface molecule structurally similar to CD28, on peripheral blood CD4+ T cells. *In vitro* studies of the CD4+ICOS^{hi} cells from the ipilimumab treated patients showed an increased effector T cell to regulatory T cell ratio, as well as an increased production on IFN-γ[55]. A phase 1 trial studied the safety of using ipilimumab in 12 presurgical patients with localized urothelial cancer and showed the treatment was tolerable in 11 patients, with grade 1-2 toxicities, rash and diarrhea, being the most common[56]. Observations of the phenotypic biomarkers and tolerability are important steps needed in order to illuminate the mechanism of action and safety profile of ipilimumab, and are paramount for determining the clinical outcomes and of this antibody in the immunotherapy of bladder cancer.

The interaction between programmed death 1 (PD-1) on T-cells and its ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273), is an immune check point explored as another viable option for checkpoint inhibition immunotherapy. PD-1, like CTLA-4, is a transmembrane protein found mainly on T cells, which serves as a regulator of lymphocytic function. *In vitro* assays show that the engagement of PD-1 with PD-L1 results in inhibition of TCR activation[57,58]. Thus, expression of PD-L1 acts to maintain immune tolerance and prevent autoimmunity. Urothelial tumors, like other solid tumors, can take advantage of this by expressing PD-L1 in order to develop immune tolerance[59,60]. Expression of PD-L1 on bladder cancer cells was associated with high grade tumors (odds ratio 2.4,95% CI 1.2-4.72, P=0.009) and was found to be extensively expressed in granulomas of recurrent tumors. Furthermore, PD-L1 acts as a marker for tumor progression, independent of tumor grade, and was associated with a higher frequency of post-operative recurrence and lower survival rate[61,62]. MPDL3280A is an anti-PD-L1 monoclonal antibody that inhibits the interaction between PD-1 and PD-L1. Administered systemically, the antibody has been studied in patients with metastatic urothelial bladder cancer in a recent phase 1 clinical trial[63]. In the evaluation of safety, 57% of 67 patients reported adverse events, while 4% of patients reported a grade 3 adverse event. No grade 4 or 5 adverse events were reported. The patients evaluated for efficacy had poor prognostic factors at baseline, including visceral metastasis in 50 patients (75%), while 48 patients (72%) had failed previous systemic therapy. After a 6 week follow up

from treatment, objective response rates were 43% in patients with PD-L1 expression on tumor infiltrating immune cells by immunohistochemistry, representing remarkable clinical activity against metastatic bladder cancer. Other check point inhibitors such as B7-H3, B7-H4, LAG3, and TIM3 have been identified in basic studies, of which some are being studied in a clinical setting. However, to date, no data has been published demonstrating the clinical efficacy of monoclonal antibodies as checkpoint inhibitors for nonmuscle invasive bladder cancer[45,64].

Vaccines

Vaccination is another form of emerging immunotherapy against NMIBC. Vaccines offer certain advantages over monoclonal antibody therapy, including decreased risk of tumor escape from targeting a single isotope and preventing tumor growth rather than requiring continual treatment of passive antibody treatment. Targets of vaccination include oncoproteins [e.g. HER2 and cancer testis antigens (CTAs)] as well as TAAs [e.g. carcinoembryonic antigen (CEA) and mucin-1 (MUC-1)]. A meta-analysis of nine studies found that HER2 expression in patients with bladder cancer was associated with higher histological grade, higher rates of lymph node metastasis, poor survival rates and poor disease free[65]. HER2 expression was positive in 27.8%-85.2% of bladder cancer making the oncoprotein a feasible vaccine target. A recently completed phase 2 trial investigated DN24-02, a form of autologous immunotherapy consisting leukapheresed APCs cultured in recombinant a HER2 antigen (NCT01353222). APC activation was observed in all of the first 30 patients who underwent three infusions in two week intervals, suggesting a boost in immunologic effect. The adverse effects were relatively minor, grades 1-2[66]. A phase 1 clinical trial is currently ongoing at the National Institutes of Health investigating an autologous AdHER2/neu dendritic cell vaccine in patients with solid metastatic tumors and as an adjuvant in postcystectomy patients with HER2+ tumors (NCT01730118). In murine models, a recombinant adenoviral ErbB-2/neu vaccine demonstrated the ability to cure subcutaneous mammary tumors and pulmonary metastases[67].

CTAs are a group of antigens that are normally expressed in male germ cells in the testis, but not in somatic tissue. Malignant cells may attain the ability to express CTAs by disruption of the regulated gene. As CTAs are immunogenic, they have become the target of developing antigen specific vaccines[68]. A study of tumor samples from 95 patients with high-grade urothelial carcinoma showed that 77% of tumors

expressed at least one CTA with MAGE-A4 and MAGE-A3 being the most common CTA expressed[69]. As a result, MAGE-A4, along with the most immunogenic CTA in urothelial carcinoma, NY-ESO-1, are both currently being studied as targets for vaccine therapy in NMIBC[70]. For example, a study evaluated the safety and immunogenicity of an NY-ESO-1 vaccine in patients with urothelial carcinoma who had previously undergone cystectomy or nephroureterectomy. Enrolled patients were administered the NY-ESO-1 vaccine plus GM-CSF along with intradermal BCG. The study found 5 of 6 patients enrolled responded with anti-NY-ESO-1 specific antibodies and 1 of 6 patients exhibited a CD8+ response. All 6 patients displayed a CD4+ response[71]. Toxicities were limited to grade 1-2 in all patients. A phase 1 study evaluating the safety of an NY-ESO-1 vaccine with or without sirolimus in patients with solid tumors is currently being conducted and has completed accrual (NCT01522820). A recently completed phase 1 vaccine trial evaluated a CTA, MAGE-A3 (NCT01498172). Patients with NMIBC received intravesical BCG along with recMAGE-A3 immunization and adjuvant AS15 (recMAGE-A3 + AS15 ASCI) in order to assess vaccine safety and assess vaccine specific T-cell response. At this time, no results are available. Another ongoing phase 2 trial is evaluating disease free survival and clinical efficacy of recMAGE-A3 + AS-15 in patients who underwent cystectomy for muscle invasive bladder cancer (NCT01435356).

The National Institutes of Health has an ongoing phase 2 trial assessing the clinical utility of PANVAC, a poxvirus based vaccine, in the treatment of NMIBC for patients who have failed at least one prior BCG course (NCT02015104). The PANVAC vaccine has shown efficacy in several other carcinomas expressing antigens such as MUC-1, CEA, and three T cell costimulatory molecules (B7.1, intracellular adhesion moldecule-1, and leukocyte function-associated antigen-3) [72,73]. Using immunohistochemistry, MUC-1 expression has been identified on 60%-70% of bladder cancers and increased expression is associated with higher grade tumors[74–76]. Similarly, CEA is expressed in approximately 76% of grade 4 tumors via immunohistochemistry[77]. As such, these immunogenic surface antigens make them plausible for use in vaccine immunotherapies.

Gene therapy

Gene-therapy refers to the genetic modification of normal or diseased cells to treat diseases, including cancers[78]. Advances in gene therapy have led to its application in pancreatic cancer[79], malignant gliomas[80], and lung cancer[81]. In bladder cancer, the use of gene therapy may be applied in the context of immunomodulation, direct oncolytic effect, and gene transfer. The entire application of gene therapy for the treatment of bladder cancer is beyond the scope of this review, and herein we will highlight some advances in gene therapy with respect to immunotherapy for superficial bladder cancer. A key component to gene therapy is the vector, or modality by which genes are delivered to the target cells. Vectors are employed to transduce a target cell with foreign DNA. Although numerous vectors have been described, the most commonly used vector in gene therapy is replication deficient adenovirus[82]. Adenovirus based gene therapy offer many advantages, including relative ease of producing, relative stability, and gene delivery capabilities, making it a commonly tested modality for gene-therapy in clinical trials[83].

In a study by Chester et al.[84], replication defective adenovirus with β-galactosidase marker gene was to transduce healthy human urothelial cells as well as neoplastic urothelial cells, demonstrating the ability to transduce human urothelial cells in *in vitro* and *ex vivo* models. Of the eight explant cultures of transitional cell carcinoma (TCC) were exposed to the adenovirus, four exhibited a near 100% transduction rate. In the same study, three fresh cold cup biopsy specimens of papillary TCC were exposed to the adenovirus. The superficial epithelial cells of the specimen demonstrated successful transduction. This study suggests the feasibility of using adenovirus as a vector for gene therapy for bladder cancer in human studies.

This principle has then been used in immunomodulating gene therapy. Transduced genes provide antitumor activity against NMIBC by sensitizing the cancer cells to the immune system. CD40L is protein found on Th1 helper cells and when bound to CD40 on APCs, is responsible for activation and antitumor activity of Th1 cells[85]. The use of the CD40L in an adenoviral vector (AdCD40L) has shown antitumor activity in a phase 1/2a trial for patients with high grade or Ta bladder cancer undergoing cystectomy[86]. Intravesical AdCD40L was administered and patient safety, along with tumor response, was monitored. Post treatment bladder biopsy samples from these patients had increased expression of CD40L on bladder epithelium as demonstrated by immunohistochemical staining. These epithelial samples were also infiltrated with CD4+ T cells. Antitumor activity was also remarkable as three of the five patients in the phase 1 trial demonstrated no tumor cells in the post treatment biopsy. Adverse events were limited to bladder spasms and mild bladder pain. As local AdCD40L therapy has shown to

be safe and immunogenic, it may play a role future bladder cancer therapy.

A phase 1 study by Dinney et al.[87] investigated the intravesical administration of recombinant adenovirus with an interferon-α2b gene (rAd-IFN-α) combined with an excipient, Syn3, in patients with BCG refractory NMIBC. The therapy was well tolerated as urgency, headache, fatigue, and nausea were the most commonly reported adverse events; no dose limiting toxicities were observed. Additionally, quantifiable levels of IFN-α were measured is all seventeen patients up to 10 days after the treatment. This therapy also demonstrated clinical activity as a complete response, as defined by a negative biopsy and no observable disease, was seen seven patients on 3-month follow up while five patients maintained complete response at 12 months. This study is the first to show evidence of safety and efficacy of intravesical recombinant adenoviral therapy for NMIBC.

Viral gene therapy is not without its obstacles. In 2003, systemic adenoviral gene therapy of a patient with ornithine transcarbamylase deficiency resulted in a fatal systemic inflammatory response syndrome, raising concerns regarding the safety of viral gene therapy[88]. However recent developments in intravesical delivery of viral vectors allows for local treatment of bladder cancer, thus avoiding complications of systemic administration[84]. Other obstacles include cellular susceptibility to viral infection. Adenovirus associated with the cell surface coxsackie-adenoviral receptor (CAR), resulting in intracellular incorporation of the virus. Neoplastic bladder epithelial cells are relatively CAR deficient, thus exhibiting increased adenoviral resistance[89–91]. Methods of increasing susceptibility, such as increasing cancer cell CAR expression[92] or modifying viral vectors[93] may pave a way for implementing viral gene therapy in clinical practice.

Adoptive T-cell therapy

Adoptive T-cell therapy, a form of passive immunotherapy, refers to the employment of autologous T-cell transfusions into tumor bearing patients in order to control tumor growth[94]. Tumors with lymphocytic infiltration have been associated with better prognosis; however, their small numbers are often not sufficient to correlate into a clinical effect[95].

In vitro, tumor infiltrating lymphocytes (TILs) can be isolated and expanded without effects of inhibitory factors found *in vivo*. Researchers found that when these cells were isolated from a lymphodepleted patient with metastatic melanoma and then infused back into the patient, 21 (49%)

of 43 patients experienced regression by RECIST criteria (Response Evaluation Criteria in Solid Tumors) criteria[96]. Grade 3-4 toxicities were observed with the lymphodepleting therapy, however no 3-4 toxicities were observed from administration of the autologous T-cells[97].

Sherif et al.[98] infused autologous T lymphocytes from the metinel node of patients with advanced urothelial carcinoma T2N1 or higher to determine if this form of immunotherapy would be safe and technically feasible. Of the six patients in which the T lymphocytes were administered, no major adverse events were observed, warranting larger trials to determine long term clinical outcomes.

However, demonstrating clinical response in patients with bladder cancer presents certain challenges as epithelial cancers contain fewer mutations than melanoma and there is little data on endogenous mutation specific T-cell response[99]. Researchers at the National Cancer Institute have attempted to undertake these challenges as they have studied adoptive T cell therapy in a patient with metastatic cholangiocarcinoma[100]. Lung metastases were extracted from the patient for both whole genomic sequencing as well as T cell extraction. Sequencing of the tumor yielded 26 nonsynomymous mutations for each a minigene construct that was generated. Multiple minigenes were used to craft tandem minigene (TMG) constructs that were used for *in vitro* transcription, creating TMG RNAs. The RNAs were transfected into autologous APCs and cultured with the TILs extracted from the patient in order to determine which TILs would react to the transfected APCs. The patient was reinfused with the TILs. Results of the study suggest regression of the cancer and stabilization of the disease.

Adoptive T cell therapy still presents challenges, such as the need to perform an invasive extract procedure and difficulty growing TILs in some patients. To overcome such challenges, Clay et al.[101] used T cells from peripheral blood and transfected the T cells using a retrovirus to create genetically modified T cells with recombinant TCRs with an affinity for the TAA MART-1 in melanoma cells. Naïve T cells were successfully transduced to express a MART-1 specific TCR and demonstrated the ability to lyse melanoma cells *in vitro*.[101] A similar study was performed with autologous T cells transduced with TCR specific to NY-ESO-1 CT antigen. Patients with NY-ESO-1 expressing tumors were treated with modified T cells. A clinical response was observed in five of the eleven patients with metastatic melanoma and four of the six patients with synovial cell carcinoma[102].

In addition to the benefit of avoiding technical difficulties, modified T cell therapy also provides the added benefit of
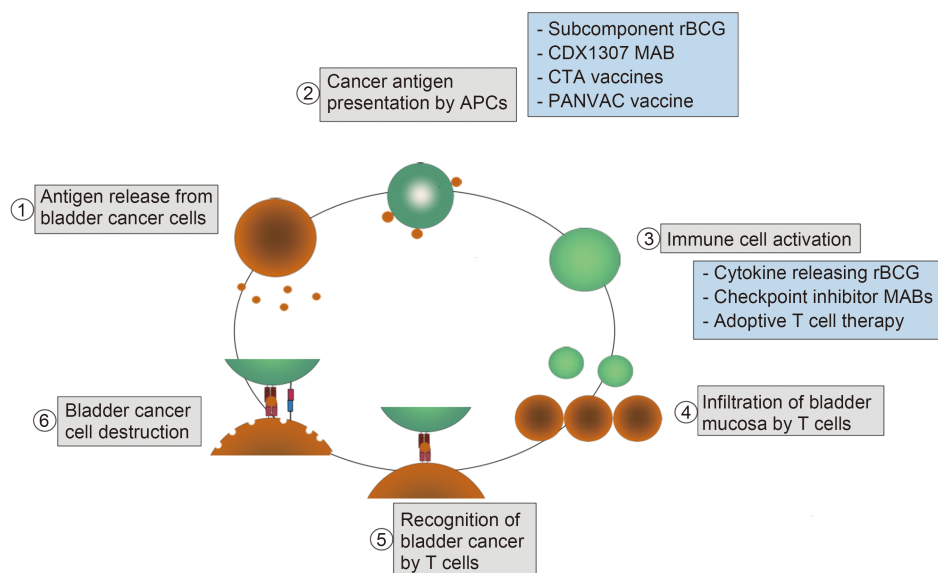
Figure 2 Immunotherapy for superficial BCa, highlighted by the blue boxes, within the cycle of cancer-immune response. Adapted from Chen et al.[104] Immunity 2013.

reducing immune escape due to MHC restrictions[96]. This has led to the development of genetically modified T cells with innate antitumor activity using chimeric antigen receptors (CARs). By grafting antigen binding domains of monoclonal antibodies to T cell signaling domains, CARs may bind directly to specific tumor antigens, bypassing stimulation from MHCs. Patients with relapsed acute lymphoblastic leukemia (ALL) were treated with CARs modified T cells specific for CD19, and a high remission rate was observed[103]. Given the exciting results modified T cell immunotherapy, it is not a far stretch that this may be an avenue for NMIBC treatment; however, research using urothelial models would be required to assess the utility and feasibility of this therapy.

Conclusions

Immunotherapy is an evolving field that offers promise in the treatment of NMIBC. Although BCG still remains the cornerstone treatment for high-grade NMIBC, the toxicity, limited efficacy in a subset of patients, and recurrence rates call for more effective treatment options. Recombinant BCG, monoclonal antibodies, vaccines, and adoptive immunotherapy are potential options aimed at addressing these shortcomings. The roles these therapies play in the cycle of cancer and immunity are displayed in **Figure 2**. Further research is necessary to bring immunotherapy further to the forefront of treating urothelial cancer. These treatment modalities may be combined with established treatments such as surgery, radiation, and chemotherapy to effectively treat bladder cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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