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Evolving Immunotherapy Strategies in Urothelial Cancer

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Abstract

The treatment of nonmuscle-invasive urothelial carcinoma with bacillus Calmette-Guérin (BCG) represents the importance of immunotherapy in the treatment of cancer. Despite its clinical efficacy, up to 30% of patients will ultimately experience progression to muscle-invasive disease. This, along with an improved understanding of the biologic pathways involved, has led to efforts to improve, enhance, or alter the immune response in the treatment of urothelial carcinoma. A number of novel therapeutic approaches currently are being pursued, including recombinant BCG to induce T helper type 1 (Th1) immune responses, nonlive *Mycobacterium* agents, targeted agents toward cancer-associated antigens, immune-modulating vaccines, and adoptive T-cell therapies. Here, we review the current and future immunotherapy treatment options for patients with urothelial cancer.

In 2013, cancer immunotherapy was declared the break-through of the year by *Science* magazine.¹ Since then, there has been a frenetic dash toward the development and implementation of immune-based therapies in all cancers, including urothelial cancer. However, immunotherapy has a long and successful history in the treatment of bladder cancer, with BCG intravesical immunotherapy as the leading therapy for nonmuscle-invasive bladder cancer (NMIBC). We will review the current and future immunotherapy treatment options for patients with urothelial cancer.

BCG

BCG is a live attenuated strain of *Mycobacterium bovis* developed initially in 1921 as a vaccine for tuberculosis. In 1976, Morales et al² reported the first successful use of intravesical BCG therapy in the treatment of recurrent superficial bladder cancer. It has been demonstrated to reduce recurrence rates and progression to muscle-invasive disease in patients with carcinoma in situ (CIS) and with superficial bladder tumors.³⁻⁵ BCG has gone on to become the standard of care in the treatment of high-grade NMIBC after transurethral resection.

Although BCG has been a mainstay treatment of NMIBC for more than 3 decades, the exact mechanism by which BCG achieves its therapeutic effect remains an area of investigation.

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Zbar and Rapp⁶ in 1974 discovered several conditions required to obtain an antitumor effect with BCG, including the ability to develop an immune response, a sufficient number of live BCG, close contact between BCG and cancer cells, and a low tumor burden.

As early as 1959, it was recognized that the antitumor effect of BCG was mediated through the activation of an immune response and induction of the inflammatory response.⁷ Additional studies have confirmed that an intact immune system, particularly the cellular system, is required for achievement of a therapeutic response. Abundant evidence indicates that intravesical BCG results in an extensive influx of inflammatory cells and cytokine production, which results in an immune response against tumor cells.

The immune response is preceded by the attachment of BCG to urothelial cells. The normal urothelium is lined with glycosaminoglycans (GAGs), which have been suggested as a primary defense mechanism against noxious elements in urine.⁸ The antibacterial properties of GAGs are secondary to its hydrophilic, highly negative charge, imposing a barrier between the urothelium and urine. After instillation into the bladder, BCG adheres to urothelial cells via fibronectin, a glycoprotein that is part of the extracellular matrix. BCG attaches to fibronectin through its fibronectin attachment protein. The importance of this interaction has been demonstrated in a mouse model, in which blocking fibronectin attachment reduced the delayed-type hypersensitivity response and antitumor activity.⁹ Internalization of BCG by urothelial, tumor, and inflammatory cells triggers an inflammatory cascade of cytokine release and immune cell recruitment. This results in an influx of granulocytes, macrophages, natural killer cells, dendritic cells, and lymphocytes. Many studies have examined the cytokines present in the urine of patients treated with BCG and found elevated amounts of interleukin (IL)-1, IL-2, IL-6, IL-8, tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, and granulocytemacrophage colony-stimulating factor (GM-CSF).^{10,11} In addition, an anti-BCG-specific immune response via antigen presentation to T cells amplifies the response. Ratliff et al¹² showed that the absence of either CD4 or CD8 T-cell subsets eliminated the BCG-mediated antitumor activity.

Although BCG is a very effective intravesical treatment for high-grade NMIBC, ultimately 20% to 50% of patients will experience recurrence after a successful induction cycle.¹³ Of these patients, 25% to 45% can benefit from salvage therapy with a second induction course.¹⁴ Side effects of intravesical BCG are common. A randomized EORTC trial assessed the dose and duration of maintenance BCG in patients with intermediate- and high-risk NMIBC.¹⁵ Of the 1,316 patients enrolled, 62.8% reported local side effects and 30.6% reported systemic side effects. The most frequent local and systemic side effects reported were chemical cystitis in 35% of patients and general malaise in 15.5% of patients.

Prolonged BCG treatment is limited in some patients by the development of severe local side effects and/or recurrent and/or progressive disease. Rarely, BCG systemic toxicity also can preclude further BCG use. Although radical cystectomy is an effective procedure for these patients, it can be accompanied by considerable morbidity and an altered quality of life.

RECOMBINANT BCG

Recombinant BCG (rBCG) options have been engineered to address the stated limitations of BCG. rBCG consists either of Th1 cytokine-secreting rBCG strains or of non-live rBCG strains that contain BCG subcomponents (see Table 1).

TH1 CYTOKINE-SECRETING RBCG

The most common Th1 cytokines incorporated into rBCG strains include IL-2, IL-12, IL-18, IFN- α , and IFN- γ .¹⁶ In vitro, IL-2 enhances the production of cytotoxic lymphocytes and the cytotoxic activity of natural killer cells and monocytes.¹⁷ In patients treated with BCG, increased IL-2 levels are noted in the urine shortly after BCG administration.¹⁸ Therefore, IL-2 rBCG strains have been engineered and studied in animal models: IL-2 rBCG strains induce a more favorable ratio of IFN- γ to IL-4,¹⁹ accelerate greater antigen-specific proliferation,²⁰ and release more Th1 cytokines than BCG.²⁰ IL-12 has a very complementary function to IL-2 and has been shown to induce tumor regression and improve survival in animal models as a single agent.²¹ Our colleagues at the National Cancer Institute (NCI) showed that intravesical IL-12 can be more successful than intravesical BCG in treating orthotopic murine tumors when it is combined with chitosan.²² Furthermore, its role in immunotherapy is vital, because IL-12 knockout (and IFN- γ knockout) mice do not respond to BCG therapy.²³ Unfortunately, a phase I trial of intravesical recombinant IL-12 demonstrated no activity in patients²⁴ (although the IL-12 in this trial was not combined with chitosan); to date, no trial results with IL-12–based rBCG are available. IL-18 is secreted from activated macrophages and, similar to IL-2 and IL-12, can synergize BCG activity by inducing Th1 cytokines.²⁵ In vitro, IL-18–based rBCG enhances macrophage cytotoxicity and cellular proliferation of IFN- γ –secreting cells.²⁶

Unlike the previous cytokines, IFN- α has been widely used in patients, often in conjunction with BCG. Preclinical work demonstrates that IFN- α can induce apoptosis by increased TNF–related apoptosis-inducing ligand (TRAIL) expression²⁷ and reduced angiogenesis in bladder tumors.²⁸ Although a large, phase II trial showed the efficacy and tolerability of BCG given with IFN- α , it is unknown whether the combination offers any benefit compared with BCG monotherapy.²⁹ The IFN- α –secreting rBCG strain is superior to BCG in the production of IFN- γ from peripheral blood mononuclear cells (PBMCs) and in the enhancement of PMBC cytotoxicity toward bladder cancer cells.^{30,31} IFN- γ is an essential cytokine for BCG efficacy, and it has been shown to reduce proliferation in bladder cancer cell lines.^{23,32} In a murine model, IFN- γ –based rBCG upregulated major histocompatibility complex (MHC) class I molecules on murine bladder cancer cells and, compared with the control rBCG strain, increased CD4⁺ T cells, IL-2, and IL-4 in the bladder and prolonged survival after intravesical administration.³³

BCG SUBCOMPONENT-BASED RBCG

Most of the evaluated subcomponents are portions of the BCG cell wall. Mycobacterial cell wall extract demonstrated excellent activity: 41.1% of patients had negative cystoscopies and

biopsies at 60 weeks after therapy.³⁴ However, it contained thimerosal, a known organomercurial preservative with the potential for neurotoxicity. A newer formulation without thimerosal, called mycobacterial cell wall complex (MCC), was subsequently evaluated in a multicenter study in 2009. Fifty-five patients (82% of whom had previously experienced treatment failure with BCG) received MCC induction and maintenance at two different doses. At 26 weeks of follow-up time, the complete response rates were 27.3% in the 4-mg group and 46.4% in the 8-mg group.³⁵ Unfortunately, a phase III trial (NCT01200992) comparing MCC with mitomycin C in patients with BCG–recurrent/refractory bladder cancer closed early because of poor accrual. Finally, the BCG cell wall skeleton has been studied further after being incorporated into other particles, because it has unfavorable characteristics for penetration of the urothelium by itself. Both a liposomal and a lipid nanoparticle formulation have demonstrated antineoplastic effects in animal models.^{36,37}

MONOCLONAL ANTIBODIES

Most excitement in immunotherapy is focused on monoclonal antibodies directed against tumor-associated antigens (e.g., beta-HCG, cytotoxic T-lymphocyte–associated antigen 4 [CTLA-4], programmed death ligand 1 [PD-L1]). Beta-HCG is expressed in 35% to 75% of bladder cancers, and expression correlates with a worse prognosis.³⁸ CDX-1307 is an interesting monoclonal antibody that functions like a vaccine in the body. It consists of the beta-HCG subunit genetically fused to the human monoclonal antibody B11 that is specific for the mannose receptor on antigen-presenting cells (APCs). On administration, CDX-1307 is internalized by APCs and, in processing, presents beta-HCG as an antigen to CD4⁺ and CD8⁺ T cells.³⁹ Thus, it has been proposed as a therapy in beta-HCG–expressing bladder cancers.

Checkpoint blockade inhibitors are extremely popular now, because they target inhibitors of the immune response. T cells engage APCs via their T-cell receptor as the primary signal; however, a secondary costimulatory signal is provided by CD28 (on the T cell) and B7 (on the APC). To prevent excessive T-cell proliferation, an inhibitory signal, CTLA-4, can be expressed by activated T cells and competes with CD28 in binding to B7 on APCs. This can mitigate a T-cell response in a normal circumstance. However, in cancer, T-regulatory cells (Tregs) are expressed in the tumor microenvironment and can constitutively express CTLA-4, which can suppress an anticancer immune response.⁴⁰ Therefore, CTLA-4 –blocking antibodies have been developed as a therapeutic strategy. These antibodies have prolonged overall survival in metastatic melanoma but cause grade 3 or 4 toxicities in the 10% to 15% range.⁴¹ A pilot presurgical trial in patients with urothelial cancer demonstrated that peripheral and tumor CD4 T cells had increased expression of inducible costimulator (ICOS) in response to an anti–CTLA-4 antibody. These CD4⁺ ICOS (hi) T cells produced IFN-gamma, recognized NY-ESO-1 as a tumor antigen, and increased the ratio of effector T cells to Tregs.⁴² A subsequent phase I trial of 12 patients with urothelial cancer demonstrated only grades 1 to 2 toxicities, and all patients expressed CD4⁺ ICOS (hi) T cells in tumor tissues and systemic circulation. These preliminary findings with the use of CTLA-4 –blocking antibodies have stimulated efforts to evaluate the efficacy of these antibodies in urothelial cancer.

As an immune response progresses, CD4 and CD8 T lymphocytes will upregulate the expression of other checkpoint inhibitors, such as the programmed death 1 (PD-1) receptor. Inflammatory conditions will prompt IFN release, which will upregulate PD-L1 and PD-L2 in peripheral tissues to maintain immune tolerance and prevent autoimmunity. Binding of PD-L1 and/or PD-L2 to PD-1 results in dephosphorylation of proximal signaling molecules downstream of the T-cell receptor complex via *src* homology region 2 domain–containing phosphatase (SHP)-1 and SHP-2 as well as augmentation of phosphatase and tensin homolog (PTEN), leading to decreased T-cell proliferation, survival, and protein synthesis.⁴³ However, many cancers take advantage of this checkpoint blockade by upregulating PD-L1 expression on their surface, and urothelial cancers have higher levels of PD-L1 relative to other tumors.⁴⁴ Among urothelial tumors, PD-L1 expression is higher among BCG treatment failures⁴⁵ and among more aggressive and metastatic tumors.⁴⁶

The PD-L1 expression data would suggest activity in metastatic tumors with a PD-L1 blockade. A recently completed phase I clinical trial evaluated the anti-PD-L1 monoclonal antibody, MPDL3280A, in patients with metastatic urothelial bladder cancer.⁴⁷ This antibody has an Fc domain modification that prevents antibody-dependent cellular cytotoxicity so that local T cells were not killed and thereby depleted. As a result, the antibody inhibits the interaction of PD-L1 with PD-1. In this trial, 72% of the patients experienced failure of two or more prior systemic regimens. The overall objective response rate (ORR) for all 65 patients was 26%. However, the ORR varied on the basis of the degree of immunohistochemistry (IHC) staining of tumor-infiltrating cells or tumor cells. Strong (2+ to 3+) PD-L1 expression by IHC (5% or greater PD-L1 positive) resulted in a 43% ORR, whereas weak (0 to 1+) PD-L1 expression by IHC (less than 5% PD-L1 positive) resulted in an 11% ORR. The median time to first response was 42 days, and the median overall survival times were 4.2 months for the strong IHC expression group and 2.7 months for the weak IHC expression group. Importantly, the main toxicity of the therapy was fatigue, and grade 3 adverse events only occurred in 4% of patients (no grade 4 or 5 toxicity noted). On the basis of the encouraging results of this trial, the U.S. Food and Drug Administration (FDA) granted MPDL3280A a breakthrough designation in June 2014.

VACCINES

HER2 status has prognostic and therapeutic value in breast cancer. Recently reported results from The Cancer Genome Atlas⁴⁸ found that HER2 alterations were almost as frequent in urothelial carcinoma compared to the TCGA breast cancer study. A meta-analysis of nine retrospective studies (2,242 patients) indicated that HER2 expression significantly correlated with poorer disease-specific survival (hazard ratio [HR] 2; 95% CI, 1.22 to 3.29; $p = 0.006$) and disease-free survival (HR 1.68; 95% CI, 1.33 to 2.14; $p < 0.0001$) of patients with bladder cancer.⁴⁹ DN24 – 02 is an investigational active cellular immunotherapy targeted at the HER2 receptor. It consists of autologous PBMCs, including APCs, which are activated *ex vivo* with a recombinant fusion protein. A randomized phase II study comparing adjuvant DN24 – 02 to the standard of care in patients with high-risk urothelial cell carcinoma met accrual and results are pending at this time. Colleagues at the National Institutes of Health Clinical Center are investigating the use of another HER2/*neu* vaccine in a phase I trial (NCT01730118). This trial investigates a novel therapeutic autologous AdHER2 dendritic

cell vaccine in patients with HER2-expressing metastatic solid tumors as well as in patients with adjuvant bladder cancer who have HER2-positive tumors. In preclinical animal models, the vaccine was documented to eradicate large established tumors through the induction of polyclonal, anti-HER2 antibodies.

M phase phosphoprotein 1 and disheveled, EGL-10, and pleckstrin (DEP) domain have roles in the growth of bladder cancer cells. In addition, human leukocyte antigen (HLA)-A24 – restricted peptide epitopes corresponding to parts of these two proteins have been identified that can induce peptide-specific cytotoxic T cells. Of the six patients with metastatic bladder cancer enrolled in a phase I trial, four developed vaccine-specific cytotoxic T cells.⁵⁰

Cancer testis antigens (CTAs) are a group of tumor-associated antigens with restricted expression in normal tissues but with expression in a variety of tumors. Sharma et al⁵¹ examined the expression patterns of nine CTA by IHC and reverse transcriptase (RT) polymerase chain reaction (PCR) in a panel of 95 high-grade bladder tumors. They found that at least one CTA was expressed in 77% of the tumors and that 61% of the tumors expressed more than one. A recombinant NY-ESO-1 protein vaccine coadministered with GM-CSF and BCG as immunologic adjuvant was tested in six patients with organ-confined disease.⁵² NY-ESO-1-specific antibody responses were induced in five patients, CD8 T-cell responses occurred in one patient, and CD4 T-cell responses were seen in all six patients. A phase II trial (NCT01435356) evaluating the efficacy of the recMAGE-A3 vaccine after cystectomy is currently recruiting; a phase I trial (NCT01498172) evaluating MAGE-A3 plus BCG has just completed, and results are pending.

PANVAC is a poxviral cancer vaccine that has demonstrated therapeutic efficacy against a variety of carcinomas. PANVAC consists of a primary vaccination with a replication-competent recombinant vaccinia vector followed by multiple boosts with a replication-incompetent recombinant fowlpox vector. These vectors contain transgenes for both human T-cell costimulatory molecules and tumor-associated antigens against Mucin-1 (MUC-1)^{53,54} and carcinoembryonic antigen (CEA).⁵⁵ MUC-1 and CEA are expressed in approximately 93% and 76% of high-grade bladder tumors, respectively. A phase II study (NCT02015104) assessing PANVAC in combination with intravesical BCG in patients who have experience treatment failure with prior BCG therapy is recruiting patients at the NCI and the Rutgers Cancer Institute of New Jersey.

ADOPTIVE T-CELL THERAPY

Adoptive T-cell therapy involves the infusion of externally manipulated T cells to give rapid immunity instead of relying on the immune system to generate T cells after being presented with an antigen. Before infusion, however, patients require nonmyeloablative leukoreductive therapy using irradiation and chemotherapy.⁵⁶ This area of cancer immunotherapy began with tumor-infiltrating lymphocytes (TILs). These TILs are extracted from tumor tissue, expanded ex vivo, and then reinfused into patients. This approach was applied recently to patients with metastatic urothelial cancer in a feasibility study. Tumor lymphocytes were obtained from lymph nodes draining metastatic tumors; after in vitro culture, the lymphocytes were reinfused into the patients without any adverse effects.⁵⁷ However, it is

unclear how effective this approach will be. A more novel approach applies extraction of TILs from tumors and whole-exomic sequencing of the tumors to identify mutations characteristic of the tumor. The TILs are probed to see if they recognize any of the mutations; then, adoptive transfer of the TILs containing mutation-specific polyfunctional T cells is performed.⁵⁸ This technology can be applied to any malignancy, and, given that urothelial cancer has the third highest rate of mutations, it is well positioned for this type of therapy. We are assisting our colleagues who are performing this work at the NCI in the application of this approach to urothelial tumors.

TIL therapy is limited in that TILs have to be retrieved through an invasive procedure and require the ability to grow *ex vivo*. As a result, genetic modification of the T-cell receptor (TCR) only requires isolation from peripheral blood, viral transduction to express a recombinant TCR specific for a tumor antigen, and reinfusion after expansion of these genetically engineered TCRs. CTAs, as mentioned earlier, are good candidates for TCR gene therapy, because they are not present in adult somatic tissues and are found with high frequency in some tumors. For example, NY-ESO-1 is expressed in 80% of patients with synovial cell carcinoma patients. In a trial of patients who had metastatic synovial cell carcinoma, four of six patients achieved objective clinical responses after treatment with a TCR directed against NY-ESO-1.⁵⁹ This technology still requires the presence of the target antigen on the tumors and is restricted to patients who have certain HLA alleles. Also, if the tumor responds by MHC downregulation, then this therapy may be limited. However, this therapy seems attractive to apply toward urothelial tumors, given their high expression of CTAs.

A novel form of adoptive immunotherapy has evolved that can avoid the MHC restriction and the immune escape phenomenon that may be seen with genetically engineered TCRs. Chimeric antigen receptor T-cell therapy entails isolation of a patient's peripheral T cells and subsequent transduction by a chimeric receptor that consists of a single-chain variable region of an antibody domain (scFv) that is specific for a desired tumor-associated antigen (TAA) with a CD3/T cell. Because the antibody portion of the chimeric receptor is binding to the TAA, the binding is non-MHC restricted. The T-cell portion engages the native T-cell receptor-mediated activation on binding. Therefore, this approach combines the cytotoxicity of a CD8⁺ T cell with MHC-independent antigen recognition of a monoclonal antibody.⁵⁶

CONCLUSION

Immunotherapy has become a popular approach to target tumors in the last decade. Urothelial cancer is a malignancy that has a long history of successful treatment with BCG. Despite this success, the high recurrence and progression rates of localized disease along with the dismal prognosis of metastatic disease warrant improvement in the current treatment. Recombinant BCG strategies are promising for BCG-refractory disease or for patients who cannot tolerate further BCG. Monoclonal antibodies, and specifically checkpoint blockade inhibitors, have been especially exciting and are the newest therapies for metastatic urothelial cancer. These therapies likely will be applied to localized, BCG-refractory disease also. Vaccines that target TAAs, such as HER2, CTA, and MUC-1/CEA are promising, given the high expression in urothelial cancer and the tolerability of these

treatments. Finally, adoptive T-cell therapy is very promising; although this area of immunotherapy has not been explored fully in urothelial cancer, the high rate of mutations in urothelial cancer and the prevalence of urothelial-specific TAAs make this a promising area of future research.

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KEY POINTS

- Bacillus Calmette-Guérin (BCG) represents one of the most successful applications of immunotherapy and currently is the most effective treatment for high-grade nonmuscle-invasive bladder cancer.
- Recombinant BCG may enhance the cytotoxic T-cell response required for BCG-mediated antitumor effects while reducing local side effects.
- Checkpoint blockade inhibitors are a novel class of immunotherapeutics agents that target and interfere with immune stop signals.
- Vaccines present the opportunity for long-term success and are the ultimate goal.
- Adoptive T-cell immunotherapy can induce rapid immunity but carries the risk of off-target toxicities.

TABLE 1**Targeted Therapies**

Agent	Target(s)
rBCG	
Th1, cytokine secreting	IL-2, IL-12, IL-18, IL- α , IL- γ
BCG, subcomponent based	Mycobacterial cell wall, BCG cell wall
Monoclonal Antibodies	
Checkpoint blockade inhibitors	CTLA-4, PD-L1
Tumor-associated antigens (CDX-1307)	β -HCG
Vaccine	
AdHER2 dendritic cell vaccine	HER2/neu
CTAs	NY-ESO-1, recMAGE-A3
PANVAC	MUC-1, CEA
Adoptive T-cell Therapy	
TIL	CTAs (eg, NY-ESO-1)
CART	TAA

Abbreviations: rBCG, recombinant bacillus Camille-Guerin; Th1, T helper cell 1; IL, interleukin; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-L1, programmed death ligand 1; HCG, human chorionic gonadotropin; CTA, cancer testis antigen; TIL, tumor-infiltrating lymphocyte; CART, chimeric antigen receptor T cell; TAA, tumor-associated antigen.