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Genitourinary Cancers (W Oh and M Galsky, Section Editors)

Immune Therapies in Non-Muscle Invasive Bladder Cancer

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Opinion statement

Non-muscle invasive bladder cancer (NMIBC) continues to be a challenging disease to manage. Treatment involves transurethral resection and, often, intravesical therapy. Appropriate patient selection, accurate staging, and morphological characterization are vital in risk-stratifying patients to those who would most benefit from receiving intravesical therapy. Bacillus of Calmette and Guérin (BCG) continues to be the first-line agent of choice for patients with intermediate- and high-risk NMIBC. Treatment should begin with the standard induction course of 6 weekly treatments. The inclusion of subsequent maintenance courses of BCG is imperative to optimal therapeutic response. While patients with intermediate-risk disease should receive 1 year of maintenance therapy, high-risk patients benefit from up to 3 years of maintenance therapy. BCG use should not be used in low-risk patients with de novo Ta, low-grade, solitary, <3-cm tumors. Conversely, patients with muscle-invasive disease should forgo intravesical immunotherapy and proceed directly to radical cystectomy. Cystectomy also should be considered in patients with multiple T1 tumors, T1 tumors located in difficult to resect locations, residual T1 on re-resection, and T1 with concomitant CIS. Although promising new immunotherapeutic agents, such as Urocidin, protein-based vaccines, and immune check point inhibitors are undergoing preclinical and clinical investigation, immunotherapy in bladder cancer remains largely reliant on intravesical BCG with surgical consolidation as the standard salvage treatment for patients with BCG failure.

Introduction

The treatment of non-muscle invasive bladder cancer (NMIBC)—comprising the majority of patients with bladder cancer—continues to be a complex issue with many nuances. Over the years, the accumulation of knowledge and data has led to the recognition that for patients with NMIBC other than in the low-risk category, immunotherapy is the most effective mode of preventing recurrences, decreasing progression, and saving both the patient's bladders and their lives. We will review the most current studies on immunotherapy for NMIBC.

Intravesical immunotherapy

Bacillus of Calmette and Guérin (BCG)

Intravesical BCG is by far the most commonly used, studied, and proven immunotherapy for NMIBC. In this section, we will focus on mechanism of action, optimal treatment strategies, and special considerations in unique patient populations.

History of BCG

Bacillus of Calmette and Guérin was developed in 1920 by Albert Calmette and Calmille Guerin as a live attenuated, avirulent strain of *Mycobacterium bovis* by serially passaging the bacteria. Whereas it was initially explored as treatment for melanoma, leukemia, etc., it was not until 1976 that Morales et al. published the first clinical trial using intravesical BCG in nine patients with superficial bladder cancer and found a decreased tumor recurrence rate [1]. Further prospective studies in the 1980s validated the clinical benefit of BCG in NMIBC tumors and in carcinoma in situ (CIS) to reduce not only recurrence but also progression [2–5]. In 1990, the Food and Drug Administrating approved BCG for the intravesical treatment of bladder CIS.

Mechanism of action

Although the clinical benefit of BCG in non-muscle invasive disease is clear, the mechanism of action in which BCG exerts its effect on tumor cells remains an area open for active investigation. It is known that effective therapy requires both an intact immunologic constitution and live BCG [6, 7]. This was recognized as early as 1959 when Old et al. demonstrated induction of the mononuclear phagocyte system in mice with transplanted tumors who were treated with BCG [8]. More modern studies with newer tools have further explained the mechanism of action.

After binding to urothelial cells via a fibronectin-dependent pathway, BCG is taken in by urothelial and inflammatory cells, which triggers a substantial inflammatory and immunologic response [9]. Moreover, tumor-associated macrophages (TAMs) and tumor-infiltrating dendritic cells (TIDCs) have been associated with a significant risk of progression to muscle-invasive cancer [10]. Furthermore, these data suggest that the presence of mature TIDCs and possibly TAMs may help risk-stratify patients at the time of first diagnosis of non-muscle-invasive bladder cancer and may be useful in tailoring follow-up and treatment

strategies. A unique characteristic of BCG-induced inflammation is the prominent early recruitment of polymorphonuclear leukocytes (PMNs), which are found in disproportionately large numbers (75 % of immune cells) in the urine after BCG instillation [11]. Neutrophils play an essential role in BCG treatment as mouse model studies have demonstrated PMN-deficiency eliminates the therapeutic effect of BCG on bladder tumors. In addition to other chemokines, PMNs secrete TNF-related apoptosis-inducing ligand (TRAIL), which preferentially induces apoptosis in tumor cells and is preferentially induced by BCG compared with other inflammatory-inciting events, such as urinary tract infections [12, 13].

After the early influx of PMNs, CD4+ T cells predominate. Similar to results in the aforementioned PMN-depleted mice, BCG efficacy is lost in athymic mice, thus demonstrating the vital role of lymphocytes [14]. Although CD4+ cells are more prevalent, CD8+ cells also have a significant role as the depletion of either abrogates the antiftumoral effect of BCG [15].

Macrophages also are found in the bladder wall and urine of BCG treated patients. Although in vitro studies have shown that BCG-stimulated macrophages have cytotoxic activity against specific bladder cancer cells, in vivo studies have not demonstrated a role of macrophages after BCG exposure [16•]. However, studies have identified tumor-associated macrophages (TAMs) in patients prior to BCG exposure that predicts recurrence after BCG [17].

Similarly, the numbers of infiltrating tumor-associated dendritic cells have been found to be associated with recurrence after BCG [18]. Although early in vitro studies have demonstrated dendritic cell activation of natural killer cells and certain T cells after BCG exposure, the role of dendritic cells in response to BCG stimulation in a live setting has not been well-delineated [19].

As is typical of an inflammatory response to bacterial infections, the cytokine profile of IL-2, IL-12, and IFN-γ seen after BCG exposure is that of a Th1 response [16•, 20]. Efforts have been made to improve BCG efficacy by amplifying the Th1 response after BCG-stimulation with the coadministration of various associated cytokines, such as IFN-α2β, IL-2, IL18, and IFN-γ.

Induction and maintenance

The typical induction course of BCG refers to 6 weekly instillations and is recommended by the American Urological Association (AUA), the European Association of Urology (EAU), National Comprehensive Cancer Network (NCCN) and the International Consultation on Urological Diseases (ICUD) [21, 22••, 23, 24]. While this schedule was empirically selected in the original studies, subsequent studies have validated the need for 6 weeks of BCG, especially in those patients who have no prior mycobacterial antigen exposure [25].

In recent years, there has been mounting evidence supporting the benefit of maintenance BCG therapy beyond the 6-week induction course. What is less clear is the optimal schedule for maintenance therapy. Lamm et al. proposed

the commonly used protocol of 3 weekly instillations of BCG at 3, 6, 12, 18, 24, 30, and 36 months from initiation of induction therapy (also referred to as the SWOG or Lamm protocol). In his study, the median recurrence-free survival improved from 35.7 months with induction therapy alone to 76.8 months with additional maintenance therapy, while worsening-free survival (no progression to ≥T2 disease or cystectomy, chemotherapy or radiation) was 111.5 months and not estimable, respectively [26]. No significant difference in survival was seen. This schedule too was empirically selected and has been the subject of much debate.

Several studies have used alternate maintenance schedules, but none have shown the same level of benefit. These alternative suboptimal maintenance schedules include 6-week induction therapy followed by monthly maintenance (Badalament et al. 1987), one instillation every 3 months (Hudson et al. 1987), and induction with yet another 6-week cycle of instillations at 6 months (Palou et al. 2001) [27–29]. Suboptimal maintenance therapy is not effective. The risk of recurrence and progression is lifelong in these patients, and induction therapy alone is not enough to produce prolonged immune stimulation necessary for optimal patient outcomes [30].

Several meta-analyses of randomized controlled trials have concluded that the addition of BCG reduces the number of tumor recurrences with an odds ratio of 0.3 for recurrences within the first year and 0.61 for all future recurrences compared to TURBT alone [31, 32]. Furthermore, BCG therapy has been shown to be superior to intravesical chemotherapy (mitomycin C (MMC), epirubicin, among others) [33]. In their individual patient data derived meta-analysis of RCTs comparing BCG to MMC, Malmström et al. found a 32 % reduction in the risk of recurrence with maintenance BCG [34]. The ability of BCG to reduce the risk of progression is clearly dependent on the addition of maintenance therapy—in fact, meta-analyses of randomized, controlled studies showed that this benefit was restricted to only patients who receive maintenance BCG [35]. In a more recent meta-analysis, Ehdaie et al. came to similar conclusions [36••]. The absence of maintenance BCG incurred a 28 % increased risk of recurrence, while its use (compared with various control groups) reduced the risk of progression by 4 % [34, 35].

The duration and dose of maintenance therapy was examined by an EORTC-GU Cancers Group study (EORTC 30962). Although there were no differences in toxicities between a third dose versus full dose of BCG, 1 year of a third dose of BCG was inferior to 3 years of a full-dose of BCG in terms of disease-free interval (although difference in disease free rates associated with dose or duration alone were not significant) [37••]. Importantly, the study when stratified by risk found that intermediate risk patients were best treated with 1 year of full-dose BCG; additional 2 years of maintenance did not improve clinical outcomes. On the other hand, high-risk patients had decreased risk of recurrence with 3 years compared with 1 year of full-dose BCG. In this study, there was no benefit in regards to progression and survival [37••].

Thus, it is now clear that for BCG to be effective, induction BCG must be followed by maintenance therapy. The optimal schedule is that initially proposed by SWOG. Based on risk-stratification data, it appears that all patients

require at least 1 year of maintenance, whereas higher risk patients benefit most from a full 3-year course.

Optimizing BCG

Who are good candidates for intravesical immunotherapy?

According to the AUA guidelines, it is a recommendation for patients with multifocal, large, or recurrent Ta tumors and patients with high-grade Ta, T1, or carcinoma in situ to undergo BCG induction therapy with maintenance therapy recommended in the latter group and an option in the former [21]. The EAU guidelines, updated in 2013, has a Grade A recommendation for 1-year of full-dose BCG treatment in intermediate risk non-muscle invasive patients (recurrent, multifocal, or >3 cm Ta, low-grade tumors without CIS) and 1- to 3-year full-dose BCG treatment in high-risk patients (presence of T1, high-grade, or CIS or multiple, recurrent, and >3 cm Ta high-grade tumors) [22••, 23]. The ICUD-EAU International Consultation on Bladder Cancer 2012 made grade A recommendations for BCG therapy in patients with Tis and Ta high-grade tumors and grade B recommendations for patients with recurrent low-grade Ta tumors that are nonresponsive to intravesical chemotherapy [23]. Finally, the International Bladder Cancer Group (IBCG) recommends BCG induction and maintenance therapy for both intermediate- and high-risk disease [38].

Who should not be recommended BCG intravesical therapy or offered upfront cystectomy instead?

For patients with low-risk disease (initial, solitary low-grade Ta tumors), BCG is generally regarded as overtreatment due to the low risk of recurrence and progression [23]. In patients with muscle-invasive disease, all guidelines are clear in their recommendations to proceed directly to cystectomy, often with neoadjuvant chemotherapy. In addition, according to the IBCG, cystectomy could be considered for patients with multiple T1 tumors, T1 tumors located in difficult to resect locations, residual T1 on re-resection, and T1 with concomitant CIS [38].

Optimal staging is important

Risk stratification for treatment of patients with non-muscle invasive disease is only relevant in the setting of appropriate and accurate staging. While BCG treatment may be overtreatment in some low-risk patients, understaged patients with missed muscle invasive component could miss out on their potentially curative window with delays in getting upfront treatment with intravesical BCG instead of surgical extirpation. As a result, the importance of a repeat transurethral resection (TUR) for select patients cannot be understated. Pathological reviews from cystectomy series for non-muscle invasive tumors have found understaging of up to 40 % in ≥pT2 disease [39, 40]. Upstaging of non-muscle invasive to muscle invasive tumors on repeat TUR have been reported to be 24-29 % [40–42].

High-risk pathologic features to consider

Re-TUR does not only identify patients with muscle-invasive disease, but also identifies patients with high-risk non-muscle invasive bladder cancer who benefit from cystectomy over conservative therapies. Numerous studies have

shown that the presence of residual T1 disease on re-resection can have important prognostic implications with up to a fourfold increase in the risk of progression to muscle invasive disease compared to absence of tumor on second TUR [41, 43, 44].

Lymphovascular invasion (LVI) is another important high-risk pathologic feature with its presence associated with high rates of progression and worse disease-specific survival [45, 46]. The depth of invasion within the lamina propria also has been identified as a risk factor. Quantifying depth of invasion in relation to muscularis mucosae, studies have found that deeper T1 invasive disease is associated with higher progression rates [47, 48]. Given the prognostic relevance of these factors, it is important to consider them when deciding on bladder preserving treatment modalities, including intravesical immunotherapy versus cystectomy. In addition to appropriate staging, histology of the bladder cancer can influence the selection of patients to undergo BCG treatment. Shapur et al. reported on a small series of variant non-muscle invasive tumor (including squamous differentiation, glandular differentiation, nested variant, and micropapillary) and found worse progression-free survival after BCG in the variant group [49]. More specifically, Kamat et al. found BCG to be largely ineffective in patients with micropapillary variant component of urothelial bladder cancer with 67 % of patients progressing to invasive disease shortly after BCG [50]. Due to the rarity of certain histologies, it remains unclear as to the relative responsiveness to and appropriateness of BCG therapy in variant tumors.

BCG dose

As previously stated, risk of recurrence and progression is lifelong in these patients, and induction therapy alone is not enough to produce prolonged immune stimulation necessary for optimal patient outcomes [30]. Dose and duration of BCG treatment are of paramount importance to optimize treatment success. In a recently published study, Oddens et al. analyzed full-dose BCG intravesical immunotherapy compared with one-third dose treatments given with a 1- to 3-year maintenance course in the EORTC 30962 study [37••]. One-third dose was suboptimal and the full dose for 3 years was most effective in reducing recurrences of high-risk disease, whereas for intermediate-risk disease, full dose for 1 year was most effective.

BCG strain

All BCG strains used for bladder cancer therapy are derived from *Mycobacterium bovis*, but because they have been serially passaged more than 200 times, they remain genetically unique with theories of differences in relative therapeutic efficacy of each [43]. In their in vitro study of bladder cancer cell lines treated with both evolutionary early strains (Japan, Moreau, and Russian) and evolutionary late strains (Connaught, Danish, Glaxo, Phipps, and Tice), Secanella-Fandos et al. found Russian and Connaught to be most efficacious to inhibit cell proliferation and induce cytokine production [51]. A recent, small, prospective, randomized trial comparing different strains of BCG also found Connaught to be more efficacious with a significantly greater 5-year recurrence-free survival rate compared with Tice but only at reducing recurrences, not progression of disease. Mouse studies in the same paper demonstrated that Connaught induced a stronger Th1 response and T-cell recruitment than Tice

[52•]. Because response to BCG is immunologically mediated, which is likely to vary between populations exposed to various antigenic stimuli prior to region specific strain use, further studies will be needed that account for all these variables before definitive conclusions can be made.

Administration

There are key components to the administration of BCG that can help to optimize its delivery and safety. In order to prevent dilution of the instilled therapy, patients should void immediately preceding treatment. Urine should be examined for the presence of gross hematuria and infection, because their presence should prompt a delay in treatment until resolution. Any residual volume should be drained before catheterized instillation of BCG. Because the treatment course may last up to 1.5-2 hours, minimizing fluid intake before treatment is important to prevent urinary dilution. Although a common practice involves having the patient turn 90° every 15 minutes to facilitate BCG adherence to the entire bladder urothelial surface, the conformational properties of the bladder makes this practice unnecessary [41, 43, 44].

Adverse effects

In the EORTC-GU cancer group study on 1- to 3-year maintenance BCG, only 7 % of patients discontinued treatment due to toxicity [37••]. Nonetheless, adverse effects of BCG are not uncommon and need to be addressed appropriately. The most common reactions—irritative symptoms and low-grade fevers—often can simply be addressed with anticholinergic/antispasmodic medications and antipyretics. Patients suffering from these symptoms following BCG should not only be treated after therapy, but prophylactically prior to subsequent instillations. More serious side effects, including prolonged fever and BCG sepsis, must be quickly managed with a combination of supportive measures, symptomatic control, and, occasionally, antimycobacterial therapy. As previously mentioned, it is imperative to prevent systemic infections by screening urine before the initiation of BCG treatment.

Patient considerations

Immunocompromised

Preclinical studies have shown that immunological competency is important for effective BCG therapy. In addition, given the mycobacterial origins of BCG, many practitioners are wary of exposing BCG to immunosuppressed patients who may have a lower threshold of succumbing to systemic infections. However, albeit in a limited number of retrospective studies, the efficacy of BCG in patients taking steroids, other immunosuppressive agents, and hematological malignancies, has not been significantly different than in their immunocompetent counterparts [53, 54]. In the same series, in a total of 69 patients, only 1 patient had self-limited fevers and myalgias and no patients suffered from BCG sepsis [53, 54].

Patients with human immunodeficiency virus (HIV) make up a unique population of immunocompromised patients, although highly active antiretroviral therapy (HAART) has largely controlled the disease where the vast majority of patients today maintain their cell-mediated immunity. The safety

and efficacy of BCG use in these patients has not been extensively reported. Although there have been case reports of the development of BCG-related bilateral interstitial pneumonitis and of empyema in HIV patients treated with intravesical BCG, others have reported no significant adverse sequelae [55, 56]. Further studies will be needed before setting guidelines for BCG use in these patients both from a safety and efficacy perspective.

Immune systems are inherently weakened in elderly patients [57]. Studies by Herr et al. and Joudi et al. suggested that patients in their 70s and 80s had decreased benefit from BCG therapy compared with younger patients, although the difference was modest [58, 59]. A recent EORTC paper demonstrated that while BCG is less efficacious in patients older than age 70 years, it is nonetheless still more effective than intravesical chemotherapy (epirubicin) [60].

Prior BCG exposure

The recent interest in BCG priming before intravesical therapy has encouraged studies looking into the immunologic and therapeutic consequences of prior BCG exposure. As previously discussed, the efficacy of BCG relies on repeated exposures to the bacteria (multiple cycles, multiple maintenance courses) and time to develop a cell-mediated immune response. In mouse studies, Biot et al. found that bladder T-cell infiltration levels after one intravesical exposure of BCG with prior subcutaneous injection of BCG ("priming") were similar to levels after multiple intravesical exposures to BCG without priming. Remarkably, they also demonstrated that prior parenteral exposure to BCG resulted in improved antitumor response of orthotopic bladder tumors to intravesical BCG [61]. As a clinical correlate, patients with positive purified protein derivative (PPD) test, indicative of a systemic immune response to BCG, before treatment had improved recurrence-free survival rates than PPD negative patients [61]. Despite these encouraging findings, prospective, randomized trials have not shown improved outcomes with the coadministration of intradermal and intravesical BCG compared with intravesical therapy alone [62, 63]. Due to the latency of developing a durable cell-mediated response after BCG exposure, a more biologically sound approach to future clinical investigations would involve "priming" patients with BCG before—and not concurrently with—initial intravesical treatment. Such studies would provide meaningful results that could dramatically alter the approach to immunotherapy in bladder cancer.

Prior radiation exposure

Given the high prevalence of prostate cancer and its treatment with primary radiotherapy, a substantial number of patients with bladder cancer are treated in the setting of a previously irradiated field. In their study of BCG treatment of CIS after previous pelvic radiotherapy, Pisters et al. postulated that prior bladder exposure to radiation may affect BCG efficacy [64]. In a study that specifically looked at BCG treatment of non-muscle invasive tumors in patients previously treated with radiotherapy for prostate cancer, a durable response to BCG was

limited to half the patients with a longer time interval from radiotherapy to BCG treatment associated with higher BCG failure rates [65].

Who fails?

Identifying prognostic factors to predict BCG response and failure is helpful in the counseling of and treatment strategies for patients. While female gender, recurrent tumors, multifocality, and presence of CIS have been found to be independently associated with risk of recurrence after BCG therapy, recurrent tumors, high-grade (vs. low-grade), and T1 (vs. Ta), and early time to recurrence correlated with risk of progression [66].

Unfortunately, clinicopathologic findings alone cannot appropriately stratify patients to receive or forego BCG therapy based on predicted response. Various molecular markers have been evaluated for their predictive capacity. Although urinary interleukins and TRAIL, tumor immunohistochemical staining for TP53, Ki-67, and retinoblastoma, and genetic polymorphisms have given contradictory findings, increased urinary IL-2 levels have been consistently shown to be associated with improved recurrence-free survival rates [67•, 68, 69]. Kamat et al. studied FISH in the setting of BCG therapy and found that patients with a positive result during treatment were up to five times more likely to have disease progression [70]. Ongoing studies differentiating responsive and recurrent tumors to BCG at the genetic, protein, and cytokine level will hopefully provide further insight into the selection of patients for BCG.

Nonimmunologic salvage treatment after BCG

For patients who are surgical candidates, radical cystectomy achieves the best oncological control of disease with prolonged survival in greater than 90 % of patients [71]. Both the AUA and EAU recommend radical cystectomy as the preferred option for BCG failure with limited evidence supporting the next best, bladder-preserving therapies. An option for patients unable or unwilling to under radical cystectomy is intravesical chemotherapy. The most commonly studied agents in this setting are intravesical gemcitabine and docetaxel. While gemcitabine has been shown to lead to progression rates of 3.5-33 % and disease-free rates of 39-60 %, patients receiving intravesical docetaxel after BCG failure have demonstrated progression rates of 5.5 % and disease-free rates of 22-61 % [71]. The subsequent discussion will examine the other immunotherapies studied in non-muscle invasive bladder cancer.

Other immunotherapies for NMIBC

Interferon±BCG

Interferon (IFN) is an important mediator of BCG/Th1 response. Hence, it has been tried alone and in combination with BCG. When used alone IFN has not been shown to be very efficacious; however, when used in combination with BCG there has been some efficacy demonstrated. However, IFN is best suited for those patients who are not able to

tolerate full dose of BCG (i.e., augmented Th1 response) or those patients who do not respond as well as expected, such as patients with recurrent tumors, multifocality, presence of CIS, and early time to recurrence [66]. In these patients, the thought is that augmenting the response will allow these patients to be salvaged. However, salvage therapy with IFN and BCG has not been shown to be effective in patients who are considered high-risk failures (i.e., failed 2 courses or more, early recurrence, etc.). Moreover, IFN and BCG has not been proven beneficial in elderly patients as Joudi et al. showed only a 22 % reduction in disease-free survival in patients older than age 80 years treated with IFN and BCG [59].

Current treatment protocols may include a repeat course of BCG with or without additional intravesical immunotherapy, such as interferon-alpha (IFN-α) or radical cystectomy [72]. IFN potentiates T-helper type 1 (TH1) immune responses in human leukocytes when combined with BCG [73]. Furthermore, IFN has been proposed as an adjunct to BCG intravesical therapy, often with a BCG dose reduction in an attempt to reduce side effects and improve efficacy [74]. Unfortunately, this combination of BCG and IFN has shown modest results at best in patients in whom BCG has failed [75, 76].

BCG plus IFN for treating superficial bladder cancer in both BCG failure (BCG-F) as well as BCG naïve (BCG-N) has been previously studied [74]. The results from this study demonstrated a 59 % and 45 % recurrence-free rate for BCG-N and BCG-F patients, respectively. The effect of the interval to relapse after BCG on the subsequent response to intravesical BCG plus IFN also has been evaluated. Patients with failure after being in remission for 12 months had a response similar to that of BCG-N patients [77]. Moreover, patients with disease recurrence more than 1 year after BCG treatment and who were treated with low-dose BCG plus IFN had response rates similar to those of BCG-N patients treated with regular-dose BCG plus interferon [77].

Bropirimine

Bropirimine is an oral interferon inducer that has substantial immunomodulatory activity [78]. Regarding clinical efficacy in urothelial carcinoma, response rates in a single-agent phase II trial have been noted in approximately 50 % of patients with up to 18 % of patients who have had prior failed BCG therapy having complete response to oral bropirimine [79]. Moreover, prior studies also have demonstrated the clinical efficacy of bropirimine when given in combination with BCG. Early laboratory studies of bropirimine showed apparent synergy between BCG and bropirimine in cellular response to stimulation as well as in vivo [80]. Based on these and the early promising clinical results of bropirimine's single-agent efficacy, Sarosdy et al. performed a Phase II clinical trial to evaluate the combination of oral bropirimine with intravesical BCG in patients with CIS of the bladder. The 5-year, progression-free survival estimate was 53 % with minimal toxicity [80]. The authors concluded that bropirimine failed to show an increase in the efficacy of intravesical BCG for controlling CIS of the bladder.

Despite its single-agent activity and the earlier laboratory evidence that bropirimine enhanced the activity of BCG, this study showed minimal enhancement of clinical BCG activity in CIS.

Keyhole limpet hemocyanin

Keyhole limpet hemocyanin (KLH) is a high-molecular-weight glycoprotein, purified from a marine snail species called *Megathura crenulata*, which induces both cell-mediated and humoral responses in animals and humans [81]. The precise mechanism of action by which KLH induces these responses remains unknown. Due to KLH's high immunogenicity and low toxicity, KLH has been used in the local treatment for patients with bladder cancer [82]. KLH has entered into clinical trials as either adjuvant or immunomonitoring tool in a variety of vaccines directed against cancer [83, 84].

The method regarding administering KLH is unique and not necessarily standardized across studies regarding preimmunization and intravesical instillations. Patients often are started with preimmunization intracutaneous (IC) injection of 1-mg KLH at intervals of 2 to 7 days until a delayed-type hypersensitivity (DTH) response is obtained [85]. Intravesical treatment is started within 2 weeks after the TURBT, independent of the preimmunization result [85]. Patients then receive a total of 16 intravesical instillations (20 mg KLH in 20 mL solvent) given once per week for 6 weeks and then once per month for 10 months (months 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12) [85].

The safety and efficacy of KLH have been compared with that of mitomycin C (MMC) as well as BCG with conflicting results. In one study, KLH had similar adverse events to MMC, however, was inferior to MMC in preventing recurrence (61 % vs. 34 %, $p<0.001$) [85]. Literature reporting on KLH and recurrence is diverse and limited by the number of patients enrolled in the studies. Jurincic et al. treated 44 patients with either KLH or MMC and found significantly fewer recurrences after KLH treatment than after MMC treatment [86]. Echarti et al. found a significantly longer recurrence-free survival in patients after KLH treatment compared with several other intravesical treatments [87]. However, treatment schedule and KLH dosage varies across studies and these results warrant further validation. Furthermore, the manner of application (intravesical, intralesional, or systemic) remains uncertain.

Interestingly, the topic of preventing progression using KLH remains uncertain [85]. Jurincic et al. found progression in three patients treated with MMC versus in one patient treated with KLH [86]. No progression was found after KLH treatment in patients with CIS, but mean follow-up was only 23.5 months [86]. Comparing KLH versus BCG, Kalble et al. found an increase in grade or stage in two patients (11.8 %) after KLH treatment versus no progression after BCG [88]. Further comparative effectiveness research is needed comparing KLH to other immune therapies for bladder cancer.

Future directions/advances in immunotherapies for NMIBC

MCC (Urocidin)

There has been interest in the intravesical instillation of mycobacterial DNA cell wall complex (MCC, Urocidin). Nonviable preparations of mycobacteria

(*M. phlei*) have been investigated for the treatment of superficial bladder cancer in limited, open-label, clinical trials. A proposed mechanism for MCC in the setting of bladder cancer include a dual immunomodulatory and apoptotic mechanism of action [89]. MCC has shown activity against a variety of tumor cells; however, limited clinical trials have demonstrated it to be active in patients who have previously failed one or more courses of chemotherapy and/or immunotherapy with BCG [89]. Morales et al. assessed the clinical efficacy and safety of MCC after intravesical administration in patients with CIS in whom prior therapy with BCG failed or in those who were treatment naïve [90]. Patients received 6 weekly instillations of 4 or 8 mg MCC followed by 3 weekly instillations at weeks 12 and 24. Most patients were previously treated with BCG. In the intent to treat population the complete response rate was 27.3 % in the 4-mg group, whereas 46.4 % of patients receiving 8 mg had a complete response with minimal side effects. More recently, in an open label, single-arm, multi-institutional clinical, Morales et al. investigated the efficacy and safety of MCC in patients with BCG-refractory disease. Patients were treated with a 6-week induction course of 8 mg of MCC followed by maintenance therapy consisting of 3 weekly instillation at 3, 6, 12, 18, and 24 months. Interim results demonstrated a 1-year, disease-free survival rate of 25 % with 35 % in patients with papillary tumors and 21 % in with CIS Therapy was well-tolerated with only 2 % of patients discontinuing treatment due to side effects [91]. The tolerance and efficacy of MCC might hold promise for the treatment of CIS; however, the true benefit will only be determined once these clinical trials mature.

Vaccines

Tumor-associated antigens have been used in various immunotherapeutic protocols, including peptide- or protein-based vaccines, cell-based vaccines, and virus-vector vaccines [92–95]. Recent progress in the treatment of malignant tumors has significantly contributed to improvements in the prognosis of cancer patients. Vaccination with tumor-associated antigens (New York esophageal squamous cell carcinoma 1 (NY-ESO)-1, LAGE-1, human epidermal growth factor receptor type 2 (Her2) and mucin 1, cell surface associated (MUC-1)) is now considered to be one of the most useful and potent immunotherapies for several types of cancers [93–95]. However, prior studies have demonstrated low frequencies of expression of these tumor antigens in bladder cancer [95–97].

Sharma et al. provided the first vaccine study in urothelial carcinoma patients, which demonstrated NY-ESO-1–specific antibody and/or T-cell responses in all vaccinated patients [98]. NY-ESO-1 protein in combination with GM-CSF and BCG was sufficient to induce antigen-specific antibody responses in five of six patients, CD4+ T-cell responses in six of six patients, and CD8+ T-cell responses in one of six patients. The oncologic efficacy of NY-ESO-1 and other potential targets in bladder cancer patients, such as MAGE-A4 and NY-ESO-1, are still in question with further studies needed to validate these preliminary findings. Further studies aimed at exploring cytokine profiles of vaccine-induced T cells, T-cell subsets, including effector and central memory T cells, and combination immunotherapeutic strategies to induce integrated

immune responses consisting of antibody, CD4+, and CD8+ T-cell responses are warranted.

Gene Therapy

The genome wide association study identified several loci associated with bladder cancer risk [99]. Varying studies have identified variant genotypes associated with survival in patients with non-muscle to muscle invasive and metastatic bladder cancer [100–103]. To date, gene therapy has been suggested as a therapeutic approach but gene expression and efficient delivery remain a significant obstacle [104, 105]. Adenoviral gene expression has been explored as a vehicle for targeted gene therapy [105]. In vitro studies have shown promising results with use of cationic polymers to enhance delivery [104]. While preliminary, further identification of variant genotypes combined with targeted delivery to susceptible genes will be at the forefront of bladder cancer therapy.

Immune check point inhibitors (CTLA-4, PD-1, PD-L1)

Combination strategies with vaccines and other immunomodulatory agents represent future treatment paradigms. The concept of immune checkpoint blockade to overcome mechanisms restricting immune responses has shown promise in clinical trials using the prototype drug known as anti-CTLA-4 antibody. The novel immunotherapeutic agent anti-CTLA-4 antibody allows for expansion of the repertoire of T cells, which may potentially react against multiple antigens [98, 106].

Our group described the first preoperative clinical trial with the anti–CTLA-4 antibody ipilimumab in patients with localized urothelial carcinoma of the bladder [107]. Six patients were treated with 3 mg/kg/dose of anti–CTLA-4 and six patients were treated with 10 mg/kg/dose of antibody. Primary end points of the study were safety and immune monitoring. With minimal toxicity, all patients had measurable immunologic pharmacodynamic effects consisting of an increased frequency of $CD4^{+}ICOS^{hi}$ T cells in tumor tissues and the systemic circulation. This trial showed that anti–CTLA-4 therapy has a tolerable safety profile in the presurgical setting and that a preoperative model can be used to obtain biological data on human immune responses, which can efficiently guide the monitoring of patients treated in the metastatic disease setting.

PD-1 and PD-L1 also have been shown to be important in inducing immunologic escape of cancer cells by inhibiting the antitumor immune response. PD-L1 is a coregulatory ligand that can inhibit immune responses by either binding to PD-1 or a putative non-PD-1 receptor on the surface of T lymphocytes to induce antigen-specific T-cell apoptosis or anergy [108]. Several studies have shown that targeting this molecule has efficacy in melanoma [109]. In urothelial carcinoma, it has been suggested that a large percentage of patients who failed BCG therapy show high PDL-1 (B7-H1) expression within the BCG granulomas adjacent recurrent tumors [108]. Thus these data suggest that the accumulation of PDL-1 expressing cells might be causative in the inhibition of

the appropriate Th1 response and cause an abrogation of the response to BCG.

Given the above interest in identifying therapies with the potential to enhance BCG response either in combination or as a single-agent therapy for those that have previously failed, there is renewed interest in checkpoint inhibitors, which have changed the landscape of immunology. The next logical next steps would involve studying whether such agents could augment the response to BCG and identifying a subset of patients more likely to respond to single versus multimodality therapies.

TRAIL

Urinary TRAIL levels have been shown to be associated with clinical efficacy with higher levels in BCG-responders versus nonresponders [110]. However, a number of bladder cancers are resistant to TRAIL. This has spurred efforts to both discover agents to sensitize cancer cells to TRAIL-induced apoptosis and identify alternative pathways of action [111]. For example, IFN-α stimulation of PMNs has been shown to increase the secretion of TRAIL after BCG exposure and also to have an independent effect on apoptosis of bladder cancer cells [112, 113]. In addition to TRAIL, tumor necrosis factor-α (TNF-α) was identified as a neutrophil-released primary mediator responsible for anticancer activity. Preclinical studies demonstrated that Smac mimetic compounds, which sensitize cancer cells to apoptosis, could potentiate the BCG-stimulated neutrophil-mediated therapeutic effect, especially in otherwise BCG-resistant cell lines [114]. Future studies identifying predictors for success to BCG treatment are warranted, which will identify patients most likely to respond.

Recombinant BCG

Based on recent increases in understanding of BCG-induced immune responses, a variety of preclinical studies have been conducted to overcome the limitations of BCG therapy [115]. One approach is the generation of Th1 cytokine-expressing recombinant forms of BCG (rBCG) or the use of non-live bacterial agents, such as killed mycobacterium or mycobacterium extracts [115]. In animal models, some cytokine-expressing rBCGs showed promising results against malignant melanoma and breast cancer; however, studies using intravesical models of bladder cancer are still limited. Given the increased understanding of immune checkpoint inhibitors a combination of BCG and CTLA4/PD-1/PDL-1 blockade with rBCG strains may be one avenue to provide a new therapeutic option, especially in those who fail BCG therapy or are resistant to therapy in the first place.

Conclusions

The treatment of NMIBC remains a complex disease for which immunotherapy forms the cornerstone of treatment strategies. Improvement in our understanding of the molecular underpinnings of bladder carcinogenesis and recognition of the clear advantage of intravesical BCG immunotherapy have resulted

in improved survival and bladder preservation for patients with NMIBC. With the rapid progress in the field of immunotherapy in bladder cancer, ongoing studies to identify predictors for treatment success and development of targeted therapies are key immediate future steps.

Compliance with Ethics Guidelines

Conflict of Interest

Philip L. Ho, Stephen B. Williams declare that they have no conflict of interest. Ashish M. Kamat received research funding and serves as consultant to Sanofi, Merck, Bioniche, FKD Therapeutics.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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