Review Article

Intravesical immunotherapy in nonmuscle invasive bladder cancer

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ABSTRACT

Introduction: Nonmuscle invasive urothelial cell carcinoma is the most frequent malignancy of the urinary bladder. The high recurrence rate (up to 80%) and risk of progression (up to 30%) reflect the need for long-term follow-up and sometimes multiple interventions. To reduce the rate of recurrences and tumor progression, intravesical immunotherapy, especially the use of Bacille Calmette-Guerin (BCG), represents the gold standard adjuvant treatment of high-risk nonmuscle invasive bladder cancer (NMIBC). This article reviews the role of BCG therapy and several promising new immunotherapeutic approaches such as mycobacterium phlei cell wall-nucleic acid complex, interleukin-10 (IL-10) antibody, vaccine-based therapy, alpha-emitter therapy, and photodynamic therapy checkpoint inhibitors.

Methods: A systematic literature review was performed using the terms (immunotherapy, NMIBC, BCG, and intravesical) using PubMed and Cochrane databases.

Results: BCG represents the most common intravesical immunotherapeutic agent for the adjuvant treatment of high-risk NMIBC. Its use is associated with a significant reduction of recurrence and progression. Patients with NMIBC of intermediate and high-risk benefit the most from BCG therapy. To achieve maximal efficacy, an induction therapy followed by a maintenance schedule should be used. Full-dose BCG is recommended to obtain ideal antitumoral activity and there is no evidence of a reduction of side effects in patients treated with a reduced dose. There are multiple new approaches and agents in immunotherapy with potential and promising antineoplastic effects.

Conclusions: The beneficial effect of BCG is well documented and established. To reduce the tumor specific mortality, it is essential to follow guideline-based treatment. In patients with BCG-failure, there are new promising alternatives other than BCG but BCG remains the gold standard at this stage.

Key words: Bacillus Calmette-Guerin, bladder cancer, immunotherapy

INTRODUCTION

Bladder cancer (BC) is the most common tumor of the urinary tract. For men, it is the fourth most common malignancy and accounts for 7% of all incident malignancies worldwide.[1] The prolonged nature of BC is similar to many chronic diseases and requires invasive and careful long-term surveillance and, in certain cases, adjuvant treatment. This results in high costs per patient ($96,000–$187,000) making BC one of the most expensive malignancies to treat.[2]

Urothelial carcinoma (UCC) represents the most common malignancy of the bladder in the Western world (90%). About 75% of patients suffering from initially diagnosed BC show a disease limited to the mucosa (pTa or carcinoma *in situ* [CIS]) or submucosa (pT1) and is therefore classified as nonmuscle invasive tumor (nonmuscle invasive BC [NMIBC]).

NMIBCs has progression to muscle-invasion in up to 30% patients. The WHO-classification into two groups

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Access this article online	
Quick Response Code:	Website: www.indianjurol.com
	DOI: 10.4103/0970-1591.166452

How to cite this article: Jokisch JF, Karl A, Stief C. Intravesical immunotherapy in nonmuscle invasive bladder cancer. Indian J Urol 2015;31:304-11.

(high- and low-grade UCC) may be associated with genetic instability as an indicator for the potential to progress. The risk group classification is based on multiple prognostic factors (European Organization of Research and Treatment of Cancer [EORTC] risk tables) and subclassifies patients into low, intermediate, and high-risk groups [Table 1].[3] Transurethral resection of the bladder tumor (TURBT) is the standard for treatment and diagnosis of BC. The aim of TURBT is to ideally remove all visible lesions within the bladder and to provide tissue for a precise histopathologic evaluation.[3] Despite complete removal, NMIBC shows a high rate of recurrence 30–85% within 2 years after initial diagnosis and stage progression in up to 30% after 5 years.[3]

Adjuvant therapies aim to reduce recurrence rates and ideally prevent progression. Based on the individual risk-stratification of a patient, intravesical chemotherapy or immunotherapy is recommended by different international guidelines (American Urological Association [AUA] and European association of urology [EAU]) [Tables 2 and 3].[3,4] Adjuvant therapies are a complex subject as evidenced by a large number of publications (over 1605 publications in PubMed [06/2015]). Despite recommendations of international guidelines, Chamie *et al*. suggested that only 1 of 4545 patients receives a strictly guideline-conforming diagnosis and treatment in the US.[5] A major variation from guidelines-conforming management occurred in the use of adjuvant Bacillus Calmette-Guerin (BCG) instillation therapy.

In order to give our patients with NMIBC the best possible treatment, it is essential to guarantee evidence-based medicine in order to minimize morbidity and possibly improve survival. This article reviews the contemporary data on intravesical immunotherapy in UCC.

METHODS

This review on intravesical immunotherapy of NMIBC is based on a selected electronic search on databases such as PubMed and Cochrane using following keywords: Immunotherapy, NMIBC, BCG, and intravesical. For the review on the topic, contemporary guidelines on the treatment of NMIBC were used (EAU and AUA).

IMMUNOTHERAPY WITH BACILLUS CALMETTE-GUERIN

In 2016, the use of BCG in patients with UCC completes 40 years (1976).[6] Today BCG-immunotherapy has proven to reduce both recurrence and progression of NMIBC and, therefore, represents an important tool in the treatment of NMIBC.[7]

Mechanism of Bacillus Calmette-Guerin

The immunomodulating effect of BCG-instillation therapy is well studied.[8] The antitumoral activity of BCG is explained by a complex interaction between a direct antineoplastic effect on malignant cells and the host's humoral immune system response provoked by local infection.[9] The effect of BCG can be summarized into three essential steps: (1) Infection of urothelium, (2) induction of an immune response, and (3) the induction of an anti-neoplastic effect. The effect is promoted not only by a direct anti-neoplastic effect through local infection of malignant lesions of the bladder but also by the activation of the host's immune response.[9]

Table 1: Risk group stratification*

Risk group stratification	Characteristics
Low-risk tumors	Primary, solitary, Ta G1 (LG), <3 cm, no CIS
Intermediate risk tumors	All tumors not defined in two adjacent categories (between the category of low- and high-risk)
High-risk tumors	Any of the following:
	T1 tumors
	G3 (HG) tumor
	CIS
	Multiple and recurrent and large (>3 cm) Ta G1/G2 tumors (all conditions must be presented in this point)
Subgroup of highest risk	Any of the following:
	T1G3 associated with concurrent CIS
	Multiple and/or large T1G3 and/or recurrent T1G3
	T1G3 with CIS in prostatic urethra, unusual histology of UCC
	BCG failure

*Table 1 was adopted from EAU-guidelines on nonmuscle-invasive bladder cancer.[3] LG=Low-grade, HG=High-grade, CIS=Carcinoma *in situ*, EAU=European association of urology, UCC=Urothelial cell carcinoma

Table 2: Instillation regimes for intermediate-risk tumors

Intermediate-risk tumors	Instillation-regime
Primary or recurrent without previous chemotherapy	Full dose BCG for 1 year
	Induction: Weekly for 6 weeks
	Maintenance: 3 weekly at month 3, 6 and 12
	Intravesical chemotherapy: Maximum of 12 month
Recurrent tumor with previous chemotherapy	Full dose BCG for 1 year
	Induction: Weekly for 6 weeks
	Maintenance: 3 weekly at month 3, 6 and 12
	For late recurrence of small pTaG1 consider to repeat of intravesical chemotherapy

*Table 1 adopted from EAU-guidelines on nonmuscle-invasive bladder cancer.[3] BCG=Bacille Calmette-Guerin, EAU=European association of urology

Table 3: High-risk tumors and its treatment

High-risk tumors	Treatment recommendation
High-risk tumors	Intravesical full-dose BCG-instillation for 1-3 years
Subpopulation of highest-risk tumors	RC is to consider, patients who refuse RC can receive 1-3 years of full-dose BCG-instillation

RC=Radical cystectomy, BCG=Bacille Calmette-Guerin

After intravesical instillation, there is internalization of BCG into neoplastic cells, which is promoted by the attachment to extracellular proteins such as fibronectin. This first reaction reflects an infection of urothelium. Inhibiting anti-fibronectin antibodies in murine models result in a lack of antineoplastic activity of BCG and therefore the process of internalization appears to be essential for the effect of BCG.[10] Furthermore, internalization of BCG leads to an increased expression of antigen presenting cells and an enhancement of major histocompatibility complexes class I on the surface of neoplastic bladder lesions. The idea of infected bladder tissue leading to an effective immune response is supported by murine models of UCC presenting antigen to BCG-specific CD4+T-lymphocytes after intravesical instillation.[11] Infection of the urothelial tissue activates a regional immune response and usually induces an immune response promoted by both TH1-cytokines (interleukin-2 [IL-2], IL-12, tumor necrosis factor and interferon-gamma [IFN-γ]) and TH2-cytokines (IL-4, IL-5, IL-6 and IL-10). This cascade of cytokines promotes an antineoplastic activity mediated by cytotoxic T-cells, natural killer cells, macrophages, and neutrophils. Therefore, there is evidence for both TH1-response (cell-mediated acquired immune response) and TH2-response (humoral immune response) in providing antineoplastic activity.[9]

Bacillus Calmette-Guerin strains

Although the EAU-guidelines on NMIBC do not consider different BCG strains to have differences in their efficacy, literature suggests that such differences may exist.[3] Rentsch *et al*. investigated most common BCG strains (Connaught and Tice) in high-risk NMIBC patients and murine models. The Connaught-strain showed a significantly higher 5-year recurrence free rate of 74% versus 48% in patients treated with the Tice strain (P = 0.0108). In the murine sample, they also presented a stronger TH1-immunresponse, which eventually could lead to a clinical benefit.[12,13] However, further clinical trials are necessary to evaluate a potential clinical impact.

Adjuvant immunotherapy with Bacillus Calmette-Guerin

The superior efficacy of BCG in the therapy of NMIBC in comparison with TURBT alone and TURBT with adjuvant chemotherapy (mitomycin C [MMC]) has been demonstrated in large studies. The 2015 EAU guidelines refer to at least 5 meta-analyses to demonstrate BCG's superiority.[3] In comparison to other agents used for instillation therapy (MMC, epirubicin, and IFN), BCG showed the best effectivity in respect to preventing recurrences.[14-16] A single BCG induction course demonstrated decreased recurrence and prevention of tumor progression.[17,18]

Besides its well-documented ability of preventing recurrence, there is evidence for reduction of progression by BCG immunotherapy. A meta-analysis showed a reduction of 27% in the progression rate of patients following any maintenance schedule of BCG after TURBT.[19] There is data that maintenance of 3 years compared to 1 year shows a prolonged recurrence-free interval but a difference in progression could not be shown.[20] Böhle and Bock proposed in their meta-analysis that maintenance of at least 1 year is needed to provide the advantages of BCG compared to MMC.[19]

In patients with CIS, BCG instillation therapy results in significantly lower rate of recurrence. A study of patients with CIS undergoing 6-weekly BCG-courses (induction-therapy) after previous TURBT showed a complete response (CR) in 71%.[21] The rate of CR was increased to 84% by further maintenance instillations in addition to BCG induction. More than 70% of the BCG-responders remained disease free for more than 5 years.[22]

A more individualized approach was presented in 2011 in a trial including high-risk patients, undergoing a common induction course (6 weeks).[23] Patients who appear to respond after the first induction therapy did not get further maintenance therapy. Maintenance therapy or re-treatment was used in the event of relapse. The results showed a higher rate of recurrence but similar progression rates as outlined in previous studies. Although 32% of patients required further BCG instillations, the trial showed that approximately 7 of 10 patients who would regularly be treated with BCG did not actually need a BCG maintenance approach.[23] Concerning the potential severe side effects of BCG, its limited availability and the health economical burden of BCG, reduction of potential BCG-overtreatment makes this approach appealing. The major problem of this approach, however, is an obviously higher recurrence rate compared to a maintenance schedule.

Although there is data suggesting that only those patients who receive BCG maintenance benefit, it remains unclear what an ideal maintenance therapy is. Both frequency of instillation and duration of therapy are not uniformly defined in the literature. The different maintenance regimens are presented in the EAU-guidelines but there is no consensus on an optimal schedule.[7,24] In order to reduce recurrence rate of high-risk patients, the EORTC proposed a 3 year maintenance regimen rather than 1 year.[20,25] These guidelines recommend full dose BCG instillation for 1–3 years in patients presenting with high-risk tumors based on the individual risk of the patient, co-morbidities, side effects, costs, and availability of BCG.[3]

Bacillus Calmette-Guerin-dose

Considering the adverse effects of BCG there have been attempts at reducing the dose in order to achieve optimal balance between efficacy and reduction of adverse events (AE). There are several trials focusing on dose reduction (i.e., one-third) and its potential correlation of a reduction of side effects.[20,26] Although a dose reduction of one-third of the

standard dose has been shown to be adequate and efficient in intermediate-risk NMIBC patients, in the high-risk patient dose reduction was associated with a higher recurrence rate. Moreover, although a dose reduction appears to provoke less local side effects, there is no reduction in severe systemic toxicity and serious AEs with dose reduction.[27] In conclusion, most of the published data suggests that a reduction of the standard dose of BCG has little beneficial impact on serious side effects but is associated with a reduced efficiency in high-risk patients.

Bacillus Calmette-Guerin toxicity

Practical experience shows that BCG-instillation is generally well tolerated by patients but serious systemic AEs and even mortality has been reported.[3] Although most of the side effects can be treated effectively, the indication of immunotherapy with BCG has to be justified and, therefore, an individual risk evaluation should be performed.[28] There is no evidence that maintenance regimens are associated with an increased risk of side effects compared to induction therapy. Most side effects that need treatment are seen in the 1st year of BCG therapy.[29] There is no convincing evidence that local side effects correlate with efficiency of immunotherapy in terms of a better outcome.[30] Severe side effects can occur after systemic absorption. Therefore, the EAU describes visible hematuria, symptomatic urinary tract infection, previous traumatic catherisation, and status post-TURBT within 2 weeks as an absolute contraindication for BCG instillation.[3] Although EAU guidelines do not recommend the use of prophylactic antibiotics with intravesical BCG therapy, there is data suggesting an 18.5% decrease in BCG side effects with concurrent use of ofloxacin.[31] Therefore, prophylactic antibiotic use should be considered in order to reduce side effects and improve patient compliance.

Combination therapy of Bacillus Calmette-Guerin and chemotherapy

The combination of BCG with other agents such as chemotherapy may improve its efficacy. A murine model study assessing the intravesical combination of BCG and Gemcitabine suggests a decreased extent of tumor appearance rate, improved survival, and a reduction of neoplastic proliferation compared to BCG instillation alone.[32] However, a meta-analysis focusing on BCG in combination with intravesical chemotherapy detected no significant beneficial effect overall.[33] And a study assessing the combination of BCG instillation and MMC showed no superiority to BCG alone.[34]

INTRAVESICAL IMMUNOTHERAPY OTHER THAN BACILLUS CALMETTE-GUERIN

Radical cystectomy (RC) is indicated for patients with high-risk NMIBC who experience failure of intravesical immunotherapy using BCG.[3] For patients with contraindication for major surgery, there are only a few promising local intravesical immunotherapy regimens.

Interferon-alpha

IFN-α is a well known immunomodulating cytokine with antiproliferative potential with evidence of significant NMIBC tumor response through instillation-therapy. IFN-α shows a moderate response in patients with NMIBC. However, its efficacy in preventing recurrence in intermediate and high-risk NMIBC is very limited. Its utilization as a single immediate-instillation agent after TURBT could not demonstrate a reduction of recurrence risk for low-risk NMIBC.[35] In a randomized controlled trial that compared TURBT alone versus adjuvant IFN-α, the IFN-α arm showed a reduction of risk in intermediate risk patients.[36] Nevertheless in comparison with a control group of MMC over 12 months the clinical response rates were significantly lower for intermediate-risk patients.[37] In addition, IFN-α was inferior to BCG-induction in high-risk NMIBC (recurrent T1 patients).[38] Although IFN-α monotherapy shows beneficial antineoplastic responses, IFN-α-monotherapy is clearly inferior to standard installation therapies in preventing recurrence of BC.[39] IFN-α-combination with BCG in BCG-naive patients showed no benefit in recurrence or progression but is suspected to increase the risk of AE of therapy. However, dual treatment in BCG-naive high-risk patients who cannot tolerate full-dose BCG-monotherapy could help to reduce the BCG-dose and its AE.[39] Therefore, further prospective comparative studies for dual-therapy and dose reduction are needed. Contemporary IFN-α is not regarded as an efficient alternative to MMC or BCG therapy.

Mycobacterium phlei cell wall-nucleic acid complex

Mycobacterium phlei cell wall-nucleic acid complex (MCNA) (formerly Urocidin™) is an immunomodulatory and antineoplastic substance containing mycobacterial cell wall components complexed with biologically active nucleic acids derivated from the mycobacterium phlei.[40] MCNA, like BCG, shows an indirect immunomodulatory effect that provokes antineoplastic cytokin-production through immune effector cells without pathogenic potential. In addition, there is a direct chemotherapeutic effect comparable with other cytotoxic agents.[40-42] A recent multicentered clinical trial on patients with high-risk NMIBC after BCG-refractory therapy showed an overall favorabe response of MCNA compared to other intravesical treatments in matchable populations (i.e., valrubicin or gemicitabine).[43] An overall disease-free survival (DFS) of 25% at 1 year and 19% at 2 years was found in the responding arm. MCNA resulted in a 39% DFS at 1 year in patients who had BCG-failure after maintenance (BCG-relapse) and 22.1% in patients who showed BCG-resistance after induction therapy (BCG-refractory). In the same time interval, the effectivity in patients with papillary tumors and CIS was 35.1% and 21%, respectively. The median DFS for

the population was 32.7 months. In responders of MCNA, a low-risk of progression and lower RC-rates were shown. In addition, MCNA has a favorable safety profile compared to AE of BCG.[3,25]

MCNA, therefore, appears to be a promising immunotherapy for a conservative treatment of high-risk NMIBC after BCG-failure. The limitation of the trial is reflected by the small patient population (129), the short duration of surveillance of 3 years and the patient-population "beyond treatment" without the ability of cross-comparing MCNA as first-line immunotherapy.

Immune checkpoint inhibitors

BC cells are capable of expressing programmed death-ligand 1 (PD-L1), a molecule that appears to block the ability of the immune system to detect and attack tumor cells.[44,45] PD-L1 is found particularly in tumor microenvironment of invasive and metastatic stages of BC. There is evidence that PD-L1 binds to PD-1, that are found on T-lymphocytes. The binding of PD-L1 and PD-1 causes T-cell-infectivity. By blocking the PD-L1 molecules, the capability of the immune system for tumor detection and eradication is restored.[46,47] MPDL3280A, a monoclonal antibody targeting PD-L1 was tested in 31 patients with metastatic UCC. The results were promising; besides a response rate of 50%, side effects were shown to be lower than in chemotherapy and also tolerated in patients suffering from renal failure.[46] To our knowledge there are no trials evaluating immune checkpoint inhibitors in NMIBC. Although PD-L1 was found mostly in MIBC and metastatic UCC the inhibition of PD-L1 in subtypes of superficial BC (i.e., high-risk) might represent a promising therapeutic tool in patients with BCG failure to study.

Interleukin-10 antibody

Recent data from an experimental study assesses the dual use of monoclonal antibody to IL-10 (anti-IL10R1) and concurrent BCG instillation in a murine model of BC.[48] TH1-response is known to mediate efficacy of BCG instillation; IL-10, on the other hand, is known to inhibit TH1-response and, therefore, is associated with BCG failure. The authors found a 22% regression rate in the combination arm of the trial compared to a 6% regression in a group of BCG treatment only.[48] Further, no metastases were found in the group of mice. Therefore the combination of IL-10R1 monoclonal antibody and BCG represents a new promising therapeutic tool in high-risk NMIBC combining local and systemic antineoplastic effect.

Vaccine-based therapy

Vaccine-based agents have recently been evaluated in NMIBC patients. This therapy focuses on vaccine-based proteins with the ability to direct the immune system against bladder tumor specific antigens. There are several vaccine-based agents, such as ALT-803, ALT-801, rec-MAGE-A3, PANVAC, and HS-410 currently in clinical trials.[45] The use of "tumor vaccines" is a promising novel immunotherapy approach but further clinical trials are necessary in order to translate these new modalities into clinical routine.

Intravesical treatment with α-emitter Bi-213-anti-epidermal growth factor receptor-monoclonal antibody

A recent pilot study from TUM Munich, Germany evaluated the intravesical instillation of α-emitter (Bi-213) in 5 patients with BCG-failure and presence of CIS. The α-emitter was coupled to specific antibodies (anti-epidermal growth factor receptor monoclonal antibody [EGFR-MAb]; cetuximab, Merck, Germany), which had showed effectiveness in previous murine models using intravesical human BC cells.[49,50] Two patients with BCG-refractory CIS underwent a single-shot intravescial instillation of Bi-213-anti-EGFR-MAb. Patients showed no signs of AE and the process of instillation of the immunoradio-conjugate was monitored by single-photon emission computerized tomography/computerized tomography. Two of five patients showed no persistent CIS and 3 patients showed recurrent CIS after the surveillance-period. Bi-213-anti-EGFR-Mab-instillation may be a promising new approach in BCG-refractory patients with CIS and is hoped to be an alternative to RC. As there were no side effects reported, further instillations are theoretically possible. To evaluate the utilization of immunoradio-conjugates in a larger population, a phase-I trial is planned.

Photodynamic therapy

Although photodynamic therapy (PDT) is a device-assisted therapy, there are similarities to intravesical immunotherapy at cellular and bimolecular level.[9] PDT was shown to be an effective alternative even in BCG-refractory UCC and is a conservative option to avoid RC.[51] The effect of PDT is based on the interaction of a special light with its photosensitized target lesion. Photosensitive molecules are instilled into the bladder, internalized by tumor cells and exposed to a specific wavelength light and oxygen which causes a cytotoxic effect. The antineoplastic effect accrues by the production of free radicals, which lead to a direct damage of cells and their apoptosis. Nevertheless, besides the direct cytotoxic effect a secondary inflammatory process evokes a distinct acute inflammation leading to an infiltration of neutrophils similar to the local tissue reaction post-BCG.[52] This is supposed to lead to an increased T-cell-mediated antineoplastic effect.[53] This phase is suspected to be an important factor of successful PDT-treatment. Therefore, the mechanism of PDT shows many similarities with immunotherapy using BCG.

In the first generation of photosensitizers for PDT, hematoporphyrin derivates were utilized. These agents showed severe AE in up to 40% of patients.[54] Photosensitizers of the second generation are based on endogenous photoactive porphyrins that are developed intracellularly by local instillation of 5-aminolevulinic

acid (5-ALA) and show no severe side effects.[55] PDT using 5-ALA or its derivates as photosensitizer presented good clinical results in several studies.[55] The application of hexaminolevulinate (HAL) showed a 2-fold increase of fluorescence intensity and dissemination in bladder tissue allowing a 20-fold lower concentration of HAL compared to 5-ALA.[56] Bader *et al*. proposed PDT using HAL as a photosensitizing agent might offer an alternative approach in the treatment of NMIBC.[55] Because of the small number of patients, further studies with a larger sample-size and a long-term follow-up are needed in order to interpret the effectivity of PDT.

CONCLUSIONS

Intravesical immunotherapy is well established and its benefits for patients suffering from NMIBC are evident. BCG still represents the gold standard of immunomodulating intravesical treatments for reduction of recurrence and progression, as well as on the improvement of tumor specific survival. However, optimal and clear instillation-schedules or protocols are still subject to debate. Despite the evident benefits of BCG and its maintenance, it is possible that a percentage of patients are over-treated. This leads not only to immense economical burden but also to potential severe side effects in patients who actually do not benefit from the therapy. Therefore, an objective predictor of success of BCG-treatment, such as biomarkers or cytokines would be useful in order to provide an optimal and efficient treatment. In patients with BCG-failure or in patients who are not able to tolerate a BCG therapy, intravesical chemotherapy is an option. However, for high-risk-patients who show BCG-refractory disease, radical cystectomy still remains the recommended treatment. The establishment and further development of alternative immunotherapies (MCNA, PTD, IFN, Bi-213-anti-EGFR-MAb) could help provide alternative treatments for patients who are not able or are not willing to undergo cystectomy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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