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The role of mycobacterial cell wall nucleic acid complex in the treatment of bacillus Calmette–Guérin failures for non-muscle-invasive bladder cancer

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Abstract

Introduction: The treatment of high-risk non-muscle-invasive bladder cancer (NMIBC) utilizes transurethral resection followed by adjuvant intravesical immunotherapy or chemotherapy. Intravesical bacillus Calmette–Guérin (BCG) is the mainstay of adjuvant immunotherapy, but there are limited nonsurgical options for patients that fail this treatment. Mycobacterial cell wall nucleic acid complex (MCNA) is an immunotherapeutic agent utilized primarily after failure of intravesical BCG. The purpose of this paper is to provide a comprehensive review of the published literature regarding MCNA.

Methods: A literature review was performed and identified studies indexed in MEDLINE® related to utilization of MCNA for patients with NMIBC.

Results: Two trials assessed the efficacy of MCNA in patients with NMIBC, comprising a total of 184 patients. Most patients had carcinoma *in situ* (CIS) with (26%) or without (52%) concomitant papillary tumors. A minority of patients had only papillary tumors (22%). Most patients (95%) previously received BCG or other intravesical therapy prior to receiving MCNA. In the largest available trial, 25% and 19% of patients had no evidence of residual cancer in 1 and 2 years following initiation of MCNA. A total of 2.3% of patients had adverse events (AEs) leading to delay or discontinuation of therapy and 66% of patients had mild drug-related AEs.

Conclusion: Based on analysis of available published data, MCNA offers a durable response for a small proportion of patients that have failed prior intravesical therapy. There still exists a large unmet need for nonsurgical treatment options for patients with NMIBC who have failed adjuvant intravesical therapies.

Keywords: *Mycobacterium*, MCNA, BCG failure, bladder cancer

Introduction

Urothelial carcinoma of the bladder is a common malignancy. There were an estimated 74,690 newly diagnosed cases and 15,580 bladder cancer associated deaths in the United States in 2014 [Siegel *et al.* 2014]. Transurethral resection (TUR) is the first step in the treatment and staging of bladder cancer, and most patients are initially diagnosed with non-muscle-invasive bladder cancer (NMIBC) [Richards *et al.* 2014]. While the definition of high-risk NMIBC varies in different guidelines, it generally includes high-grade Ta, T1, and carcinoma *in situ* (CIS) [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013; Clark *et al.* 2013]. For high-risk NMIBC, most guidelines recommend a repeat TUR in 1–6 weeks for therapeutic and staging purposes [Hall *et al.* 2007; Babjuk *et al.* 2013]. Adjuvant intravesical therapy with immunotherapeutic and chemotherapeutic agents can prevent recurrence and progression for patients with high-risk NMIBC [Clark *et al.* 2013]. Bacillus Calmette–Guérin (BCG) is the most common adjuvant intravesical treatment [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013; Clark *et al.* 2013].

While results may vary depending on dosage and treatment regimen, the disease-free survival

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(DFS) for patients treated with common BCG regimens is approximately 70% at 5 years [Sylvester *et al.* 2002]. Patients who fail intravesical therapy with BCG are at risk for progression to muscle-invasive or metastatic disease [Schrier *et al.* 2004; Witjes, 2006]. The gold standard treatment for these patients is radical cystectomy [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013; Clark *et al.* 2013]. However, this is a major oncologic procedure associated with significant morbidity and mortality [Hollenbeck *et al.* 2007]. Valrubicin is the only US Food and Drug Administration (FDA)-approved adjuvant intravesical chemotherapy for BCG-refractory CIS [Steinberg *et al.* 2000], but it has limited long-term efficacy. There is no standard second-line intravesical therapy for high-risk BCG-refractory NMIBC at this time [Yates *et al.* 2012]. The most common available second-line intravesical options are interferon-alpha (IFNα) and BCG+IFNα, and also include valrubicin, gemcitabine, mitomycin C (MMC), epiribicin, docetaxel, paclitaxel, and mycobacterial cell wall nucleic acid complex (MCNA) [Witjes, 2006; Laudano *et al.* 2010; Yates *et al.* 2012; Mckiernan *et al.* 2014; Patel *et al.* 2015].

MCNA is derived from *Mycobacterium phlei* and has demonstrated efficacy against NMIBC in the BCG-refractory setting [Morales *et al.* 2001, 2009, 2015]. This study assesses the role of MCNA for patients with NMIBC. A literature search was performed for relevant English language MEDLINE® indexed studies.

Non-muscle-invasive bladder cancer

NMIBC is heterogeneous, and includes Ta, T1, and CIS tumors. These tumors are further risk stratified by several different guidelines. The American Urologic Association (AUA) and International Bladder Cancer Group (IBCG) define high-risk NMIBC as CIS, high-grade Ta, or high-grade T1 tumors, while the European Association of Urology (EAU) additionally includes recurrent low-grade Ta tumors >3 cm or tumors with a high calculated recurrence or progression scores [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013]. High-risk tumors are known to have significant rates of recurrence and progression to metastatic disease and death [Cookson *et al.* 1997]. All of the aforementioned guidelines, as well as the National Comprehensive Cancer Network (NCCN), advocate for the use of adjuvant intravesical therapy for high-risk tumors [Clark *et al.* 2013]. There is currently no role for use of adjuvant intravesical therapy for low-risk tumors, and no universal guideline exists for intermediate-risk tumors [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013].

BCG

The two major categories of adjuvant intravesical therapy include immunotherapy and chemotherapy. BCG is considered the gold standard first line intravesical immunotherapy for patients with high-risk tumors, particularly for CIS [Hall *et al.* 2007; Brausi *et al.* 2011; Babjuk *et al.* 2013; Clark *et al.* 2013]. BCG was first described as a treatment for bladder cancer in 1976 by Morales and colleagues [Morales *et al.* 1976]. BCG is derived from attenuated strains of *Mycobacterium bovis*. The powdered form of these attenuated bacteria is reconstituted in normal saline for bladder instillation.

BCG is thought to stimulate the immune system. It initially binds to urothelium via fibronectin and integrin α5β1 [Zhao *et al.* 2000]. The mycobacteria are then selectively internalized by bladder cancer cells due malignant alterations in cellular transport related to PTEN deletion and RAS oncogene activation [Huang *et al.* 2012; Redelman-Sidi *et al.* 2013]. Following internalization, there is upregulation of MHC class II proteins, as well as secretion of various inflammatory cytokines. Recruitment of granulocytes, CD4 and CD8 lymphocytes, natural killer (NK) cells, and macrophages is followed by further release of inflammatory cytokines. Finally, there is immune-mediated cytotoxic activity from granulocytes, CD8 lymphocytes, NK cells, and macrophages [Redelman-Sidi *et al.* 2014]. These inflammatory cells in the bladder wall and urine drive the local inflammatory response and tumor cell death [Bohle *et al.* 1990; De Boer *et al.* 1991]. In addition to these immune-mediated effects, BCG has been shown to have direct cytotoxic effects [Pook *et al.* 2007].

BCG can have significant local and systemic side effects. Irritative voiding symptoms are common, but BCG can also lead to obstruction at the bladder neck or ureteral orifices due to scarring. Local symptoms are the most frequent reason for BCG treatment cessation [Brausi *et al.* 2014]. The most severe local reactions to BCG require systemic treatment and cessation of BCG [Lamm *et al.* 1992]. Low-grade fever and malaise

are present in up to 25% of patients, and can be managed by temporarily withholding treatment. Major systemic reactions due to disseminated BCG infection require permanent discontinuation of BCG, with prompt administration of broad antibiotics, antimycobacterials, and corticosteroids [Rischmann *et al.* 2000].

BCG has demonstrated efficacy in reducing recurrence rates and progression of NMIBC in multiple comparative trials [Sylvester *et al.* 2002; Sylvester *et al.* 2005]. BCG is more effective for CIS compared with Ta tumors, and is FDA approved for the treatment of CIS [Lamm, 1992; Lamm *et al.* 2000]. Variation in the observed magnitude of progression rate reduction achieved with induction BCG has been attributed to differences in demographics, sample size, follow-up time, and augmentation with various maintenance regimens [Babjuk *et al.* 2013]. In order to maximize benefit and minimize morbidity from BCG, there have been numerous trials and analyses assessing for the optimal dosing, induction, and maintenance regimen [Lamm *et al.* 2000; Palou *et al.* 2001]. However, there is no universally adopted, standard regimen [Ehdaie *et al.* 2013]. A 6-week BCG induction course is used since it was initially shown to be an optimal duration of therapy by Morales and colleagues [Morales *et al.* 1976]. If there is persistent Ta or CIS disease, an additional 6-week induction course can be used to provide a response in up to 50% of patients [O'Donnell and Boehle, 2006; Babjuk *et al.* 2013]. Three-week maintenance courses for 1–3 years following induction have been shown to reduce cancer recurrence and progression in multiple studies, although the optimal duration of maintenance BCG remains unclear [Hinotsu *et al.* 2011; Oddens *et al.* 2013]. With regard to dosing, full-dose BCG has been shown to decrease recurrence rates compared with 1/3 and 1/6 dose BCG [Ojea *et al.* 2007; Oddens *et al.* 2013].

BCG-refractory NMIBC

Approximately 20–50% of patients will fail BCG therapy [Brake *et al.* 2000]. There is various terminology used when tumor is diagnosed after intravesical BCG treatment [Herr and Dalbagni, 2003]. BCG-refractory disease is most commonly present and is defined as the absence of disease-free status at 6 months after induction BCG. BCG relapse refers to tumor recurrence after greater than 6 months of disease-free status. BCG intolerance refers to discontinuation of treatment due to side effects. Finally, BCG resistance refers to disease persistence at 3 months but resolution at 6 months [O'Donnell and Boehle, 2006]. Of these groups, BCG-refractory patients have been shown to have markedly higher rates of progression and disease-specific mortality compared with BCG-relapsing patients [Shirakawa *et al.* 2012]. Subsequent treatment options for patients with any of these BCG failures include radical cystectomy, repeat induction course of BCG, or alternative intravesical therapy. Of these options, immediate radical cystectomy prior to development of muscle-invasive disease provides the greatest survival benefit, with >90% disease-specific survival in these patients [Herr and Sogani, 2001]. A repeat induction course of BCG provides complete response in approximately 50% of patients. However, if the patient then develops muscle-invasive disease, subsequent radical cystectomy only confers 70% disease-specific survival [Herr and Sogani, 2001; Babjuk *et al.* 2013]. There is still significant morbidity and mortality after radical cystectomy, especially for the elderly, highly comorbid population of bladder cancer patients [Lowrance *et al.* 2008]. This has created a need for improved intravesical therapy for patients who fail intravesical BCG [Witjes, 2006].

Mycobacterial cell wall extract

The search for a compound with a similar therapeutic effect as BCG but without the risks from using a live bacterium began in 1974. In early experiments, *Mycobacterium bovis* cell walls were emulsified with some success in guinea pig hepatoma and mouse leukemia models, but these experiments were abandoned after several years due to mixed results [Meyer *et al.* 1974; Pearson *et al.* 1975; Ribi *et al.* 1975]. Morales and colleagues initially used emulsified BCG cell walls for animal prostate cancer models, which showed promising results [Morales *et al.* 1991, 1997]. *Mycobacterium phlei* was then utilized as a live attenuated strain for BCG administration. *M. phlei* is found in drinking water and is not pathogenic to mammals. The promise of emulsified BCG cell walls in animal models lead to the development and use of mycobacterial cell wall extract (MCWE) in a similar model [Morales *et al.* 1995]. MCWE was developed from emulsion of the cell walls of *M. phlei* in mineral oil and thimerosal. In 1996, Chin and colleagues reported significant tumor regression on MRI with MCWE use for orthotopic and heterotopic bladder tumors in mice [Chin *et al.* 1996].

In 2001, a trial of MCWE for CIS of the bladder was performed in 61 patients [Morales *et al.* 2001]. The single-arm trial included patients diagnosed with CIS within 4 weeks of enrollment. A total of 46% of patients previously received BCG induction, with at least 3 months since last BCG administration. A 4 mg MCWE oil emulsion was reconstituted in 50 cm^3 of saline and was instilled in the bladder for at least 2 hours. Patients underwent a 6-week induction course with MCWE, followed by maintenance therapy with one dose every month for 1 year. Mandatory cystoscopies were performed every 3 months. Positive clinical response was determined by negative or suspicious urinary cytology, negative bladder biopsies, and no visual endoscopic evidence of CIS. Clinical response rates were 62.5% at 1 year and 49.3% at 2 years. However, there were only 16 patients remaining in the trial after 1 year due to treatment failure and discontinuation due to adverse effects. Surprisingly, an identical percentage of patients responded to MCWE with history of prior BCG compared to those without prior BCG, peaking interest in the use of this agent as a second-line agent after BCG failure.

Mycobacterial cell wall nucleic acid complex

Shortly after completion of the MCWE trial in 2001, a related compound was formulated and developed. MCNA is composed of the *M. phlei* cell wall as well as 5–10% of its DNA [Filion and Phillips, 1997]. The mycobacterial DNA is composed of preserved short oligonucleotides that complex to the cell wall. The cell wall portion of the compound allows for incorporation into tumor cell wall, while the DNA portion is thought to mediate its therapeutic effect [Filion and Phillips, 2001]. Multiple studies demonstrated that MCNA stimulates interleukin (IL)-6, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), and other inflammatory cytokines [Filion *et al.* 1999, 2000]. While BCG is postulated to primarily exert its effect through indirect immune response and subsequent production of inflammatory cytokines, MCNA also provides a direct cytotoxic and chemotherapeutic effect [Filion and Phillips, 2001]. The DNA portion of the compound is postulated to directly induce apoptosis, independent of the indirect inflammatory cascade [Reader *et al.* 2001]. Finally, MCNA is isolated in water and does not contain thimerosal, which may diminish toxicity risks [Morales *et al.* 2009, 2015].

MCNA phase II and III trials

There are two published single-arm series assessing the efficacy of MCNA (Table 1) [Morales *et al.* 2009, 2015]. In 2009, the first of these trials included 55 patients with CIS [Morales *et al.* 2009]. Similar to the 2001 MCWE trial, the rationale for choosing patients with CIS was that BCG has a known superior effect in patients with this tumor type [Lamm, 1992]. Furthermore, the 2009 trial aimed to show improved results with MCNA compared with MCWE, since this newer compound was postulated to be more effective given inclusion of DNA and less toxic given its lack of thimerosal [Morales *et al.* 2009]. The study was performed at 13 sites in Canada and Australia. Patients were required to have previously documented CIS and prior positive urine cytology within 60 days. In addition, 35% of patients had associated Ta or T1 tumors that were previously resected. Patients were given either 4 or 8 mg of MCNA emulsion during a 6 week induction followed by 3-week maintenance courses at 12 and 24 months. Complete response was defined as negative cystoscopy, biopsies, and urine cytology assessed at 12 and 26 months.

The study showed a moderate complete response rate in all of the subgroups assessed. When performing intention to treat analysis, patients receiving 4 mg of MCNA had a 27% complete response rate at 12 and 26 months, while patients receiving 8 mg of MCNA had a 46% complete response rate at these time points. Identical numbers of patients at both of these time points suggests an impressive durability of response. This implies that effectiveness of treatment at 1 year bodes a reassuring prognosis. Additional analyses were performed to show that patients who had been previously treated with BCG had a similar response compared with BCG-naïve patients. The major weaknesses of this study include a small sample size and a highly heterogeneous patient population. There were no more than nine patients in any single group when considering treatment dose (4 mg or 8 mg), tumor composition (CIS only, CIS with Ta or T1 tumors, Ta or T1 tumors only), and treatment history (BCG naïve, BCG induction only, multiple BCG cycles, prior chemoradiation, prior chemoradiation with BCG). Limited follow-up also hindered this study. Mean and median follow-up were not stated in the study, and authors stated that only 11 of the original 30 patients in the 8 mg MCNA group were available for follow-up at 12 months.

Table 1. Publications of mycobacterial cell wall nuclear acid complex treatment efficacy.

Authors	Design	Patients	Treatment	Median follow-up	Overall 1-year disease-free survival	Major adverse effects (%)
Morales *et al.* [2009]	Single-arm prospective multicenter trial	*n* = 55, BCG naïve and BCG failures, CIS ± papillary	4 mg *versus* 8 mg dose. Six-week induction plus 1-week maintenance monthly × 12 months	Unknown, limited	4 mg – 27% 8 mg – 46%	4 mg – 32% 8 mg – 33%
Morales *et al.* [2015]	Phase III single-arm prospective multicenter trial	*n* = 129, BCG failures only, CIS ± papillary and papillary only	8 mg dose. Six-week induction plus 3-week maintenance at 3, 6, 12, 18, 24 months	34 months	25%	14%

BCG, bacillus Calmette–Guérin; CIS, carcinoma *in situ*.

Mild adverse events (AEs) were ubiquitous, with 98% of patients noting at least one symptom. Serious AEs were present in 32% and 33% of patients receiving 4 and 8 mg of MCNA, respectively. However, the only serious AEs attributable to MCNA were UTI, hematuria, and severe clot retention.

A larger phase III trial was published in 2015 with 129 patients, which addressed some concerns regarding sample size and follow-up time [Morales *et al.* 2015]. The study was performed across 25 sites in North America from 2006 through 2011. Patients were given a 8 mg dose of MCNA with a more rigorous instillation protocol, consisting of a 6-week induction and 3-week maintenance courses of MCNA at 3, 6, 12, 18, and 24 months. Complete response was assessed by cystoscopy and cytology every 3 months with intermittent biopsies. Patients were included with history of CIS only (59 patients), Ta–T2 tumors only (38 patients), or CIS with other Ta–T2 tumors (32 patients). Regardless of presence of CIS, there were 56 patients (43.4%) with Ta tumors, nine patients (7%) with T1 tumors, and five patients (3.9%) with T2 tumors. All patients previously received BCG, with 107 BCG-refractory patients and 22 BCG-relapsing patients. There were 20–30 patients who previously received either one BCG induction course without maintenance, one BCG induction course with maintenance, two BCG induction courses, or three or more BCG induction courses.

Overall DFS rates were favorable in this study, highlighted by 25% DFS at 1 year and 19% DFS at 2 years. Median follow-up was 34 months, which was significantly longer than median follow-up time in prior studies. As expected, improved one year DFS was seen in patients with BCG-relapsing disease (39%) compared with BCG-refractory disease (22%). Patients with only papillary tumors fared better than patients with CIS (35% *versus* 21% DFS), which could have been due to heterogeneous duration of prior BCG exposure in CIS patients. Considering prior BCG instillation history, patients with a single induction course followed by maintenance therapy experienced the greatest 2 year DFS (29%). Similar to the results of the 2009 trial, MCNA response was durable. Patients who were disease free at 1 year had median disease-free duration of 32.7 months. Of the 43% of patients that underwent cystectomy, 31% had muscle-invasive disease on final pathology.

Fewer patients (66%) experienced overall AEs compared with the 2009 trial, although many of these effects were subjective. There were rare patients (6%) that discontinued treatment due to AEs, and the authors did not directly attribute most of these discontinuations to MCNA.

Discussion

MCNA compares favorably to other second-line intravesical therapies for BCG failure. Valrubicin

is currently the only FDA-approved second-line intravesical agent for patients with BCG refractory CIS who are unfit or unwilling to undergo radical cystectomy [Steinberg *et al.* 2000]. In the phase III trial which led to approval of this medication, valrubicin was shown to have a 21% response rate at 6 months, but conferred only 8% DFS in 2 years. The efficacy and durability of response to valrubicin is lower compared with that of MCNA and several other more contemporary agents. Gemcitabine has also been studied for BCG failures, demonstrating a 28% response rate at 1 year [Skinner *et al.* 2013]. It is important to note that the composition of patients in this study included a higher proportion of patients with BCG-relapsing disease, who are known to have favorable outcomes compared with patients with BCG-refractory disease [Herr and Dalbagni, 2003]. BCG+IFNα has been shown to have 45% 2-year recurrence-free survival for a series of patients with prior BCG failure [Joudi *et al.* 2006].

The treatment of patients after BCG failure is highly nuanced, and must account for comorbid conditions, the stage and extent of previous tumors, type of BCG failure, and specific intolerances to prior therapies. MCNA has been shown to have far lower toxicity compared with BCG, especially with respect to severe reactions. This is likely secondary to the compound's composition with nonviable bacterial components, as opposed to attenuated mycobacteria in BCG. BCG is discontinued in up to one-third of patients [Rischmann *et al.* 2000], while the discontinuation rate of MCNA is only 1.6% [Morales *et al.* 2015]. However, the most important outcome to consider when assessing adjuvant second-line intravesical therapies is disease progression. Patients failing MCNA had a 12% yearly progression rate to muscle-invasive disease, which was similar to previously cited rates for those with BCG failure [Hall *et al.* 2007]. This suggests that failure of a course of MCNA does not increase the risk of progression. The durability of response to MCNA (median DFS of 34 months for patients who respond at 1 year) is promising and provides a rationale for choosing this agent in appropriately selected patients.

There are a variety of novel adjuvant intravesical agents for patients who fail BCG. Similar to BCG and MCNA, many of these new treatments function by manipulation of the immune system. CG0070 is an oncolytic adenovirus that has selectivity and cytotoxic properties specific to bladder tumor cells. The complete response rate of this agent has been shown to be as high as 63% [Burke *et al.* 2012]. Checkpoint inhibitors such as PD-1 and PD-L1 inhibitors have shown dramatic effect in the metastatic tumor setting and are currently being studied in the setting of NMIBC [Powles *et al.* 2014]. There are a myriad of other targeted therapies, cytotoxic agents, vaccine-based therapies, and genetic therapies that are currently being investigated with promising results [Boehm and Svatek, 2015]. MCNA and other BCG modifications can compete with these agents, but further studies are needed to better refine their formulation and treatment regimens. MCNA was a significant improvement compared with MCWE, and there is the potential for continued evolution of this type of therapy.

Conclusion

BCG failure for patients with high-risk NMIBC is a common and serious problem. Although standard subsequent treatment is radical cystectomy, alternative intravesical treatments can be explored in appropriately selected patients. Trials must be designed carefully due to significant risk of disease progression in these patients. Based on existing evidence, MCNA appears to have a role as an effective treatment for patients with BCG failure. Comparisons of the safety and efficacy of MCNA to other second-line intravesical therapeutic agents are generally favorable. Further studies are needed to fully assess MCNA's role in the continuously evolving landscape of intravesical treatment for NMIBC.

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Conflict of interest statement

Dr. Steinberg discloses that he is a consultant for EndoPharmaceuticals, Genentech, Telesta, Cold Genesys, Heat Biologics, Baxter, Photocure, Karl Storz, Taris Biomedical, Abbott Molecular.

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