Review Article

BCG Immunotherapy in the Treatment of Urinary Bladder Cancer

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ABSTRACT

Bladder cancer has become a common cancer globally and ranks as the ninth most frequently diagnosed cancer worldwide with the highest incidence rates observed in men in Southern and Western Europe, North America as well as in certain countries in Northern Africa and Western Asia. Bladder cancer ranks as 13th in terms of death ranks, with mortality rates decreasing in most developed countries. Intravesical immunotherapy with bacilli Calmette-Geurin (BCG) was partially responsible for this improvement in survival and is expected that patients treated with optimal BCG treatment regimens will have a long term reduction in tumor recurrence, tumor progression and cancer mortality. The addition of intravesical therapy is based on prognostic factors, including grade of disease, rapidity of recurrences, and the presence of carcinoma in situ (CIS). BCG elicits a massive influx of inflammatory cells and cytokines that leads to a response against neoplastic cells. The first intravesical BCG dose was empirically determined to be 120 mg. BCG inductions with 6-week instillations must be followed by 3-week maintenance instillations. BCG has potential side effects that may lead to treatment cessation and incomplete treatment courses. Local side effects of BCG include cystitis, hematuria, contracted bladder, urethral obstruction, granulomatous prostatitis, and epididymo-orchitis. Systemic side effects include systemic BCG and allergic reactions. The keys to management of these adverse events are education, prevention, and awareness to optimize treatment outcomes.

Keywords: BCG, Carcinoma insitu, Intravesical therapy, immunotherapy.

INTRODUCTION

Primary malignancies of the urinary bladder represent a spectrum of disease from superficial, well differentiated disease, which does not significantly impact patient survival, to highly malignant tumors for which long-term survival may be dismal.[1]

A three-tiered system is most often used for the grading of Transitional cell carcinoma. Grade 1 tumors are well-differentiated, grade 2 are moderately differentiated, and grade 3 are poorly differentiated. Most invasive bladder tumors are high grade. Carcinoma in situ (CIS) is defined as high grade, noninvasive, transitional cell carcinoma, often involving large portions of the bladder urothelium.[2] Now, all invasive tumors confined to the bladder are classified as T2 tumors, with T2a denoting superficial muscle invasion and T2b denoting deep muscle invasion. Pathologic T3 tumors are now defined as those that extend beyond the bladder.[3]

The standard treatment of Ta bladder cancer is complete endoscopic resection with or without intravesical therapy.[4] Intravesical therapy is indicated when there is diffuse bladder surface involvement, frequent recurrences, or short intervals between recurrences. Treatment of CIS involves transurethral biopsy to verify the diagnosis followed by intravesical immunotherapy or chemotherapy.[5]

BCG, an attenuated strain of Mycobacteriuim bovis, is the most commonly used form of intravesical immunotherapy [6] with tumor-free rates of 54% after one or two cycles of therapy and is most frequently utilized for CIS and recurrent superficial disease. [5]

Dosage and Mode of Instillation

The first intravesical BCG dose was empirically determined to be 120 mg.[7] BCG instillation usually start a minimum of 2 week after transurethral resection to allow healing of the urothelium and to avoid systemic side effects.[8-9] The 6 weekly instillation is the first reported regimen is considered to be the standard induction course and is very effective.[10] BCG induction course can be repeated in two situations. The first in BCG recurrence defined as disease recurrence while off treatment after a period of being disease free,[11] the second situation applies to CIS that persists after an initial induction course. [6,12,13] Maintenance has been defined as a crucial factor for BCG efficacy. Maintenance therapy has resulted in statistically significant improvement in patients survival compared with that of induction only. The widely accepted maintenance schedule is based on Southwest oncology group regimen, starting with the series of six weekly induction followed by 3 weekly instillation at 3 months and every 6 month for 3 years. This schedule has markedly increased and extended the benefit of BCG.[8]

Mechanism of Action

A functional host immune system is necessary prerequisite for successful BCG therapy. The effects of intravesical BCG depend on the induction of a complex inflammatory cascade event in the bladder mucosa.[14-15] Instillation of BCG into the bladder establishes a localized

infection that involves both attachment and internalization into normal and malignant urothelial cells via a fibronectin dependent process resulting in an array of pro-inflammatory cytokine and chemokine release such as IL-1,IL-6,IL-8,tumour necrosis factor(TNF)-α, and granulocyte-macrophage colony stimulating factor(GM-CSF).[16-18] An influx of various types of leukocyte into the bladder wall takes place including neutrophils, monocytes/macrophages, lymphocytes, natural killer (NK) cells, and dendritic cells (DC). These infiltrating leukocytes are activated and produce a variety of additional pro-inflammatory cytokines and chemokines and also form BCG-induced granuloma structures in the bladder wall.[19-21] Subsequently, a large number of leukocyte types such as neutrophils, T cells, and macrophages are expelled into the bladder lumen and appear in patients' voided urine.[22-25]In addition, transient massive cytokines and chemokines can be detected in voided urine including IL-1β, IL-2, IL-6, IL-10, IL-12, IL-18, interferon (IFN)-γ, TNFα, GM-CSF, macrophage colony-stimulating factor (M-CSF), macrophage-derived chemokine (MDC), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory-protein- (MIP-) 1α, interferon-inducible protein (IP)-10, monokine induced by γ-interferon (MIG), and eosinophil chemo attractant activity.[24,26-31] The development of a predominant Th1 cytokine profile (e.g., IFN-γ, IL-2, and IL12)is associated with the therapeutic effects of BCG, whereas the presence of a high level of Th2 cytokines (e.g., IL-10) is associated with BCG failure.[27,29,30]

Multiple immune cell types participate in the inflammatory response induced by BCG in the bladder. Macrophages serve as the first line of defense in mycobacterial infection.[32] The killing of bladder cancer cells by macrophages relies on direct cell-to-cell contact and release of various soluble effector factors such as cytotoxic cytokines TNF-α and IFN-γ and apoptotic mediators such as nitric oxide (NO).[33-35, 36] Th1 cytokines enhance the induction of macrophage cytotoxicity whereas Th2 cytokines inhibit the induction of macrophage cytotoxicity.[34,35]

Neutrophils are central mediators of the innate immunity in BCG infection.[37] Neutrophils are the primary source of TNF-related apoptosis-inducing ligand TRAIL found in the urine after BCG instillation.[38, 39] TRAIL is a member of the TNF family that induces apoptosis in malignant cells but not in normal cells.[38]

Recruitment of other immune cell types including CD4+ T cells,CD8+ T cells, NK cells, and DC [19,20] types are crucial for BCG immunotherapy of bladder cancer, as depletion of these cell types failed to develop effective anti bladder cancer responses.[40,41] DC, together with macrophages, trigger an anti-BCG-specific immune response via antigen presentation to T cells that also amplifies the BCG-induced antitumor immunity. T cells and NK cells are cytotoxic toward bladder cancer cells upon activation. They kill target cells via the major histocompatibility complex (MHC) pathways.[42-44]

Specialized cell populations called BCG-activated killer (BAK) cells are $CD3^{-}CD8^{+}CD56$. BAK cells kill bladder cancer cells through the perforin-mediated lysis pathway and effectively lyse NK cell-resistant bladder cancer cells.[45-47]

In addition to the ability of BCG to elicit host immune responses, evidence supports a direct effect of BCG on the biology of UCC. In vitro studies have shown that BCG is anti-proliferative and even cytotoxic to UCC. [42, 48]

Side Effects and Management

Side effects that occur after immunotherapy with BCG in bladder cancer patients can be categorized as either local or systemic effects.

➢ **LOCAL EFFECTS**

These effects are confined to the bladder or organs that are in contact with the BCG bacilli.

- **Cystitis**

  Cystitis caused by BCGs are drug induced or BCG cystitis manifested as urinary frequency, urgency and pain at the bladder site. These symptoms usually start 2 to 4 hours after instillation and subside in 6 to 48 hours without therapy. Increased diuresis is advocated as it enhances the evacuation of mycobacteria. Relief by symptomatic management with spasmolytics, anticholinergics, antiphlogistics (oxybutynin, phenazopyridine, propantheline bromide or NSAIDS) and analgesics are advised for serious side effects.[49] It is always worthwhile to make a culture of the urine and start the therapy thereafter, if cystitis lasts longer than 48 hours. Postponement of instillation, subsequent dose reduction or the use of quinolone antibiotic should be the treatment options.[50]

- **Hematuria**

  It is often associated with drug induced cystitis. It is important to perform a urine culture to exclude hemorrhagic cystitis. Suspend instillations until urine clears. Catheterization and bladder irrigation for clots may be required.[51]

- **Contracted bladder**

  A rare and serious complication due to extravasation of intravesical therapy and is most likely to be associated with multiple TURBTs and maintenance instillations. Management includes dose reduction, withholding therapy, hydro-distention and, on occasion, cystectomy.[52]

- **Urethral obstruction**

  It is a serious complication of BCG therapy. Obstruction is generally temporary and self-limiting after the cessation of the therapy.

Percutaneous drainage or stenting of the kidney may be required. The presence of carcinoma in situ and muscle-invasive bladder cancer should be excluded.[53]

- **Granulomatous prostatitis**

  It is a common occurrence in BCG treated patients and can be considered as a complication resulting from BCG contaminated urine. Granulomatous prostatitis is most often asymptomatic.[53-54]Prostate specific antigen levels are often elevated and ultrasound typically shows diffuse hypoechoic zones.

  In patients with symptoms of acute granulomatous prostatitis, a combination of 300 mg isoniazid and rifampin for 3 months, plus high dose fluoroquinolones and steroids for 6 persistent symptoms. Suspension of instillation is also required.[50]

- **Epididymo-orchitis**

  Epididymo orchitis is also caused by BCG contaminated urine and which occurs during instillation is primarily due to gram negative bacilli resulting from catheterization while later appearance of the condition is related to mycobacteria.[55]

  Treatment should include isoniazid and rifampin[50] and also with high dose fluoroquinolones is also recommended.[56-57] For persistent symptoms steroids following fluoroquinolone therapy is required. Orchiectomy rarely may be required in case of an abcess formation secondary to epididdymo orchitis.

➢ **Systemic Effects**

Systemic reactions are less frequent than local reaction but are more likely to be severe.

- Systemic bacillus calmette guerin reactions

  It usually occurs immediately after instillation. It is generally associated with high grade fever and may progress to multiple organ failure.

  Management of severe systemic reactions involves cessation of BCG therapy and treatment with isoniazid, rifampin and ethambutol for 6 months plus early high dose fluoroquinolones and high dose corticosteroids as long as symptoms persists. For the treatment of gram negative bacilli or enterococcus an empirical nonspecific antibiotic is also recommended.[50]

- **Allergic reactions**

  May involve skin rashes and arthralgia

  Treatment involves anti histamines and anti-inflammatory agents. For severe reactions BCG may be discontinued and the addition of isoniazid, rifampin plus corticosteroids should be considered.[50]

Contraindications

Absolute contraindications to BCG as per European Association of Urology guidelines are[58]:

- Within 2 weeks of transurethral resection
- Macroscopic hematuria
- Traumatic catheterization
- Symptomatic UTI

CONCLUSION

Bladder cancer includes a broad spectrum of diseases. The majority of cases are superficial diseases that may have little impact on patient survival. Management of these tumors consists of endoscopic resection and continued surveillance to ensure that progression does not occur, or is detected early if it does occur. Current optimal BCG regimens that use maintenance therapy significantly improve long-term results, and reduction in the dose of BCG administered may further improve the safety of intravesical BCG.Recognition of risk factors, particularly traumatic catheterization or concurrent cystitis that result in systemic BCG absorption as well as the prompt and appropriate treatment of early side effects should significantly decrease the incidence of severe toxoicity.

REFERENCES

1. Margaret CM, Jobe CM et al.Bladder cancer:diagnosis and management of bladder cancer.BJU International, vol.120(6), 2017, (pg.755-765).
2. Farrow GM, Utz DC, Rife CC. Morphological and clinical observations of patients with early bladder cancer treated with total cystectomy. Cancer Res, vol.36, 1976, (pg.2495)
3. Fleming ID. AJCC Staging Manual. 5th ed., Philadelphia: Lippincott-Raven; 1997.
4. Heniey MM, Proppe K, Pr-ouit CG Jr. Invasive bladder cancer: tumor configuration, lymphatic invasion, and survival. / Urol, vol.130, 1983, (pg.895-897).
5. Coplen DE, Marctus MD, Myers JA, et al. Long-term follow-tip of patients treated with 1 or 2, 6-week courses of intra vesical bacillus Calmette-Guerin: Analysis of possible predictors of response free tumor. J Urol, vol.144, 1990, (pg.652).
6. Lamm DL, Blumenstein BA, Crawford D, et al. A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette-Guerin for transitional cell carcinoma of the bladder. N EnglJMed, vol.325, 1991,(pg.1205-1209).
7. Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guérin in the treatment of superficial bladder tumors.J. Urol, Vol.116, 1976,(pg.180–183).
8. Lamm DL, Blumenstein BA, Crissman JD,et al. Maintenance bacillus Camette-Geurin immunotherapy for recurrent TA,T1 and carcinoma in situ transitional cell carcinoma of the bladder:a randomized Southwest Oncology Group Study. J urol, vol.163, 2000,(pg.1124-1129).

9. Van Der Meijden APM,Sylvester AJ et al.Maintenance bacillus Calmette Guerin for TaT1 bladder tumors is not associated with increased toxicity :results from a European Organisation for Research and Treatment of Cancer Genito –Urinary Group phase trial . Eur Urol, vol.44,2003.
10. Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guérin in the treatment of superficial bladder tumors. J. Urol, vol.116, 1976, (pg.180–183).
11. Bui TT, Schellhammer PF. Addititonal bacillus Calmette-Guerin therapy for recurrent transitional cell carcinoma after an initial complete response.Urology, vol.49, 1997, (pg.687-90).
12. De Reijke TM, Kurth KH,Sylvester RJ, et al. Bacillus Calmette- Guerin versus epirubicin for primary, secondary or concurrent carcinoma in situ of the bladder: results of a European Organisation for the research and treatment of cancer-Genito-Uruinary Group phase 3 trial. J Urol, vol.173, 2005, (pg.405-9).
13. Sylvester RJ,Van der Meijden A,Witjes JA,et al.High grae Ta urothelial carcinoma and carcinoma in situ of the bladder.Urology, vol.66(1), 2005, (pg.90-107).
14. Brandau S, Suttmann H. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer a success story with room for improvement. Biomedicine and Pharmacotherapy vol.61 (6), 2007, (pg.299-305).
15. Alexandroff AB, Nicholson S, Patel PM, Jackson AM. Recent advances in bacillus Calmette-Guerin immunotherapy in bladder cancer. Immunotherapy, vol.2 (4), 2010, (pg.551-560).
16. Kavoussi LR, Brown EJ, Ritchey JK, Ratliff TL. Fibronectin-mediated Calmette-Guerin bacillus attachment to murine bladder mucosa. Requirement for the expression of an antitumor response. Journal of Clinical Investigation, vol.85 (1), 1995, (pg.62-67).
17. Becich MJ, Carroll S, Ratliff TL. Internalization of bacille Calmette-Guerin by bladder tumor cells. The Journal of Urology, vol.145 (6), 1995, (pg.1316-1324).
18. Bevers RF, Kurth KH, Schamhart DH. Role of urothelial cells in BCG immunotherapy for superficial bladder cancer. British Journal of Cancer, vol.91 (4),2004(pg.607-612).
19. Böhle A, Gerdes J, Ulmer AJ, Hofstetter AG, Flad HD. Effects of local bacillus Calmette-Guerin therapy in patients with bladder carcinoma on immunocompetent cells of the bladder wall. The Journal of Urology, vol.144 (1), 1990(pg.53-58).
20. Prescott S, James K, Hargreave TB, Chisholm GD, Smyth JF. Intravesical Evans strain BCG therapy quantitative immunohistochemical analysis of the immune response within the bladder wall. The Journal of Urology, vol.147 (6), 1992, (pg.1636-1642).
21. Saban MR, Simpson C, Davis C, Wallis G, Knowlton N, Frank MB, Centola M, Gallucci RM, Saban R. Discriminators of mouse bladder response to intravesical Bacillus Calmette-Guerin (BCG). BMC Immunology, vol.8, 2007, (pg.6).
22. De Boer EC, de Jong WH, van der Meijden AP, Steerenberg PA, Witjes F, Vegt PD, Debruyne FM, Ruitenberg EJ. Leukocytes in the urine after intravesical BCG treatment for superficial bladder cancer. A flow cytofluorometric analysis. Urological Research, vol.19 (1), 1991, (pg.45-50).
23. De Boer EC, de Jong WH, van der Meijden AP, Steerenberg PA, Witjes JA, Vegt PD, Debruyne FM, Ruitenberg EJ. Presence of activated lymphocytes in the urine of patients with superficial bladder cancer after intravesical immunotherapy with bacillus Calmette-Guérin. Cancer Immunology and Immunotherapy, vol.33 (6), 1991, (pg.411-416).
24. De Boer EC, de Jong WH, Steerenberg PA, van der Meijden AP, Aarden L, Debruyne FM, Ruitenberg EJ. Leukocytes and cytokines in the urine of superficial bladder cancer patients after intravesical immunotherapy with bacillus Calmette-Guerin. In Vivo, vol.5 (6), 1991, (pg.671-677).
25. Simons MP, O'Donnell MA, Griffith TS. Role of neutrophils in BCG immunotherapy for bladder cancer. Urologic Oncology, vol.26 (4), 2008, (pg.341-345).
26. Böhle A, Nowc C, Ulmer AJ, Musehold J, Gerdes J, Hofstetter AG, Flad HD. Elevations of cytokines interleukin-, interleukin- and tumor necrosis factor in the urine of patients after intravesical bacillus Calmette-Guerin immunotherapy. The Journal of Urology, vol.144 (1), 1990, (pg.59-64).
27. De Reijke TM, De Boer EC, Kurth KH, Schamhart DH. Urinary cytokines during intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer processing, stability and prognostic value. The Journal of Urology, vol.155 (2), 1996, (pg.477-482).
28. Taniguchi K, Koga S, Nishikido M, Yamashita S, Sakuragi T, Kanetake H, Saito Y. Systemic immune response after intravesical instillation of bacille Calmett-Guérin ”CG for superficial bladder cancer. Clinical and Experimental Immunology, vol.115 (1), 1999, (pg.131-135).
29. Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, Abbou CC, Chopin DK. Prognostic value of a T helper urinary cytokine response after intravesical bacillus.
30. Immunotherapy of Urinary Bladder Carcinoma: BCG and Beyond Calmette-Guerin treatment for superficial bladder cancer. The Journal of Urology, vol.167 (1), 2002, (pg.364-367).
31. Nadler R, Luo Y, Zhao W, Ritchey JK, Austin JC, Cohen MB, O'Donnell MA, Ratliff TL. Interleukin induced augmentation of delayed-type hypersensitivity DTH enhances Mycobacterium bovis bacillus Calmette-Guérin BCG mediated antitumour activity. Clinical and Experimental Immunology, vol.131 (2), 2003, (pg.206-216).
32. Luo Y, Chen X, O'Donnell MA. Mycobacterium bovis bacillus Calmette-Guérin BCG induces human CC- and CXC-chemokines in vitro and in vivo. Clinical and Experimental Immunology, vol.147 (2),2007,(pg.370-378).
33. Heldwein KA, Liang MD, Andresen TK, Thomas KE, Marty AM, Cuesta N, Vogel SN, Fenton MJ. TLR and TLR serve distinct roles in the host immune response against Mycobacterium bovis BCG. Journal of Leukocyte Biology, vol.74 (2),2003,(pg.277-286).
34. Luo Y, Yamada H, Chen X, Ryan AA, Evanoff DP, Triccas JA, O'Donnell MA. Recombinant Mycobacterium bovis bacillus Calmette-Guérin BCG expressing mouse IL- augments Th1 immunity and macrophage cytotoxicity.Clinical and Experimental Immunology, vol.137(1), 2004, (pg.24-34).
35. Luo Y, Yamada H, Evanoff DP, Chen X. Role of Th-stimulating cytokines in bacillus Calmette-Guérin BCG-induced macrophage cytotoxicity against mouse bladder cancer MBT-2 cells. Clinical and Experimental Immunology, vol.146 (1), 2006, (pg.181-188).
36. Luo Y, Han R, Evanoff DP, Chen X. Interleukin- inhibits Mycobacterium bovis bacillus Calmette-Guérin BCG-induced macrophage cytotoxicity against bladder cancer cells. Clinical and Experimental Immunology, vol.160 (3), 2010, (pg.359-368).

37. Jansson OT, Morcos E, Brundin L, Lundberg JO, Adolfsson J, Söderhäll M, Wiklund NP. The role of nitric oxide in bacillus Calmette-Guérin mediated anti-tumour effects in human bladder cancer. British Journal of Cancer, vol.78 (5), 1998, (pg.588-592).

38. Godaly G, Young DB. Mycobacterium bovis bacille Calmette Guerin infection of human neutrophils induces CXCL secretion by MyD-dependent TLR2 and TLR4 activation. Cellular Microbiology, vol.7 (4), 2005, (pg.591-601).

39. Ludwig AT, Moore JM, Luo Y, Chen X, Saltsgaver NA, O'Donnell MA, Griffith TS. Tumor necrosis factor-related apoptosis-inducing ligand a novel mechanism for Bacillus Calmette-Guérin-induced antitumor activity. Cancer Research, vol.64 (10),2004, (pg.3386-3390).

40. Kemp TJ, Ludwig AT, Earel JK, Moore JM, Vanoosten RL, Moses B, Leidal K, Nauseef WM, Griffith TS. Neutrophil stimulation with Mycobacterium bovis bacillus Calmette-Guerin BCG results in the release of functional soluble TRAIL/Apo-2L Blood, vol.106 (10), 2005, (pg.3474-3482).

41. Ratliff TL, Ritchey JK, Yuan JJ, Andriole GL, Catalona WJ. T-cell subsets required for intravesical BCG immunotherapy for bladder cancer. The Journal of Urology, vol.150 (3), 1993, (pg.1018-1023).

42. Brandau S, Riemensberger J, Jacobsen M, Kemp D, Zhao W, Zhao X, Jocham D, Ratliff TL, Böhle A. NK cells are essential for effective BCG immunotherapy. International Journal of Cancer, vol.92 (5), 2001, (pg.697-702).

43. Pryor K, Goddard J, Goldstein D, Stricker P, Russell P, Golovsky D, Penny R. Bacillus Calmette-Guerin BCG enhances monocyte- and lymphocyte-mediated bladder tumour cell killing. British Journal of Cancer, vol.71 (4), 1995, (pg.801-807).

44. Suttmann H, Jacobsen M, Reiss K, Jocham D, Böhle A, Brandau S. Mechanisms of bacillus Calmette-Guerin mediated natural killer cell activation. The Journal of Urology, vol.172 (4), 2004, (pg.1490-1495).

45. Liu W, O'Donnell MA, Chen X, Han R, Luo Y. Recombinant bacillus Calmette-Guérin BCG expressing interferon-alpha 2B enhances human mononuclear cell cytotoxicity against bladder cancer cell lines in vitro. Cancer Immunology and Immunotherapy, vol.58 (10), 2009, (pg.1647-1655).

46. Böhle A, Thanhäuser A, Ulmer AJ, Ernst M, Flad HD, Jocham D. Dissecting the immunobiological effects of Bacillus Calmette-Guérin BCG in vitro evidence of a distinct BCG-activated killer BAK cell phenomenon. The Journal of Urology, vol.150 (6), 1993,(pg.1932-1937).

47. Brandau S, Böhle A, Thanhäuser A, Ernst M, Mattern T, Ulmer AJ, Flad HD. In vitro generation of bacillus Calmette-Guérin-activated killer cells. Clinical Infectious Diseases, vol.31 (Suppl 3), 2000, (pg.S94-S100).

48. Brandau S, Suttmann H, Riemensberger J, Seitzer U, Arnold J, Durek C, Jocham D, Flad HD, Böhle A. Perforin-mediated lysis of tumor cells by Mycobacterium bovis Bacillus Calmette-Guérin-activated killer cells. Clinical Cancer Research, vol.6 (9), 2000, (pg.3729-3738).

49. Pook SH, Rahmat JN, Esuvaranathan K, Mahendran R. Internalization of Mycobacterium bovis, Bacillus Calmette Guerin, by bladder cancer cells is cytotoxic. Oncology Reports, vol.18 (5), 2007, (pg.1315-1320).

50. Brosman, S.A., Lamm, D.L.,van der Meijden, A.P.M. and Debruyne, F.M.J.: A practical guide to the use of intravesical BCG for the management of stage Ta,T1, and CIS transitional cell cancer.In:EORTC Genitourinary Group Monograph 6:BCG in Superficial Bladder Cancer.Edited by F.M.J.Debruyne,L.Denis and A.P.M. van der Meijden. New York: Alan R.Liss, Inc.pp., 1989, (pg.311-323).

51. Rischmann P, Desgrandschamp F, Malavaud B, Chopin DK. BCG intravesical instillations: recommendations for side-effects management .Eur Urol, vol.37(Suppl 1),2000,(pg.33-36).

52. Witjes, J., Palou, J., Soloway, M., Lamm, D., Brausi, M., Spermon, J., Persad, R., Buckley, R., Akaza, H., Colombel, M. and Böhle, A. Clinical Practice Recommendations for the Prevention and Management of Intravesical Therapy–Associated Adverse Events. European Urology Supplements, 7(10)2008,(pg.667-674).

53. Nieder AM, Sved PD, Stein JP, Skinner DJ, Soloway MS.Cystoprostatectomy and orthotopic ileal neobladder reconstruction for management of bacille Calmette Guerin-induced bladder contractures. Urology, vol.65,2005,(pg.909-12).

54. Lamm DL.Complications of bacillus Calmette- Guerin immunotherapy. Urol Clin North Am, vol.19,1992,(pg.565-72).

55. Steg A, Leleu C, Debre B, Boccon-Gibod L,Sicard D.Systemic bacillus Calmette-Guerin infection : “BCGitis” in patients treated by intravesical bacillus Calmette-Geurin therapy for bladder cancer. Eur Urol, vol.16,1989,(pg.161-4).

56. Falkensammer C, Gozzi C, Hager M, et al. Late occurrence of bilateral tuberculous-like epididymo-orchitis after intravesical bacilli Calmette-Guerin therapy for superficial bladder carcinoma. Urology, vol.65,2005,(pg.175).

57. Durek C, Rush-Gerdes S, Jocham D, Bohle A. Interference of modern antibacterials with bacillus Calmette-Guerin viability. J Urol, vol.162,1992,(pg.1959-62).

58. Durek C, Rush-Gerdes S, Jocham D, Bohle A. Sensitivity of BCG to modern antibiotics. Eur Urol Suppl, vol.37(Suppl 1),2003,(pg.21-5).

59. Babjuk M, Burger M, Zigeuner R, et al; European Association of Urology. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol, vol. 64(4),2013,(pg.639–653).

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