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Advances and Future Prospects in Improving Intravesical BCG Immunotherapy of Non-muscle Invasive Bladder Cancer

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Abstract—*Bladder cancer is one of the most common diseases worldwide with no improvement in associated mortality since last four decades. Intravesical BCG therapy can prevent the progression of NMIBC to muscle invasive form but the treatment fails and the disease relapses in most cases. Using immunopotentiators in combination with BCG vaccine holds promise for decreasing the rate of progression and recurrence of NMIBC.*

1. INTRODUCTION

Human urinary bladder cancer is the ninth most common malignancy worldwide with men four times more likely to be affected than women [52]. While 430,000 new cases of bladder cancer (BC) were diagnosed worldwide in 2012 [18], as many as 74,690 new cases and about 15,580 deaths occurred due to this cancer in US alone in 2014. Although cancer was considered to be more of a developed world issue for long, it was reported that more than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the world, and these proportions will increase further by 2025 [18]. BC is more of a concern for developing countries as this cancer particularly requires routine monitoring and treatment and so is associated with the highest economic burden among all cancers [8].

Approximately 75 % of all newly diagnosed BCs are non-muscle invasive bladder cancer (NMIBC). NMIBC is defined as a tumor that invades up to the lamina propria, but not into the bladder smooth muscle layer. According to the tumor-node-metastasis (TNM) classification, NMIBC can be of three types: stage Ta (noninvasive papillary carcinoma confined to the mucosa), stage T1 (invasive into the subepithelial connective tissue or lamina propria), and Tis (carcinoma in situ; CIS). Approximately 70% of NMIBCs diagnosed are at stage Ta, 20% at stage T1, and 10% at stage Tis. NMIBC in absence appropriate therapy may progress into high grade muscle invasive bladder cancer (MIBC) like stage T2, T3 or T4. Stage T2 cancer is muscle invasive, while the cancer that invades through the muscle layer into surrounding fat is classified as Stage T3 BC and the one that invades beyond the fat into nearby organs is classified as Stage T4 BC [11]. The primary treatment of NMIBC involves transurethral resection of the bladder tumor (TURBT). Unfortunately, the rate of recurrence post TURBT is as high as 70%, while up to 30% cases progress to MIBC. But till date, TURBT is the gold standard initial intervention for bladder tumor as it has a diagnostic and prognostic use in addition to therapeutic benefit.

2. INTRAVESICAL THERAPY OF NMIBC

Adjuvant intravesical therapy is used post TURBT for prophylaxis to prevent recurrence and progression of Stage Ta or T1 BC into MIBC as well as for the treatment of Stage Tis cancer. These therapeutic agents are administered to the bladder via catheterization delivering it directly to the tumor cells, while minimizing toxicities and so side effects associated with systemic drug absorption. The decision for the choice of agents and schedule of administration in intravesical therapy largely varies with the grade, stage, number and size of tumors and whether the tumor is an initial tumor or a recurrence in addition to the patient's characteristics. While a number of intravesical agents are available for the treatment of NMIBC, they can be divided into two broad categories: immunotherapeutic and chemotherapeutic agents. Immunotherapeutic agents such as Bacillus Calmette Guerin (BCG) and cytokines elicit local immune response against tumor cells, while chemotherapeutic agents such as mitomycin C (MMC), thiotepa, gemcitabine, doxorubicin, epirubicin etc interfere with the cell cycle of tumor cells. Clinical trials for administration of electromotive drug to enhance the efficacy of intravesical chemotherapy are currently underway in US [11]. The intravesical therapy, however, can never be viewed as an alternative to TURBT in patients with Stage Ta or T1 disease.

Various clinical studies have demonstrated that intravesical BCG therapy after TURBT is more effective than MMC chemotherapy followed by TURBT in preventing tumor recurrence in intermediate- and high-risk NMIBC [39]. A

recent comprehensive report on comparative effectiveness of chemotherapeutic versus immunotherapeutic agents by Chou et al also showed that intravesical BCG therapy is associated with decreased risk of BC recurrence than that associated with the chemotherapy using doxorubicin, epirubicin and thiotepa [11]. However, certain adverse effects are observed with BCG therapy and its failure to reduce the rate of recurrence and progression or develop resistance to treatment as seen in >40% of NMIBC cases is one of the major challenges in clinical research [36].

This review will highlight the available literature on mechanism of action, and key limitations of the conventional intravesical BCG therapy while focussing on advances in recombinant BCG based strategies in improving the efficacy and safety of immunotherapy of NMIBC.

3. CLINICAL USE OF BCG IN BC IMMUNOTHERAPY

In 1929, Pearl noted during autopsy studies that Tuberculosis (TB) patients had lower rates of cancer [46]. Approximately twenty years later, the inhibitory effects of BCG, the only vaccine available for human TB, on the rate of cancer were confirmed in animal studies, while the first report of the prophylactic use of intravesical BCG for prevention of BC recurrence in human patients came as late as 1976 [40]. Since then, intravesical BCG therapy remains widely used and recognized as a gold standard therapeutic tool for NMIBC post TURBT.

Originally, Morales et al suggested administration of an intravesical dose of 120 mg per week for 6 or 8 weeks for treatment of NMIBC [40]. Lately, maintenance therapy in addition to this induction therapy has been proposed to improve the efficacy of BCG therapy. The optimal regimen, however, in terms of frequency and duration of induction and maintenance doses is variable. The most commonly used protocol as given by Southwest Oncology Group (SWOG) recommends maintenance therapy consisting of intravesical BCG instillation per week for 3 weeks for 3, 6, 12, 18, 24, 30, or 36 months following TURBT [32]. Prolonged courses of BCG were found to be associated with decreased risk of recurrence of BC versus induction therapy in patients for high risk NMIBC, but also, an increased risk of adverse events [11].

Because of varied growth conditions and continuous passaging over the years across different parts of the world, the original 1921 BCG strain accumulated mutations and >14 sub-strains exist today [49]. *In vitro* results showed that the immunostimulatory activities of early strains (e.g., BCG Russia, Moreau, Sweden and Japan) are more than the late strains (e.g. BCG, Tice, Connaught, Danish and Glaxo) [23]. Rentsch *et al* compared the efficacy of two commonly used strains for prevention of recurrence of NMIBC, BCG Tice and BCG Connaught and found higher efficacy with BCG Connaught. The observation is supported by the fact that BCG Connaught induced stronger T helper type 1 (Th1) biased responses and is more immunogenic as compared to the other strain in an *in vivo* model [49]. Very recently, the results in phase III clinical trials have shown superior efficiency of BCG Connaught compared to BCG Tice and BCG Danish compared to BCG Tice in attaining recurrence-free survival [42]. BCG-Moreau is an effective substrain for adjuvant instillation therapies of NMIBC, which may serve as an equally effective alternative during worldwide shortage of BCG-TICE and Connaught [25].

4. IMMUNOLOGICAL BASIS OF ANTITUMOR ACTION OF

BCG

The antitumor action of BCG involves both innate and adaptive immune response involving activation of multiple immune cells as well as urothelial cells. The immune response against BCG gradually declines after induction therapy, which justifies the essentiality of a maintenance therapy in the treatment regimen. Intravesically administered BCG bacilli adhere to fibronectin in the bladder mucosa through a fibronectin attachment protein. Following attachment, bacteria are internalized and processed into cancerous as well as normal urothelial cells leading to primary innate immune response that involves the release of an array of cytokines and chemokines including IL-1, IL-6, IL-8 and TNF-α. This results in influx and activation of neutrophils, monocytes/macrophages, T lymphocytes and natural killer (NK) cells. The chemoattractants released by neutrophils recruit other immune cells like CD4+ T cells, CD8+ T cells, NK cells and dendritic cells (DC). Activated DCs, together with macrophages, trigger an anti-BCG-specific immune response via presentation of antigens to T cells that further amplifies immune response [37]. Activated leucocytes then produce a number of proinflammatory cytokines like IL-1, IL-2, IL-6, IL-10, IL-12, IL-18, IFN-γ, TNF-α etc and eliminate cancerous urothelial cells that internalized BCG. Importantly, it has been noted that a T helper 1 (Th1) biased cytokine profile shift (e.g., IFN-γ, IL-2, and IL12) is essential for a successful immunotherapy of BC using BCG, while a T helper 2 (Th2) biased cytokine (e.g., IL-10) profile is correlated with BCG failure (as reviewed in [58]).

5. KEY LIMITATIONS OF BCG IMMUNOTHERAPY IN

NMIBC

Although BCG therapy is a significantly successful therapy against NMIBC, it has a potential to produce local or systemic side effects that account for discontinuation of treatment in one-third of the cases. Frequent local effects include cystitis-like irritative voiding symptoms, frequency and hematuria. Systemic effects are less common but occur with greater severity. Skin rash, arthralgia occur in < 1% of patients, while fever, malaise and other flu-like symptoms may occur in up to

one quarter of patients. The most severe systemic complication that occurs in >0.1% is disseminated BCG infection, also called BCG sepsis that manifests as miliary TB (as reviewed in [45]).

The complete response rate to BCG therapy in patients with high-risk NMIBC can be >80%, but unfortunately, most of the patients having high-risk NMIBC suffer from recurrence or progression into MIBC. It has been estimated that 40-50% of patients treated with BCG for high-risk disease suffer from recurrence within one year of treatment. The term 'BCG failure' has been expanded into four different types namely BCG refractory, BCG resistant, BCG relapsing, and BCG intolerant [34]. A recent report demonstrated that NMIBC with pyuria exhibited a higher overall recurrence rate compared with NMIBC without pyuria [4].

6. USE OF RBCG IN CANCER IMMUNOTHERAPY

Considering the problem of intravesical BCG therapy failure in a significant proportion of cases, there is an imperative need for the development of more effective treatment options for NMIBC. BCG bacteria can be genetically engineered for expression of foreign genes of interest in order to enhance the efficacy as well as safety for use in cancer immunotherapy. Antigens expressed by these recombinant BCG (rBCG) strains can elicit both humoral and cell-mediated immune responses, including CD4+ and CD8+ T lymphocyte responses. With the aim of increasing the antitumor potential of BCG, various candidate genes have been used for construction of rBCG since the last two decades. The antigenic proteins expressed in BCG for this purpose can be broadly classified into two categories namely, *exogenous factors* including cytokines, tumor-associated antigen (TAA) genes and toxins from other bacteria; and *endogenous factors* including immuno-stimulatory mycobacterial surface antigens. In some of the reports, purified recombinant proteins have been used for intravesical administration in combination with BCG as adjuvant. The results with the use of rBCG expressing exogenous or endogenous proteins are more promising than combination therapy using BCG plus recombinant protein, reason being BCG can adhere to bladder wall for up to several months through fibronectin and is consequently internalized by bladder mucosa epithelial cells and macrophages [15, 48]. During its attachment to the bladder wall, rBCG can continue to express the factor while exogenously administered proteins cannot be sustained at amounts enough for induction of immune responses.

7. BCG AND EXOGENOUS FACTORS IN CANCER IMMUNOTHERAPY

IL-2

Interleukin-2 (IL-2), a Th1 cytokine can stimulate the production of cytotoxic T lymphocytes (CTLs) as well as also enhance the cytotoxicity of NK-cells and monocytes. Animals immunized with rBCG strain expressing IL-2 (rBCG-IL-2) showed significantly higher IFN-γ production by splenocytes as compared to native BCG [44]. Both IL-2 and IFN-γ are predominant cytokines in the urine of patients undergoing intravesical BCG therapy [37]. Intravesical delivery of recombinant IL-2 has shown satisfactory results in T1 papillary bladder carcinoma following TURBT, in which marker lesions regressed without much toxicity [13]. In NMIBC cases, who were untreated or had failed treatment with other intravesical agents, IL-2 based therapy was found to be an effective and safer option [19, 55]. Also, splenocytes from mice vaccinated with rBCG expressing human IL-2 in combination with a mycobacterial antigenic protein, ESAT-6 (early secreted antigenic target-6 kDa), rBCG-ESAT-6-IL-2 displayed higher CTL activity against tumor cells [16].

IL-18

Interleukin-18 (IL-18) is one of the immunoregulatory cytokines produced by macrophages that plays a significant role in both innate and adaptive immune response [56]. Intraperitoneal administration of purified IL-18 has led to retardation of primary tumor growth and suppression of lymph-node metastasis in tumor-bearing guinea pigs [52]. Also, the variations in promoter sequence of gene encoding IL-18 which may lead to altered IL-18 production and/or activity has been correlated with an individual's susceptibility to BC [26]. IL-18 has also been demonstrated to synergize with BCG for induction of a T-helper-type 1 (Th1) immune response. The splenocytes isolated from mice infected with an rBCG strain that secretes mouse IL-18 (rBCG-IL-18), in response to BCG antigen, produced higher levels of IFN-γ and GM-CSF, decreased levels of Th2 biased cytokines and stimulated proliferation and differentiation of cells secreting IFN-gamma. The recombinant strain also enhanced BCG-induced macrophage cytotoxicity against BC MBT-2 cells [38]. The *in vivo* antitumor efficacy of rBCG-IL-18 strain against BC remains unexplored.

IFN-α

Interferon- α (IFN-α) is an immunomodulatory cytokine that potentiates Th1 immune response by inhibiting IL-10 in addition to its anti-proliferative effect [28]. The combination BCG-IFN-α therapy is one of the most viable treatment options for patients with BCG-refractory and relapsing NMIBC. A phase II clinical trial showed that the low dose BCG plus recombinant IFN-α2B combination therapy can be particularly used for patients with previous BCG failures, those with Stage Tis cancer, and the elderly. Because of this treatment, 45–53% of patients who had failed prior BCG therapy could remain disease free at 24-month median follow up [29], while the same combination therapy in another patient population showed no better efficacy and toxicity over BCG monotherapy [5]. In another approach, human peripheral blood mononuclear cells (PBMCs) stimulated with rBCG strain secreting human IFNα-2b (rBCG-IFNα-2b) showed

enhanced proliferation and exhibited enhanced cytotoxicity towards human BC cell lines T24 and T5637. It showed to be a more promising agent than BCG monotherapy for BC patients as it could be used in low clinical dosage and so lesser side effects [14]. Since, rBCG-IFNα-2b strain continuously synthesizes and secretes IFNα-2b, one-time intravesical treatment is expected to induce a strong and prolonged immune activity against cancer. The combination of BCG and exogenous IFNα-2b is less clinically desirable due to short half-life of IFNα-2b, which therefore needs repeated intravesical administration [14]. Most importantly, rBCG-IFNα-2b strain was recently shown to have substantial antitumor efficacy in an orthotopic mouse model of BC. Mice administered with rBCG-IFNα-2b strain displayed prolonged survival with higher reduction in average bladder weight and higher monocyte and T lymphocyte levels as compared to BCG or BCG plus hIFNα-2b [54].

IFN-γ

Interferon-γ (IFN-γ) is another Th1 cytokine that is known to exert cytostatic and cytotoxic effects on tumor cells including BC cells [22]. In addition to being one of the major cytokines detected in the urine following BCG treatment, IFN-γ has been demonstrated to be involved in BCG-induced antitumor responses [43, 50] as also evident by the observation that higher levels of IFN-γ and IL-2 produced by PBMC on treatment with rBCG-IFNα-2b is crucial for the enhanced cytotoxicity [35]. Intravesical rIFN-γ monotherapy induced localized cellular immune response and imparted superior therapeutic efficacy over chemotherapy with MMC for NMIBC cases who underwent TURBT [21].

Treatment of murine BC cells with IFN-γ-secreting rBCG (rBCG-IFN-γ) strain upregulated the cellular expression of MHC class I molecules. Intravesical instillation of an rBCG-IFN-γ in mice model of orthotopic bladder tumor resulted in the local expression of IL-2 and IL-4 cytokines in addition to augmented influx of CD4+ cells into the bladder as compared to BCG monotherapy. Most importantly, IFN-γ based rBCG is a promising treatment option as a low-dose treatment regimen significantly prolonged animal survival [3].

Pertussis toxin (S1PT)

Nascimento et al. constructed an rBCG strain expressing pertussis toxin (PT) active domain subunit S1 (rBCG-S1PT) fused with the signal sequence and under the control of the upregulated M. fortuitum b-lactamase promoter [41]. Intravesical instillation of an orthotopic BC animal model with rBCG-S1PT elicited a stronger Th1-biased immune response than naïve BCG. The cytotoxicity assays showed that the splenocytes from animals treated with rBCG-S1PT strain were more effective in destroying MB49 tumor cells. Furthermore, rBCG-S1PT treated mice displayed significantly reduced average bladder weight and prolonged survival as compared to naïve BCG [2]. S1PT based rBCG could therefore be a useful substitute for wildtype BCG against NMIBC.

UC-1

Mucin-1 (MUC-1), a heavily glycosylated protein on epithelial cell surface is abnormally overexpressed in underglycosylated form in various epithelial-derived tumor cells, including breast, lung, ovarian, prostate and pancreatic tumors. Since underglycosylated MUC1 in malignant cells unmasks novel peptide and carbohydrate epitopes, it serves as a candidate TAA for development of vaccine for breast cancer and other epithelial adenocarcinomas. Despite their antitumor effect in animal models, some of these vaccines have been observed to induce only weak immune responses in humans. In an attempt to develop a live MUC1-cytokine coexpressing vaccine, Chung et al. constructed a rBCG strain that expresses a truncated form of MUC-1 as well as human IL-2, designated as BCG-hIL2-MUC1. Since N-glycosylation does not take place in BCG, it expressed MUC1 in malignant form. The SCID mice reconstituted with human peripheral blood lymphocytes (hu-PBL-SCID) were vaccinated with BCG-hIL2-MUC1 strain prior to challenge with human breast tumor cells to check the immunoprotective capability of this construct. As compared to BCG or MUC1 peptide, immunization of hu-PBL-SCID mice with BCG-hIL2-MUC1 strain inhibited the tumor growth while primary tumor formation was also prevented in certain animals [12]. Certain other rBCG strains expressing MUC-1 in combination with colony stimulation factor or CD80 significantly inhibited the growth of MCF-7 tumors implanted in hu-PBL-SCID mice [58].

Recently, in order to enhance the immunogenicity of MUC1, Fang et al generated a recombinant protein, MUC1-MBP consisting of MUC1 peptide in fusion with E. coli maltose-binding protein (MBP). Using BCG as an adjuvant, MUC1-MBP/BCG immunization was reported to induce a Th1 immune profile, stimulated MUC1‑specific CTL killing activity as well as inhibited growth of MUC1 bearing B16 cells in mice [17]. The efficacy of MUC1-MBP/BCG for prophylactic purpose has not been evaluated yet in animal model for BC.

PANVAC

A phase II randomized, prospective study to determine the efficacy of BCG in combination with a subcutaneous vaccine, PANVAC in adults with high grade NMIBC, who failed atleast one induction course of BCG initiated at the National Cancer Institute in 2013 and is currently underway (ClinicalTrials.gov; NCT no. NCT02015104). PANVAC, is a unique poxviral vector based ‘priming vaccine’ that has demonstrated therapeutic efficacy against a variety of carcinomas. It comprises of a replication-competent recombinant vaccinia vector, followed by ‘booster vaccines’ consisting of replication-incompetent recombinant fowlpox vector. It has been shown to induce a CD4 and CD8 antigen-

specific immune response against TAAs, carcinoembryonic antigen (CEA) and mucin-1 (MUC-1) which are overexpressed in high-grade BC. In addition, this vaccine also contains transgenes for three human T cell co-stimulatory molecules that can potentially augment an immune response namely B7-1, ICAM-1, and LFA-3.

8. BCG AND ITS ENDOGENOUS FACTORS IN CANCER IMMUNOTHERAPY

ESAT-6

ESAT-6, an immunodominant antigen has been intensively studied as a virulence factor of *Mycobacterium tuberculosis (M. tb)*. As mentioned earlier, rBCG-ESAT-6-IL-2 displayed higher immunogenicity than native BCG in an *in vivo* model [16]. Two BCG independent approaches have also been used to evaluate antitumor efficacy of ESAT-6 in animal models. In the first approach, He X et al engineered a viable tumor vaccine, B16F10/ESAT-6-GPI-IL-21, in which ESAT-6 acts as a heterogenetic antigen on the surface of B16F10 cells and IL-21 acts as an adjuvant. In mouse model of melanoma, this vaccine induced powerful antitumor responses to autologous tumors by eliciting immune responses, inhibiting TGF-β generation and promoting mechanisms that target inhibition of epithelial–mesenchymal transition in a process that can block tumor metastasis [24]. Very recently, Koyama et al developed a novel strategy to block immune escape of tumor cells by transfecting them with a therapeutic complex comprising of plasmid DNA encoding ESAT-6 in combination with polyethyleneimine and chondroitin sulfate. This complex induces presentation of the TB antigen on tumor cell surfaces, which can be recognized by APCs as a "danger signal" stimulating antitumor immune response, particularly amplified against TAAs thereby blocking immune escape of the tumor. When injected intratumorally into syngeneic melanoma tumor-bearing mice, the terenary therapeutic complex induced significant tumor growth suppression and complete tumor regression in combination with IL-2 gene [31]. The antitumor efficacy of this complex in combination with BCG as an adjuvant however remains unexplored.

Ag85B

An auxotrophic recombinant BCG strain overexpressing Ag85B, a major secretory antigen shared by BCG and *M. tb* (BCGΔleuD/Ag85B), has been reported to enhance the cytotoxicity to human bladder carcinoma cell line 5637. Interestingly, the expression level of pro-apoptotic, antioxidant and cell cycle-related genes increased after BCG ΔleuD/Ag85B treatment, whereas the mRNA levels of anti-apoptotic genes decreased. This is the first report of use of an auxotrophic BCG strain as a proposed therapy for BC. Since an auxotrophic complementation as a selectable marker is more suitable than antibiotic resistance genes for use in practical therapy, BCG ΔleuD/Ag85B therapy would be a safer option in the treatment of superficial BC [7].

Two other immunodominant mycobacterial antigens, namely Mpt64 [57] and PstS1 [51] have been used as exogenous recombinant proteins in orthotopic mice models of tumor and revealed substantial antitumor efficacy with enhanced Th1 type immune reponses. The competence of rBCG overexpressing PstS1, a promising TB vaccine [10] in immunotherapy against cancer has not been investigated so far.

Poly rBCG

Having known antitumor efficacy of BCG expressing endogenous mycobacterial antigens, Lee CF et al evaluated the efficacy of a combination of four rBCG DNA vaccines encoding Ag85A, Ag85B, Mpt64, PstS3, and ESAT6 (poly-rBCG) with murine IL-12 (mIL-12) in tumor cell killing and treating mice with bladder tumors. To improve antitumor efficacy, poly-rBCG plus mIL-2 preparation was introduced in vivo by electroporative gene immunotherapy (EPGIT) that led to significant inhibition of tumor growth and prolonged mouse survival. Electroporation of poly-rBCG + mIL12 resulted in complete tumor eradication in seven of eight mice model with subcutaneous tumor (P0.01) within 28 days [33]. This strategy presents better possibilities for highly effective treatment of BC using rBCG.

9. USING DC BASED IMMUNOTHERAPY FOR NMIBC

For the development of more specific and safer vaccines for cancer treatment, therapeutic designs are shifting towards the use of DC-based immunotherapy. The discovery of immunopotentiators for DC-based immunotherapy is particularly in demand as tumor cells escape immune system and have poorly immunogenic TAAs. The antigenic components on the surface of microbes called pathogen-associated molecular patterns are recognized by cells of the innate immune system through Toll like receptors (TLRs) during infection [1]. Certain mycobacterial antigens have been shown to activate DCs through interaction with TLRs and stimulate the differentiation and activation of cytotoxic T lymphocytes [9, 47]. Infection of human DCs with *M. tb* results in their maturation reflected by increased surface expression of maturation markers as well as secretion of elevated levels of inflammatory cytokines [27]. One of the TLR4 agonists, *M. tb* HspX has recently been reported to be highly effective in the induction of antitumor immune responses in DC-based cancer immunotherapy, in both *in vitro* and *in vivo* models [30].

In a study published in 2010, it was demonstrated that two cell wall-associated/secretory pro-glu polymorphic GC-rich sequence (PE_PGRS) proteins from *M. tb*, PE_PGRS 17 (Rv0978c) and PE_PGRS 11 (Rv0754), recognize TLR2, induce maturation and activation of human DCs, and enhance the ability of DCs to stimulate CD4+ T cells [5]. *M. tb* PE_PGRS33 protein has been proposed as an ideal target for

future TB vaccine design [20]. In view of antitumor immune responses of DCs, I suggest to explore the efficacy of rBCG overexpressing PE_PGRS family proteins namely PE_PGRS 17, PE_PGRS_11 and PE_PGRS33 as antitumor agents. This strategy is expected to enhance the immune responses to otherwise, less immunogenic, tumor associated antigens (TAA) as well. Further research should be encouraged towards identification of novel immunopotentiators for DCs and evaluation of therapeutic potential of DC based vaccines for the management of NMIBC.

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