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Platinum Opinion

Unraveling the Mechanism of the Antitumor Activity of Bacillus Calmette-Guérin

Roger Li^{a,b,*}, Scott M. Gilbert^a, Ashish M. Kamat^c

^a Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA; ^b Department of Immunology, H. Lee Moffitt Cancer Center, Tampa, FL, USA; ^c Department of Urology, MD Anderson Cancer Center, Houston, TX, USA

Although it has been the mainstay treatment for high-risk non-muscle-invasive bladder cancer (NMIBC) for more than four decades, our understanding of the mechanism of bacillus Calmette-Guérin (BCG) in controlling urothelial carcinoma continues to evolve [1]. Interrogations of post-BCG tissue and urine samples have revealed a complex and multifaceted immune response, with various cell types implicated in achieving therapeutic efficacy [1]. It is believed that intravesical BCG induces urothelial and antigen-presenting cells to produce cytokines and chemokines, leading to recruitment of additional cellular components of the innate immune response [1]. Moreover, numerous studies have demonstrated the critical importance of the activation of adaptive immunity for BCG response [1]. It is here, however, where the picture becomes blurred. Whether adaptive immunity is directed towards neoantigens specific to the tumor cells or the BCG particles taken up by tumor cells via micropinocytosis remains unclear [2]. To address this, Biot et al [3] showed that intravesical BCG led to expansion of BCG-specific T cells in the bladder-draining lymph nodes, which then trafficked into the tumor microenvironment within the bladder. Furthermore, a priori subcutaneous BCG immunization improved T-cell infiltration of the tumor microenvironment, leading to enhanced tumor elimination. On the basis of these results, they concluded that activation of BCG-specific T cells was essential for the ensuing antitumor response.

In contrast to this model, a recent study by Antonelli et al [4] showed that although adoptively transferred BCG-specific T cells trafficked to the bladder-draining lymph nodes as previously described, they conferred no additional antitumor activity when administered in conjunction with

BCG. Instead, adoptive transfer of tumor-specific T cells collected from mice cured of bladder cancer with intravesical BCG to another tumor-bearing, treatment-naïve mouse resulted in tumor rejection. Furthermore, it was found that BCG works by boosting the effector function of tumor-specific CD4⁺ T cells via enhanced activation/differentiation and attenuated checkpoint blockade (Fig. 1) [4]. These results convincingly demonstrated the tumor specificity of the effector CD4⁺ T cells central to the antitumor activity seen after intravesical BCG. Rather than serving as a direct target to enhance homing of T cells, BCG played an auxiliary role in supplementing the pre-existing adaptive antitumor response.

That the efficacy of intravesical BCG is underpinned by activation of CD4⁺ T cells is seemingly distinct from the CD8⁺ T-cell-driven immunoreactivity seen with other forms of immunotherapy, including checkpoint inhibition (CPI) [5] and oncolytic virotherapy (OV) [6]. The new mechanistic insight can therefore be used to personalize therapeutic strategies for high-risk NMIBC. For instance, in a recent immunohistochemical study of NMIBC samples collected before BCG treatment, 25–28% of nonresponders simultaneously expressed high levels of PD-L1 on tumor-infiltrating CD8⁺ T cells and lacked CD4⁺ T cells in the tumor microenvironment [7]. Given its dependence on a pre-existing CD4⁺ T-cell response, it is no surprise that BCG failed to confer a therapeutic benefit. Instead, CPI administration may reconstitute the potency of CD8⁺ T cells in these tumors, unleashing their immunoreactive potential.

Owing to the divergent mechanisms of action, BCG is less likely to synergistically potentiate the effects of CPI, although additive effects may be seen. To truly achieve synergy with CPI, combination agents capable of recruiting

* Corresponding author. Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center & Research Institute, 12902 USF Magnolia Drive, Tampa, FL 33606, USA. Tel. +1 813 7458343; Fax: +1 813 7456882.
E-mail address: roger.li@moffitt.org (R. Li).

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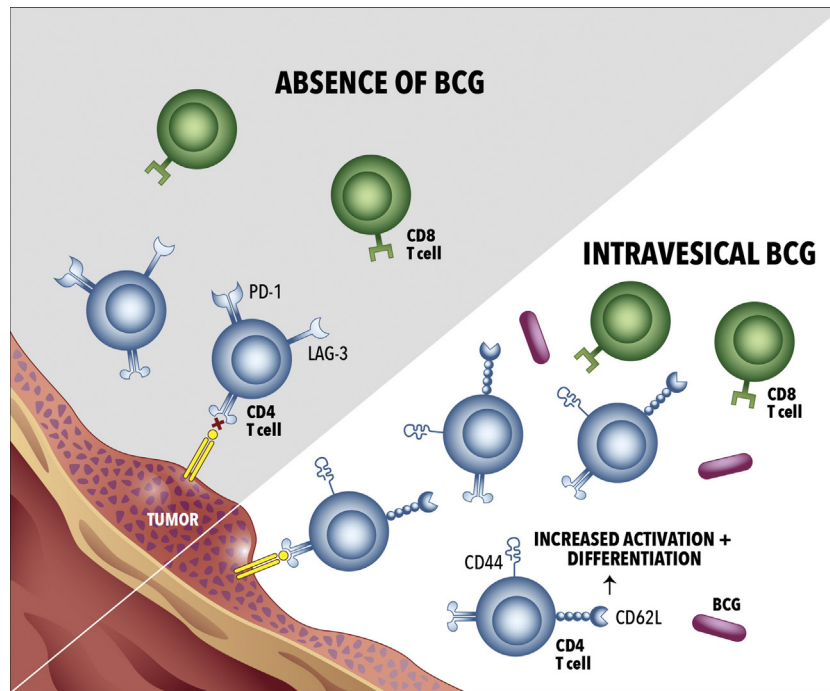


Fig. 1 – Intravesical bacillus Calmette-Guérin (BCG) treatment leads to proliferation, activation, and differentiation of CD4⁺ T cells. In a recent study conducted in syngeneic MB49 orthotopic immunocompetent mice [4], intravesical BCG treatment led to an increase in the proportion of CD4⁺ T cells, with increased proliferation and differentiation, measured by Ki-67, CD44, and CD62L expression, and a decrease in the expression of checkpoint markers including PD-1 and LAG-3. In comparison, no significant differences were observed in CD8⁺ T cells after BCG treatment.

tumor-reactive CD8⁺ T cells, such as rAd-IFN α /Syn3 and OV, are needed. A recently completed phase 3 clinical trial demonstrated that rAd-IFN α /Syn3 monotherapy had a complete response rate of 53.4% at 3 mo and 24.3% at 12 mo among 103 patients with carcinoma in situ containing BCG-unresponsive disease [8]. In preclinical and clinical correlative studies, tumor-infiltrating lymphocytes, particularly CD8⁺ lymphocytes, increased after treatment [9]. Oncolytic adenovirus CG0070 also had antitumor activity in a cohort of bladder cancer patients unsuccessfully treated with BCG [10]. Intriguingly, a recent clinical trial combining a different OV, talimogene laherparepvec (T-VEC), and pembrolizumab yielded an overall response rate of 62% in the setting of advanced melanoma [6]. Further analyses revealed that T-VEC increased CD8⁺ T-cell infiltration and PD-L1 protein expression and IFN- γ gene expression in the tumor microenvironment before the administration of pembrolizumab. Importantly, treatment response did not appear to be associated with baseline CD8⁺ T-cell infiltration. Together, these findings suggest that OV is capable of transforming the tumor microenvironment by recruiting tumor-infiltrating lymphocytes. Whether the success seen with this combination strategy can be replicated in bladder cancer remains to be seen.

In parallel to our deepening understanding of BCG-elicited adaptive immunity, much progress has also been made in uncovering its effects on innate immune cells. A phenomenon termed *trained immunity*, whereby BCG induces epigenetic changes in monocytes and natural killer cells leading to enhanced innate immune response, has

recently been highlighted in the protection against multiple viral infections. Grounded in these findings, BCG vaccination is currently being tested as a defense measure against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the viral pathogen behind the COVID-19 pandemic.

As we continue to unravel the complex mechanisms driving the enhanced immunity induced by BCG vaccination, we will also begin to understand the reasons for treatment failure and to better select novel agents to achieve therapeutic success. These are truly exciting times for bladder cancer patients, clinicians, and researchers as we continue our quest to conquer this disease.

Conflicts of interest: Roger Li serves on clinical trial protocol committees for CG Oncology and BMS, and is a scientific advisor/consultant for BMS and Fergene. Ashish M. Kamat is a scientific advisor/consultant for Merck, BMS, Eisai, Arquer, MDx Health, Photocure, Astra Zeneca, IBCG, TMC Innovation, Theralase, BioClin Therapeutics, FKD Industries, Cepheid, Medac, Asieris, Pfizer, Abbott Molecular, US Biotest, Ferring, Imagin, Cold Genesys, Roviand, Sessen Bio, CEC Oncology, and Nucleix, and holds intellectual property rights in CyPRIT (Cytokine Panel for Response to Intravesical Immunotherapy). Scott M. Gilbert has nothing to disclose.

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