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BCG Unleashed: Using the Immune Arsenal Against Bladder Cancer

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ABSTRACT
The strain of *Mycobacterium bovis* is BCG, which is a live attenuated strain mainly used as a vaccine against tuberculosis. BCG has been used for cancer treatment since clinical evidence was published in 1976. BCG is primarily used to treat high-risk non-muscle invasive bladder cancer, representing a significant clinical challenge due to high recurrence rates and progression potential. BCG remains the standard intravesical immunotherapy for high-risk NMIBC. According to data from model studies on humans and animals, the BCG vaccine triggers both innate and adaptive immune responses, which kill tumor cells. This review synthesizes the historical development of BCG therapy, elucidates the current understanding of its immunological mechanisms, and evaluates clinical efficacy and safety based on existing trials.
Keywords- BCG, Bladder cancer, Immunotherapy, Recombinant BCG, Novel BCG strain.

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I. INTRODUCTION

BCG administered intravenously has been the therapy of choice for intermediate- and high-risk non-small cell lung cancer (NMIBC) for more than four decades, making it the gold standard. Its significance as a therapy that preserves the bladder is supported by substantial data, since it slows the recurrence and development of non-muscle-invasive bladder cancer (NMIBC) [1]. The transitional cell epithelium is the origin of more than 90% of bladder cancers; around 80% of these tumors are non-muscle-invasive bladder cancers (NMIBC), which are characterized as being contained inside the mucosa or submucosa [2]. Bladder cancer is the sixth most frequent form of cancer in the United States, accounting for more than 14,000 fatalities annually. It is projected that more than 73,000 instances of the disease were detected in the year 2012. In addition to being the ninth most frequent cancer in the world, bladder cancer is also one of the most common tumors that have been found in the urinary system [3]. Nearly three-quarters of bladder cancer cases are identified at the early, superficial stages. The vast majority of the superficial urothelial cell carcinomas (TCCs) are papillary bladder carcinomas, and the remaining ten percent of these carcinomas are referred to as carcinoma in situ (CIS), which is characterized by high-grade, diffuse lesions that extend over the whole surface [4]. Investigators have been attempting to comprehend the mechanism of action of BCG as an anticancer modality ever since the first report of its intravenous use in the year 1976. Both the treatment plan and the dosage of BCG are decided by experience. The BCG treatment consists of a single course of six intravenous instillations that are administered once per week [4].

II. BACKGROUND HISTORY

2.1 Early Development of BCG

One of the weakened variants of *Mycobacterium bovis* is Bacillus Calmette-Guérin. BacillusCalmette–Guérin (BCG) was first produced at the Pasteur Institute in 1921 by Albert Calmette and Camille Guérin [5]. They began the development of a tuberculosis vaccine by utilizing a virulent strain of M bovis that had been isolated from the milk of an infected cow, in response to the escalating tuberculosis epidemic. Upon commencing their research in 1908, the duo observed that the virulence of M bovis diminished with each subsequent culture. The harmless strain of Mycobacterium bovis was identified as BCG in 1921 after being isolated in 231 subcultures on a medium of cooked potatoes preserved in ox bile [6] [7]. Since its first use by Morales in 1976, BCG has evolved into the therapy of choice for non-muscle invasive bladder cancer (NMIBC) [8]. It is recommended that patients with high-risk malignancies receive full-dose intravesical BCG for 1–3 years. However, BCG, a live organism, may induce infections with adverse effects ranging from mild cystitis to fatal sepsis in rare circumstances [4].

2.2 Transition to Bladder cancer immunotherapy

In 1921, Albert Calmette and Camille Guérin established that the isolated bacillus was not only non-pathogenic in animal models but also conferred protection against TB in vaccinated humans. Subsequently, large-scale manufacture of BCG began for the prevention of TB in humans, and it remains the only commercially accessible vaccination for this disease. During that period, the use of a combination of two bacteria, Serratia marcescens and Streptococcus pyogenes, was explored for cancer therapy, while the potential utilization of the recently produced and safe BCG presented an innovative treatment alternative for some cancer patients. While several studies indicated the potential effectiveness of the new BCG in treating various cancer types, it was not until the 1970s that BCG received approval as an immunotherapeutic therapy for individuals with bladder cancer (BC) [9] [10]. In the early stages of research on the effects of BCG on bladder cancer, it was shown that BCG stimulated both the innate and acquired immune systems of the bladder. In addition to activating the immune system to fight bladder cancer, the findings demonstrated that BCG had a direct impact on the tumor cells, causing them to undergo apoptosis, necrosis, and oxidative stress, among other consequences [3] [11] [12].

III. Mechanism of action

It is still not entirely known how BCG treatment works to treat bladder cancer. While many theories have been advanced on how BCG works, the two most common are the following: first, BCG interacts directly with urothelial and bladder cancer cells. Second, an innate immune response is activated; and third, BCG-specific and tumor-specific T cell immunity are initiated [5].

3.1 Interaction of BCG with the bladder wall

An initial stage for BCG is to interact with the luminal surface of the bladder. BCG Interaction in Bladder Wall.
To begin, BCG must come into contact with the bladder's luminal surface [5]. To infiltrate urothelial cells, bacteria like BCG must overcome the mutual repulsion caused by the negatively charged glycosaminoglycans found on the surface of normal urothelium [13]. BCG can bind to urothelial cells, which might help with subsequent immune responses. The process by which BCG binds to the urothelium involves the joining of mycobacterial fibronectin attachment proteins (FAPs) to host fibronectin. This leads to the attachment of BCG to urothelial cells through integrin α5β1 [4], which is then taken up by bladder cancer cells through micropinocytosis. This ultimately activates the urothelium and triggers inflammatory reactions within the bladder. Urothelial cell uptake of BCG is not necessary for BCG's effectiveness, according to definitive in vivo studies [5]. Recent research provided circumstantial support for this process by showing that BCG expressing the mannose-binding protein FimH exhibited enhanced adhesion to and internalization by urothelial cells, as well as a stronger anti-tumor response compared to wild-type BCG [14].

3.2 Induce Innate Immune Activation

At first, BCG activates the body's innate immune system. Both healthy and malignant cells in the bladder may absorb it via its interactions with the epithelium. This triggers the release of cytokines and chemokines, attracting immune cells to the location [15] [16] [17].

3.2.1 Pattern Recognition Receptors

By attaching to host pattern recognition receptors (PRRs), pathogen-associated molecular patterns (PAMPs) trigger an innate immune response. Host PRRs can identify several PAMPs produced by BCG. The Toll-like Receptors (TLRs) TLR2, TLR4, and TLR9 are among them; they activate the MyD88 signaling pathway, which controls the synthesis of cytokines that promote inflammation [17]. Multiple mycobacterial lipid components may activate toll-like receptor 2 (TLR2), which is expressed on many innate immune cells, including monocytes, polymorphonuclear cells (PMNs), B cells, and T cells [18] [19]. Myeloid cells express Toll-like receptor 4, which binds heat-shock proteins produced by mycobacteria [20]. Dendritic cells, macrophages, and natural killer (NK) cells express Toll-like receptor 9, which may be triggered by DNA from mycobacteria. Additionally, a MyD88 defective mouse had no response to BCG in a subcutaneous model of BCG therapy, suggesting that TLR signalling is involved in BCG's effectiveness [21]. Not only do TLRs respond to BCG PAMPs, but so can complement receptors [22], NOD-Like Receptor NOD2 [23], Dectin-1, DC-SIGN, and other C-type lectin-like receptors [24] [25].

3.2.2 Macrophages

As BCG binds to the urothelium, it is taken up by APCs and bladder cancer cells. These cells then release chemokines and cytokines, which entice granulocytes and mononuclear cells to the bladder [26]. Epithelioid and Gigantocellular granulomas, which include macrophages, dendritic cells, lymphocytes, neutrophils, and fibroblasts, are characteristic of these occurrences and are discovered in the bladder wall after BCG instillation [26] [27]. Using human urothelial cancer cell lines,

researchers have shown that BCG increases cytokine production. This includes IL-6, IL-8, GM-CSF, and TNF [28]. In investigations conducted on humans, cytokines and chemokines such as (IL-1β), (IL-8), (IL-15), (IL-18), 'CXC-chemokine ligand 10' (CXCL10), 'GM-CSF', 'CC-chemokine ligand 2' (CCL2), and CCL3 were detected in urine samples taken after BCG instillation [28] [29]. These chemokines and cytokines may be detected in the urine within one day following BCG instillation, with maximal expression occurring between two and eight hours after instillation [26][28].

3.2.3 Natural Killer Cells

Natural killer (NK) cells are a subset of the innate lymphoid cells (ILCs) family [30], which is experiencing fast expansion. In vitro, natural killer (NK) cells exhibit cytotoxicity against bladder cancer cells that have been infected with BCG [31]. This cytotoxicity may also be seen in the immune infiltrate of bladder tumors in mice. In animal models of bladder cancer, the survival advantage afforded by BCG is rendered ineffective when the cells are depleted using an antibody targeting NK1.1. This suggests that either NK cells or NKT cells are necessary for the effectiveness of BCG [32].

3.3 Adaptive immune response

Additionally, when macrophages take up BCG by phagocytosis, antigen-presenting cells work to break it down into its component antigens. These cells then activate CD4+ and CD8+ T cells by interacting with components that make up the major histocompatibility complex class II (MHC-II). Surprisingly, researchers discovered that, in addition to typical APC, bladder cancer cells induced by BCG showed significant surface expression of MHCII, which turned the tumor cells into APC [4] [33] [34]. This protection against MHC-II molecule degradation occurs when BCG stimulates TLR, which in turn causes MARCH 1 ubiquitin ligase expression to be down-regulated.

In this way, the immunological response is boosted. Once the antigen is presented to T cells by APC, CD4+ +T cells will secrete cytokines such as IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, TNF-α, IFN-γ, GM-CSF, and others [35] [36].

IV. The success of immunotherapy with PD-1/PDL-1 inhibitors in advanced-stage bladder cancer

The Food and Drug Administration (FDA) has authorized five immunotherapeutic medications, three of which target PD-L1 and two of which target PD-1, for the treatment of bladder and other urothelial carcinomas (UCs) [37].

4.1 Effect of PD-1/ PD-L1 on BCG immunotherapy

PD-1/PD-L1 inhibitors are a category of immunotherapeutic agents that function by obstructing the interaction between PD-1 (programmed cell death protein 1) on T cells and PD-L1 (programmed death-ligand 1) on cancer cells. In a normal immune system, this contact would inhibit T cell function. These medications improve the immune system's capacity to identify and destroy cancer cells by blocking this mechanism. On tumor cells, you may find the crucial immunological checkpoint known as programmed death ligand-1 (PD-L1), which is identical to the PD-L1 receptor found on T cells. Programmed death-1 (PD-1) allows T cells to connect to tumor cell surface PD-L1, and when combined, the two cause T cells to mistake the cell for a normal cell and stop attacking tumor cells. Consequently, drugs that target this checkpoint have been a major focus of tumor immunotherapy research over the last several years [38]. Furthermore, both in vivo and in vitro studies on bladder cancer treatment with a combination of anti-PD-L1 and BCG demonstrated that tumor-infiltrating CD8+ + T cells increased in number and activity after the combination drug therapy, while MDSCs decreased in number. Immune fatigue and the pre-treatment adaptive immune response may be caused in part by certain BCG failures. The term "pre-treatment adaptive immune response" describes the process by which CD8+ + T cells are recruited to produce INF-γ after the pre-BCG therapy. This starts the anti-tumor response, which then elevates PD-L1 to avoid being recognized by immune cells [3].

4.2 Early Clinical Trials

4.2.1 Anti-PD-L1 immunotherapies

According to Powles's 2014 report, atezolizumab was the first anti-PD-1/PD-L1 antibody medication evaluated for immunotherapy of bladder and urothelial cancers [39] [40]. Compared to a historical chemotherapy control with a 10% overall objective response rate (ORR), a multicentre phase II study with atezolizumab (IMvigor 210), which achieved a 15% overall objective response rate (ORR), was statistically higher [41]. First-line atezolizumab therapy was beneficial for individuals who were ineligible for cisplatin, according to results in IMvigor 210. When it comes to treating locally advanced or metastatic UC, atezolizumab has two indications: first-line (for patients who are not eligible for cisplatin) and second-line (for those who are). A 2018 phase III study demonstrated that atezolizumab offers a prolonged response duration and fewer adverse effects compared to chemotherapy [42]. The phase

I/II multicentre study findings led to the FDA's rapid approval of durvalumab as a second-line treatment for locally advanced/metastatic UC [43], as described in two separate publications. Avelumab is the third FDA-approved anti-PD-L1 antibody for second-line treatment of locally advanced/metastatic bladder cancer [44].

4.2.2 Anti-PD-1 immunotherapies

Phase I/II CheckMate 032 and phase II CheckMate 275 are two multicentre clinical studies that have demonstrated the safety and effectiveness of nivolumab for locally advanced/metastatic UC. In contrast to CheckMate 275, which did find a significant correlation between PD-L1 expression and therapy response, CheckMate 032 failed to do so [45] [46]. A phase Ib clinical study, KEYNOTE-012, first examined the safety and effectiveness of pembrolizumab in treating post-platinum locally progressed or metastatic UC [47]. Pembrolizumab was shown to be effective in treating advanced UC in a phase III study called KEYNOTE-045. The overall response rate (ORR) was substantially greater than chemotherapy in both the total patient group ($p = 0.001$) and in patients with a combined PD-L1 positive score of 10% or more ($p = 0.0034$). Both the overall OS ($p = 0.0004$) and in the population with high PD-L1 expression ($p = 0.005$) were considerably longer in the pembrolizumab group compared to the chemotherapy group. Additionally, a phase II trial found that pembrolizumab is beneficial as a first-line therapy for cisplatin-ineligible locally advanced or metastatic UC, with PD-L1 expression being associated with response [39].

4.3 Examination of current BCG usage recommendations

There are several organisations that have put out recommendations for how to administer BCG to patients with non-muscular inflammatory bladder cancer (NMIBC). These include AUA (the American Urological Association), EAU (the European Association of Urology), the NCCN (National Comprehensive Cancer Network), IBCG (the International Bladder Cancer Group), and the ICUD (International Consultation on Urological Diseases). Each of these groups has put forth its own set of recommendations [48] [49] [50] [51].

4.3.1 Benign Tumor/low-grade Tumor

When it comes to small, solitary, superficial low-grade tumors, there is consensus that BCG is not necessary because the risk of disease progression is so low. However, when it comes to multiple, large, or recurrent low-grade tumors (the intermediate-risk category), BCG therapy—with or without maintenance-is—is seen as optional according to the EAU and AUA guidelines and the IBCG [48] [51] [49].

4.3.2 Malignant tumor/higher-grade tumor

Although there is some variance in the suggested period of treatment, overall, the guidelines for the use of BCG in high-grade tumors are rather similar. According to the AUA, EUA, and IBCG, all high-grade tumors should be treated with BCG induction, followed by maintenance for one to three years [48] [51] [50].

There is a lack of clear information about the impact of maintenance BCG on disease development in Ta high-grade tumors, which is why it is not included in ICUD recommendations. Only carcinoma in situ (CIS) is ICUD-recommended for maintenance, and the guideline specifies that if the first assessment does not reveal a response, reinduction of BCG instillations should be considered [52] [53].

V.Safety and Efficacy

The risk of adverse effects with BCG is higher than from intravesical chemotherapy. Serious adverse effects, however, are uncommon and often manageable. Irritative urinary symptoms, including dysuria, frequency of urine, and urgency of urine, are the most prevalent adverse effects of BCG. Typically, these symptoms won't last forever. Patients who continue to have these symptoms should be checked for bacterial cystitis and given antibiotics if it is found to be present [5]. In cases with BCG infection, antituberculosis medication therapy is recommended. This treatment includes two more months of two antimicrobials after the first four months of daily antimicrobials [9]**.** Concerning BCG treatment, another important concern is the effectiveness of the various sub-strains. There doesn't seem to be a clear winner among the strains, according to some research. Comparing BCG versus intravesical chemotherapy, many randomized controlled trials (RCTs) and meta-analyses have shown that BCG lowers the chance of tumor recurrence. According to several studies, the recurrence decrease rates might reach 30-50% [9].

VI.The Failure of BCG Treatment

Typically, the term "BCG failure" refers to the return or advancement of the bladder tumor while the patient is undergoing treatment. There are four types of BCG failure: refractory, relapsing, intolerant, and unresponsive [54]. The differentiation among these categories is crucial, as each signifies a unique response to therapy: BCG refractory indicates the persistence of high-grade tumors following one induction and one maintenance course of BCG; BCG relapse refers to the revival of a tumor after a disease-

free interval; and BCG intolerance denotes the inability to endure at least one complete induction course of BCG. Each category involves specific follow-up treatment strategies [55] [56]. Emphasizing two parts of the categorization will help us understand BCG failure better. On the first induction round of BCG, 25-60% of patients do not respond; however, on the second round, administered within six months, they will respond. For better disease therapy and clinical trial design may be achieved with a clear understanding of what constitutes a BCG failure [57] [58] [59].

VII.Future of Non-invasive BCG Treatment

Several viable tactics are used to rationalize the utilization of BCG. These strategies include the development of various administration schedules as well as the manipulation of BCG to enhance its immunotherapeutic impact [9]**.**

7.1 Combination Therapy with BCG for NMIBC

Some BCG combination therapies have been created to improve effectiveness and reduce side effects, because intravesical BCG is more effective and has less toxicity than chemotherapy. Reduced BCG dose allowed patients to better manage adverse effects and finish the BCG with the chemotherapy regimen. In terms of enhancing recurrence-free survival, overall survival, and disease-specific survival, a meta-analysis of 13 RCTs showed that combining BCG with chemotherapy was considerably better than intravesical BCG instillation [60]. The two groups did not vary in toxicity terms. The most frequent chemotherapeutic medicines for NMIBC, including Mitomycin C, Epirubicin, and Pirarubicin, were included in the meta-analysis for their effectiveness [61] [62]. Combining BCG with immune checkpoint inhibitors may be an effective therapy for NMIBC, particularly for individuals who have been determined to have BCG failure. Malignant malignancies, particularly bladder tumors, have extensively used immune checkpoint inhibitors, such as PD-1/PD-L1 antagonist antibodies. Combining anti-PD-1 antibodies with BCG for NMIBC therapy was supported by higher PD-L1 expression in tumor tissues after intravesical BCG treatment, which was associated with a decreased five-year recurrence. A humanized monoclonal antibody targeting PD-1, pembrolizumab, has been used in the treatment of several advanced malignancies. The effectiveness and side effects of treating individuals with NMIBC with BCG plus pembrolizumab were examined in a clinical trial [61] [63] [64].

7.2 Current Alternatives to BCG

Rescue individuals who do not react to BCG treatment have been the primary focus of research on improving NMIBC therapy, as BCG is effective in preventing progression and recurrence events for most people. As carriers for particular tumor growth inhibitory medicines or immunostimulatory components, viruses and other bacteria that are not BCG are among the alternative treatment choices. Other alternatives include chemotherapeutic drugs, innovative delivery mechanisms for existing treatments, chemotherapy, and systemic immunotherapies [9]. At 6 months, 21% of patients treated with intravesical valrubicin in a phase 2 trial involving 90 patients with recurrent CIS following BCG instillations had a full response; however, at a median follow-up of 30 months, the disease-free rate was only 8%. Additionally, valrubicin was associated with an increase in local bladder symptoms in comparison to other treatments [65]. Relapsing and refractory individuals have shown modest success when administered gemcitabine, an inhibitor of DNA synthesis. In a study of 25 patients with resistant NMIBC who did not respond to BCG therapy, the safety and effectiveness of injecting both gemcitabine and docetaxel were assessed; the HG-RFS at 1 and 2 years were 49% and 34%, respectively [54] [66].

By blocking cytokine release and T cell activity, immunotherapy proteins PD-1 and PD-L1 enable tumors to evade the anticancer response [67]. PD-L1 expression on tumor cells was shown to be associated with the advancement of the tumor, and the development of inhibitors of PD-1 and PD-L1 represents a significant step forward in the treatment of patients who are not responding to BCG immunotherapy [68]. An expression of PD-L1 was shown to be related to a worse survival rate along with the failure of BCG immunotherapy in a retrospective study of sixty-five patients with primary CIS [54].

VIII.Novel BCG strains

Very little research has attempted to address the question of whether or not various BCG sub-strains vary in terms of safety and toxicity. In recent years, the toxicity of BCG Tice, Moreau, and RIVM was compared in 844 patients. It was found that BCG Tice caused more local and mild systemic adverse effects than the other BCG strains that were tested. Patients who received BCG RIVM had more severe complications, and patients who received two different strains had severe complications right after the treatment switch. The switching of sub-strains during treatment decreased the first adverse events seen in another trial that compared BCG Connaught with BCG Japan. Based on these results, more

research is needed to determine if BCG strains are safe for patients with NMIBC [69].

IX.CONCLUSION

Malignancy of the bladder is the most prevalent malignancy among men. It is possible to classify 75% of bladder cancer patients as having non-muscle invasive bladder cancer, and 25% of patients as having superficial bladder cancer, one of two forms of the disease. Only patients with NMIBC, particularly those with high-risk NMIBC, are given BCG therapy. Two to four weeks after the removal of the bladder tumor is when the BCG therapy starts. In the first, or "induction," phase of BCG treatment, patients get injections once weekly for six weeks. The second, called "maintenance," phase lasts for ten months and consists of monthly injections. Several new combination therapies, including immunotherapy, chemotherapy, and the RITE approach, which combines BCG immunotherapy with other drugs, have recently been developed to treat patients who do not respond to BCG alone.

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