Oncol Ther
https://doi.org/10.1007/s40487-026-00445-8

REVIEW

Beyond the BCG Paradox: Biological Rationale and Clinical Evidence for Combining Intravesical BCG with Immune Checkpoint Inhibitors in High-Risk Non-muscle-Invasive Bladder Cancer

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Received: March 18, 2026 / Accepted: May 13, 2026

ABSTRACT

Bladder cancer (BC) is the most common malignancy of the urinary tract, with more than 75% of cases diagnosed as non-muscle-invasive bladder cancer (NMIBC). Intravesical Bacillus Calmette–Guérin (BCG) remains the gold standard adjuvant treatment for high-risk NMIBC owing to its ability to induce a robust local immune response within the bladder tumor microenvironment. Nevertheless, a substantial proportion of patients experience disease recurrence or progression, highlighting the

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Published online: 11 June 2026

need for improved therapeutic strategies. This narrative review examines the biological rationale and available clinical evidence supporting the combination of intravesical BCG with immune checkpoint inhibitors (ICIs) in high-risk NMIBC. A literature review was conducted to analyze the immunological mechanisms underlying BCG-induced antitumor activity, adaptive immune resistance, and the potential role of ICIs in modulating the tumor microenvironment. Clinical trials were evaluated with particular attention to patient populations, study design, safety profiles, and efficacy end points. From a biological perspective, BCG acts as an immunological primer by recruiting and activating innate and adaptive immune cells, while immune checkpoint inhibitors may counteract functional exhaustion of BCG-induced antitumor responses. However, translation of this rationale into consistent clinical benefit remains challenging. Early-phase studies have reported variable response rates in selected patient populations, particularly in BCG-unresponsive disease. In contrast, recent phase III data have shown that upfront combination strategies do not necessarily translate into improved oncological outcomes. Overall, while the combination of BCG and immune checkpoint inhibition is supported by a strong immunological rationale, current clinical evidence remains heterogeneous and largely derived from early-phase or ongoing trials, precluding definitive conclusions regarding long-term oncological benefit and durable bladder preservation. Despite a strong immunological rationale, the combination of BCG with immune checkpoint inhibitors remains investigational. Its adoption into routine clinical practice will require mature phase III evidence, validated predictive biomarkers, and a clear demonstration of superiority over established bladder-preserving treatment strategies.

PLAIN LANGUAGE SUMMARY

Bladder cancer is a common condition, and in many cases it does not spread into the muscle of the bladder. In these patients, a treatment called Bacillus Calmette–Guérin is commonly used to stimulate the immune system to fight cancer cells. However, this treatment does not work for everyone, and the disease may return or worsen over time. New therapies have been developed to help the immune system better recognize and attack cancer. This review explores whether combining these treatments could improve patient outcomes. Although there is strong scientific reasoning behind this approach, current studies show mixed results, with only limited benefit thus far. Further research is needed to understand which patients may benefit most and how best to use these treatments together.

Keywords: Non-muscle-invasive bladder cancer; Bacillus Calmette–Guérin; Tumor microenvironment; Immune checkpoint inhibitors; PD-1/PD-L1; BCG-unresponsive disease; Immunological priming; Adaptive immune resistance; Bladder preservation; Cancer immunotherapy

Key Summary Points

Why carry out this study?

High-risk non-muscle-invasive bladder cancer (NMIBC) is associated with high recurrence and progression rates despite intravesical Bacillus Calmette–Guérin (BCG), and a significant proportion of patients develop BCG-unresponsive disease.

There is a critical unmet need for more effective bladder-preserving therapeutic strategies.

What was learned from the study?

The combination of BCG with immune checkpoint inhibitors is supported by a strong biological rationale, but current clinical evidence remains heterogeneous, with recent phase III data demonstrating only a statistically significant but modest benefit for upfront combination strategies, without clear improvements in progression or cystectomy-free survival.

These findings suggest that patient selection, treatment timing, and biomarker-driven approaches will be essential to optimize the role of combination immunotherapy in NMIBC.

INTRODUCTION

Bladder cancer (BC) is one of the most prevalent urological malignancies worldwide, with a steadily rising incidence, ranking as the 10th most commonly diagnosed cancer. Although the primary risk factors include tobacco smoking, urinary infections, and occupational exposures, the disease is notably more frequent in men, with incidence rates approximately four times higher than in women [1–3]. More than 75% of initial cases of BC are diagnosed as non-muscle invasive bladder cancer (NMIBC), a form that does not involve the muscular layer of the bladder. If inadequately treated, NMIBC has the potential to progress to muscle-invasive bladder cancer (MIBC) in 30–40% of cases [4–8]. Considering the limited array of choice in the treatment of BC, except for surgery, the imperative need to develop medical therapies as alternatives to radical cystectomy is evident [17]. Originally developed in the early twentieth century as a tuberculosis vaccine, Bacillus Calmette–Guérin (BCG) was introduced by Morales et al. in 1976 for the treatment of NMIBC [4, 9–13]. It works by stimulating both the local innate and the adaptive immune response, thereby inhibiting tumor growth [18]. Clinical guidelines (EAU, AUA, NCCN) recommend adjuvant BCG therapy for high-risk NMIBC, particularly for patients with T1 tumors and high-grade (G3) disease [14–17]. Despite advances in immunotherapy research, BCG continues to play a crucial role in NMIBC management [20–24]. However, its efficacy is not without limitations: up to 30–40% of patients treated with BCG may experience recurrence, and approximately 30% of these cases progress to MIBC [19]. This high failure rate translates into a significant clinical burden, as patients with BCG-unresponsive disease face limited bladder-preserving options and often require radical cystectomy—a procedure associated with substantial morbidity and quality-of-life impairment. This clinical reality underscores the "BCG paradox:" an initial, robust immune activation that often fails to translate into durable tumor control, leading to recurrence and progression [25, 26, 28, 30]. This failure is not merely a lack of immune response, but a consequence of the tumor's adaptive resistance. The concept of adaptive immune resistance—whereby tumor-infiltrating T cells upregulate inhibitory checkpoints such as PD-1/PD-L1 in response to ongoing inflammation—provides a compelling biological framework for combining BCG with immune checkpoint inhibitors (ICIs). Moreover, it remains unclear whether BCG failure is primarily due to tumor-induced immunosuppression or to an incomplete and dysfunctional immune response induced by BCG itself [30–34]. To address this paradox, a dynamic view of resistance is required, focusing on the evolving interactions within the tumor microenvironment (TME) [27, 29, 30, 35]. The TME is not a static entity but a dynamic ecosystem that influences both the induction and regulation of the immune response against the tumor. [33, 34, 38]. It promotes mechanisms of immune counter-regulation, where immunosuppressive factors such as cytokines, regulatory T cells, and abnormal dendritic cells prevail, thereby hindering an effective response to BCG therapy [35–37, 39]. The study of TME suggests a potential role for ICIs in the treatment of NMIBC, although results have been variable [39–41]. The efficacy of ICIs may depend on the need for an immunologically primed TME, which in NMIBC tends to be less immunogenic and more immunosuppressive compared with

advanced cancer. Given that BCG can convert an immunologically "cold" or "ignorant" microenvironment into an inflamed, T-cell-infiltrated milieu, it represents an ideal partner to sensitize NMIBC to subsequent or concurrent ICI therapy. A strategy to enhance ICI efficacy could involve combining these drugs with other therapies, such as BCG, to strengthen the immune response and optimize therapeutic outcomes [35, 39–41]. In this context, combining BCG with ICIs has been proposed as a strategy to enhance immunological priming and restore effective antitumor immunity within the bladder tumor microenvironment. BCG recruits and activates immune cells, while ICIs block inhibitory signals and prevent the functional exhaustion of the immune response, thereby enabling a more durable and effective antitumor effect [35, 37, 41]. However, translating this sound biological rationale into consistent clinical benefit has proven challenging, as evidenced by the modest outcomes of recent phase III trials such as POTOMAC. This review critically examines the immunological basis for BCG-ICI combinations and synthesizes the available clinical evidence, with a focus on patient selection, treatment sequencing, and future directions. In the following sections, the immunological mechanisms underlying the effectiveness of BCG, tumor resistance, and the potential of combining BCG with immune checkpoint inhibitors (ICIs) were examined, while also addressing the clinical challenges associated with these therapeutic strategies [41]. The aim of this narrative review is to critically evaluate the biological rationale and current clinical evidence supporting the combination of intravesical Bacillus Calmette–Guérin with immune checkpoint inhibitors in high-risk non-muscle-invasive bladder cancer, with particular focus on treatment sequencing, patient selection, and future clinical implications.

METHODOLOGY

This narrative review was carried out in order to summarize and critically discuss the biological basis and existing clinical data of intravesical BCG combined with ICIs in high-risk NMIBC. The literature search was done in PubMed/MEDLINE, Scopus, Google Scholar, and Web of Science databases, and it included publications up to October 2024. To incorporate recently published phase III trial data and emerging evidence, the search was subsequently updated to include relevant studies published through March 2026. This ensured that key recent publications were appropriately captured and discussed. The search terms comprised various combinations of: "non-muscle-invasive bladder cancer," "BCG," "immune checkpoint inhibitors," "PD-1", "PD-L1," "immunotherapy," "tumor microenvironment," and "BCG-unresponsive disease." Original articles, clinical trials (phase I–III), translational studies and relevant systematic or narrative reviews published in English were considered for inclusion. Extra attention was given to studies investigating the immunological basis of BCG's effects, adaptive immune resistance, and clinical trials of combined or sequential BCG and ICIs regimens. Only when necessary to provide a background to the ongoing phase III trials, were conference abstracts and unpublished data included. Of note, a small number of 2025 and 2026 publications were manually incorporated during revision to reflect the most current clinical evidence. Studies were chosen on the basis of their relevance to the topic, the clarity of patient populations, and study end points, in addition to their contribution to the understanding of the biological mechanisms or clinical outcomes. Owing to the variation in the data available and the fact that most of the trials are early-phase or ongoing, the authors decided to qualitatively synthesize the data instead of doing a formal meta-analysis. The interpretation of the results explicitly took into account the limitations of the current evidence, including differences in study design, patient selection, and follow-up duration. Several methodological limitations should be acknowledged. The authors selected and interpreted the studies included in their narrative review according to their personal judgment, which created a different process from the systematic review and meta-analysis methods. The literature discussed in this study does not include all available evidence because some evidence remains unavailable, and some evidence was chosen through

selective processes. The clinical evidence under examination comes from early-stage clinical trials and current research projects, which include different patient groups and assess different treatment outcomes while conducting short-term patient monitoring.

Ethical approval: This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

COMPARATIVE IMMUNOBIOLOGY—MECHANISMS OF ACTION OF BCG AND PD-1/PD-L1 INHIBITORS

The Bladder Tumor Microenvironment: Immunological Architecture and Functional Plasticity

The bladder TME is a highly specialized immunologic system formed by the ongoing exposure to antigens, mechanical stress, and the peculiar barrier role of the urothelium. Under physiological conditions, the bladder is in a state of immune surveillance, which is counterbalanced by tolerance mechanisms in order to suppress excessive immunity activation. Resident innate immune cells, such as macrophages, dendritic cells, mast cells, and innate lymphocytes, together with a very limited number of tissue-resident immunologic memory cells, consisting of $CD8^+$ and $CD4^+$ T cells, play a pivotal role in the regulation of this process [42, 43]. Throughout tumorigenesis, there is a disturbance in this balance. High-grade NMIBC is marked by an active and diverse tumor microenvironment in which the presence of immune activation and immune suppression occurs concomitantly. There may be immune infiltration and occasional lymphocytic aggregates in early lesions that point towards a partial immune recognition. This immune infiltration can be disorganized and immunologically not very effective against the tumor cells [44]. Immunologically speaking, NMIBC cases rarely express the typical "immune-cold" phenotype. Rather, they usually exhibit the "primed but constrained" phenotype. This condition is characterized by inflammation with incomplete and ineffective clearance of tumor. This continuous exposure to antigens, as well as tissue damage, can lead to immune adaptation rather than immune eradication. Tumor cells are actively involved in this immune adaptation by regulating cytokine gradients, reducing the function of antigen presentation machinery, and turning on the production of immunosuppressive cell pools such as regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), and dysfunctional macrophages with the M2 phenotype. Notably, the bladder TME shows significant immunological plasticity and dynamically changes according to the progression of the tumor and the treatment. The instilled therapies, especially BCG, further modulate this milieu through the engagement of the immune response and the activation of compensatory inhibitors. Such an ever-changing milieu can explain the variability of treatment responses and the need to appreciate the nature of the bladder TME when developing immunotherapeutic approaches [45].

Immunological Effects of BCG: From Trained Innate Immunity to Adaptive Dysfunction

BCG Immunotherapy: BCG Immunotherapy is believed to have antitumor effects owing to a complicated multi-layered immune reaction involving innate as well as adaptive immunity [46, 47]. BCG, when instilled intravesically, adheres to the urothelium as a result of fibronectin interactions and enters the urothelial cells as well as the antigen-presenting cells, activating the pattern recognition receptors TLR2, TLR4, TLR9, and NOD receptors, thus inducing the innate immune system [48, 49]. The initial response phase involves the activation of innate immune cells. Neutrophils, macrophages, natural killer cells, and dendritic cells quickly accumulate within the bladder wall. They produce proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and CXCL10. Apart from the classical activation response, another phenomenon called

"trained immunity" occurs. This involves the innate immune activation that results from the immune reprogramming that occurs. This reprogramming results from the bacterial effects, perhaps due to metabolic alterations. This results in these immune cells being highly responsive [50]. However, the efficacy of BCG ultimately relies on the effective transition between innate activation and adaptive immune priming. BCG-stimulation of dendritic cells, in conjunction with exposure to tumor antigens, leads to the maturation of dendritic cells, which then traffic to lymphoid aggregates, giving rise to a Th1-biased immune response. This resultant Th1 bias is essential for the induction and activation of $CD8^+$ cytotoxic T cells, as these cells are the main initiators of the eradication of the tumor cells. IFN-γ significantly contributes to this mechanism through augmenting antigen presentation and cytotoxic differentiation, hence ensuring immune control of tumors [51, 52]. This powerful immune cascade, however, often fails to promote sustained tumor rejection by BCG. Several mechanisms lead to the functional dysfunction of BCG. There can be reduced expression of MHC class I antigens, compromised interferon signaling, or resistance to apoptosis in the T cells. Simultaneously, there is an inflammatory environment in the TME that promotes the compensation of immunity by the TME, with the help of Tregs and MDSCs, or the production of inhibitory cytokines [53]. A key component of immune evasion in patients treated with BCG involves the process of adaptive immune resistance, which occurs as a direct result of the ongoing inflammatory cytokine expression in the bladder microenvironment [54]. Even though the cytokine IFN-γ is critical to the process of antitumor immunity because of its involvement in augmenting immune responses, such as the mechanisms of enhanced presentation of antigens and the promotion of a Th1 immune response, as well as supporting cytotoxic T-cell responses, the continuous secretion of IFN-γ has a paradox [55]. Repeated or chronic exposure to BCG, with continuous IFN-γ signaling, leads to increased PD-L1 expression not only on tumor cells, but also on infiltrating immune cells, such as macrophages and dendritic cells, in a physiological negative feedback mechanism intended to suppress tissue injury due to excessive IFN-γ stimulation. However, in the tumor environment, there is attenuation of effector T-cell function due to engagement of the PD-1/PD-L1 pathway [56]. In terms of function, T-cells in this chronically inflamed environment become transformed from an "activated" phenotype into an "exhausted" phenotype. This can be described by characteristics including attenuation of proliferation ability, impaired secretion of effector cytokines such as IFN-γ and TNF-α, reduced cytotoxic function, and upregulation of inhibitory receptors. Crucially, the exhausted T-cells can still be "present" within the tumor microenvironment despite being "incapable" of generating effective "antitumor" pressure [56, 57]. Consequently, the bladder TME assumes the characteristics of the "immune-inflamed but functionally suppressed" stage, in which the immune cell infiltration is maintained or increased, but the ability to suppress the tumor is lost. This clinical situation corresponds to the unresponsiveness of the BCG treatment in the face of ongoing immune activation, which immediately explains the lack of success of additional BCG instillations in restoring the antitumor response. On the other hand, this situation corresponds to an immune dysfunction state mediated by adaptive resistance [58]. This phenomenon represents the biological core of the so-called "BCG paradox": a therapy capable of inducing a strong inflammatory and immune response that nevertheless frequently fails to achieve durable tumor eradication. The coexistence of immune activation and functional immune suppression within the bladder tumor microenvironment, therefore, provides a conceptual framework for combination strategies aimed at restoring effective antitumor immunity. In this context, immune checkpoint inhibition emerges as a biologically coherent approach, capable of reversing adaptive immune resistance and sustaining the cytotoxic immune response initially triggered by BCG.

PD-1/PD-L1 Blockade: Immunological Prerequisites and Intrinsic Constraints

Immune checkpoint agents that target the PD-1/PD-L1 axis work by reactivating the functional

ability of exhausted T-cells within the TME [59, 60]. Physiologically speaking, the PD-1/PD-L1 axis has a pivotal role in maintaining immune tolerance within the peripheral tissues. In cancer, on the other hand, this regulatory pathway can be aberrantly used by tumor cells as a means of escaping immune surveillance [61]. At a cellular level, binding of PD-1 on activated T cells to its ligand PD-L1 transmits inhibitory signals for attenuating the activation of the T-cell receptor, cell proliferation, cytokine secretion, and latent cytotoxicity against tumor cells. Permanent activation of this pathway leads to a condition of functional exhaustion in these cells, indicated by decreased antitumor responses despite their persistence in the microenvironment surrounding cancer cells. Immune checkpoint inhibitors block such interactions to reactivate exhausted cells in cancer therapy [62, 63]. Importantly, the blockade of PD-1/PD-L1 does not trigger an "innate" immune response from scratch. Efficacy requires the presence of an existing, antigen-specific adaptive immune response that is suppressed rather than absent. From an immunologic viewpoint, the following conditions precedent to the inhibition of the immune regulatory "checkpoint" must be satisfied: sufficient infiltration of antitumor T cells, functional machinery for antigen presentation, and functional pathways for interferon that can trigger the immune response even in the absence of inhibitory stimuli [64]. Within the setting of NMIBC, these criteria are not consistently fulfilled. While there is immune infiltration, the effector T cells are commonly dysfunctional, absent from the tumor surface, or confined within a microenvironment that limits cellular metabolism. Further, the tumor cells can be resistant via mechanisms such as reduction in major histocompatibility complex expression, antigen processing and presentation, or pathways for interferon response. Such aspects make it difficult for revived effector T cells to target these neoplastic cells. In addition, the bladder tumor microenvironment has higher numbers of immunosuppressive cell populations including regulatory T-cells, myeloid-derived suppressor cells, and tumor-associated macrophages bearing an immunoregulatory phenotype. These cells work oppositely to the cytotoxic immune response through inhibitory cytokine production and cellular interactions [65]. Biologically, these factors are known to be responsible for the recently observed limited response rates in NMIBC to immune checkpoint inhibitors used singly. Tumors in whom there is a lack of sufficient priming or those exhibiting intense immune dysfunction would not likely benefit from immune checkpoint blockade used singly. Intratumoral blockade of PD-1/PD-L1 would increase PD-1 binding affinity to its ligand but would not counteract impaired antigen recognition. Taken together, these findings suggest that immune checkpoint inhibitors will be most successful in NMIBC when administered in a permissive immunological milieu—a setting in which there is preexisting infiltration by effector immune cells that are functionally suppressed in their antitumor activity. This theoretical model supports a combined approach that pairs immune checkpoint blockade with immunostimulatory agents, such as BCG, that can augment antigen secretion, immune cell infiltration, and adaptive immune priming. By synchronizing immune activation with immune release, combination regimens attempt to address the natural shortcomings of immune checkpoint monotherapy [66, 67].

CLINICAL EVIDENCE FOR COMBINING BCG AND IMMUNE CHECKPOINT INHIBITORS

The biological synergy between BCG and ICIs has prompted numerous clinical trials aiming to translate this rationale into tangible patient benefit. The following sections critically examine the available evidence from these studies, highlighting the heterogeneity in patient populations, trial designs, and outcomes.

The Biological and Clinical Rationale for Combination Therapy

The mechanistic differences and functional complementarities between BCG and PD-1/PD-L1 inhibitors form the biological

foundation for their combination. BCG acts as an immunological primer, promoting antigen release, APC activation, and T-cell recruitment [49, 68]. However, this inflammation simultaneously induces adaptive immune resistance, primarily through IFN-γ-driven upregulation of PD-L1, leading to T-cell exhaustion [56, 57]. Blocking the PD-1/PD-L1 axis can counteract this resistance, "releasing the brakes" on BCG-primed, but functionally exhausted, T cells, thereby sustaining cytotoxic pressure [67, 69]. This synergy is particularly relevant in BCG-unresponsive disease, where an immune infiltrate is often present but functionally compromised. However, the question of optimal timing for the combination remains open. Concomitant or sequential strategies could exploit different immunological windows, with significant implications in terms of efficacy and toxicity [67]. These aspects have a direct impact on the design of clinical trials and the future integration of such approaches into clinical practice. Clinical trials exploring these strategies include heterogeneous populations, comprising BCG-naive high-risk patients, previously patients treated with BCG, and more frequently, patients with BCG-unresponsive disease who are not candidates for or reluctant to undergo radical cystectomy [70, 71, 73]. The heterogeneity in clinical data requires a critical analysis that considers variations in patient selection, definitions of response, and end points. (see Table 1) Clinically relevant outcomes include the complete response rate, duration of response, recurrence-free and progression-free survival, as well as the ability to preserve bladder function while avoiding or delaying radical cystectomy [73–75].

Table 1 Key mechanisms of immune evasion underlying BCG failure and the biological rationale for adding immune checkpoint inhibitors

Mechanism of immune escape	Biological consequence in bladder TME	Clinical impact	Proposed therapeutic strategy	Related studies/ Approaches
IFN-γ-induced upregulation of PD-L1	Functional inhibition of $CD8^+$ T cells; immunological exhaustion	Initial response followed by early recurrence	PD-1/PD-L1 axis blockade	KEYNOTE-676, CheckMate 7G8
Incomplete activation of adaptive immunity	Persistent but noncytotoxic inflammation	Functional failure of BCG	Combination BCG + checkpoint inhibitors	KEYNOTE-676, NIVO + BCG
Expansion of Tregs and MDSC	Immunosuppressive environment; cytotoxic inhibition	Reduced duration of response	Integration of immunomodulators (IL-15 agonists)	QUILT-3.032
Defects in antigen presentation	Reduced tumor recognition	Primary resistance to BCG	Adaptive strategies based on biomarkers	ADAPT-Bladder
Alterations in interferon signaling pathways	Reduced sensitivity to cell-mediated immunity	Secondary resistance	Combinatorial and sequential approaches	SWOG Studies
"Immune-inflamed but inhibited" microenvironment	T infiltrate present but nonfunctional	Inefficacy of intensified BCG	Sequential BCG → checkpoint blockade	CheckMate 7G8

BCG Bacillus Calmette–Guérin, *CD8* cluster of differentiation 8, *IFN-γ* interferon-gamma, *IL-15* interleukin-15, *MDSC* myeloid-derived suppressor cells, *PD-1* programmed cell death protein-1, *PD-L1* programmed death-ligand 1, *TME* tumor microenvironment, *Tregs* regulatory T cells

Clinical Trials of BCG and PD-1/PD-L1 Inhibitor Combinations

Intravesical BCG coupled with ICIs has been the focus of several clinical trials for high-risk NMIBC. The primary objective is to enhance the local control of the disease whilst maintaining bladder function. However, the different studies vary greatly in terms of the patient populations, timing of treatment, and clinical end points; thus, their results need to be interpreted carefully. Several phase III trials are investigating this combination. KEYNOTE-676 (NCT03711032) is evaluating pembrolizumab plus BCG versus BCG alone in BCG-naive patients, with results eagerly awaited [76]. Similarly, CheckMate 7G8 (NCT04149574) is testing nivolumab with BCG in a broader population of BCG-naive and -treated patients [77]. It is worth noting that, unlike the POTOMAC trial, which enrolled only BCG-naive patients, CheckMate 7G8 includes a broader population of both BCG-naive and patients treated with BCG, which may influence trial timelines and outcomes. The first mature data from a phase III trial, the POTOMAC study, provided important insights into combination therapy [78]. This trial investigated the addition of durvalumab to BCG induction and maintenance therapy in BCG-naive, high-risk patients with NMIBC. The results demonstrated a statistically significant improvement in disease-free survival (DFS) with the combination versus BCG alone (hazard ratio [HR] 0.84, 95% CI 0.71–0.99, $p = 0.036$). However, the magnitude of benefit was modest and secondary end points—including prevention of progression to muscle-invasive disease and avoidance of radical cystectomy—did not show statistically significant improvement. These findings provide a critical reality check: while biological synergy exists, the clinical benefit of upfront combination therapy is limited in unselected BCG-naive populations. The POTOMAC results create a need to investigate how to best combine checkpoint inhibition with BCG therapy, emphasizing the importance of patient selection, biomarker-driven approaches, and optimized therapeutic sequencing rather than uniform treatment intensification. Several non-mutually exclusive explanations may account for the lack of clinical benefit observed. First, the biological activity of PD-L1 blockade may depend on the presence of a sufficiently primed immune microenvironment, which may not yet be fully established at the time of initial BCG administration in treatment-naïve patients. The combination of BCG and checkpoint inhibition fails because it fails to use the adaptive immune resistance period that develops through ongoing immune stimulation. The neutral result emerged because of the patient selection process. BCG-naive populations include a substantial proportion of patients who are already likely to respond to BCG alone, thereby reducing the potential incremental benefit of adding systemic immunotherapy. The trial design elements had an impact on study outcomes. The assessment of disease-free survival or recurrence-free survival end points needs extended patient monitoring to detect immunological effects that emerge after checkpoint blockade. The possibility of drug-specific factors remains as an unproven assumption. The differences in pharmacodynamic properties between PD-1 and PD-L1 inhibitors and how they interact with local intravesical immune activation affect the level of therapeutic synergy they create with BCG. Taken together, these considerations suggest that the failure of the POTOMAC trial should not necessarily be interpreted as evidence against the biological validity of BCG-checkpoint inhibitor combinations, but rather as an indication that treatment timing, patient selection, and tumor immunobiology are likely critical determinants of therapeutic success. In contrast to these large, ongoing phase III trials in BCG-naive patients, early-phase studies have focused on the high-unmet-need population with BCG-unresponsive disease. Besides the phase III trials, there have been additional efforts in phase I/II trials to combine BCG and ICIs for a more selective group of patients, particularly those with BCG-unresponsive disease who refuse or are not suitable for radical cystectomy. Phase I and II trials of BCG combined with pembrolizumab or nivolumab have established the practicality of these treatment modalities and

have shown promising complete response rates in certain subpopulations. Nevertheless, these trials suffer from several limitations, such as small sample sizes, nonuniform inclusion criteria, and short duration of follow-up, which means that it is not possible to make definite statements about response durability, effectiveness in preventing progression, or long-term bladder preservation rate. Additionally, the use of adaptive and biomarker-driven strategies is also being explored. The ADAPT-Bladder study is one of the attempts to personalize immunotherapy escalation based on tumor and microenvironment features, thus not applying the same treatment intensification to all [79]. While conceptually appealing, the result data remains preliminary and insufficient to be used in everyday clinical practice. Similarly, combination approaches incorporating immunomodulators beyond PD-1/PD-L1 blockade, such as the IL-15 super agonist N-803 tested in QUILT-3.032, indicate that the joint activation of the innate and adaptive immune systems may provide alternative or complementary strategies, especially in BCG-unresponsive NMIBC [80]. Overall, it is currently known that the biological reason for the combination of BCG and immune checkpoint inhibitors is very convincing, but the lack of solid phase III data providing a clear clinical benefit is evident. (see Table 2) The negative or neutral findings of the POTOMAC trial, for example, point out that combination immunotherapy should not be taken for granted as being universally effective and underline the necessity of biomarker-driven patient selection, optimized sequencing, and longer follow-up before the widespread use of this strategy in clinical practice [78].

Preliminary Evidence: Efficacy and Safety

Collectively, available evidence indicates that the combination of intravesical BCG and immune checkpoint inhibitors can provide clinical benefits in a selected subpopulation of high-risk patients with NMIBC, particularly in cases of BCG-unresponsive or recurrent disease [81, 82]. Complete response rates reported in preliminary studies with pembrolizumab or nivolumab in association with BCG are encouraging, especially in patients with mucosal-limited disease and without muscle-invasive progression. The duration of response remains, however, a still-undefined parameter. Limited follow-up in the majority of studies makes it difficult to determine whether initial responses translate into durable oncological control and a real reduction in the risk of progression or cystectomy, an aspect particularly relevant considering the objective of bladder preservation [82]. Regarding safety, according to preliminary data, the BCG-checkpoint inhibitor combination is generally feasible but associated with an articulated toxicity profile, reflecting the overlap of local and systemic adverse events. BCG-related toxicities primarily affect the lower urinary tract with irritative symptoms such as dysuria, pollakiuria, urinary urgency, and hematuria, frequently reported during treatment and often cumulative with the number of instillations [84–87]. In a minority of patients, more serious complications can manifest, such as granulomatous prostatitis, orchiepididymitis, or systemic dissemination of the mycobacterium [88–90]. ICIs, on the other hand, cause systemic immune-mediated adverse events from nonselective activation of the immune response [91]. Common manifestations include dermatological, gastrointestinal, and endocrinological toxicities. Events such as autoimmune hepatitis, interstitial pneumonia, nephritis, or myocarditis are rare but potentially severe and require early recognition and specialist management [92–95]. A significant difficulty in combination strategies is the discrimination between local BCG toxicity and initial manifestations of immune-mediated adverse events affecting the urinary tract. Symptoms such as dysuria or pollakiuria can reflect either local inflammation or immune-mediated involvement.(see Table 3) Moreover, the management of these events might require the use of immunosuppressive therapies, with consequent repercussions on the overall efficacy of the immunotherapeutic strategy [96, 97].

Table 2 Principal clinical studies evaluating combination strategies between BCG and immunotherapy in high-risk NMIBC

Study/trial	NCT Number	Phase	Population	Experimental intervention	Control arm	Primary end points	Study status	Rationale/key message
KEYNOTE-676	NCT03711032	III	High-risk NMIBC, BCG-naive	BCG + pembrolizumab	Standard BCG	RFS, duration of response, progression	Ongoing, results awaited	Testing if PD-1 blockade can enhance BCG priming in the first-line setting
CheckMate 7G8	NCT04149574	III	High-risk NMIBC, BCG-naive + BCG treated	BCG + nivolumab	Standard BCG	RFS, event-free survival	Ongoing, results awaited	Evaluating if PD-1 blockade can overcome adaptive resistance in a mixed BCG-exposed population
Nivolumab + BCG (early-phase)	NCT03519256 (representative)	I/II	BCG-unresponsive NMIBC	BCG + nivolumab	None / historic	CR rate, safety	Completed	Early-phase study demonstrating feasibility and safety in BCG-unresponsive disease
ADAPT-Bladder	NCT03775031	II	High-risk NMIBC (biomarker-driven)	Adaptive immunotherapy ± BCG	Variable	CR rate, response biomarkers	Ongoing	Pioneering biomarker-driven, adaptive trial design to personalize immunotherapy

Table 2 continued

Study/trial	NCT Number	Phase	Population	Experimental intervention	Control arm	Primary end points	Study status	Rationale/key message
QUILT-3.032	NCT03022825	II	BCG-unresponsive NMIBC (CIS ± Ta/T1)	BCG + N-803 (IL-15 superagonist) ± nivolumab	None / indirect comparison	CR rate, duration of response	Completed / follow-up	Exploring alternative immunomodulation (IL-15) beyond PD-1/PD-L1 to enhance BCG response
POTOMAC	NCT03528694	III	High-risk NMIBC, BCG-naive	BCG + durvalumab	Standard BCG	DFS, progression	Completed and published, statistically significant DFS benefit but modest magnitude	Landmark phase III trial demonstrating that while upfront PD-L1 blockade with BCG improves DFS, the benefit is modest and secondary end points were not significantly improved, highlighting need for optimized patient selection

Table 2 continued

Study/trial	NCT Number	Phase	Population	Experimental intervention	Control arm	Primary end points	Study status	Rationale/key message
SWOG Studies (various)	Multiple (e.g., NCT04164082, NCT02324582)	II/III	High-risk NMIBC / BCG-exposed	BCG + immunotherapy (sequential or combined)	Standard of care	RFS, safety	Ongoing	Aiming to standardize trial designs and endpoints for combination regimens

Direct comparisons between studies are limited by heterogeneity in patient populations, study design, therapeutic sequencing, and follow-up duration

BCG Bacillus Calmette–Guérin, *CIS* carcinoma in situ, *CR* complete response, *DFS* disease-free survival, *IL-15* interleukin-15, *NCT* National Clinical Trial, *NMIBC* non-muscle-invasive bladder cancer, *PD-1* programmed cell death protein-1, *PD-L1* programmed death-ligand 1, *RFS* recurrence-free survival, *SWOG* Southwest Oncology Group

Positioning BCG-Checkpoint Inhibitor Combinations within the Current Therapeutic Landscape

The therapeutic landscape of NMIBC, especially in the case of BCG-unresponsive disease, has changed dramatically over the last 10 years. Radical cystectomy is still the oncological standard for patients who fail BCG therapy [98], but several bladder-sparing options have been approved by the regulatory authorities, thus providing alternative strategies for patients who are either unwilling or unfit for surgery. Therefore, any discourse on BCG plus ICIs combinations should be seen in the context of this rapidly growing treatment paradigm. Pembrolizumab monotherapy was the first systemic immunotherapy that the U.S. Food and Drug Administration (FDA) approved for patients with BCG-unresponsive carcinoma in situ, and this was based on the results of the KEYNOTE-057 trial [99]. Complete response rates were clinically meaningful in a subset of patients; however, the duration of the response was limited, and many responders eventually relapsed. These results demonstrated the value of systemic PD-1 blockade as a bladder-sparing option, but at the same time, they showed that there is still a need for better strategies to achieve long-lasting disease control. A variety of bladder-preserving interventions have recently been added to the arsenal through nadofaragene firadenovec intravesical gene therapy by delivering interferon-α into the bladder through a nonreplicating adenoviral vector and thus aims to stimulate a localized and long-lasting immune response in the bladder [100]. Clinical results have indicated that a portion of BCG-unresponsive patients with NMIBC can experience durable responses, thus confirming that intravesical immunomodulation is still a valid alternative to systemic therapy. Meanwhile, combination regimens of BCG and new immunostimulatory agents have exhibited significant potential. The mixture of BCG and IL-15 super agonist N-803 is a biologically very different approach that boosts innate and adaptive immune responses without directly targeting immune checkpoints

Table 3 Toxicity profile of BCG-immunotherapy combination strategies

Type of toxicity	Intravesical BCG	Checkpoint inhibitors (PD-1/PD-L1)	Combination BCG + CPI	Clinical implications
Local urinary toxicity	Dysuria, pollakiuria, urgency, hematuria	Rare (immune-mediated cystitis)	Frequent; difficult etiological distinction	Necessitates strict clinical monitoring
Local inflammatory complications	Granulomatous prostatitis, orchioepididymitis	Not typical	Possible symptomatic overlap	Differential evaluation
Systemic toxicity	Rare (disseminated BCG)	Rash, colitis, endocrinopathies, hepatitis, pneumonitis	Cumulative risk	Multidisciplinary management
Immune-mediated adverse events	Not applicable	Potentially severe (G3–G4)	Possible need for steroids	Impact on efficacy
Need for immunosuppression	Rare	Not infrequent	Critical during combination therapy	Careful risk–benefit balance
Treatment interruption	Occasional	Dependent on severity	More frequent in combination trials	Accurate patient selection

BCG Bacillus Calmette–Guérin, *CPI* checkpoint inhibitor, *G3–G4* Grade 3 to Grade 4, *PD-1* programmed cell death protein-1, *PD-L1* programmed death-ligand 1

[101]. The technique has obtained good response rates in BCG-unresponsive illness and has helped expand the idea of immunotherapy beyond PD-1/PD-L1 blockade only. In fact, combinations of BCG with immune checkpoint inhibitors within this setting should be considered as experimental therapies rather than the existing standard of care. BCG ICI combinations, unlike authorized bladder-sparing therapies, currently have no mature phase III data that clearly demonstrate the modalities of the benefit (durable response, progression prevention, or cystectomy-free survival) that would be expected. Compliance with local safety and systemic toxicity may additionally represent the same substance causing more than one adverse effect. Every case of a patient who potentially can benefit from the treatment has to be carefully selected, and the risk–benefit ratio considered. Therefore, the availability of multiple FDA-approved bladder-preserving options leads to several good reasons for a cautious and evidence-based positioning of BCG checkpoint inhibitor combinations. In other words, instead of a panacea, these tactics could eventually be only workable in such patient subsets that will probably be determined by immune activation and resistance biomarkers. Trials that are presently running and those that will come in the future will be instrumental in clarifying the extent to which BCG ICI combinations might confer benefits over the other therapies within the contemporary NMIBC treatment landscape (Fig. 1).

DISCUSSION

This narrative review critically examines the biological rationale and existing clinical evidence regarding the combination of intravesical BCG and ICIs in high-risk NMIBC [70, 81]. While the biological rationale is convincing, the existing clinical evidence should be cautiously assessed in view of the recent findings from the phase III clinical trials and the changing landscape of treatments. From a biological perspective, the combination of BCG and PD-1/PD-L1 inhibitors is well justified. Indeed, the use of BCG

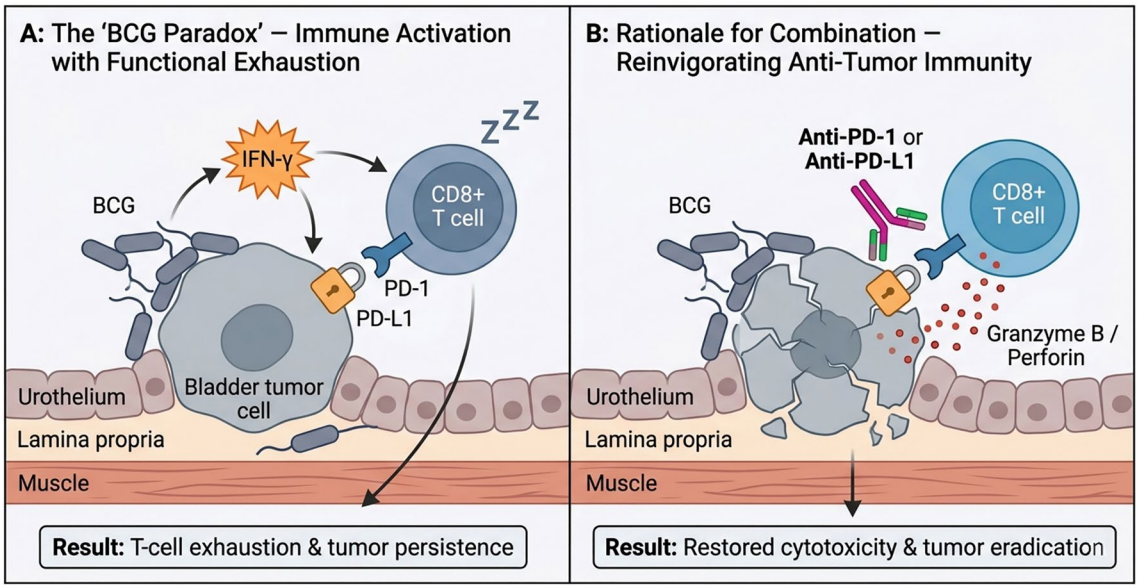

Fig. 1 Mechanistic rationale for combining BCG with PD-1/PD-L1 blockade to overcome T-cell exhaustion in bladder cancer. **A** The "BCG paradox": BCG-induced immune activation drives IFN-γ production and PD-L1 upregulation, leading to PD-1-mediated $CD8^+$ T-cell exhaustion and persistent tumor. (B) Combination with anti-PD-1 or anti-PD-L1 antibodies blocks the inhibitory checkpoint, restoring $CD8^+$ T-cell cytotoxic function (Granzyme B/Perforin) and enabling tumor eradication across the urothelium, lamina propria and muscle layers. Abbreviations: BCG, Bacillus Calmette–Guérin; CD8, cluster of differentiation 8; IFN-γ, interferon-gamma; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1. During the preparation of this work, the authors used Gemini (Google, Gemini 1.5 Pro, May 2024) to refine the visual alignment and layout consistency of Fig. 1. No scientific content, data or conceptual elements were generated or modified using Gemini. The authors reviewed and refined the figure to ensure scientific accuracy and take full responsibility for the content

is characterized by its strong immunostimulatory effects on the bladder mucosa, leading to increased amounts of antigens released and innate immune cell infiltration with activation of the adaptive antitumor immune response within the bladder tumor microenvironment. Nonetheless, immune stimulation is often followed by the phenomenon of "inhibitory feedback," where the immune system turns against itself through mechanisms involving increased expression of immune checkpoints, functional exhaustion of effector T cells, and other immune checkpoints. In the context of this biological phenomenon, the use of immune checkpoint inhibitors represents a logical step aimed at reversing the phenomenon of immune adaptation and restoring cytotoxic activity of effector T cells against the bladder cancer cells, which are no longer recognized by the immune system within the unique immune context created in the bladder mucosa after the instillation of the BCG agent. While the biological rationale is robust and well-substantiated, the challenge of translating the concept into a favorable clinical benefit has been daunting indeed. In view of the revised clinical trials, the recent findings in the interventional studies involving combinations of ICI and intravesical instillation of the BCG agent in the treatment of NMIBC remain mixed indeed. In the category of the hypothesis-testing studies, the KEYNOTE 676 and CheckMate Trial 7G8 are yet to be finalized on efficacy end points, while the POTOMAC Trial demonstrated that upfront addition of durvalumab to BCG provided a statistically significant but modest improvement in disease-free survival without clear benefits in preventing progression or reducing cystectomy rates. This provides a critical reality check that biological plausibility alone is insufficient to predict meaningful

therapeutic success and that modest improvements in surrogate end points may not translate into clinically meaningful benefits for patients. These findings imply that uniform upfront combination therapy for all BCG-naive patients does not represent a major advance over standard BCG alone [76–78]. As explained in the methodology adopted within this review, there is no evidence available to allow the performance of a quantitative analysis or draw definitive conclusions with regard to long-term end points such as progression prevention, cystectomy-free survival rates, or bladder preservation. Considering this evidence gap, it is important to be cautious before attempting to draw conclusions based on response rates. Finally, there is a critical role played by the rational integration of BCG/ICI combination treatments within the modern therapeutic algorithm of NMIBC. In recent years, several bladder-sparing treatments have been approved for the use of BCG-refractory patients. These include systemic pembrolizumab treatments, intravesical gene therapy using nadofaragene firadenovec, as well as immunomodulation with the N-803 agent [100]. Given the availability of several evidence-based bladder-preserving therapies, new BCG-ICI combinations must demonstrate a clear incremental benefit before being integrated into clinical practice. Indeed, it is being recognized with greater frequency within contemporary research almanacs of urologic oncology that patients with NMIBC exhibit a complex heterogeneity of immunologic characteristics. This is such that certain patients will exhibit similarly varied consideration with regard to the application of treatments based primarily upon a new set of platform principles. Biomarkers reflecting tumor microenvironment composition, immune activation, interferon signaling, and post-BCG immunological remodeling may prove essential to identify patients most likely to benefit from checkpoint inhibition [44, 60]. Future strategies should therefore move away from uniform treatment intensification toward biomarker-driven and adaptive therapeutic approaches. Safety considerations further reinforce the need for caution. The combination of intravesical BCG with systemic ICIs introduces the potential for overlapping local and immune-mediated toxicities, complicating clinical management and potentially limiting treatment adherence. Given that bladder preservation is pursued with curative intent in high-risk NMIBC, the balance between therapeutic benefit and treatment-related risk must be carefully evaluated on an individual basis [83, 84]. In summary, this review highlights a persistent gap between biological rationale and clinical validation for BCG-ICI combination therapy in NMIBC. This gap reflects a broader challenge in immuno-oncology: effective immunotherapy often depends less on the presence of immune activation than on the precise timing and biological context in which it is therapeutically exploited. While immunological insights strongly support continued investigation, current evidence does not justify widespread clinical adoption. Progress in this field will depend on mature phase III data, refined patient selection, integration of predictive biomarkers, and a clear demonstration of added value over existing approved bladder-sparing therapies.

MAJOR UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

Though there is a strong biological plausibility supporting the integration of intravesical BCG with ICIs, there are several important questions that remain unanswered and need to be answered before they can be applied on a wider scale. The discrepancies between highly plausible immunobiology and the inconsistent results at the clinical level reflect the complexities being experienced during modulation of the immune system in patients with NMIBC [102]. One of the most relevant unresolved issues concerns optimal patient selection. The group NMIBC represents highly heterogeneous tumors, and it appears increasingly plausible that it is inappropriate to rely solely on the presence/absence or degree of immune cell infiltration as a criterion to select patients with NMIBC to receive treatment with checkpoint inhibitors [103]. Future studies should integrate multiplex immunohistochemistry, transcriptomic signatures (e.g., IFN-γ gene signatures, T-cell exhaustion profiles), and perhaps even circulating immune

biomarkers to identify those patients with a "primed but constrained" immune microenvironment most likely to benefit from combination therapy. Another important challenge is associated with treatment sequence and timing. The modest magnitude of benefit observed in initial combination trials, as demonstrated by the POTOMAC trial, provides evidence that an upfront combination approach—administering checkpoint inhibitors concurrently with BCG from the first induction course—may not universally offer clinically meaningful advantage over BCG alone [78]. This negative result does not necessarily disprove the biological synergy between BCG and ICIs; rather, it challenges the assumption that "more immunotherapy earlier" is always better. An alternative and biologically compelling hypothesis is that a sequential treatment strategy might be more effective. In such an approach, BCG would first be administered to serve as an immunological primer, inducing inflammation, antigen release, and T-cell recruitment within the bladder tumor microenvironment. Only after this priming phase—potentially at the time of maximal adaptive resistance (when PD-L1 upregulation and T-cell exhaustion become dominant) or upon evidence of early recurrence—would ICIs be introduced to "release the brakes" on the BCG-primed, but functionally exhausted, T cells. This sequential paradigm acknowledges that immune checkpoint blockade does not generate de novo immune responses but rather reinvigorates preexisting ones. It also aligns with the observation that patients who initially respond to BCG and then fail may have a more immunologically active tumor microenvironment than those who are primarily refractory. Future trials should therefore consider comparing upfront combination regimens versus sequential designs, potentially incorporating a run-in phase with BCG alone followed by randomization to ICI or placebo based on early immune dynamics or clinical response. Furthermore, this strategy could be explored through subsequent research within a particular group of patients according to specific biomarkers. Another challenge associated with this treatment strategy involves patient safety. The strategy could pose a high chance of both immune-related toxicities owing to intravesical immunotherapy, alongside immune-related toxicities due to immune checkpoint inhibition [97, 104]. Clinically, distinguishing between local BCG-induced cystitis and the onset of systemic immune-related adverse events (such as immune-mediated colitis, pneumonitis, or dermatitis) will become increasingly complex with combination regimens. This diagnostic challenge requires multidisciplinary expertise and clear algorithms for management, including the judicious use of immunosuppressive agents including corticosteroids, which could theoretically counteract the desired antitumor immune response. Finally, it is necessary to make a careful comparison between new and existing therapies in the ever-changing environment of NMIBC treatment. New clinical studies should focus on proving efficacy and demonstrating exactly how new strategies provide a clear benefit over existing standards of treatment, such as improved response rate, overall survival, or disease-free survival. The availability of multiple FDA-approved bladder-sparing options for BCG-unresponsive disease—including pembrolizumab monotherapy, nadofaragene firadenovec, and N-803 plus BCG—sets a high bar for any new combination. Future trials must be designed not only to demonstrate superiority over historical controls but also to establish comparative effectiveness against these approved agents, ideally through head-to-head randomized designs with patient-reported outcomes and health-economic analyses. Briefly, despite the growing interest in BCG with checkpoint inhibitors, it remains to be seen exactly how these agents will be used. It will be important to resolve these questions in well-controlled studies before it will be possible to say that these strategies provide any worthwhile benefit to patients with NMIBC.

CONCLUSIONS

In NMIBC, BCG is still the main adjuvant therapy for high-risk disease owing to its unparalleled ability to provoke a strong local immune reaction in the bladder tumor microenvironment [105]. However, even after BCG therapy, disease recurrence and progression continue

to be major clinical issues; the necessity for novel therapeutic approaches is evident [106]. Immune checkpoint inhibitors provide a mechanism of action that is biologically complementary and may be capable of boosting BCG-mediated antitumor immune response through the elimination of adaptive immune resistance [107]. Nonetheless, available clinical data are heterogeneous. Early-phase studies, as well as ongoing trials, deliver valuable mechanistic information, but recently published phase III results, including those from upfront combination strategies, have shown only modest clinical benefit, without consistent improvements in progression or bladder preservation. Thus, biological synergy does not reliably translate into meaningful patient benefit. Currently, BCG checkpoint inhibitor combinations should only be considered as experimental treatments. At present, BCG-ICI combinations remain experimental and are not standard of care, especially given the availability of approved bladder-sparing therapies for BCG-unresponsive disease. Future progress requires reliable predictive biomarkers, optimized treatment sequencing, and mature phase III data demonstrating durable oncological benefit and bladder preservation. In summary, the future of BCG-ICI combinations in high-risk NMIBC lies in biomarker-driven patient selection and therapeutic sequencing, not universal upfront combination therapy. Integrating translational insights into clinical trial design will be essential to identify who benefits most, thereby preserving patient quality of life while improving oncological outcomes.

Author Contributions. Michele Musone: Conceptualization, methodology, investigation, writing—original draft preparation. Biagio Barone: Conceptualization, supervision, project administration. Stefano Chianese: Methodology, investigation, writing—review and editing. Paolo Conforti: Investigation, data curation, visualization. Antonio Madonna: Methodology, investigation, writing—review and editing. Silvestro Imperatore: Investigation, resources, writing—review and editing. Fabrizio Dinacci: Investigation, visualization. Raffaele Balsamo: Writing—review and editing. Giuseppe Lucarelli: Writing—review and editing. Roberto Falabella: Writing—review and editing. Vincenzo Francesco Caputo: Writing—review and editing. Antonio Luigi Pastore: Writing—review and editing. Andrea Fuschi: Writing—review and editing. Matteo Ferro: Writing—review and editing. Lorenzo Romano: Writing—review and editing. Marco Fabiano: Writing—review and editing. Celeste Manfredi: Writing—review and editing. Gian Maria Busetto: Writing—review and editing. Valerio Santarelli: Writing—review and editing. Francesco Del Giudice: Writing—review and editing. Felice Crocetto: Conceptualization, supervision, resources, writing—review and editing. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship. All authors have contributed significantly to the work, have approved the final version of the manuscript, and consent to its submission for publication.

Funding. No funding or sponsorship was received for this study or publication of this article. The Rapid Service Fee was funded by the authors.

Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed.

Declarations

Conflict of Interest. Michele Musone, Biagio Barone, Stefano Chianese, Paolo Conforti, Antonio Madonna, Silvestro Imperatore, Fabrizio Dinacci, Raffaele Balsamo, Giuseppe Lucarelli, Roberto Falabella, Vincenzo Francesco Caputo, Antonio Luigi Pastore, Andrea Fuschi, Matteo Ferro, Lorenzo Romano, Marco Fabiano, Celeste Manfredi, Gian Maria Busetto, Valerio Santarelli, Francesco Del Giudice, and Felice Crocetto have nothing to disclose.

Ethical Approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

REFERENCES

1. Ferro M, Tătaru OS, Musi G, Lucarelli G, Abu Farhan AR, Cantiello F, et al. Modified Glasgow prognostic score as a predictor of recurrence in patients with high grade non-muscle invasive bladder cancer undergoing intravesical bacillus Calmette-Guerin Immunotherapy. Diagnostics (Basel, Switzerland). 2022;12(3):586. https://doi.org/10.3390/diagnostics12030586.

2. Contieri R, Hurle R, Paciotti M, Casale P, Saita A, Porpiglia F, et al. Accuracy of the European Association of Urology (EAU) NMIBC 2021 scoring model in predicting progression in a large cohort of HG T1 patients with NMIBC treated with BCG. Minerva Urol Nefrol. 2023;75(2):180–7. https://doi.org/10.23736/S2724-6051.22.04953-9.

3. Barone B, Finati M, Cinelli F, Fanelli A, Del Giudice F, De Berardinis E, et al. Bladder cancer and risk factors: data from a multi-institutional long-term analysis on cardiovascular disease and cancer incidence. J Pers Med. 2023;13(3):512. https://doi.org/10.3390/jpm13030512.

4. Ferro M, Chiujdea S, Musi G, Lucarelli G, Del Giudice F, Hurle R, et al. Impact of age on outcomes of patients with pure carcinoma in situ of the bladder: multi-institutional cohort analysis. Clin Genitourin Cancer. 2022;20(2):e166–72. https://doi.org/10.1016/j.clgc.2021.12.005.

5. Del Giudice F, Abu-Ghanem Y, Nair R, Mensah E, Kam J, Ibrahim Y, et al. Contemporary trends and predictors associated with adverse pathological upstaging among non-metastatic localized clinical T2 Muscle-invasive bladder cancers undergoing radical cystectomy: outcomes from a single tertiary centre in the United Kingdom. Cancers. 2025;17(9):1477. https://doi.org/10.3390/cancers17091477.

6. Del Giudice F, Santarelli V, Spurna K, Ali Kirmani SG, Bhatti NH, Abu-Ghanem Y, et al. Contemporary trends and predictors of pT0 in radical cystectomy specimens among non-muscle and muscle-invasive bladder cancer patients: a propensity score-matched analysis from a single tertiary centre in the United Kingdom. Cancers. 2025;17(19):3110. https://doi.org/10.3390/cancers17193110.

7. Del Giudice F, Asero V, Bologna E, Scornajenghi CM, Carino D, Dolci V, et al. Efficacy of different bacillus of Calmette-Guérin (BCG) strains on recurrence rates among intermediate/high-risk non-muscle invasive bladder cancers (NMIBCs): single-arm study systematic review, cumulative and network meta-analysis. Cancers. 2023;15(7):1937. https://doi.org/10.3390/cancers15071937.

8. Ferro M, Del Giudice F, Carrieri G, Busetto GM, Cormio L, Hurle R, et al. The impact of SARS-CoV-2 pandemic on time to primary, secondary resection and adjuvant intravesical therapy in patients with high-risk non-muscle invasive bladder cancer: a retrospective multi-institutional cohort analysis. Cancers. 2021;13(21):5276. https://doi.org/10.3390/cancers13215276.

9. Porreca A, Di Nicola M, Lucarelli G, Dorin VM, Soria F, Terracciano D, et al. Time to progression is the main predictor of survival in patients with high-risk nonmuscle invasive bladder cancer: Results from a machine learning-based analysis of a large multi-institutional database. Urol Oncol. 2024;42(3):69.e17-69.e25. https://doi.org/10.1016/j.urolonc.2024.01.001.

10. Chen X, He F, Zhang W, Fu Y, Cao Z. Emerging trends of BCG immunotherapy for bladder cancer in last decade: a bibliometric and visualization analysis. Front Oncol. 2023;13:1092969. https://doi.org/10.3389/fonc.2023.1092969.

11. Chen J, Gao L, Wu X, Fan Y, Liu M, Peng L, et al. BCG-induced trained immunity: history, mechanisms and potential applications. J Transl Med. 2023;21(1):106.

12. Lobo N, Brooks NA, Zlotta AR, Cirillo JD, Boorjian S, Black PC, et al. 100 years of Bacillus Calmette–Guérin immunotherapy: from cattle to COVID-19. Nat Rev Urol. 2021;18(10):611–22. https://doi.org/10.1038/s41585-021-00481-1.

13. Kamat AM, Bellmunt J, Galsky MD, Konety BR, Lamm DL, Langham D, et al. Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of bladder carcinoma. J Immunother Cancer. 2017;5(1):68. https://doi.org/10.1186/s40425-017-0271-0.

14. Contieri R, Tan WS, Grajales V, Hensley PJ, Martini A, Bree K, et al. Influence of lamina propria invasion extension on T1 high-grade non-muscle-invasive bladder cancer in patients undergoing BCG or radical cystectomy. BJU Int. 2024;133(6):733–41. https://doi.org/10.1111/bju.16293.

15. D'Andrea D, Soria F, Grotenhuis AJ, Cha EK, Malats N, Di Stasi S, et al. Association of patients' sex with treatment outcomes after intravesical Bacillus Calmette–Guérin immunotherapy for T1G3/HG bladder cancer. World J Urol. 2021;39(9):3337–44. https://doi.org/10.1007/s00345-021-03653-1.

16. Lobo N, Hensley PJ, Bree KK, Nogueras-Gonzalez GM, Navai N, Dinney CP, et al. Updated European Association of Urology (EAU) prognostic factor risk groups overestimate the risk of progression in patients with non-muscle-invasive bladder cancer treated with Bacillus Calmette–Guérin. Eur Urol Oncol. 2022;5(1):84–91. https://doi.org/10.1016/j.euo.2021.11.006.

17. Flaig TW, Spiess PE, Abern M, et al. NCCN Guidelines® Insights: Bladder Cancer, Version 3.2024. J Natl Compr Canc Netw. 2024;22(4):216–25. https://doi.org/10.6004/jnccn.2024.0024. (Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bladder Cancer V.1.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed March 10, 2026. To view the most recent and complete version of the guideline, go online to NCCN.org).

18. D'Andrea D, Mostafid H, Gontero P, Shariat S, Kamat A, Masson-Lecomte A, et al. Unmet need in non-muscle-invasive bladder cancer failing Bacillus Calmette–Guérin therapy: a systematic review and cost-effectiveness analyses from the International Bladder Cancer Group. Eur Urol Oncol. 2025;8(1):216–29. https://doi.org/10.1016/j.euo.2024.10.012.

19. Sylvester RJ. Natural history, recurrence, and progression in superficial bladder cancer. ScientificWorldJournal. 2006;6:2617–25. https://doi.org/10.1100/tsw.2006.404.

20. Tabayoyong WB, Kamat AM, O'Donnell MA, McKiernan JM, Ray-Zack MD, Palou J, et al. Systematic review on the utilization of maintenance intravesical chemotherapy in the management of non-muscle-invasive bladder cancer. Eur Urol Focus. 2021;4(4):512–21. https://doi.org/10.1016/j.euf.2018.08.019.

21. Racioppi M, Di Gianfrancesco L, Ragonese M, Palermo G, Sacco E, Bassi PF. ElectroMotive drug administration (EMDA) of Mitomycin C as first-line salvage therapy in high risk "BCG failure" non muscle invasive bladder cancer: 3 years follow-up outcomes. BMC Cancer. 2018;18(1):1224. https://doi.org/10.1186/s12885-018-5134-7.

22. Fuge O, Vasdev N, Allchorne P, Green JS. Immunotherapy for bladder cancer. Res Rep Urol. 2015;7:65–79. https://doi.org/10.2147/RRU.S63447.

23. Chevalier MF, Nardelli-Haefliger D, Domingos-Pereira S, Jichlinski P, Derré L. Immunotherapeutic strategies for bladder cancer. Hum Vaccin Immunother. 2014;10(4):977–81. https://doi.org/10.4161/hv.27621.

24. Tan QL, Zhou CY, Cheng L, Luo M, Liu CP, Xu WX, et al. Immunotherapy of *Bacillus Calmette-Guérin* by targeting macrophages against bladder cancer in a NOD/scid IL2Rg-/- mouse model. Mol Med Rep. 2020;22(1):362–70. https://doi.org/10.3892/mmr.2020.11090.

25. Han J, Gu X, Li Y, Wu Q. Mechanisms of BCG in the treatment of bladder cancer-current understanding and the prospect. Biomed Pharmacother. 2020;129:110393. https://doi.org/10.1016/j.biopha.2020.110393.

26. Huang X, Wang X, He Z, Huang Y, Hu B, Chen W, et al. Mechanisms, clinical trials, and new treatments for BCG-unresponsive in nonmuscle invasive bladder cancer. Cancer Med. 2025;14(18):e71243. https://doi.org/10.1002/cam4.71243.

27. Maas M, Hilsendecker A, Pertoll A, Stühler V, Walz S, Rausch S, et al. PD-L1 expression in high-risk non-muscle-invasive bladder cancer is influenced by intravesical *Bacillus Calmette-Guérin* (BCG) therapy. Cancers. 2024;16(7):1356. https://doi.org/10.3390/cancers16071356.

28. Komura K, Hirosuna K, Tokushige S, Tsujino T, Nishimura K, Ishida M, et al. The impact of FGFR3 alterations on the tumor microenvironment and the efficacy of immune checkpoint inhibitors in bladder cancer. Mol Cancer. 2023;22(1):185. https://doi.org/10.1186/s12943-023-01897-6.

29. Yolmo P, Rahimi S, Chenard S, Conseil G, Jenkins D, Sachdeva K, et al. Atypical B cells promote cancer progression and poor response to *Bacillus Calmette–Guérin* in non-muscle invasive bladder cancer. Cancer Immunol Res. 2024;12(10):1320–39. https://doi.org/10.1158/2326-6066.CIR-23-1114.

30. Zlotta AR, Fleshner NE, Jewett MA. The management of BCG failure in non-muscle-invasive bladder cancer: an update. Can Urol Assoc J. 2009;3(6 Suppl 4):S199–205. https://doi.org/10.5489/cuaj.1196.

31. Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer—A current perspective. Nat Rev Urol. 2014;11(3):153–62. https://doi.org/10.1038/nrurol.2014.15.

32. Shah KA, Shah SR, Khan WA, Nisar S, Zaidi SF, Asif M, et al. Comparison of intravesical *Bacillus Calmette–Guérin* (BCG) and Mitomycin C for the treatment of non-muscle-invasive bladder cancer. Cureus. 2025;17(6):e86524. https://doi.org/10.7759/cureus.86524.

33. Lamm DL, Blumenstein BA, Crissman JD, Montie JE, Gottesman JE, Lowe BA, et al. Maintenance *Bacillus Calmette-Guérin* immunotherapy for recurrent TA, T1 and carcinoma in situ transitional cell carcinoma of the bladder: a randomized Southwest Oncology Group study. J Urol. 2000;163(4):1124–9.

34. Kamat AM, Li R, O'Donnell MA, Black PC, Roupret M, Catto JW, et al. Predicting response to intravesical *Bacillus Calmette-Guérin* immunotherapy: are we there yet? A systematic review. Eur Urol. 2018;73(5):738–48. https://doi.org/10.1016/j.eururo.2017.10.003.

35. Li R, Sundi D, Zhang J, Kim Y, Sylvester RJ, Spiess PE, et al. Systematic review of the therapeutic efficacy of bladder-preserving treatments for non-muscle-invasive bladder cancer following intravesical *Bacillus Calmette–Guérin*. Eur Urol. 2020;78(3):387–99. https://doi.org/10.1016/j.eururo.2020.02.012.

36. Askeland EJ, Newton MR, O'Donnell MA, Luo Y. Bladder cancer immunotherapy: BCG and beyond. Adv Urol. 2012;2012:181987. https://doi.org/10.1155/2012/181987.

37. Steinberg RL, Thomas LJ, O'Donnell MA. *Bacillus Calmette-Guérin* (BCG) treatment failures in non-muscle invasive bladder cancer: what truly constitutes unresponsive disease. Bladder cancer (Amsterdam, Netherlands). 2015;1(2):105–16. https://doi.org/10.3233/BLC-150015.

38. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558–62. https://doi.org/10.1038/nature13904.

39. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med. 2017;376(11):1015–26. https://doi.org/10.1056/NEJMoa1613683.

40. Li F, Wang Y, Xie K, Fang Y, Du Y, Hou L, et al. The efficacy and safety of PD-1/PD-L1 immune checkpoint inhibitors in treating advanced urothelial cancer: a meta-analysis of clinical trials. Aging Albany NY. 2021;13(16):20468–80. https://doi.org/10.18632/aging.203429.

41. Powles T, Smith K, Stenzl A, Bedke J. Immune checkpoint inhibition in metastatic urothelial cancer. Eur Urol. 2017;72(4):477–81. https://doi.org/10.1016/j.eururo.2017.03.047.

42. Balar AV, Kamat AM, Kulkarni GS, Uchio EM, Boormans JL, Roumiguié M, et al. Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. Lancet Oncol. 2021;22(7):919–30. https://doi.org/10.1016/S1470-2045(21)00147-9.

43. Hatogai K, Sweis RF. The tumor microenvironment of bladder cancer. Adv Exp Med Biol. 2020;1296:275–90. https://doi.org/10.1007/978-3-030-59038-3_17.

44. Andolfi C, Bloodworth JC, Papachristos A, Sweis RF. The urinary microbiome and bladder cancer: susceptibility and immune responsiveness. Bladder Cancer. 2020;6(3):225–35. https://doi.org/10.3233/BLC-200277.

45. Lu L, Wang F, Wang Z, Guo S, Jiang M, Ma Z. Immune snapshots along the inflammation-to-cancer road in bladder urothelium. Front Immunol. 2025;16:1685237. https://doi.org/10.3389/fimmu.2025.1685237.

46. Liatsos GD, Mariolis I, Hadziyannis E, Bamias A, Vassilopoulos D. Review of BCG immunotherapy for bladder cancer. Clin Microbiol Rev. 2025;38(1):e0019423. https://doi.org/10.1128/cmr.00194-23.

47. Ghodoussipour S, Bivalacqua T, Bryan RT, Li R, Mir MC, Palou J, et al. A systematic review of novel intravesical approaches for the treatment of patients with non-muscle-invasive bladder cancer. Eur Urol. 2025;88(1):33–55. https://doi.org/10.1016/j.eururo.2025.02.010.

48. Wang Y, He M, Li J, Li L. Immunotherapeutic strategies for invasive bladder cancer: a comprehensive review. Front Immunol. 2025;16:1591379. https://doi.org/10.3389/fimmu.2025.1591379.

49. Del Giudice F, Santarelli V, Łaszkiewicz J, Li S, Krajewski W, Nowak Ł, et al. The influence of immunocompromised status on recurrence and progression

free survival among nonmuscle invasive bladder cancers (NMIBCs) undergoing transurethral resection of bladder tumor (TURBT) and adjuvant intravesical *Bacillus* Calmette Guerin (BCG): analysis of USA insurance claim data. Urol Oncol. 2025;43(12):693.e9-693.e18. https://doi.org/10.1016/j.urolonc.2025.07.005.

50. Ranti D, Yu H, Salomé B, Bang S, Duquesne I, Wang YA, et al. HLA-E and NKG2A mediate resistance to BCG immunotherapy in non-muscle-invasive bladder cancer. Biorxiv Preprint Serv Biol. 2025. https://doi.org/10.1101/2024.09.02.610816.

51. Lima MZT, Bastos DA, Mattedi RL, Dzik C, Jardim DLF, Coelho R, et al. Infiltrating natural killer cells influence the efficacy of BCG immunotherapy in non-muscle-invasive bladder cancer. Pathol Res Pract. 2025;270:155997. https://doi.org/10.1016/j.prp.2025.155997.

52. Alonso JCC, Gonçalves JM, de Arruda Camargo GC, de Oliveira G, da Silva Santos A, Cintra AA, et al. Dual molecular axis of resistance and immunoresponsiveness to BCG in pT1 non-muscle invasive bladder cancer: insights from HER-2, SERBP1, HABP4, and IFN-γ profiling. Tissue Cell. 2026;98:103137. https://doi.org/10.1016/j.tice.2025.103137.

53. Prabagar MG, McQueney M, Bommireddy V, Siegel R, Schieven GL, Lu K, et al. STING agonist VB-85247 induces durable antitumor immune responses by intravesical administration in a non-muscle-invasive bladder cancer. Cancer Res. 2025;85(7):1287–96. https://doi.org/10.1158/0008-5472.CAN-24-1022.

54. Meghani K, Yu Y, Frydenlund N, Li EZ, Choy B, Abdulkadir SA, et al. Genomic and transcriptomic profiling of high-risk bladder cancer reveals diverse molecular and microenvironment ecosystems. Eur Urol. 2025. https://doi.org/10.1016/j.eururo.2025.09.4138.

55. Köseoğlu H, Yalçin B, Eroğlu T, Yücetaş U, Mungan U. Qualitative interferon-γ release assay for predicting response to BCG instillations in bladder cancer. Arch Esp Urol. 2025;78(8):1015–21. https://doi.org/10.56434/j.arch.esp.urol.20257808.133.

56. Ariafar A, Mansourabadi Z, Alipoor H, Faghih Z. Gamma-delta T cells in bladder cancer draining lymph nodes. Iran J Immunol IJI. 2024;21(4):271–8. https://doi.org/10.22034/iji.2024.103549.2846.

57. Gerald T, Woldu SL, Halstuch D, Ber Y, Lifshitz K, Cook G, et al. Impact of baseline PD-L1 status in BCG naive nonmuscle invasive bladder cancer on outcomes and changes after BCG exposure. Urol Oncol. 2025;43(6):391.e1-391.e10. https://doi.org/10.1016/j.urolonc.2025.01.015.

58. de Jong FC, Kvikstad V, Hoedemaeker RF, van der Made ACJ, van der Bosch TP, van Casteren NJ, et al. PD-L1 expression in high-risk non-muscle invasive bladder cancer is not a biomarker of response to BCG. World J Urol. 2025;43(1):57. https://doi.org/10.1007/s00345-024-05392-5.

59. Oualla K, Alem M, Teoh J. Systemic immunotherapy for BCG unresponsive nonmuscle-invasive bladder cancer. Curr Opin Urol. 2025;35(6):636–44. https://doi.org/10.1097/MOU.0000000000001334.

60. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71. https://doi.org/10.1038/nature13954.

61. Aliazis K, Christofides A, Shah R, Yeo YY, Jiang S, Charest A, et al. The tumor microenvironment's role in the response to immune checkpoint blockade. Nat Cancer. 2025;6(6):924–37. https://doi.org/10.1038/s43018-025-00986-3.

62. Kawashima S, Togashi Y. Resistance to immune checkpoint inhibitors and the tumor microenvironment. Exp Dermatol. 2023;32(3):240–9. https://doi.org/10.1111/exd.14716.

63. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321–30. https://doi.org/10.1038/nature21349.

64. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med. 2013;5(200):200ra116. https://doi.org/10.1126/scitranslmed.3006504.

65. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov. 2019;18(3):197–218. https://doi.org/10.1038/s41573-018-0007-y.

66. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24(5):541–50. https://doi.org/10.1038/s41591-018-0014-x.

67. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016;375(9):819–29. https://doi.org/10.1056/NEJMoa1604958.

68. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707–23. https://doi.org/10.1016/j.cell.2017.01.017.

69. Moliva JI, Turner J, Torrelles JB. Immune responses to *Bacillus* Calmette–Guérin vaccination: why do they fail to protect against *Mycobacterium tuberculosis*? Front Immunol. 2017;8:407. https://doi.org/10.3389/fimmu.2017.00407.

70. Kamat AM, Apolo AB, Babjuk M, Bivalacqua TJ, Black PC, Buckley R, et al. Definitions, end points, and clinical trial designs for bladder cancer: recommendations from the society for immunotherapy of cancer and the international bladder cancer group. J Clin Oncol. 2023;41(35):5437–47. https://doi.org/10.1200/JCO.23.00307.

71. Szabados BE, Guerrero-Ramos F, Grande E, Grivas P, Grünwald V, Miguel MC, et al. On the horizon: a global multidisciplinary perspective on delivering emerging therapies for patients with BCG-naïve high-risk NMIBC. Oncol Therapy. 2025;13(2):275–91. https://doi.org/10.1007/s40487-025-00334-6.

72. Longoni M, Scilipoti P, Soria F, Pradere B, Krajewski W, D'Andrea D, et al. Oncological outcomes in bacillus Calmette–Guérin-naïve High-risk non-muscle-invasive bladder cancer patients: a systematic review on current treatment strategies and future perspectives. Eur Urol Oncol. 2025;8(5):1375–92. https://doi.org/10.1016/j.euo.2025.03.007.

73. Turdo A, Tulone G, Di Bella S, Porcelli G, D'Accardo C, Gaggianesi M, et al. Heightened IDO1 levels predict *Bacillus Calmette–Guèrin* failure in high-risk non-muscle-invasive bladder cancer patients. Cell Death Discov. 2025;11(1):203. https://doi.org/10.1038/s41420-025-02489-7.

74. Artiles Medina A, Subiela JD, Tagalos Muñoz A, Tato Díez M, Mínguez Ojeda C, López Curtis D, et al. Clinical outcomes of BCG infections in patients undergoing intravesical immunotherapy for non-muscle-invasive bladder cancer: a systematic review and meta-analysis. Minerva Urol Nephrol. 2025;77(1):13–24. https://doi.org/10.23736/S2724-6051.24.05910-X.

75. Taylor JI, Kamat AM, O'Donnell MA, Annapureddy D, Howard J, Tan WS, et al. Long-term outcomes of bladder-sparing therapy vs radical cystectomy in BCG-unresponsive non-muscle-invasive bladder cancer. BJU Int. 2025;135(2):260–8. https://doi.org/10.1111/bju.16509.

76. Gabriel PE, Roumiguié M, Marcq G, Allory Y, Audenet F, Bajeot AS, et al. The evolving landscape of systemic immunotherapy for bacillus Calmette-Guérin-naïve High-risk non-muscle-invasive bladder cancer: at the edge of a tsunami? Eur Urol Oncol. 2025;8(4):1174–81. https://doi.org/10.1016/j.euo.2025.06.003.

77. Bristol-Myers Squibb. A phase 3, randomized, double-blind trial of nivolumab in combination with intravesical BCG versus standard of care BCG alone in participants with high-risk non-muscle invasive bladder cancer that is persistent or recurrent after treatment with BCG. ClinicalTrials.gov. 2023. Retrieved September 7, 2023, from https://clinicaltrials.gov/study/NCT04149574.

78. De Santis M, Palou Redorta J, Nishiyama H, Krawczyński M, Seyitkuliev A, Novikov A, et al. Durvalumab in combination with BCG for BCG-naive, high-risk, non-muscle-invasive bladder cancer (POTOMAC): final analysis of a randomised, open-label, phase 3 trial. Lancet. 2025;406(10516):2221–34. https://doi.org/10.1016/S0140-6736(25)01897-5.

79. Hahn NM, O'Donnell MA, Efstathiou JA, Zahurak M, Rosner GL, Smith J, et al. A phase 1 trial of Durvalumab in combination with *Bacillus Calmette–Guerin* (BCG) or external beam radiation therapy in patients with BCG-unresponsive non-muscle-invasive bladder cancer: The Hoosier Cancer Research Network GU16-243 ADAPT-BLADDER study. Eur Urol. 2023;83(6):486–94. https://doi.org/10.1016/j.eururo.2023.01.017.

80. Chamie K, et al. Quality of life in QUILT 3.032 study: Patients with BCG-unresponsive non-muscle invasive bladder cancer (NMIBC) receiving IL-15RαFc superagonist N-803 plus BCG. J Clin Oncol. 2023;41:495–495. https://doi.org/10.1200/JCO.2023.41.6_suppl.495.

81. Keane KG, Redfern A, Lim J, Hayne D. The role of immune checkpoint inhibitors in the management of NMIBC: a scoping review of current status and future direction. Expert Rev Anticancer Ther. 2025. https://doi.org/10.1080/14737140.2025.2587295. (**Advance online publication.**).

82. Ferro M, Barone B, Crocetto F, Lucarelli G, Busetto GM, Del Giudice F, et al. Predictive clinico-pathological factors to identify BCG, unresponsive patients, after re-resection for T1 high grade non-muscle invasive bladder cancer. Urol Oncol Semin Orig Investig. 2022;40(11):490.e13-490.e20. https://doi.org/10.1016/j.urolonc.2022.05.016.

83. Necchi A, Roumiguié M, Kamat AM, et al. Pembrolizumab monotherapy for high-risk non-muscle-invasive bladder cancer without carcinoma in situ and unresponsive to BCG (KEYNOTE-057): a single-arm, multicentre, phase 2 trial. Lancet Oncol. 2024;25(6):720–30. https://doi.org/10.1016/S1470-2045(24)00178-5.

84. Balar A, Kamat A, Kulkarni G, et al. Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. Lancet Oncol. 2021;22:919–30.

85. Lahoud J, Okullo A, Rutherford C, Smith DP, Costa DSJ, Tait MA, et al. Symptoms and side effects of *Bacille Calmette-Guerin* therapy for non-muscle invasive bladder cancer as reported by patients: a systematic review. Cancers Basel. 2025;17(2):160. https://doi.org/10.3390/cancers17020160.

86. Albakr A, Narayan VM, Tyson M, Shoskes D, Vasavada S. Pharmacotherapy for bladder spasm and irritative lower urinary tract symptoms associated with intravesical therapy for bladder cancer: a review of available evidence. Transl Androl Urol. 2025;14(10):3402–12. https://doi.org/10.21037/tau-2025-483.

87. Gubbiotti M, Rubilotta E, Bacchiani M, Cocci A, Rosadi S, Serni S, et al. Intravesical administration of highly concentrated hyaluronic acid and chondroitin sulfate as add-on therapy for chemical cystitis induced by *Bacillus Calmette–Guérin* (BCG) immunotherapy. Int Urol Nephrol. 2025;57(7):2033–9. https://doi.org/10.1007/s11255-025-04375-8.

88. Obaid K, Akpınar Ç, Karaburun MC, İbiş MA, Göğüş Ç, Türkölmez K, et al. A randomized prospective trial comparing *Bacillus Calmette-Guérin* (BCG) RIVM and Russian strains in non-muscle invasive bladder cancer: efficacy and side effects. Urol Oncol. 2025;43(12):695.e1-695.e9. https://doi.org/10.1016/j.urolonc.2025.08.028.

89. Kovačic B, Rybár Ľ, Hesko P, Krajčovič T, Kollárik B, Kubíková E. Granulomatous prostatitis following BCG therapy presenting as a PI-RADS 5 lesion on MRI in the transitional zone: a case report. Radiol Case Rep. 2025;21(1):118–21. https://doi.org/10.1016/j.radcr.2025.09.016.

90. Yu R, Qin QJ, Haines GK, Oh WK, Lewis S. Intravesical BCG therapy-induced granulomatous prostatitis in a patient with non-muscle invasive bladder cancer and prostate adenocarcinoma. Urology Case Rep. 2025;60:103020. https://doi.org/10.1016/j.eucr.2025.103020.

91. Hotta Y, Yoshioka N, Sakamoto A, Inagaki M, Ito R, Shiraki K, et al. Late-onset disseminated BCG infection with hepatosplenomegaly after intravesical BCG immunotherapy in a non-immunocompromised patient. Clin J Gastroenterol. 2025;18(2):337–42. https://doi.org/10.1007/s12328-024-02086-8.

92. Sun Y, Zhang Z, Jia K, Liu H, Zhang F. Autoimmune-related adverse events induced by immune checkpoint inhibitors. Curr Opin Immunol. 2025;94:102556. https://doi.org/10.1016/j.coi.2025.102556.

93. De Perna ML, Rigamonti E, Zannoni R, Espeli V, Moschovitis G. Immune checkpoint inhibitors and cardiovascular adverse events. ESC Heart Fail. 2025;12(4):2404–16. https://doi.org/10.1002/ehf2.15281.

94. De Martin E, Fulgenzi CAM, Celsa C, Laurent-Bellue A, Torkpour A, Lombardi P, et al. Immune checkpoint inhibitors and the liver: balancing therapeutic benefit and adverse events. Gut. 2025;74(7):1165–77. https://doi.org/10.1136/gutjnl-2024-332125.

95. Herrmann SM, Abudayyeh A, Gupta S, Gudsoorkar P, Klomjit N, Motwani SS, et al. Diagnosis and management of immune checkpoint inhibitor-associated nephrotoxicity: a position statement from the American Society of Onco-nephrology. Kidney Int. 2025;107(1):21–32. https://doi.org/10.1016/j.kint.2024.09.017.

96. Triantafyllou E, Gudd CLC, Possamai LA. Immune-mediated liver injury from checkpoint inhibitors: mechanisms, clinical characteristics and management. Nat Rev Gastroenterol Hepatol. 2025;22(2):112–26. https://doi.org/10.1038/s41575-024-01019-7.

97. Chen Z, Chen X, Li D, Jian J, Yao C, Wei X, et al. Comparison of treatments for preventing lower urinary tract symptoms after BCG immunotherapy of bladder tumors : a systematic review and network meta-analysis. BMC Urol. 2025;25(1):19. https://doi.org/10.1186/s12894-024-01675-6.

98. Akbulut I, Ödemiş İ, Atalay S. Analysis of local and systemic side effects of bacillus Calmette–Guérin immunotherapy in bladder cancer: a retrospective study in Türkiye. PeerJ. 2025;13:e18870. https://doi.org/10.7717/peerj.18870.

99. Farré A, Huguet J, Basile G, Diéguez L, Izquierdo P, Sánchez R, et al. Oncological outcomes for patients with European Association of Urology definitions of BCG failure treated with radical cystectomy. Actas Urológicas Españolas (English Edition). 2025;49(9):501834. https://doi.org/10.1016/j.acuroe.2025.501834.

100. Necchi A, Roumiguié M, Kamat AM, Shore ND, Boormans JL, Esen AA, et al. Pembrolizumab monotherapy for high-risk non-muscle-invasive bladder cancer without carcinoma in situ and unresponsive to BCG (KEYNOTE-057): a single-arm, multicentre, phase 2 trial. Lancet Oncol. 2024;25(6):720–30. https://doi.org/10.1016/S1470-2045(24)00178-5.

101. Boorjian SA, Alemozaffar M, Konety BR, Shore ND, Gomella LG, Kamat AM, et al. Intravesical Nadofaragene Firadenovec gene therapy for BCG-unresponsive non-muscle-invasive bladder cancer: a single-arm, open-label, repeat-dose clinical trial. Lancet Oncol. 2021;22(1):107–17. https://doi.org/10.1016/S1470-2045(20)30540-4.

102. Chamie K, Chang SS, Rosser CJ, Kramolowski E, Gonzalgo ML, Sexton WJ, et al. N-803 plus BCG treatment for BCG-naïve or -unresponsive non-muscle invasive bladder cancer: a plain language review. Future Oncol. 2024;20(31):2307–17. https://doi.org/10.1080/14796694.2024.2363744.

103. Meng L, Wu H, Wu J, Ding P, He J, Sang M, et al. Mechanisms of immune checkpoint inhibitors: insights into the regulation of circular RNAS involved in cancer hallmarks. Cell Death Dis. 2024;15(1):3. https://doi.org/10.1038/s41419-023-06389-5.

104. Mazzucchelli R, Cormio A, Zanelli M, Zizzo M, Palicelli A, Galosi AB, et al. Tumor-infiltrating immune cells in non-muscle-invasive bladder cancer: prognostic implications, predictive value, and future perspectives. Appl Sci. 2025;15(22):12032. https://doi.org/10.3390/app152212032.

105. Adan MM, Mao X, Ding L, Abdullahi KM, Li G. Efficacy and safety of BCG and immune checkpoint inhibitors in non-muscle invasive bladder cancer: a meta-analysis with exploratory chemotherapy comparisons. Oncol Lett. 2025;30(1):348. https://doi.org/10.3892/ol.2025.15094.

106. Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Dominguez Escrig JL, et al. European Association of Urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma in situ). Eur Urol. 2022;81(1):75–94. https://doi.org/10.1016/j.eururo.2021.08.010.

107. Sylvester RJ, van der Meijden AP, Lamm DL. Intravesical *Bacillus* Calmette–Guerin reduces the risk of progression in patients with superficial bladder cancer: a meta-analysis of the published results of randomized clinical trials. J Urol. 2002;168(5):1964–70. https://doi.org/10.1016/S0022-5347(05)64273-5.

108. de Jong FC, Rutten VC, Zuiverloon TCM, Theodorescu D. Improving Anti-PD-1/PD-L1 therapy for localized bladder cancer. Int J Mol Sci. 2021;22(6):2800. https://doi.org/10.3390/ijms22062800.