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Review

Decoding Immune Mechanisms in BCG-unresponsive Non-muscle Invasive Bladder Cancer

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Abstract

Non-muscle-invasive bladder cancer (NMIBC) accounts for roughly 75% of all bladder cancer cases. For patients with intermediate- and high-risk disease, intravesical Bacillus Calmette-Guérin (BCG) remains the standard treatment, yet it fails in up to 40% of cases. While radical cystectomy is the most effective salvage option, it carries significant morbidity and long-term quality-of-life consequences, highlighting the urgent need for bladder-sparing alternatives. Advancing such therapies requires a deep understanding of the immunologic mechanisms within the tumor microenvironment (TME). This review offers a concise overview of the immunologic mechanisms underlying BCG therapy, along with a detailed examination of the multifactorial immune evasion mechanisms that contribute to its failure in NMIBC. Within the TME, ten principal mechanisms of immune suppression have been identified. These include the activity of myeloid-derived suppressor cells, tumor-associated macrophages, regulatory T cells, and tolerogenic dendritic cells, as well as signaling pathways such as programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1), the natural killer group 2A/human leukocyte antigen-E (NKG2A/HLA-E) checkpoint, and the release of immunomodulatory molecules within the TME. Further contributors to immune evasion include cluster of differentiation 6/activated leukocyte cell adhesion molecule (CD6-ALCAM) signaling, effector T cell exhaustion, and cancer-associated fibroblasts. Collectively, these mechanisms disrupt antigen presentation, suppress cytotoxic immune responses, and facilitate tumor progression, ultimately undermining the efficacy of BCG therapy. In parallel, we highlight emerging intravesical immunotherapies for BCG-unresponsive NMIBC with carcinoma in situ, including nadofaragene firadenovec (Adstiladrin), nogapendekin alfa inbakicept-pmln (Anktiva), cretostimogene grenadenorepvec (CG0070), and detalimogene voraplasmid (EG-70). These agents employ diverse platforms, including gene therapy, cytokine stimulation, oncolytic virotherapy, and plasmid-based immune activation, to enhance antitumor responses. While early and late-phase clinical trials have shown promising response rates and favorable safety profiles for these novel agents, direct comparisons remain limited due to the reliance on single-arm study designs. The lack of comparative data, coupled with the absence of predictive biomarkers of response, complicates treatment selection. Our review underscores that developing effective therapies for BCG-unresponsive disease will require combination strategies targeting multiple immune escape mechanisms that shape immune dynamics within the TME.

Keywords: non muscle invasive bladder cancer; urinary bladder neoplasms; Bacillus Calmette-Guérin; BCG unresponsive disease; tumor microenvironment; immune evasion; immunotherapy

1. Introduction

Bladder cancer is the ninth most common malignancy worldwide, with approximately 75% of cases classified as non-muscle-invasive bladder cancer (NMIBC), for which intravesical therapy remains a cornerstone of treatment. According to major urological guidelines, patients with intermediate- and high-risk NMIBC should receive intravesical Bacillus Calmette-Guérin (BCG) as induction therapy for six weeks, followed by maintenance for up to one to three years [1,2]. However, BCG fails in up to 40% of cases [3], and the only guideline-endorsed option in this setting remains radical cystectomy (RC) [1,2], a major surgical procedure associated with a complication rate exceeding 60% within 90 days, including major complications in over 15% of cases [4]. For patients who are unfit for or decline surgery, bladder-sparing therapy (BST) offers a viable alternative without compromising survival. Recent data show no significant difference in overall survival (HR: 1.40, p = 0.4) or cancer-specific survival (HR: 0.88, p = 0.9) between BST and early RC in highly selected BCG-unresponsive NMIBC [5]. BST options include intravesical chemotherapy, gene therapy, such as nadofaragene firadenovec, and cytokine-based immunotherapy, including nogapendekin alfa inbakicept-pmln, an interleukin-15 (IL-15) superagonist. Each of these modalities has demonstrated clinical efficacy in the management of BCG-unresponsive disease [6].

For over four decades, BCG has remained the cornerstone of intravesical therapy for NMIBC, primarily attributed to its ability to elicit robust local innate and adaptive immune responses within the tumor microenvironment (TME). However, recent insights have significantly broad-

ened our understanding of its mechanism of action. BCG can be internalized by urothelial carcinoma cells, enabling them to acquire antigen-presenting capabilities, modulate Programmed Death-Ligand 1 (PD-L1) expression on both tumor and immune cells, and orchestrate a reshaping of the local immune landscape [7]. Additional evidence suggests that prolonged BCG exposure may lead to sustained stimulation of cytotoxic $CD8^+$ T cells, ultimately driving them toward an exhausted or anergic state and facilitating tumor immune escape [8].

Despite its long-standing clinical use, the immunological interplay between BCG and the TME remains incompletely understood. These insights have inspired the development of novel therapeutic strategies for BCG-unresponsive disease. For example, the rationale for using immune checkpoint inhibitors stems from the observation that PD-L1 is frequently overexpressed in tumors resistant to BCG [7]. Similarly, nogapendekin alfa inbakicept-pmln, a recombinant IL-15 superagonist designed to activate and expand NK and $CD8^+$ T cells within the TME, has shown promise in this setting, particularly when combined with BCG to enhance antitumor immunity [9]. Interestingly, recent data suggest that BCG rechallenge in patients with BCG-unresponsive NMIBC may yield acceptable outcomes, which is unexpected given the prior failure of BCG therapy [10]. This paradox may reflect the intricate and dynamic immunological mechanisms at play within TME, or alternatively, highlight the heterogeneity of BCG-unresponsive disease, characterized by distinct immune signatures.

A deeper understanding of immune mechanisms in NMIBC not only facilitates the development of novel therapeutics but also improves the ability to predict treatment response. Systemic inflammatory markers, such as the neutrophil-to-lymphocyte ratio have been investigated as potential predictors of BCG efficacy, reflecting underlying immune activity [11]. Additionally, urinary cytokine profiles may serve as non-invasive biomarkers of immune activation and therapeutic response in NMIBC [12].

The immune landscape of NMIBC is multifaceted, shaped by factors such as field cancerization and heterogeneous tumor lineage characteristics, which may precede intravesical therapy and influence treatment response [7]. A thorough understanding of the TME is essential for advancing the clinical management of NMIBC. In this review, we synthesize key immune mechanisms implicated in NMIBC, revisit BCG-induced immunologic responses previously characterized by our team [7], and examine pathways of immune evasion that contribute to BCG-unresponsive disease. We also highlight emerging immunotherapeutic strategies and their mechanisms of action that are either approved or currently under regulatory evaluation for patients with BCG-unresponsive NMIBC.

2. Literature Review

2.1 Definitions and Terminology of BCG Failure in NMIBC

Historically, failure of intravesical BCG therapy in NMIBC has been described using heterogeneous terms, including BCG-refractory, BCG-relapsing, and BCG-exposed. These terms broadly refer to persistent high-grade disease despite BCG, recurrence after an initial response, or prior BCG exposure without meeting failure criteria, respectively, but their inconsistent application has limited comparability across studies [13].

To harmonize patient classification and better identify tumors unlikely to respond to additional BCG, contemporary guidelines from the U.S. Food and Drug Administration (FDA), American Urological Association (AUA), and European Association of Urology (EAU) have adopted the unified category of BCG-unresponsive NMIBC. Under the FDA definition, BCG-unresponsive disease encompasses persistent high-grade T1 disease at the first post-treatment assessment (typically performed approximately three months after BCG initiation), recurrence of carcinoma in situ (CIS) within 12 months, or recurrence of high-grade Ta or T1 disease within six months, despite adequate BCG exposure. Adequate therapy is defined as completion of at least five of six induction instillations, in combination with either a minimum of two of three maintenance doses or at least two of six re-induction instillations [14].

Accordingly, this review focuses primarily on patients with BCG-unresponsive NMIBC, with legacy terminology referenced only when discussing historical studies conducted prior to standardization.

2.2 BCG-induced Immune Cascade Overview

2.2.1 Innate Immunity Post BCG Instillations

BCG instillation initiates a potent innate immune response marked by the recruitment and activation of macrophages, neutrophils, natural killer (NK) cells, and dendritic cells (DCs). Macrophage polarization is central to the immunologic outcome: M1 macrophages promote antitumor activity through pro-inflammatory cytokine release and direct tumoricidal effects, whereas M2 macrophages facilitate immune evasion and tumor progression via immunosuppressive signaling. The dynamic interchange between M1 and M2 phenotypes is regulated by cytokines and interactions with regulatory T cells (Tregs) within the TME. Neutrophils contribute to tumor cell death through the release of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and tumor necrosis factor-alpha (TNF-α), and further amplify immune responses by activating NK cells, T cells, and macrophages via secretion of cytokines. BCG-activated NK cells ($CD3^-/CD56^+$) mediate direct cytotoxicity against tumor cells through the release of perforin and interferon-gamma (IFN-γ), which promotes the recruitment and differentiation of $CD8^+$ T cells. NK cells also en-

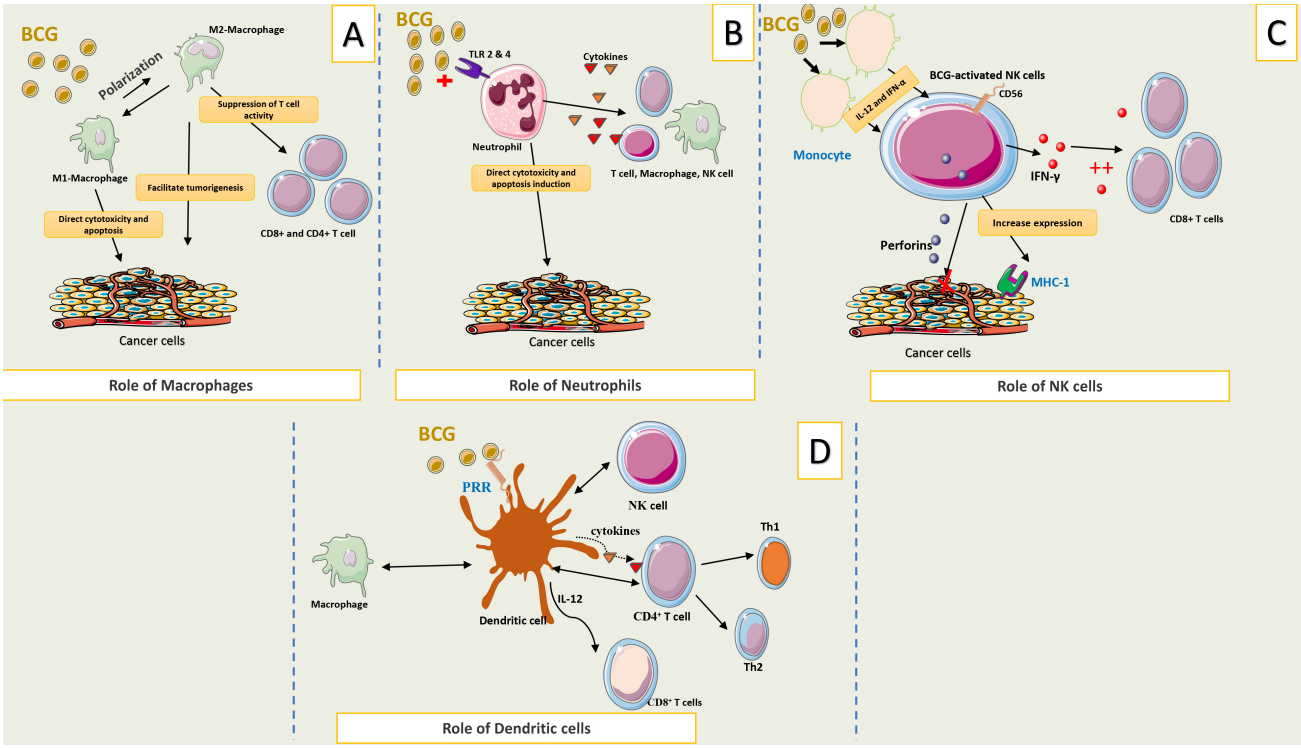

Fig. 1. Brief description of innate immune response following BCG instillation. (A) Macrophages exhibit a dual role in the bladder tumor microenvironment, with their antitumor or pro-tumorigenic effects largely dependent on their polarization state. M2 macrophages promote immune evasion and tumor progression, while M1 macrophages support antitumor immunity. (B) Neutrophils contribute to tumor cell death through the release of TRAIL and TNF-α, and further enhance immune activation by stimulating T cells, NK cells, and macrophages through the secretion of various cytokines. (C) BCG-activated NK cells ($CD3^{-}/CD56^{+}$) exert direct cytotoxicity on tumor cells via perforin release and indirectly promote $CD8^{+}$ T-cell activation through IFN-γ secretion. NK cells also upregulate MHC class I expression on tumor cells, rendering them more vulnerable to $CD8^{+}$ T-cell-mediated cytotoxicity. (D) Dendritic cells serve as an important link between innate and adaptive immunity by activating NK cells and presenting antigens to $CD8^{+}$ T cells. BCG enhances DC survival via NF-κB-mediated anti-apoptotic pathways. The influence of DCs on $CD4^{+}$ T-cell differentiation remains unclear and may require additional signals beyond cytokine profiles. BCG, Bacillus Calmette-Guérin; NK cells, Natural killer cells; TRAIL, Tumor necrosis factor-related apoptosis-inducing ligand; TLRs, Toll-like receptors; IL, Interleukin; TNF-α, Tumor necrosis factor alpha; IFN-α, Interferon-alpha; IFN-γ, Interferon gamma; MHC-I, Major histocompatibility complex class I; DCs, dendritic cells; Th1, type 1 T helper; Th2, type 2 T helper; PRRs, Pattern recognition receptors; CD, Cluster of differentiation.

hance $CD8^{+}$ T-cell responses by upregulating major histocompatibility complex class I (MHC-I) expression on tumor cells, increasing their susceptibility to cytotoxic killing. DCs serve as a critical link between innate and adaptive immunity by activating NK cells and presenting tumor antigens to $CD8^{+}$ T cells, and they sustain $CD8^{+}$ T-cell function through interleukin-12 (IL-12) secretion. BCG further promotes DCs survival via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), dependent anti-apoptotic pathways, although its role in $CD4^{+}$ T-cell differentiation remains incompletely understood [7].

Fig. 1 summarizes the key components and interactions involved in the innate immune response following BCG instillation.

2.2.2 Adaptive Immunity Post BCG Instillations

The adaptive immune response to BCG therapy involves the coordinated activation of multiple immune cell subsets, including $CD4^{+}$ and $CD8^{+}$ T cells, Tregs, gamma delta T cells ($\gamma\delta$ T cells), and B cells. Th1-polarized $CD4^{+}$ T cells play a central role in initiating antitumor immunity, while $CD8^{+}$ cytotoxic T cells contribute to direct tumor cell lysis. Tregs, characterized by FOXP3 and CD25 expression, suppress $CD8^{+}$ T-cell activity and are enriched in BCG-refractory tumors, potentially contributing to immune escape. $\gamma\delta$ T cells represent a distinct T-cell subset that differs structurally and functionally from conventional T cells. They recognize antigens independently of MHC molecules, enhance DCs function, and secrete IFN-γ, thereby supporting antitumor responses and improving BCG efficacy. B cells may also exert a protective role within the TME, as

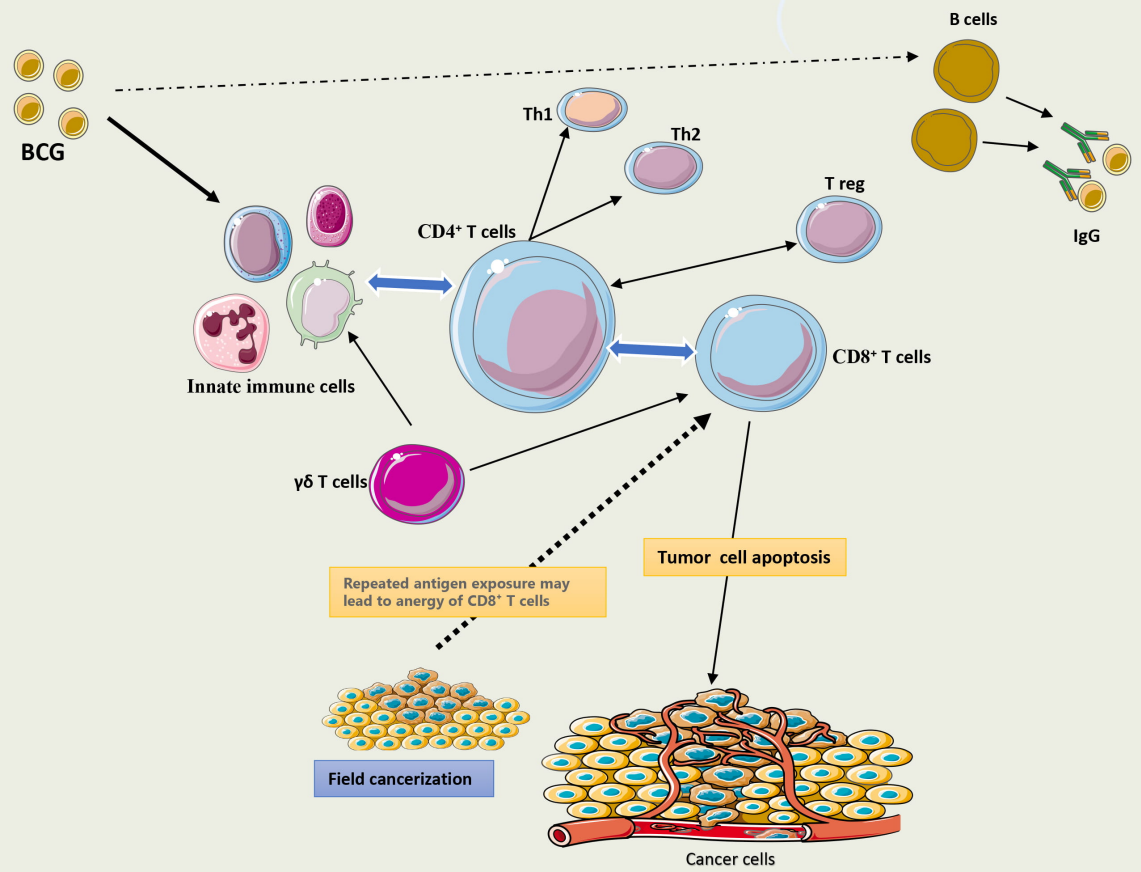

Fig. 2. Brief description of adaptive immune response following BCG instillation. BCG therapy activates a range of innate immune cells, including natural killer cells, macrophages, neutrophils, and dendritic cells. Dendritic cells function as key antigen-presenting cells, driving $CD4^+$ T-cell differentiation toward a Th1 phenotype and initiating IFN-γ secretion. IFN-γ enhances tumor immunogenicity by promoting apoptosis and increasing tumor antigen presentation, while $\gamma\delta$ T cells contribute to immune activation. Although B cells may produce anti-BCG antibodies, their role in adaptive immunity remains incompletely understood. BCG, Bacillus Calmette-Guérin; Treg, regulatory T cell; IFN-γ, Interferon-gamma; $\gamma\delta$ T cells, Gamma delta T cells; Th1, type 1 T helper; Th2, type 2 T helper; IgG, Immunoglobulin G. The dotted black arrow represents repeated stimulation of immune cells.

high B-cell infiltration has been associated with reduced recurrence rates. However, the contribution of humoral immunity remains uncertain due to conflicting data regarding the role of B cells in tumor response [7].

Fig. 2 summarizes the key cellular components and interactions involved in the adaptive immune response following BCG instillation.

2.3 Mechanisms of Immune Evasion Contributing to BCG Failure

2.3.1 Immunosuppressive Functions of Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs), which are immature precursors of neutrophils and basophils, undergo abnormal expansion in response to tumor-derived signals that inhibit their differentiation. Unlike transient expansion during acute inflammation, cancer-associated MDSCs remain persistently activated and inhibit T-cell responses [15]. Beyond their immunosuppressive functions, MDSCs contribute to tumor progression by enhancing angiogenesis, remodeling the extracellular matrix, and supporting tumor growth [16]. Accordingly, bladder cancer patients demonstrate increased circulating MDSCs and robust infiltration of these cells within tumor tissue [17]. In a clinical study of NMIBC patients undergoing BCG therapy, urine samples collected before and four hours after BCG instillation revealed that a low T-cell to MDSC ratio (<1) correlated with higher recurrence rates and a Th2-skewed cytokine profile [18].

BCG has been shown to induce the production of chemokines such as C-X-C motif chemokine ligand 8 (CXCL8) and C-C motif chemokine ligand 22 (CCL22) by urothelial cells, macrophages, and dendritic cells, thereby promoting the recruitment of MDSCs to the tumor microenvironment [19]. Furthermore, combining BCG with anti-PD-L1 therapy has been shown to reduce MDSC infiltration within the tumor, highlighting the complex dynamics of immune modulation in the TME [20]. Together, these observations suggest that BCG's capacity to reshape

the TME through modulation of MDSC populations may play an important role in determining its therapeutic efficacy.

2.3.2 Immunosuppressive Functions of Tumor-associated Macrophages

Tumor-associated macrophages (TAMs) play a central role in tumor progression, particularly when polarized toward the M2 phenotype. M2-TAMs suppress $CD8^+$ T-cell activity through PD-L1 expression and the secretion of immunosuppressive cytokines such as IL-10 and TGF-β1, promoting regulatory T-cell recruitment. They also remodel the extracellular matrix, secrete VEGF to drive angiogenesis, and interact with cancer and endothelial cells to form tumor microenvironment of metastasis (TMEM) structures that facilitate metastatic dissemination [21]. TGF-β secreted by TAMs inhibits the activity of NK cells, DCs, and effector T cells, while promoting the differentiation of $CD4^+$ T cells into a Th2 phenotype, further enhancing immunosuppression [22].

There are four distinct subtypes of M2-TAMs, each with unique phenotypic characteristics. Some subtypes secrete epidermal growth factor (EGF), which activates epidermal growth factor receptor (EGFR) signaling in tumor cells, enhancing pseudopod formation and promoting metastasis. Importantly, TAMs can facilitate DNA repair in tumor cells following chemotherapy-induced damage, thereby contributing to the development of drug resistance [23]. TAMs originate from hematopoietic stem cells in the bone marrow or from erythromyeloid progenitors in the yolk sac and fetal liver, and their functional roles may vary depending on their origin [24]. Clinically, a high density of TAMs infiltrating the TME has been associated with poor outcomes in patients with NMIBC undergoing BCG therapy. In a study involving 71 patients with NMIBC, higher TAM counts within tumors were associated with decreased recurrence-free survival [25]. Another study involving 99 NMIBC patients showed that high TAM infiltration in the tumor stroma was linked to a twofold increased risk of BCG failure [26]. Additional data suggest that the presence of M2-polarized TAMs within the TME may compromise the therapeutic effectiveness of BCG in NMIBC, with their abundance being linked to tumor recurrence as demonstrated by immunofluorescence-based profiling of M1 and M2 macrophage subsets [27].

2.3.3 Immunosuppressive Functions of Regulatory T Cells

Regulatory T cells (Tregs) are immunosuppressive $CD4^+CD25^+$ lymphocytes that arise either as thymus-derived natural Tregs or from naïve $CD4^+$ T cells in the periphery. Their differentiation is driven by T-cell receptor recognition of self-antigens presented by MHC molecules, and they are characterized by high expression of the transcription factor FOXP3 and dependence on IL-2 for survival [28].

Tregs suppress antitumor immunity through multiple complementary mechanisms, including secretion of immunosuppressive cytokines such as TGF-β and IL-10, which inhibit NK cells, DCs, effector T cells, and $\gamma\delta$ T cells [29]. They also induce apoptosis of antigen-presenting cells (APC) via perforin and granzyme B release and limit effector T-cell proliferation through competitive consumption of IL-2 [30]. In addition, Tregs express immune checkpoint molecules, including such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and lymphocyte activation gene-3 (LAG-3), which suppress APC activation by engaging CD80/CD86. Treg-derived ATP is converted to adenosine by CD39 and CD73, further suppressing immune function through A2A receptor signaling on T cells and Antigen-presenting cells (APCs) [29,30]. Although PD-1 is expressed on Tregs, its functional role within the TME remains incompletely defined. Emerging evidence suggests that PD-1 blockade may enhance Treg suppressive activity, implicating PD-1 signaling as a potential modulator of Treg-mediated immune suppression in cancer [31].

In patients with NMIBC treated with intravesical BCG, increased intratumoral Treg infiltration has been associated with shorter recurrence-free survival, suggesting a potential predictive role for Treg density in tumor recurrence [25]. Consistently, elevated levels of $PD\text{-}L1^+$ Tregs were detected in urine during BCG therapy, indicating that BCG may promote Treg recruitment and generate an alternative source of PD-L1-expressing cells that could facilitate recurrence [32]. Histologic analyses of BCG-unresponsive tumors further demonstrate dense Treg infiltration in non-responding lesions [33].

2.3.4 Immunosuppressive Function Through the PD-1/PD-L1 Pathway

The PD-1/PD-L1 axis represents a central immune-regulatory pathway within the bladder cancer TME. Tumor-mediated engagement of PD-1 on effector T cells by PD-L1 suppresses T-cell activation and facilitates immune evasion [34]. BCG therapy induces PD-L1 expression on tumor cells and APCs, including macrophages and DCs, through IL-6- and IL-10-mediated Signal Transducer and Activator of Transcription 3 (STAT3), activation, as well as toll-like receptor 4 (TLR4)-dependent extracellular signal-regulated kinases (ERK) signaling [35]. In parallel, BCG stimulates immune cells to release IFN-γ, a cytokine known to influence PD-L1 regulation through its interaction with IFN-γ receptors expressed on tumor cells. Collectively, these mechanisms promote PD-1/PD-L1 interactions that drive $CD8^+$ T-cell dysfunction and impair antitumor immunity [36].

In bladder cancer models, BCG increases PD-L1 expression on tumor cells, and its combination with anti-PD-L1 therapy enhances both the number and function of $CD8^+$ T cells [20,37]. Clinically, PD-L1 expres-

sion correlates with high-grade tumors, disease progression, and BCG unresponsiveness [38,39]. Importantly, in a cohort of NMIBC patients identified as BCG-unresponsive, tumor specimens collected before and after BCG instillations revealed PD-L1 overexpression. Interestingly, these tumors also showed dense infiltration by $CD8^+$ T cells, suggesting that BCG may impair T cell function without affecting their recruitment to the TME [40]. A recent study analyzing tissue microarrays from 432 patients with BCG-naïve HR-NMIBC found that only 7% of patients had tumors that expressed PD-L1. PD-L1 positivity was defined as membranous staining in $\geq$5% of tumor-infiltrating immune cells within the tumor area. Importantly, PD-L1 expression was not associated with treatment failure following adequate BCG therapy. However, it was linked to more aggressive clinical features, including higher-risk disease and advanced tumor stage. A notable limitation of the study was the use of a single antibody across all tumor samples, which may not adequately capture microenvironmental heterogeneity or allow for nuanced analysis of PD-L1 expression in relation to effector immune cell populations [41].

2.3.5 Immunosuppressive Function Through Tolerogenic Dendritic Cells

In high-grade bladder cancer tissues, the malignant phenotype-associated glycan sialyl-Tn (STn), a tumor-associated antigen, is markedly overexpressed. This overexpression is associated with increased infiltration of DCs within the TME, which exhibit an immature phenotype characterized by low expression of MHC class II, CD80, and CD86. These immature DCs demonstrate impaired antigen-presenting capacity and fail to effectively activate immune effector cells, thereby contributing to a diminished antitumor immune response [42].

In a cohort of 94 bladder cancer patients treated with intravesical BCG, primary tumors from patients who experienced disease recurrence exhibited reduced STn expression. Although STn may impair DC-mediated antitumor immunity, it could potentially enhance BCG internalization into tumor cells, supporting the hypothesis that BCG may exert a direct cytotoxic effect on cancer cells. In certain cases, this direct cellular effect may compensate for, or even surpass, the antitumor immune response elicited by BCG therapy. However, these findings should be interpreted with caution due to the limited sample size and the possibility that the observed outcomes may reflect a direct tumoricidal effect of BCG, rather than an immune-mediated mechanism, in a subset of cases [43].

2.3.6 Immunosuppressive Function Through the Release of Immunomodulatory Molecules

One key mechanism in cancer progression is the upregulation of cyclooxygenase-2 (COX-2) within tumor tissues, which enhances the inflammatory response through increased production of prostaglandin E2 (PGE2), a protumorigenic molecule. PGE2 impairs the function of APCs, promotes a Th2-skewed immune response, and contributes to immunosuppression. Additionally, PGE2 exerts paracrine effects within the TME by inducing angiogenesis, primarily through the upregulation of VEGF secretion [44].

In a bladder cancer model, PGE2 has been shown to be overproduced within the TME, leading to increased infiltration of MDSCs and inhibition of APC function, collectively contributing to a weakened antitumor immune response [45]. *In vitro* studies have shown that COX-2 inhibitors suppress bladder cancer cell proliferation in a COX-2-dependent fashion. Additional data suggest that COX-2 inhibitors may also exert antitumor effects through mechanisms beyond PGE2 inhibition, including the direct induction of cancer cell apoptosis [46]. Celecoxib was evaluated as an adjuvant therapy for bladder cancer in a randomized clinical trial involving patients with intermediate- or high-risk NMIBC, known as the BOXIT trial. Participants (n = 472) received standard-of-care treatment, BCG induction and maintenance for high-risk disease, and mitomycin C induction for intermediate-risk disease, and were randomized 1:1 to receive either celecoxib 200 mg twice daily or placebo for two years. While recurrence-free rates were similar between the two groups, time to recurrence in patients with T1 tumors was longer in the celecoxib arm. Despite comparable induction and maintenance protocols, not all patients completed the full treatment course as indicated. Furthermore, COX-2 expression levels in tumor tissues were not assessed, which may have influenced the observed outcomes [47]. As PGE2 primarily exerts its effects PGE2 receptor (EP), selective inhibition of these receptors may enhance antitumor strategies and offer a safer alternative to celecoxib, particularly given the cardiovascular safety concerns associated with its use in elderly cancer patients [46].

NANOG, a transcription factor essential for maintaining stem cell pluripotency, may contribute to tumor progression when aberrantly expressed in the cytoplasm of cancer cells. This atypical localization has been implicated in the upregulation of HDAC1 (Histone Deacetylase 1), an epigenetic modifier known to repress immune-related gene transcription. One consequence of this regulatory axis is the suppression of CXCL10, a chemokine that plays a key role in recruiting $CD8^+$ cytotoxic T lymphocytes to the TME. In patients with NMIBC receiving intravesical BCG therapy, elevated expression of NANOG and/or HDAC1 is associated with unfavorable clinical outcomes, including reduced recurrence-free and progression-free survival. From an immunological perspective, tumors co-expressing these markers exhibit diminished infiltration of $CD8^+$ T cells and a lower abundance of granzyme B^+ cytotoxic cells, indicative of a more immunosuppressive TME and compromised anti-tumor immunity [48].

2.3.7 Immunosuppressive Function Mediated Through the NKG2A/HLA-E Immune Checkpoint Axis

Natural Killer Group 2A (NKG2A) is an inhibitory receptor expressed on NK cells, $CD8^+$ T cells, and other subsets of T cells. It forms a heterodimeric complex with CD94, another membrane protein also found on immune effector cells. This NKG2A/CD94 complex specifically interacts with HLA-E, a non-classical MHC class I molecule that is frequently overexpressed on cancer cells. The nature of the peptide presented by HLA-E critically influences the outcome of this interaction, potentially delivering either inhibitory or stimulatory signals to immune cells. Notably, cancer cells may present peptides via HLA-E that engage the NKG2A/CD94 receptor complex on cytotoxic immune cells, such as NK cells and $CD8^+$ T cells, leading to their functional inhibition and contributing to immune evasion [49,50]. Upon engagement, the intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) of NKG2A become phosphorylated, leading to suppression of activating signals from receptors such as NKG2D and the T-cell receptor (TCR), thereby inhibiting the cytotoxic activity of effector immune cells [51]. However, the interaction between NKG2A and HLA-E within the TME is complex and modulated by various factors. These include other HLA molecules and cytokines such as IFN-γ, which upregulates both NKG2A and HLA-E, and IL-15, which specifically enhances NKG2A expression. Additionally, $CD4^+$ T cells may interact with NKG2A via Qa-1, the murine homolog of human HLA-E, leading to inhibition of NK cell activity [49].

Recent studies have investigated the role of the NKG2A/HLA-E axis in patients with NMIBC treated with intravesical BCG. In BCG-unresponsive cases, tumors were heavily infiltrated by $NKG2A^+$ NK and T cells, and the addition of autologous tumor organoids restored antitumor activity, suggesting a potential therapeutic role for NKG2A blockade in overcoming BCG failure [52].

Monalizumab is a humanized monoclonal antibody that blocks NKG2A, thereby enhancing NK cell cytotoxic function. It can be combined with cetuximab, an anti-EGFR antibody, to promote antibody-dependent cellular cytotoxicity (ADCC), or with durvalumab, an anti-PD-L1 antibody, to further boost NK and $CD8^+$ T cell-mediated cytotoxicity. This therapeutic strategy has shown promising results in metastatic colorectal cancer and non-small cell lung cancer [49,53], with potential for extension to NMIBC and advanced bladder carcinoma.

2.3.8 Immune Suppression Mediated Through the CD6-ALCAM Signaling Pathway

CD6 is a surface glycoprotein expressed on T lymphocytes, some NK cells, and subsets of B cells. It binds to CD166, also known as activated leukocyte cell adhesion molecule (ALCAM), which is expressed on tumor cells, APCs, and endothelial cells. CD6 plays a key role in T cell activation, proliferation, and adhesion to APCs and endothelial cells. Additionally, CD6 can modulate TCR-mediated signaling and, under certain conditions, exert a negative regulatory effect on T cell responses [54].

A study using single cell RNA sequencing of NMIBC tumor in patients, both BCG-naïve and BCG-unresponsive disease, has revealed elevated expression of the CD6-ALCAM immune-regulatory pathway prior to BCG therapy. These findings suggest that pre-existing inflammatory signaling within the TME may contribute to resistance to BCG [55]. These findings suggest that inhibition of the CD6-ALCAM axis may restore antitumor immunity and represents a promising therapeutic strategy to overcome BCG unresponsiveness.

In a separate study on T-cell lymphoma, intratumoral injection of a CD6-targeted antibody-drug conjugate (CD6-ADC) led to regression of subcutaneous nodules, demonstrating potent antitumor activity through CD6-ALCAM pathway inhibition [56]. These insights may inform future in vitro and clinical investigations targeting CD6-ALCAM signaling in BCG-unresponsive NMIBC.

2.3.9 Immune Suppression Mediated by Effector T Cell Exhaustion

Chronic stimulation of $CD8^+$ T cells leads to functional exhaustion, characterized by reduced cytotoxicity and diminished secretion of TNF-α, granzyme B, and IFN-γ [57]. This exhausted state is marked by upregulation of inhibitory receptors, including PD-1 and CTLA-4, which are highly expressed in tumors from BCG-unresponsive patients and in infiltrating $CD8^+PD\text{-}1^+$ T cells [58]. In bladder cancer, field cancerization may further exacerbate exhaustion by increasing tumor mutational burden and neoantigen exposure, thereby sustaining immune activation and promoting T-cell dysfunction [59].

Strandgaard *et al.* [60] performed a multiomics analysis of urinary and tumor samples collected before and after BCG therapy in a cohort of 156 patients with NMIBC. They identified an immune activation signature following BCG therapy, characterized predominantly by elevated levels of IFNγ-inducible chemokines, including CXCL9, CXCL10, and CXCL11. Their findings also revealed that patients with high-grade recurrences exhibited elevated urinary levels of immunoinhibitory proteins (CD70, CD5, PD-1) and significantly higher $CD8^+$ T cell exhaustion scores ($p < 0.05$), based on genetic profiling correlated with T cell exhaustion [60].

Collectively, these findings suggest that BCG-unresponsive disease is characterized by a paradoxical immune landscape in which intense pro-inflammatory signaling coexists with profound T-cell exhaustion following BCG stimulation. In contrast, durable responders appear to harbor a more favorable baseline TME, defined by lower CD4:CD8 and Th2:Th1 ratios [61].

Table 1. Summary of clinical trials evaluating emerging immunomodulatory therapies in BCG-unresponsive disease.

Parameter	CG0070 (Cretostimogene)	N-803 + BCG	Nadofaragene firadenovec	Intravesical EG-70 (Detalimogene voraplasmid)
	BOND-003	QUILT-3.032	NCT02773849	LEGEND
Trial phase	III	II	III	II
Therapy type	Oncolytic adenovirus	IL-15 superagonist + BCG	Adenoviral gene therapy	Non-viral plasmid-based immunotherapy
Route	Intravesical	Intravesical	Intravesical	Intravesical
Sample size (CIS cohort)	112	84	103	26
CR (any time), (CIS cohort)	75%	71%	55%	71%
CR at 3 mo, (CIS cohort)	68%	55%	53%	67%
CR at 12 mo, (CIS cohort)	46%	45%	24%	N/A
CR at 24 mo, (CIS cohort)	42%	37%	19%	N/A
Grade $\geq$III TRAE	0%	2%	4%	0%

NMIBC, non-muscle-invasive bladder cancer; BCG, Bacillus Calmette-Guérin; CIS, carcinoma in situ; CR, complete response; TRAE, treatment-related adverse event.

2.3.10 Immune Suppression Mediated by Cancer Associated Fibroblasts

Cancer-associated fibroblasts (CAFs) in the bladder cancer TME arise from resident fibroblasts activated by tumor-derived factors, particularly TGF-β1 and insulin-like growth factor 1 (IGF-1). Once activated, CAFs promote tumor growth by secreting interleukin-1β (IL-1β) and TGF-β1, remodeling the extracellular matrix (ECM) to increase tissue stiffness, and facilitating invasion. CAFs further drive disease progression by releasing monocyte chemoattractant protein-1 (MCP-1/CCL2) and hepatocyte growth factor (HGF), which enhance metastasis and modulate immune cell recruitment [62].

In addition, CAFs release extracellular vesicles (EVs) that suppress $CD8^+$ T cell proliferation and reduce the secretion of key pro-inflammatory cytokines, including IFN-γ, IL-2, and TNF-α, thereby modulating the immune response. These EVs also carry PD-L1, which interacts with PD-1 receptors on $CD8^+$ T cells, impairing their cytotoxic function [63]. CAFs themselves can express PD-L1, further contributing to immunosuppression through PD-1/PD-L1 signaling [64].

Following BCG therapy, fibroblasts contribute to granuloma formation and inflammatory signaling within the TME, yet their direct modulation by BCG remains unstudied, representing an important knowledge gap given their role in immune evasion and therapeutic resistance [65].

Figs. 3,4 summarize the key mechanisms of immune evasion that contribute to BCG failure in patients with NMIBC.

2.4 Integrated Immune Evasion Network in BCG-unresponsive Disease

Rather than acting independently, the ten suggested immune escape mechanisms identified in BCG-unresponsive disease operate as an integrated and self-reinforcing immune evasion network within the TME. BCG-induced chemokine release promotes the recruitment of suppressive myeloid cells, including myeloid-derived suppressor cells and M2-polarized tumor-associated macrophages, which in turn secrete immunosuppressive cytokines and angiogenic factors that impair antigen presentation and favor regulatory T-cell accumulation. These suppressive immune circuits are further reinforced by checkpoint signaling through the PD-1/PD-L1 axis and alternative inhibitory pathways such as NKG2A/HLA-E and CD6-ALCAM, which collectively attenuate cytotoxic lymphocyte function despite continued immune infiltration. In parallel, tumor- and stromal-derived immunomodulatory mediators, including PGE_2 and NANOG-HDAC1-dependent epigenetic repression of CXCL10, restrict effective immune cell recruitment and polarization. Chronic antigen exposure within this suppressive environment ultimately drives effector T-cell exhaustion, while cancer-associated fibroblasts augment immune exclusion through extracellular matrix remodeling and PD-L1-mediated suppression. Together, these mechanisms form a hierarchical and interconnected network (Fig. 5) that simultaneously impairs immune activation, effector function, and immune persistence, providing a biological explanation for BCG-unresponsive disease and highlighting why targeting isolated pathways is unlikely to yield durable responses.

2.5 Emerging Immunomodulatory Therapies for BCG-unresponsive Disease

Our aim in this section is not to compare the efficacy of emerging therapies for BCG-unresponsive disease (Table 1). Rather, given that the available evidence is derived from heterogeneous single-arm clinical trials, response rates are presented descriptively to illustrate overall efficacy within the context of individual studies. These

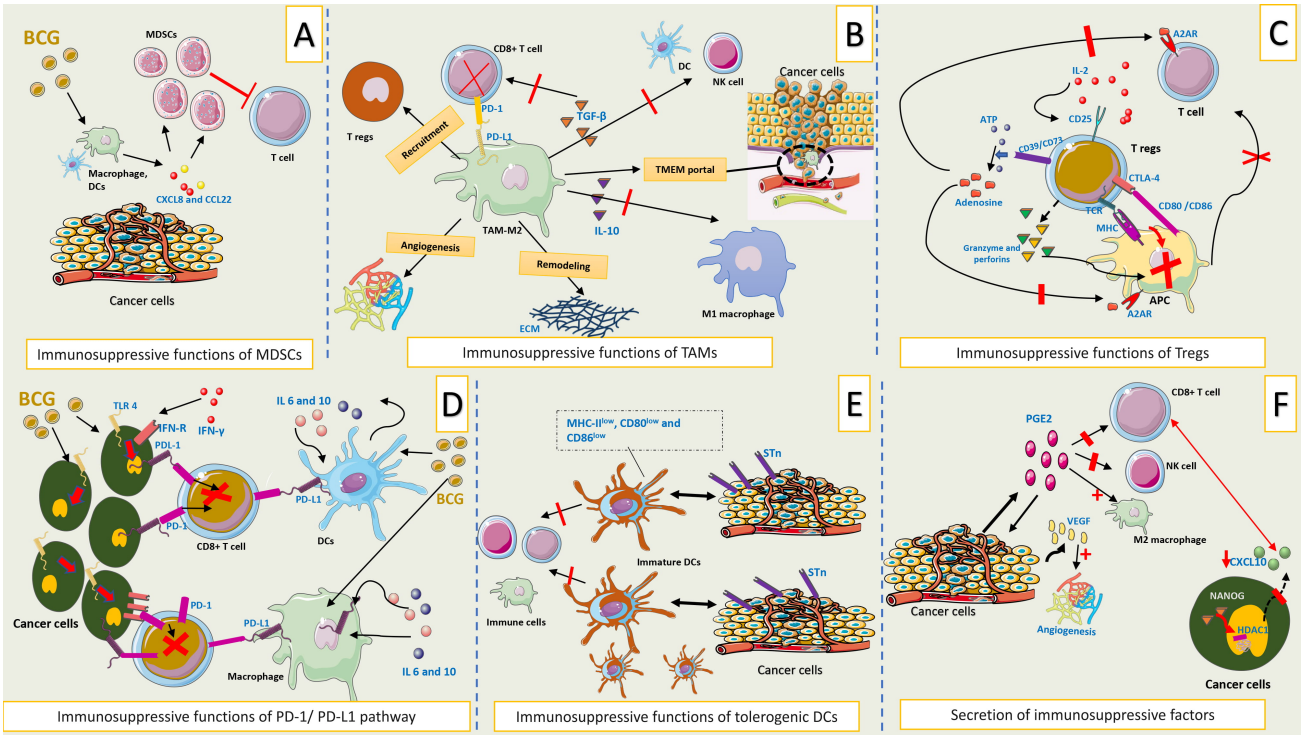

Fig. 3. Mechanisms of immune evasion contributing to BCG failure in NMIBC. (A) Immunosuppressive functions of MDSCs. BCG stimulates the release of chemokines such as CXCL8 and CCL22 from urothelial cells, macrophages, and dendritic cells. These chemokines recruit MDSCs to the tumor microenvironment, where they remain persistently activated and suppress T-cell responses, contributing to immune evasion. (B) Immunosuppressive functions of TAMs. M2-polarized TAMs contribute to tumor progression by suppressing immune responses and remodeling the extracellular matrix within the tumor microenvironment. These cells express PD-L1 and secrete immunosuppressive cytokines such as IL-10 and TGF-β, which inhibit effector immune cells and promote the recruitment of regulatory T cells. Additionally, they release pro-angiogenic factors like VEGF, facilitating neovascularization and metastatic spread. TAMs also engage in direct interactions with cancer and endothelial cells to form tumor microenvironment of metastasis (TMEM) structures, which support tumor cell dissemination. (C) Immunosuppressive functions of regulatory T cells. Tregs express CTLA-4, which binds to CD80/CD86 on APCs. This interaction inhibits APC activation and reduces their ability to stimulate effector T cells. Tregs express high levels of CD25, the alpha chain of the IL-2 receptor. By consuming IL-2, they deprive other T cells of this crucial growth factor, limiting their proliferation and survival. Tregs release ATP, which is enzymatically converted to adenosine by CD39 and CD73. Adenosine binds to A2A receptors on T cells and APCs, suppressing their activation and cytokine production. Tregs can induce apoptosis in APCs and effector T cells by releasing granzyme B and perforin. (D) Immunosuppressive functions of PD-1/ PD-L1 pathway. BCG binds to TLR4 on tumor cells, activating the ERK signaling cascade and inducing PD-L1 overexpression. Concurrently, BCG activates APCs, including macrophages and DCs, leading to the secretion of IL-6 and IL-10. These cytokines induce STAT3 phosphorylation, which subsequently enhances PD-L1 expression on APCs. BCG also stimulates immune cells to secrete IFN-γ, which acts on tumor cells via IFN-γ receptors, contributing to additional PD-L1 expression. The cumulative effect of these pathways facilitates PD-1/PD-L1 interactions, leading to $CD8^+$ T cell dysfunction and impaired anti-tumor immune responses. (E) Immunosuppressive functions of tolerogenic DCs. In high-grade bladder cancer tissue, the malignant phenotype-associated glycan STn is overexpressed. This overexpression correlates with an increased presence of immature DCs in the tumor microenvironment, characterized by low expression of MHC-II, CD80, and CD86. These immature DCs are functionally impaired in their ability to activate other immune cells, unlike mature DCs, thereby contributing to a reduced antitumor immune response. (F) Immunosuppressive activity mediated by the secretion of immunomodulatory molecules into the tumor microenvironment. Increased PGE2 production in tumor microenvironment impairs APCs function, skews immunity toward a Th2 phenotype, suppresses antitumor responses, and induces angiogenesis via VEGF secretion. In the bladder cancer TME, cytoplasmic NANOG expression in cancer cells promotes transcription of HDAC1, leading to downregulation of CXCL10, a chemokine critical for recruiting $CD8^+$ T cells. BCG, Bacillus Calmette-Guérin; MDSCs, Myeloid-derived suppressor cells; CXCL8, C-X-C motif chemokine ligand 8; CCL22, C-C motif chemokine ligand 22; DCs, Dendritic cells; TAM, Tumor-associated macrophages; VEGF, Vascular Endothelial Growth Factor; ECM, extracellular matrix; PD-L1, Programmed death-ligand 1; IL, Interleukin; TGF-β, Transforming growth factor-beta; Tregs, regulatory T cells; CD, Cluster of differentiation; CTLA-4, Cytotoxic T-Lymphocyte-Associated Protein 4; APCs, Antigen-presenting cells; MHC, Major Histocompatibility Complex; PD-1, Programmed Death-1; TLR4, Toll-like receptor 4; STAT3, Signal transducer and activator of transcription 3; ERK, Extracellular signal-regulated kinases; IFN-R, IFN receptor; IFN-γ, Interferon-gamma; STn, Sialyl-Tn antigen (cancer-associated glycan); PGE2, prostaglandin E2; Th2, type 2 T helper; HDAC1, Histone Deacetylase 1; NANOG, Nanog Homeobox (a transcription factor involved in stem cell pluripotency); CXCL10, C-X-C Motif Chemokine Ligand 10; NK cells, Natural killer cells; $CD8^+$ T cells, Cluster of differentiation 8 positive T cells. Note that the red horizontal line indicates functional blockade, and the 'X' symbols denote inhibition or cessation of the corresponding function.

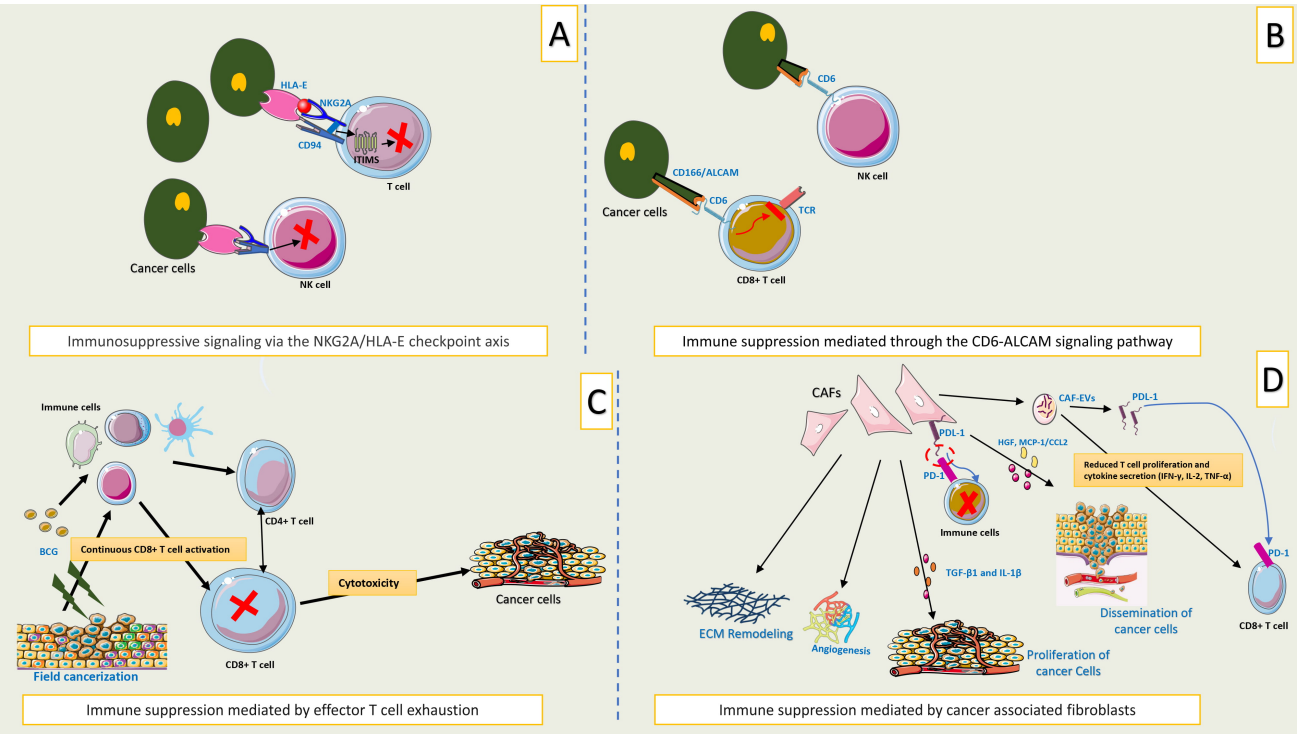

Fig. 4. Mechanisms of immune evasion contributing to BCG failure in NMIBC (continued). (A) Immunosuppressive signaling via the NKG2A/HLA-E checkpoint axis. This involves the inhibitory receptor NKG2A, which is expressed on NK cells and $CD8^+$ T cells. NKG2A forms a heterodimer with CD94 that binds to HLA-E, a non-classical MHC class I molecule frequently overexpressed on cancer cells. Peptides presented by HLA-E modulate this interaction, triggering phosphorylation of the ITIMs within NKG2A, thereby suppressing cytotoxic immune responses in the tumor microenvironment. (B) Immune suppression mediated through the CD6-ALCAM signaling pathway. Immune suppression in bladder cancer may be mediated through the CD6-ALCAM signaling pathway, wherein $CD6^+$ T cells and NK cells interact with ALCAM-expressing tumor cells. This interaction modulates TCR signaling and may negatively regulate T cell activation and proliferation within the tumor microenvironment. (C) Immune suppression mediated by effector T cell exhaustion. Prolonged stimulation of $CD8^+$ T cells by tumor antigens leads to functional exhaustion, characterized by reduced cytokine secretion and increased expression of inhibitory receptors on T cells, such as PD-1 and CTLA-4. (D) Immune suppression mediated by cancer associated fibroblasts. CAFs within the tumor microenvironment promote tumor progression by remodeling the ECM and secreting cytokines and growth factors that enhance cancer cell proliferation and metastasis. They also suppress $CD8^+$ T cell function through the release of EVs carrying PD-L1 and by directly expressing PD-L1, leading to impaired cytotoxic activity and diminished pro-inflammatory cytokine production. TCR, T Cell Receptor; NKG2A, Natural killer group 2A; NK cells, Natural killer cells; $CD8^+$ T cells, Cluster of differentiation 8 positive T cells; CD, Cluster of differentiation; HLA-E, Human leukocyte antigen-E; MHC, Major histocompatibility complex; ITIMs, Immunoreceptor tyrosine-based inhibitory motifs; ALCAM, Activated leukocyte cell adhesion molecule; PD-1, Programmed cell death protein 1; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; CAF, Cancer-associated fibroblast; TGF-β1, Transforming growth factor beta 1; IL-1β, Interleukin-1 beta; ECM, Extracellular matrix; MCP-1/CCL2, Monocyte chemoattractant protein-1/Chemokine (C-C motif) ligand 2; HGF, Hepatocyte growth factor; EV, Extracellular vesicle; IFN-γ, Interferon gamma; PD-L1, Programmed death-ligand 1; TNF-α, Tumor necrosis factor alpha. Note that the red horizontal line indicates functional blockade, and the 'X' symbols denote inhibition or cessation of the corresponding function.

studies vary substantially in trial design, eligibility criteria, endpoints, and duration of follow-up.

2.5.1 Intravesical Nadofaragene Firadenovec

Nadofaragene firadenovec (Adstiladrin) is an intravesical gene therapy that employs a non-replicating adenoviral vector to deliver the human interferon alpha-2b (IFNα2b) gene directly into urothelial cells. The formulation includes Syn3, a surfactant that enhances viral uptake and transgene delivery, resulting in localized production of IFNα2b to stimulate anti-tumor immune responses [66]. IFNα2b promotes apoptosis in cancer cells by upregulating TRAIL (TNF-related apoptosis-inducing ligand), which activates caspase-8 and caspase-10 through death receptor-mediated signaling [67,68]. Within the TME, IFNα2b also inhibits angiogenesis by suppressing the expression of ba-

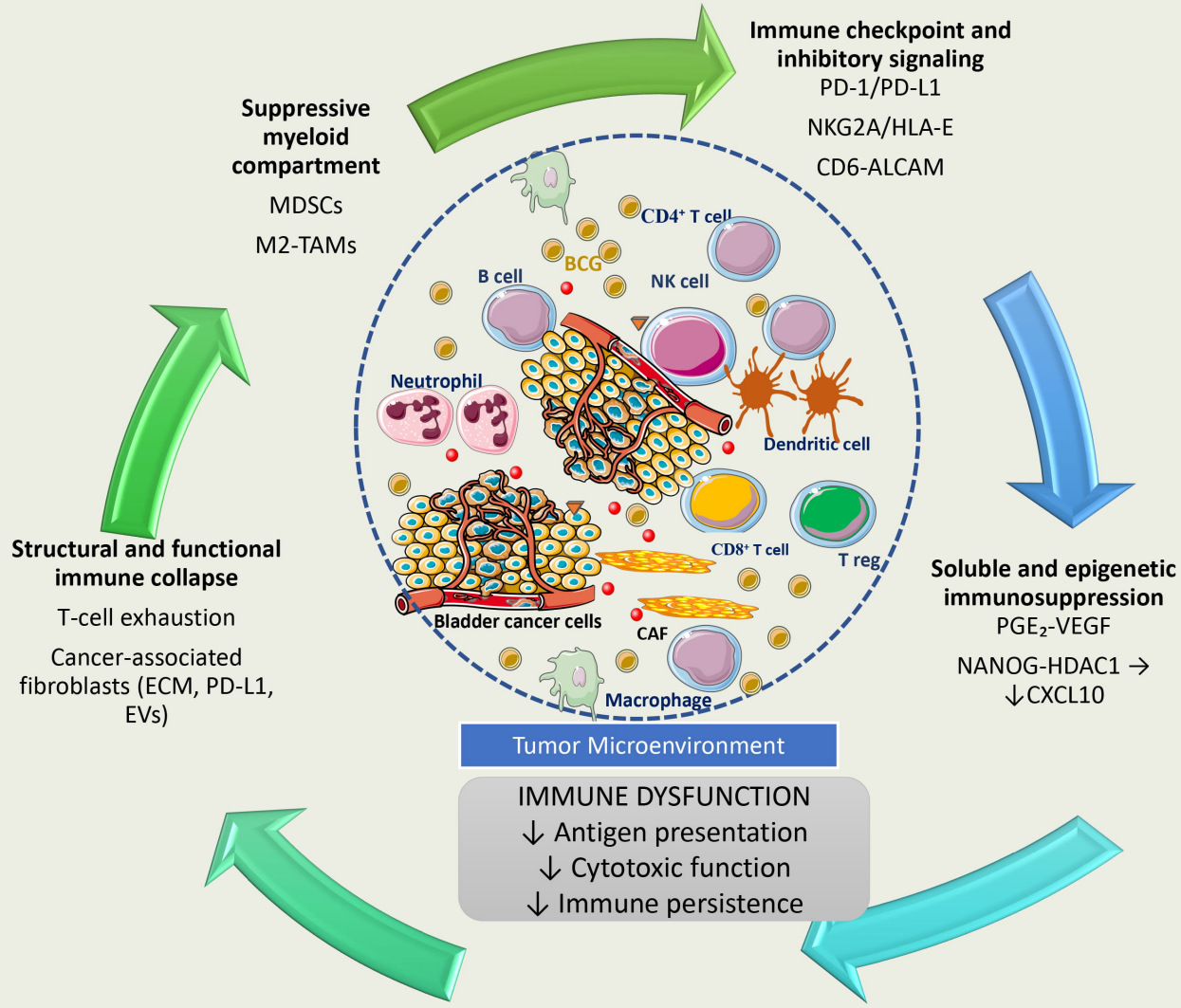

Fig. 5. Integrated immune evasion in BCG-unresponsive disease. This schematic illustrates how multiple immune escape mechanisms operate as an interconnected and self-reinforcing network within the TME of BCG-unresponsive NMIBC. Suppressive myeloid cells, immune checkpoint and inhibitory signaling, soluble and epigenetic immunosuppression, and stromal-mediated immune exclusion interact dynamically to undermine effective antitumor immunity. These pathways converge on shared functional outcomes, impaired antigen presentation, reduced cytotoxic immune activity, and loss of immune persistence, ultimately contributing to resistance to BCG therapy. BCG, Bacillus Calmette-Guérin; NMIBC, non-muscle-invasive bladder cancer; TME, tumor microenvironment; MDSCs, Myeloid-derived suppressor cells; M2-TAMs, M2-polarized tumor-associated macrophages; DCs, Dendritic cells; ECM, extracellular matrix; PD-L1, Programmed death-ligand 1; NKG2A, Natural killer group 2A; HLA-E, Human leukocyte antigen E; CD6, Cluster of differentiation 6; ALCAM (CD166), Activated leukocyte cell adhesion molecule; PGE_2, Prostaglandin E_2; VEGF, Vascular endothelial growth factor; Tregs, regulatory T cells; PD-1, Programmed Death-1; EVs, Extracellular vesicles; HDAC1, Histone Deacetylase 1; NANOG, Nanog Homeobox (a transcription factor involved in stem cell pluripotency); CXCL10, C-X-C Motif Chemokine Ligand 10.

sic fibroblast growth factor (bFGF) in cancer cells [69]. Beyond its direct cytotoxic effects, IFNα enhances the function of DCs and NK cells, increases MHC class I expression on tumor cells, and facilitates $CD8^+$ T cell-mediated killing through improved antigen recognition. Since bladder cancer cells frequently downregulate MHC I, the activation of NK cells by IFNα2b provides an additional and potent mechanism of action for nadofaragene firadenovec [70].

The Phase III multicenter clinical trial (NCT02773849) evaluating the efficacy of intravesical nadofaragene firadenovec led to its FDA approval for patients with high-risk BCG unresponsive disease with CIS [71]. Patients received 75 mL of nadofaragene firadenovec at a concentration of 3×10^{11} viral particles/mL, administered once every three months for up to 12 months, with continued treatment permitted in the absence of high-grade recurrence. A total of 103 patients with BCG-unresponsive

CIS were included in the efficacy analysis. The complete response (CR) rate at any time was 55%, with CR rates of 24% and 19% at 12 and 24 months, respectively. Grade III treatment-related adverse events (TRAEs) occurred in 4% of patients, including approximately 3% with urinary symptoms (urgency, incontinence, bladder spasms) and 1% with syncope or hypertension. No TRAEs exceeding Grade III were reported [71,72].

2.5.2 Intravesical IL-15 Superagonist (Nogapendekin Alfa Inbakicept-pmln)

Nogapendekin alfa inbakicept-pmln (Antkiva) (N-803), also known as IL-15 superagonist or ALT-803, is a cytokine complex composed of IL-15, the sushi domain of the interleukin-15 receptor alpha (IL-15Rα), and the Fc portion of IgG1. This engineered fusion enhances the biological activity and half-life of IL-15, thereby amplifying its anti-tumor effects through activation of $CD8^+$ T cells and NK cells [73]. IL-15 exerts its immunostimulatory effects by interacting with a receptor complex consisting of IL-15Rα (CD25), IL-2Rβ (CD122), and IL-2Rγ (CD132). Notably, N-803 mimics the physiological trans-presentation of IL-15, allowing it to selectively stimulate immune cells that express IL-2R$\beta\gamma$, such as NK cells and $CD8^+$ T cells, while bypassing IL-2Rα, which is highly expressed on Tregs. This selective receptor engagement minimizes Treg expansion and preserves anti-tumor immunity [74]. Recent evidence suggests that intravesical BCG therapy induces trained immunity, a form of innate immune memory that enhances responsiveness within the TME. This trained response is more robust than that of naïve immune cells [75]. The synergistic potential of combining BCG with N-803 lies in the ability of N-803 to further amplify this trained immunity, potentially improving the efficacy of intravesical immunotherapy for NMIBC.

In the phase II, open-label, multicenter QUILT 3.032 study, patients with BCG-unresponsive NMIBC with CIS received intravesical therapy consisting of 400 µg of N-803 combined with 50 mg of BCG. Treatment was administered once weekly for six weeks, followed by additional courses once weekly for three weeks at months 4, 7, 10, 13, and 19. Among the CIS cohort (n = 84), the CR rate at any time was 71%. CR rates at 12 and 24 months were 45% and 37%, respectively. In a safety analysis of patients who received intravesical N-803 monotherapy (n = 10), one patient experienced a Grade III TRAE (cerebrovascular accident). No adverse events exceeding Grade III were reported [76].

2.5.3 Intravesical Cretostimogene Grenadenorepvec (CG0070)

Cretostimogene grenadenorepvec also known as CG0070 is a tumor-selective oncolytic adenovirus designed to target bladder cancer cells with dysregulated retinoblastoma (Rb) protein pathways. The virus selectively replicates in these malignant cells, leading to direct tumor cell lysis. This process releases tumor-associated antigens, including neoantigens, into the local TME. Concurrently, the virus delivers a transgene encoding granulocyte-macrophage colony-stimulating factor (GM-CSF), which is synthesized by infected tumor cells. GM-CSF promotes the recruitment and activation of APC, such as DCs and macrophages, facilitating the uptake and presentation of neoantigens to T lymphocytes [77,78]. This dual mechanism, tumor destruction and immune stimulation, enhances both local and systemic anti-tumor immune responses.

In the phase III BOND-003 trial, patients with BCG-unresponsive NMIBC with CIS (n = 112) received intravesical cretostimogene grenadenorepvec (CG0070) at a dose of 1×10^{12} viral particles. Treatment consisted of weekly instillations for six weeks, followed by maintenance courses of three weekly doses every three months during year one, and every six months during year two. The CR rate at any time was 75%, with estimated CR rates of 46% and 42% at 12 and 24 months, respectively. No grade III TRAEs were reported in the study [79].

2.5.4 Intravesical EG-70 (Detalimogene Voraplasmid)

EG-70 (detalimogene voraplasmid) is a non-viral plasmid-based immunotherapy administered directly into the bladder. Following instillation, the plasmid is primarily internalized by bladder epithelial cells. Once inside, the plasmid is transcribed, leading to the expression of two key immunostimulatory components: IL-12, a cytokine that promotes adaptive immune responses, and double-stranded RNA (dsRNA) mimics, which activate retinoic acid-inducible gene I (RIG-I), initiating innate immune signaling pathways [80]. EG-70 comprises a plasmid, a dually derivatized chitosan polymer that complexes with the plasmid, and a polyethylene glycol-polyglutamic acid coating that surrounds the resulting complex [81].

In the phase II LEGEND trial involving patients with BCG-unresponsive CIS NMIBC (n = 26), EG-70 was administered intravesically at a dose of 40 mg per treatment, delivered in a 50 mL volume at a concentration of 0.8 mg/mL. The treatment schedule included four instillations given during weeks 1, 2, 5, and 6 of a 12-week induction cycle. Patients who remained free of disease progression after this initial cycle were eligible to receive up to three additional 12-week cycles of therapy. Preliminary results demonstrated an overall CR rate of 71%, with CR rates of 67% at 3 months and 47% at 6 months. Notably, no TRAEs of grade III or higher were reported [82].

Fig. 6 illustrates emerging immunomodulatory therapies for BCG-unresponsive NMIBC, along with their proposed mechanisms of action.

2.6 Conceptual Framework for Immune Evasion Mechanisms Contributing to BCG-unresponsive Disease

Multiple immune escape mechanisms have been implicated in resistance to intravesical BCG therapy, al-

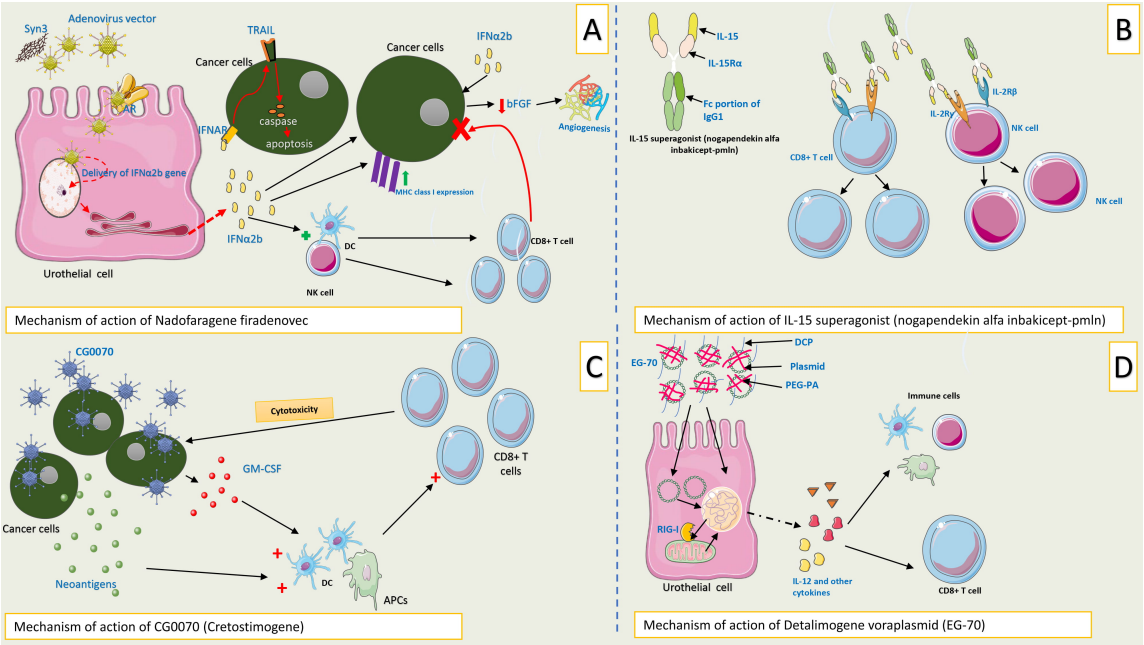

Fig. 6. Mechanisms of action of emerging immunomodulatory therapies for BCG unresponsive NMIBC. (A) Mechanism of action of intravesical nadofaragene firadenovec: Following bladder instillation, the adenoviral vector utilizes Syn3, a surfactant, to facilitate entry into urothelial cells. Once internalized, the vector delivers the IFNα2b gene to the nucleus, resulting in local overexpression of interferon alpha-2b. IFNα2b binds to interferon receptors (IFNAR) on tumor cells, inducing the expression of TRAIL, which activates caspase-8 and caspase-10, thereby triggering apoptosis. It also suppresses angiogenesis by downregulating bFGF expression in tumor cells. Additionally, IFNα2b enhances the function of DCs and NK cells, strengthening both innate and adaptive immune responses to cancer. Furthermore, IFNα2b upregulates MHC class I molecules on tumor cells, increasing their immunogenicity and promoting $CD8^+$ T cell-mediated cytotoxicity. (B) Mechanism of action of intravesical IL-15 superagonist, N-803 (nogapendekin alfa inbakicept-pmln; Anktiva). N-803 is a fusion cytokine complex that enhances IL-15 activity and stability by combining IL-15, the sushi domain of IL-15Rα, and the Fc portion of IgG1. It activates $CD8^+$ T cells and NK cells by mimicking the trans-presentation mechanism of IL-15, specifically targeting these immune effector cells through their expression of the IL-2 receptor $\beta\gamma$ complex (IL-2R$\beta\gamma$). (C) Mechanism of action of intravesical Cretostimogene grenadenorepvec (CG0070). Cretostimogene grenadenorepvec selectively replicates in bladder cancer cells with Rb pathway defects, causing tumor cell lysis and release of neoantigens into the tumor microenvironment. Infected cells also produce GM-CSF, which recruits antigen-presenting cells to initiate a systemic anti-tumor immune response. This dual mechanism enhances tumor cytotoxicity by promoting $CD8^+$ T cell-mediated immune responses. (D) Mechanism of action of intravesical EG-70 (detalimogene voraplasmid). EG-70 is a non-viral plasmid-based immunotherapy delivered intravesically, where it is taken up by bladder epithelial cells, leading to the expression of interleukin-12 and double-stranded RNA mimics. These molecules activate innate immune pathways via RIG-I and stimulate adaptive immunity by promoting $CD8^+$ T cell-mediated tumor clearance. NMIBC, non-muscle invasive bladder cancer; IFNα2b, Interferon alpha-2b; Syn3, Surfactant used to enhance adenoviral transduction; IFNAR, Interferon alpha and beta receptors; TRAIL, TNF-related apoptosis-inducing ligand; bFGF, Basic fibroblast growth factor; DCs, Dendritic cells; NK cells, Natural killer cells; MHC class I, Major histocompatibility complex class I; $CD8^+$ T cells, Cluster of differentiation 8 positive T cells; AR, Adenovirus receptor; IL-15, Interleukin-15; IL-15Rα, IL-15 receptor alpha; IL-2Rβ, IL-2 receptor beta; IL-2Rγ, IL-2 receptor gamma; Fc, Fragment crystallizable; CG0070, cretostimogene grenadenorepvec; Rb, Retinoblastoma; GM-CSF, Granulocyte-macrophage colony-stimulating factor; DCP, Derivatized chitosan polymers; PEG-PA, polyethylene glycol-polyglutamic acid; RIG-I, retinoic acid-inducible gene I; EG-70, detalimogene voraplasmid. Note that the red solid arrows represent the direct cytotoxic effect on cancer cells exerted by activated $CD8^+$ T cells, while the red dashed arrows indicate the delivery of genetic material into the nucleus. The solid black arrows denote the impact on the target cell.

though the relative contribution of individual pathways varies among experimental and clinical studies. In this review, we examine ten core immune evasion processes identified through a comprehensive synthesis of the literature, prioritizing pathways supported by reproducible preclinical or translational evidence, associations with reduced responsiveness to BCG or adverse clinical outcomes, and relevance as established or emerging therapeutic targets in NMIBC.

These mechanisms should not be considered isolated or mutually exclusive. Rather, resistance to BCG reflects a complex and interconnected immune landscape encompassing the proposed ten evasion processes. The framework presented here is intended to provide a clinically grounded and translationally relevant model for understanding BCG-unresponsive disease, rather than a definitive or exhaustive mechanistic classification.

Notably, much of the literature linking immune dysregulation to BCG-unresponsive disease is based on observational clinical data or findings from preclinical experimental models. Consequently, for many proposed pathways, the current evidence indicates correlation rather than definitive causal relationships. While mechanistic studies *in vitro* and *in vivo* offer valuable insight and biological plausibility, their direct applicability to human BCG-unresponsive disease has yet to be fully established. Future prospective, biomarker-guided studies and interventional trials will be critical to validate these pathways, define their relative contributions, and guide the rational development of targeted therapeutic strategies.

2.7 Linking Immune Escape Pathways to Emerging Immunomodulatory Therapies for BCG Unresponsive Disease

As discussed above, BCG-unresponsive NMIBC results from the interplay of multiple immune evasion mechanisms that evolve across time and space within the TME. Although emerging intravesical immunotherapies activate antitumor immunity through diverse modalities, none currently addresses the full spectrum of resistance pathways, accounting for both their observed efficacy and inherent limitations.

Defects in antigen presentation and tolerogenic dendritic cell states (Fig. 3E) are most directly addressed by cretostimogene grenadenorepvec (CG0070) and detalimogene voraplasmid (EG-70). CG0070 promotes immunogenic tumor cell lysis and delivers GM-CSF, enhancing dendritic cell recruitment and priming, thereby counteracting deficient antigen presentation. EG-70 activates innate immune sensing through RIG-I signaling and induces IL-12 expression, favoring Th1 polarization and potentially reversing tolerogenic DC phenotypes. In contrast, nadofaragene firadenovec indirectly enhances antigen presentation by upregulating MHC class I expression via interferon-α signaling, while nogapendekin alfa inbakicept-pmln primarily amplifies downstream effector responses rather than antigen priming itself.

Effector lymphocyte dysfunction and exhaustion (Fig. 4C) are most effectively targeted by nogapendekin alfa inbakicept-pmln, which selectively expands NK cells and $CD8^+$ T cells while limiting regulatory T-cell proliferation. This mechanism directly counteracts effector cell exhaustion-associated functional decline, particularly in the setting of BCG-induced chronic immune stimulation. Nadofaragene firadenovec also enhances cytotoxic lymphocyte activity through interferon-mediated NK-cell activation, although it does not directly reverse exhaustion programs driven by persistent antigen exposure.

Regulatory T-cell- and checkpoint-mediated suppression (Fig. 3C,D) are only partially addressed by current intravesical agents. While nogapendekin alfa inbakicept-pmln minimizes Treg expansion by avoiding IL-2 receptor α signaling, none of the approved intravesical therapies directly inhibit PD-1/PD-L1 signaling. Similarly, alternative immune checkpoints, including NKG2A/HLA-E (Fig. 4A) and CD6–ALCAM (Fig. 4B) pathways, are not directly targeted, despite growing evidence of their role in BCG unresponsiveness.

Myeloid-driven immunosuppression (Fig. 3A,B), including MDSC recruitment and M2-polarized TAM activity, remains a major unmet therapeutic need. While GM-CSF delivery by CG0070 and interferon signaling induced by nadofaragene firadenovec may indirectly modulate myeloid function, none of the available intravesical immunotherapies specifically deplete or reprogram suppressive myeloid populations. Similarly, CAF-mediated immune suppression (Fig. 4D) is not directly addressed by existing agents.

Soluble immunosuppressive mediators (Fig. 3F), such as PGE_2-mediated signaling that suppresses APCs function, are also largely unaffected by current intravesical therapies, potentially limiting immune cell recruitment and persistence despite immune activation.

2.8 Challenges and Future Directions

An important point to consider is that advanced age has been identified as a potential factor contributing to reduced responsiveness to BCG therapy in patients with NMIBC. Clinical data indicate that older individuals ($\geq$80 years) exhibit significantly lower response rates to BCG at 24 months compared to younger patients aged 61–70 years (39% vs. 61%) [83]. This diminished efficacy is thought to stem from age-associated immune dysregulation, driven by two key processes: First, aging promotes chronic low-grade activation of the innate immune system, partly due to the accumulation of sterile breakdown products such as damage-associated molecular patterns (DAMPs), including cell-free DNA and oxidized proteins, and mislocalized microorganism-associated molecular patterns (MAMPs) like lipopolysaccharide (LPS). These stimuli chronically activate resident immune cells, including TAMs and tumor-infiltrating neutrophils (TINs), inducing the secretion of pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α) and elevated reactive oxygen species (ROS) production. Second, adaptive immune function declines with age due to immunosenescence, characterized by a reduced number and diversity of naïve $CD4^+$ and $CD8^+$ T cells [84]. This should be taken into consideration when discussing the efficacy of novel or emerging immune modulatory agents for BCG-unresponsive NMIBC.

In addition, BCG efficacy may be diminished in immunosuppressed patients, including transplant recipients, individuals with autoimmune conditions, and those receiving systemic chemotherapy. This is biologically plausible given the reliance of BCG on host immune activation. While BCG appears to be safe in these populations, lower efficacy, particularly in transplant recipients, has been reported, though available data are limited by small sample sizes and short follow-up [85]. Importantly, the retained activity of BCG in some immunosuppressed patients suggests the presence of immune-independent mechanisms, including direct cytotoxic effects on tumor cells, as previously discussed by our group [7]. Consequently, BCG-unresponsive disease may reflect tumor-intrinsic resistance mechanisms in addition to impaired immune stimulation.

To date, two novel intravesical therapies have received FDA approval for the treatment of BCG-unresponsive NMIBC with CIS: nadofaragene firadenovec (Adstiladrin) in 2022 and nogapendekin alfa inbakicept-pmln (Anktiva) in 2024 [86]. While both agents have demonstrated promising efficacy, long-term outcomes remain under investigation. As additional therapies are expected to gain regulatory approval, clinicians will increasingly face the challenge of selecting the most appropriate treatment in the absence of predictive biomarkers. The reliance on single-arm trials for regulatory approval further complicates direct comparisons between available agents [87]. Caution is warranted when considering these therapies for patients with BCG-unresponsive papillary disease without CIS, as robust data in this subgroup are still lacking. In such cases, clinicians should engage in shared decision-making with patients, discussing the current evidence, potential benefits, tolerability, and the off-label nature of use. A comparative assessment of efficacy outcomes among emerging immunomodulatory therapies for BCG-unresponsive NMIBC suggests that relying on a single immune-enhancing mechanism may be suboptimal. This underscores the importance for clinicians to critically evaluate the underlying mechanisms of action rather than passively interpreting outcome data.

A deeper understanding of the immune mechanisms underlying BCG failure, along with the immunologic basis of emerging therapies in this context, is essential for advancing treatment strategies. This is particularly complex due to the dynamic interactions within the TME, which we have highlighted in this review. Given the heterogeneity of immune signatures across bladder tumors, an optimal therapeutic strategy should address the diverse mechanisms of immune evasion contributing to BCG unresponsive disease, ten of which have been identified as key contributors in our analysis. Ultimately, combination therapies capable of addressing several of these mechanisms simultaneously may offer the most promising path forward. For example, combining an intravesical immune-priming therapy such as cretostimogene grenadenorepvec or detalimogene voraplasmid with systemic PD-1/PD-L1 inhibition may simultaneously enhance antigen presentation while alleviating checkpoint-mediated T-cell dysfunction. Alternatively, pairing intravesical interferon-based therapies with agents targeting myeloid-driven or NK-cell inhibitory pathways (e.g., TAM reprogramming or NKG2A blockade) could produce more durable antitumor immunity by addressing both immune activation and suppression within the TME.

3. Conclusions

BCG-unresponsive NMIBC presents a multifactorial clinical and immunological challenge, driven by diverse mechanisms of immune escape within the TME. Emerging intravesical therapies, including gene-based, cytokine-driven, and oncolytic platforms, offer promising bladder-sparing alternatives. However, the absence of predictive biomarkers for treatment response and the lack of head-to-head comparative data among these novel agents complicate clinical decision-making. Future strategies should prioritize combination approaches that target multiple immune evasion pathways implicated in BCG failure. This multi-targeted strategy may enhance therapeutic efficacy and help overcome resistance to single-agent therapies. Immunologists must play a central role in guiding therapeutic development and translating immunologic insights into clinical practice for the treatment of BCG-unresponsive disease. A multidisciplinary approach is essential to optimize outcomes and advance personalized care in this evolving therapeutic landscape.

Author Contributions

MAC: Conceptualization, methodology, literature collection, original draft writing. ID: literature collection, manuscript review and editing. MAO: Conceptualization, methodology, manuscript review and editing. All authors: Reviewed and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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