

## Treatment Strategies for BCG Unresponsive Non-muscle Invasive Bladder Cancer

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### Abstract

*Bacillus Calmette-Guérin* (BCG) is the standard treatment for patients with non-muscle invasive bladder cancer (NMIBC). Although this therapy has been effective, BCG resistance poses a significant challenge, highlighting the need for alternative treatment options. Possible alternative treatments include intravesical chemotherapy, immunotherapy, antibody-drug conjugates, device-assisted therapies, gene therapy, and radiotherapy. Although radical cystectomy is recommended after BCG failure, its high morbidity and considerable impact on patients' lives underscore the necessity of developing new treatment strategies. This review provides an outline of the current knowledge and ongoing research on alternative treatments for BCG-unresponsive high-risk NMIBC, aiming to improve patient outcomes. Considering the current global shortage of BCG, it is essential to prioritize alternative therapies as treatment options for patients with BCG-unresponsive NMIBC.

**Key words** non-muscle invasive bladder cancer, BCG failure, immunotherapy, intravesical chemotherapy, gene therapy, clinical trials

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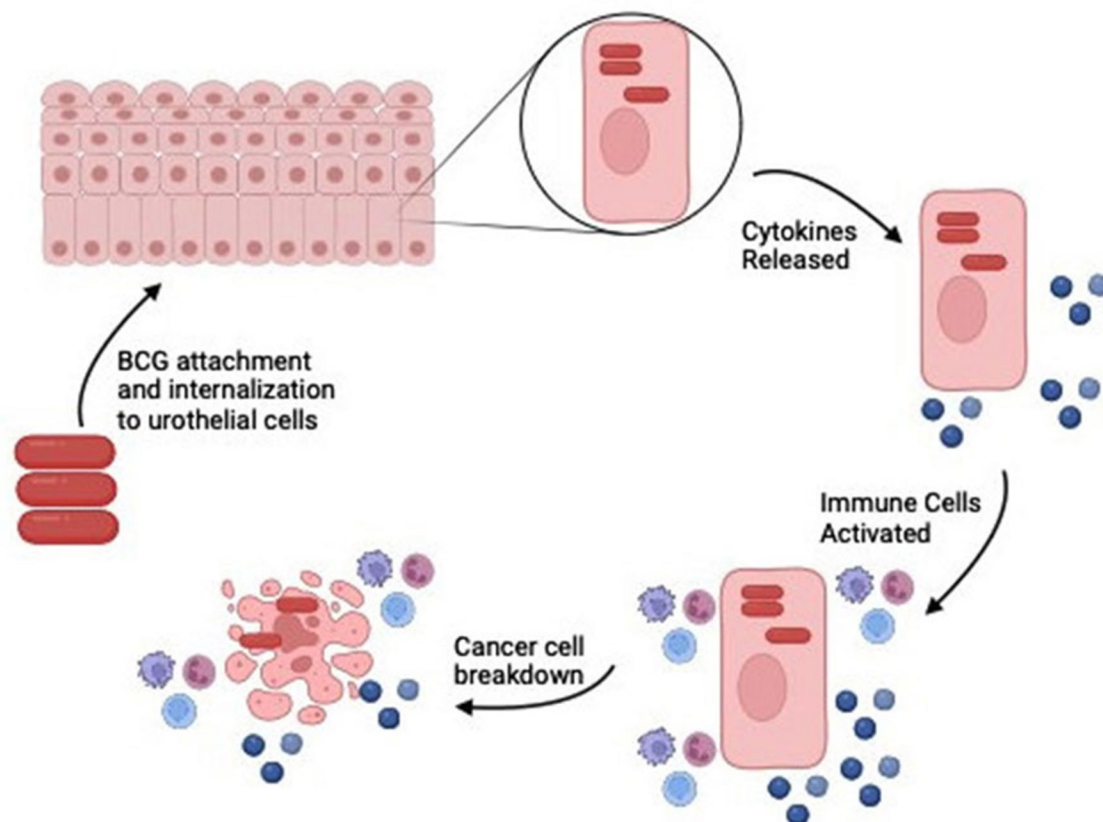
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## Introduction

Bladder cancer is among the most common types of cancer, ranking as the sixth most prevalent worldwide. Non-muscle invasive bladder cancer (NMIBC) is a specific form that has not penetrated the deeper muscle layers of the bladder. It accounts for approximately 75% of bladder cancer cases and can progress to a more severe condition if left untreated. In the Ta stage of NMIBC, tumors affect only the innermost lining of the bladder, known as the urothelium, and extend into the underlying connective tissues [1]. Carcinoma in situ represents the most aggressive form of NMIBC and can invade the muscle layer if not treated adequately. After diagnosis, patients are classified as low, intermediate, high, or very high-risk based on the cancer's aggressiveness. The characteristics of high-risk NMIBC are defined by both the American Urological Association (AUA) and the European Association of Urology (EAU) (Table 1). Treatments, such as BCG, are recommended based on risk factors and the patient's specific circumstances to manage the impact of NMIBC. The criteria for identifying BCG-unresponsive NMIBC and for determining adequate BCG treatment are outlined (Table 2).

For over 40 years, Bacillus Calmette-Guérin (BCG) has been a cornerstone in the treatment of bladder cancer. Originally

developed from *Mycobacterium bovis*, which was isolated from an infected organism to create a tuberculosis vaccine, BCG has emerged as a promising option for both clinicians and patients with NMIBC [2]. BCG exerts cytotoxic effects on bladder cancer cells through various mechanisms, including immune system activation, apoptosis, necrosis, and oxidative stress [3]. Upon instillation into the bladder, BCG binds to cancer cells, triggering a strong immune response. This activation involves T-cells and cytokine production, which in turn stimulates natural killer (NK) cells, macrophages, and neutrophils. These immune cells work together to eliminate tumor cells in the bladder (Figure 1). BCG can also directly induce apoptosis and necrosis by activating death receptor-mediated signaling pathways and upregulating proteases. The treatment generates oxidative stress by releasing reactive oxygen species, which contribute to cell death. BCG therapy is considered successful if no tumor recurrence is observed for two years, at which point the patient is considered tumor-free. However, additional rounds of BCG may be necessary if the tumor recurs. Its effectiveness in reducing bladder cancer has been demonstrated through a meta-analysis of 24 randomized controlled trials [4]. The results showed that BCG treatment reduced the odds of bladder cancer progression by about 27% over 2.5 years. While BCG is effective in curing 35% of patients, 30%



**Figure 1.** BCG induces an immune response by activating immune cells to treat NMIBC. Following treatment BCG is attached and internalized to the urothelial cells. The presence of BCG triggers the recruitment and activation of a variety of immune cells, including macrophages, dendritic cells, and neutrophils. These cells present antigens and secrete cytokines, which further activate T-cells and natural killer (NK) cells. This cascade of immune responses leads to the production of pro-inflammatory cytokines such as interferon-gamma (IFN- $\gamma$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ), which play crucial roles in attacking and destroying cancer cells.

**Table 1. High-risk NMIBC characteristics defined by the American and European Urologic Association.**

Characteristic	American Urologic Association	European Urologic Association
Carcinoma in situ	Yes	Yes
High-grade T1 Tumor	Yes	Any high-grade tumor
Any T1 tumor	Indefinite	Yes
Recurrent or multifocal or large (>3cm) high-grade Ta tumors	Yes	Indefinite
Any tumor following BCG failure	Yes	Indefinite
Lymphovascular invasion or non-urothelial histology	Yes	Indefinite
High-grade tumor involving prostatic urethra	Yes	Indefinite
T1 HG with CIS	Indefinite	Highest risk
Multiple, large, or recurrent T1 high-grade tumors	Indefinite	Highest risk
T1 with CIS in prostatic urethra	Indefinite	Highest risk
Some variant histology or lymphovascular invasion	Indefinite	Highest risk

to 40% may experience BCG failure, which increases the risk of tumor recurrence and progression. Additionally, BCG treatment can lead to toxic side effects, including cystitis, severe flu-like symptoms, prostatitis, and other systemic infections. These issues highlight the need for alternative treatments for patients who do not respond to BCG therapy [5, 6]. Although radical cystectomy is the primary treatment option following BCG failure, it may not be suitable for all patients or could be declined due to its considerable impact on quality-of-life [7, 8]. This review explores the range of treatment options available for high-risk NMIBC patients who have experienced BCG failure and are either unable or unwilling to undergo radical cystectomy.

**Treatment strategies for BCG-unresponsive NMIBC**

Several therapies employing various mechanisms of action have shown positive results in both BCG-naïve and BCG-unresponsive settings. These treatments include intravenous and intravesical

immunotherapy, viral- and bacterial- based intravesical therapies, combination intravesical chemotherapy regimens, and innovative methods of intravesical chemotherapy administration including devise-assisted therapies and radiotherapy. The effectiveness and tolerability of these emerging treatments for NMIBC are promising, offering potential alternatives to radical cystectomy. The current management landscape for BCG-unresponsive disease is detailed in **Table 3**.

*Intravesical chemotherapy*

Intravesical chemotherapy involves administering cytotoxic drugs directly into the bladder to target cancer cells and prevent tumor recurrence. [29, 30] Drugs used for this treatment include valrubicin, gemcitabine, gemcitabine with mitomycin C, docetaxel, gemcitabine with docetaxel, and paclitaxel as combination (**Figure 2**).

Valrubicin is used in intravesical therapy for NMIBC. It

**Table 2. Criteria of BCG-unresponsive non-muscle invasive bladder cancer and adequate BCG treatment.**

BCG-unresponsive non-muscle invasive bladder cancer	Adequate BCG treatment
Persistent or recurrent CIS alone or with Ta/T1 disease within 12 months of adequate BCG therapy	At least five of six doses of the initial induction course and at least two of three doses of the maintenance treatment
Recurrent high-grade Ta/T1 disease within 6 months of completion of adequate BCG therapy	At least five of six doses of the initial induction course and at least two of six doses of the second induction course
T1 high-grade disease on the first evaluation following an induction BCG course	-

**Table 3. Treatment options post-BCG failure for NMIBC.**

Type of treatment/therapy	Variable	Route of administration	FDA approval	Mechanism of action	Ref.
Intravesical chemotherapy	Valrubicin	Intravesical	Yes	Arrests the cell cycle in the G2 phase by inhibiting topoisomerase II and the fusion of nucleotides in DNA	10
Intravesical chemotherapy	Gemcitabine	Intravesical	No	Causes apoptosis in tumour cells by blocking ribonucleotide reductase and inhibiting DNA synthesis	11
Intravesical chemotherapy	Docetaxel	Intravesical	No	Inhibits certain proteins and microtubule breakdown which prevents cell division, leading to apoptosis	12
Intravesical chemotherapy	Gemcitabine + Mitomycin C	Intravesical	No	Mitomycin c is administered after gemcitabine	13, 14
Intravesical chemotherapy	Gemcitabine + Docetaxel	Intravesical	No	Docetaxel is administered after gemcitabine	15
Intravesical chemotherapy	Paclitaxel	Intravesical	No	Stabilizes microtubules to prevent cell division, leading to apoptosis	16
Immunotherapy	Pembrolizumab	Intravenous	Yes	Binds to programmed cell death 1 receptor to prevent the deactivation of T-cells	17
Immunotherapy	Nivolumab	Intravenous	No	Targets programmed cell death 1 receptor to maintain T-cell activation	18
Immunotherapy	Atezolizumab	Intravenous	No	Attaches to protein programmed death-ligand 1 to trigger an immune response	19
Immunotherapy	Durvalumab	Intravenous + intravesical	No	Blocks the binding of PD-L1 with its receptor, to produce an immune response against cancer cells	20
Device-Assisted Therapy	Chemohyperthermia	Intravesical	No	The tumor is heated to make it easier for the inserted drug to kill cancer cells	21
Device-Assisted Therapy	Electromotive drug administration	Catheter insertion	No	Applies electric currents to allow chemotherapy drugs to reach cancer cells in high concentrations	22
Antibody-Drug Conjugates	Vicinium	Intravesical	No	Attaches to epithelial cell adhesion molecules to release cytotoxic drugs, killing cancer cells	23
Antibody-Drug Conjugates	N-803	Intravesical	Yes	Connects to the IL-15 receptor to activate immune cells, helping them recognize and destroy cancer cells	24
Gene therapy	Nadofaragene Firadenovec	Intravesical	Yes	Delivers a protein-encoding gene that enhances the immune system and destroys cancer cells	25
Gene therapy	CG0070	Intravesical	No	Replicates in cancer cells with dysfunctional retinoblastoma protein to boost immune system and induce apoptosis	26
Radiotherapy	External Beam Radiotherapy	External	No	Sends x-rays or protons through advanced imaging technologies to target the cancer cells in the bladder	27
Radiotherapy	Brachytherapy	Internal	No	Radioactive source placed in bladder to damage cancer cell DNA and induce apoptosis	28



**Figure 2. Intravesical chemotherapy drugs and their combinations approved or are currently being studied in bladder cancer.**

integrates into the DNA and disrupts nucleotide incorporation, effectively halting the cell cycle during the G2 phase [31]. This FDA-approved drug inhibits the enzyme topoisomerase II, which is crucial for unwinding double-stranded DNA during replication. By suppressing this enzyme, valrubicin causes DNA damage, leading to the death of cancer cells. In a phase III study, 90 patients with multiple failed courses of intravesical therapy, including one round of BCG, were treated with six weekly instillations of 800 mg valrubicin. After 30 months, 21% of these patients achieved a complete response, with 7 remaining disease-free and 14 showing superficial Ta disease at the last evaluation [32]. The potential of valrubicin was further assessed in a retrospective study [33], involving 113 NMIBC patients who had experienced BCG failure and were not candidates for cystectomy. The study found event-free survival (EFS) rates of 51.6% at 3 months, 30.4% at 6 months, and 16.4% at 12 months following valrubicin instillations, with patients also reporting local bladder symptoms. Valrubicin is considered an effective short-term alternative to more invasive treatments like cystectomy and is associated with relatively mild side-effects, as demonstrated in numerous studies. However, due to its limited long-term effectiveness, there is currently no ongoing research focused on this treatment. Resistance to valrubicin often develops as cancer cells overexpress reductive enzymes, such as carbonyl reductases and aldo-keto reductases, which convert the drug into less cytotoxic metabolites [34]. These metabolites are then efficiently expelled from the cells by ABC transporters, reducing the drug's efficacy and contributing to resistance. This situation underscores the need for continued research into alternative chemotherapy drugs to improve long-term outcomes for patients who are unresponsive to BCG.

Gemcitabine is a widely used intravesical chemotherapy drug most commonly used for treating high-risk and BCG-unresponsive NMIBC patients. Once inside the cell, gemcitabine undergoes phosphorylation to form its active metabolites, including gemcitabine diphosphate and gemcitabine triphosphate [11]. Gemcitabine diphosphate inhibits ribonucleotide reductase, an enzyme essential for DNA synthesis, thereby disrupting DNA replication. Concurrently, gemcitabine triphosphate is incorporated into DNA, blocking the addition of new nucleotides and inhibiting DNA synthesis. The combined effects of ribonucleotide reductase inhibition and the interruption of DNA synthesis lead to cancer cell apoptosis. A comparative study [35] evaluated the effectiveness of intravesical gemcitabine versus BCG in 117 patients. After administering 1 g of gemcitabine, patients were randomly assigned to receive either BCG or intravesical gemcitabine weekly for 6 weeks. The study found that the efficacy of both treatments was relatively similar, but gemcitabine was associated with significantly fewer side effects compared to BCG (13.6% for gemcitabine

versus 44.8% for BCG). In a controversial Phase II study [13] conducted by the Southwest Oncology Group, the effectiveness of gemcitabine was assessed. In this study, 47 patients received 2 g of gemcitabine weekly for 6 weeks, followed by monthly treatments for a year. Despite this regimen, the durable response rate remained below 30% even after 12 months. Gemcitabine presents a promising alternative for patients with BCG failure, especially for those seeking a less toxic treatment option. When administered intravesically, gemcitabine generally has fewer side-effects compared to valrubicin. Although both drugs demonstrate similar efficacy, gemcitabine is considered more versatile for NMIBC due to its broader mechanism of action. Exploring the combination of gemcitabine with other drugs has been on rise and could provide new opportunities for developing more effective and long-lasting treatments for NMIBC.

Docetaxel significantly affects bladder cancer by stabilizing microtubules, which are made up of tubulin units that help maintain cell structure and shape. This stabilization interferes with the normal dynamic process of microtubule assembly and disassembly, thereby stopping cell division and inhibiting cancer cell proliferation [36]. Additionally, docetaxel binds to the apoptosis-inhibiting protein B-cell leukemia 2 (Bcl2), promoting cancer cell death. In a study conducted by Shantharam et al. [12], 13 patients received docetaxel chemotherapy at a dose of 75 mg. The recurrence-free survival rates were 75% at 6 months, 50% at both 12 and 18 months, and 25% at 24 months. Additionally, a separate group of 54 patients who received weekly docetaxel treatments for 6 weeks had recurrence-free survival rates of 40% at one year and 25% at three years [37]. Docetaxel demonstrates a promising initial response in treatment, but the recurrence-free survival rate significantly declines over time as tumor cells develop resistance to the drug. Further research is necessary to clarify the mechanisms behind this resistance, particularly since docetaxel works by stabilizing microtubules. Understanding these resistance mechanisms could pave the way for developing combination therapies that leverage docetaxel's initial effectiveness while addressing and overcoming resistance.

Combining gemcitabine and mitomycin C takes advantage of their complementary mechanisms to enhance cancer cell eradication. Gemcitabine incorporates into DNA, inhibiting DNA synthesis and blocking ribonucleotide reductase, while mitomycin C induces apoptosis by cross-linking DNA. This synergistic approach allows both drugs to work together more effectively against cancer cells [11, 38]. Breyer et al. [39] administered a combination of gemcitabine (1000 mg in 50 ml sterile water) followed by mitomycin C (40 mg in 20 ml sterile water) to 10 patients who were resistant to BCG. After a median follow-up of 26.5 months, the study reported that 6 patients (60%)

remained recurrence-free, while 4 patients (40%) experienced tumor recurrence. Additionally, a multi-institutional analysis [40] involving 47 patients evaluated this combination therapy, with 1 g of gemcitabine administered for 90 minutes followed by 40 mg of mitomycin C for 90 minutes. The results showed that 30% of patients remained recurrence-free after a median follow-up of 26 months. Gemcitabine and mitomycin C are generally well-tolerated, with only mild urinary symptoms, such as rashes, which typically resolve with treatment. However, the recurrence-free rate for this combination therapy does not match the success rates of other NMIBC treatments. Although the combined approach was anticipated to reduce the likelihood of cancer cells developing resistance, its overall success rate remains modest. Due to its limited efficacy, there are currently no ongoing studies investigating this treatment.

Sequential intravesical gemcitabine and docetaxel, first described in 2015, has emerged as an effective and well-tolerated therapy for patients who experience BCG failure. This approach leverages the distinct mechanisms of both drugs, allowing them to complement each other in targeting and treating tumor cells. By combining gemcitabine's ability to inhibit DNA synthesis and docetaxel's stabilization of microtubules, this regimen enhances the overall therapeutic effect against bladder cancer. A retrospective study conducted by Steinberg et al. [15] assessed the sequential treatment of gemcitabine and docetaxel. In this study, 45 predominantly male patients with BCG failure received 6 weekly instillations of 1 g of gemcitabine in 50 ml of sterile water, followed immediately by 37.5 mg of docetaxel in 50 ml of saline. The treatment success rates were 66% at the first surveillance, 54% at one year, and 34% at two years. Another retrospective study [41] evaluated the efficacy and safety of sequential gemcitabine and docetaxel treatment in 60 patients with BCG failure over a 6-week period. The treatment success rates, defined as the absence of recurrence, progression, cystectomy, or death, were 83% at the first surveillance, 69% at one year, and 55% at two years. Although the success rate decreases over time, the combination of gemcitabine and docetaxel shows reasonable efficacy as an alternative treatment after BCG failure. The initial effectiveness of docetaxel, along with gemcitabine's dual mechanism of action, contributes to this outcome. However, the limitations of small sample sizes and short follow-up periods in both studies underscore the need for further research to confirm the treatment's efficacy and to explore its broader applicability.

Paclitaxel, similar to docetaxel, is a taxane drug that stabilizes microtubules, thereby disrupting cell division—a critical process for cancer cell survival and proliferation [42]. By inhibiting microtubule dynamics, paclitaxel induces apoptosis in cancer cells, effectively slowing or halting disease progression. A Phase II study assessed the impact of paclitaxel on 28 NMIBC patients who had experienced recurrence and failed at least one course of BCG [43]. After treatment with 500 mg/100 ml of paclitaxel, 36% of the patients achieved a complete response, providing promising prospects for managing NMIBC in patients with extensive prior treatments. The impact of paclitaxel was also evaluated in an additional 28 patients, resulting in a 35.7% complete response rate immediately after treatment [44]. Paclitaxel exhibited low toxicity, and the complete response rate remained favorable even after a one-year follow-up. While paclitaxel provides a viable treatment option by targeting microtubules similar to docetaxel, it is less potent, which accounts for the differences in efficacy between the two drugs. Current research is investigating the potential role of paclitaxel in treating NMIBC utilizing various carrier molecules. Exploring the combination of paclitaxel and docetaxel as a therapy could be a promising future direction, as their combined use may enhance microtubule stabilization and improve treatment outcomes.

In summary, valrubicin, gemcitabine, docetaxel, and paclitaxel each hold promise as treatments for NMIBC, though their efficacy and success rates differ. Despite initial successes, challenges such as drug resistance and long-term effectiveness remain. Exploring combination therapies, such as gemcitabine and docetaxel, could offer a promising alternative with potentially improved long-term outcomes compared to BCG. Additionally, investigating the combination of docetaxel and paclitaxel is a potential future direction, as this approach may enhance microtubule stabilization and provide a more durable impact on NMIBC patients.

### *Immunotherapy*

For NMIBC, immunotherapy represents a promising treatment option due to the high mutation rate in tumor cells, which can trigger a strong immune response [45-47]. Immune checkpoint inhibitors such as pembrolizumab, atezolizumab, durvalumab, and nivolumab play a crucial role in enhancing this immune response. These inhibitors work by blocking specific proteins that tumors use to evade the immune system. For example, following BCG infection, the immune evasion protein programmed death-ligand 1 (PD-L1) is often upregulated on T cells. Immune checkpoint inhibitors target and block PD-L1, thereby enhancing the immune response against NMIBC.

Pembrolizumab administered intravenously represents a promising non-surgical alternative for treating NMIBC. Pembrolizumab is a humanized monoclonal antibody that targets PD-1, blocking its interaction with PD-L1 and thereby enhancing the immune response by activating T-cells. The PD-1 receptor on T-cells, when bound to its ligands, such as PD-L1, inhibits T-cell activity and allows tumor cells to evade anti-tumor immunity. By preventing this interaction, pembrolizumab helps to restore T-cell activity and promote a more effective immune response against cancer cells. [48] The KEYNOTE-057 study [17, 49], conducted across 54 sites in 14 countries, assessed the efficacy of pembrolizumab in NMIBC patients resistant to BCG. The study involved 101 eligible patients who received 200 mg of pembrolizumab intravenously every 3 weeks for up to 24 months or until disease recurrence. The results showed a 41% complete response rate after 3 months, with 46% of responders remaining disease-free for 12 months or longer. Grade 3 or 4 treatment-related adverse effects were observed in 13% of patients, while 8% experienced serious events. The trial is ongoing to further explore pembrolizumab's role in NMIBC treatment. A phase I trial conducted by Meghani et al. [50] investigated the effects of intravesical administration of pembrolizumab in 9 NMIBC patients, exploring an under-researched method of treatment. Although the trial was halted due to COVID-19, the results showed 67% recurrence-free rates at 6 months and 22% at 1 year. The treatment was associated with mild side-effects and one potentially treatment-related death. Pembrolizumab effectively blocks PD-1, inducing a durable immune response with good tolerability. It offers potential for positive long-term outcomes, addressing a common challenge in intravesical chemotherapy. Intravesically delivered pembrolizumab also shows safety and feasibility and can evoke a lasting immune response. Both delivery methods are currently under further investigation, but intravenous administration remains the primary focus, as the drug's FDA approval was based on intravenous clinical studies.

Nivolumab is a fully human monoclonal antibody that, like pembrolizumab, targets PD-1 to keep T-cells active and capable of destroying cancer cells [51]. The phase II CheckMate 9UT study evaluated the efficacy of nivolumab both as a monotherapy and in combination with BCG [52]. The study found that nivolumab offers promising survival rates with minimal side-effects, such as fatigue. While nivolumab has been approved for muscle-invasive bladder

cancer, its role in treating NMIBC is still under investigation. Despite both drugs blocking the same receptor, pembrolizumab has more clinical evidence supporting its efficacy, which may be attributed to differences in their molecular structures. Future studies comparing nivolumab and pembrolizumab could provide valuable insights into the effectiveness of PD-1 immune checkpoint inhibitors.

Atezolizumab is a humanized monoclonal antibody that targets PD-L1, preventing it from binding to its receptor PD-1 [53]. By blocking this interaction, atezolizumab facilitates an immune response by allowing T-cells to remain active and uninhibited, thereby enhancing their ability to target and destroy cancer cells. A single-arm phase II trial evaluated patients receiving atezolizumab intravenously every 3 weeks for a year [54]. The study found a 27% complete response rate after 6 months and a 49% event-free survival rate at 18 months. However, 90.2% of patients experienced adverse events during the trial. Another study assessed the effects of atezolizumab alone versus in combination with BCG in patients who were unresponsive to BCG therapy [55]. The study found that 33% of patients in the atezolizumab-only group experienced severe side-effects, whereas no severe side-effects were reported in the combination group. Six months after treatment, the complete response rate was 33% for the atezolizumab-only group and 42% for the combination group. While atezolizumab is a promising therapy, it has shown moderate efficacy and is associated with serious side-effects, making it crucial to weigh its benefits against potential harms. The combination of atezolizumab with BCG demonstrated a higher success rate and fewer side-effects compared to atezolizumab alone. This finding underscores the need for further research into combination therapies, such as pairing atezolizumab with other treatments like chemotherapy and BCG, to improve efficacy, reduce toxicity, and enhance disease-free rates.

Similar to atezolizumab, a fully human monoclonal antibody, targets PD-L1 to block its interaction with the PD-1 receptor [56]. By preventing this binding, durvalumab helps to reactivate T-cells, thereby enhancing the immune response and potentially reducing tumor progression. A phase II study assessed the effectiveness of 1500 mg of durvalumab administered intravenously every 4 weeks for 12 months in 17 patients who were unresponsive to BCG [57]. The study found a 12% complete response rate at 6 months, and 41% of patients experienced immune-related adverse events. The observed resistance to durvalumab may be attributed to challenges with the immune system's ability to fully restore its activity and effectively target cancer cells, a phenomenon known as incomplete activation. Although both atezolizumab and durvalumab target the same ligand, there is less clinical research supporting the efficacy of durvalumab. Further investigation into durvalumab, particularly when administered intravesically, could help address systemic side-effects and improve its therapeutic potential. Additionally, studying durvalumab in combination with other checkpoint inhibitors or treatments might provide clues to potential resistance issues and enhance treatment outcomes.

In summary, immune checkpoint inhibitors—such as pembrolizumab, nivolumab, atezolizumab, and durvalumab—show promise for treating NMIBC. Future research should focus on comparative studies to evaluate the relative efficacy of different checkpoint inhibitors and explore combination therapies involving immunotherapy, chemotherapy, BCG, or radiation. Additionally, investigating intravesical delivery methods for immune checkpoint inhibitors could help mitigate adverse side-effects. Identifying and analyzing biomarkers associated with immunotherapy responses may further enhance patient outcomes and optimize treatment strategies.

#### *Antibody-drug conjugates*

Antibody-drug conjugates (ADCs) are complex molecules composed of an antibody, a linker, and a cytotoxic drug. These ADCs bind to specific antigens on the surface of cancer cells. Once internalized in the cell, the cytotoxic drug is released and exerts its toxic effects, leading to the destruction of the cancer cells [58]. Vicinium is a fusion protein designed to target cancer cells by binding to the overexpressed epithelial cell adhesion molecule (EpCAM) on their surface [59]. Once internalized in the cancer cell, vicinium releases the cytotoxic agent *Pseudomonas* exotoxin A, which inhibits protein synthesis and leads to cell death. Kowalski et al [60]. evaluated the efficacy and tolerability of vicinium in 46 bladder cancer patients. The study found that the complete response rate at 3 months was 44%, and the disease-free rate was 16%, indicating promising results. A phase III trial evaluated vicinium administered intravesically twice a week for 6 weeks, followed by weekly doses for an additional 6 weeks. The trial demonstrated a 40% complete response rate at 3 months and a 52% disease-free rate at 12 months, further supporting the protein's efficacy. However, despite these promising results, vicinium was not approved by the FDA in 2021 due to insufficient clinical data, leading to a decrease in research interest regarding its potential for treatment of NMIBC [61].

The recently FDA-approved protein N-803 binds to the IL-15 receptor on the surface of CD8+ T cells and NK cells [62]. This binding activates and enhances the production of these immune cells, which are crucial for immune responses. The increased and activated immune cells are then better equipped to detect and destroy cancer cells. The approval of N-803 was supported by data from the phase II/III Quilt-3.032 study, which investigated its use in combination with BCG for patients with high-grade NMIBC, specifically carcinoma in situ (CIS), who were unresponsive to BCG alone [63]. The study reported a complete response rate of 71% after a median follow-up of 26.6 months, highlighting the protein's significant efficacy. Another study explored the effects of combining BCG with N-803 on 9 patients. In this study, patients received weekly doses of 50 mg of BCG for 6 weeks, along with escalating doses of N-803 (100, 200, or 400 micrograms per installation) [64]. Remarkably, after 6 years of treatment, 100% of the patients remained disease-free. Given the risks and benefits of combining BCG with N-803, the favorable outcomes make it a compelling option. The clinical trials and data demonstrate that the combination is both significantly effective and durable. Compared to vicinium, which was not granted FDA approval, N-803 has proven to be a more effective and promising treatment for NMIBC. ADCs like vicinium and N-803 target cancer cells using distinct mechanisms, offering varying levels of efficacy while minimizing damage to normal tissues [65]. Future research should focus on elucidating the specific mechanisms of ADCs, particularly vicinium, to enhance their effectiveness and optimize treatment strategies. Understanding these pathways in greater detail could lead to improved designs and more effective therapies.

#### *Device-assisted therapy*

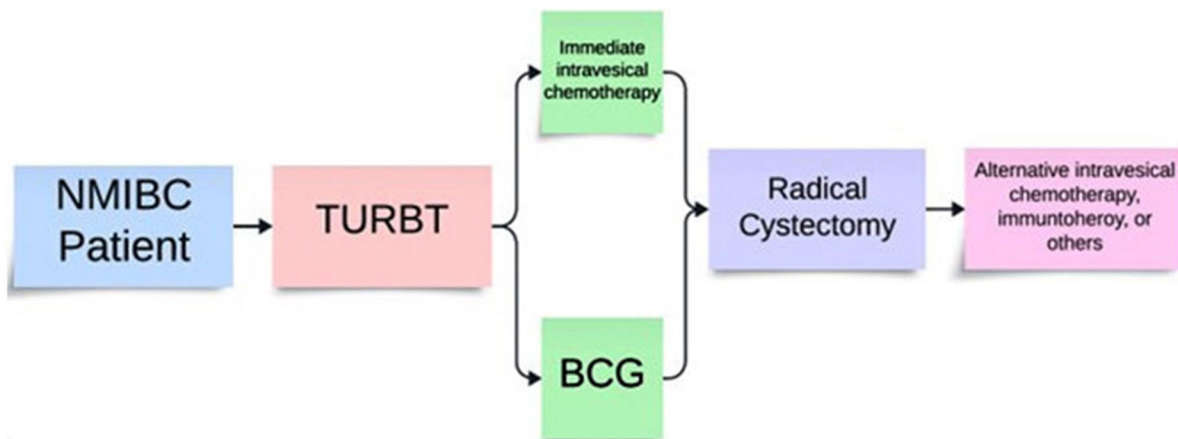
Device-assisted therapies offer innovative methods to enhance the effectiveness of cancer treatments by using physical devices to improve drug delivery or activation [66]. Chemohyperthermia, electromotive drug administration, and photodynamic therapy are examples of device-assisted therapies for NMIBC.

Chemohyperthermia combines chemotherapy with localized heat to enhance the treatment of bladder cancer. This approach aims to improve drug efficacy and reduce recurrence rates by leveraging the synergistic effects of heat and chemotherapy. Mitomycin C has demonstrated lower efficacy and a higher recurrence rate compared to other chemotherapy drugs such as

**Table 4. Clinical trials on treatment options for NMIBC.**

Clinical Trial Number	Study Title	Treatment	Eligibility Criteria	Estimated Completion Date
NCT06255964	A Study of IAP0971 in Combination with Bacillus Calmette Guerin in High Risk Non-muscular Invasive Bladder Cancer	IAP0971 vs BCG	Age: 18 or older; High-risk NMIBC; Adequate organ function; Failed BCG therapy; Negative Pregnancy	2026-12-07
NCT05519241	A Phase I Intravesical PPM Therapy for NMIBC	PLZ4-coated paclitaxel-loaded nanoscale micelle (PPM)	Some type of NMIBC; BCG-unresponsive; Absolute neutrophil count (AGC/ANC) 1,500/uL; Platelets 100,000/uL [Patients may be transfused to meet this requirement]; Hemoglobin 8 g/dL	2026-08-31
NCT04172675	A Study of Erdafitinib Versus Investigator Choice of Intravesical Chemotherapy in Participants Who Received Bacillus Calmette-Guérin (BCG) and Recurred with High-Risk Non-Muscle-Invasive Bladder Cancer (NMIBC)	Erdafitinib vs Gemcitabine vs Mitomycin C	Histologically confirmed, recurrent, non-muscle-invasive urothelial carcinoma of the bladder; Refuses or not eligible for cystectomy; Adequate bone marrow, liver, and renal function; Negative pregnancy test; BCG-unresponsive	2025-03-01
NCT04752722	LEGEND Study: EG-70 in NMIBC Patients BCG-Unresponsive and High-Risk NMIBC Incompletely Treated with BCG or BCG-Naïve	EG-70	Male or non-pregnant, non-lactating female, 18 years or older; BCG-unresponsive; NMIBC with carcinoma in situ (CIS) with or without coexisting papillary Ta/T1 tumors who are ineligible for or have elected not to undergo cystectomy; Failed checkpoint inhibitor treatment	2027-05
NCT05957757	RC48 Combined with Tislelizumab for Bladder Sparing Treatment in NMIBC With BCG Treatment Failure and HER2 Expression	RC48 + Tislelizumab	Age: Older than 18 years; Histologically confirmed recurrent, non-muscle invasive bladder cancer; When BCG recurred after treatment, the presence of HER2 expression was detected; Refuse or be unsuited for radical cystectomy	2026-06-08
NCT02202772	Intravesical Cabazitaxel, Gemcitabine, and Cisplatin (CGC) in the Treatment Urothelial Carcinoma of the Bladder (CGC)	Cabazitaxel + Gemcitabine + Cisplatin	High grade Ta papillary lesion(s); Persistent high risk bladder cancer; Eligible radical cystectomy candidates must refuse it; Patients enrolled in other clinical trials must have received their last treatment 6 weeks prior to enrollment	2028-04
NCT06427291	Clinical Study of T3011 Intravesical Instillation for Treatment of NMIBC Patients	Herpes virus T3011 injection	Participants who voluntarily sign the written ICF; Male or female; Age: Older than 18; No visible tumors after TURBT; Expected survival is greater than 3 months	2025-12-31
NCT03933826	CISTO: Comparison of Intravesical Therapy and Surgery as Treatment Options for Bladder Cancer(CISTO)	Intravesical therapy vs surgery	Current and history high-grade NMIBC; Attempted BCG therapy; Age: 18 years or older; Received at least 1 instillation of any intravesical agent in the last 12 months	2025-03-31
NCT04706598	Safety and Efficacy of Camrelizumab for High-risk NMIBC Failing BCG Treatment	Camrelizumab	Age: 18 years or older; BCG-unresponsive; Consent to tissue specimen retrieval and testing; Adequate normal organ and marrow function	2024-12





**Figure 3. Recommended order of treatment for NMIBC according to NCCN guidelines.**

gemcitabine, as evidenced by a Phase III trial [67]. To address this limitation, mitomycin C is often administered through device-assisted therapies such as chemohyperthermia [68]. This technique involves delivering the chemotherapy drug and then applying localized heat to the tumor, typically raising the temperature to 40-44°C. Methods for applying this heat include intravesical microwave-induced heating, conductive heating, or loco-regional heating [69]. The elevated temperature increases the permeability of cancer cell membranes, allowing the drug to penetrate more effectively. Additionally, the heat enhances the cytotoxic effects of the chemotherapy drug, thereby improving its ability to kill cancer cells. Chemohyperthermia with mitomycin C was evaluated against BCG in an open-label, multicenter trial [70]. The results showed that the device-assisted therapy achieved a recurrence-free survival rate of 78.1%, compared to 64.8% for BCG, highlighting the potential advantages of chemohyperthermia in improving treatment outcomes. A Phase II trial [71] compared the efficacy and safety of chemohyperthermia combined with mitomycin C versus mitomycin C alone, yielding contradictory results. At 24 months, the disease-free rates for chemohyperthermia with mitomycin C and the drug alone were 61% and 60%, respectively. While chemohyperthermia demonstrated similar efficacy to mitomycin C alone, it was associated with more short-term adverse effects and a lower treatment completion rate. While chemohyperthermia has demonstrated a higher success rate as an alternative treatment after BCG failure, it cannot yet be recommended as a standard treatment over intravesical chemotherapy. Its potential benefits are tempered by a higher incidence of short-term adverse effects and a lower treatment completion rate. Further studies exploring chemohyperthermia with mitomycin C and other drugs are needed to provide more definitive conclusions and assess its overall efficacy and safety in treating NMIBC.

Electromotive drug administration (EMDA) is a technique designed to enhance the delivery and efficacy of chemotherapy drugs like mitomycin C [72, 73]. The process involves inserting a catheter into the bladder and applying electric currents to facilitate the movement of the drug through the bladder lining. This electrical stimulation increases the drug's concentration at the site of the cancer cells, potentially improving its effectiveness in treating NMIBC. Busseto et al. [74] conducted a study on 80 NMIBC patients, comparing outcomes between those who

received a second round of BCG alone and those who received a second round of BCG combined with EMDA of mitomycin C. After a median follow-up of 38 months, the results showed that the group treated with EMDA and mitomycin C had a higher recurrence-free survival rate compared to the group that received only BCG. This suggests that EMDA can enhance the efficacy of mitomycin C and potentially improve outcomes for patients with NMIBC who have failed prior BCG treatments. Another study compared the impact of EMDA of mitomycin C combined with BCG versus BCG alone [75]. The results indicated that while the NMIBC progression rate was slightly higher for the BCG-only group compared to the group receiving EMDA with mitomycin C, the difference was not statistically significant. This suggests that EMDA might offer some advantage in terms of progression rates, but further research is needed to confirm its overall efficacy and benefits in combination with BCG therapy.

In summary, device-assisted therapies such as chemohyperthermia and EMDA represent promising approaches to enhance the efficacy of treatments for NMIBC. While the outcomes are not impressively high, it shows better results compared to BCG alone. More research on these techniques compared to other types of treatments such as immunotherapy or intravesical chemotherapy could provide additional data on the efficacy and safety of EMDA. For future purposes, identifying specific patient subgroups that would benefit the most from these therapies could help in tailoring treatment plans to maximize patient outcomes.

#### *Gene therapy*

Gene therapy for NMIBC involves delivering nucleic acids to cancer cells to modify their genes and produce therapeutic effects [76]. The manipulation of genes through nadofaragene firadenovec or CG0070 is conducted to produce therapeutic benefits for cancer patients.

Nadofaragene firadenovec is engineered to treat NMIBC by leveraging the immune system's capabilities. The therapy labeled as nadofaragene firadenovec uses a viral vector to deliver the gene encoding interferon alpha-2b (IFN- $\alpha$ 2b) protein directly into tumor cells within the bladder [77]. This protein activates various immune cells to target and attack cancer cells, stimulates

the production of cytokines, block angiogenesis, and induces apoptosis (programmed cell death) in cancer cells, leading to their destruction. In a phase III trial [78], 151 patients received 75 ml of nadofaragene firadenovec at the start, and then at 3, 6, and 9 months to evaluate its effect on patient outcomes. At the 3-month mark, 53.4% of patients exhibited a complete response. Of those who responded completely at 3 months, 45.5% maintained their complete response at 12 months. The most frequently reported drug-related side effect was micturition urgency, with no treatment-related deaths occurring. This study's findings led to the FDA approval of nadofaragene firadenovec in 2022 [79]. This gene therapy offers a promising alternative for patients who have failed BCG treatment, with several advantages over traditional options. Its recent approval underscores its effectiveness, but further research is needed to validate its long-term benefits.

The CG0070 adenovirus, which carries a granulocyte-macrophage colony-stimulating factor (GM-CSF) gene, targets cancer cells with dysfunctional retinoblastoma (RB) protein [80]. This therapy stimulates the immune system to generate anti-tumor responses. In a phase II trial [81], involving 45 patients with carcinoma in situ, CG0070 demonstrated a notable 50% complete response rate at 6 months with manageable toxicity. Current research is ongoing to assess the safety of this promising treatment, under clinical trial number NCT06253845. The encouraging results from previous studies, combined with ongoing investigations, highlight CG0070's potential as a viable option for treating NMIBC. Expanding research could offer a less toxic alternative for patients who prefer not to undergo or are ineligible for radical cystectomy, paralleling the recent acceptance of nadofaragene firadenovec. In conclusion, gene therapy represents a promising advancement in the treatment of NMIBC, demonstrating encouraging results. Ongoing research into nadofaragene firadenovec and CG0070 could further solidify their roles, potentially leading to widespread endorsement by clinicians.

### Radiotherapy

While radiotherapy is a well-established cancer treatment, its application in managing NMIBC has not been extensively studied. However, both external beam radiotherapy and brachytherapy have shown promising results in specific circumstances.

External beam radiotherapy is used in patients who have failed BCG or intravesical chemotherapy [82]. This method employs advanced imaging technologies to direct x-rays or protons precisely at the bladder, minimizing damage to surrounding normal tissues. Current research is evaluating the effects of intravesical durvalumab alone, durvalumab combined with BCG, and durvalumab combined with external beam radiotherapy [83]. The 3-month complete response rates for these treatments are 33%, 85%, and 50%, respectively. These results highlight the potential of external beam radiotherapy, though other treatment combinations currently show higher efficacy. Despite its relatively lower response rate compared to other treatments, external beam radiotherapy holds promise for improved outcomes when used in conjunction with other drugs and therapies.

Brachytherapy involves placing a radioactive source directly in the bladder to target cancerous cells [84]. This method allows for higher doses of radiation to be delivered to the tumor while minimizing exposure to surrounding tissues. The radiation damages the DNA of cancer cells, leading to apoptosis. A study comparing brachytherapy with and without external beam radiation to radical cystectomy followed 301 patients over 28 years [85]. With a median follow-up of 10 years, disease-specific survival rates were 67% for brachytherapy and 65% for radical cystectomy. While the outcomes are similar, brachytherapy shows a slightly better result than surgery. Although radiotherapy is not

commonly used for NMIBC, combining it with other treatments, such as intravesical chemotherapy, could yield significant benefits.

### Ongoing clinical trials on naïve and bcg unresponsive NMIBC

Numerous clinical trials are investigating new treatment options for patients with BCG-unresponsive and naïve NMIBC (Table 4). These trials focus on patients who wish to preserve their bladder or are too frail to undergo radical surgery. The recently standardized definition of BCG-unresponsive NMIBC involves multiple factors, which can complicate patient selection. In the absence of an established salvage therapy for comparison, the FDA has established a drug registration pathway that only requires a single-arm phase II trial. The FDA recommends that a clinically significant outcome should include an initial complete response rate of 40–50% at 6 months for CIS and a durable response rate of at least 30% at 18–24 months, with the lower bound of the 95% confidence interval exceeding 20% [86].

### Conclusion and future directions

NMIBC at elevated risk remains a significant challenge, especially for patients who do not respond to BCG therapy. Given the substantial impact of radical cystectomy—both in terms of the physical and emotional well-being of patients—there is a critical need to explore and develop alternative treatment options. This article provides a comprehensive overview of NMIBC, including current treatment options and the existing gaps in care. FDA-approved therapies for NMIBC now include intravesical treatments such as valrubicin, pembrolizumab, N-803, and nadofaragene firadenovec, among others. These therapies offer various mechanisms of action and may be selected based on individual patient needs. In addition to FDA-approved treatments, there are several experimental therapies under investigation. According to the National Comprehensive Cancer Network (NCCN) guidelines, the preferred sequence of therapies for NMIBC patients is illustrated in Figure 3. However, this recommended treatment sequence may be adjusted based on individual patient characteristics and the risk of cancer progression. The choice of therapy is therefore tailored to each patient's unique situation and is made collaboratively by the clinician and the patient. Future research should focus on several key areas to improve NMIBC treatment. One promising direction is the investigation of combination therapies, such as integrating chemotherapy with BCG, or combining immunotherapy and radiotherapy. Exploring these combinations may lead to more effective treatment strategies. Additionally, identifying biomarkers for NMIBC could pave the way for personalized therapy, enhancing treatment efficacy and improving patient outcomes by tailoring interventions to the specific characteristics of each patient's cancer.

### Ethical policy

Clinical trials performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consents were obtained from individual participants included in the study.

### Availability of data and materials

All data generated or analysed during this study are included in this publication.

### Author contributions

Conceptualization: AG and SG; Methodology: AG, SV, and SG; Data Curation: AG and SG; Writing to Original Draft Preparation: AG; Writing to Review & Editing: AG, SV, and SG; Visualization: AG and SG; Supervision: SG; Project Administration: SG.

### Competing interests

All authors declare no competing interests.

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