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Immunotherapies to Nano-Immunotherapies: Advances in Immune Targeting in Bladder Cancer

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Abstract

Bladder cancer is among most common malignancies worldwide, with significant morbidity and mortality. Conventional treatment strategies for bladder cancer include transurethral resection, radical cystectomy and chemotherapy. However, the complex immune landscape of bladder cancer involves innate and adaptive immune components that either promote or suppress tumor progression. Upregulation of checkpoint molecules like PD-L1 and recruitment of immunosuppressive cells, contribute to immune evasion and treatment resistance. Immune checkpoint inhibitors such as nivolumab, pembrolizumab, avelumab, and atezolizumab have shown promising results in clinical trials and have been approved for metastatic and high-risk bladder cancer. Additionally, Bacillus Calmette-Guérin (BCG) immunotherapy has long been in use as bladder cancer treatment. Furthermore, natural killer cell-based therapies and novel immune targets like TIGIT and CD155 are under investigation to enhance anti-tumor immunity. However, challenges such as toxic side-effects, variable response rates and the need for predictive biomarkers persists. Nanotechnology offers promising solutions to improve immunotherapy outcomes. Recent advances include the use of gold nanoparticles, TLR agonist-loaded nanoparticles, and exosome-based delivery systems to boost immune responses. Additionally, nanovaccine strategies incorporating tumor-associated antigens and immune adjuvants show potential for personalized cancer immunotherapy. Here, we discuss the immune landscape of bladder cancer, explore the emerging immunotherapies being used as bladder cancer treatment, and discuss the advantages of using nanoparticles as carriers of immunotherapies against bladder cancer. By optimizing combination strategies, identifying novel immunotherapeutic targets, and leveraging nanotechnology for precision medicine, future holds great promise in improving the efficacy of immunotherapies and alleviating bladder cancer burden.

Key words bladder cancer, nano-immunotherapies, BCG immunotherapy, nanovaccines, checkpoint molecules

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Introduction

Bladder cancer ranks among the most common cancer types globally, with more than 430,000 new diagnoses annually, 75% of which are men [1]. Based on the TNM classification, non-muscle-invasive bladder cancer (NMIBC), encompassing carcinoma in situ, Ta, and T1 stages, constitutes 70% of all bladder cancer cases. In contrast, the remaining 30% are categorized as muscle-invasive bladder cancer (MIBC) at stages T2 to T4 [2]. Over the past two decades, groundbreaking advancements in immunotherapy have transformed cancer treatment. The management of non-metastatic bladder cancer typically involves tumor resection through transurethral resection, followed by chemotherapy or bacillus Calmette-Guérin (BCG) immunotherapy. If a patient fails to respond to BCG, radical cystectomy is recommended due to the elevated risk of disease progression [3, 4]. MIBC is conventionally managed with neoadjuvant cisplatin-based chemotherapy, followed by radical cystectomy and pelvic lymphadenectomy [4, 5]. However, the poor prognosis associated with MIBC underscores the potential of immunotherapies to improve patient outcomes and provides opportunities for novel treatment combinations [6, 7]. Notably, nanoparticles have revolutionized the field of cancer treatment in recent years with more and more nanotechnology-based therapeutic formulations getting approved as treatment for diverse cancer types [8]. These particles are small, ranging from 1 to 100 nm in diameter, but have exceptional stiffness, stability, and porosity, making them highly advantageous for use in drug delivery systems [9]. In addition, they significantly enhance the effectiveness of cancer treatments by improving therapeutic outcomes through increased bioavailability, enhanced permeability and retention [10], and more precise tissue targeting [11-13]. Similarly, nanoparticle-based drug delivery systems have also been tested to deliver immunotherapies in bladder cancer. Here, we discuss the immune landscape of bladder cancer, explore the emerging immunotherapies being used as bladder cancer treatment, and discuss the advantages of using nanoparticles as carriers of immunotherapies against bladder cancer.

Immune landscape of bladder cancer

Innate immune system is the first line of defense against cancer. It includes key effector cells such as macrophages, neutrophils, dendritic cells, and natural killer cells. These components play a crucial role in recognizing and eliminating malignant cells [14, 15]. Damage-associated molecular patterns and tumor-derived DNA activate dendritic cells and induce the production of interferon gamma (IFN-γ) [14]. Higher numbers of dendritic cells infiltrating human bladder cancer are linked to the progression to muscle invasive type, indicating that dendritic cells may have a detrimental role in bladder cancer [16]. Macrophages are present in normal human bladders, but their numbers increase significantly at every stage of tumor development [14]. During the initial phases of tumor progression, M1 macrophages, known for their pro-inflammatory properties, execute cytotoxic activities by engaging in phagocytosis, producing reactive oxygen species, and secreting inflammatory cytokines [17, 18]. These cells possess significant functional adaptability, allowing them to transition into M2 macrophages, which are associated with tissue repair and immunosuppressive functions [18, 19]. Notably, M2 macrophages do not secrete C-X-C motif chemokine ligand 9 (CXCL9) or CXCL10, which are involved in recruiting anti-tumor Th1 lymphocytes [20]. Tumor-associated macrophages are mostly M2 type, and correlate with poor survival outcomes in bladder cancer, as high tumor-associated macrophage counts have been linked to worse prognosis [20, 21]. Natural killer cells, which are innate cytotoxic lymphocytes, play crucial roles in the initial immune response against cancer [22]. Natural killer cells possess significant cytotoxic capabilities, which are governed by an intricate interplay of stimulatory and inhibitory molecular pathways. The delicate balance between these opposing signals determines their activation and cytotoxic function against target cells [23]. Under physiological conditions, the bladder microenvironment lacks resident natural killer cells, suggesting that their presence is not a constitutive feature of healthy urothelial tissues. However, their precise involvement in the pathophysiology of bladder cancer remains incompletely understood, with conflicting evidence regarding their potential contributions to tumor surveillance, immune evasion, and disease progression [24, 25]. Higher natural killer cell infiltration was linked to larger tumor sizes in NMIBC patients who experienced relapse after two years [26], suggesting a potential detrimental role for these cells. Healthy bladder does not harbor neutrophils, but these cells are actively recruited in response to chemotactic cytokines such as CXCL1, CXCL5, and interleukin (IL-8). Once infiltrated, these immune cells accumulate in substantial numbers and frequently acquire immunosuppressive properties that contribute to tumor immune evasion [27]. In bladder cancer, both an increased neutrophil-to-lymphocyte ratio [28] and elevated tumor-associated neutrophil densities [29] have been identified as prognostic biomarkers, correlating with diminished therapeutic efficacy, reduced overall survival and higher recurrence rates. This underscores the pivotal role of tumor-associated neutrophils in disease progression. Moreover, similar to macrophages, neutrophils exhibit phenotypic plasticity and can be driven toward a tumor-promoting N2 subtype through exposure to transforming growth factor-beta (TGF-β) [30]. These cells then contribute to tumor progression and invasion by reshaping the extracellular matrix and triggering angiogenesis in the early stages of tumor development, while also influencing tumor cell biology in later stages [31].

In terms of adaptive immune responses, recent research in both human and mouse models has uncovered specific actions of various T cell subsets, most of which seem to negatively impact the host, such as insufficient elimination of cancer cells, and/or increased inflammation [32]. Extensive research has been conducted to characterize T cell-mediated immune responses in bladder cancer models, with the objective of advancing targeted immunotherapeutic strategies. A diverse array of T cell subsets has been identified, each playing distinct immunological roles in bladder cancer. These include cytotoxic CD8+ T cells, which exert pro-inflammatory and antitumor effects, regulatory T cells (Tregs) that contribute to immunosuppression, and CD4+ helper T (Th) cells, with a notable presence of Th1-polarized subsets [33]. This heterogeneity in T cell populations within bladder tumors underscores the complexity of immune interactions shaping disease progression and therapeutic outcomes [34]. In the tumor microenvironment, T-cells release IFN-γ, which enhances antigen presentation by dendritic cells via CD40/CD40L, promotes cytotoxic T cell function, and shifts macrophages to an M1 pro-inflammatory state [34]. In bladder cancer murine models, an increase in IFN-γ producing Th1 cells infiltration has been observed. Moreover, neutralizing IFN-γ nullifies the anti-tumor effects of the therapy, highlighting the crucial role of Th1 cells [35]. An elevated density of CD4+ T cells in the tumor has been linked to poor prognosis in NMIBC [36, 37]. Factors released within the tumor microenvironment play a significant role in attracting Tregs from the bloodstream, which subsequently weakens tumor immune surveillance. This immunosuppressive effect is primarily mediated through the secretion of IL-10 and TGF-β [34, 38], which contribute to dampening anti-tumor immune responses. Additionally, these factors may facilitate the depletion or functional impairment of key anti-tumor effector cells and antigen-presenting cells, further weakening immune surveillance

[34]. This adaptive shift in T cell phenotype exacerbates immunosuppression, ultimately hindering the activation of nascent anti-tumor immunity and promoting tumor progression. T cells upregulate the expression of programmed cell death protein 1 (PD-1) upon activation, which interacts with its PD-L1 present on the majority of tumor cells, thus impairing the T cells' anti-tumor functions by restricting their effector activities [39].

Immune evasion and immunotherapy

To circumvent immune detection, tumors release or encourage the release of immunosuppressive and anti-apoptotic molecules [40]. In reaction to these immunosuppressive signals, the tumor microenvironment recruits additional immune effector cells from the bloodstream, including neutrophils and FoxP3+ Tregs [14]. These cells promote immunosuppressive nature of the tumor microenvironment by upregulating the expression of immune checkpoint molecules such as PD-L1, and by promoting tumor-associated macrophage expansion, which further contribute to immune evasion. In addition, these cells also elevate the production of prostaglandin E2 (PGE2), thereby altering the extracellular matrix and facilitating tumor progression [40]. This complex network of interactions perpetuates a hostile environment for anti-tumor immunity, allowing cancer cells to thrive. In the context of BCG treatment, immune evasion occurs through a variety of mechanisms, such as the downregulation of multi-histocompatibility complex 1 (MHC-I) expression [41] or the upregulation of immune checkpoint proteins like PD-L1 and CD155 on tumor cells [42, 43]. In bladder cancer, CD155 expression correlates with worse prognosis and accelerated tumor progression [44, 45]. Hence, immune evasion is a critical parameter of the tumor microenvironment that needs to be therapeutically targeted to enhance the anti-tumor immunity and improve patient outcomes.

Immunotherapy works by activating immune system, and has thus become a viable option as a first-line treatment [46, 47] or as part of combination therapy strategies alongside other therapeutic modalities [48]. Immune checkpoint inhibitors aim to boost immune defenses against cancer. This approach leads to improved cancer cell eradication and the establishment of durable anti-tumor immunity [49]. These advancements have significantly broadened the scope of immunotherapy, offering new hope for patients with various malignancies. Although clinical outcomes have been somewhat limited, ongoing clinical trials and experimental models are investigating novel approaches to amplify anti-tumor T cell responses. These include the use of monoclonal antibodies that prevent PD-1's interaction with PD-L1, thereby improving T cell activity against tumors [39, 50]. The most significant breakthroughs over the past decade has been the development of immune checkpoint inhibitors targeting CTLA-4 and PD-1/PD-L1 [49, 51]. Additionally, other immune checkpoint molecules like tumor necrosis factor receptor 2 (TNFR2) [52], are also being targeted to further modulate T lymphocyte function and improve therapeutic efficacy. The success of immunotherapy with immune checkpoint inhibitors largely depends on how responsive the tumor is to these inhibitors, with factors such as the genomic diversity

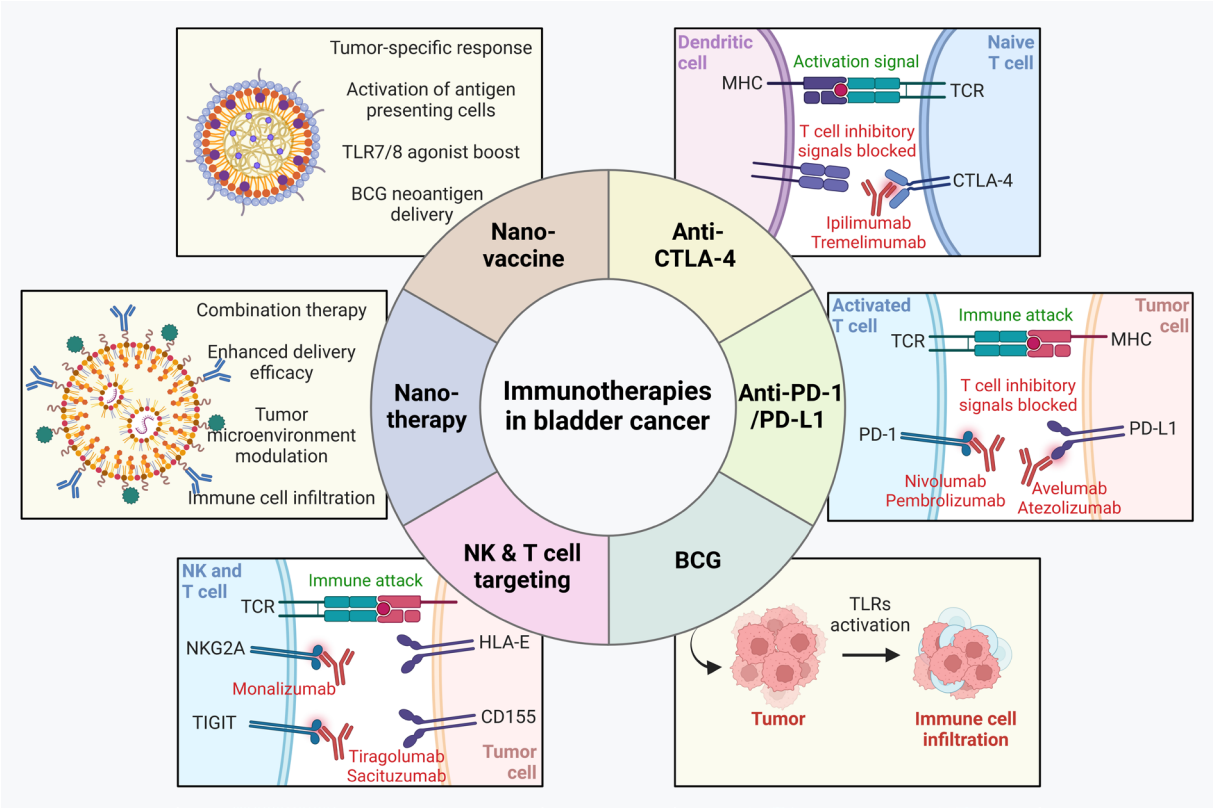

Figure 1. Immunotherapies in bladder cancer. Various immunotherapies including anti-CTLA4, anti-PD1/PD-L1, BCG and those targeting natural killer (NK) and T cells have been approved for treatment in bladder cancer. In addition, nanoimmunotherapies and nano-vaccines are making key progresses in pre-clinical settings with potential to be translated into clinics in future.

Table 1. Immunotherapies in bladder cancer.

Drug / Approach	Target	Clinical trials	Key findings	FDA approval status	References
Ipilimumab	CTLA-4	CheckMate 032, CheckMate 901	Combination with nivolumab shows improved response	Not approved yet for bladder cancer	[55, 56]
Tremelimumab	CTLA-4	DANUBE, NCT0281242	Combination with durvalumab did not improve survival over chemotherapy	Not yet approved	[57, 58]
Nivolumab	PD-1	CheckMate 275, CheckMate 274	Improved survival in platinum-resistant and high-risk MIBC patients	Approved for metastatic bladder cancer and adjuvant therapy in MIBC	[59-62]
Pembrolizumab	PD-1	KEYNOTE-052, KEYNOTE-045	Significant response in platinum-refractory patients	Approved for advanced or metastatic bladder cancer	[63-65]
Avelumab	PD-L1	JAVELIN Solid Tumor, JAVELIN Bladder 100	Improved disease control in maintenance therapy after chemotherapy	Approved for maintenance therapy in advanced bladder cancer	[66-68]
Atezolizumab	PD-L1	IMvigor130	Effective as a first-line option for cisplatin-ineligible patients	Approved but later restricted due to trial findings	[69]
BCG	Immune activation via TLR2, TLR4, TLR9	-	Standard therapy for NMIBC, induces T cell and NK cell responses	Approved for NMIBC	[73, 74]
Monalizumab	NKG2A	COAST, ENHANCE	Blocks NK cell inhibition, under investigation for combination therapy	Not yet approved	[91, 92]
Tiragolumab	TIGIT	NCT05394337	Tested in combination with PD-1 inhibitors (atezolizumab) for urothelial carcinoma	Not yet approved	[93]
Sacituzumab	TIGIT	NCT03547973	Shown to improve survival in metastatic bladder cancer	Approved for metastatic bladder cancer	[94, 95]
KIR2DL5 Inhibitors	KIR2DL5/ CD155	Preclinical studies	Blocking KIR2DL5 enhances NK cell activity	Not yet approved	[96]

of the tumor, host germline genetics, microbiome composition, and PD-L1 expression levels influencing their effectiveness [53]. Different immunotherapies being used and tested as bladder cancer treatment are discussed in the following sections in detail (**Figure 1**).

Anti-CTLA-4 immunotherapies

Various anti-CTLA-4 immunotherapies are in clinical use in bladder cancer (**Figure 1, Table 1**). An anti CTLA-4 antibody, ipilimumab, was the first FDA-approved immune checkpoint inhibitor for stage III and IV melanoma [54]. In case of bladder cancer, CheckMate 032 trial (NCT01928394) tested combination of ipilimumab and nivolumab in patients with advanced or metastatic disease. Comparing the outcomes of combination therapy to monotherapy with each drug individually, the trial showed that combining these drugs have potential to significantly reduce tumor burden along with a favorable safety profile and extended response duration, underscoring the long-lasting effects and substantial therapeutic benefits of this dual-immunotherapy approach [55]. In the CheckMate 901 trial (NCT03036098), nivolumab and ipilimumab were combined as neoadjuvant therapy for MIBC patients before radical cystectomy, and the results indicated strong pathologic responses in patients [56]. While the role of tremelimumab in bladder cancer has been less defined, it has been explored in several clinical settings. One such study, the DANUBE phase III trial (NCT02516241), evaluated its use, both as a monotherapy and in combination with tremelimumab, in patients with stage IV untreated, unresectable, locally advanced, or metastatic bladder cancer, comparing these treatments to standard chemotherapy [57]. Unfortunately, the trial did not meet the endpoint of improving overall survival. However, another trial (NCT0281242) is investigating the use of such combination as neoadjuvant therapy in high-risk MIBC patients. Preliminary results suggest a favorable safety profile, with early indications of promising efficacy [58]. These findings highlight that, although combination immunotherapy may not always surpass chemotherapy in advanced bladder cancer, it could offer potential benefits in the neoadjuvant setting for certain patient populations.

Anti-PD-1/PD-L1 immunotherapies

Various anti-PD-1/PD-L1 immunotherapies have been approved for clinical use in bladder cancer (**Figure 1, Table 1**). Nivolumab is an

FDA-approved anti-PD-1 monoclonal antibody, which got approval for patients with platinum-resistant metastatic bladder cancer based on findings from CheckMate 275 clinical trial (NCT02387996) [59]. The trial results showed that nivolumab induce anti-tumor impact in metastatic bladder cancer patients who had already undergone treatment with platinum-based chemotherapy regimens [60]. Further analysis in 2020 reinforced the initial findings, showing that nivolumab continued to offer sustained anti-cancer activity and prolonged tumor responses in individuals with platinum-refractory metastatic disease [61]. Despite its therapeutic benefits, nivolumab treatment was associated with adverse effects in certain patients, with some experiencing severe complications, including fatalities linked to acute respiratory failure, pneumonitis, and cardiovascular events. To further assess the efficacy of nivolumab, a Phase III CheckMate-274 trial, evaluated the use of nivolumab as an adjuvant therapy in with high-risk MIBC patients who had undergone radical cystectomy. The trial revealed that the nivolumab-treated group had a median disease-free survival of 20.8 months, significantly outperforming that of 10.8 months in placebo group [62]. Pembrolizumab is another anti-PD-1 monoclonal antibody which was evaluated for its efficacy in advanced disease patients who were deemed unsuitable for treatment with cisplatin-based chemotherapy. The results showed a notable response rate of 47% among participants exhibiting elevated PD-L1 expression, whereas those with low PD-L1 levels demonstrated a significantly lower response of 21% [63], suggesting that PD-L1 expression may serve as a potential biomarker for predicting the response to pembrolizumab therapy in this patient population. In the pivotal KEYNOTE-045 study with five years of patient follow-up, pembrolizumab monotherapy showed sustained and robust therapeutic benefit for individuals diagnosed with platinum-resistant metastatic bladder cancer. Similarly, the KEYNOTE-052 trial, which evaluated pembrolizumab as a first-line therapeutic option for patients unable to receive cisplatin-based chemotherapy, yielded comparable favorable results. Even after extended observation, no new safety concerns emerged, further solidifying pembrolizumab's status as a safe and highly effective treatment choice for these cohorts [63]. Conversely, the PLUMMB trial (NCT02560636), which explored the combination of pembrolizumab and radiation therapy in MIBC patients, faced early challenges. The study was temporarily halted after severe toxicities were observed in the initial treatment cohort, prompting a reevaluation of the safety of this combination regimen [64, 65]. Avelumab is another FDA-approved anti-PD-L1 antibody for metastatic bladder cancer patients who are non-responsive to platinum-based chemotherapy [66]. The JAVELIN solid tumor trial reported an overall survival of 13.7 months and a response rate of 18.2% for avelumab in patients with metastatic bladder cancer [67]. The JAVELIN Bladder 100 Phase III trial (NCT02603432) investigated the use of avelumab as a first-line maintenance therapy for patients with advanced bladder cancer who had not shown disease progression after completing chemotherapy [68]. Long-term follow-up has consistently demonstrated substantial efficacy, reinforcing avelumab's established role in prolonging disease control for patients. Atezolizumab, another FDA-approved anti-PD-L1 antibody for metastatic bladder cancer patients non-responsive to platinum-based chemotherapy. Atezolizumab has been used both as a first-line treatment for cisplatin-ineligible patients and as a second-line therapy following chemotherapy failure, as shown in the IMvigor130 trial (NCT02807636) [69]. Durvalumab is another anti-PD-L1 antibody that blocks the PD-1/PD-L1 interaction [70, 71]. It has been tested alongside other immunotherapies. The NIAGARA trial (NCT03732677) is testing durvalumab combined with chemotherapy in MIBC patients to improve pathological response and survival. Overall, anti-PD-1/PD-L1 therapies represent promising immunotherapies against bladder cancer.

BCG immunotherapy

BCG was first successfully used in 1976 (**Figure 1, Table 1**) [72], and has been associated with achieving positive outcomes in 55 to 65% of cases involving papillary tumors and 70 to 75% in cases of carcinoma in situ [73, 74]. BCG interacts with urothelial cells, either through non-specific mechanisms or via the fibronectin adhesion protein (FAP) [75], with a particular affinity for poorly differentiated cells [76]. Nevertheless, once BCG is internalized by bladder cancer cells, it triggers an increase in the production of nitric oxide (NO) [77], which could contribute to the cytotoxic effects on urothelial cancer cells. Although the bladder cancer associated therapeutic effects of BCG are being explored [78], it is well-recognized that BCG activates both innate and adaptive immune responses. These interactions primarily involve toll-like receptors (TLRs) such as TLR2, TLR4, and TLR9 [79]. In addition to activating these cells, BCG immunotherapy activates the recruitment of circulating macrophages and antigen-presenting cells to the bladder region [80, 81], where they undergo activation and release pro-inflammatory cytokines, including IL-6, IL-12, and TNF-α. These cytokines play a crucial role in promoting macrophage polarization toward the M1 phenotype and fostering the development of Th1 cells, which are essential for effective anti-tumor immunity [82, 83]. BCG treatment induces the recruitment of T cells to the bladder mucosa. The presence of increased CD4+ T cells has been strongly associated with improved responses to BCG therapy [80, 84]. Th17 cells induced in response to BCG release cytokines which are essential not only for immune defense against fungi and extracellular bacteria but also for promoting inflammatory responses [85]. In addition to their influence on T cell differentiation, BCG therapy also activates natural killer cells, prompting them to release pro-inflammatory cytokines such as IL-1β and IL-6, further enhancing the immune response [86]. After BCG therapy, a significant expansion of the CD56high natural killer cell subpopulation has been observed, showcasing functional maturity and antitumor cytotoxicity [87]. These findings emphasize the crucial role of natural killer cells in bladder cancer and encourage further research into their potential for therapeutic applications. Additionally, analyzing the genetic characteristics of natural killer cell receptor interactions with ligands from both the host and tumor may offer valuable information regarding patient prognosis and contribute to the development of more individualized therapeutic strategies [88].

Natural killer and T cells targeting immunotherapies

NKG2A is a receptor predominantly expressed on natural killer cells and CD8+ T cells, where it interacts with its frequently overexpressed ligand, HLA-E [89]. This binding between NKG2A and HLA-E suppresses these immune cell function. Blocking NKG2A can partially reverse this inhibition, enhancing the functional activity of natural killer cells and CD8+ T cells. This effect is also dependent on the presence of HLA-E, highlighting a potential therapeutic approach to overcome immune evasion in tumors [90], while supporting the idea of combining NKG2A blockade with other immune checkpoint inhibitors in clinical trials, particularly for tumors with high levels of HLA-E. Various natural killer and T cell targeting immunotherapies are being tested for clinical use in bladder cancer (**Figure 1, Table 1**). Monalizumab, a pioneering IgG4 antibody targeting NKG2A, is currently undergoing clinical evaluation. The COAST trial (NCT03822351) is investigating the effectiveness of combining monalizumab with durvalumab [91]. The ENHANCE trial (NCT06503614), a phase II study, will assess the same combination in NMIBC and is expected

to begin recruitment soon [92]. TIGIT is a relatively new immune checkpoint receptor with inhibitory properties found on natural killer and T cells that interacts with CD155 [93]. Tiragolumab and Sacituzumab are monoclonal antibodies designed to inhibit the interaction between TIGIT and its ligands CD112/CD155. Ongoing clinical trials, including the Phase I study NCT05394337, are investigating the safety of combining monoclonal antibodies targeting NKG2A with neoadjuvant PD-1 inhibitors (such as Atezolizumab) and TIGIT inhibitors (like Tiragolumab) in patients with high-risk urothelial carcinoma who are not candidates for cisplatin-based chemotherapy. Despite these efforts, recent findings from two large Phase III trials involving TIGIT blockade have not demonstrated any significant improvements in cancer outcomes. KIR2DL5, which is also known as CD158f, represents a new member of family of functional killer-cell receptors. Its ligand, CD155, has emerged as a key target in cancer immunotherapy, with particular relevance in MIBC [94, 95]. As an inhibitory receptor, KIR2DL5 interacts with CD155, forming inhibitory synapses that dampen natural killer cells [96]. The overexpression of CD155 is associated with an increased likelihood of recurrence in NMIBC. Blocking the KIR2DL5/CD155 interaction using monoclonal antibodies has demonstrated promising results by enhancing natural killer cell-mediated tumor cytotoxicity, especially against CD155-expressing tumors, and reducing tumor growth in humanized mouse models [96]. Interestingly, research has highlighted KIR2DL5 as a potential alternative pathway for suppressing natural killer and T cell activity. KIR2DL5 shares its ligand, CD155, with TIGIT, and its inhibition may provide a different mechanism to overcome immune evasion and enhance anti-tumor immunity. This highlights the potential benefit of combining therapies that target both of these inhibitory receptors for a more comprehensive approach to immune modulation [96].

Nano-immunotherapy

The primary goal of immunotherapy is to stimulate the immune system and counteract the mechanisms that tumors employ to evade immune surveillance. Nanoparticles have shown significant promise in increasing their specificity for cancer cells, and reducing their associated side effects [97]. Immune checkpoint inhibitors are primarily used in two ways: once approach is as neoadjuvant therapy to improve complete remission rates, which in turn can increase the chances of bladder preservation [98]. The second approach involves administering the therapy post-surgery or after radiotherapy. This strategy may help extend both progression-free and overall survival for patients [99]. Despite the promise of immunosuppressive therapy in improving outcomes for advanced bladder cancer patients, several challenges persist, such as side-effects, low response rates, and limited efficacy due to the complex nature of the tumor microenvironment [100]. Developing advanced nanoplatforms to optimize the delivery and boost the effectiveness of cancer immunotherapies has gained much attention recently [101]. For instance, the use of macrophage-derived exosome-mimetic nanovesicles (EMVs) to deliver a combination of a CD73 inhibitor (AB680) and a PD-L1 inhibitor has been investigated in a mouse model of bladder cancer, where it showed enhanced tumor-targeting capabilities. The combined treatment notably boosted T cell activation and facilitated their infiltration into the tumor site, enhancing the anti-tumor immune response (**Figure 1, Table 2**) [102, 103]. Another strategy utilized a reactive oxygen species-sensitive polymer, PHPM, to co-encapsulate copper ion carriers, including elesclomol and copper, forming nanoparticles known as NP@ESCu. These nanoparticles facilitate immune response enhancement by inducing cuproptosis, a copper-dependent form of cell death, while also being conjugated with an anti-PD-L1 antibody. The study demonstrated that NP@ESCu nanoparticles effectively released copper, inducing cell death in bladder cancer cells. Additionally, NP@ESCu nanoparticles upregulated PD-L1 expression, thereby boosting the efficacy of the αPD-L1 treatment [104]. Another novel approach is the conjugated gold nanoparticles (GNPs) with a bacterial peptide (LLO 91–99), derived from listeriolysin O (LLO), to create the GNP-LLO 91–99 nanovaccine, further exploring potential mechanisms to enhance anti-tumor immunity. The GNP-LLO 91–99 demonstrated substantial effectiveness in treating bladder cancer by addressing the immunosuppressive tumor microenvironment via increasing the infiltration of cytotoxic T cells and dendritic cells into the tumor, while simultaneously reducing the presence of immunosuppressive Tregs and myeloid-derived suppressor cells. Additionally, the GNP-LLO 91–99 nanovaccine promoted the activity of immune checkpoint inhibitors, underscoring the potential of this nanosystem as an innovative immunotherapy strategy for bladder cancer [105]. However, it is important to note that while immunotherapy has shown promise, it is also associated with significant local and systemic side effects [106]. To address these challenges, a novel macrophage-targeted delivery system has been developed, utilizing a specialized nanoparticle known as MNC-ICG-NIG@SiO2 (MINS). This innovative nanoparticle comprises magnetic nanoclusters conjugated with CpG at its core, encased by a silica (SiO2) shell that incorporates indocyanine green and nigericin, with modifications made through Se-Se bonds. Controlled release of its components trigger immune activation specifically within the tumor microenvironment. When administered intravenously, BCG therapy induces localized inflammation within the tumor, which helps to enhance the targeted accumulation of MINS@MΦ (macrophages), thus facilitating a more precise and potent immune response against the tumor. This accumulation is further activated by laser irradiation, causing the indocyanine green to generate reactive oxygen species, rupture the Se-Se bonds, and release nigericin to trigger autoimmolation. By modulating cytokine levels, MINS enhances BCG immunotherapy efficacy, offering improved therapeutic potential for bladder cancer [107]. Overall, these approaches exemplifies the potential of nanotechnology in overcoming barriers to effective cancer immunotherapy, and their clinical translation holds promise to effectively eradicate advanced tumors in bladder cancer.

Nanovaccines

Anti-cancer vaccines trigger a tumor-specific cytotoxic T-lymphocyte response, crucial for identifying and eliminating cancer cells. This immune response depends on the activation of antigen-presenting cells, such as dendritic cells and macrophages [108, 109]. By stimulating these cells, the vaccines help train the immune system to recognize and target tumor-specific antigens, enhancing the body's ability to fight cancer more effectively. The presence of tumor-associated antigens in the immune environment helps to strengthen and broaden the adaptive immune response, ultimately improving the body's ability to recognize and attack cancer cells [110]. Nanovaccines have the ability to deliver tumor-specific antigens or tumor associated antigens precisely to the immune system, while also addressing immune escape mechanisms within the tumor microenvironment by using immune adjuvants to counteract immune suppression [109, 111]. Delivery systems act as carriers that enhance antigen presentation by increasing the bioavailability of antigens and directing their delivery to lymph nodes or antigen-presenting cells [112]. These systems are crucial for improving the efficiency of vaccines by ensuring that the immune system is properly stimulated. TLR agonists have garnered attention as a promising class of vaccine adjuvants. TLR 7/8 agonists, in particular, have shown encouraging

Table 2. Preclinical nanoimmunotherapies and nanovaccines in bladder cancer.

Approach	Target / Mechanism	Key findings	References
Macrophage-derived exosome-mimetic nanovesicles (EMVs) with AB680 + PD-L1 inhibitor	CD73 inhibition + PD-L1 blockade	Enhanced T cell activation and tumor infiltration	[102, 103]
Reactive oxygen species (ROS)-sensitive nanoparticles (NP@ESCu) with cuproptosis induction	Copper ion release + PD-L1 upregulation	Induced cancer cell death and improved immune response	[104]
Gold nanoparticles (GNPs) conjugated with listeriolysin O peptide (GNP-LLO 91–99 nanovaccine)	Tumor microenvironment modulation	Increased cytotoxic T cell infiltration and improved response to checkpoint inhibitors	[105]
MNC-ICG-NIG@SiO2 (MINS) macrophage-targeted delivery system	BCG therapy enhancement + autoimmunity modulation	Improved BCG therapy response through cytokine regulation	[107]
TLR7/8 agonist-loaded polymeric nanoparticles	Dendritic cell activation	Enhanced CD8+ T cell response and reduced metastasis	[108, 113, 114]
BCG cell wall skeleton-based nanovaccine with neoantigens (M27, M30)	Cancer-specific immune activation	60% tumor elimination and synergy with PD-L1 inhibitors	[115]

results in boosting cancer therapies by promoting a stronger immune response, enhancing the overall effectiveness of cancer vaccines (**Figure 1, Table 2**) [113, 114]. One example of enhancing anti-cancer immunotherapy involves encapsulating a TLR-7/8 agonist nanoparticles. When administered subcutaneously, these nanoparticles travel to the draining lymph nodes, where they effectively activate and expand dendritic cells. This activation not only stimulates the recruitment of antigen-specific CD8+ T cells but also amplifies the cytotoxic T lymphocyte response, leading to significant therapeutic improvements in bladder cancer via reducing metastatic spread of the diasease [108]. Furthermore, a novel nanovaccine combining peptide neoantigens (M27 and M30) with nanoscale BCG cell wall skeleton, has shown promising results in melanoma treatment, highlighting the potential of nanovaccine-based therapies in oncology. This innovative nanovaccine effectively stimulated both innate immune responses and tumor-specific immunity [115]. While there have been significant advances in nanoparticle-based immunotherapies, much of the current research remains focused on conventional CD8+ T cell-mediated cytotoxic responses. There is still limited exploration into how nanoparticles could be further optimized to improve cancer immunotherapy beyond enhancing cytotoxic T cell activity [34].

Conclusion and future prospect

Immunotherapy for bladder cancer has evolved significantly, moving beyond BCG and immune checkpoint inhibitors. Alternative approaches are emerging with promising potential. Use of cytokine-based therapies is limited due to considerable side effects. Ongoing studies are focused on refining these treatments to reduce toxicity while improving their efficacy, particularly when combined with other therapies [116]. Vaccine-based treatments, such as the Ty21a vaccine, are gaining attention for the treatment of NMIBC. These vaccines have the potential to stimulate robust anti-tumor immune responses, offering an alternative treatment strategy [117]. Additionally, dendritic cell vaccines are being explored to activate T cells against tumor-specific antigens. Some studies are also investigating the potential of combining these vaccines with other immunotherapeutic approaches to enhance their effectiveness [118, 119]. This highlights the growing role of targeted therapies in managing bladder cancer, particularly in challenging, refractory cases. The identification of new co-stimulatory and co-inhibitory receptors and the exploration of their roles have led to the development of innovative immunotherapies. Despite being a significant breakthrough for advanced cancer therapy, most patients still experience limited benefit. Moving forward, the focus of research in advanced bladder cancer treatment should be directed at introducing novel immunogenic therapeutic targets and combining the therapies against them with existing ones, with the goal of improving outcomes for a larger proportion of patients.

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Non applicable.

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

Author contributions

BR and DR searched academic literature, wrote the draft manuscript, drew the figures and submitted the final manuscript online.

Competing interests

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