Am J Clin Exp Urol 2026;14(5):170-188
www.ajceu.us /ISSN:2330-1910/AJCEU0175672

Review Article

Inflammation in bladder cancer landscape: biological insights and clinical significance

Shevanka De Silva[1*], Anish Korrapati[1*], Sudeh Izadmehr[2*], Edgar Gonzalez-Kozlova[3], Matthew D Galsky[2], Ketan Badani[1], Navneet Dogra[4], Natasha Kyprianou[1,4,5]

[1]Department of Urology, Icahn School of Medicine, Mount Sinai, New York, USA; [2]Division of Hematology/Medical Oncology, Icahn School of Medicine, Mount Sinai, New York, USA; [3]Department of Immunology, Icahn School of Medicine, Mount Sinai, New York, USA; [4]Department of Pathology and Cell Based Medicine, Icahn School of Medicine, Mount Sinai, New York, USA; [5]Department of Oncological Sciences, Icahn School of Medicine, Mount Sinai, New York, USA. [*]Equal contributors and co-first authors.

Received July 28, 2026; Accepted October 20, 2026; Epub October 25, 2026; Published October 30, 2026

Abstract: The inflammatory state of the bladder can be due to a multitude of factors such as smoking history, bladder microbiome, exposure to carcinogens, diet, and upregulation of certain genes. Moreover, clinical evidence supports that men have a higher chance of developing bladder cancer while women can be diagnosed at a later stage due to a misdiagnosis of a common urinary tract infection or an abnormal uterine bleeding condition. There are several sex-specific contributors to the difference in presentation in bladder cancer such as hormonal influence, Y chromosome loss, and bladder microbiome diversity that influences the treatment response. Chronic inflammation is a common mechanism that links this disparity as seen by how it creates the tumor microenvironment and shapes the disease presentation, progression, and response to treatment. Gene signatures have recently been developed with predictive value for the prognosis of patients with bladder cancer, while models are being evaluated to determine responsiveness of radical cystectomy, immunotherapy, and chemotherapy based on the tumor microenvironment. EVs can serve as a non-invasive diagnostic tool, as malignant cells shed EVs in the urine and can be monitored to profile the biological state of the bladder. Novel biomarkers for bladder cancer that are cost-effective and efficient are currently being pursued to better predict the extent of bladder tumor invasive progression, as well as a diagnostic tool to differentiate between male and female urothelial carcinoma. In this review, we examine the contribution of chronic inflammation as it shapes the bladder tumor microenvironment and impacts the clinical disparity observed in bladder cancer between males and females. Furthermore, we discuss the effects of such disparity as well as the inflammatory microbiome on the clinical management of the disease. Finally, we review the current understanding of the benefits of EVs as a potential non-invasive predictive and screening tool for bladder cancer.

Keywords: Inflammation, bladder tumors, tumor microenvironment, sex disparity, extracellular vesicles

Introduction

Bladder cancer (BC) is the sixth most diagnosed cancer in the United States and the ninth most common worldwide. Men are more likely to be diagnosed with bladder cancer than women, however, women are more likely to be diagnosed at a later stage of the cancer with a worse prognosis [1]. This is often due to bladder cancer being misdiagnosed with more common diagnoses such as uterine conditions or urinary tract infections in women. There is a multitude of factors that contribute to the disparity between BC diagnosis in men and women for bladder cancer with women being disproportionally affected. Common differences that contribute to the disparity, include the bladder microbiome, exposure to aniline dye use from hair products, while smoking remains a higher risk factor among men. In 2021, smoking was the primary reason for 26.48% of global bladder cancer deaths, most prominently in East Asia. Compared to other urological cancers, bladder cancer had a global incidence of 540,000 (95% CI 4.95-5.83), while kidney cancer and prostate cancer incidences were 388,000 (95% CI 3.65-4.07), and 1,324,000 (95% CI 12.17-14.00), respectively [1].

https://doi.org/10.62347/BXAB9246

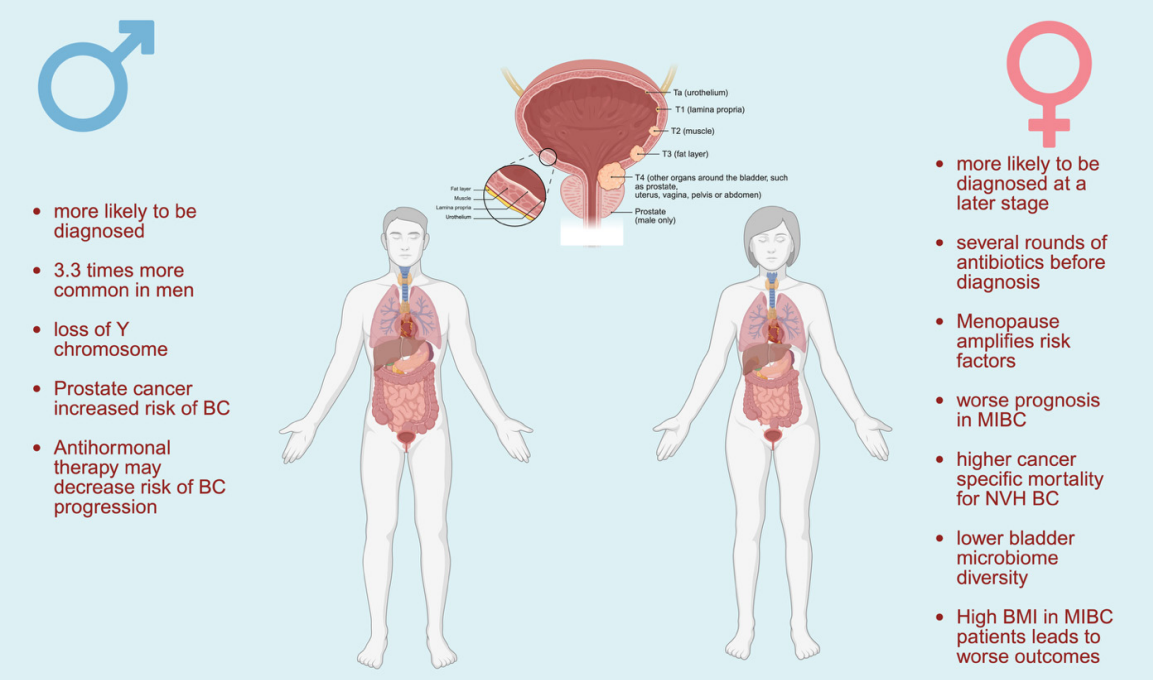


Figure 1. Sex disparities in bladder cancer: the central image of tumor staging of bladder cancer with Ta stage at the urothelium, T1 stage in the lamina propria, T2 invading the surrounding muscle, T3 invading the fat layer, and T4 invading surrounding organs such as the prostate, urethra, vagina, and pelvis or abdomen. Men are more likely to be diagnosed with bladder cancer and have 3.3 times higher risk of developing the cancer compared to women. The loss of the Y chromosome in men leads to a progression of the cancer. Prostate cancer, the most common cancer in men, is a risk factor for bladder cancer. Women are more likely to be diagnosed with a higher stage of bladder cancer often due to a misdiagnosis of a UTI which requires several rounds of antibiotics before further diagnostic tests. Women have worse prognosis in muscle-invasive bladder cancer, especially with a higher BMI, and a higher cancer-specific mortality in non-variant histology bladder cancer. Women have a lower bladder microbiome diversity compared to men.

Chronic inflammation is a central driver of bladder tumorigenesis, and these patients consistently show evidence of elevated systemic inflammatory markers such as C-reactive protein, Interleukin-6, and composite indices such as the Systemic Immune Inflammation Index (SII) or Dietary Inflammatory Index (DII) compared to the general population. These markers can be prognostic and are useful to monitor in patients with certain oncological diseases as they can be helpful in predicting survival outcomes as well at treatment responsiveness by understanding how the tumor microenvironment evades the immune system's natural protective defense mechanisms. These mechanisms that create the inflammatory state and how they differ between men and women are further described.

In 2022, Cedars-Sinai Medical Center held the first conference, Gender Medicine Symposium, focusing on the role of sex and gender differences that impact bladder cancer progression, patient survival and treatment response [2]. For female patients, physicians initially prescribe antibiotics in suspicion of urinary tract infections due to their commonality amongst the population without significant investigation of further urological disease since a high number of this patient population are referrals to an urologist. This delay has harmful effects on female patients due to a high probability of detection of advanced stage of bladder cancer (schematically summarized on **Figure 1**). The role of sex chromosomes has already been well established in the context of bladder cancer as seen by sex chromosomal disorders such as Klinefelter or Turner syndrome which impact androgen circulation [2]. Ongoing studies will improve overall care for female patients towards establishing a less invasive screening test for bladder cancer [2].

Analysis of urine cytology in patients post gender affirming surgery, revealed that 8 out of 32

patients had atypical urothelial cells on cytology; all 32 patients were confirmed to be negative for high-grade urothelial carcinoma. Of the 8 patients, 7 patients exhibited prominent small nucleoli and irregular nuclear membranes [3]. This might pose a challenge for the clinicopathological management of the disease, with overdiagnosis of urothelial malignancies resulting in an unnecessary increase in invasive procedures (cystoscopy) [3]. Tumor biology techniques using genomics, transcriptomic, and spatial proteomic domains were applied to define sex specific mortality; however this study does not account for hormonal, microbiology, and immunological responses within the TME [4]. Muscle invasive bladder cancer (MIBC) did exhibit gender-related differences in clinical outcomes. Thus, females experienced a worse overall prognosis in MIBC compared to males with data from The Genomic Cancer Atlas. This study also showed a correlation with Body Mass Index (BMI) and the progression of MIBC, highlighting the role of obesity and metabolism in disease progression. Females with a BMI over 24, had a worse overall survival compared with males. In females with a BMI less than 24, the disease-free survival was worse than their male counterparts. Females also had a worse overall survival and disease-free survival with stage III disease [5]. Non-urothelial variant histology bladder cancer (Squamous cell carcinoma, Adenocarcinoma, and Neuroendocrine carcinoma) makes up between 10-25% of all bladder cancers and has a higher cancer specific mortality in females. Females were also found to have a higher frequency of T1 stage tumor compared to males especially for Squamous cell carcinoma. This study concluded that the female sex may be an independent marker for T1 stage and high cancer-specific mortality in non-urothelial variant histology bladder cancer [6].

The loss of the Y chromosome occurs in about 40% of male bladder cancers. The Y chromosome has genes that regulate epigenetic modification as well as immune modulation; its loss in bladder cancer leads to a worse overall prognosis due to $CD8^+$ cell dysfunction within the tumor microenvironment. There was also a higher number of tumor associated macrophages as well as an increase in immune checkpoint molecules. While this can lead to a worse outcome, the loss of the Y chromosome confers a better response to PD-1 inhibitors due to the highly immunogenic environment created within the microenvironment. Although the T-cells are dysfunctional, the inflamed tumor can be targeted more by these check point inhibitors [7]. The progression of bladder cancer is different due to anatomical differences between male and female patients [8]. However, there was no difference found in the response of intravesical Bacillus Calmette-Guérin (BCG) due to gender with a similar risk of recurrence between men and women with 57.5% and a 52% respectively [8].

From chronic inflammation to established tumor microenvironment

Chronic bladder inflammation is a sustained response that reshapes the tissue into a permissive tumor microenvironment. The constant urothelial injury increases cytokine and chemokine signaling, especially IL-6 and NF-kB [9]. This results in immune cell activation into the bladder wall and allows for macrophage recruitment that creates an immunosuppressive environment. The bladder wall undergoes stromal remodeling through cancer associated fibroblasts (CAFs) and myofibroblasts (myoCAFs) [10]. The presence of an immunogenic environment results in several inflammatory cascades such as hypoxia-inducible factor-1α (HIF-1α) which upregulates PKM2 through ALYREF and increases the tumor cell metabolism [11]. Likewise, macrophages secrete TGF-β that also increases PKM2 expression via the STAT3 cascade that results in higher transcription of PD-L1 [12]. This links chronic inflammation signaling to understanding the importance of the tumor microenvironment as it results in immune checkpoint regulation and reduced immunotherapy response with lower T-cell infiltration.

Mechanisms of chronic inflammation in bladder tumorigenesis

Chronic inflammatory conditions of the bladder create a tumor-promoting microenvironment that contributes to carcinogenesis through diverse mechanisms including sustained cytokine signaling, oxidative stress, DNA damage, and immune dysregulation. Environmental exposures that induce chronic urothelial injury further amplify these inflammatory processes. 4-Aminobiphenyl (4-ABP) is a known carcinogen that has been banned in Europe but can

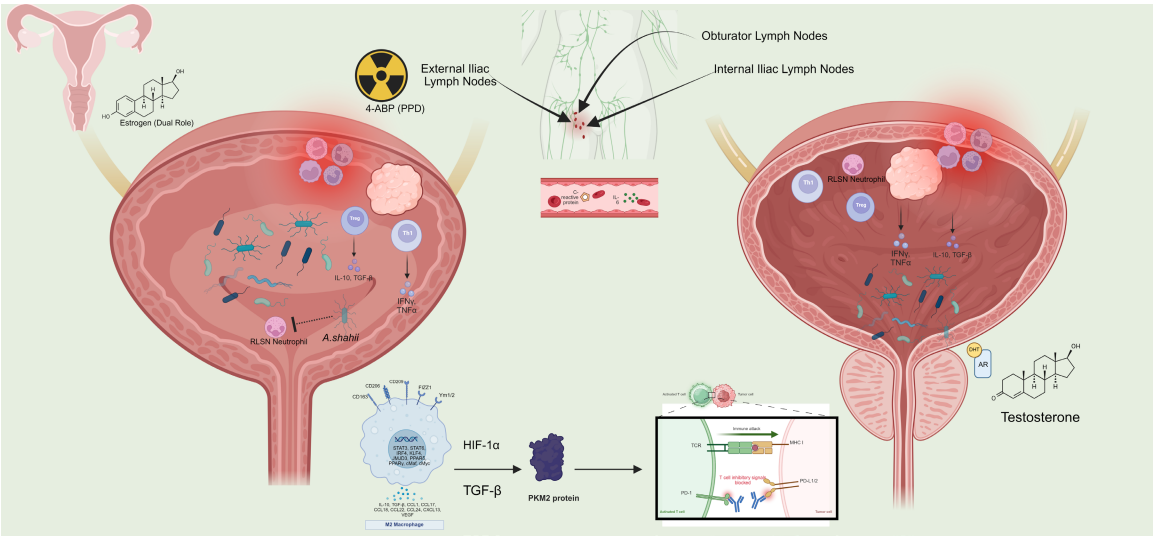


Figure 2. Pro-inflammatory state of bladder cancer: the differences in bladder morphology between females (left) and males (right). The pelvic lymph nodes (obturator, external iliac, and internal iliac) are depicted as the site of spread and metastasis of bladder cancer. The female bladder tumor microenvironment can be influenced by the lower diversity in bacteria. Estrogen plays a protective role in preventing bladder cancer but also aids in tumorigenesis. Due to anatomical distance, males with prostate cancer have an increased risk of bladder cancer which could also be due to androgen deprivation therapy which lowers the conversion of testosterone to DHT. RLSNs accumulate in male TMEs but are suppressed via Lurasidone produced by the gut bacteria Alistipes shahii. The HIF-1α/ALYREF/PKM2 and TGF-β/STAT3/PD-L1 signaling pathways lead to immune evasion, tumor cell migration, and tumor invasion. CRP and IL-6 are shown in a blood vessel as systemic inflammatory markers.

still be found in trace amounts in the hair dye para-phenylenediamine (PPD) (shown in **Figure 2**). The Office of Environmental Health Hazard Assessment (OEHHA) gave 4-ABP a No Significant Risk Level of 0.03 micrograms a day based on animal carcinogenicity studies. The manufacturing of PPD can result in different levels of 4-ABP in the dye and can lead to bladder cancer due to dermal exposure, however, current exposure level from these products does not suggest that using PPD hair dyes causes an increased risk of bladder cancer [13].

Tobacco smoking is the most important modifiable risk factor for bladder cancer because it exposes the urothelium to numerous carcinogenic compounds. An analysis done on the impact of smoking on bladder cancer patients in China from 1990 to 2021 showed in these patients, mortality rises rapidly in males as they age after 75 but increase gradually in females [14]. This team also highlighted the fact that in their model, they predicted a significant decrease in smoking-related bladder cancer that is more prominent in males. The difference in patient outcomes in diverse populations reflected the nation's public health policies and access to healthcare [14]. Beyond its direct carcinogenic effects, smoking promotes systemic inflammation through persistent activation of inflammatory cytokines, including IL-6, which stimulates hepatic production of C-reactive protein (CRP).

As a low-cost, widely available blood-based biomarker, CRP has emerged as a prognostic marker in bladder cancer [15]. A systematic review and meta-analysis including 6,789 patients across 28 studies demonstrated that elevated pretreatment CRP was associated with significantly worse overall survival across both localized and advanced disease settings [16]. Notably, elevated CRP has been associated with poorer clinical outcomes among patients treated with immune checkpoint inhibitors, supporting its potential utility for risk stratification. Recent mechanistic studies have provided biological context for these associations. Across multiple clinical trial cohorts of patients treated with immune checkpoint inhibitors, elevated plasma CRP and IL-6 levels were associated with increased intratumoral macrophage infiltration, enrichment of immunosuppressive SPP1+ macrophages, reduced T-cell activity, and resistance to immunotherapy [9]. In con-

trast, CXCL9+ macrophages exhibited enhanced T-cell activation and improved response to checkpoint blockade. These findings suggest that CRP may reflect more than nonspecific systemic inflammation and could serve as a readily accessible surrogate of an immunosuppressive tumor microenvironment (TME).

The tumor microenvironment (TME) and the microbiome

Initiation of a pro-inflammatory state in the bladder could be due to a microbial dysbiosis event [17]. Differences in bladder microbiome and urinary microorganisms have been hypothesized as a screening method for treatment. However, in a 2020 study analyzing different species in urine and tissues samples of bladder cancer patients, they did not find significant differences in Shannon diversity and richness of microbiome in transurethral catheterized urine and tissue samples. Certain microorganisms within the TME can lead to an increase in certain elements such as nitrates. After renal filtration, there could be an increase in various toxins that interact with the microbiome and increase or decrease the risk of bladder cancer [18]. Polycyclic Aromatic Hydrocarbon degrading bacteria Sphingomonas, Acinetobacter, Micrococcus, Pseudomonas, and Ralstonia were the most consistently found organisms in bladder cancer patients. However, differences in bacterial population were more related to geography rather than the disease [19]. The female bladder TME can be influenced by the lower diversity in bacteria and the protective effect of estrogen on bladder cancer (**Figure 2**) [20]. Another study found that patients with non-muscle invasive bladder cancer, high grade tumors had a lower diversity in bacteria within the bladder microbiome especially in women who had a significantly lower diversity than men. In women, there was a significant difference between diversity in urine bacteria between high grade and low-grade tumors, but this relationship was not found among male urine samples. High grade tumor female microbiomes included Salmonella, Romboutsia, and Enterobacter while male microbiomes included the species Aeribacillus, Peptococcus, Alcaligenes, Actinomyces, Pseudomonas, Acinetobacter, Proteus [20]. The sex-specific microbiome compositions give us an insight to understanding, and could eventually explain, the sex disparity in tumor grade and inflammatory burden.

A specific immune cell, RETNLG+LCN2+ senescence-like neutrophils (RLSNs), that accumulate specifically in male bladder TME, resulting in a local immunosuppressive effect. This effect was more pronounced in males than females due to higher concentration of the intestinal bacteria Alistipes shahii in females which produces Lurasidone, an FDA-approved antipsychotic drug (**Figure 2**). Lurasidone increases iron availability by binding to LCN2 proteins and induces apoptosis of RLSNs, thus presenting a therapeutic value of lurasidone in male patients with bladder tumors [21].

Diet and inflammation

A 2020 study examined Dietary Inflammatory Index (DII) scores and found that higher scores were associated with a higher pro-inflammatory state and a higher risk of bladder cancer (ORs 4.89, 95% CIs 2.09-11.25 $P < 0.001$). The Dietary Inflammatory Index estimates how certain foods and nutrients influences levels of inflammatory markers such as Interleukin 6 (IL-6), C-Reactive Protein (CRP), and Tumor Necrosis Factor-Alpha (TNF-α). This study used 112 bladder cancer patients and 292 control subjects while also obtaining 109 bladder cancer patients and 319 control subjects from the Nutrition Examination Survey (NHANES) database from 1999 to 2020. The bladder cancer group consumed more pro-inflammatory foods such as grains, red meat, fats, carbohydrates, and soybean oil compared to the control group however, no difference in nutrient consumption was observed between the groups. The study used the NHANES database to match subjects based on confounders (age, sex, smoking, alcohol consumption, education occupation, exercise and energy) and saw a 2.00 times higher risk for bladder cancer (95% CIs: 1.03-3.87). Subjects with higher DII scores were 5.82 times (95% CIs: 2.43-9.69) more likely to develop bladder cancer [22]. A meta-analysis completed in 2024 evaluated Systemic Immune Inflammation Index (SII) scores. They observed pre-treatment bladder cancer patients with higher SII scores had worse survival outcomes. Cancer-free survival and high SII were significantly associated (pooled HR = 1.68; 95% CI, 1.14-2.47) [23]. The inflammation indices had

a significant value as potential biomarkers for MIBC. These indices can also help differentiate NMIBC cancer and MIBC before final pathology. Furthermore, it can be useful in predicting how effective the responsiveness of BCG therapy with future studies focusing on efficacy on immune checkpoint inhibitors [24].

The postoperative neutrophil-to-albumin ratio (NAR) for patients undergoing radical cystectomy could further predict prognosis for bladder cancer patients. A recent 2026 study investigated this marker and found elevated NAR was correlated with invasive pathology, lymph node metastasis, and advanced tumor stage. Patients with elevated NAR also had a shorter overall survival and recurrence-free survival compared to patients with a low NAR. This marker serves as a systemic inflammation marker factoring in nutritional status as well and can serve as a tool for postoperative management and risk assessment. A high NAR reflects neutrophilia and hypoalbuminemia, which gives an insight into the patients' nutritional and proinflammatory state, leukocyte count and serum albumin. The outcomes of using the neutrophil to albumin ratio can assist in deciding for neoadjuvant therapy postoperatively in radical cystectomy patients [25]. Similarly, another research team identified the substrate ABZ-Met-Val-Lys-Trp-ANB-NH2 to identify proteases in urine from bladder cancer. This acted as a marker for human neutrophil elastase activity which showed increased proteolytic activity of neutrophils due to a high inflammatory state of the bladder. These findings support the use of ABZ-Met-Val-Lys-Trp-ANB-NH2 as a marker of epithelial neoplasms as well as inflammation [26].

Autonomic regulation

There is evidence identifying Triggering Receptor Expressed on Myeloid cells (TREM) ratios, as an inflammatory biomarker as well as identify the association of higher Heart Rate Variability (HRV) and sympathetic stimulation with bladder cancer [27]. It was demonstrated that among bladder cancer patients, there was a higher ratio of TREM-1 (pro-inflammation) to TREM-2 (immune suppression) expression on CD-14 + monocytes while also seeing a lower concentration of anti-inflammatory mediators, such as Interleukin-10 (IL-10), and a higher concentration of pro-inflammatory cytokines (**Figure 1**). Moreover, there was an association between a higher systolic blood pressure and heart rate in bladder cancer patients compared to controls. The higher HRV parameters are associated with increased sympathetic activity while also having a decreased parasympathetic modulation. Lymphocyte to Monocyte ratio and Platelet to Monocyte ratio also served as inflammatory makers and had lower ratios in bladder cancer patients. They also investigated the vicious cycle of various negative emotions (fatigue, anxiety, depression) that exacerbate one another and create a sedentary lifestyle that enables a more pro-inflammatory state [27]. This is supported by evidence suggesting a negative correlation between physical function with TREM1/TREM2 ratio, and a positive correlation with Brain Derived Neurotrophic Factor (BDNF) [27]. Studies to evaluate the association between mental stress and inflammation on bladder cancer, led to the identification of an effect of HRV of stress on inflammation. The Perceived Stress Scale was positively correlated with Transforming Growth Factor Beta (TGF-β) in patients with high life satisfaction. Patients with low HRV had a negative relationship with TGF-β and positive correlation with TNF-α, implicating a role of HRV in autonomic regulation on stress and inflammation, and ultimately a poor prognosis. Stress management and vagal nerve activity could therefore be targeted to improve outcomes in bladder cancer patients [28].

Significance of inflammation in therapeutic targeting

Immunotherapy

In recent years, there have been improvements in immunotherapy for bladder cancer that can be influenced by inflammatory cells [29]. Siglec15 is a sialic acid binding immunoglobulin-like lectin that helps foster a noninflammatory bladder tumor microenvironment due to its role in reducing $CD8^+$ cell infiltration. Siglec15 downregulates several pro-inflammatory cytokines which consequently facilitates immune evasion by the malignancy. It was shown that Siglec15 overexpression is therefore linked to a worse survival in patients with nasopharyngeal adenocarcinoma and glioblastoma [29]. These findings suggest that Siglec15 can be a target to fight immunotherapy-resistant cancer. Anti-Siglec15 therapy may also

increase efficacy of other immune checkpoint inhibitors such as Programmed Death 1 (PD-1) inhibitors [29]. Cyclooxygenase (COX) is normally present in low levels in tissues but elevated in a setting of inflammation or in cancer. In a tumor microenvironment, the activation of the COX-2 enzyme, can contribute to tumor progression as well as chemotherapy resistance. A more recent study designed a prodrug DNP that releases cytotoxic cisplatin combined with the anti-inflammatory COX-2 inhibitor naproxen. Compared to cisplatin alone, DNP increased cytotoxicity and increased intracellular platinum concentration [30]. The prodrug significantly increased cancer cell apoptosis and demonstrated strong inhibition of tumor growth. In addition, this drug also had an enhanced lipophilicity which improved the penetration and retention of the drug to induce tumor cell apoptosis [30]. For muscle invasive bladder cancer, trimodality therapy (Transurethral Resection of Bladder Tumor (TURBT), radiation with cisplatin or 5-Fluorouracil) was found to be an oncologically safe and effective method to treat the cancer compared to radical cystectomy. The overall survival in the two groups in this retrospective study was similar, but the trimodality therapy group had a slight advantage due to postoperative complications in the radical cystectomy group. 5-year survival free of recurrence in the trimodality therapy cohort for non-muscle invasive recurrence was 82% (95% CI 78-86) and for muscle-invasive recurrence 89% (95% CI 85-93) [31]. A 2023 study built a predictor model, titled Slide Based Lymph Node Predictor, which identified subtle patterns in whole images of lymph nodes in patients that underwent radical cystectomy for MIBC [32]. These investigators found that the lymphocytic inflammation surrounding the stroma of the lymph node was a key predictor of lymph node metastatic invasion. This is critical in guiding clinicians in lymph node dissection as well as in neoadjuvant therapy postoperatively since lymph node metastasis highly correlates with disease recurrence and mortality [32]. A new potential therapy emerged from the isolation of the E.coli 536 species. The supernatant of the strain had a potent suppressing function on inflammatory cytokines such as IL-1 beta, CCL2, and CX3L1 while also increasing gene expression of NQ01 which has antioxidant effects. Additionally, the compound was able to reduce c-MYC expression as well as raise the BAX/BCL2 ratio to induce apoptosis [33]. Similarly, another model was developed to risk stratify patients based on long non-coding RNA (LncRNA) which influences the response of bladder cancer to chemotherapy. High risk patients with high levels of LncRNA related to immunity and invasion had high levels of immune checkpoint activation and infiltration. These RNA molecules also helped differentiate between “hot” and “cold” tumors, like prostate cancer, which may have potential predictive value of the response to immunotherapy [34].

Molecular link of inflammation to bladder tumor progression

Inflammation can promote immune cell recruitment, enhance antigen presentation, and support the activation of cytotoxic T cells. However, persistent inflammation can induce T-cell exhaustion, upregulate immune checkpoint molecules such as PD-L1 expression, and promote resistance through the expansion of immunosuppressive cell populations. It has been widely recognized that inflammation has a context-dependent role on immunotherapy response in bladder cancer [35]. Acute inflammation can enhance anti-tumor immunity and improve responses to immune checkpoint inhibitors, whereas chronic inflammation often promotes immune suppression and therapeutic resistance. Based on these immune characteristics, bladder tumors are classified as existing along a continuum of three immune states: immune-inflamed (“hot”), immune-excluded, and immune-desert (“cold”) phenotypes [35]. Immune-inflamed tumors are characterized by high infiltration of activated $CD8^+$ T-cells, dendritic cells, natural killer cells, together with elevated interferon-γ (IFN-γ) responsive genes and chemokines, such as CXCL9 and CXCL10. These tumors often show improved responses to checkpoint inhibitors such as pembrolizumab and atezolizumab. In contrast, chronic inflammation can drive immune exhaustion, recruitment of suppressive immune populations, and activation of pathways such as IL-6/STAT3 and TGF-β signaling. Persistent inflammatory signaling contributes to T-cell dysfunction, expansion of regulatory T cells and myeloid-derived suppressor cells, and stromal remodeling that limits immune-cell trafficking into tumors [36]. A subset of bladder

tumors have a cold or immune-desert phenotype characterized by a low density of intratumoral $CD8^+$ T cells and dendritic cells, resulting in inadequate priming and activation of adaptive immune responses [35]. Compared with inflamed tumors, immune-desert tumors generally exhibit low PD-L1 expression and limited neoantigen-specific T-cell responses, reducing the likelihood of response to immune checkpoint blockade. Immune-excluded tumors contain abundant immune cells that remain confined to the tumor stroma or invasive margin rather than penetrating the tumor parenchyma. Stromal barriers, extracellular matrix remodeling, CAFs, and TGF-β signaling are thought to limit T-cell infiltration, resulting in reduced responsiveness to immune checkpoint inhibitors despite the presence of immune cells.

Inflammatory gene signatures

Four inflammation-related genes (MEST, FASN, KRT6B, and RGS2) could serve as potential markers for prognosis of bladder cancer. Using these genes, a model was built to determine the prognosis of patients with bladder cancer. The study highlighting these specific gene signatures was also able to identify three risk groups in which the low-risk group was most suitable for immunotherapy and the high-risk group was more suitable for chemotherapy. The high-risk group contained a high concentration of stromal cells and immune cells such as macrophages, Th1 cells, Tfh cells, and Treg cells while Th2 cells were more commonly seen in the low-risk group. Among those in the high-risk group, cell division proteins were increased such as keratin filaments, cytoskeleton components, and intermediate filaments [37]. For the low-risk group, immune-related genes were more strongly associated [37]. Similarly, a study analyzing the effect of inflammation, fatty acid metabolism, and hypoxia on bladder cancer prognosis found that patients in their low-risk cohort responded better to immunotherapy while patients in their high-risk cohort responded better to chemotherapy drugs such as Gemcitabine, Methotrexate, and Vinorelbine [38]. A prognostic model with six inflammation related genes identified that the genes TNFRSF12A, IL1R1, ELN and CYP26B1 were unfavorable while NR1H3 and ITIH4 were protective. In this model, high risk score patients had significantly worse overall survival and were associated with a cold tumor microenvironment, indicating poor response to immunotherapy [39].

The interstitial cystitis-related gene coiled coil domain-containing 8 (CCDC8) was seen to be an oncogenic factor for bladder cancer. Elevated expression of CCDC8 was correlated with lymph node metastasis, advanced tumor stage, and worse overall prognosis. CCDC8 functions by interacting with E3 ubiquitin ligase scaffold protein CUL7 which acts on proteosome degradation of P53, which activates both P21 and the BAX suppressor. Thus, CCDC8 acts as an oncogenic factor as it allows for tumor growth, migration, and tumor cell survival [40]. CELF2 is an RNA binding protein that plays a role in alternative splicing and mRNA stability. C-X-C motif chemokine ligand 5 (CXCL5) is an inflammatory cytokine that acts as a neutrophilic chemoattractant and can increase tumor progression and angiogenesis. CELF2 expression was seen to be downregulated in many cancers including bladder cancer which causes progression and invasion of bladder cancer cells, via a downstream suppressive effect of the CXCL5-AKT signaling pathway [41]. CELF2 is located on chromosome 10p, opening another avenue to analyze genes that lead to bladder tumor progression and new therapeutic treatments [41].

Smoking has been implicated in enhancing the progression of bladder cancer through the CKAP2L and FOXM1 pathway. Functional knockdown of CKAP2L impaired cell proliferation, invasion, and migration in pre-clinical bladder cancer models. In this study, Polymerase Chain Reaction (PCR) determined high binding of FOXM1 to the CKAP2L promoter in the presence of the inflammatory condition of smoking [42]. This suggests that in patients who have a significant smoking history, the inflammatory state may drive bladder cancer progression through transcriptional activity of CKAP2L-FOXM1 axis. This pathway provides a mechanistic link between tumor progression and a modifiable risk factor.

Hypoxia-inducible factor-1 alpha (HIF-1α) was seen to upregulate, indirectly through m5c modification, pyruvate kinase M2 (PKM2) expression by activating ALYREF (shown in **Figure 2**). This pathway was crucial to glucose meta-

bolism, lactate production, and intracellular ATP production of bladder cancer and can be a new therapeutic target since PKM2 and ALYREF were predictive of poorer survival in bladder cancer patients [11]. In a different study, Mannose was seen to bind to PKM2 which inhibited the enzyme and reduced the production of the lactate. This led to a downstream activation of the Nuclear Factor Kappa Beta (NF-κB) pathway, inducing pyroptosis. This inflammation induced cell death can turn a tumor into a highly immunogenic environment, called a hot tumor, which enhances immune checkpoint inhibitor efficacy [43]. TGF-β, secreted by M2-tumor-associated macrophages (TAMs), also promoted PKM2 expression through STAT3 transcription, which in turn promoted PD-L1 transcription (**Figure 2**). This TGF-β pathway, through the JAK-STAT dimerization, led to immune evasion, tumor cell migration, and tumor invasion. The blockade of TGF-β and PKM2 had promising results to enhance anti-tumor immune responses [12]. The RNA protein, HNRNPA1-K350, was identified as a key protein in glycolysis-dependent tumor progression due to lactylation of the protein. In the setting of glycolysis, the increased lactate production resulted in the lactylation of HNRNPA1-K350 and promoted PKM2 expression. The suppression of this pathway may also be a new approach for bladder cancer therapy [44].

Regulator of cullins 1 (ROC1) promotes a high tumor grade, metastasis, and poorer prognosis through interacting with NF-κB signaling. ROC1 activates NF-κB through ubiquitation of p-IκBα which results in p65 nuclear translocation and promotes transcription of genes related to metastasis such as urokinase-type plasminogen activator receptor (u-PAR), vascular cell adhesion molecule 1 (VCAM1), matrix metalloproteinase 9 (MMP9), intercellular adhesion molecule 1 (ICAM1). These proteins that are upregulated due to the interaction between NF-κB and ROC1 allow for bladder cancer invasion and migration [45]. In radical cystectomy patients, Interleukin 6 (IL-6) and IL-6 receptor plasma levels were studied preoperatively to determine if it can serve as a biomarker for oncological outcomes. The study discovered that elevated IL-6 and IL-6R levels were associated with an increased risk of having a stage pT3 disease or higher and lymph node metastasis. These markers were also both correlated with worse overall survival and could serve clinicians as a marker that these patients undergoing radical cystectomy, would require multimodal therapy [46].

Gender based biological differences in bladder cancer

Hormonal influence

The most common cancer diagnosed in men is prostate cancer and due to the anatomical closeness of the prostate to the bladder, there may be a link in the cancers. In 2020, there were 132,414 new bladder cancer cases in females and 440,864 new cases in men (3.3 times more in men than women). Another study analyzed data from genome-wide association studies looking at the presence of prostate cancer and bladder cancer as well as the testosterone levels in men. Their results showed that genetically predisposed prostate cancer had an increased risk of developing bladder cancer and clinicians should take measures early on to monitor the progression. Other treatment strategies for prostate cancer patients, such as radiotherapy or androgen deprivation therapy [47], have been implicated to increase the risk of bladder cancer but there have been no conclusive results [48]. One study showed that out of 48,776 prostate cancer patients, the 5-year risk for bladder cancer was 1.1% for patients with chemical castration (HR of 1.13, 95% CI 0.98; 1.31), 0.7% in patients with surgical castration (0.95, 95% CI 0.62; 1.47), and for patients with no antihormonal treatment, the risk was 1.3% (1.18, 95% CI 1.09; 1.28). Patients receiving antihormonal treatment had a slightly lower incidence of bladder cancer compared to patients without prostate cancer [49]. Males with prostate cancer have an increased risk of bladder cancer which could be due to androgen deprivation therapy, impacting the dynamic between the bladder and androgens. EPPK1 knockdown was shown to have highly significant inhibitory effects on bladder cancer cell proliferation [50]. EPPK1 was heavily promoted in the presence of Dihydrotestosterone (DHT) in mice which led to increased tumor growth and elevated JUP expression. This study further highlighted that DHT does have a role in bladder cancer progression, but not through the typical androgen receptor (AR) pathway [50].

Pre vs post menopausal women and bladder cancer

In female patients there is evidence to suggest that earlier menopause may also be a risk factor for bladder cancer [51]. Estrogen has a dual role since it can be protective from bladder cancer, however, estrogen can promote tumor progression through interaction with a different receptor [51]. In a prospective study done in Japan evaluating 194 urothelial cancer cases, the researchers found that an earlier age at menopause, either natural or surgical, increased risk of bladder cancer. Women under the age of 44 that have undergone menopause had more than double the risk of bladder cancer compared to women that had undergone menopause between the ages of 49-51 (HR, 2.09; 95% CI, 1.04-4.20) [52]. A retrospective study done in China observed that post-menopausal status amplified certain risk factors for bladder cancer. Smoking history and history of urinary tract infections in post-menopausal women carried an increased risk of bladder cancer possibly due to the decreased levels of estrogen that normally protect the vaginal lining from carcinogenesis and chronic inflammation which affects the bladder microbiome [53]. This study, however, did not identify menopausal status as an independent predictor of bladder cancer risk [53]. For post-menopausal women, smoking cessation played a pivotal role in decreasing the risk for bladder cancer as seen by a 25% decline within the first 10 years and a continued decreased risk as time progressed. Post-menopausal women who quit smoking have a lower risk of bladder cancer compared to women who kept smoking (HR = 0.61; 95% CI, 0.40-0.94). This further addresses the importance of advocating for smoking cessation in older women due to the loss of the protective effects of estrogen [54].

In a study of Egyptian women, BMI was found to affect the risk of bladder cancer in post-menopausal women by enhancing the risk of low estrogen exposure. In this population, early menopause (under age 45) had a higher risk of bladder cancer in obese women (AOR = 2.90, 95% CI = 1.40-5.98). This study also identified parity as a risk factor since the group with the highest risk for bladder cancer was women that had their first child over the age of 18 and the number of pregnancies was less than or equal to 6. However, overweight and obese women had a lower risk of bladder cancer (AOR = 0.59, 95% CI = 0.43-0.81, and AOR = 0.26, 95% CI = 0.18-0.38, respectively) [55]. Similarly, a meta-analysis done in 2020, found that both early menopause and nulliparity were associated with a higher risk of bladder cancer [56]. Furthermore, physical activity appeared to have an inverse relationship with bladder cancer risk in post-menopausal women in a dose-dependent manner. Women who did 15 or more metabolic equivalent (MET) hours per week of physical activity had a lower risk of bladder cancer compared to women who were physically inactive (HR = 0.74, 95% CI: 0.59-0.94). Post-menopausal women who walked over 8.75 hours per week or more or 11.25 hours of moderate to vigorous physical activity also had a lower risk of bladder cancer compared to the physically inactive group (HR = 0.79, 95% CI: 0.63-0.98 and HR = 0.76, 95% CI: 0.61-0.94, respectively) [57]. Collectively, these results provide us with meaningful implications on the risk of bladder cancer due to modifiable risk factors such as increasing physical activity and the cessation of smoking that can offset the loss of estrogen's protective effect after menopause.

Clinical and therapeutic landscape of bladder cancer

Bladder cancer is characterized by high recurrence rates, substantial molecular heterogeneity, and variable clinical outcomes [58]. Current management depends on disease stage, ranging from local intravesical therapies for NMIBC to multimodal approaches incorporating surgery, radiation, chemotherapy, immunotherapy, targeted therapies, and antibody-drug conjugates (ADCs) for MIBC and metastatic disease. Recent advances in molecular characterization and biomarker-guided therapy have facilitated more personalized treatment strategies and improved patient stratification. For intermediate- and high-risk NMIBC, TURBT followed by intravesical BCG therapy remains the standard of care [59]. However, the emergence of BCG-unresponsive tumors has driven the development of alternative therapeutic approaches, including immune checkpoint inhibitors (e.g. pembrolizumab) and novel intravesical gene therapies (nadofaragene firadenovec) [60]. For cisplatin-eligible patients with MIBC, radical

Table 1. Features of inflammatory landscape of luminal and basal cells in bladder cancer

Subtype	Inflammatory State	Molecular Features	Response to Therapy
Basal	Hot/Inflamed	High immune infiltration Increased cytotoxic T cells ($CD8^+$ T cells), NK cells, macrophages, Upregulated IFN-γ responsive gene signatures and antigen presentation pathways Enriched TP53/RB1 alterations Accounts for 35% of MIBC	Good response to cisplatin-based neoadjuvant chemotherapy may exhibit greater sensitivity to immune checkpoint inhibitors due to an inflamed tumor microenvironment
Luminal	Cold/Immune Desert	Low immune infiltration Reduced IFN-γ signaling Lower PD-L1 expression Frequent FGFR3 alterations	Lower sensitivity to immune checkpoint inhibitors than basal tumors Tumors with susceptible FGFR2/3 alterations may benefit from FGFR-targeted therapy (e.g., erdafitinib)

cystectomy with neoadjuvant cisplatin-based chemotherapy remains the standard treatment [61]. While platinum-based chemotherapy remains an important component of treatment, immune checkpoint inhibitors targeting PD-1/PD-L1, FGFR-targeted therapies for patients with susceptible FGFR alterations, and ADCs such as enfortumab vedotin have significantly improved outcomes. Perioperative systemic therapy, including neoadjuvant cisplatin-based chemotherapy with or without immunotherapy, has expanded treatment options for some patients. For selected individuals, bladder-preserving trimodality therapy consisting of maximal TURBT followed by concurrent chemoradiation represents an established alternative to radical cystectomy [10, 62]. Combination regimens integrating immunotherapy with chemotherapy or targeted agents have become treatment options for many patients with advanced bladder cancer, reflecting the transition toward biologically informed and personalized management of bladder cancer.

In NMIBC, these tumors contained more microvascular alterations with higher amounts of cancer associated fibroblasts (CAF) and myofibroblasts (myoCAF) with higher variation in immune cell distribution [63]. MyoCAF was found to be a valuable prognostic marker as higher infiltration of these cells within the TME had higher rates of progression and recurrence while M2-like macrophages and myoCAFs were also shown to be enriched in high grade NMIBC TMEs [63]. Using spatial analysis, CD163+ macrophages had a greater distance from myoCAF in low grade (G1) tumors while G2 tumors showed a close distance between the cells. G3 tumors again showed further separation between these macrophages and myoCAFs indicating the possible co-evolution in intermediate NMIBC of these cells [63]. Moreover, a more recent study tested the need for perioperative therapy in patients with MIBC. These patients, who could not take cisplatin-based chemotherapy, are sent straight to surgery for radical cystectomy with pelvic node dissection. Adding enfortumab vedotin to pembrolizumab was shown to have a greater overall survival and greater pathological response compared to surgery alone [64]. In cisplatin-ineligible metastatic bladder cancer patients, the drug combinations of gemcitabine/carboplatin with nivolumab versus gemcitabine/oxaliplatin with nivolumab were tested to see which combination had a higher response rate. Oxaliplatin has been known to improve tumor sensitivity to drugs such as nivolumab through immunomodulation of cancer cells, but the oxaliplatin arm did not improve response compared with the carboplatin arm [65]. The group treated with carboplatin demonstrated a substantially higher objective response rate (69.6% vs. 33.3%) and showed sustained adaptive immune activation with increased plasma levels of IFNγ, CXCL9, CXCL10, indicating immune activity within the TME. In contrast, the group treated with oxaliplatin, exhibited lower IFNγ activity and increased angiopoietin 1 receptor, nitric oxide synthase 3, and IL-6, biomarker profiles associated with angiogenesis, inflammation, and a more immunosuppressive TME [65].

Additionally, luminal and basal tumors the two major molecular classes of MIBC, have varied molecular characteristics, therapeutic responses and immune microenvironments (**Table 1**) [47, 58]. Luminal tumors resembling differentiated urothelium, are often associated with a more favorable prognosis, and are enriched for

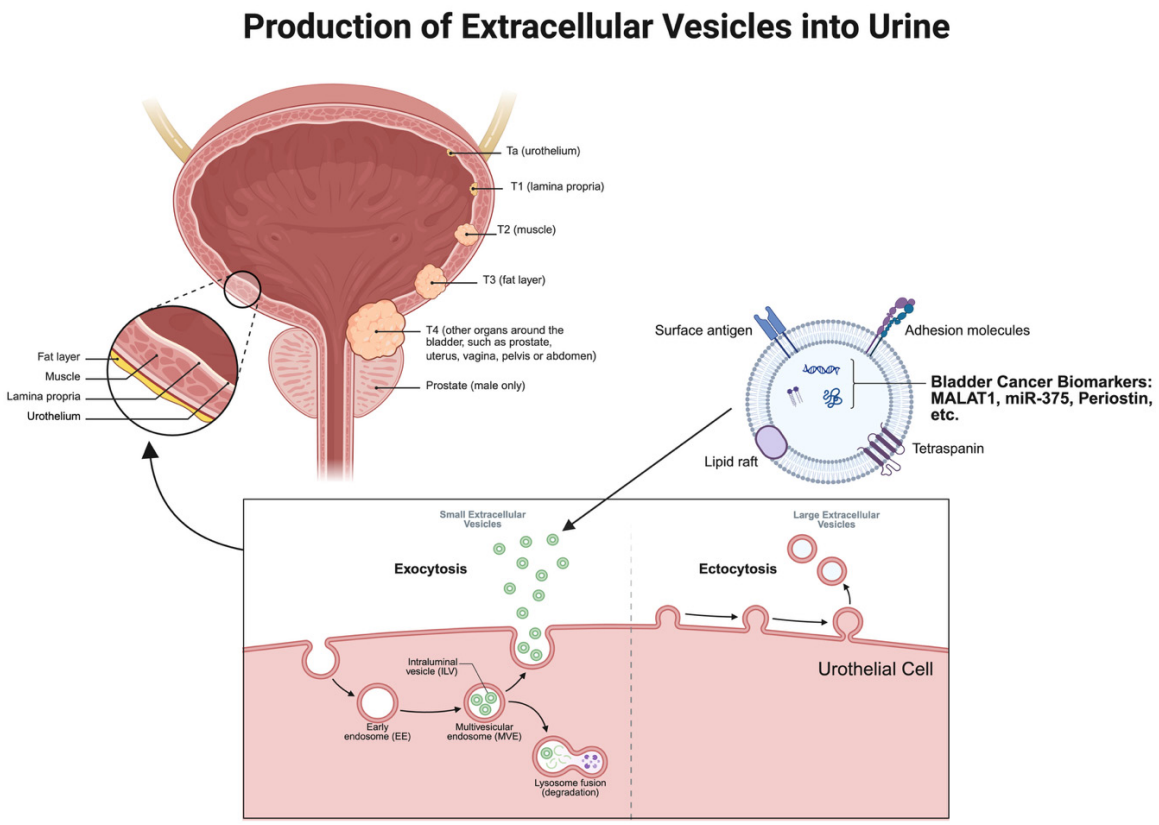


Figure 3. Mechanisms of EV release into urine by urothelial cells. Diagram showing the two distinct pathways by which extracellular vesicles are generated in urothelial cells along with the stages of bladder cancer (Right of bladder diagram). (Left) Small EVs are produced through the classical endosomal pathway, when the plasma membrane invaginates to form an early endosome (EE). Soon, the EE matures into the late sorting endosome (LSE). Then, the membrane of the LSE invaginates, forming many intraluminal vesicles (ILVs). Now known as a multivesicular endosome (MVE), the MVE will either fuse with the plasma membrane to release the ILVs (now considered exosomes) or with a lysosome to degrade its contents. (Right) Alternatively, large EVs are formed by budding from the plasma membrane through ectocytosis. Both types of EVs are shed directly into urine and carry bladder-specific biomarkers including MALAT1, miR-375, and Periostin. These biomarkers can indicate the presence of bladder cancer and may change in expression during different stages of the disease.

FGFR3 alterations, making patients with susceptible FGFR3 alterations eligible for FGFR-targeted therapy [47]. In contrast, basal tumors exhibit a more aggressive phenotype but are often more responsive to cisplatin-based neoadjuvant chemotherapy [47]. Basal tumors are characterized by increased immune cell infiltration and elevated expression of inflammatory and immune checkpoint markers, including PD-L1, reflecting a more inflamed TME that may contribute to improved responsiveness to immune checkpoint blockade [66]. These subtype-specific differences highlight the close interplay between tumor biology and inflammation in bladder cancer and the clinical need for molecularly informed therapeutic strategies.

Extracellular vesicles in bladder cancer: diagnostic significance

Extracellular vesicles (EVs) are membrane-enclosed nanoparticles released by all studied cells. The most recent MISEV 2023 guidelines from the International Society of Extracellular Vesicles (ISEV) define three types of extracellular particles: EVs, extracellular particles (EPs), and non-vesicular extracellular particles (NEVPs) [67]. EPs are considered an umbrella term for both EVs and NEVPs, such as exomeres, which often co-purify with EVs. EVs themselves can be separated into groups: small EVs and large EVs [68]. Small EVs, such as exosomes, mostly arise from the endosomal pathway (as shown in **Figure 3**) while large

EVs are largely formed through budding from the cell membrane itself (**Figure 3**). Further studies have identified novel sub 50 nanometer (nm) EPs, termed SECmeres and other subpopulations of extracellular vesicles and particles (EVPs) [69-71]. EVPs primarily function in cell-cell communication, carrying DNA, proteins, messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA (lncRNA), and other molecular cargo between cells [71-73]. Moreover, novel unannotated RNAs with tissue specificity have been discovered in EVs [74]. This transfer of biomolecules allows cells to impact gene expression and signaling pathways of their recipients. In the context of cancer, tumor-derived EVs are major contributors to tumor progression because they facilitate communication between malignant cancer cells and the TME [75]. These EVs transfer tumor-promoting molecules such as oncogenic proteins, mutated DNA, and miRNAs to nearby stromal, endothelial, and immune cells (**Figure 3**).

EVs may provide a particularly important role in detecting the invasion and metastatic progression of bladder cancer, leading to better clinical outcomes. Since bladder-derived EVs are directly shed into urine by the urothelium, they can be used to determine the current molecular and biological state of bladder cells by analyzing their molecular cargo [76]. This includes EVs released by malignant urinary cells, which have been shown to release greater amounts of EVs than normal urothelial cells [77]. The presence of EVs in urine may therefore potentially be used to detect bladder cancer in patients. Additionally, the progression of the disease and effectiveness of treatment can both be observed with EVs as they offer the ability to understand changes in the contents of urinary cells over time. This is especially valuable in bladder cancer, where high recurrence rates necessitate frequent non-invasive medical surveillance [78].

EV-based signatures are primarily extracted from the nucleic acids and proteins within EVs released by bladder tumor cells. When identified, these biomarkers express different molecular states when cancer is present, making them useful to distinguish malignant and non-malignant conditions. Examples include lncRNAs, circular RNAs (circRNAs), microRNAs, mRNAs, DNA, and proteins [71]. As bladder EVs originate directly from cancerous tumors, these signatures provide reliable information about the TME and the progression of the tumor. Among these biomarkers in bladder cancer, miRNAs, short non-coding RNAs that regulate gene expression, are the most thoroughly researched in EVs [79]. Increased expression of miR-155 has been linked to tumor cell proliferation and a consequential induction in tumor aggressiveness [80]. MiR-21 is commonly overexpressed in bladder cancer and is considered a promoter of tumor migration and proliferation [81]. Other miRNAs, including miR-126, miR-375, and miR-146a, have also been associated with the progression of bladder cancer and may serve as potential EV-based biomarkers [82]. Besides miRNAs, two promising biomarkers of bladder cancer-derived EVs are lncRNAs and protein signatures. LncRNAs are RNA transcripts longer than 200 nucleotides that act as regulators of gene transcription [83]. Urothelial carcinoma-associated 1 (UCA1) is a lncRNA that is associated with tumor proliferation and bladder cancer recurrence by strengthening oncogenic signaling pathways [84]. Additional lncRNA biomarkers include MALAT1, TUG1, and PCAT1, which are linked to increased levels of tumor cell proliferation, metastasis, and tumor angiogenesis [85]. Protein signatures such as EDIL-3 and periostin are also relevant EV-derived biomarkers for bladder cancer. EDIL-3 is a glycoprotein primarily involved in the angiogenesis of cancerous tumors, which stimulates tumor growth by supplying nutrients and oxygen through the bloodstream [86]. Furthermore, periostin is an extracellular matrix protein that assists in remodeling the TME by interacting with integrins to facilitate tumor cell adhesion and migration [87]. In fact, elevated periostin expression has been observed to lead to more aggressive tumors in muscle-invasive bladder cancer [87]. Finally, a significant advantage of analyzing EV-based biomarkers compared to other sources of biomarkers like free-circulating nucleic acids is their greater molecular stability. The lipid bilayer membrane of EVs protects their contents from enzymatic degradation in bodily fluids, allowing them to remain stable in urine and maintain molecular integrity during laboratory analysis [88]. Overall, tumor-derived EVs in the bladder carry valuable molecular cargo, including miRNA, lncRNA, and protein signatures, that show strong potential as biomarkers for

Table 2. Value of bladder cancer EVs as new biomarker signatures (lncRNA, proteins, miRNA)

Biomarker Type	Biomarker Name	Sample Source	References
lncRNA	MALAT1	Urine	[85]
lncRNA	UCA1	Urine	[84]
lncRNA	PCAT-1	Urine	[85]
lncRNA	HOTAIR	Urine	[83]
miRNA	miR-375	Urine	[82]
miRNA	miR-21-5p	Urine	[81]
miRNA	miR-93-5p	Urine	[82]
miRNA	miR-146a-5p	Urine	[82]
miRNA	miR-155-5p	Urine	[80]
miRNA	miR-17-5p	Urine	[82]
miRNA	miR-126-3p	Plasma	[82]
Protein	Periostin	Urine	[87]
Protein	EDIL-3	Urine	[86]
Protein	CEACAM1	Urine	[71]
Protein	CEACAM5	Urine	[71]
Protein	CEACAM6	Urine	[71]

bladder cancer diagnosis and surveillance (as summarized on **Table 2**).

Conclusions and future directions

Inflammatory pathways in urological cancers give new insights into different therapeutic approaches to better treat patients. One must consider differences in bladder microbiome contributing to a different inflammatory state of the bladder and differential progression of bladder cancer between men and women. The role of diet in inflammation has been studied across various pathologies and contributes to worse outcomes in bladder cancer patients as seen by DII and SII scores. Inflammation can also be used to determine the relevant therapeutic strategy as it produces an immunogenic environment that is full of immune cells where immunotherapeutic drugs can be the most efficacious. This poses a new challenge to identify biomarkers to better predict the responsiveness of certain therapies. Future studies will focus on closing the disparity between males and females in treating bladder cancer, by investigating EVs and their cargo as therapeutic targets and as a non-invasive diagnostic tool.

Acknowledgements

This work was supported by NIH CA232574-01A1; CA 296581-01A1 (Kyprianou), CA224-319 (Galsky), DK124165 (Galsky), and CA196521 (Gonzalez-Kozlova).

Disclosure of conflict of interest

MDG has served as a consultant to Samsung, Merck, Astra Zeneca, GlaxoSmithKline, and Genentech.

Abbreviations

BC, Bladder Cancer; MIBC, Muscle-Invasive Bladder Cancer; NMIBC, Non-Muscle-Invasive Bladder Cancer; PPD, Paraphenylenediamine; 4-ABP, 4-Aminobiphenyl; OEHHA, Office of Environmental Health Hazard Assessment; EVs, Extracellular Vesicles; TME, Tumor Microenvironment; DII, Dietary Inflammatory Index; NHANES, National Health and Nutrition Examination Survey; SII, Systemic Immune Inflammation Index; lncRNA, Long non-coding RNA; HRV, Heart Rate Variability; TREM, Triggering Receptor Expressed on Myeloid cells; COX, Cyclooxygenase; RLSNs, RETNLG+ LCN2+ senescence-like neutrophils; BDNF, Brain-derived neurotrophic factor; IFN-γ, Interferon-γ; TGF-β, Transforming Growth Factor Beta; TNF-α, Tumor Necrosis Factor-Alpha; NF-κB, Nuclear Factor Kappa B; NAR, Neutrophil-to-Albumin ratio; CCDC8, Coiled coil domain-containing 8; PKM2, Pyruvate kinase M2; TAMs, Tumor-associated macrophages; ROC1, Regulator of cullins 1; u-PAR, Urokinase-type plasminogen activator receptor; VCAM1, Vascular cell adhesion molecule 1; MMP9, Matrix metalloproteinase 9; ICAM1, Intercellular adhesion molecule 1; HIF-1α, Hypoxia-inducible factor-1 alpha; PD-1, Programmed Death 1; IL-6, Interleukin 6; AR, Androgen Receptor; DHT, Dihydrotestosterone; BCG, Bacillus Calmette-Guérin; BMI, Body Mass Index; CRP, C-Reactive Protein; CXCL5, C-X-C motif chemokine ligand 5; PCR, Polymerase Chain Reaction; IL-10, Interleukin-10; TURBT, Transurethral Resection of Bladder Tumor; EE, Early endosome; ILVs, Intraluminal vesicles; LSE, Late Sorting Endosome; MVE, Multivesicular endosome; AOR, Adjusted Odds Ratio; ADCs, Antibody-drug conjugates; CAFs, Cancer-

associated fibroblasts; CI, Confidence Interval; FGFR, Fibroblast growth factor receptor; HR, Hazard Ratio; PD-L1, Programmed death-ligand 1; UTI, Urinary Tract Infection.

Address correspondence to: Dr. Natasha Kyprianou, Department of Urology, Icahn School of Medicine, Mount Sinai, 1 Gustave L. Levy Pl, New York, NY 10029, USA. E-mail: natasha.kyprianou@mountsinai.org

References

[1] Zi H, Liu MY, Luo LS, Huang Q, Luo PC, Luan HH, Huang J, Wang DQ, Wang YB, Zhang YY, Yu RP, Li YT, Zheng H, Liu TZ, Fan Y and Zeng XT. Global burden of benign prostatic hyperplasia, urinary tract infections, urolithiasis, bladder cancer, kidney cancer, and prostate cancer from 1990 to 2021. Mil Med Res 2024; 11: 64.

[2] Theodorescu D, Li Z and Li X. Sex differences in bladder cancer: emerging data and call to action. Nat Rev Urol 2022; 19: 447-449.

[3] Khorsandi N, Ding CC, VandenBussche CJ, Verduzco CS, Greenland N and Vohra P. Urine cytology in patients with gender confirmation surgery and hormone therapy: evaluation of urine cytology performance in an underserved patient population. J Am Soc Cytopathol 2023; 12: 267-274.

[4] Lobo N, Duan Z, Sood A, Zhao H, Lindskrog SV, Dyrskjot L, Giordano SH, Williams SB, Bree KK and Kamat AM. Sex disparity in non-muscle-invasive bladder cancer: pitfalls of large population-based data sets and lessons from an integrated analysis. Eur Urol Oncol 2025; 8: 608-611.

[5] Zhao C, Li K, Zhu M, Wang F, Zhang K, Fan C and Wang J. The impact of patient sex on characteristic-adjusted bladder cancer prognosis. J Cancer Res Ther 2021; 17: 1241-1247.

[6] Flammia RS, Chierigo F, Wurnschimmel C, Wenzel M, Horlemann B, Tian Z, Borghesi M, Leonardo C, Tilki D, Shariat SF, Anceschi U, Chun FKH, Terrone C, Saad F, Gallucci M and Karakiewicz PI. Sex-related differences in non-urothelial variant histology, non-muscle invasive bladder cancer. Cent European J Urol 2022; 75: 240-247.

[7] Abdel-Hafiz HA, Schafer JM, Chen X, Xiao T, Gauntner TD, Li Z and Theodorescu D. Y chromosome loss in cancer drives growth by evasion of adaptive immunity. Nature 2023; 619: 624-631.

[8] Fadel J, Simonyan D, Fradet V, Lodde M, Lacombe L, Fradet Y and Toren P. Analysis of sex-based differences to Bacillus Calmette-Guerin for non-muscle invasive bladder cancer. Urol Oncol 2022; 40: 539.e531-539.e538.

[9] Tran MA, Cho BA, Izadmehr S, Yoo SK, Youssef D, Anker JF, Farkas AM, Figueiredo I, Lee K, Ananthanarayanan A, Balan S, Onkar S, Banchereau R, Gupta S, Chhibber A, Wang L, Beaumont KG, Li Z, Garcia-Barros M, Bicak M, Cordon-Cardo C, Brody R, Kim-Schulze S, Yuen K, Mariathasan S, Atiq SO, Adeyelu TT, Elliott A, Barata P, McKay RR, Gnjatic S, Gonzalez-Kozlova E, Mehrazin R, Horowitz A, Sfakianos JP, Chowell D, Galsky MD and Bhardwaj N. A tumor-promoting inflammatory SPP1+ Macrophage-IL6-CRP axis drives immune dysfunction in bladder cancer. Cancer Discov 2026; 16: 1126-1151.

[10] Galsky MD, Mortazavi A, Milowsky MI, George S, Gupta S, Fleming MT, Dang LH, Geynisman DM, Walling R, Alter RS, Kassar M, Wang J, Gupta S, Davis N, Picus J, Philips G, Quinn DI, Haines GK 3rd, Hahn NM, Zhao Q, Yu M and Pal SK. Randomized double-blind phase II study of maintenance pembrolizumab versus placebo after first-line chemotherapy in patients with metastatic urothelial cancer. J Clin Oncol 2020; 38: 1797-1806.

[11] Wang JZ, Zhu W, Han J, Yang X, Zhou R, Lu HC, Yu H, Yuan WB, Li PC, Tao J, Lu Q, Wei JF and Yang H. The role of the HIF-1alpha/ALYREF/PKM2 axis in glycolysis and tumorigenesis of bladder cancer. Cancer Commun (Lond) 2021; 41: 560-575.

[12] Yu Y, Liang Y, Xie F, Zhang Z, Zhang P, Zhao X, Zhang Z, Liang Z, Li D, Wang L, Chen Y, Sun L, Niu H and Wang Y. Tumor-associated macrophage enhances PD-L1-mediated immune escape of bladder cancer through PKM2 dimer-STAT3 complex nuclear translocation. Cancer Lett 2024; 593: 216964.

[13] More SL, Fung ES, Mathis C, Schulte AM and Hollins D. Dermal exposure and hair dye: assessing potential bladder cancer risk from permanent hair dye. Regul Toxicol Pharmacol 2023; 138: 105331.

[14] Zuo J, Chen J, Tan Z, Zhu X, Wang H, Fu S and Wang J. Analysis of long-term trends and 15-year predictions of smoking-related bladder cancer burden in china across different age and sex groups from 1990 to 2021. Discov Oncol 2025; 16: 408.

[15] Saito K and Kihara K. C-reactive protein as a biomarker for urological cancers. Nat Rev Urol 2011; 8: 659-666.

[16] Fujiwara Y, Karol AB, Joshi H, Reford E, Izadmehr S, Doroshow DB and Galsky MD. C-reactive protein (CRP) as a prognostic biomarker in patients with urothelial carcinoma: a system-

atic review and meta-analysis. Crit Rev Oncol Hematol 2024; 197: 104352.
[17] Heidar NA, Bhat TA, Shabir U and Hussein AA. The urinary microbiome and bladder cancer. Life (Basel) 2023; 13: 812.
[18] Mansour B, Monyók Á, Makra N, Gajdács M, Vadnay I, Ligeti B, Juhász J, Szabó D and Ostorházi E. Bladder cancer-related microbiota: examining differences in urine and tissue samples. Sci Rep 2020; 10: 11042.
[19] Bukavina L, Isali I, Ginwala R, Sindhani M, Calaway A, Magee D, Miron B, Correa A, Kutikov A, Zibelman M, Ghannoum M, Retuerto M, Ponsky L, Markt S, Uzzo R and Abbosh P. Global meta-analysis of urine microbiome: colonization of polycyclic aromatic hydrocarbon-degrading bacteria among bladder cancer patients. Eur Urol Oncol 2023; 6: 190-203.
[20] Bilski K, Żeber-Lubecka N, Kulecka M, Dąbrowska M, Bałabas A, Ostrowski J, Dobruch A and Dobruch J. Microbiome sex-related diversity in non-muscle-invasive urothelial bladder cancer. Curr Issues Mol Biol 2024; 46: 3595-3609.
[21] Zhu Q, Zhang G, Cao M, Huang H, He D, Zang Z, Xing J, Zhan M, Pei S, Deng X, Li J, Meng G, Xu J, Dai D, Hu G, Zheng M, Liu C, Qin J and Xiao Y. Microbiota-shaped neutrophil senescence regulates sexual dimorphism in bladder cancer. Nat Immunol 2025; 26: 722-736.
[22] Teng C, Lu W, Che J, Wu Y, Meng D and Shan Y. Association of pro-inflammatory diet, smoking, and alcohol consumption with bladder cancer: evidence from case-control and NHANES studies from 1999 to 2020. Nutrients 2024; 16: 1793.
[23] Cao W, Shao Y, Zou S, Wang N and Wang J. Prognostic significance of systemic immune-inflammation index in patients with bladder cancer: a systematic review and meta-analysis. Medicine (Baltimore) 2022; 101: e30380.
[24] Jaromin M, Kutwin P, Konecki T, Popiela D, Kamecki M and Kurowski M. Inflammation indices as predictive markers of muscle-invasive bladder cancer. Cancers (Basel) 2025; 18: 136.
[25] Li W, Xiang C, Xie G and Zhang Y. Postoperative neutrophil-to-albumin ratio as a prognostic marker for patients with bladder cancer undergoing radical cystectomy: a retrospective observational cohort study. Int J Gen Med 2026; 19: 595401.
[26] Gruba N, Stachurski L and Lesner A. Elastolytic activity is associated with inflammation in bladder cancer. J Biochem 2021; 170: 547-558.
[27] Mikolaskova I, Zvarik M, Szaboova K, Tibenska E, Durmanova V, Suchankova M, Kollarik B, Hesko P, Palacka P, Bucova M and Hunakova L. Association of sympathovagal imbalance with increased inflammation and impaired adaptive immunity in bladder cancer patients. Int J Mol Sci 2024; 25: 12765.
[28] Mikolaskova I, Gidron Y, Durmanova V, Suchankova M, Bucova M and Hunakova L. Mental distress and inflammation in bladder cancer: the nerve makes things less vague. Brain Behav Immun Health 2025; 46: 100995.
[29] Deng D, Xiao J, Liu J, Li H, Hu M, Zhou B, Liang H, Fan B, Chen J, Kuang X, Nie Z, Hu J and Zu X. Evasion of immunosurveillance by the up-regulation of Siglec15 in bladder cancer. J Hematol Oncol 2024; 17: 117.
[30] Li Y, Liu S, Zhou M, Zhao Z, Song D, Guo H and Yang R. A COX-2-Targeted Platinum(IV) prodrug induces apoptosis and reduces inflammation in bladder cancer models. Pharmaceuticals (Basel) 2025; 18: 1185.
[31] Zlotta AR, Ballas LK, Niemierko A, Lajkosz K, Kuk C, Miranda G, Drumm M, Mari A, Thio E, Fleshner NE, Kulkarni GS, Jewett MAS, Bristow RG, Catton C, Berlin A, Sridhar SS, Schuckman A, Feldman AS, Wszolek M, Dahl DM, Lee RJ, Saylor PJ, Michaelson MD, Miyamoto DT, Zietman A, Shipley W, Chung P, Daneshmand S and Efstathiou JA. Radical cystectomy versus trimodality therapy for muscle-invasive bladder cancer: a multi-institutional propensity score matched and weighted analysis. Lancet Oncol 2023; 24: 669-681.
[32] Zheng Q, Jian J, Wang J, Wang K, Fan J, Xu H, Ni X, Yang S, Yuan J, Wu J, Jiao P, Yang R, Chen Z, Liu X and Wang L. Predicting lymph node metastasis status from primary muscle-invasive bladder cancer histology slides using deep learning: a retrospective multicenter study. Cancers (Basel) 2023; 15: 3000.
[33] Mehmandar-Oskuie A, Tohidfar M, Hajikhani B and Karimi F. Anticancer effects of cell-free culture supernatant of Escherichia coli in bladder cancer cell line: new insight into the regulation of inflammation. Gene 2023; 889: 147795.
[34] Xiong X, Chen C, Li X, Yang J, Zhang W, Wang X, Zhang H, Peng M, Li L and Luo P. Identification of a novel defined inflammation-related long noncoding RNA signature contributes to predicting prognosis and distinction between the cold and hot tumors in bladder cancer. Front Oncol 2023; 13: 972558.
[35] Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, Coussens LM, Gabrilovich DI, Ostrand-Rosenberg S, Hedrick CC, Vonderheide RH, Pittet MJ, Jain RK, Zou W, Howcroft TK, Woodhouse EC, Weinberg RA and Krummel MF. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 2018; 24: 541-550.

[36] Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, Kadel EE III, Koeppen H, Astarita JL, Cubas R, Jhunjhunwala S, Banchereau R, Yang Y, Guan Y, Chalouni C, Ziai J, Şenbabaoğlu Y, Santoro S, Sheinson D, Hung J, Giltnane JM, Pierce AA, Mesh K, Lianoglou S, Riegler J, Carano RAD, Eriksson P, Höglund M, Somarriba L, Halligan DL, van der Heijden MS, Loriot Y, Rosenberg JE, Fong L, Mellman I, Chen DS, Green M, Derleth C, Fine GD, Hegde PS, Bourgon R and Powles T. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 2018; 554: 544-548.

[37] Xiao Y, Xu D, Bao E, Liu Z, Zhou X, Li X and Li L. Identification of inflammation related gene signatures for bladder cancer prognosis prediction. Sci Rep 2024; 14: 28867.

[38] Xiao Y, Yang J, Yang M, Len J and Yu Y. The prognosis of bladder cancer is affected by fatty acid metabolism, inflammation, and hypoxia. Front Oncol 2022; 12: 916850.

[39] Wang Y, Tang Y, Liu Z, Tan X, Zou Y, Luo S and Yao K. Identification of an inflammation-related risk signature for prognosis and immunotherapeutic response prediction in bladder cancer. Sci Rep 2024; 14: 1216.

[40] Wang J, Wang J, Meng L, Wang X, Yan Z, Pan H, Wu J, Zhou Q, Ye L, Wu J, Zhang Y and Wang J. Interstitial cystitis-related gene CCDC8 accelerates tumorigenesis by participating in CUL7-mediated degradation of P53 in bladder cancer. Oncogene 2026; 45: 745-756.

[41] Dong S, Wang L, Liu X, Hou D, Liu Q, Zheng J and Wang H. CELF2 inhibits bladder cancer progression by decreasing the stability of CXCL5. Life Sci 2025; 370: 123585.

[42] Wu F, Wu S, Huang Y, Xiao X, Yu H, Bai X, Zhang C, Feng Z, Li L, Mei Y, Li X and Gou X. Smoking promotes the progression of bladder cancer through FOXM1/CKAP2L axis. J Transl Med 2025; 23: 785.

[43] Jin H, Wu P, Lv C, Zhang S, Zhang Y, Li C, Gao R, Shan G, Bi H, Chang H, Liu X and Zeng Y. Mannose inhibits PKM2 lactylation to induce pyroptosis in bladder cancer and activate antitumor immune responses. Commun Biol 2025; 8: 689.

[44] Wang T, Ma X, Wang Y, Liu H, Ding G, Li Y, Yuan H, Gao J, Sun F, Guo Y, Ma J and Wu J. Lactate-driven lactylation of HNRNPA1 orchestrates PKM2 splicing and glycolytic reprogramming in bladder cancer. J Exp Clin Cancer Res 2025; 44: 331.

[45] Wu Q, Zhou X, Li P, Ding M, You S, Xu Z, Ye J, Chen X, Tan M, Wang J, Wang W and Qiu J. ROC1 promotes the malignant progression of bladder cancer by regulating p-IkappaBalpha/NF-kappaB signaling. J Exp Clin Cancer Res 2021; 40: 158.

[46] Schuettfort VM, Pradere B, Trinh QD, D'Andrea D, Quhal F, Mostafaei H, Laukhtina E, Mori K, Sari Motlagh R, Rink M, Karakiewicz PI, Chlosta P, Yuen-Chun Teoh J, Lotan Y, Scherr D, Abufaraj M, Moschini M and Shariat SF. Impact of preoperative plasma levels of interleukin 6 and interleukin 6 soluble receptor on disease outcomes after radical cystectomy for bladder cancer. Cancer Immunol Immunother 2022; 71: 85-95.

[47] Choi W, Czerniak B, Ochoa A, Su X, Siefker-Radtke A, Dinney C and McConkey DJ. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. Nat Rev Urol 2014; 11: 400-410.

[48] Ren W and Zhu Y. Causal associations between the presence of prostate cancer or testosterone levels and bladder cancer risk: a Mendelian randomization study. Clin Genitourin Cancer 2025; 23: 102334.

[49] Hyldgaard JM, Nørgaard M, Hjort PE and Jensen JB. Bladder cancer incidence and mortality among men with and without castration therapy for prostate cancer - a nation-wide cohort study. Acta Oncol 2024; 63: 746-754.

[50] Yang L, Huang W, Bai X, Wang H, Wang X, Xiao H and Li Y. Androgen dihydrotestosterone promotes bladder cancer cell proliferation and invasion via EPPK1-mediated MAPK/JUP signalling. Cell Death Dis 2023; 14: 363.

[51] Hyldgaard JM and Jensen JB. The inequality of females in bladder cancer. APMIS 2021; 129: 694-699.

[52] Minami T, Inoue M, Yamaji T, Iwasaki M, Tsugane S and Sawada N. Reproductive factors in the risk of bladder cancer and upper urinary tract cancer: the japan public health center-based prospective study. Cancer Epidemiol Biomarkers Prev 2023; 32: 1572-1582.

[53] Zhang J, Jia H and Han H. Emerging drivers of female bladder cancer: a pathway to precision prevention and treatment. Front Oncol 2025; 15: 1497637.

[54] Li Y, Tindle HA, Hendryx MS, Xun P, He K, Liang X and Luo J. Smoking cessation and the risk of bladder cancer among postmenopausal women. Cancer Prev Res (Phila) 2019; 12: 305-314.

[55] Amr S, Wolpert BJ, St George DM, James I and Loffredo CA. Body mass index modifies bladder cancer risk associated with low estrogen exposure among Egyptian women after menopause. Cancer Causes Control 2019; 30: 249-258.

[56] Xu X, Mo Q, Shen H, Wang S and Liu B. Reproductive and hormonal factors and bladder can-

cer risk: a prospective study and meta-analysis. Aging (Albany NY) 2020; 12: 14691-14698.
[57] Li Y, Hendryx MS, Xun P, He K, Shadyab AH, Lane DS, Nassir R, Stefanick ML, Wactawski-Wende J, Pal SK and Luo J. Physical activity and risk of bladder cancer among postmenopausal women. Int J Cancer 2020; 147: 2717-2724.
[58] Kamoun A, de Reyniès A, Allory Y, Sjödahl G, Robertson AG, Seiler R, Hoadley KA, Groeneveld CS, Al-Ahmadie H, Choi W, Castro MAA, Fontugne J, Eriksson P, Mo Q, Kardos J, Zlotta A, Hartmann A, Dinney CP, Bellmunt J, Powles T, Malats N, Chan KS, Kim WY, McConkey DJ, Black PC, Dyrskjøt L, Höglund M, Lerner SP, Real FX and Radvanyi F; Bladder Cancer Molecular Taxonomy Group. A consensus molecular classification of muscle-invasive bladder cancer. Eur Urol 2020; 77: 420-433.
[59] Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Dominguez Escrig JL, Gontero P, Liedberg F, Masson-Lecomte A, Mostafid AH, Palou J, van Rhijn BWG, Rouprêt M, Shariat SF, Seisen T, Soukup V and Sylvester RJ. European association of urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and Carcinoma in Situ). Eur Urol 2022; 81: 75-94.
[60] Balar AV, Kamat AM, Kulkarni GS, Uchio EM, Boormans JL, Roumiguie M, Krieger LEM, Singer EA, Bajorin DF, Grivas P, Seo HK, Nishiyama H, Konety BR, Li H, Nam K, Kapadia E, Frenkl T and de Wit R. Pembrolizumab monotherapy for the treatment of high-risk non-muscle-invasive bladder cancer unresponsive to BCG (KEYNOTE-057): an open-label, single-arm, multicentre, phase 2 study. Lancet Oncol 2021; 22: 919-930.
[61] Galsky MD, Daneshmand S, Izadmehr S, Gonzalez-Kozlova E, Chan KG, Lewis S, Achkar BE, Dorff TB, Cetnar JP, Neil BO, D'Souza A, Mamtani R, Kyriakopoulos C, Jun T, Gogerly-Moragoda M, Brody R, Xie H, Nie K, Kelly G, Horowitz A, Kinoshita Y, Ellis E, Nose Y, Ioannou G, Cabal R, Del Valle DM, Haines GK, Wang L, Mouw KW, Samstein RM, Mehrazin R, Bhardwaj N, Yu M, Zhao Q, Kim-Schulze S, Sebra R, Zhu J, Gnjatic S, Sfakianos J and Pal SK. Gemcitabine and cisplatin plus nivolumab as organ-sparing treatment for muscle-invasive bladder cancer: a phase 2 trial. Nat Med 2023; 29: 2825-2834.
[62] Powles T, Valderrama BP, Gupta S, Bedke J, Kikuchi E, Hoffman-Censits J, Iyer G, Vulsteke C, Park SH, Shin SJ, Castellano D, Fornarini G, Li JR, Gümüş M, Mar N, Loriot Y, Fléchon A, Duran I, Drakaki A, Narayanan S, Yu X, Gorla S, Homet Moreno B and van der Heijden MS; EV-302 Trial Investigators. Enfortumab vedotin and pembrolizumab in untreated advanced urothelial cancer. N Engl J Med 2024; 390: 875-888.
[63] Cañizo CG, Guerrero-Ramos F, Perez Escavy M, Lodewijk I, Suárez-Cabrera C, Morales L, Nunes SP, Munera-Maravilla E, Rubio C, Sánchez R, Rodriguez-Izquierdo M, Martínez de Villarreal J, Real FX, Castellano D, Martín-Arriscado C, Lora Pablos D, Rodríguez Antolín A, Dueñas M, Paramio JM and Martínez VG. Characterisation of the tumour microenvironment and PD-L1 granularity reveals the prognostic value of cancer-associated myofibroblasts in non-invasive bladder cancer. Oncoimmunology 2025; 14: 2438291.
[64] Vulsteke C, Adra N, Danchaivijitr P, Sabadash M, Rodriguez-Vida A, Zhang Z, Atduev V, Göger YE, Rausch S, Kang SH, Loriot Y, Bedke J, Galsky MD, O'Donnell PH, von Amsberg G, Alimohamed N, Sulimka G, Gupta S, Paramonov V, Nakane K, Mihm M, Meng C, Huang CD, Ramamurthy C, Homet Moreno B and Ullén A; KEYNOTE-905/EV-303 Investigators. Perioperative enfortumab vedotin and pembrolizumab in bladder cancer. N Engl J Med 2026; 394: 1257-1269.
[65] Li Z, Izadmehr S, Hoffman-Censits J, Maughan B, Mayer T, Tan A, Brody R, Xie H, Nie K, Kelly G, Ioannou G, Cabal R, Tuballes K, Guo R, Figueiredo I, Del Valle DM, Mehrazin R, Yu M, Zhao Q, Kim-Schulze S, Sfakianos JP, Gupta S, Hahn NM, Gonzalez-Kozlova E, Gnjatic S and Galsky MD. Gemcitabine plus nivolumab with carboplatin or oxaliplatin in cisplatin-ineligible patients with metastatic urothelial carcinoma: a randomized phase II trial. Clin Cancer Res 2026; 32: 2911-2920.
[66] Guo CC, Lee S, Lee JG, Chen H, Zaleski M, Choi W, McConkey DJ, Wei P and Czerniak B. Molecular profile of bladder cancer progression to clinically aggressive subtypes. Nat Rev Urol 2024; 21: 391-405.
[67] Correction to "minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches". J Extracell Vesicles 2024; 13: e12451.
[68] Raposo G and Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200: 373-383.
[69] Gonzalez-Kozlova E, Tichkule S, Nose Y, Chen TY, Reznik E, Santiago JV, Korrapati A, Soleymani T, Kosoy R, Figueiredo I, Lee D, Hoffman GE, Kyprianou N, Gordon RE, Cordon-Cardo C, Rangaraju S, Seyfried NT, Haroutunian V, Fullard JF, Roussos P and Dogra N. SECmeres outperform extracellular vesicles as potential blood RNA biomarkers for Alzheimer's disease. Nat Commun 2026; 17: 5453.
[70] Soleymani T, Chen TY, Gonzalez-Kozlova E and Dogra N. The human neurosecretome: extra-

cellular vesicles and particles (EVPs) of the brain for intercellular communication, therapy, and liquid-biopsy applications. Front Mol Biosci 2023; 10: 1156821.
[71] Miceli RT, Chen TY, Nose Y, Tichkule S, Brown B, Fullard JF, Saulsbury MD, Heyliger SO, Gnjatic S, Kyprianou N, Cordon-Cardo C, Sahoo S, Taioli E, Roussos P, Stolovitzky G, Gonzalez-Kozlova E and Dogra N. Extracellular vesicles, RNA sequencing, and bioinformatic analyses: challenges, solutions, and recommendations. J Extracell Vesicles 2024; 13: e70005.
[72] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ and Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654-659.
[73] Ma X, Yang R, Li H, Zhang X, Zhang X and Li X. Role of exosomes in the communication and treatment between OSCC and normal cells. Heliyon 2024; 10: e28148.
[74] Dogra N, Chen TY, Gonzalez-Kozlova E, Miceli R, Cordon-Cardo C, Tewari AK, Losic B and Stolovitzky G. Extracellular vesicles carry transcriptional 'dark matter' revealing tissue-specific information. J Extracell Vesicles 2024; 13: e12481.
[75] Cavallari C, Camussi G and Brizzi MF. Extracellular vesicles in the tumour microenvironment: eclectic supervisors. Int J Mol Sci 2020; 21: 6768.
[76] Rimmer MP, Gregory CD and Mitchell RT. Extracellular vesicles in urological malignancies. Biochim Biophys Acta Rev Cancer 2021; 1876: 188570.
[77] Bebelman MP, Janssen E, Pegtel DM and Crudden C. The forces driving cancer extracellular vesicle secretion. Neoplasia 2021; 23: 149-157.
[78] Campistol M, Lozano F, Carrion A, Raventós CX, Morote J and Trilla E. Active surveillance in non-muscle invasive bladder cancer: a systematic review. Cancers (Basel) 2025; 17: 1714.
[79] Urabe F, Kimura T, Ito K, Yamamoto Y, Tsuzuki S, Miki J, Ochiya T and Egawa S. Urinary extracellular vesicles: a rising star in bladder cancer management. Transl Androl Urol 2021; 10: 1878-1889.
[80] Hussain QM, Al-Hussainy AF, Sanghvi G, Roopashree R, Kashyap A, Anand DA, Panigrahi R, Shavazi N, Taher SG, Alwan M, Jawad M and Mushtaq H. Dual role of miR-155 and exosomal miR-155 in tumor angiogenesis: implications for cancer progression and therapy. Eur J Med Res 2025; 30: 393.
[81] Gan L, Zheng L, Zou J, Luo P, Chen T, Zou J, Li W, Chen Q, Cheng L, Zhang F and Qian B. MicroRNA-21 in urologic cancers: from molecular mechanisms to clinical implications. Front Cell Dev Biol 2024; 12: 1437951.
[82] Enokida H, Yoshino H, Matsushita R and Nakagawa M. The role of microRNAs in bladder cancer. Investig Clin Urol 2016; 57 Suppl 1: S60-76.
[83] Cao J. The functional role of long non-coding RNAs and epigenetics. Biol Proced Online 2014; 16: 11.
[84] Li F and Hu CP. Long non-coding RNA urothelial carcinoma associated 1 (UCA1): insight into its role in human diseases. Crit Rev Eukaryot Gene Expr 2015; 25: 191-197.
[85] Arriaga-Canon C, De La Rosa-Velázquez IA, González-Barrios R, Montiel-Manríquez R, Oliva-Rico D, Jiménez-Trejo F, Cortés-González C and Herrera LA. The use of long non-coding RNAs as prognostic biomarkers and therapeutic targets in prostate cancer. Oncotarget 2018; 9: 20872-20890.
[86] Ke B, Jin P, Wang XJ, Liu N, Liang H and Zhang RP. Oncogenic and immunological role of EDIL3 in human tumours: from pan-cancer analysis to validation in gastric cancer. Heliyon 2024; 10: e32291.
[87] Silvers CR, Liu YR, Wu CH, Miyamoto H, Messing EM and Lee YF. Identification of extracellular vesicle-borne periostin as a feature of muscle-invasive bladder cancer. Oncotarget 2016; 7: 23335-23345.
[88] Di Bella MA and Taverna S. Extracellular vesicles: diagnostic and therapeutic applications in cancer. Biology (Basel) 2024; 13: 716.