# Unleashing the power of urine-based biomarkers in diagnosis, prognosis and monitoring of bladder cancer (Review)

XUEBIN WAN[1,2*], DAN WANG[3*], XIAONI ZHANG[2], MINGYAN XU[2], YUYING HUANG[4], WENJIAN QIN[1] and SHIFU CHEN[1,2]

[1]Department of Biomedical and Health Engineering, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, P.R. China; [2]Department of Research and Development, HaploX Biotechnology, Co., Ltd., Shenzhen, Guangdong 518057, P.R. China; [3]Department of Molecular Microbiology and Genetics, Institute for Microbiology and Genetics, University of Goettingen, Göttingen D-37077, Germany; [4]Department of Pediatrics, Guizhou Provincial People's Hospital, Guiyang, Guizhou 550002, P.R. China

Received September 23, 2024; Accepted January 13, 2025

DOI: 10.3892/ijo.2025.5724

**Abstract.** Bladder cancer (BCa) is a prevalent malignant neoplasm of the urinary tract with high incidence rate, frequent recurrence and rapid disease progression. Conventional approaches for diagnosing, prognosticating and monitoring BCa often rely on invasive procedures such as cystoscopy and tissue biopsy, which are associated with high costs and low patient compliance for follow-up. Liquid biopsies have advantages, such as being non-invasive, real-time, and reproducible, in obtaining diverse biomarkers derived from cellular, molecular, proteomic and genetic signatures in urine or plasma samples. Although plasma-based biomarkers have been clinically validated, urine provides greater specificity for directly assessing biological materials from urological sources. The present review summarizes advancements and current limitations in urinary protein, genetic and epigenetic biomarkers for disease progression and treatment response of BC, compares performance and application scenarios of urine and blood biomarkers and explores how urinary biomarkers may serve as an alternative or complementary tool to traditional diagnostic methods. The integration of urine-based or plasma-based biomarkers into existing diagnostic workflows offers promising avenues for improving accuracy and efficiency of diagnosis in the management of BCa. Notably, the emergence of synthetic biomarkers and urine metabolites, combined with artificial intelligence or bioinformatic technologies, has promise in the screening of potential targets. Continued research and validation efforts are needed to translate these findings into routine clinical practice, ultimately improving patient outcomes and decreasing the burden of BCa.

**Contents**



## 1. Introduction

Bladder cancer (BCa) is the most frequently occurring neoplasm in the urinary tract, originating from the urothelium. It is a widespread malignancy that carries notable morbidity and mortality rates. Annually, there are >500,000 new cases and more than 200,000 deaths reported worldwide This disease primarily affects individuals aged ≥55 years (1,2). In general, compared with muscle-invasive BCa (MIBC), non-MIBC (NMIBC) exhibits more favorable prognosis and a decreased propensity for extravesical spread. Low grade NMIBC carries a higher risk of recurrence. Specifically, the 5-year recurrence rates range from 31 to 78%, while the rates of progression are between 1 and 45% (3). A total of 60-70% of patients with NMIBC encounter frequent recurrences following transurethral resection of bladder tumor. The high recurrence rate can be attributed to incomplete piecemeal resection and subsequent tumor re-implantation (4). In addition, patients diagnosed with MIBC at stage T2-T4 display a poor treatment response and prognosis. This is primarily due to the tumor susceptibility to rapid growth and distant metastasis, which leads to an unfavorable clinical outcome and a decreased lifespan of ~15 months (5). Currently,

*Correspondence to:* Professor Wenjian Qin or Dr Shifu Chen, Department of Biomedical and Health Engineering, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, 1068 Xueyuan Avenue, Nanshan, Shenzhen, Guangdong 518055, P.R. China
E-mail: wj.qin@siat.ac.cn
E-mail: sf.chen@siat.ac.cn

*Contributed equally

*Key words:* bladder cancer, liquid biopsy, urinary biomarker, genetic signature, disease progression, treatment response

cystoscopy is the recommended standard procedure for diagnosing and continuously monitoring treatment outcomes of BCa (6). This is key for patients at a high risk of recurrence or progression, who require regular surveillance (3,7).

Regular follow-up cystoscopy is crucial for the early detection of any new or recurrent tumors, particularly when monitoring patients with NMIBC who have recurrence rates up to 74.3% (8). Furthermore, high-risk patients requires more frequent cystoscopy, usually scheduled every 3-4 months within the first 2 years of follow-up (9). However, discomfort and potential complications, invasiveness, cost, accessibility and the frequency of follow-up examinations all contribute to the complexity of implementing and maintaining regular surveillance (10-12). While cystoscopy combined with biopsy serves an indispensable role in disease monitoring and recurrence prevention, the development of emerging liquid biopsy-based biomarkers has decreased reliance on cystoscopy. These non-invasive approaches may revolutionize management options for BCa, leading to improved patient outcomes and decreased healthcare costs (13). Efforts have been made to develop non-invasive diagnostic tools for urinary tract cancer by utilizing urine (14) as urine comes into direct contact with urothelial tumor cells and reflects the local tumor microenvironment. Analysis of urine composition, including cell-specific characteristics, protein/antigen biomarkers and genetic mutations, holds great promise for revolutionizing the diagnosis and monitoring of bladder conditions. Urine biomarkers with high sensitivity and negative predictive value (NPV) may effectively exclude the presence of BCa (15-17), decreasing the need for additional diagnostic tests performed in the operating theatre.

As an adjunct measure for detecting BCa, urine cytology serves a key role in the objective assessment of cell characteristics, including morphology, nuclear features and cellularity. Urine cytology enables the classification of lesions into cyto-diagnostic categories, including non-diagnostic cases, neoplastic high-grade urothelial carcinoma (UC), atypical urothelial cells, those suspicious for high-grade UC, high-grade UC, and other malignancies. This classification provides valuable insight into the response of tumors to therapeutic interventions, such as chemotherapy or radiation (18,19). Currently, urine cytology demonstrates high specificity (80-95%) in detecting high-grade UC. However, its sensitivity is inadequate, especially for detecting low-grade UC (overall sensitivity, 4-31%; well differentiated grade 1, 12%; moderately differentiated, grade 2, 26%) (20,21). Recent research has focused on evaluating the effectiveness of urine cytology as a screening method for monitoring low-risk NMIBC (22,23). Among urine cytology tests conducted, only a small percentage (2.9%) of patients in all risk groups consistently show positive results consistent with cystoscopy and pathological findings (22,23). Non-invasive urine cytology and urography present >30% variation in sensitivity and specificity, depending on the location and grading of the UC. High-grade tumors, including carcinoma *in situ*, can show a positivity rate up to 84%. By contrast, low-grade tumors exhibit a considerably lower sensitivity, with a positivity rate of 16% (24,25).

The present review discusses the utilization of urine-based liquid biopsy technology to address concerns regarding the high costs and low patient compliance associated with follow-up procedures. It systematically summarizes urinary biomarkers, including protein assays, characterization of nucleic acids (such as DNA and RNA) and emerging potential biomarkers, as well as innovative diagnostic tool (Table I). The present reviewed aimed to evaluate advancements in urinary biomarkers, with an emphasis on performance variability and the ongoing challenges in the diagnosis, prognosis and monitoring of patients with BCa. Additionally, the present review compares the efficacy of urine and blood biomarkers in the detection of urological tumors, especially in BCa.

## 2. Non-invasive approaches

*Food and Drug Administration (FDA)-approved urinary biomarkers*

*Nuclear matrix protein (NMP)22.* At present, several protein-based tests, such as NMP22 (MDxHealth), bladder tumor antigen (BTA) (Polymedco Inc. Cortlandt Manor, NY, USA), UroVysion (Abbott Laboratories) and ImmunoCyt (Diagnocure Inc., Québec, Canada), have been approved by the United States FDA and are commercially available for the diagnosis and surveillance of BCa (26). NMP22, a key component of the nuclear mitotic apparatus protein, serves a vital role in cellular processes, including DNA replication, RNA transcription and gene expression. Understanding of the NMP22 regulatory network is key for the proper functioning and regulation of cells; disruption in these mechanisms can have notable implications for cancer proliferation. The concentration of NMP22 in malignant transitional cells is 80-fold higher in BCa compared with normal urothelial cells (27). The NMP22 test is initially approved for surveillance of NMIBC in 1996. It utilizes quantitative ELISA for detection, which demonstrates a sensitivity of 69% and a specificity of 77% (28). In 2020, an additional point-of-care (POC) test, BladderChek, developed by Matritech Inc., is also approved for use (sensitivity, 58%; specificity, 88%) (29). The performance of NMP22 assays has been confirmed through several meta-analyses (30,31), demonstrating overall sensitivity of 73 and specificity of 79% (sensitivity, 60% and specificity, 79% for low-grade upper tract UC (UTUC); sensitivity and specificity, both 79% for high-grade UTUC) (32). The expression of proteins is dynamic and can vary based on factors including tumor stage and grade, heterogeneity, size and presence of infections or inflammation. Existing research indicates an association between the sensitivity and specificity of NMP22 and tumor aggressiveness (33-35). Recent studies have demonstrated that NMP22 exhibits greater sensitivity, NPV and accuracy in detecting recurrence compared with cytology (36,37). In low-grade BCa tumors, the sensitivity of NMP22 is 81%, whereas cytology achieves a sensitivity of 23%. Combination of NMP22 and cytology demonstrates significantly higher sensitivity (91.63% vs. 81.83% vs. 53.96%) and NPV (87.59% vs. 77.46% vs. 61.02%) than either NMP22 or cytology alone (38,39).

*BTA.* The urinary tests for BTA, which include BTA stat (Bion Diagnostic Sciences, Inc.) and BTA TRAK (Bard Diagnostics), have obtained FDA approval as non-invasive diagnostic approaches (40,41). BTA stat and BTA TRAK are single-step immunochromatographic qualitative assays for diagnosis and monitoring of BCa (42). BTA stat test is a rapid

Table I. Overview of current urinary biomarker tests in BCa.

| Test | Biomarker | Genetic material | Methods | Clinical use | Approval | SEN, % | SPN,% | Limitations | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|
| Cystoscopy | NA | NA | Endoscopic inspection | Diagnosis; surveillance | Flexible cystoscope, FDA | Overall, 87-100; WLC, 71 | Overall, 64-100; WLC, 72 | Low specificity; invasive; high cost | (6-13) |
| Urine cytology | NA | CTC | Giemsa or HE staining | Complementary diagnosis | NA | Overall, 12-90; low-grade lesions, 4-31 | Overall, 51-98; high-grade tumor, 80-95 | Low sensitivity for low-grade tumors | (18-25) |
| BladderChek BC | NMP22 | Protein | ELISA, POC | Surveillance combined with cystoscopy | FDA, CE | ELISA, 70-81; POC, 52-59 | ELISA, 73-89; POC, 87-89 | Low specificity for low-grade tumors (low NPV) | (28-39) |
| BTA stat BTA TRAK | hCFHrp | Protein/ antigen | POC, ELISA | Surveillance combined with cystoscopy | FDA, CE | POC, 40-72; ELISA, 54-75 | POC, 29-86; ELISA, 64-82 | Low Specificity (low NPV) | (40-47) |
| UroVysion | Chromosomes 3, 7, 17 and 9p21 locus | Sediment | FISH | Diagnosis | FDA, CE | 69-94 | 63-100 | High cost; time-consuming; false- positive results (primary and secondary adenocarcinoma), low NPV | (48-51) |
| ImmunoCyt/ Ucyt+ | Carcinoembryonic antigen and 2 mucins | Sediment | Immunohist ochemistry | Diagnosis | FDA | 50-100 | 69-79 | Low specificity; high cost; complicated operation | (61-66) |
| Specific protein biomarkers | CK20, CDKN2A, ERBB2, PTEN, VEGF, MMP-9, survivin, BRDT, CYBP, GARS, HDGF, MMP23B | Protein | NGS, MS, proteomics, ELISA, western blot, machine learning | Diagnosis; surveillance | NA | 36-71 | 61-91 | Insufficient sensitivity to detect low-grade tumors; low concentration of protein markers in urine; false positives due to inflammation and hematuria | (69-85) |
| Mutational biomarkers (UroSEEK, Uromonitor-V2, XpertBC) | *Ras, ERBB2, MDM2, CCND1, C-MYC, EGFR, TP53, p21, PTEN, FGFR3, PIK3CA, STK15, FHIT, TERT, ATM* | cfDNA/ctDNA | NGS, Sanger sequencing, qPCR | Diagnosis; surveillance of recurrence | Uromonitor-V2 approved by FDA/CE | UroSEEK, 96; Uromonitor 73.5; XpertBC, 46. 2 | UroSEEK, 88; Uromonitor 93.2; XpertBC 77 | Variability in diagnostic performance (single mutation or mutation panel);low specificity and false positive results; study design, population characteristics, analytic technique, and urine DNA heterogeneity influence accuracy | (88-118) |

Table I. Continued.

| Test | Biomarker | Genetic material | Methods | Clinical use | Approval | SEN, % | SPN,% | Limitations | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|
| Methylation biomarkers (UriFind, AssureMD, Bladder Epicheck) | *TERT, FGFR3, OTX1, ONECUT2, VIM TWIST1, IPF1, GALR1, TAL1, PENK, TJP2* | cfDNA/ ctDNA | qPCR, NGS | Diagnosis; surveillance | UriFind approved by FDA | UriFind, 91.2; AssureMDX, 93; Epicheck, 55 | UriFind, 85.7; AssureMDX, 86; Epicheck, 81 | Low specificity; false-positive results, suboptimal reproducibility due to tumor heterogeneity; insufficient number of validated clinical cohorts; short follow-up time | (119-137) |
| CNV-based biomarkers (UroCAD; UC Detector) | CDKN2A, RBI, PDE4D, FHIT, CREBBP, YWHAZ, PPARG, YAP1, MYC, E2F3, FRS2 | cfDNA/ ctDNA | NGS; qPCR | Diagnosis | NA | UroCAD, 80.4; UC Detector, 86.5 | UroCAD, 94.9; UC Detector, 94.7 | Low sensitivity for low-grade tumors; lack of sufficient urine exfoliated cells; insufficient number of validated clinical trials | (141-146) |
| MSI-based biomarkers | BAT-25, BAT-26, NR-21, NR-24, or D5S346, D2S123, D17S250 | cfDNA/ ctDNA | PCR; immunohist ochemistry; NGS | Immunotherapy; diagnosis | MSI/dMM R as pan-cancer biomarkers approved by FDA/NMPA | 83-95 | 89-100 | Low sensitivity and specificity; loci and number of sites affect positivity rate; complicated operation; | (151-164) |
| miR biomarkers | miR-29b-3p, miR-31, miR-141, miR-34b, miR-10b, miR-103, miR-145, miR-182 | RNA | RT-qPCR; NGS | Diagnosis; prognosis | NA | 75-93 | 63-91 | Suboptimal reproducibility; lack of standardization of samples (whole urine, supernatant, pellet or EV) and protocols (centrifugation, reference genes) | (185-190) |
| lncRNA | PCAT-1, ANRIL, MKLN1-AS, TALAM1, TTN-AS1, UCA1, LNMAT2, BCYRN1, BLACAT2, MIR205HG, GAS5 | RNA | RT-qPCR | Diagnosis; prognosis | NA | 43-96 | 48-90 | Lack of a validation cohort; different follow-up times, sample type heterogeneity; different cut-off value | (194-205) |

Table I. Continued.

| Test | Biomarker | Genetic material | Methods | Clinical use | Approval | SEN, % | SPN,% | Limitations | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|
| mRNA (CxBladder, Xpert Bladder) | KLHDC7B, CASP14, PRSS1, IGFBP5, HOXA13, MDK, CDK1, CXCR2, ABL1, CRH, IGF2, UPK1B, ANXA10 | RNA | RT-qPCR; machine learning | Diagnosis; surveillance | CE | CxBladder, 74; Xpert Bladder, 91 | CxBladder, 82; Xpert Bladder, 96 | Low specificity; insufficient number of validated clinical trials | (206-211) |
| circRNA | circ102336, circ0008399, circ0058063, circKDM4C, circITCH, circACVR2A | RNA | RT-qPCR; NGS | Diagnosis; prognosis | NA | None | None | Most circRNAs have low specificity and sensitivity; insufficient number of validated clinical trials; mediated signaling pathways have crosstalk | (212-217) |

BCa, bladder cancer; SEN, sensitivity; SPN, specificity; cfDNA, circulating free DNA; ctDNA, circulating tumor DNA; FDA, Food and Drug Administration; HE, hematoxylin and eosin; CE, Conformity with European; WLC, white light cystoscopy; CTC, circulating tumor cell; NPV, negative predictive value; NMP22, nuclear matrix protein 22; BTA, bladder tumor antigen; hCFHrp, human complement factor H-related protein; FISH, fluorescence *in situ* hybridization; MS, mass spectrometry; NGS, next-generation sequencing; NA, not applicable; POC, point-of-care; CNV, copy number variation; UC, urothelial carcinoma; MSI, microsatellite instability; dMMR, deficient mismatch repair; NMPA, National Medical Products Administration; miRNA, microRNA; RT-q, reverse transcription-quantitative; lncRNA, long non-coding RNA; circRNA, circular RNA; UBC, urinary bladder cancer; miR, miRNA; KLHDC7B, kelch domain containing 7B; CASP14, caspase 14; PRSS1, protease serine 1; TERT, telomerase reverse transcriptase; ONECUT 2, one cut homeobox 2; OTX1, orthodenticle homeobox 1; BAT, bethesda tutorial; NR, non-repetitive; RBI, retinoblastoma inhibitory gene; PDE4D, phosphodiesterase 4D; FHIT, fragile histidine triad diadenosine triphosphatase; FGFR3, fibroblast growth factor receptor 3; UCA1, urothelial cancer associated 1; LNMAT2, lymph node metastasis-associated transcript 2; BCYRN1, brain cytoplasmic RNA 1; MIR205HG, MIR205 host gene; GAS5, growth arrest specific 5; CDKN2A, cyclin dependent kinase inhibitor 2A; ERBB2, erb-B2 receptor tyrosine kinase 2.

test in which a urine sample is applied to a test strip. This strip contains specific monoclonal antibodies that bind to the targeted antigen if H-related protein (hCFHrp) is detected (43). hCFHrp plays a key role in regulating activity of the alternate complement pathway, allowing malignant cells to escape from immune surveillance. Complement factor H in the bloodstream has the potential to interfere with the precision of BTA tests, resulting in the occurrence of false positive outcomes (44). This effect is particularly prominent in individuals who have benign urological conditions (45). Sensitivity and specificity of the BTA test in detecting BCa range from 57 to 83% and 60 to 92%, respectively (40,46,47). Due to variability in its specificity (29-86%), BTA is not recommended for the initial diagnosis of BCa. However, it can be utilized as a component of surveillance protocols to monitor changes in antigen levels over time, particularly when combined with cystoscopy.

*UroVysion assay.* UroVysion is a specialized fluorescence *in situ* hybridization (FISH) test to identify three chromosome enumeration probes for chromosomes 3, 7 and 17, as well as a single locus-specific identifier probe for the 9p21 locus (48,49). The selection of probes exhibit a sensitivity of 75% in identifying chromosomal aberrations, particularly in urothelial tumors (50). Chromosomal instabilities and aneuploidies frequently occur during progression of tumors, with a notable impact on chromosomes 3, 7 and 17 (51). Additionally, homozygous deletions in the 9p21 region are commonly observed in primary BCa, leading to inactivation of potential tumor suppressor genes, such as cyclin dependent kinase inhibitor 2 (*CDKN2*, *CDKN2B*) and methylthioadenosine phosphorylase (52,53). Several studies have investigated the effectiveness of UroVysion in detection of UC in comparison with urine cytology (54,55). The sensitivity of UroVysion has been reported to vary between 69 and 94%, whereas the specificity ranges from 63 to 100% in detecting UC (56). However, UroVysion tends to produce more false positive results in comparison with cytology. Recent cohort studies have highlighted the need of assessing supplementary chromosomal probes due to the considerable variability in sensitivity observed in both high- (83-100%) and low-grade tumors (36-55%) (57-59).

*ImmunoCyt (UCyt+).* ImmunoCyt (UCyt+) assay has been approved by the FDA since February 2000 as an approved supplementary tool for the management of BCa alongside urine cytology and cystoscopy (60). ImmunoCyt test can identify specific tumor-associated antigens in urine based on immunofluorescence using monoclonal antibodies M344, LDQ10 and 19A211 (61). The sensitivity and specificity of ImmunoCyt assay are 50-100% and 69-79%, respectively, and the positive predictive value (PPV) and NPV are 26-67% and 91-96%, respectively (61,62).

A number of studies have examined the diagnostic accuracy of ImmunoCyt and cytology, revealing notable differences (55-58). ImmunoCyt exhibits lower specificity (65.7% vs. 90.6%), positive (2.578 vs. 3.862) and negative likelihood ratio (0.385 vs. 0.459) and area under the curve (AUC) (0.791 vs. 0.824) compared with cytology (63). While ImmunoCyt demonstrates higher sensitivity (72.5% vs. 56.6%), it may not possess the same level of accuracy as cytology in relation to other factors such as endogenous and exogenous interference, as well as the methods of detection and sample handling. On the other hand, several studies confirm that ImmunoCyt improves overall performance of cytology, particularly for small (<1 cm), superficial and low histological grade (tumor *in situ*, Ta, T1 and T2) tumors (64,65). Cytology tests demonstrate sensitivity of 29% and NPV of 88%. When combined with ImmunoCyt, these values improve to 84 and 95%, respectively (65). These results suggest the potential utility of the ImmunoCyt test in decreasing the need for frequent follow-up cystoscopies. This test is also suitable for continuous monitoring of low-risk patients with BCa, thereby allowing early detection of disease progression and prompt intervention when necessary. ImmunoCyt test plays a key role in predicting painless hematuria, surpassing the effectiveness of traditional cytology, especially in cases where imaging and cystoscopy results are negative (66).

*Specific protein biomarkers.* A large number of protein-specific biomarkers or molecular signatures are identified using proteomic techniques in urine (67), blood serum (68,69) and plasma (70,71). Advanced methodologies such as mass spectrometry (MS) and machine learning have notably enhanced the ability to identify these biomarkers, particularly in urine samples (72-75). Studies have been performed to determine potential prognostic or predictive value of multiple protein candidates, including UBC Rapid immunoassay (CK8, CK18) (IDL Biotech), CK20, cyclin dependent kinase inhibitor 2A (CDKN2A), erb-B2 receptor tyrosine kinase 2 (ERBB2), phosphatase and tensin homolog (PTEN), vascular endothelial growth factor (VEGF), matrix metallopeptidase 9 (MMP-9) and Survivin, to enhance the assessment of disease progression and risk of BCa recurrence (75-81). Similarly, a quantitative proteomic approach using isotope tags for relative and absolute quantification (iTRAQ) labeling has identified 21 candidate proteins in urinary samples (82). Of these markers, bromodomain testis associated (BRDT), calcyclin binding protein (CYBP), glycyl-tRNA synthetase (GARS) and heparin binding growth factor (HDGF) with a high discrimination ability in UC patients, have shown substantially elevated expression in the UC cohort compared with normal groups. The AUC values for the combined panel of these biomarkers are 0.962 for distinguishing between UC and normal groups, and 0.860 for differentiating between UC and control groups (82). Machine learning has been employed to identify various protein markers for constructing a BCa diagnostic model, which comprises 14 markers found in serum and urine supernatants, achieving 86% sensitivity and 82% specificity and surpasses FISH in detecting low-risk BCa (64).

Use of ELISA and western blotting has enabled the measurement and verification of specific proteins of interest in urine samples obtained from patients with BCa (83). In one study, researchers analyzed genome-wide gene and protein expression via quantitative PCR (qPCR), western blotting and ELISA in 66 patients with BCa and 70 individuals without tumors of the urinary system (84). The results demonstrated a significant increase in protein expression of MMP23B, a member of the MMP family, in both urine and plasma compared with tissue biopsies. There is an association between the level of urinary MMP23B and risk stratification, suggesting MMP23B could be a potential non-invasive biomarker for diagnosis of BCa (84,85).

*Limitations and variability*. Urine protein tests typically show greater sensitivity than cytological analysis but lower specificity. For example, the BTA assay exhibits a higher sensitivity (67% vs. 43%) and a lower specificity (77% vs. 90%) compared with urine cytology (86). To date, no single biomarker has replaced cystoscopy and urine cytology in clinical practice. These urinary tests are limited by lack of specificity and high proportion of false positive results, with specificity ranging from 50 to 80% and false-positive rates between 16.7 and 30.5% (87,88). False positives can be caused by conditions such as inflammation, kidney and bladder stone disease and hematuria (89). The sensitivity of protein-based biomarkers typically increases with the advancement in tumor stage or grade (90). To the best of our knowledge, there are few prospective studies investigating urine biomarkers in low-grade NMIBC and small papillary lesions and the available cross-sectional studies appear to overestimate the sensitivity of these biomarkers (91,92). In addition, the concentration of potential protein biomarkers in urine is typically low, and detection can be influenced by various factors, including sex, age, hormonal status, diet and physical activity (93). Considering the limitations of urinary protein-based markers, it is advisable to analyze these markers alongside a panel of biomarkers or in combination with clinical tests. An integrated approach is likely to enhance the detection rate of BCa and improve effectiveness of screening programs for high-risk populations.

Despite the benefits and potential value of protein markers in predicting, diagnosing and determining the prognosis of BCa, the field of urinary proteomics faces challenges due to lack of standardized protocols for the collection, storage, processing and analysis of samples (64). This absence of standardization leads to inconsistencies and lack of reproducibility in experimental results. Advances in proteomics sequencing technology have enhanced the capability to generate comprehensive datasets at the single-cell level, even with minimal sample quantities (72,94). The incorporation of artificial intelligence (AI) and machine learning into analysis of proteomics data is expected to advance the early detection of superficial BCa and enhance the precision of diagnosis.

*Genomic and epigenetic alternations based on urinary DNA*. BCa is a common malignant tumor characterized by genomic abnormalities that influence tumorigenesis and progression through the accumulation of mutational factors (95). In addition to protein biomarkers released into urine by tumor cells, DNA-based analysis of genetic material, such as point mutations, methylation patterns, copy number variation (CNV) and microsatellite instability (MSI), as well as the molecular characterization and fragmentation of cell-free DNA (cfDNA), reveal an association between genetic/epigenetic signatures and high mutational burden and widespread variations affecting multiple chromosomes (15,96). Next-generation sequencing (NGS) and machine learning approaches facilitate identification of the most frequently mutated genes and methylated regions, as well as the potential mechanisms of action in BCa (97). The systematic analysis of big data, in conjunction with advancements in bioinformatics technology, enhances assessment of the characteristics of driver genes and sequence characterizations to establish associations between cancer subtype, prognosis, grading, staging and treatment responses. Consequently, it provides valuable insights and guidance for early diagnosis of BCa, identification of therapeutic targets and the monitoring of follow-up care.

*Mutation-based biomarkers*. Driver variants can be detected through molecular testing and provide valuable information regarding the presence and progression of UC. Numerous somatic hotspot mutations can be identified utilizing diverse technological platforms, including NGS, machine learning, real-time qPCR and Sanger sequencing (98,99). Several commercially available assays, such as UroSEEK (Memorial Sloan Kettering Cancer Center), XpertBC (Cepheid) and Uromonitor-V2 (U-monitor, Porto, Portugal), are employed for early detection and prognostic monitoring (100-104). The aforementioned tests are generally designed to evaluate combinations of common oncogenes and tumor suppressor genes. For example, oncogenes such as *Ras*, *ERBB2*, MDM2 proto-oncogene, cyclin D1 (*CCND1*), myelocytomatosis proto-oncogene (*C-MYC*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) and epidermal growth factor receptor (*EGFR*), have been identified as potential diagnostic markers for BCa (105). Mutations or expression deletions in tumor suppressor genes, such as *TP53*, *p21*, *PTEN*, fibroblast growth factor receptor 3 (*FGFR3*), serine/threonine-protein kinase 15 and fragile histidine triad diadenosine triphosphatase (*FHIT*), have shown promise as prognostic markers for BCa disease (106). Mutations in the *FGFR3* and *PIK3CA* genes are commonly found in superficial papillary bladder tumors. The overall mutation rate of *FGFR3* is 57.50% (23/40), which is significantly higher compared with patients with benign bladder disease (107). The activation of the PI3K signaling pathway serves a key role in promoting the aggressive behavior of tumor with *FGFR3* mutation (108). Therefore, evaluation of *PIK3CA* requires the concurrent assessment of *FGFR3* to determine its prognostic value, which holds potential as a therapeutic strategy for BCa (109). Furthermore, p53 protein expression is prevalent in BCa tissues, with a notable association between p53 expression and the grade and stage of tumors. The incidence of recurrence is notably higher in patients exhibiting positive *TP53* gene expression compared with those with negative expression (110). Alterations in *TP53* gene expression may influence the evolutionary and progressive aspects of BCa (111). Moreover, mutation classifiers including *TP53*, *PIK3CA* and ataxia telangiectasia mutated, have the capability to assess tumor mutational burden. This assessment can subsequently guide therapeutic decisions regarding use of immune checkpoint inhibitors (ICIs) across different risk levels in BCa (112). Telomerase reverse transcriptase (*TERT*) promoter mutations represent the most common genetic alterations in the urinary system and have been identified in 70-80% of uroepithelial BCa cases. *TERT* promoter mutations (C228T and C250T) occur in 50-70% and 8-15% of patients, respectively (102,113). *TERT* promoter mutations are demonstrated in urinary DNA, present high sensitivity and specificity in detecting various types of UC. The overall performance of urine supernatant cfDNA shows sensitivity of 80.7% and a specificity of 96.6%, with NPV of 92.5% (114).

UroSEEK classifier, which integrates genetic alterations from 11 commonly affected genes, exhibits a sensitivity of 92.7% and a specificity of 90.7% for the diagnosis of UC (115). Moreover, it has a high accuracy rate of 91.8% in low-grade

UC (116). Mutations in *TERT*, *FGFR3* and *KRAS* genes have a sensitivity of 93.3% and a specificity of 80% for diagnosing BCa (117). A pilot study involving 16 patients with BCa revealed that gene mutations detected in both the urine supernatant and sediment show a higher concordance with cancerous tissue compared with those identified in plasma (118); AUC values are 0.94 in urine supernatant (panel, *TERT*, *FGFR3*, *TP53*, *PIK3CA* and *KRAS*) and 0.91 in urine sediment cohort (panel, *TERT*, *FGFR3*, *TP53*, *HRAS, PIK3CA*, *KRAS* and *ERBB2*), respectively. The detection of somatic mutations in cfDNA in urine holds promise as a diagnostic approach for identifying BCa in patients with hematuria (118).

*Methylation-based biomarkers*. Several studies have provided evidence that BCa transformation is frequently accompanied by widespread changes in epigenetic landscape including DNA methylation and histone modifications, which occur prior to formation of tumors (119,120). A comprehensive analysis of the BCa epigenome has revealed epigenetic alterations in methylation-specific PCR targeting orthodenticle homeobox 1 (*OTX1*), one cut homeobox 2 (*ONECUT2*) and twist family bHLH transcription factor 1 (*TWIST1*) (121). AssureMDx (MDxHealth) assay is developed to design a panel consisting of mutational statuses (*TERT*, *FGFR3*, *HRAS*) and methylation patterns (*OTX1*, *ONECUT2*, and *TWIST1*) in urinary exfoliated cells (122). Notably, there are distinct differences in DNA methylation patterns between NMIBC and MIBC, with DNA hypomethylation being commonly observed in low-grade, non-invasive uroepithelial tumors (123). Specifically, methylation levels of insulin promoter factor 1 (*IPF1*), galanin receptor 1 (*GALR1*), T-cell acute lymphocytic leukemia 1 (*TAL1*), proenkephalin (*PENK*) and tight junction protein 2 (*TJP2*), as determined through bisulfite sequencing, are noticeably higher in MIBC compared with NMIBC samples (124). The continued presence of these abnormal DNA methylation changes in urine samples following surgical excision of the tumor indicates their potential as biomarkers assessing the probability of early detection, tumor recurrence and treatment efficacy (125). The aforementioned study indicates the possibility of comprehensively studying the entire process of tumor development, including precancerous lesions, through the detection of methylation patterns in urinary tumor DNA (utDNA) (126). Urinary DNA methylation profiles are valuable in effectively stratifying the risk of BCa. The sensitivity of these profiles for diagnosing high-grade BCa is 100% (95% CI: 82.5-100%), while for low-grade BCa, it is 62% (95% CI: 51.3-72.7%) (126,127).

Analysis of DNA methylation pattern can be utilized to predict the likelihood of molecular residual disease (MRD). Methylation-based recurrence monitoring models offer an advantage over mutation-based recurrence monitoring by providing earlier indications of MRD (128-130). UCseek classifier (Beijing Institute of Genomics & Peking University First Hospital), which uses methylation and CNV features of urine samples to construct methylation models (118). This approach shows good performance in accurate non-invasive diagnosis and recurrence monitoring of UC. It demonstrates an accuracy of 91.8% for low-grade UCs and recurrence monitoring accuracy (90.91% vs. 59.09%), which is better than that of cystoscopy (118). Compared with other urinary biomarkers, Bladder Epicheck (Nucleix Ltd.) provides an advantage in ruling out the recurrence of BCa, as its effectiveness is uncompromised by previous or ongoing treatments (131). The reliability of hematuria screening as an early indicator for diagnosing BCa faces challenges due to variations in clinical presentation. The incidence of BCa is between 10 and 20% in patients presenting with gross hematuria and between 2 and 5% in individuals with microscopic hematuria (132). The assessment of cancer risk in populations with hematuria reveals promising outcomes when using UriFind (AnchorDx Medical Company, Ltd.), a diagnostic tool that leverages the methylation of the dual genes *ONECUT2* and vimentin. UriFind exhibits a diagnostic sensitivity of 91.2% and a specificity of 85.7%, underscoring its strong performance (133). Methylation genes or combinations, including *TERT*, *FGFR3*, *TWIST* and orthodenticle homeobox 1 (*OTX1*), have been extensively investigated but are not yet available for clinical use (134,135). Although urine methylation studies in detecting MRD are still in the stage of large-scale clinical observation (36,136,137), urine testing may have advantages (such as non-invasive and reproducible procedure, a low protein and cellular content, and detection of multiple diseases and cancer type) compared with blood testing.

*CNV-based biomarkers*. Malignant tumor cells exhibit genetic instability and frequently display copy number alteration (CNA) involving entire chromosomes or segments. CNV serves a key role in the tumor progression and metastasis of BCa; alternations can result in upregulation of oncogenes or the loss of function of tumor suppressor genes, promoting uncontrolled cell proliferation and tumor progression (138). These genetic abnormalities disrupt normal cellular function and enhance the ability to proliferate, invade surrounding tissue and metastasize. Studies have revealed that consistent CNV features are identified in multiple cells of patients with UC (139,140). A total of 57 CNVs have been identified in 131 cases of high-grade MIBC. Among these CNVs identified by whole genome and RNA sequencing, notable copy number deletions are observed in CDKN2A, retinoblastoma inhibitory gene, phosphodiesterase 4D, FHIT and CREB binding protein, while copy number gains are displayed in tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ), peroxisome proliferator activated receptor gamma (PPARG), yes-associated protein 1 (YAP1), MYC and E2F transcription factor 3 (E2F3) (141). This analysis offers perspective on mutations and regions of CNV across multiple pathways that are frequently dysregulated in BCa. Further analysis of exome sequencing data from tumor tissue obtained from 120 patients with UC highlighted notable disparities in CNV between normal uroepithelial and tumor tissue and suggests that the accumulation of mutations may not be adequate to drive transformation of normal epithelium into malignant tumors (142). Studies have revealed a noteworthy association between fibroblast growth factor receptor substrate 2 CNV and protein expression in BCa, demonstrating unfavorable prognosis characterized by decreased overall survival and heightened risk of disease recurrence or progression (143,144). A novel diagnostic model, UroCAD (Hongyuan Biotech, Inc.), has been developed using an optimized low-coverage whole genome sequencing (WGS) technology. The test demonstrates superiority over cytology as a non-invasive method for diagnosis and monitoring of UC, exhibiting notably higher

sensitivity (80.4% vs. 33.9%) while maintaining comparable specificity (94.9% vs. 100.0%) (145). Low-depth WGS has been performed on a cohort of 168 patients diagnosed with urological tumors to assess CNA; machine learning techniques have been used to develop multidimensional algorithmic model UC Detector (Beijing Institute of Genomics & Peking University First Hospital), designed to identify 50 distinct CNA features associated with UC (sensitivity, 86.5%; specificity, 94.7%) (146).

*MSI-based biomarkers*. Microsatellites, also referred to as short tandem repeats, are repetitive DNA sequences ubiquitously present across the human genome. These sequences are characterized by high levels of polymorphic variability (147). MSI phenotype has importance in BCa tumorigenesis and development due to its ability to cause the insertion and deletion of repetitive sequences (148). There have been advancements in analysis of genome-wide MSI status through NGS methods (149). Microsatellite analysis (MSA) not only enables identification of valuable biomarker targets but also facilitates detection of MSI-positive cancerous lesions for guiding immunotherapy in BCa (150). Studies on a cohort of 149 patients with 15 deficient mismatch repair/high MSI (MSI-H) tumors have been performed and the FDA has granted approval for utilization of MSI as a pan-cancer biomarker for guiding pembrolizumab treatment (151,152). MSI-H status is associated with a high mutational load, Lynch syndrome (LS) and a durable response to ICIs in upper tract UC (153). Additionally, MSI typing effectively distinguishes patients with different prognoses, with those exhibiting MSI-H having a more favorable prognosis compared to those with low microsatellite instability (154).

An approach has been developed for detection of MSI using amplicon-based NGS technology instead of tissue biopsy. This approach involves mapping plasma circulating tumor DNA (ctDNA) fragments to the genome (155). Similarly, other groups have examined the association between MSI and clinicopathological features of bladder or renal cell carcinoma, which can be determined by detecting MSI in urine and identifying targeted loss of heterozygosity (LOH) deletions during tumor cell transformation (156,157). MSA can be an effective tool for BCa stratification and evaluate the likelihood of recurrence. This technique demonstrates a notable sensitivity in detecting both low- and high-grade lesions. Specifically, it exhibits sensitivities of 67, 86 and 93% for G1, G2 and G3 lesions, respectively (157). Another study has explored use of MSA assay for detecting recurrent transitional cell carcinoma of the bladder in 21 patients; MSA assay is able to detect recurrence 4-6 months earlier than cystoscopy (158). The sensitivity of MSA in detecting BCa tends to improve with the stage and grade, while the accuracy of results can be affected by complications such as inflammation. Combination of LOH analysis with other BCa diagnostic methods, such as cytology or FISH, has demonstrated enhanced accuracy compared with single assays (159). Integrating LOH and cytological analysis of urine samples, in conjunction with cystoscopy, offers a novel paradigm for monitoring recurrence in urinary tract malignancies. Several clinical trials have confirmed that MSA exhibits greater sensitivity than cytology in detecting and predicting recurrent BCa (160,161). The sensitivity of MSA ranges from 75 to 96%, whereas cytology shows a sensitivity range of 13-50% (162). Moreover, MSA has high diagnostic performance, with an accuracy rate of 83.3% and specificity of 100%, particularly in patients with superficial disease or low-grade tumors (163). The performance is notably superior to other diagnostic tests, such as cytology and BTA tests and UBC tests (148,164).

*cfDNA status and fragmentation patterns*. Concentration of cfDNA in patients is significantly higher compared with that found in a healthy population, particularly in individuals with advanced and progressing disease stages (165). Patients with liver cancer show the highest concentration of cfDNA (46.0±35.6 ng/ml), while those with lung cancer exhibit the lowest concentration (5.23±6.4 ng/ml). The concentrations of cfDNA are significantly correlated with both the type and stage of cancer (166). This makes urinary cfDNA (ucfDNA) an ideal candidate for investigation of urological malignancies, such as BCa and kidney cancer (167). Further studies have demonstrated that the specific molecular characteristics, particularly concentration and integrity of ucfDNA, may serve as potent discriminators differentiating patients with BCa from healthy controls (168,169). DNA originating from normal apoptotic cells is typically highly fragmented, which distinguishes it from the DNA of necrotic cancer cells, which maintains structural integrity. The integrity of ucfDNA is >40-fold higher in patients diagnosed with BCa than healthy controls (170). Investigations have been conducted into variations in concentration of ucfDNA in relation to BCa and specific benign urological conditions (171,172). The total quantity of ucfDNA exhibits a notable discrepancy between pTa group (~40,000 ng for 25 BCa patients) and pT1-pT4 (around 360,000 ng for 40 BCa patients) (165). These findings suggest that ucfDNA could potentially serve as a promising biomarker for distinguishing stages of BCa. In addition, patients with BCa display ucfDNA concentrations >250 ng/ml. By contrast, <40% of healthy individuals with negative cystoscopy results show ucfDNA concentration levels >250 ng/ml (173). The levels of ucfDNA may vary depending on number and size of tumors, grade, stage, presence of urinary tract infection and leukocyturia. ucfDNA demonstrates limited sensitivity in detection of poorly staged and graded BCa, such as those in patients with pTa; therefore, there is a continuing need for the development of standard methods for quantification and amplification (174).

Distribution of ucfDNA fragments does not occur at random, but rather demonstrates a patterned organization. Cells undergoing apoptosis predominantly release ucfDNA that is highly fragmented (185-200 bp). The size distribution of ucfDNA fragments exhibits prominent peaks within the 40-120 bp spectrum, with a mode value of 81 bp (175). Each peak is regularly spaced by 10 bp intervals, suggesting a possible transient defense against total degradation (176). ucfDNA fragments exhibit distinct characteristics compared with those in plasma, including increased degradation and decreased length (175). Despite the high fragmentation observed in ucfDNA, it remains uncertain whether the profile of these fragments accurately mirrors the inherent genomic architecture. Owing to potential interference from cfDNA of non-tumor origin, which may be released into the urine from the systemic circulation, the concentration of ctDNA in urine becomes diluted, resulting in reduced levels of detectable

ctDNA. Long degradation periods may lead to an abundance of short fragments of ucfDNA. ucfDNA fragments >250 bp in length correspond to the sequences of three specific oncogenes: *c-Myc*, human epidermal growth factor receptor 2 and brain enriched myelin associated protein 1 (177). These oncogenes are frequently amplified in various stages of BCa, which include premalignant conditions, primary invasive high-grade and metastatic BCa (178). Therefore, to enhance the capacity and precision of BCa detection using this non-invasive marker, further exploration is warranted to comprehend the precise mechanism and origin of ucfDNA fragments. Evaluation of the relationship between ucfDNA content and tumors, particularly through analysis of the ratio between longer and shorter DNA fragments in conjunction with the concentration at a specific fragment size may hold potential as an effective tool for BCa diagnosis.

Fragmentation pattern of ucfDNA faces several challenges, including a notable proportion of false positives and a detection efficacy inferior to that of its blood-based counterparts (179). Urine exhibits higher activity levels of DNA enzymes compared with blood, thus elevating the probability of cfDNA degradation (180,181). Secondly, the abundance of microorganisms interferes with chemical and physical properties of urine samples, as well as the accuracy of test results (182). Thirdly, enzymatic activities of both deoxyribonuclease I and II demonstrate higher values in urine than in blood (183). This leads to a decreased half-life of the ucfDNA samples, ranging from 2.6 to 5.1 h. Consequently, the ucfDNA exhibits a heightened susceptibility to degradation and extensive fragmentation. These factors impede the extensive application of ucfDNA in BCa detection. At present, morning urine samples are preferred due to advantages including its higher concentration, reduced external interference, decreased risk of contamination, and minimized variation in urine composition for urine-based liquid biopsies (184). To maintain the integrity and stability of ucfDNA at room temperature, EDTA is used (176,180). EDTA serves a pivotal role in preventing the degradation of ucfDNA, thereby enhancing the likelihood of accurate testing outcomes. Additionally, there is a lack of standardized methodologies for both the preservation and analysis of ucfDNA samples.

*Other promising RNA-biomarkers*. MicroRNAs (miRNAs or miRs), a class of small RNA molecule, are characterized by their non-coding nature and size, typically ranging from 18 to 25 bp. Aberrantly expressed microRNAs serve as both oncogenes and tumor suppressors in different types of malignant tumor (185). These miRNAs regulate malignant biological properties of tumors, including progression and metastasis of urinary tumors. Levels of miRNA 15a, miRNA-200a (miR-200a) and miRNA-210-3p (miR-210-3p) in the urine of patients with renal cell carcinoma (RCC) are increased following surgical intervention (186). This is associated with tumor size and suggests the potential utility of these miRNAs as molecular markers for prognostic assessment and therapeutic monitoring of RCC. The downregulation of miR-29b-3p and miR-31 expression is associated with clinicopathological features and survival prognosis of patients with BCa (187,188). In addition, miRNA-10b (miR-10b) is detected in urine at an early stage of BCa, suggesting its potential for early screening and diagnosis. It exhibits a specificity of 91.1% and sensitivity of 80.9%, which is higher than that of urine cytology (specificity, 85%; sensitivity, 75%) (189). A panel comprising miR-141, miR-34b, miR-10b and miR-103 exhibits enhanced diagnostic efficacy for BCa, achieving sensitivity of 75% and a specificity of 63.5% compared to the detection of individual miRNA (190). To evaluate the predictive potential of miRNAs found in urine, a study has been performed to identify differentially expressed miRNAs in bladder tumors through transcriptome sequencing; miRNA-145 (miR-145) and miRNA-182 (miR-182) demonstrate superior diagnostic performance compared with traditional methods such as urine cytology (sensitivity, 44%; specificity, 85%) and cystoscope (sensitivity, 85-90%), achieving sensitivity of 93% and a specificity of 86% (191). Despite these findings, the diagnostic efficacy of individual or various combinations of miRNAs has yet to be fully elucidated.

The role of circulating mRNAs, including mRNA, circular RNA (circRNA) and non-coding RNA as markers in BCa has garnered considerable interest (192-194). The urinary biomarker urothelial cancer associated 1 (UCA1), a BCa-specific long non-coding RNA (lncRNA), is associated with malignant characteristics of bladder tumors (195) and serves a crucial role in facilitating progression of BCa through the regulation of glutamine-driven tricarboxylic acid cycle anaplerosis (196). Meta-analysis indicates that UCA1 exhibits a sensitivity of 84% and a specificity of 87% for detection of BCa (197). Transcriptomic data derived from 700 patient samples treated with ICIs corroborates that a machine learning approach using network-guided biomarkers enhances prediction of overall survival in patients with BCa undergoing ICI therapy (198). Urinary exosomes serve as abundant sources of lncRNAs such as prostate cancer associated transcript 1 (PCAT-1), anti-ribosome protein intergenic lncRNA (ANRIL), muskelin 1-antisense RNA (MKLN1-AS), tumor associated lipid metabolism 1 (TALAM1) and titin-antisense 1 (TTN-AS1) that exhibit notable enrichment and high expression in patients with BCa compared to healthy controls (199). Studies report sensitivity ranging from 43 to 96% and specificity between 48 and 90% (200,201). Additionally, the integrated AUC is 0.87, indicating a strong overall diagnostic performance for BCa. lncRNAs display the highest overall sensitivity, with integrated sensitivity value of 78%. Conversely, miRNAs demonstrate the highest overall specificity, achieving an integrated specificity value of 81% (202,203). lncRNAs such as lymph node metastasis-associated transcript 2 (LNMAT2) and brain cytoplasmic RNA 1 (BCYRN1) are important factors in lymph node (LN) metastasis of BCa and are associated with a poor prognosis (204,205). Furthermore, long intergenic non-protein coding RNA 958 promotes LN metastasis, proliferation, lymphatic vessel formation, distant metastasis and invasion in BCa (206).

Urinary exosome-specific markers, including mRNA kelch domain containing 7B, caspase 14 (CASP14), protease serine1 (PRSS1) and lncRNAs MIR205 host gene (MIR205HG) and growth arrest specific 5 (GAS5), have been found to be associated with BCa stage, grade and degree of hematuria (207). A model based on the combination of these markers shows sensitivity of 88.5% and a specificity of 83.3% for diagnosing BCa at early stage (207). To date, the commercially available mRNA-based urine tests for BCa are CxBladder (Pacific Edge

Ltd.) and Xpert BC (Cepheid, Sunnyvale, CA, USA) tests (208). CxBladder uses reverse transcription-qPCR to detect expression levels of several urinary targets, including insulin like growth factor binding protein 5, homeobox A13 (HOXA13), midkine (MDK), cyclin dependent kinase 1 (CDK1) and C-X-C motif chemokine receptor 2 (CXCR2). This approach is suitable for excluding low-risk BCa with hematuria, diagnosing the urinary tract-related disease and monitoring treatment progress. The sensitivity of the CxBladder test is up to 82% in patients with hematuria (209). XpertBC targets mRNA signatures such as acute basophil leukemia 1, corticotropin releasing hormone (CRH), insulin like growth factor 2 (IGF2), uroplakin 1B (UPK1B) and annexin A10 (ANXA10). It offers simplicity, speed and automated detection, with sensitivity of around 80% (210). Studies have demonstrated that circRNAs are key regulators in BCa and prostate and renal cancers, exerting their effects on proliferation, metastasis, apoptosis, metabolism and drug resistance (211,212). By modulating expression of miRNAs and associated proteins, circRNAs contribute to the complex molecular landscape of cancers. For example, circ102336, circ0008399 and circ0058063 have been found to exert effects on drug resistance through the regulation of miRNAs expression and associated proteins. Specifically, the sensitivity of cisplatin-resistant BCa cells to cisplatin is enhanced by modulating the expression levels and stability of circRNAs, either through promotion or inhibition (194,213). In addition, circRNA lysine demethylase 4C, circRNA itchy E3 ubiquitin protein ligase (circITCH) and circRNA activin A receptor type 2A (circACVR2A) enhance BCa invasion and metastasis via the miRNA-200bc-3p (miR-200bc-3p)/zinc finger e-box binding homeobox 1 (ZEB1), miRNA-17 (miR-17)/miRNA-224 (miR-224) and miRNA-626 (miR-626)/EYA transcriptional coactivator and phosphatase 4 (EYA4) axes (214-216). circRNAs have a distinct advantage in discovery of novel clinical diagnostic biomarkers due to their increased stability compared with linear RNAs. Unlike linear RNAs, circRNAs are resistant to nuclease degradation, rendering them more resilient (217). A multi-omics analysis incorporated mRNA, miRNA, lncRNA and methylation data, utilizing an innovative clustering approach using 10 distinct machine learning methods to develop risk prediction models and identify potential biomarkers; these biomarkers could enhance prognosis and inform immunotherapy strategies for MIBC (218).

*Challenges in clinical translation*. Urological tumors are characterized by a large number of genetic variants in urine, making urine promising as a carrier of genetic material such as DNA (cfDNA and exosome DNA) as well as RNAs (Fig. 1). In recent years, there has been a growing focus on investigating patterns of point mutations, methylation changes, fragmentation and structural variants (219,220). The aforementioned studies support the hypothesis that novel genome type analysis can be used not only as a diagnostic tool, but also for predicting the likelihood of recurrence or progression following treatment in clinical practice. European Association of Urology and FDA-approved methods, such as NMP22, UroVysion, *FGFR3*, and microsatellite assays in comparison to cystoscopy and cytology, demonstrate higher sensitivity but lower specificity, particularly in cases of low-grade, early or recurrent BCa (221). For example, FISH test does not fully meet clinical standards and it has value in specific scenarios including the initial diagnosis of patients experiencing hematuria, recurrence monitoring following treatment and assessment of patients with atypical cellular abnormalities (222). The specificity of most biomarkers is <90%, with some <80%. The PPV of these markers is attributed to their specificity and prevalence. While most markers exhibit sensitivity ranging from 80 to 90% for high-grade disease, the failure to detect 10-20% of high-grade tumors is considered unacceptable.

## 3. Urine and blood biomarkers

The discovery of fetal genetic data in the urine of pregnant patients was the first empirical evidence corroborating the ability of cfDNA to traverse the glomerulus, subsequently becoming a constituent of ucfDNA (223,224). Urine ctDNA can be derived from two primary sources. Firstly, ctDNA is filtered through the glomeruli, facilitating transfer from the bloodstream into the urine. Secondly, ctDNA detected in urine can originate from tumor cells directly shed by the urinary tract system (225). Studies have shown that both urine supernatants and sediments exhibit a notably higher cumulative mutation rate compared with DNA extracted from plasma (181,226,227). The aforementioned findings suggest consistency in the mutational data conveyed by utDNA and ctDNA within ucfDNA. There are notable molecular and genetic differences in ucfDNA that is derived from patients with BCa, which reflect the genomic content of tumor cells. Ability to detect genomic characterization in both blood (serum and plasma) and urine (sediment and supernatant) indicates that liquid biopsy-based biomarkers have potential for diagnosing and monitoring disease conditions, including chronic disease and tumors (228).

Urine may be a superior source of markers compared with blood (229). Patients with NMIBC exhibit a notable elevation in the concentration of cfDNA in both urine and blood samples. However, blood samples have greater efficacy in indicating disease recurrence and metastatic tumors compared with urine samples in patients who have undergone radical cystectomy (230). Certain miRNAs such as miRNA-451a (miR-451a) and miRNA-486-5p (miR-486-5p) are upregulated in extracellular vesicles derived from urine samples in individuals with BCa, while their expression remains unchanged in plasma samples (231). The aforementioned findings indicate that a notable proportion of circulating miRNA is either retained by the kidneys through unidentified mechanisms or degraded in urine. To the best of our knowledge, the exploration of protein markers in the blood of patients with BCa has received less attention than urine-based studies (232). Conversely, markers associated with DNA/RNA-based biomarkers are more frequently investigated in clinical trials. This may be attributed to the challenges in enriching and analyzing proteins (233,234). However, the use of targeting strategies for MS holds promise in enhancing detection of protein markers in blood samples.

Total cfDNA concentration in urine of patients with cancer patients is higher compared with both the general population and patients diagnosed with benign urological conditions (171). This increase is particularly prominent in

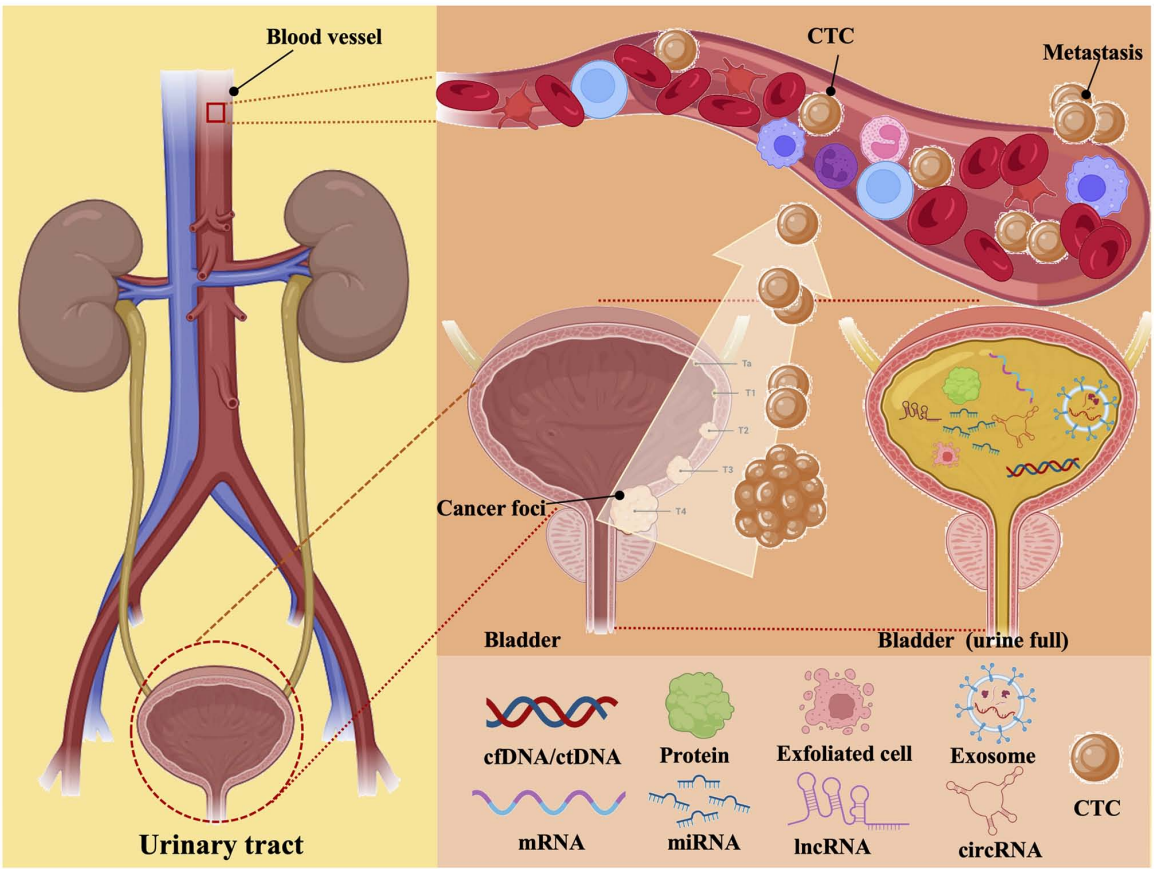


Figure 1. Biomarkers in the urinary tract system. Biomarkers in liquid biopsies occur primarily in urine and blood samples. Urinary biomarkers such as ctDNA, cfRNAs (mRNA, circRNA and non-coding RNA), proteins, exosomes and urinary exfoliated cells have potential for the diagnosis, prognosis and treatment of BCa. In addition, CTCs are released from the primary tumor and enter the circulatory system, partially facilitating metastasis. BCa, bladder cancer; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; CTC, circulating tumor cell; cfRNA, circulating free RNA; miRNA, microRNA; lncRNA, long non-coding RNA; circRNA, circular RNA.

patients with advanced or progressive disease recurrence. Studies on UC show that mutations detected in urine samples are associated with those found in both plasma and tissue samples (235,236). A total of 168 somatic mutations have been identified in 25 cases of UC using targeted sequencing, with approximately half of the mutations are identified in both the urine supernatant and precipitates, whereas 2% of mutations identified in the tumor are observed in the plasma samples (237). Another study reports high concordance between urine cfDNA and tissue DNA detection in patients with UC with a sensitivity of 86.7% and a specificity of 99.3% (238). Consequently, it is advisable to prioritize urine samples over plasma samples for higher detection rates in patients with these conditions. Similar findings are observed in PCa; methylation levels of glutathione s-transferase pi 1, ras association domain family member 2 (*RASSF2*), histone cluster 1 H4 family member K (*HIST1H4K*) and transcription factor AP-2 epsilon (*TFAP2E*) are detected in both urine and plasma samples. The aforementioned urinary methylation biomarkers with AUC ranging from 0.82 to 0.94, exhibit higher sensitivity to PCa compared with plasma DNA (AUC, 0.30-0.53) (239). Evidence contrary to the aforementioned results suggests that plasma DNA is more sensitive to PCa than urine testing (240), whereas additional findings propose there is no obvious difference (241). The sample size and criteria for enrollment may not be sufficient to demonstrate differences in performance between urine and blood samples. Differences in the number and performance of urine and blood biomarkers suggest that not all ctDNA found in both sample types originates from the same tumor. It is more likely that plasma ctDNA originates from a metastatic lesion, while changes in uDNA may indicate a primary lesion in a urological tumor (242). Due to homeostatic mechanisms, changes in blood are rapidly eliminated, only resulting in substantial modifications at the point of decompensation. Overall, the performance of biomarkers in urine and blood tests may be influenced by histological grade, advanced pathological stage, presence of carcinoma *in situ*, surgical procedure, marker types, individual differences in background signal and identification of cancer characteristics (243). Blood and urine markers may serve as both complementary and independent assays, offering distinct advantages and disadvantages depending on clinical application scenario (Table II).

## 4. Discussion

BCa imaging techniques such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI), along with urine cytology, have emerged as non-invasive alternatives

Table II. Differences between urinary and blood biomarkers in BCa.

| Aspect | Urine | Blood |
|---|---|---|
| Biological characteristics | Unstable; more precise reflection of early biomarker changes *in vivo*; higher levels of specific biomarkers (such as small molecules); easily detected; may contain non-specific biomarkers | More stable; early biomarker changes may be removed; low impact of microorganisms |
| Collection | Closer contact with bladder tumor; higher concentrations of specific biomarkers; easy to collect; low invasiveness of collection; high volume; suitable for continuous high sampling at home; patient acceptance | Contact with most tumors; specialized operation; limited quantity; commonly used for routine testing |
| Tumor signals | DNA (genetic, epigenetic); cfDNA; tumor cells shed from the urinary tract (≥1 kb); ctDNA (10-250 bp), low content; exfoliated cells; sediments; large variations in protein composition, abundance, stored for a long time; cfRNA (mRNA, miRNA, lncRNA); extracellular vesicles (e.g., exosomes) | DNA (genetic, epigenetic); plasma/serum cfDNA (150-200 bp); cfDNA (133-160 bp); low ratio of ctDNA to cfDNA; susceptible to dilution by DNA from non-tumor sources; CTC; cfRNA (mRNA, miRNA, lncRNA); proteins or peptide metabolites undergo metabolic processes in the bloodstream at varying rates; rate of metabolism influences the duration for which these metabolites remain present in the circulatory system |
| Advantages | ctDNA may have more genetic and epigenetic changes; cytology is suitable for high-grade tumor detection; sensitivity >90%; urine (sediment cell, mRNA, protein) is used in most current commercial BCa diagnostic and monitoring kits, with higher sensitivity and PPV; DNA/RNA and protein may have higher performance due to BCa associated organs; well-validated, reproducible technologies; non-invasive and applications for early detection and longitudinal monitoring | ctDNA of plasma/serum samples can be stored for a longer period; higher available concentrations; CTC biomarkers with high specificity; predict earlier relapse or metastasis following primary treatment; well-validated, reproducible technologies; more stable |
| Limitations | High levels of nuclease in urine and cfDNA is easily degraded; interference of genomic DNA affects cfDNA concentration; contamination with high levels of pathogenic microorganisms affects the accuracy; instability and low abundance of cfRNA | DNA/RNA contamination background of blood cell in plasma in genitourinary tract; low volume of CTCs in peripheral blood; detection may not be accurate for initial screening; ctDNA source does not fully reflect the biology of the primary tumor or BCa metastasis; amount of ctDNA detected in plasma is small or may not perform as well; human-derived proteins are complex and challenging to analyze and detect; heterogeneity and number of exosomes may result in false negatives or positives |
| Sensitivity/ specificity | Some biomarkers may be more sensitive for early detection of BCa; available biomarkers for low-risk BCA are relatively inadequate, have low specificity and are more accurate at initial diagnosis than at follow-up; sensitivity and specificity are insufficient for clinical use; limited availability of specific markers; specific biomarkers with high sensitivity or specificity; non-invasive BCa assays have false positive rates; FDA/CE-approved methods show low specificity and limited clinical utility; apply to standardize the detection of urinary tract cancers and to expand application to diseases such as diabetes, kidney disease, bladder infections | Superior for MIBC follow-up and metastatic tumors; low detection rate of early BCa; specificity and sensitivity require further improvement; exosomes and cfRNA lack standardized isolation methods and reproducible and practical application in the clinic |

Table II. Continued.

| Aspect | Urine | Blood |
|---|---|---|
| Clinical trials | Diagnosis, surveillance and prognosis; surveillance in adjunct to cystoscopy; require further validation and research for clinical practice | Diagnosis, surveillance and prognosis; establish performance for clinical application |

BCa, bladder cancer; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; CTC, circulating tumor cell; miRNA, microRNA; lncRNA, long non-coding RNA; cfRNA, circulating free RNA; FDA, Food and Drug Administration; CE, Conformity with European; MIBC, muscle-invasive bladder cancer.

to cystoscopy. However, there are limitations associated with these procedures. For example, ultrasound exhibits relatively low sensitivity and specificity, while CT and MRI have limited capability in detecting small and flat lesions (244,245). Similarly, urine cytology exhibits a high specificity but is accompanied by a low sensitivity in its diagnostic capabilities. Furthermore, low-grade malignant BCa often presents a high leakage rate, which can contribute to false positive results in patients experiencing severe hematuria and urinary tract infection (22). With technological advancements, liquid biopsy utilizing urine is anticipated to emerge as a dependable, precise and non-intrusive biomarker for diagnosis and monitoring of BCa, offering improved personalized diagnosis and treatment alternatives for patients.

Over the past decade, research has primarily focused on development of non-invasive biomarkers for BCa derived from urine. These biomarkers originate from both urine supernatants and sediment, and include tumor exfoliated cells, genetic material (DNA and RNA), proteins, extracellular vesicles/exosomes, as well as novel available markers currently being researched (Fig. 2) (246). Diagnostic tests NMP22, BTA, ImmunoCyt, and UroVysion are used as adjuncts to cystoscopy because they offer greater sensitivity than urine cytology. However, these tests frequently lack of specificity and are susceptible to false positive results, particularly in the context of benign urological conditions (83). High-throughput NGS technology has enhanced the identification of urinary biomarkers originating from cancer cells. Consequently, assays such as Cxbladder, XpertBC, UriFind, and Uromonitor have been developed (208,247). This multi-target approach has improved detection sensitivity and dynamic monitoring of tumor progression or recurrence; however, the scope of these assays remains inadequate to address the vast genomic heterogeneity of NMIBC. The accuracy of clinical diagnosis and surveillance is affected by potential genomic differences between primary and residual lesions, as well as by false positives for somatic mutations in cfDNA present in normal and benign patient cells. While biomarker tests show promise, they should be used in combination with cystoscopy and imaging.

Patients diagnosed with high-risk NMIBC are advised to undergo cystoscopy every 3 months and Bacillus Calmette-Guérin (BCG) is the standard initial treatment. Despite the stringent surveillance protocol, ~50% of patients experience recurrence, which results in a more unfavorable prognosis (248). The implementation of biomarker testing alongside cystoscopy may support a decrease in the frequency of surveillance cycles for cystoscopy. Urinary biomarkers have potential as a diagnostic tool to rule out patients with hematuria, enhance the diagnostic capabilities of cystoscopy for patients with positive results, monitor post-treatment and assess the risk of postoperative recurrence or progression following initial intravesical therapy, such as BCG. These assessments guide the necessity for a second line of treatment or radical surgery. However, the current role of urinary biomarkers is limited and requires additional support from prospective trials. The diagnostic accuracy is influenced by the acceptable threshold for a positive result and various sample processing methods. Further research is needed to standardize sample collection (whole urine, supernatant, pellet or extracellular vesicles) and processing protocols (centrifugation techniques and selection of reference genes), as well as validation of biomarker performance in clinical practice.

The advent of high-throughput technologies, such as NGS and MS, has accelerated the discovery of biomarkers (74,249,250). Use of these methodologies is crucial in screening and identifying potential biomarkers by employing large dataset analyses and machine learning algorithms. These biomarkers, when coupled with bioinformatics and AI (machine and deep learning) techniques, have been effectively validated for the screening of potential drug targets (251). Likewise, bioinformatic analytical approaches are key tools for the detection of highly sensitive and disease-specific biomarkers, facilitating early diagnosis and treatment of BCa (252).

In conclusion, liquid biopsy using urine holds promise in BCa. Future research is expected to explore new biomarkers and targets, using urine as a carrier and employing AI techniques for marker analysis and data processing. For example, application of molecular optical probes is intended to produce synthetic biomarkers. This process involves the interaction of these probes with disease biomarkers to produce artificial urinary biomarkers. These artificial urine biomarker probes (AUBPs) are designed to address limitations associated with the diagnostic performance of endogenous markers. The development of AUBPs has the potential to broaden the spectrum of biomarkers and diseases detectable through urinalysis (253,254), facilitate signal amplification and enable cost-effective follow-up monitoring. In conjunction with the advancement of smart toilets, development of portable urine biomarkers could potentially

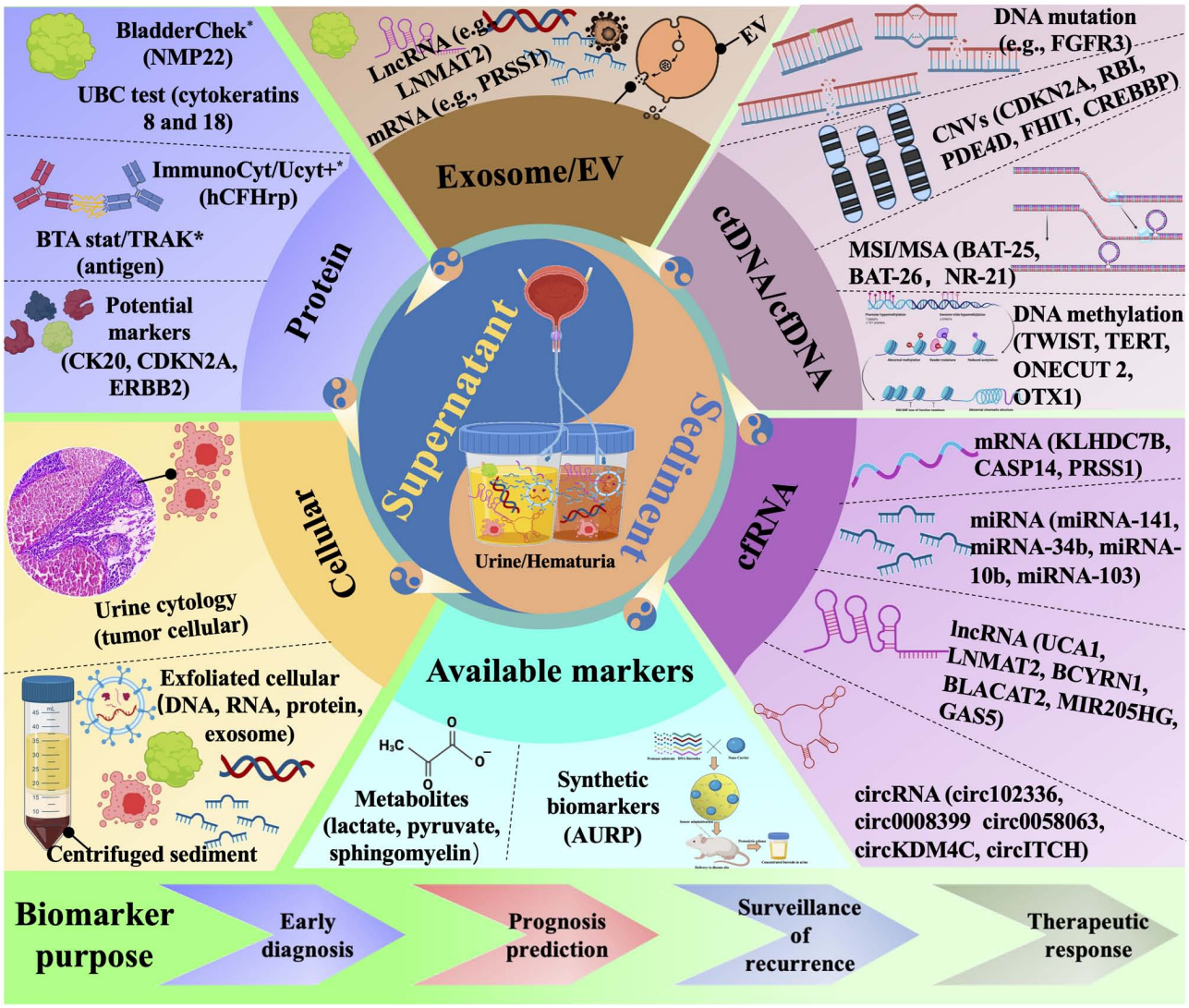


Figure 2. Urinary biomarkers for BCa diagnosis, prognosis and surveillance. Non-invasive biomarkers for BCa primarily originate from urine supernatant and sediment, encompassing tumor exfoliated cells, genetic material (DNA and RNA), protein and EVs/exosomes. Biomarkers serve an important role in the process of early diagnosis, prognosis prediction, recurrence monitoring and response to treatment in BCa. *Food and Drug Administration-approved. BCa, bladder cancer; NMP22, nuclear matrix protein 22; BTA, bladder tumor antigen; hCFHrp, human complement factor H-related protein; lncRNA, long non-coding RNA; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; cfRNA, circulating free RNA; EV, extracellular vesicle; MSI, microsatellite instability; CNV, copy number variation; CNA, copy number alteration; MSA, microsatellite analysis; circRNA, circular RNA; AURP, artificial urine biomarker probe; UBC, urinary bladder cancer; miR, miRNA; KLHDC7B, kelch domain containing 7B; CASP14, caspase 14; PRSS1, protease serine 1; TERT, telomerase reverse transcriptase; ONECUT 2, one cut homeobox 2; OTX1, orthodenticle homeobox 1; BAT, bethesda tutorial; NR, non-repetitive; RBI, retinoblastoma inhibitory gene; PDE4D, phosphodiesterase 4D; FHIT, fragile histidine triad diadenosine triphosphatase; FGFR3, fibroblast growth factor receptor 3; UCA1, urothelial cancer associated 1; LNMAT2, lymph node metastasis-associated transcript 2; BCYRN1, brain cytoplasmic RNA 1; MIR205HG, MIR205 host gene; GAS5, growth arrest specific 5; CDKN2A, cyclin dependent kinase inhibitor 2A; ERBB2, erb-B2 receptor tyrosine kinase 2.

become a pivotal aspect of future BCa monitoring and treatment. A portable analytical system employing biosensors for real-time monitoring of urine has been developed, offering both user-friendly operation and suitability for home testing; this system exhibits a diagnostic sensitivity of 100% and specificity of 92% (255). Synthetic biomarker and urine metabolite analysis have the capability to facilitate non-invasive disease detection and monitoring in urine, offering high sensitivity and ability to conduct multiplexing testing (256,257). The integration of multidisciplinary approaches to diagnostic and follow-up protocols, as well as clinical risk stratification, emphasizes the potential role of these technologies for early detection, accurate staging and targeted therapy for BCa.

**Acknowledgements**

Not applicable.

**Funding**

The present study was supported by Research Project on the Industrial Application of Basic Research on Synthetic Biological Devices to Intervene in Bladder Cancer and Shenzhen Academician Expert Workstation (grant no. CJGJZD20200617102403009), Development of Quantitative Gene Mutation Detection Technology for Targeted Tumor Drugs (grant no. JSGG20201103153801005) and Liquid Biopsy-Based Molecular Diagnostic Product

Development Project for Real-Time Tumor Detection (grant no. 2023B1111040002).

**Availability of data and materials**

Not applicable.

**Authors' contributions**

XW and DW conceived the study and wrote the manuscript. XZ constructed figures and tables. MX and YH constructed figures and tables. WQ and SC conceived the study and revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Richters A, Aben KKH and Kiemeney LALM: The global burden of urinary bladder cancer: An update. World J Urol 38: 1895-1904, 2020.
3. Tan WS, Rodney S, Lamb B, Feneley M and Kelly J: Management of non-muscle invasive bladder cancer: A comprehensive analysis of guidelines from the United States, Europe and Asia. Cancer Treat Rev 47: 22-31, 2016.
4. Teoh JY, Kamat AM, Black PC, Grivas P, Shariat SF and Babjuk M: Recurrence mechanisms of non-muscle-invasive bladder cancer-a clinical perspective. Nat Rev Urol 19: 280-294, 2022.
5. Facchini G, Cavaliere C, Romis L, Mordente S, Facchini S, Iovane G, Capasso M, D'Errico D, Liguori C, Formato R, *et al*: Advanced/metastatic bladder cancer: Current status and future directions. Eur Rev Med Pharmacol Sci 24: 11536-11552, 2020.
6. Devlies W, de Jong JJ, Hofmann F, Bruins HM, Zuiverloon TCM, Smith EJ, Yuan Y, van Rhijn BWG, Mostafid H, Santesso N, *et al*: The diagnostic accuracy of cystoscopy for detecting bladder cancer in adults presenting with haematuria: A systematic review from the European Association of Urology Guidelines Office. Eur Urol 10: 115-122, 2024.
7. Zuiverloon TCM, de Jong FC and Theodorescu D: Clinical decision making in surveillance of non-muscle-invasive bladder cancer: The evolving roles of urinary cytology and molecular markers. Oncology (Williston Park) 31: 855-862, 2017.
8. Chamie K, Litwin MS, Bassett JC, Daskivich TJ, Lai J, Hanley JM, Konety BR and Saigal CS; Urologic Diseases in America Project: Recurrence of high-risk bladder cancer: A population-based analysis. Cancer 119: 3219-3227, 2013.
9. Rezaee ME, Lynch KE, Li Z, MacKenzie TA, Seigne JD, Robertson DJ, Sirovich B, Goodney PP and Schroeck FR: The impact of low-versus high-intensity surveillance cystoscopy on surgical care and cancer outcomes in patients with high-risk non-muscle-invasive bladder cancer (NMIBC). PLoS One 15: e0230417, 2020.
10. Burke DM, Shackley DC and O'Reilly PH: The community-based morbidity of flexible cystoscopy. BJU Int 89: 347-349, 2002.
11. Van Der Aa MN, Steyerberg EW, Sen EF, Zwarthoff EC, Kirkels WJ, van der Kwast TH and Essink-Bot ML: Patients' perceived burden of cystoscopic and urinary surveillance of bladder cancer: A randomized comparison. BJU Int 101: 1106-1110, 2008.
12. Matulewicz RS, DeLancey JO and Meeks JJ: Cystoscopy. JAMA 317: 1187, 2017.
13. Talukdar S, Emdad L, Das SK, Sarkar D and Fisher PB: Noninvasive approaches for detecting and monitoring bladder cancer. Expert Rev Anticancer Ther 15: 283-294, 2015.
14. Yaxley JP: Urinary tract cancers: An overview for general practice. J Family Med Prim Care 5: 533-538, 2016.
15. Zhang X and Zhang Y: Bladder cancer and genetic mutations. Cell Biochem Biophys 73: 65-69, 2015.
16. Thompson D, Lawrentschuk N and Bolton D: New approaches to targeting epigenetic regulation in bladder cancer. Cancers (Basel) 15: 1856, 2023.
17. Eissa S, Swellam M, Sadek M, Mourad MS, EI Ahmady O and Khalifa A: Comparative evaluation of the nuclear matrix protein, fibronectin, urinary bladder cancer antigen and voided urine cytology in the detection of bladder tumors. J Urol 168: 465-469, 2002.
18. Sale MS, Kulkarni VV, Kulkarni PV and Patil CA: Efficacy of modified cell block cytology compared to fine needle aspiration cytology for diagnostic oral cytopathology. Biotech Histochem 96: 197-201, 2021.
19. Raskin RE and Meyer D (eds): General Categories of Cytologic Interpretation. Canine and Feline Cytology. A Color Atlas and Interpretation Guide. 2nd edition. Elsevier, Amsterdam, pp16-33, 2009.
20. Sullivan PS, Chan JB, Levin MR and Rao J: Urine cytology and adjunct markers for detection and surveillance of bladder cancer. Am J Transl Res 2: 412-440, 2010.
21. Xing J and Reynolds JP: Diagnostic advances in urine cytology. Surg Pathol Clin 11: 601-610, 2018.
22. Feiertag N, Barry E, Abramson M, Park JY, Kovac E, Aboumohamed A, Watts K and Sankin A: Urine cytology rarely escalates clinical management in the surveillance of non-muscle-invasive bladder cancer. Clin Genitourin Cancer 21: 258-264, 2023.
23. Lozano F, Raventós CX, Carrion A, Dinarés C, Hernández J, Trilla E and Morote J: Xpert bladder cancer monitor for the early detection of non-muscle invasive bladder cancer recurrences: Could cystoscopy be substituted? Cancers (Basel) 15: 3683, 2023.
24. Palou J, Rodríguez-Rubio F, Huguet J, Segarra J, Ribal MJ, Alcaraz A and Villavicencio H: Multivariate analysis of clinical parameters of synchronous primary superficial bladder cancer and upper urinary tract tumor. J Urol 174: 859-861; discussion 861, 2005.
25. Raitanen MP, Aine R, Rintala E, Kallio J, Rajala P, Juusela H and Tammela TL; FinnBladder Group: Differences between local and review urinary cytology in diagnosis of bladder cancer. An interobserver multicenter analysis. Eur Urol 41: 284-289, 2002.
26. Oliveira MC, Caires HR, Oliveira MJ, Fraga A, Vasconcelos MH and Ribeiro R: Urinary biomarkers in bladder cancer: Where do we stand and potential role of extracellular vesicles. Cancers (Basel) 12: 1400, 2020.
27. Kundal VK, Pandith AA, Hamid A, Shah A, Kundal R and Wani SM: Role of NMP22 bladder check test in early detection of bladder cancer with recurrence. Asian Pacific J Cancer Prev 11: 1279-1282, 2010.
28. Soloway MS, Briggman JV, Carpinito GA, Chodak GW, Church PA, Lamm DL, Lange P, Messing E, Pasciak RM, Reservitz GB, *et al*: Use of a new tumor marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urol 156: 363-367, 1996.
29. Miyake M, Goodison S, Giacoia EG, Rizwani W, Ross S and Rosser CJ: Influencing factors on the NMP-22 urine assay: An experimental model. BMC Urol 12: 23, 2012.
30. Liang Q, Zhang G, Li W, Wang J and Sheng S: Comparison of the diagnostic performance of fluorescence in situ hybridization (Fish), nuclear matrix protein 22 (nmp22), and their combination model in bladder carcinoma detection: A systematic review and meta-analysis. Onco Targets Ther 12: 349-358, 2019.
31. Dong Y, Zhang T, Li X, Yu F, Yu H and Shao S: Urine biomarkers for the diagnosis of bladder cancer: A network meta-analysis. Urol J 18: 623-632, 2021.

32. Gong YW, Wang YR, Fan GR, Niu Q, Zhao YL, Wang H, Svatek R, Rodriguez R and Wang ZP: Diagnostic and prognostic role of BTA, NMP22, survivin and cytology in urothelial carcinoma. Transl Cancer Res 10: 3192-3205, 2021.
33. Egawa S and Kuruma H: Search for biomarkers of aggressiveness in bladder cancer. Eur Urol 50: 20-22, 2006.
34. Tang X, Cao Y, Liu J, Wang S, Yang Y and Du P: The diagnostic and prognostic value of nuclear matrix protein 22 in bladder cancer. Transl Cancer Res 9: 7174-7182, 2020.
35. Frantzi M, Makridakis M and Vlahou A: Biomarkers for bladder cancer aggressiveness. Curr Opin Urol 22: 390-396, 2012.
36. Białek Ł, Bilski K, Dobruch J, Krajewski W, Szydełko T, Kryst P and Poletajew S: Non-invasive biomarkers in the diagnosis of upper urinary tract urothelial carcinoma-A systematic review. Cancers (Basel) 14: 1520, 2022.
37. Ecke TH, Scislowski M, Hassan N, Saura M, Hallmann S, Koch S, Vuolle S, Malakoutikhah M, Kopra K and Härmä H: Luminophore chemistry for detection of urinary bladder cancer-comparison to cytology and urinary rapid tests (BTA stat®, NMP22® BladderChek® and UBC® Rapid Test). Anticancer Res 42: 5249-5256, 2022.
38. Kumar A, Kumar R and Gupta NP: Comparison of NMP22 BladderChek test and urine cytology for the detection of recurrent bladder cancer. Jpn J Clin Oncol 36: 172-175, 2006.
39. Sajid MT, Zafar MR, Ahmad H, Ullah S, Mirza ZI and Shahzad K: Diagnostic accuracy of NMP 22 and urine cytology for detection of transitional cell carcinoma urinary bladder taking cystoscopy as gold standard. Pak J Med Sci 36: 705-710, 2020.
40. Sarosdy MF, Hudson MA, Ellis WJ, Soloway MS, deVere White R, Sheinfeld J, Jarowenko MV, Schellhammer PF, Schervish EW, Patel JV, *et al*: Improved detection of recurrent bladder cancer using the bard BTA stat test. Urology 50: 349-353, 1997.
41. Thomas L, Leyh H, Marberger M, Bombardieri E, Bassi P, Pagano F, Pansadoro V, Sternberg CN, Boccon-Gibod L, Ravery V, *et al*: Multicenter trial of the quantitative BTA TRAK assay in the detection of bladder cancer. Clin Chem 45: 472-477, 1999.
42. Heicappell R, Wettig IC, Schostak M, Muller M, Steiner U, Sauter T and Miller K: Quantitative detection of human complement factor H-related protein in transitional cell carcinoma of the urinary bladder. Eur Urol 35: 81-87, 1999.
43. Raitanen MP, Marttila T, Kaasinen E, Rintala E, Aine R and Tammela TL: Sensitivity of human complement factor H related protein (BTA stat) test and voided urine cytology in the diagnosis of bladder cancer. J Urol 163: 1689-1692, 2000.
44. Heicappell R, Müller M, Fimmers R and Miller K: Qualitative determination of urinary human complement factor H-related protein (hcfHrp) in patients with bladder cancer, healthy controls, and patients with benign urologic disease. Urol Int 65: 181-184, 2000.
45. Raitanen MP, Marttila T, Nurmi M, Ala-Opas M, Nieminen P, Aine R and Tammela TL; Finnbladder Group: Human complement factor H related protein test for monitoring bladder cancer. J Urol 165: 374-377, 2001.
46. Miyake M, Goodison S, Rizwani W, Ross S, Bart Grossman H and Rosser CJ: Urinary BTA: Indicator of bladder cancer or of hematuria. World J Urol 30: 869-873, 2012.
47. Ecke TH, Arndt C, Stephan C, Hallmann S, Lux O, Otto T, Ruttloff J and Gerullis H: Preliminary results of a multicentre study of the UBC rapid test for detection of urinary bladder cancer. Anticancer Res 35: 2651-2655, 2015.
48. Reid-Nicholson MD, Ramalingam P, Adeagbo B, Cheng N, Peiper SC and Terris MK: The use of Urovysion fluorescence in situ hybridization in the diagnosis and surveillance of non-urothelial carcinoma of the bladder. Mod Pathol 22: 119-127, 2009.
49. Dalquen P, Kleiber B, Grilli B, Herzog M, Bubendorf L and Oberholzer M: DNA image cytometry and fluorescence in situ hybridization for noninvasive detection of urothelial tumors in voided urine. Cancer 96: 374-379, 2002.
50. Zhou H, Yan Y and Song L: The clinical application of fluorescence in situ hybridization in diagnosing urothelial carcinoma. Clin Lab 62: 2001-2009, 2016.
51. Ainthachot S, Sa-ngiamwibool P, Thanee M, Watcharadetwittaya S, Chamgramol Y, Pairojkul C and Deenonpoe R: Chromosomal aberrations, visualized using UroVysion® fluorescence in-situ hybridization assay, can predict poor prognosis in formalin-fixed paraffin-embedded tissues of cholangiocarcinoma patients. Hum Pathol 126: 31-44, 2022.
52. Stadler WM and Olopade OI: The 9p21 region in bladder cancer cell lines: Large homozygous deletions inactivate the CDKN2, CDKN2B and MTAP genes. Urol Res 24: 239-244, 1996.
53. Cairns P, Tokino K, Eby Y and Sidransky2 D: Homozygous deletions of 9p21 in primary human bladder tumors detected by comparative multiplex polymerase chain reaction. Cancer Res 54: 1422-1424, 1994.
54. Moonen PM, Merkx GF, Peelen P, Karthaus HF, Smeets DF and Witjes JA: UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. Eur Urol 51: 1275-1280, 2007.
55. Pycha S, Trenti E, Mian C, Schwienbacher C, Hanspeter E, Palermo M, Pycha A, Danuser H and D'Elia C: Diagnostic value of Xpert® BC detection, bladder epicheck®, Urovysion® FISH and cytology in the detection of upper urinary tract urothelial carcinoma. World J Urol 41: 1323-1328, 2023.
56. Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC, Cheville JC, Sebo TJ, Ramakumar S, Stewart CS, *et al*: A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol 164: 1768-1775, 2000.
57. Halling KC and Kipp BR: Bladder cancer detection using FISH (UroVysion assay). Adv Anat Pathol 15: 279-286, 2008.
58. Laudadio J, Keane TE, Reeves HM, Savage SJ, Hoda RS, Lage JM and Wolff DJ: Fluorescence in situ hybridization for detecting transitional cell carcinoma: Implications for clinical practice. BJU Int 96: 1280-1285, 2005.
59. Youssef RF, Schlomer BJ, Ho R, Sagalowsky AI, Ashfaq R and Lotan Y: Role of fluorescence in situ hybridization in bladder cancer surveillance of patients with negative cytology. Urol Oncol 30: 273-277, 2012.
60. Messing EM, Teot L, Korman H, Underhill E, Barker E, Stork B, Qian J and Bostwick DG: Performance of urine test in patients monitored for recurrence of bladder cancer: A multicenter study in the United States. J Urol 174: 1238-1241, 2005.
61. Fradet Y and Lockhard C: Performance characteristics of a new monoclonal antibody test for bladder cancer: ImmunoCyt trade mark. Can J Urol 4: 400-405, 1997.
62. Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S and Kassouf W: Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urol Oncol 33: 66.e25-e31, 2015.
63. He H, Han C, Hao L and Zang G: ImmunoCyt test compared to cytology in the diagnosis of bladder cancer: A meta-analysis. Oncol Lett 12: 83-88, 2016.
64. Kong T, Qu Y, Zhao T, Niu Z, Lv X, Wang Y, Ding Q, Wei P, Fu J, Wang L, *et al*: Identification of novel protein biomarkers from the blood and urine for the early diagnosis of bladder cancer via proximity extension analysis. J Transl Med 22: 314, 2024.
65. Têtu B, Tiguert R, Harel F and Fradet Y: ImmunoCyt/uCyt+™ improves the sensitivity of urine cytology in patients followed for urothelial carcinoma. Mod Pathol 18: 83-89, 2005.
66. Schmitz-Dräger BJ, Beiche B, Tirsar LA, Schmitz-Dräger C, Bismarck E and Ebert T: Immunocytology in the assessment of patients with asymptomatic microhaematuria. Eur Urol 51: 1582-1588, 2007.
67. Alberice JV, Amaral AF, Armitage EG, Lorente JA, Algaba F, Carrilho E, Márquez M, García A, Malats N and Barbas C: Searching for urine biomarkers of bladder cancer recurrence using a liquid chromatography-mass spectrometry and capillary electrophoresis-mass spectrometry metabolomics approach. J Chromatogr A 1318: 163-170, 2013.
68. Gómez BB, López-Cortés R, Casas-Nebra FJ, Vázquez-Estévez S, Pérez-Fentes D, Chantada-Vázquez MDP, Bravo SB and Núñez C: Detection of circulating serum protein biomarkers of non-muscle invasive bladder cancer after protein corona-silver nanoparticles analysis by SWATH-MS. Nanomaterials (Basel) 11: 2384, 2021.
69. Schwamborn K, Krieg RC, Grosse J, Reulen N, Weiskirchen R, Knuechel R, Jakse G and Henkel C: Serum proteomic profiling in patients with bladder cancer. Eur Urol 56: 989-996, 2009.
70. Nedjadi T, Benabdelkamal H, Albarakati N, Masood A, Al-Sayyad A, Alfadda AA, Alanazi IO, Al-Ammari A and Al-Maghrabi J: Circulating proteomic signature for detection of biomarkers in bladder cancer patients. Sci Rep 10: 10999, 2020.
71. Thomas S, Hao L, Ricke WA and Li L: Biomarker discovery in mass spectrometry-based urinary proteomics. Proteomics Clin Appl 10: 358-370, 2016.
72. Ahn JH, Kang CK, Kim EM, Kim AR and Kim A: Proteomics for early detection of non-muscle-invasive bladder cancer: Clinically useful urine protein biomarkers. Life (Basel) 12: 395, 2022.

73. Wang YT, Shi T, Srivastava S, Kagan J, Liu T and Rodland KD: Proteomic analysis of exosomes for discovery of protein biomarkers for prostate and bladder cancer. Cancers (Basel) 12: 2335, 2020.
74. Ren R, Wang H, Xie L, Muthupandian S and Yang X: Identify potential urine biomarkers for bladder cancer prognosis using NGS data analysis and experimental validation. Appl Biochem Biotechnol 195: 2947-2964, 2023.
75. Jazayeri MH, Aghaie T, Nedaeinia R, Manian M and Nickho H: Rapid noninvasive detection of bladder cancer using survivin antibody-conjugated gold nanoparticles (GNPs) based on localized surface plasmon resonance (LSPR). Cancer Immunol Immunother 69: 1833-1840, 2020.
76. Kumar B, Koul S, Petersen J, Khandrika L, Hwa JS, Meacham RB, Wilson S and Koul HK: p38 Mitogen-activated protein kinase-driven MAPKAPK2 regulates invasion of bladder cancer by modulation of MMP-2 and MMP-9 activity. Cancer Res 70: 832-841, 2010.
77. Koshkin VS, Boyiddle C, Schwartz N, Yu J, Yu KS, Kang A, Bloudek L, Fang Q, Schafer JM, Baker AF, *et al*: Systematic literature review and testing of HER2 status in urothelial carcinoma (UC). J Clin Oncol 41: 556-556, 2023.
78. Zhang HH, Qi F, Zu XB, Cao YH, Miao JG, Xu L and Qi L: A proteomic study of potential VEGF-C-associated proteins in bladder cancer T24 cells. Med Sci Monit 18: BR441-BR449, 2012.
79. Zhang Z, Xu H, Ji J, Shi X, Lyu J, Zhu Y, Yu H and Wang F: Heterogeneity of PTEN and PPAR-γ in cancer and their prognostic application to bladder cancer. Exp Ther Med 18: 3177-3183, 2019.
80. Chen Z, Guo Y, Zhao D, Zou Q, Yu F, Zhang L and Xu L: Comprehensive analysis revealed that CDKN2A is a biomarker for immune infiltrates in multiple cancers. Front Cell Dev Biol 9: 808208, 2021.
81. Mi Y, Zhao Y, Shi F, Zhang M, Wang C and Liu X: Diagnostic accuracy of urine cytokeratin 20 for bladder cancer: A meta-analysis. Asia Pac J Clin Oncol 15: e11-e19, 2019.
82. Chen CJ, Chou CY, Shu KH, Chen HC, Wang MC, Chang CC, Hsu BG, Wu MS, Yang YL, Liao WL, *et al*: Discovery of novel protein biomarkers in urine for diagnosis of urothelial cancer using iTRAQ proteomics. J Proteome Res 20: 2953-2963, 2021.
83. Tsai CH, Chen YT, Chang YH, Hsueh C, Liu CY, Chang YS, Chen CL and Yu JS: Systematic verification of bladder cancer-associated tissue protein biomarker candidates in clinical urine specimens. Oncotarget 9: 30731-30747, 2018.
84. Allione A, Pardini B, Viberti C, Giribaldi G, Turini S, Di Gaetano C, Guarrera S, Cordero F, Oderda M, Allasia M, *et al*: MMP23B expression and protein levels in blood and urine are associated with bladder cancer. Carcinogenesis 39: 1254-1263, 2018.
85. Gobin E, Bagwell K, Wagner J, Mysona D, Sandirasegarane S, Smith N, Bai S, Sharma A, Schleifer R and She JX: A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. BMC Cancer 19: 581, 2019.
86. Lokeshwar VB, Schroeder GL, Selzer MG, Hautmann SH, Posey JT, Duncan RC, Watson R, Rose L, Markowitz S and Soloway MS: Bladder tumor markers for monitoring recurrence and screening comparison of hyaluronic acid-hyaluronidase and BTA-stat tests. Cancer 95: 61-72, 2002.
87. Lokeshwar VB, Habuchi T, Grossman HB, Murphy WM, Hautmann SH, Hemstreet GP III, Bono AV, Getzenberg RH, Goebell P, Schmitz-Dräger BJ, *et al*: Bladder tumor markers beyond cytology: International consensus panel on bladder tumor markers. Urology 66 (Suppl 1): S35-S63, 2005.
88. Friedrich MG, Hellstern A, Toma MI, Hammerer P and Huland H: Are false-positive urine markers for the detection of bladder carcinoma really wrong or do they predict tumor recurrence? Eur Urol 43: 146-151, 2003.
89. Todenhöfer T, Hennenlotter J, Khs U, Tews V, Gakis G, Aufderklamm S, Stenzl A and Schwentner C: Influence of urinary tract instrumentation and inflammation on the performance of urine markers for the detection of bladder cancer. Urology 79: 620-624, 2012.
90. Li A, Zhang T, Zheng M, Liu Y and Chen Z: Exosomal proteins as potential markers of tumor diagnosis. J Hematol Oncol 10: 175, 2017.
91. Song JB, Morrissey JJ, Mobley JM, Figenshau KG, Vetter JM, Bhayani SB, Kharasch ED and Figenshau RS: Urinary aquaporin 1 and perilipin 2: Can these novel markers accurately characterize small renal masses and help guide patient management? Int J Urol 26: 260-265, 2019.
92. Benderska-Söder N, Hovanec J, Pesch B, Goebell PJ, Roghmann F, Noldus J, Rabinovich J, Wichert K, Gleichenhagen J, Käfferlein HU, *et al*: Toward noninvasive follow-up of low-risk bladder cancer-Rationale and concept of the UroFollow trial. Urol Oncol 38: 886-895, 2020.
93. Aitekenov S, Gaipov A and Bukasov R: Review: Detection and quantification of proteins in human urine. Talanta 223: 121718, 2021.
94. Goecks J, Jalili V, Heiser LM and Gray JW: How machine learning will transform biomedicine. Cell 181: 92-101, 2020.
95. Li Y, Sun L, Guo X, Mo N, Zhang J and Li C: Frontiers in bladder cancer genomic research. Front Oncol 11: 670729, 2021.
96. Sarhadi VK and Armengol G: Molecular biomarkers in cancer. Biomolecules 12: 1021, 2022.
97. Shi R, Wang X, Wu Y, Xu B, Zhao T, Trapp C, Wang X, Unger K, Zhou C, Lu S, *et al*: APOBEC-mediated mutagenesis is a favorable predictor of prognosis and immunotherapy for bladder cancer patients: evidence from pan-cancer analysis and multiple databases. Theranostics 12: 4181-4199, 2022.
98. Le NQK, Kha QH, Nguyen VH, Chen YC, Cheng SJ and Chen CY: Machine learning-based radiomics signatures for egfr and kras mutations prediction in non-small-cell lung cancer. Int J Mol Sci 22: 9254, 2021.
99. Gao J, Wu H, Shi X, Huo Z, Zhang J and Liang Z: Comparison of next-generation sequencing, quantitative PCR, and sanger sequencing for mutation profiling of EGFR, KRAS, PIK3CA and BRAF in clinical lung tumors. Clin Lab 62: 689-696, 2016.
100. Chauhan PS, Shiang A, Alahi I, Sundby RT, Feng W, Gungoren B, Nawaf C, Chen K, Babbra RK, Harris PK, *et al*: Urine cell-free DNA multi-omics to detect MRD and predict survival in bladder cancer patients. NPJ Precis Oncol 7: 6, 2023.
101. Sieverink CA, Batista RPM, Prazeres HJM, Vinagre J, Sampaio C, Leão RR, Máximo V, Witjes JA and Soares P: Clinical validation of a urine test (uromonitor-V2®) for the surveillance of non-muscle-invasive bladder cancer patients. Diagnostics (Basel) 10: 745, 2020.
102. Hosen MI, Sheikh M, Zvereva M, Scelo G, Forey N, Durand G, Voegele C, Poustchi H, Khoshnia M, Roshandel G, *et al*: Urinary TERT promoter mutations are detectable up to 10 years prior to clinical diagnosis of bladder cancer: Evidence from the golestan cohort study. EBioMedicine 53: 102643, 2020.
103. Soputro NA, Gracias DN, Dias BH, Nzenza T, O'Connell H and Sethi K: Utility of urinary biomarkers in primary haematuria: Systematic review and meta-analysis. BJUI Compass 3: 334-343, 2022.
104. Springer SU, Chen CH, Rodriguez Pena MDC, Li L, Douville C, Wang Y, Cohen JD, Taheri D, Silliman N, Schaefer J, *et al*: Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy. Elife 7: e32143, 2018.
105. Baffa R, Letko J, McClung C, LeNoir J, Vecchione A and Gomella LG: Molecular genetics of bladder cancer: Targets for diagnosis and therapy. J Exp Clin Cancer Res 25: 145-160, 2006.
106. Mertens LS, Claps F, Mayr R, Bostrom PJ, Shariat SF, Zwarthoff EC, Boormans JL, Abas C, van Leenders GJLH, Götz S, *et al*: Prognostic markers in invasive bladder cancer: FGFR3 mutation status versus P53 and KI-67 expression: A multi-center, multi-laboratory analysis in 1058 radical cystectomy patients. Urol Oncol 40: 110.e1-110.e9, 2022.
107. Kim YS, Kim K, Kwon GY, Lee SJ and Park SH: Fibroblast growth factor receptor 3 (FGFR3) aberrations in muscle-invasive urothelial carcinoma. BMC Urol 18: 68, 2018.
108. Calderaro J, Rebouissou S, De Koning L, Masmoudi A, Hérault A, Dubois T, Maille P, Soyeux P, Sibony M, de la Taille A, *et al*: PI3K/AKT pathway activation in bladder carcinogenesis. Int J Cancer 134: 1776-1784, 2014.
109. Hao L, Fang J, Xu R, Liu S, Luo G and Wang X: Significance of the FGFR3 mutation in Chinese patients with bladder cancer. Transl Androl Urol 12: 761-769, 2023.
110. Sakr SA, Mahran HA, Fahmy AM, El-Kholy MA and Meawad M: Expression of c-erb-B2 gene in bladder cancer of Egyptian patients and its correlation with p53 and bcl-2. Biomed Pharmacother 76: 73-81, 2015.
111. George B, Datar RH, Wu L, Cai J, Patten N, Beil SJ, Groshen S, Stein J, Skinner D, Jones PA and Cote RJ: p53 gene and protein status: The role of p53 alterations in predicting outcome in patients with bladder cancer. J Clin Oncol 25: 5352-5358, 2007.
112. Landolfi JA and Terio KA: Transitional cell carcinoma in fishing cats (Prionailurus viverrinus): Pathology and expression of cyclooxygenase-1, -2, and p53. Vet Pathol 43: 674-681, 2006.

113. Pakmanesh H, Anvari O, Forey N, Weiderpass E, Malekpourafshar R, Iranpour M, Shahesmaeili A, Ahmadi N, Bazrafshan A, Zendehdel K, *et al*: TERT promoter mutations as simple and non-invasive urinary biomarkers for the detection of urothelial bladder cancer in a high-risk region. Int J Mol Sci 23: 14319, 2022.
114. Avogbe PH, Manel A, Vian E, Durand G, Forey N, Voegele C, Zvereva M, Hosen MI, Meziani S, De Tilly B, *et al*: Urinary TERT promoter mutations as non-invasive biomarkers for the comprehensive detection of urothelial cancer. EBioMedicine 44: 431-438, 2019.
115. Eich ML, Rodriguez Pena MDC, Springer SU, Taheri D, Tregnago AC, Salles DC, Bezerra SM, Cunha IW, Fujita K, Ertoy D, *et al*: Incidence and distribution of UroSEEK gene panel in a multi-institutional cohort of bladder urothelial carcinoma. Mod Pathol 32: 1544-1550, 2019.
116. Wang P, Shi Y, Zhang J, Shou J, Zhang M, Zou D, Liang Y, Li J, Tan Y, Zhang M, *et al*: UCseek: Ultrasensitive early detection and recurrence monitoring of urothelial carcinoma by shallow-depth genome-wide bisulfite sequencing of urinary sediment DNA. EBioMedicine 89: 104437, 2023.
117. Ramos P, Brás JP, Dias C, Bessa-Gonçalves M, Botelho F, Silva J, Silva C and Pacheco-Figueiredo L: Uromonitor: Clinical validation and performance assessment of a urinary biomarker within the surveillance of patients with non-muscle-invasive bladder cancer patients. J Urol: Nov 19, 2024 (Epub ahead of print).
118. Ou Z, Li K, Yang T, Dai Y, Chandra M, Ning J, Wang Y, Xu R, Gao T, Xie Y, *et al*: Detection of bladder cancer using urinary cell-free DNA and cellular DNA. Clin Transl Med 9: 4, 2020.
119. Zhang T, Du E, Liu Y, Cheng J, Zhang Z, Xu Y, Qi S and Chen Y: Anticancer effects of zinc oxide nanoparticles through altering the methylation status of histone on bladder cancer cells. Int J Nanomedicine 15: 1457-1468, 2020.
120. Porten SP: Epigenetic alterations in bladder cancer. Curr Urol Rep 19: 102, 2018.
121. van Kessel KEM, Van Neste L, Lurkin I, Zwarthoff EC and Van Criekinge W: Evaluation of an epigenetic profile for the detection of bladder cancer in patients with hematuria. J Urol 195: 601-607, 2016.
122. Sathianathen NJ, Butaney M, Weight CJ, Kumar R and Konety BR: Urinary biomarkers in the evaluation of primary hematuria: A systematic review and meta-analysis. Bladder Cancer 4: 353-363, 2018.
123. Tripathi K, Goel A, Singhai A and Garg M: Promoter hypomethylation as potential confounder of Ras gene overexpression and their clinical significance in subsets of urothelial carcinoma of bladder. Mol Biol Rep 48: 2183-2199, 2021.
124. Wolff EM, Chihara Y, Pan F, Weisenberger DJ, Siegmund KD, Sugano K, Kawashima K, Laird PW, Jones PA and Liang G: Unique DNA methylation patterns distinguish noninvasive and invasive urothelial cancers and establish an epigenetic field defect in premalignant tissue. Cancer Res 70: 8169-8178, 2010.
125. Locke WJ, Guanzon D, Ma C, Liew YJ, Duesing KR, Fung KYC and Ross JP: DNA methylation cancer biomarkers: Translation to the clinic. Front Genet 10: 1150, 2019.
126. Xiao Y, Ju L, Qian K, Jin W, Wang G, Zhao Y, Jiang W, Liu N, Wu K, Peng M, *et al*: Non-invasive diagnosis and surveillance of bladder cancer with driver and passenger DNA methylation in a prospective cohort study. Clin Transl Med 12: e1008, 2022.
127. Shindo T, Shimizu T, Nojima M, Niinuma T, Maruyama R, Kitajima H, Kai M, Itoh N, Suzuki H and Masumori N: Evaluation of urinary DNA methylation as a marker for recurrent bladder cancer: A 2-center prospective study. Urology 113: 71-78, 2018.
128. Ko K, Kananazawa Y, Yamada T, Kakinuma D, Matsuno K, Ando F, Kuriyama S, Matsuda A and Yoshida H: Methylation status and long-fragment cell-free DNA are prognostic biomarkers for gastric cancer. Cancer Med 10: 2003-2012, 2021.
129. Lutfi A, Afghan MK, Swed B and Kasi PM: False-positive liquid biopsy assays secondary to overlapping aberrant methylation from non-cancer disease states. Case Rep Oncol 16: 1536-1541, 2023.
130. Shields MD, Chen K, Dutcher G, Patel I and Pellini B: Making the rounds: Exploring the role of circulating tumor DNA (ctDNA) in non-small cell lung cancer. Int J Mol Sci 23: 9006, 2022.
131. D'Andrea D, Soria F, Zehetmayer S, Gust KM, Korn S, Witjes JA and Shariat SF: Diagnostic accuracy, clinical utility and influence on decision-making of a methylation urine biomarker test in the surveillance of non-muscle-invasive bladder cancer. BJU Int 123: 959-967, 2019.
132. Tan WS, Feber A, Sarpong R, Khetrapal P, Rodney S, Jalil R, Mostafid H, Cresswell J, Hicks J, Rane A, *et al*: Who should be investigated for haematuria? Results of a contemporary prospective observational study of 3556 patients. Eur Urol 74: 10-14, 2018.
133. Davalos V and Esteller M: Cancer epigenetics in clinical practice. CA Cancer J Clin 73: 376-424, 2023.
134. Wu J, Lin Y, Yang K, Liu X, Wang H, Yu T, Tao R, Guo J, Chen L, Cheng H, *et al*: Clinical effectiveness of a multitarget urine DNA test for urothelial carcinoma detection: A double-blinded, multicenter, prospective trial. Mol Cancer 23: 57, 2024.
135. Maas M, Todenhöfer T and Black PC: Urine biomarkers in bladder cancer-current status and future perspectives. Nat Rev Urol 20: 597-614, 2023.
136. Iisager L, Ahrenfeldt J, Keller AK, Nielsen TK, Fristrup N and Lyskjær I: KIDNEY-PAGER: Analysis of circulating tumor DNA as a biomarker in renal cancer-an observational trial. Study protocol. Acta Oncol 63: 51-55, 2024.
137. Schardt J, Roth B and Seiler R: Forty years of cisplatin-based chemotherapy in muscle-invasive bladder cancer: Are we understanding how, who and when? World J Urol 37: 1759-1765, 2019.
138. Soave A, Kluwe L, Yu H, Rink M, Gild P, Vetterlein MW, Marks P, Sauter G, Fisch M, Meyer CP, *et al*: Copy number variations in primary tumor, serum and lymph node metastasis of bladder cancer patients treated with radical cystectomy. Sci Rep 10: 21562, 2020.
139. Soave A, Chun FK, Hillebrand T, Rink M, Weisbach L, Steinbach B, Fisch M, Pantel K and Schwarzenbach H: Copy number variations of circulating, cell-free DNA in urothelial carcinoma of the bladder patients treated with radical cystectomy: A prospective study. Oncotarget 8: 56398-56407, 2017.
140. Keck B, Ellmann C, Stoehr R, Weigelt K, Goebell PJ, Kunath F, Taubert H, Hartmann A, Wullich B and Wach S: Comparative genomic hybridization shows complex genomic changes of plasmacytoid urothelial carcinoma. Urol Oncol 32: 1234-1239, 2014.
141. Cancer Genome Atlas Research Network: Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 507: 315-322, 2014.
142. Li R, Du Y, Chen Z, Xu D, Lin T, Jin S, Wang G, Liu Z, Lu M, Chen X, *et al*: Macroscopic somatic clonal expansion in morphologically normal human urothelium. Science 370: 82-89, 2020.
143. Luo T, Yi X and Si W: Identification of miRNA and genes involving in osteosarcoma by comprehensive analysis of microRNA and copy number variation data. Oncol Lett 14: 5427-5433, 2017.
144. Wu S, Ou T, Xing N, Lu J, Wan S, Wang C, Zhang X, Yang F, Huang Y and Cai Z: Whole-genome sequencing identifies ADGRG6 enhancer mutations and FRS2 duplications as angiogenesis-related drivers in bladder cancer. Nat Commun 10: 720, 2019.
145. Zeng S, Ying Y, Xing N, Wang B, Qian Z, Zhou Z, Zhang Z, Xu W, Wang H, Dai L, *et al*: Noninvasive detection of urothelial carcinoma by cost-effective low-coverage whole-genome sequencing from urine-exfoliated cell DNA. Clin Cancer Res 26: 5646-5654, 2020.
146. Ge G, Peng D, Guan B, Zhou Y, Gong Y, Shi Y, Hao X, Xu Z, Qi J, Lu H, *et al*: Urothelial carcinoma detection based on copy number profiles of urinary cell-free DNA by shallow whole-genome sequencing. Clin Chem 66: 188-198, 2020.
147. Ellegren H: Microsatellites: Simple sequences with complex evolution. Nat Rev Genet 5: 435-445, 2004.
148. Moon C, Gordon M, Moon D and Reynolds T: Microsatellite instability analysis (MSA) for bladder cancer: Past history and future directions. Int J Mol Sci 22: 12864, 2021.
149. Zheng K, Wan H, Zhang J, Shan G, Chai N, Li D, Fang N, Liu L, Zhang J, Du R, *et al*: A novel NGS-based microsatellite instability (MSI) status classifier with 9 loci for colorectal cancer patients. J Transl Med 18: 215, 2020.
150. Zheng J, Miao F, Wang Z, Ma Y, Lin Z, Chen Y, Kong X, Wang Y, Zhuang A, Wu T and Li W: Identification of MDM2 as a prognostic and immunotherapeutic biomarker in a comprehensive pan-cancer analysis: A promising target for breast cancer, bladder cancer and ovarian cancer immunotherapy. Life Sci 327: 121832, 2023.

151. Malapelle U, Parente P, Pepe F, De Luca C, Pisapia P, Sgariglia R, Nacchio M, Gragnano G, Russo G, Conticelli F, *et al*: Evaluation of micro satellite instability and mismatch repair status in different solid tumors: A multicenter analysis in a real world setting. Cells 10: 1878, 2021.
152. Kasi PM, Bucheit LA, Liao J, Starr J, Barata P, Klempner SJ, Gandara D, Shergill A, Madeira da Silva L, Weipert C, *et al*: Pan-cancer prevalence of microsatellite instability-high (MSI-H) identified by circulating tumor DNA and associated real-world clinical outcomes. JCO Precis Oncol 7: e2300118, 2023.
153. Ma YT, Hua F, Zhong XM, Xue YJ, Li J, Nie YC, Zhang XD, Ma JW, Lin CH, Zhang HZ, *et al*: Clinicopathological characteristics, molecular landscape, and biomarker landscape for predicting the efficacy of PD-1/PD-L1 inhibitors in Chinese population with mismatch repair deficient urothelial carcinoma: A real-world study. Front Immunol 14: 1269097, 2023.
154. Chang L, Chang M, Chang HM and Chang F: Microsatellite instability: A predictive biomarker for cancer immunotherapy. Appl Immunohistochem Mol Morphol 26: e15-e21, 2018.
155. Hirotsu Y, Nagakubo Y, Amemiya K, Oyama T, Mochizuki H and Omata M: Microsatellite instability status is determined by targeted sequencing with MSIcall in 25 cancer types. Clin Chim Acta 502: 207-213, 2020.
156. Zekri AR, Khaled HM, Mohammed MB, Diab FM, Abdellateif MS, El Deeb S, Badr AM, Mohanad M, Abdallah SO and Bahnassy AA: Microsatellite instability profiling in Egyptian bladder cancer patients: A pilot study. Curr Probl Cancer 43: 100472, 2019.
157. Awadalla A, Harraz AM, Abol-Enein H, Laymon M, Ahmed AE, Abdel-Rahim M, Zekri AN and Shokeir AA: Prognostic influence of microsatellite alterations of muscle-invasive bladder cancer treated with radical cystectomy. Urol Oncol 40: 64.e9-64.e15, 2022.
158. Steiner G, Schoenberg MP, Linn JF, Mao L and Sidransky D: Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 3: 621-624, 1997.
159. Wild PJ, Fuchs T, Stoehr R, Zimmermann D, Frigerio S, Padberg B, Steiner I, Zwarthoff EC, Burger M, Denzinger S, *et al*: Detection of urothelial bladder cancer cells in voided urine can be improved by a combination of cytology and standardized microsatellite analysis. Cancer Epidemiol Biomarkers Prev 18: 1798-1806, 2009.
160. Carpagnano GE, Costantino E, Palladino GP, Lacedonia D, Martinelli D, Orlando S and Foschino-Barbaro MP: Microsatellite alterations and cell-free dna analysis: Could they increase the cytology sensitivity in the diagnosis of malignant pleural effusion? Rejuvenation Res 15: 265-273, 2012.
161. Wagner SJ, Reisenbüchler D, West NP, Niehues JM, Zhu J, Foersch S, Veldhuizen GP, Quirke P, Grabsch HI, van den Brandt PA, *et al*: Transformer-based biomarker prediction from colorectal cancer histology: A large-scale multicentric study. Cancer Cell 41: 1650-1661, 2023.
162. Van Rhijn BW, Van Der Poel HG and Van Der Kwast TH: Urine markers for bladder cancer surveillance: A systematic review. Eur Urol 47: 736-748, 2005.
163. Bartoletti R, Dal Canto M, Cai T, Piazzini M, Travaglini F, Gavazzi A and Rizzo M: Early diagnosis and monitoring of superficial transitional cell carcinoma by microsatellite analysis on urine sediment. Oncol Rep 13: 531-537, 2005.
164. Frigerio S, Padberg BC, Strebel RT, Lenggenhager DM, Messthaler A, Abdou MT, Moch H and Zimmermann DR: Improved detection of bladder carcinoma cells in voided urine by standardized microsatellite analysis. Int J Cancer 121: 329-338, 2007.
165. Brisuda A, Pazourkova E, Soukup V, Horinek A, Hrbáček J, Capoun O, Svobodova I, Pospisilova S, Korabecna M, Mares J, *et al*: Urinary cell-free DNA quantification as non-invasive biomarker in patients with bladder cancer. Urol Int 96: 25-31, 2016.
166. Bryzgunova OE, Konoshenko MY and Laktionov PP: Concentration of cell-free DNA in different tumor types. Expert Rev Mol Diagn 21: 63-75, 2021.
167. Green EA, Li R, Albiges L, Choueiri TK, Freedman M, Pal S, Dyrskjøt L and Kamat AM: Clinical utility of cell-free and circulating tumor DNA in kidney and bladder cancer: A critical review of current literature. Eur Urol Oncol 4: 893-903, 2021.
168. Ralla B, Stephan C, Meller S, Dietrich D, Kristiansen G and Jung K: Nucleic acid-based biomarkers in body fluids of patients with urologic malignancies. Crit Rev Clin Lab Sci 51: 200-231, 2014.
169. Li P, Ning J, Luo X, Du H, Zhang Q, Zhou G, Du Q, Ou Z, Wang L and Wang Y: New method to preserve the original proportion and integrity of urinary cell-free DNA. J Clin Lab Anal 33: e22668, 2019.
170. Tse RT, Zhao H, Wong CY, Cheng CK, Kong AW, Peng Q, Chiu PK, Ng CF and Teoh JY: Urinary cell-free DNA in bladder cancer detection. Diagnostics (Basel) 11: 306, 2021.
171. Augustus E, van Casteren K, Sorber L, van Dam P, Roeyen G, Peeters M, Vorsters A, Wouters A, Raskin J, Rolfo C, *et al*: The art of obtaining a high yield of cell-free DNA from urine. PLoS One 15: e0231058, 2020.
172. Piao XM, Jeong P, Kim YH, Byun YJ, Xu Y, Kang HW, Ha YS, Kim WT, Lee JY, Woo SH, *et al*: Urinary cell-free microRNA biomarker could discriminate bladder cancer from benign hematuria. Int J Cancer 144: 380-388, 2019.
173. Zancan M, Galdi F, Di Tonno F, Mazzariol C, Orlando C, Malentacchi F, Agostini M, Maran M, Del Bianco P, Fabricio AS, *et al*: Evaluation of cell-free DNA in urine as a marker for bladder cancer diagnosis. Int J Biol Markers 24: 147-155, 2009.
174. do Nascimento Alves SIPM, Lavalhegas Hallack M, Moreira Perez M, da Costa Aguiar Alves B, da Silva LH and Afonso Fonseca FL: Application of the Z-scan technique for the detection of CFCDNA (cell-free circulating DNA) and urine DNA (uDNA) in patients with bladder cancer. Photodiagnosis Photodyn Ther 26: 131-133, 2019.
175. Nel I, Münch C, Shamkeeva S, Heinemann ML, Isermann B and Aktas B: The challenge to stabilize, extract and analyze urinary cell-free DNA (ucfDNA) during clinical routine. Diagnostics (Basel) 13: 3670, 2023.
176. Markus H, Zhao J, Contente-Cuomo T, Stephens MD, Raupach E, Odenheimer-Bergman A, Connor S, McDonald BR, Moore B, Hutchins E, *et al*: Analysis of recurrently protected genomic regions in cell-free DNA found in urine. Sci Transl Med 13: aaz3088, 2021.
177. Casadio V, Calistri D, Salvi S, Gunelli R, Carretta E, Amadori D, Silvestrini R and Zoli W: Urine cell-free DNA integrity as a marker for early prostate cancer diagnosis: A pilot study. Biomed Res Int 2013: 270457, 2013.
178. Casadio V, Calistri D, Tebaldi M, Bravaccini S, Gunelli R, Martorana G, Bertaccini A, Serra L, Scarpi E, Amadori D, *et al*: Urine Cell-Free DNA integrity as a marker for early bladder cancer diagnosis: Preliminary data. Urol Oncol 31: 1744-1750, 2013.
179. Nuzzo PV, Berchuck JE, Korthauer K, Spisak S, Nassar AH, Abou Alaiwi S, Chakravarthy A, Shen SY, Bakouny Z, Boccardo F, *et al*: Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. Nat Med 26: 1041-1043, 2020.
180. Zhou Q, Liu F, Guo L, Chen R, Yuan X, Li C, Shu L, Liu H, Zhou Y, Wu Y, *et al*: A novel urine cell-free DNA preservation solution and its application in kidney transplantation. Nephrology (Carlton) 26: 684-691, 2021.
181. Mouliere F, Smith CG, Heider K, Su J, van der Pol Y, Thompson M, Morris J, Wan JCM, Chandrananda D, Hadfield J, *et al*: Fragmentation patterns and personalized sequencing of cell-free DNA in urine and plasma of glioma patients. EMBO Mol Med 13: e12881, 2021.
182. Chang A, Mzava O, Lenz JS, Cheng AP, Burnham P, Motley ST, Bennett C, Connelly JT, Dadhania DM, Suthanthiran M, *et al*: Measurement biases distort cell-free DNA fragmentation profiles and define the sensitivity of metagenomic cell-free DNA sequencing assays. Clin Chem 68: 163-171, 2022.
183. Yasuda T, Nadano D, Awazu S and Kishi K: Human urine deoxyribonuclease II (DNase II) isoenzymes: A novel immunoaffinity purification, biochemical multiplicity, genetic heterogeneity and broad distribution among tissues and body fluids. Biochim Biophys Acta 1119: 185-193, 1992.
184. Nardelli C, Aveta A, Pandolfo SD, Tripodi L, Russo F, Imbimbo C, Castaldo G and Pastore L: Microbiome profiling in bladder cancer patients using the first-morning urine sample. Eur Urol Open Sci 59: 18-26, 2023.
185. Zhang B, Pan X, Cobb GP and Anderson TA: microRNAs as oncogenes and tumor suppressors. Dev Biol 302: 1-12, 2007.

186. Elballal MS, Sallam AM, Elesawy AE, Shahin RK, Midan HM, Elrebehy MA, Elazazy O, El-Boghdady RM, Blasy SH, Amer NM, *et al*: miRNAs as potential game-changers in renal cell carcinoma: Future clinical and medicinal uses. Pathol Res Pract 245: 154439, 2023.
187. Xie Y, Ma X, Chen L, Li H, Gu L, Gao Y, Zhang Y, Li X, Fan Y, Chen J and Zhang X: MicroRNAs with prognostic significance in bladder cancer: A systematic review and meta-analysis. Sci Rep 7: 5619, 2017.
188. Pan D, Du Y, Li R, Shen A, Liu X, Li C and Hu B: miR-29b-3p increases radiosensitivity in stemness cancer cells via modulating oncogenes axis. Front Cell Dev Biol 9: 741074, 2021.
189. Eissa S, Matboli M, Hegazy MG, Kotb YM and Essawy NO: Evaluation of urinary microRNA panel in bladder cancer diagnosis: Relation to bilharziasis. Transl Res 165: 731-739, 2015.
190. Nekoohesh L, Modarressi MH, Mowla SJ, Sadroddiny E, Etemadian M, Afsharpad M, Zolfaghari F, Barzegari M, Saffari M, Oskooei VK, *et al*: Expression profile of miRNAs in urine samples of bladder cancer patients. Biomark Med 12: 1311-1321, 2018.
191. Suarez-Cabrera C, Estudillo L, Ramón-Gil E, Martínez-Fernández M, Peral J, Rubio C, Lodewijk I, Martín de Bernardo Á, García-Escudero R, Villacampa F, *et al*: BlaDimiR: A urine-based miRNA score for accurate bladder cancer diagnosis and follow-up. Eur Urol 82: 663-667, 2022.
192. Li HJ, Gong X, Li ZK, Qin W, He CX, Xing L, Zhou X, Zhao D and Cao HL: Role of long non-coding RNAs on bladder cancer. Front Cell Dev Biol 9: 672679, 2021.
193. Wang X, Song D, Zhu B, Jin Y, Cai C and Wang Z: Urinary exosomal mRNA as a biomarker for the diagnosis of bladder cancer. Anticancer Drugs 35: 362-370, 2024.
194. Zhang ZH, Wang Y, Zhang Y, Zheng SF, Feng T, Tian X, Abudurexiti M, Wang ZD, Zhu WK, Su JQ, *et al*: The function and mechanisms of action of circular RNAs in urologic cancer. Mol Cancer 22: 61, 2023.
195. Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F and Liu Y: Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. FEBS J 281: 1750-1758, 2014.
196. Zhao H, Wu W, Li X and Chen W: Long noncoding RNA UCA1 promotes glutamine-driven anaplerosis of bladder cancer by interacting with hnRNP I/L to upregulate GPT2 expression. Transl Oncol 17: 101340, 2022.
197. Wang Z, Wang X, Zhang D, Yu Y, Cai L and Zhang C: Long non-coding RNA urothelial carcinoma-associated 1 as a tumor biomarker for the diagnosis of urinary bladder cancer. Tumor Biol 39: 1010428317709990, 2017.
198. Kong JH, Ha D, Lee J, Kim I, Park M, Im SH, Shin K and Kim S: Network-based machine learning approach to predict immunotherapy response in cancer patients. Nat Commun 13: 3703, 2022.
199. Abbastabar M, Sarfi M, Golestani A, Karimi A, Pourmand G and Khalili E: Tumor-derived urinary exosomal long non-coding RNAs as diagnostic biomarkers for bladder cancer. EXCLI J 19: 301-310, 2020.
200. Bao Z, Zhang W and Dong D: A potential prognostic lncRNA signature for predicting survival in patients with bladder urothelial carcinoma. Oncotarget 8: 10485-10497, 2017.
201. Zhao L, Li J, Xue Z and Wang J: Exosomal noncoding RNAs as noninvasive biomarkers in bladder cancer: A diagnostic meta-analysis. Clin Transl Oncol 26: 1497-1507, 2024.
202. Bian B, Li L, Ke X, Chen H, Liu Y, Zheng N, Zheng Y, Ma Y, Zhou Y, Yang J, *et al*: Urinary exosomal long non-coding RNAs as noninvasive biomarkers for diagnosis of bladder cancer by RNA sequencing. Front Oncol 12: 976329, 2022.
203. Yu Y, Zhang W, Li A, Chen Y, Ou Q, He Z, Zhang Y, Liu R, Yao H and Song E: Association of long noncoding RNA biomarkers with clinical immune subtype and prediction of immunotherapy response in patients with cancer. JAMA Netw Open 3: e202149, 2020.
204. Lin YH: The effects of intracellular and exosomal ncRNAs on cancer progression. Cancer Gene Ther 30: 1587-1597, 2023.
205. Chen C, Luo Y, He W, Zhao Y, Kong Y, Liu H, Zhong G, Li Y, Li J, Huang J, *et al*: Exosomal long noncoding RNA LNMAT2 promotes lymphatic metastasis in bladder cancer. J Clin Invest 130: 404-421, 2020.
206. He W, Zhong G, Jiang N, Wang B, Fan X, Chen C, Chen X, Huang J and Lin T: Long noncoding RNA BLACAT2 promotes bladder cancer-associated lymphangiogenesis and lymphatic metastasis. J Clin Invest 128: 861-875, 2018.
207. Huang H, Du J, Jin B, Pang L, Duan N, Huang C, Hou J, Yu W, Hao H and Li H: Combination of urine exosomal mRNAs and lncRNAs as novel diagnostic biomarkers for bladder cancer. Front Oncol 11: 667212, 2021.
208. Laukhtina E, Shim SR, Mori K, D'Andrea D, Soria F, Rajwa P, Mostafaei H, Compérat E, Cimadamore A, Moschini M, *et al*: Diagnostic accuracy of novel urinary biomarker tests in non-muscle-invasive bladder cancer: A systematic review and network meta-analysis. Eur Urol Oncol 4: 927-942, 2021.
209. Li KD, Chu CE, Patel M, Meng MV, Morgan TM and Porten SP: Cxbladder monitor testing to reduce cystoscopy frequency in patients with bladder cancer. Urol Oncol 41: 326.e1-326.e8, 2023.
210. Fasulo V, Paciotti M, Lazzeri M, Contieri R, Casale P, Saita A, Lughezzani G, Diana P, Frego N, Avolio PP, *et al*: Xpert bladder cancer monitor may avoid cystoscopies in patients under 'active surveillance' for recurrent bladder cancer (BIAS Project): Longitudinal cohort study. Front Oncol 12: 832835, 2022.
211. Tortora F, La Civita E, Trivedi P, Febbraio F, Terracciano D and Cimmino A: Emerging RNA-based therapeutic and diagnostic options: Recent advances and future challenges in genitourinary cancers. Int J Mol Sci 24: 4601, 2023.
212. Yang L, Zou X, Zou J and Zhang G: Functions of circular RNAs in bladder, prostate and renal cell cancer (Review). Mol Med Rep 23: 307, 2021.
213. Jiang YK, Shuai YJ, Ding HM, Zhang H, Huang C, Wang L, Sun JY, Wei WJ, Xiao XY and Jiang GS: Erratum to: ARID1A inactivation increases expression of circ0008399 and promotes cisplatin resistance in bladder cancer. Curr Med Sci 43: 1260, 2023.
214. Dong W, Bi J, Liu H, Yan D, He Q, Zhou Q, Wang Q, Xie R, Su Y, Yang M, *et al*: Circular RNA ACVR2A suppresses bladder cancer cells proliferation and metastasis through miR-626/EYA4 axis. Mol Cancer 18: 95, 2019.
215. Yang C, Yuan W, Yang X, Li P, Wang J, Han J, Tao J, Li P, Yang H, Lv Q and Zhang W: Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21, PTEN expression. Mol Cancer 17: 19, 2018.
216. Ma X, Ying Y, Sun J, Xie H, Li J, He L, Wang W, Chen S, Shen H, Yi J, *et al*: circKDM4C enhances bladder cancer invasion and metastasis through miR-200bc-3p/ZEB1 axis. Cell Death Discov 7: 365, 2021.
217. Liang Z, Guo W, Fang S, Zhang Y, Lu L, Xu W and Qian H: CircRNAs: Emerging bladder cancer biomarkers and targets. Front Oncol 10: 606485, 2021.
218. Chu G, Ji X, Wang Y and Niu H: Integrated multiomics analysis and machine learning refine molecular subtypes and prognosis for muscle-invasive urothelial cancer. Mol Ther Nucleic Acids 33: 110-126, 2023.
219. Harsanyi S, Novakova ZV, Bevizova K, Danisovic L and Ziaran S: Biomarkers of bladder cancer: Cell-free DNA, epigenetic modifications and non-coding RNAs. Int J Mol Sci 23: 13206, 2022.
220. Martin-Way D, Puche-Sanz I, Cozar JM, Zafra-Gomez A, Gomez-Regalado MDC, Morales-Alvarez CM, Hernandez AF, Martinez-Gonzalez LJ and Alvarez-Cubero MJ: Genetic variants of antioxidant enzymes and environmental exposures as molecular biomarkers associated with the risk and aggressiveness of bladder cancer. Sci Total Environ 843: 156965, 2022.
221. Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Dominguez Escrig JL, Gontero P, Liedberg F, Masson-Lecomte A, Mostafid AH, *et al*: European association of urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma in situ). Eur Urol 81: 75-94, 2022.
222. Chen AA and Grasso M: Is there a role for FISH in the management and surveillance of patients with upper tract transitional-cell carcinoma? J Endourol 22: 1371-1374, 2008.
223. Koide K, Sekizawa A, Iwasaki M, Matsuoka R, Honma S, Farina A, Saito H and Okai T: Fragmentation of cell-free fetal DNA in plasma and urine of pregnant women. Prenat Diagn 25: 604-607, 2005.
224. Wong FC and Lo YM: Prenatal diagnosis innovation: Genome sequencing of maternal plasma. Annu Rev Med 32: 419-432, 2016.
225. Tivey A, Church M, Rothwell D, Dive C and Cook N: Circulating tumour DNA-looking beyond the blood. Nat Rev Clin Oncol 19: 600-612, 2022.

226. Wu Z, Yang Z, Li CS, Zhao W, Liang ZX, Dai Y, Zhu Q, Miao KL, Cui DH and Chen LA: Differences in the genomic profiles of cell-free DNA between plasma, sputum, urine, and tumor tissue in advanced NSCLC. Cancer Med 8: 910-919, 2019.
227. Lo YM, Chan KC, Sun H, Chen EZ, Jiang P, Lun FM, Zheng YW, Leung TY, Lau TK, Cantor CR and Chiu RW: Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med 2: 61ra91, 2010.
228. Chen G, Jia G, Chao F, Xie F, Zhang Y, Hou C, Huang Y, Tang H, Yu J, Zhang J, *et al*: Urine- and blood-based molecular profiling of human prostate cancer. Front Oncol 12: 759791, 2022.
229. Saha MK, Massicotte-Azarniouch D, Reynolds ML, Mottl AK, Falk RJ, Jennette JC and Derebail VK: Glomerular hematuria and the utility of urine microscopy: A review. Am J Kidney Dis 80: 383-392, 2022.
230. He Y, Pan C, Zhang Y, Lv M and Yang B: Nomogram for customized recurrence prediction in primary non-muscle-invasive bladder cancer based on routine blood and urine parameters. BMC Urol 24: 67, 2024.
231. Teixeira-Marques A, Lourenço C, Oliveira MC, Henrique R and Jerónimo C: Extracellular vesicles as potential bladder cancer biomarkers: Take it or leave it? Int J Mol Sci 24: 6757, 2023.
232. Latosinska A, Frantzi M, Vlahou A and Mischak H: Clinical applications of capillary electrophoresis coupled to mass spectrometry in biomarker discovery: Focus on bladder cancer. Proteomics Clin Appl 7: 779-793, 2013.
233. Claps F, Rossin G, van Rhijn BWG, Mir MC, Mertens LS, Ongaro L, Traunero F, Iachimovsky AI, Piasentin A, Vedovo F, *et al*: The utility of inflammatory serum markers in the assessment of perioperative morbidity after radical cystectomy for bladder cancer. Medicina (Kaunas) 59: 926, 2023.
234. López-Cortés R, Gómez BB, Vázquez-Estévez S, Pérez-Fentes D and Núñez C: Blood-based protein biomarkers in bladder urothelial tumors. J Proteomics 247: 104329, 2021.
235. Lee DH, Yoon H, Park S, Kim JS, Ahn YH, Kwon K, Lee D and Kim KH: Urinary exosomal and cell-free DNA detects somatic mutation and copy number alteration in urothelial carcinoma of bladder. Sci Rep 8: 14707, 2018.
236. Kobayashi M, Abe H, Arai K, Murakami S and Kamai T: Circulating tumor cells and cell-free tumor DNA analyses in urothelial cancer using the LiquidBiopsy platform. Curr Urol 16: 99-106, 2022.
237. Hirotsu Y, Yokoyama H, Amemiya K, Hagimoto T, Daimon H, Hosaka K, Oyama T, Mochizuki H and Omata M: Genomic profile of urine has high diagnostic sensitivity compared to cytology in non-invasive urothelial bladder cancer. Cancer Sci 110: 3235-3243, 2019.
238. Zhang R, Zang J, Xie F, Zhang Y, Wang Y, Jing Y, Zhang Y, Chen Z, Shahatiaili A, Cai MC, *et al*: Urinary molecular pathology for patients with newly diagnosed urothelial bladder cancer. J Urol 206: 873-884, 2021.
239. Payne SR, Serth J, Schostak M, Kamradt J, Strauss A, Thelen P, Model F, Day JK, Liebenberg V, Morotti A, *et al*: DNA methylation biomarkers of prostate cancer: Confirmation of candidates and evidence urine is the most sensitive body fluid for non-invasive detection. Prostate 69: 1257-1269, 2009.
240. Jerónimo C, Usadel H, Henrique R, Silva C, Oliveira J, Lopes C and Sidransky D: Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer. Urology 60: 1131-1135, 2002.
241. Goessl C, Müller M, Heicappell R, Krause H and Miller K: DNA-based detection of prostate cancer in blood, urine, and ejaculates. Ann N Y Acad Sci 945: 51-58, 2001.
242. Smith CG, Moser T, Mouliere F, Field-Rayner J, Eldridge M, Riediger AL, Chandrananda D, Heider K, Wan JCM, Warren AY, *et al*: Comprehensive characterization of cell-free tumor DNA in plasma and urine of patients with renal tumors. Genome Med 12: 23, 2020.
243. Christensen E, Nordentoft I, Birkenkamp-Demtröder K, Elbæk SK, Lindskrog SV, Taber A, Andreasen TG, Strandgaard T, Knudsen M, Lamy P, *et al*: Cell-free urine and plasma DNA mutational analysis predicts neoadjuvant chemotherapy response and outcome in patients with muscle-invasive bladder cancer. Clin Cancer Res 29: 1582-1591, 2023.
244. Crozier J, Papa N, Perera M, Ngo B, Bolton D, Sengupta S and Lawrentschuk N: Comparative sensitivity and specificity of imaging modalities in staging bladder cancer prior to radical cystectomy: A systematic review and meta-analysis. World J Urol 37: 667-690, 2019.
245. Bandyk MG, Gopireddy DR, Lall C, Balaji KC and Dolz J: MRI and CT bladder segmentation from classical to deep learning based approaches: Current limitations and lessons. Comput Biol Med 134: 104472, 2021.
246. Humayun-Zakaria N, Ward DG, Arnold R and Bryan RT: Trends in urine biomarker discovery for urothelial bladder cancer: DNA, RNA, or protein? Transl Androl Urol 10: 2787-2808, 2021.
247. Sun T, Hutchinson L, Tomaszewicz K, Caporelli ML, Meng X, McCauley K, Fischer AH, Cosar EF and Cornejo KM: Diagnostic value of a comprehensive, urothelial carcinoma-specific next-generation sequencing panel in urine cytology and bladder tumor specimens. Cancer Cytopathol 129: 537-547, 2021.
248. Claps F, Pavan N, Ongaro L, Tierno D, Grassi G, Trombetta C, Tulone G, Simonato A, Bartoletti R, Mertens LS, *et al*: BCG-unresponsive non-muscle-invasive bladder cancer: Current treatment landscape and novel emerging molecular targets. Int J Mol Sci 24: 12596, 2023.
249. Frantzi M, Latosinska A, Flühe L, Hupe MC, Critselis E, Kramer MW, Merseburger AS, Mischak H and Vlahou A: Developing proteomic biomarkers for bladder cancer: Towards clinical application. Nat Rev Urol 12: 317-330, 2015.
250. Xu X, Huang F, Cao M, Chen X, Wang H, Jiang H, Yu Y, Shen M, Yang Y, Wang B, *et al*: Cross-platform comparison of next-generation sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for detecting KRAS/NRAS/BRAF/PIK3CA mutations in cfDNA from metastatic colorectal cancer patients. J Clin Lab Anal 35: e23818, 2021.
251. Raina R, Nada A, Shah R, Aly H, Kadatane S, Abitbol C, Aggarwal M, Koyner J, Neyra J and Sethi SK: Artificial intelligence in early detection and prediction of pediatric/neonatal acute kidney injury: Current status and future directions. Pediatr Nephrol 39: 2309-2324, 2024.
252. Lima T, Henrique R, Vitorino R and Fardilha M: Bioinformatic analysis of dysregulated proteins in prostate cancer patients reveals putative urinary biomarkers and key biological pathways. Med Oncol 38: 9, 2021.
253. Cheng P, Wang R, He S, Yan P, Huang H, Chen J, Shen J and Pu K: Artificial Urinary biomarkers for early diagnosis of acute renal allograft rejection. Angew Chem Int Ed Engl 62: e202306539, 2023.
254. Xu C, Xu M, Hu Y, Liu J, Cheng P, Zeng Z and Pu K: Ingestible artificial urinary biomarker probes for urine test of gastrointestinal cancer. Adv Mater 36: e2314084, 2024.
255. Yang Y, Wang J, Huang W, Wan G, Xia M, Chen D, Zhang Y, Wang Y, Guo F, Tan J, *et al*: Integrated urinalysis devices based on interface-engineered field-effect transistor biosensors incorporated with electronic circuits. Adv Mater 34: e2203224, 2022.
256. Hao L, Zhao RT, Welch NL, Tan EKW, Zhong Q, Harzallah NS, Ngambenjawong C, Ko H, Fleming HE, Sabeti PC and Bhatia SN: CRISPR-Cas-amplified urinary biomarkers for multiplexed and portable cancer diagnostics. Nat Nanotechnol 18: 798-807, 2023.
257. Shi H, Li X, Zhang Q, Yang H and Zhang X: Discovery of urine biomarkers for bladder cancer via global metabolomics. Biomarkers 21: 578-588, 2016.