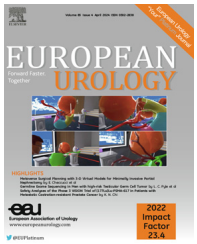
available at www.sciencedirect.com
journal homepage: www.europeanurology.com



European Association of Urology

Platinum Opinion

The Elusive Horizon: Biomarkers in Urothelial Carcinoma

Joshua A. Linscott [a], *Joshua J. Meeks* [b,c], *Lars Dyrskjøt* [d,e], *Roger Li* [a,f,*]

[a] *Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA;* [b] *Departments of Urology and Biochemistry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA;* [c] *Jesse Brown VAMC, Chicago, IL, USA;* [d] *Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark;* [e] *Department of Clinical Medicine, Aarhus University, Aarhus, Denmark;* [f] *Department of Immunology, H. Lee Moffitt Cancer Center, Tampa, FL, USA*

1. Introduction

Urothelial cancer (UC) is unique in that it has a constant interface with urine, which provides direct diagnostic and monitoring opportunities. As early as 1945, Papanicolaou and Marshall attempted to use urine cytology to diagnose UC. In the ensuing decades, many urinary biomarkers for prediction of cancer recurrence were developed, yet none has garnered widespread clinical adoption. The use of urinary biomarkers to detect and monitor UC seems straightforward, so why is success elusive? We reviewed the significant efforts behind the development of three generations of urinary biomarkers for predicting recurrence of non–muscle-invasive bladder cancer (NMIBC) and here we attempt to distil lessons to inform future efforts.

2. First-generation biomarkers

First-generation biomarkers included assays of overexpressed proteins (NMP22, BTA), cell-surface proteomic changes (Immunocyt/uCyt+), or chromosomal alterations (UroVysion) observed in UC (Table 1). Used as adjuncts to standard-of-care cystoscopy, these tests superseded the sensitivity achieved with urine cytology (particularly for low-grade UC) and were consequently granted US Food and Drug Administration approval. However, these assays lacked specificity as they were frequently confounded by false-positive results in the setting of benign urologic conditions such as infections, urolithiasis, and hematuria [1]. Moreover, the diagnostic properties of some first-generation biomarkers were imprecise, with interpretation of results highly expertise-dependent, further hampering their clinical implementation [2]. Finally, assays of cellular properties (eg, Urovysion and Immunocyt) were intrinsically dependent on the abundance of the cellular content within the testing substrate, which is notoriously unreliable.

3. Second-generation biomarkers

Rapid improvements in genomic sequencing technology facilitated the second wave of urinary biomarker development. Multiplex, high-throughput assays such as CxBladder, Xpert BC Monitor, Uromonitor (mRNA expression), AssureMDx (DNA methylation/mutation), and Epicheck (methylation) were constructed on the basis of unique molecular features of NMIBC (Table 1) [3]. Although the detection sensitivity improved with this multitargeted approach, such tests with limited panels remained inadequate to cover the vast genomic heterogeneity for NMIBC [4,5]. A recent network meta-analysis of five commercially available urinary biomarkers used for cancer surveillance by Laukhtina et al. [6] revealed pooled sensitivity results that were mostly insufficient, ranging between 57% and 74%, with some notable exceptions according to preliminary data (Cxbladder 91%, Uromonitor 93%). These results have led to prospective studies further examining some second-generation tests for specific applications. Ultimately, whether the diagnostic characteristics of these targeted assays are sufficient for clinical use will depend on the results from large randomized prospective studies, some of which are already under way (NCT03988309, NCT05796375).

4. Urinary circulating tumor DNA

The advent of next-generation sequencing (NGS) allowed comprehensive molecular analyses of tumor samples,

* Corresponding author. Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center, 12901 USF Magnolia Drive, Tampa, FL 33612, USA. Tel. +1 813 745 8210; Fax: +1 813 745 6882.
E-mail address: roger.li@moffitt.org (R. Li).

https://doi.org/10.1016/j.eururo.2024.01.004

Table 1 – Performance of UB assays in diagnosing recurrence of non–muscle-invasive bladder cancer

Test	Approach	Target	Sample	Source	Size	SSY	SPY	PPV	NPV
First-generation UBs									
NMP22	Protein expression	1 protein	WU	MA	19 studies	0.69	0.77	NR	NR
BTA Trak	Protein expression	1 protein	WU	MA	4 studies	0.65	0.74	NR	NR
Immunocyt	Cell-surface proteins	2 proteins	WU	MA	14 studies	0.78	0.78	NR	NR
UroVysion	CHR alteration	4 CHRs	WU	MA	11 studies	0.63	0.87	NR	NR
ADxBladder	Protein expression	1 protein	WU	PA	3 studies	0.57	0.61	0.24	0.87
Second-generation UBs									
Bladder Epicheck	DNA methylation	15 loci	WU	PA	5 studies	0.74	0.85	0.51	0.94
CxBladder Monitor	mRNA panel	5 genes	WU	PA	2 studies	0.92	0.59	0.33	0.97
Xpert bladder cancer	mRNA panel	5 genes	WU	PA	10 studies	0.72	0.78	0.42	0.93
Uromonitor	DNA mutation	2 genes	cfDNA	PAs	2 studies	0.94	0.59	0.33	0.97
Next-generation UBs									
UroAmp	NGS hybrid capture	60 genes	WU	SS	86 UC pts	0.89	0.74	0.63	0.93
uCAPP	NGS hybrid capture	460 genes	cfDNA	SS	37 UC pts	0.84	0.96	NR	NR

cfDNA = cell-free DNA; CHR = chromosome; MA = meta-analysis; NGS = next-generation sequencing; NR = not reported; PA = pooled analysis; NPV = negative predictive value; PPV = positive predictive value; pts = patients; SPY = specificity; SS = single study; SSY = sensitivity; UB = urinary biomarker; UC = urothelial carcinoma; WU = whole urine.

advancing our understanding of the complexity of UC. Whole-exome and whole-genome sequencing of cell-free DNA (cfDNA; DNA released by both cancer and normal cells on cell death) extracted from the urine of UC patients can be used to detect somatic alterations unique to the tumor (circulating tumor DNA, ctDNA). Alternatively, NGS of tumor tissue can inform the design of personalized genomic panels used to interrogate urine. In theory, these whole-scale genomic assays should not only overcome the low sensitivity seen with previous generations of urinary biomarkers but also provide biological insight into the mechanisms underpinning cancer recurrence and/or treatment response.

In practice, several challenges remain. First, the exquisite sensitivity of ctDNA and the consequences of overdetection need to be emphasized. Somatic mutations can be detected in urothelium that appears histologically normal, especially in patients with a history of bladder cancer [7]. This "field cancerization" phenomenon could lead to shedding of altered cfDNA in patients without clinical cancer recurrence, raising concerns for false-positive results, which could lead to unnecessary procedures and patient anxiety. Robust data must be generated to understand the mutational landscape unique to the cfDNA profiles associated with normal versus cancerous bladders. As illustrated by Dudley et al. [8] in the development of uCAPP-Seq (Urine Cancer Personalized Profiling by Deep Sequencing), although mutations may overlap, a threshold for the ctDNA fraction may be set to better distinguish between normal and diseased states. Patients testing positive for ctDNA in the absence of a clinical cancer diagnosis may be at high risk of recurrence and vigilant surveillance is mandated in these cases. Furthermore, the benefit of early intravesical therapy in patients with molecular but not clinical disease recurrence warrants further investigation in the NMIBC setting, akin to the TOMBOLA trial assessing treatment of metastatic bladder cancer at the time of biochemical relapse following radical cystectomy (NCT04138628).

It is also conceivable that potential genomic differences between the initial tumor biopsy and any residual disease may hamper the accuracy of tumor-informed assays. To address this concern, Rose et al. [9] compared the mutational landscape between tumor samples harvested from the initial biopsy and repeat transurethral resection of bladder tumor in a cohort of patients with high-risk NMIBC and found concordance of 83%. However, with selective pressure from various treatments (intravesical or systemic), tumor clonality may fluctuate over time and with treatment response. Tumor-informed assays based on alterations detected in the baseline tumor biopsy should therefore be augmented with panel assays that encompass hotspot alterations common to UC in order to capture newly emerging clones.

Finally, the quality of the urine samples and the processing and storage methods are of the utmost importance. Procedures must be optimized to avoid the introduction of unintended variations into interpretation of the results, and must be standardized within prospective trials that test both the feasibility and the diagnostic accuracy of the tests [10]. Specific clinical applications of the assays should only be implemented after their performance characteristics are thoroughly understood, such as high specificity and positive predictive value for surveillance of low-grade NMIBC, and high sensitivity and negative predictive value for detection of high-grade NMIBC.

Precision urinary ctDNA tests have the potential to accurately distinguish bladder cancer from other common inflammatory processes for the diverse molecular heterogeneity seen within the disease. Despite their exciting prospects, questions remain regarding the ability of urinary ctDNA tests to differentiate signals from cancer versus field cancerization, a well-known phenomenon in diseased bladders. In addition, logistical hurdles pertaining to clinical application and cost effectiveness need to be addressed. Ultimately, clinical benefit has to be unequivocally demonstrated in well-designed prospective trials before clinical application. Only with such meticulous efforts will we finally be able to reach the elusive horizon (Fig. 1).

Conflicts of interest: Roger Li has received research support from Predicine, Veracyte, CG Oncology, Valar Labs, and Merck; serves on clinical trial protocol committees for CG Oncology, Merck, and Janssen; and is a scientific advisor/consultant for BMS, Merck, Fergene, Arquer Diagnos-

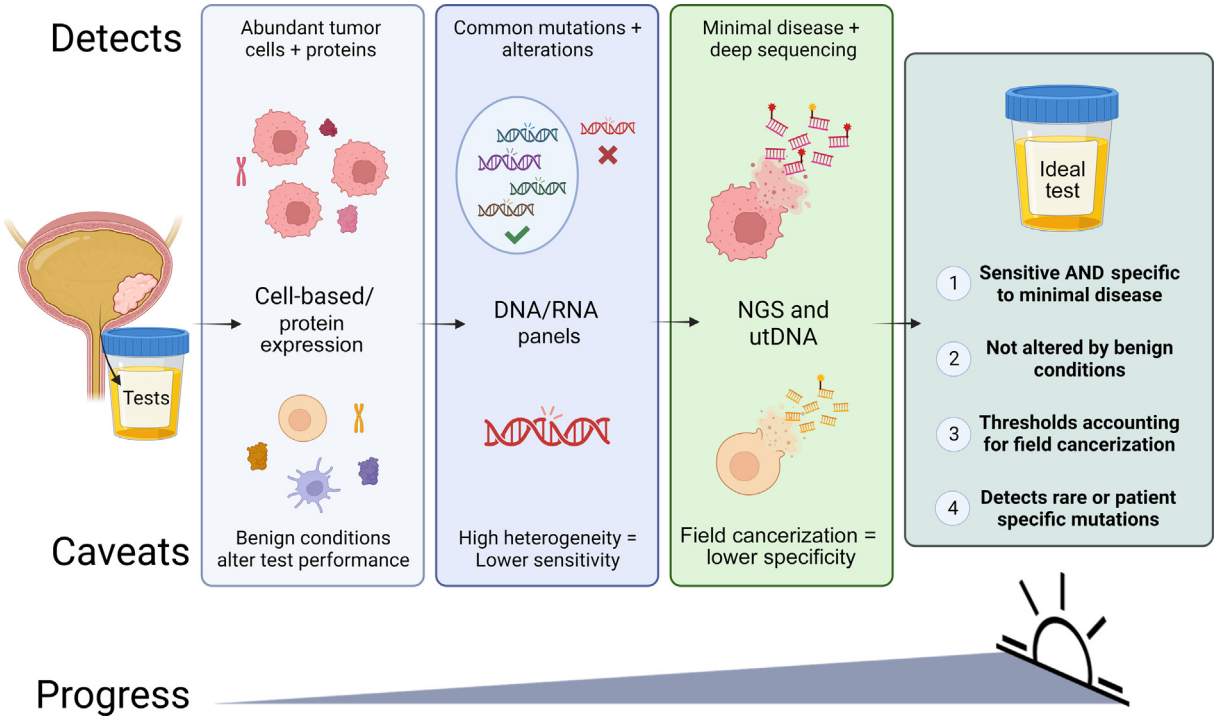


Fig. 1 – Approaches explored and inherent caveats in relation to urinary biomarkers while searching for the ideal test. NGS = next-generation sequencing; utDNA = urinary tumor DNA.

tics, Urogen Pharma, Lucence, CG Oncology, Janssen, and Thericon. Joshua J. Meeks is a consultant for Merck, AstraZeneca, Janssen, BMS, UroGen, Prokarium, Imvax, Pfizer, Seagen/Astellas, and Ferring; has received research funding from VHA, the National Institutes of Health, and the Department of Defense; has received compensation for talks/educational courses from the American Urological Association, OncLive, Olympus, and UroToday; has participated in clinical trials run by Southwest Oncology Group, Genentech, Merck, AstraZeneca, and Incyte; and holds two patents (T1 and TCGA classifier). Lars Dyrskjøt has sponsored research agreements with C2i, AstraZeneca, Natera, Photocure, and Ferring; is a consultant for Ferring and UroGen; and is Chairman of the Board for BioXpedia A/S. Joshua A. Linscott has nothing to disclose.

References

[1] Miyake M, Goodison S, Rizwani W, Ross S, Grossman HB, Rosser CJ. Urinary BTA: indicator of bladder cancer or of hematuria. World J Urol 2012;30:869–73. https://doi.org/10.1007/s00345-012-0935-9.
[2] Mischinger J, Guttenberg LP, Hennenlotter J, et al. Comparison of different concepts for interpretation of chromosomal aberrations in urothelial cells detected by fluorescence in situ hybridization. J Cancer Res Clin Oncol 2017;143:677–85. https://doi.org/10.1007/s00432-016-2310-5.
[3] Maas M, Todenhöfer T, Black PC. Urine biomarkers in bladder cancer — current status and future perspectives. Nat Rev Urol 2023;20:597–614. https://doi.org/10.1038/s41585-023-00773-8.
[4] Hedegaard J, Lamy P, Nordentoft I, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 2016;30:27–42. https://doi.org/10.1016/j.ccell.2016.05.004.
[5] Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017;171:540–556.e25. https://doi.org/10.1016/j.cell.2017.09.007.
[6] Laukhtina E, Shim SR, Mori K, 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 2021;4:927–42. https://doi.org/10.1016/j.euo.2021.10.003.
[7] Strandgaard T, Nordentoft I, Birkenkamp-Demtröder K, et al. Field cancerization is associated with tumor development, T-cell exhaustion, and clinical outcomes in bladder cancer. Eur Urol 2024;85:82–92. https://doi.org/10.1016/j.eururo.2023.07.014.
[8] Dudley JC, Schroers-Martin J, Lazzareschi DV, et al. Detection and surveillance of bladder cancer using urine tumor DNA. Cancer Discov 2019;9:500–9. https://doi.org/10.1158/2159-8290.CD-18-0825.
[9] Rose KM, Murthy P, Gould B, et al. Cell-free urinary tumor DNA to detect minimal residual disease prior to repeat-transurethral resection of bladder tumor in non–muscle-invasive bladder cancer: a prospective study. J Clin Oncol 2023;41(6 Suppl): LBA445. https://doi.org/10.1200/JCO.2023.41.6_suppl.LBA445.
[10] Lotan Y, Black PC, Caba L, et al. Optimal Trial design for studying urinary markers in bladder cancer: a collaborative review. Eur Urol Oncol 2018;1:223–30. https://doi.org/10.1016/j.euo.2018.04.010.