Cancer of the urinary bladder is incredibly aggressive and is highly resistant to many of today’s currently available cancer therapies. In 2019 alone, an estimated 80,470 newly diagnosed cases of bladder cancer (BCa) and 17,670 deaths from BCa is expected to occur in 2019 in the United States (1). In addition, since 2002, the absolute numbers of cases and deaths from BCa have increased by 45% and 35%, respectively (2,3). BCa is still the fourth most common cancer in men, affecting males at a 4:1 higher rate than females and remains the 8th most common cause of cancer deaths in men and women in the United States. Furthermore, BCa has one of the highest recurrence rates of any tumor type (4). Interestingly, in developed countries, BCa is noted as the 4th most common cancer in men and the 7th most frequent in cancer related death, while in developing countries, it is the 7th most frequent cancer in men (3). Due to the prolonged natural history of BCa (~2 million survivors worldwide) combined with the protracted and invasive nature of follow-up and treatment strategies, BCa is now one of the most expensive cancers to handle on a per-patient basis (~$2.2 billion annually in just the US) (4). Therefore, BCa is a major problem worldwide.

Today, there is a rapid development of molecular profiling techniques. The establishment of major international cancer genome consortia, a catalog of molecular profile data acquired from excised tumor tissue samples, continues to amass at a welcome pace. Although BCa is relatively understudied, The Cancer Genome Atlas (TCGA) has reported an initial study on this cancer (5). The analysis was restricted to muscle-invasive BCa (MIBC) obtained from 131 cases. Excised tumors were analyzed for somatic mutations, DNA copy number variants, RNA and protein expression, and DNA methylation. A number of studies have used this foundation to classify tumors into molecular subgroups and subtypes based on differentiation (6), alignment with other cancers (7), as well as to propose the basal/luminal phenomenon observed in other solid tumors (8-10). To date, no clinically useful biomarkers have been derived from this data.

Voided urine cytology (VUC), which has been utilized and has remained virtually unaltered since the 1950s, remains the most widely used urine-based assay for diagnosis today. VUC can be a challenging test to perform, since it is dependent on the skills and experience of a highly trained cytopathologist. Median sensitivity reported for VUC was 35% with an accompanying median specificity of 94% (11). Thus, VUC has insufficient predictive power to be applied to the management of individual patients. Furthermore, because of these substantial limitations, only approximately 10% of patients presenting with suspected BCa are evaluated with VUC (12), which is the recommended procedure in published guidelines.

Unfortunately, few new diagnostic assays have penetrated and persisted in this space. Springer et al. reported on a urine-based molecular assay for detecting and surveilling BCa (UroSEEK). This new assay is designed to detect alterations in 11 genes (TERT, FGFR3, PIK3CA, TP53, HRAS, KRA, KRAS, ERBB2, CDKN2A, MET, MLL and VHL) that include most common genetic alterations in BCa. When linked with cytology, UroSEEK had a sensitivity of.
95% and a specificity of 93% within their early detection cohort (13). Building upon this, recently, Eich et al. reported a fascinating large, multi-institute study of 527 BCa cases. In this study, UroSEEK was applied to 527 formalin fixed paraffin embedded samples, including 373 noninvasive and 154 invasive BCa from transurethral resections or cystectomy performed between 1991–2016 to illustrate that the genetic alterations seen in the shed urothelial is indicative of the genetic alterations in the actual tumors. The authors discovered that 92% of all bladder tumors were found positive for at least one genetic alteration in their panel. TERT promoter mutations were identified in 70% of all cases, with the most common alteration being g.1295228C > T, and second most as g.1295250C > T. Within the 10 genes included in the UroSeqS assay, FGFR3 and PIK3CA mutations were found to have occurred significantly more often in low-grade noninvasive papillary carcinoma tumors compared with high-grade noninvasive papillary carcinomas and carcinomas in situ (P<0.0001), while the reverse was true for TP53 (P<0.0001).

Genetic alterations of UroSEEK were not associated with recurrence rates, yet interestingly, genetic alterations of UroSEEK were associated with a reduction in disease progression (96% vs. 81%, P=0.016) (14).

The study by Eich exhibits a strong sample size derived from four large international centers and includes an independent cohort of 188 unrelated healthy individuals from which DNA from white blood cells was obtained. It would have been ideal if urine, tumor and white blood cells came from the same individuals to minimize variability. Nonetheless, the investigators should be commended for their steadfast work to validate their urine-based diagnostic signature. Based on these results, the authors believed they have confirmed the comprehensive coverage of the panel, and in doing so, shows its capability as a noninvasive urine-based assay for the future.

As shown from the data, detecting BCa using diagnostic biomarkers continues to be a significant challenge. Because of this, development of accurate assays for the non-invasive detection of BCa remains an active area. Previously, we concentrated on single biomarkers (e.g., NMP22 and BTA) (15,16). However, single biomarkers are limited by the undisputed reality that not all BCa cases, or even all cases in one category of lesions (e.g., low stage or low-grade), will harbor any single molecular change. Thus, the concept that the presence or absence of one molecular biomarker will aid clinical evaluation has shown to be insufficient for BCa detection. Fortunately, the emergence of high-throughput technologies has greatly enabled future and current DNA, RNA, protein and metabolite biomarker discoveries.

Recently, several groups have begun to identify panels of diagnostic biomarkers. For example, Hoque et al. found that 69% of BCa patients had methylation in at least one of four genes (CDKN2A, ARF, MGMT, GSTP1), and that the control cases had no such methylation detectable (17). In combining the data from all four genes, a logistic prediction model was derived with a sensitivity of 82% and specificity of 96%. Chung et al. selected 10 candidate hypermethylated genes from data collected from tumor tissue and tested them in voided urine samples by quantitative methylation-specific RT-PCR to identify a multigene predictive model comprised of five target genes (MYO3A, CA10, NKX6-2, DBC1, and SOX11). Sensitivity and specificity of this model were 85% and 95%, respectively (18). Further examples include RNA signatures proposed by Holyoake et al. (19), Hanke et al. (20), Mengual et al. (21) possessing sensitivities ranging from 80–92% and specificities ranging from 85–99%. Holyoake’s work has progressed to a clinical assay in New Zealand, Singapore and Australia with fledgling presence in the US (Cxbladder™). Dudley et al. (22) reported detecting a median of 6 mutations per bladder tumor with a sensitivity of 93% and specificity from 96–100% in a cohort of 118 patients with early-stage BCa. Lastly, our group has identified 10 proteins (APOE, ANG, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1 VEGA) within a BCa-associated diagnostic signature (Oncuria™), which has been shown to possess a sensitivity of 85% and specificity of 81% (23) and is currently in three large multicenter prospective studies (NCT03193541, NCT03193528 and NCT03193515). It must be noted that many of these studies have small sample sizes, limited populations analyzed (i.e., few benign confounding conditions included) and/or have not undergone extensive validation. Thus, high quality, comprehensive, translational BCa biomarker discovery and validation research have been limited.

Currently, no robust non-invasive assay is available for the early detection of BCa. Nevertheless, early detection is an important goal for patients at risk for BCa. At presentation, more than 70% of BCa cases have been found to be non-muscle-invasive BCa (NMIBC), whilst the outstanding 30% are MIBC or metastatic (24). When detected early (i.e., NMIBC), the 5-year survival rate is approximately 94%, compared to at best a 50% 5-year survival rate when the disease is noted to be MIBC and less than a 20% 5-year survival rate when the disease is metastatic (4,25).
As absolute numbers of BCa cases and deaths continue to grow, there is an increased yet still severely unfulfilled need to identify tumors early to improve disease outcomes. Ideally, such an assay would be non-invasive and could help ‘rule-in’ which patients may require more invasive, time consuming, and expensive procedures, e.g., cystoscopy. Such an assay would be a multiplex assay and in addition to its early detection prowess, one should assess its ability to predict disease recurrence and progression, as Eich et al. set out to do in their study. Thus, greater attention to BCa diagnostic signatures in urine, tissue and blood must be stressed as we continue to explore biomarkers for this devastating disease.

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Footnote
Conflicts of Interest: Charles J. Rosser is an officer for the Nonagen Bioscience Corp. R Wong has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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