INTRODUCTION

Bladder cancer (BC) is the sixth most prevalent cancer worldwide in both men and women, with incidence and mortality increasing year by year. BC can originate in the urothelial cells lining inside the organ or other parts of the urinary tract.

In general, most types begin as non-muscle invasive urothelial carcinoma of the urinary bladder (UCB), which are localized to a region and do not invade the muscular wall and can be treated effectively. However, recurrence rates are high, with progression to muscle invasion UCB seen in 15% of patients.

Given the high rate of recurrence, regular follow-ups are necessary to monitor progression. Guidelines from the European Association of Urology (EAU) and the American Urological Association (AUA) suggest a combination of cystoscopy, cytology and imaging for surveillance of patients with UCB, procedures that can be invasive and expensive. This intensive follow-up program makes BC one of the most expensive cancers to monitor.

Consequently, finding novel diagnostic strategies, that are non-invasive as well as cost-effective, can improve the quality of life of patients with BC. This white paper focuses on urine as sample type in BC research and how urinary biomarkers can be used for initial detection and follow-up of the disease. Given the function of urine as a region and do not invade the muscular wall and can be treated effectively. Consequently, novel diagnostic strategies, that are non-invasive as well as cost-effective, can improve the quality of life of patients with BC. This white paper focuses on urine as sample type in BC research and how urinary biomarkers can be used for initial detection and follow-up of the disease. Given the function of the bladder and its close proximity to the urinary tract, urine as a sample type is particularly exciting as it can contain a reliable source of cancer biomarkers.

Biomarker detection in urine for BC is also attractive as it allows non-invasive collection, as well as offers the possibility of repeated sampling. Table 1 shows the range of commercially urinary biomarker tests and kits that are available for the detection of BC.

Cystoscopy is the gold standard method for BC diagnosis, and involves inserting a cystoscope to observe any abnormal changes in the bladder. While cystoscopy can offer sensitivity of up to 90%, it can miss detection of small tumors. Additionally, the procedure is invasive, can be highly uncomfortable for the patient and requires the experience of a urologist or nurse.

Urine cytology involves observing a urine sample under a microscope to check for abnormal cells. Together with cystoscopy, this test is used in the diagnosis and follow-up of BC patients. Benefits of urine cytology are that it is non-invasive, inexpensive and offers a specificity of up to 98% for high-grade tumor detection. However, urinary cytology has an overall low sensitivity (less than 40%), making it challenging to use as a diagnostic tool for BC suspicion. Additionally, voided urine cytology requires trained cytopathologists and can be subject to inter-observer variability.

As cystoscopy and urine cytology may not always be practical or feasible and is a costly procedure to follow repeatedly, finding alternative ways to detect as well as monitor BC is actively being researched.

There is an increased interest in non-invasive urinary biomarkers for initial detection and follow-up of the disease. Given the function of the bladder and its close proximity to the urinary tract, urine as a sample type is particularly exciting as it can contain a reliable source of cancer biomarkers.

Table 1: Commercially available urinary biomarker tests for bladder cancer detection

<table>
<thead>
<tr>
<th>Test</th>
<th>FDA/CE</th>
<th>Starting material</th>
<th>Biomarker type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>uCyt</td>
<td>Y Y</td>
<td>Exfoliated cells</td>
<td>Antigens/Metabolites</td>
<td>73%</td>
<td>66%</td>
</tr>
<tr>
<td>NMP22</td>
<td>Y Y</td>
<td>Exfoliated cells</td>
<td>Peptides</td>
<td>40%</td>
<td>99%</td>
</tr>
<tr>
<td>UroVysion</td>
<td>Y Y</td>
<td>Exfoliated cells</td>
<td>DNA</td>
<td>72%</td>
<td>83%</td>
</tr>
<tr>
<td>BTA stat/BTA Track</td>
<td>Y Y</td>
<td>Exfoliated cells</td>
<td>Proteins</td>
<td>70%</td>
<td>75%</td>
</tr>
<tr>
<td>Callbladder</td>
<td>N N</td>
<td>Exfoliated cells</td>
<td>mRNA</td>
<td>82%</td>
<td>85%</td>
</tr>
<tr>
<td>Xpert Detection</td>
<td>N Y</td>
<td>Exfoliated cells</td>
<td>mRNA</td>
<td>78%</td>
<td>85%</td>
</tr>
<tr>
<td>Uromonitor</td>
<td>N Y</td>
<td>Exfoliated cells</td>
<td>Tumor cell DNA</td>
<td>74%</td>
<td>93%</td>
</tr>
</tbody>
</table>

TYPES OF BIOMARKERS IN URINE

Current FDA-approved tests have poor sensitivity or specificity, especially for low-grade and early-stage BC tumors and recurrent diagnoses. Therefore, other urinary biomarkers for BC diagnostics are currently being investigated.

Possible biomarker candidates for BC in urine include exfoliated bladder cancer cells (EBCCs), cell-free DNA (cfDNA) and exosomes. Exosomes, which are a class of extracellular vesicles released by all cells, and containing DNA, RNA, and proteins, are particularly promising as they represent a fingerprint of the cell of origin. Recent advancements in OMics technologies, including genomics, epigenomics, proteomics, transcriptomics, and metabolomics have also improved our understanding of the molecular landscape causing cancers.

Genomic biomarkers

One particularly interesting biomarker that has shown to be a game-changer for disease monitoring and early detection of recurrence in BC is telomerase reverse transcriptase (TERT) promoter mutations. These mutations are extremely specific to BC and are not present in inflammatory or urinary infections, which are current drawbacks with the current non-invasive assays. These biomarkers are currently being included in BC urine-based tests, Uromonitor, Uromonitor-V2, and UroSEEK.
In low-grade non-muscle invasive bladder cancer (NMIBC) tumors, mutations in the fibroblast growth factor receptor 3 (FGFR3) oncogene are frequent. One study showed the sensitivity for detecting BC by the FGFR3 mutation was 58%⁹. Alternatively, in high-grade NMIBC tumors, mutations in p53 genes, which can cause dysregulation of the RAS-MAK (mitogen-activated protein kinase) pathway are seen more often. Mutations in these genes are a strong indicator for BC. Mutations in RAS (Rat sarcoma) oncogenes occur in 13% of all BC tumors, providing valuable urinary biomarker candidates⁷.

Epigenetic biomarkers
Epigenetic factors also play an important role in the development of BC and can be useful biomarkers for disease detection and monitoring. Several studies have revealed the role of DNA methylation and methylated genes in influencing gene expression, ultimately leading to the development and progression of BC. However, further validation of these markers is required⁴.

Transcriptomic biomarkers
Several studies have shown the potential of miRNAs in the detection of BC. While some studies used a single and others a panel of up and/or downregulated miRNAs, all of them showed sensitivity ranging from 72% to 90% and specificity ranging from 82% to 90%⁶.

A recent meta-analysis concluded IncRNAs in urine may serve as non-invasive diagnostic biomarkers for BC, but more work is needed in this space¹⁴.

Proteomic biomarkers
Several urinary protein biomarkers for BC have been identified. One study comparing 46 patients with BC and 40 healthy controls reported urinary calprotectin can detect the cancer type with 80% sensitivity and 92% specificity. The median calprotectin level was 10-fold higher in patients than healthy controls¹¹.

Other studies reported similar findings with a higher abundance of proteins in urine samples of BC patients (p < 0.05) compared with matched controls¹⁰.

For example, urinary proteins, stathmin-1 (also known as oncoprotein-18) and CD147 (also known as basigin or EMMPRIN) have also shown potential in BC detection. In a group of 30 patients and 30 controls, stathmin-1 had a sensitivity and specificity of 90% and 87% respectively, while CD147 had 97% and 100% respectively¹⁰. Additionally, increased levels of Reg-1 (lithostathine-1-alpha) were also found in urine of BC patients¹⁰.

Urithelial bladder carcinoma 1 (BLCA-1) and urithelial bladder carcinoma 4 (BLCA-4) are nuclear matrix proteins (NMPs) which can be elevated early in the development of BC, thereby can be used to detect the cancer in its initial stages, even before the appearance of a visible tumor⁸.