Summary

Bladder cancer (BC) is the fifth most common type of cancer in most western countries, and the seventh leading cause of cancer related deaths in Denmark. Most BC patients are men, but more women present with higher-stage tumors and have an overall worse prognosis than men. Hematuria is the most common symptom of BC, and warrants u*rologic* evaluation, in all cases where a simple urinary tract infection can be ruled out. However, less than 10% of patients with hematuria are diagnosed with BC, leading to vast resources being used to examine healthy persons. Furthermore, most patients who are diagnosed with bladder tumors experience recurrence, warranting life-long surveillance. Therefore, BC is the most expensive cancer form to treat, from diagnosis to death. Transurethral cystoscopy, which is considered the golden standard for detecting bladder tumors, has an estimated sensitivity of 71% using white light and 92% using fluorescence, and may therefore miss tumors. In 25% of all BC patients, the tumor is not discovered before it has become invasive, and the median survival for these patients is only 14-15 months. Hence, early detection of BC is essential for down-staging tumors and reducing mortality.

In paper I, we present a disposable device for filtration of urine, which has the potential for non-invasive BC detection when used in combination with downstream DNA testing. A high amount of normal cells in urine may reduce the chance of detecting tumor cells, especially for samples with few tumor cells present. The device provides a means for urine filtration in order to capture tumor cells and eliminate smaller-sized cells in urine, as well as provide easy and safe storage of the filter. Comparison of the standard procedure for urine processing, sedimentation, with filtration of urine showed that filtration resulted in an enrichment of tumor DNA. The performance of this device was further investigated by analysis of urine samples from 59 persons suspected of having bladder tumors. Using a panel of eight DNA methylation and mutation markers, a positive signal was detected in 29 of 31 urine samples from patients with biopsy-verified bladder tumors. Furthermore, positive methylation markers were detected in 13 of 26 urine samples, from patients who had no bladder tumor detectable by cystoscopy. Seven of these

persons were previous BC patients, suggesting that a pre-malignant lesion may be present in the bladder of these patients. In one of these samples a positive *FGFR3* mutation signal was also detected. This patient was diagnosed with a Ta tumor at follow-up examination six months after the urine sample was given, indicating that the urine based method might detect bladder tumors before they are visible by cystoscopy.

In the study described in paper II, we examined whether bladder tumor driver mutations could be detected in urine before clinical diagnosis of BC. Pre-diagnostic urine samples from 606 persons, who were diagnosed with BC between 1.2 months and 18.2 years after urine sampling, were tested for the presence of mutations in *TERT* and *FGFR3*. We were able to detect mutations in 153 of the urine samples up to 17.7 years prior to diagnosis, with the highest detection rate (above 60%) for patients diagnosed within the first three years after urine sampling. Our results show that mutations in *TERT* and *FGFR3* are early events in the development of BC, which indicates that these markers can be used for early detection of BC. We found that bladder tumors with invasive potential could be detected earlier than non-invasive tumors, and that women had detectable markers in urine earlier than men, which is important for down-staging tumors and improving the prognosis for women. We also analyzed 299 urine samples from participants who have not presented with any malignancies for 15 to 19 years after urine sampling. Two persons had detectable mutant DNA in the urine, and might represent cases with pre-malignant bladder lesions, which either have not progressed to a tumor or have regressed spontaneously.

The third paper describes a blinded prospective study, where the performance of the urine filtration device described in paper I was evaluated by comparison to golden standard cystoscopy. A total of 475 patients with macroscopic hematuria who had been referred to urologic evaluation, were included in the study. Patients were examined by cystoscopy, computed tomography (CT) urography and urine cytology; 99 patients were diagnosed with bladder tumors and 376 had normal findings. Patients delivered a urine sample before and/or after flexible cystoscopy, and these samples were processed using the urine filtration device. Using a panel of six methylation and two mutation markers, we detected tumor DNA in urine

from 96 of the 99 patients with bladder tumors, resulting in a sensitivity of 97% for the test. The sensitivity of the test was lower when applied to one sample per patient, obtained either before or after cystoscopy (92% and 94%, respectively). This corroborates previous findings that pooling urine samples may increase the chance of detecting tumor cells using DNA-based methods. An analysis of the tumors in the three patients with a false negative test revealed that the tumors did not display any of the markers in the panel, necessitating an improvement of the marker panel for future studies, in order to insure complete coverage of bladder tumors. We also found positive markers in urine from 87 patients, who had no visible tumor at cystoscopy. Follow-up of these patients revealed three cases with BC (diagnosed between 0.5 and 1.3 years after initial examination), indicating that the urine-DNA test may detect bladder tumors before they are visible by cystoscopy.

The work presented here demonstrates the potential of filtration-based urine-DNA testing for non-invasive detection of BC. Further use could be screening programs for early detection of BC, as DNA markers for BC can be detected in urine years and even decades before the appearance of symptoms.