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# Superior molecular pathology of urothelial bladder cancer using urinary cell-free DNA

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Predicine 慧渡医疗

## INTRODUCTION

Current methods for diagnosing and monitoring bladder cancer patients are often invasive and/or lack sensitivity and specificity. Urine- and blood-based circulating cell-free DNA (cfDNA) assays have been developed for the noninvasive detection and surveillance of urothelial bladder cancer (UBC). A direct comparison of their diagnostic performance in the real-world setting is lacking.

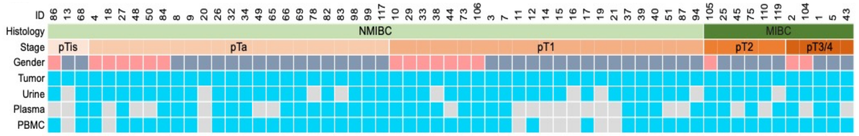
Here, we applied the 152-gene PredicineCARE liquid biopsy assay for targeted sequencing and somatic alteration identification using matched tumor tissue, urine, blood and PBMC samples from patients with non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Results demonstrated that urine-based molecular pathology can provide valid and complete genetic information that represents a faithful surrogate for genotyping UBC.

## METHODS

### Patients and samples

59 eligible cases with pathologically confirmed disease were prospectively enrolled and consented to Institutional Review Board-approved protocols.

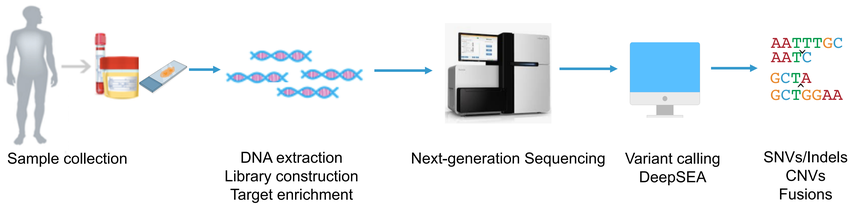
Tissue samples from 59 patients, urine samples from 50 patients, and plasma samples from 36 patients were analyzed in this study.




### Targeted NGS assay

The 152-gene PredicineCARE liquid biopsy assay was applied for somatic alteration identification in tissue-based tumor DNA (tDNA), urinary tumor DNA (utDNA), blood circulating tumor DNA (ctDNA), and genomic DNA from PBMC. NGS assays were completed at the Huidu Laboratory in Shanghai, China.

Fig 1. Workflow for PredicineCARE, a targeted NGS assay for tissue, urine and blood.




## RESULTS

**Figure 1.** Frequently mutated genes detected from tissue tumor DNA (tDNA), urinary tumor DNA (utDNA) and blood circulating tumor DNA (ctDNA) in NMIBC and MIBC. Mutations detected in utDNA and tDNA are highly concordant and recapitulate tissue-based mutational landscape reported in literature [1-3].

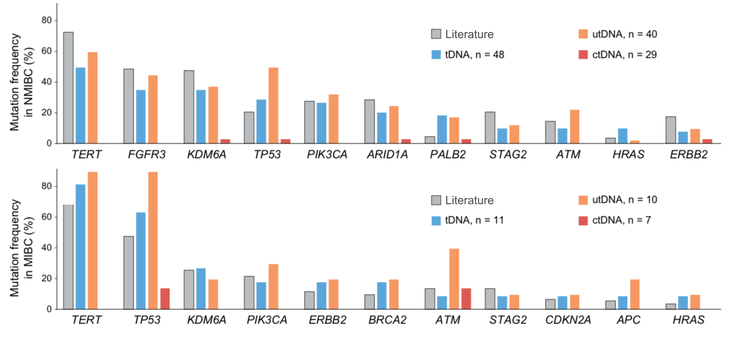



**Figure 2.** Superior performance for variant detection in utDNA vs. ctDNA in bladder cancer. Venn plots showed the proportion of overlapping variants detected in tDNA and cfDNA. Bar plots show the variant-level sensitivity, specificity, PPV, NPV, and accuracy of liquid biopsies using tDNA-informed results as the gold standard.

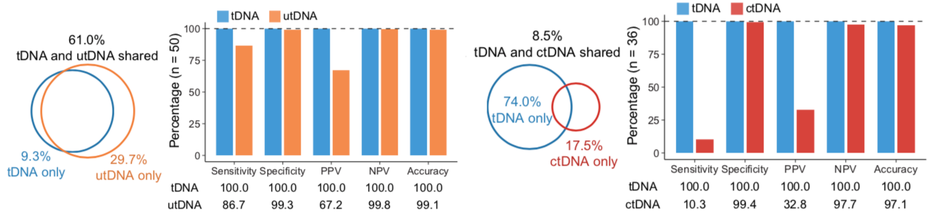



**Figure 3.** Dynamic perioperative changes of mutations in a representative patient with paired pre- and post-operation observed in utDNA but not ctDNA. Reduction in the VAF of TERT and TP53 variants in utDNA after curative-intent surgery.

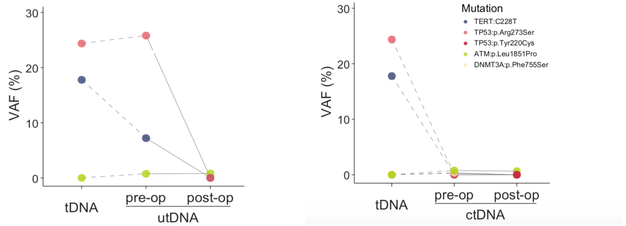



**Figure 4.** Kaplan-Meier analysis of relapse-free survival in 50 patients according to the mutation status of *APC* and *PIK3CA* genes from tDNA (upper) or utDNA (lower) testing. *P-values* were based on log-rank tests.

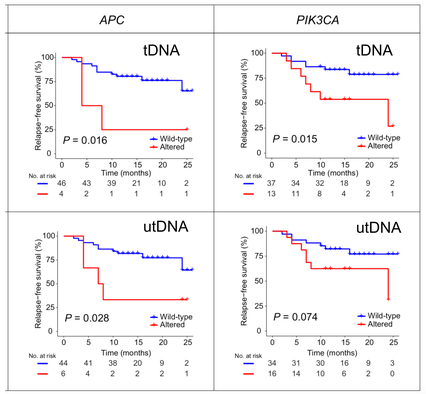
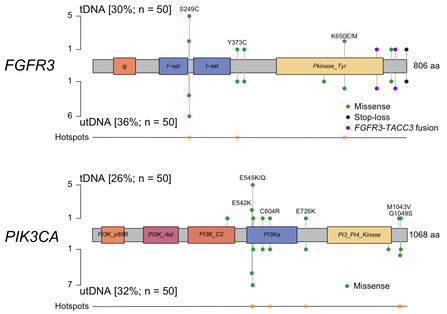



**Figure 5.** Lollipop view of the mutation and fusion pattern for actionable genes *FGFR3* and *PIK3CA* in tDNA and utDNA.




## CONCLUSIONS

- Mutational profiling of utDNA but not ctDNA exhibited multidimensional concordance with that of corresponding tumor tissue in bladder cancer.
- Our results demonstrated the potential clinical utility of urine cfDNA-based molecular profiling for treatment selection, therapy monitoring and minimal residual disease in bladder cancer.

1. *Robertson AG, et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell. 2017;171:540-56.e25.*
2. *Pietzak EJ, et al. Next-generation Sequencing of Nonmuscle Invasive Bladder Cancer Reveals Potential Biomarkers and Rational Therapeutic Targets. Eur Urol. 2017;72:952-9.*
3. *Rachakonda PS, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proceedings of the National Academy of Sciences. 2013;110:17426.*