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Development and clinical application of PredicineBEACON next-generation minimal residual disease assay for genitourinary cancers.

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INTRODUCTION

Tumor-informed minimal residual disease (MRD) assay has been studied in muscle invasive bladder cancer (MIBC) in IMvigor010 study. However, the current methods require the use of tumor tissue and lacking actionable insights. In this study, a tumor-agnostic and actionable minimal residual disease (MRD) assay (PredicineBEACON) is developed with high sensitivity and capability to detect actionable mutations in blood- or urine-based circulating tumor DNA.

METHODS

PredicineBEACON tumor-agnostic MRD assay includes three components, i.e., baseline mutation identification, personalized panel design, and ultra-deep next-generation sequencing of cancer variants. Step 1: whole exon sequencing (WES) with boosted depth in 600 cancer-related genes is performed using baseline tumor tissue or liquid biopsy (blood or urine) to identify somatic mutations. Step 2: Up to fifty somatic mutations are selected for personalized MRD panel design, in combination with the use of a fixed core panel of 500 actionable/hotspot variants. Step 3: MRD and actionable mutations were called from ultra-deep sequencing (100,000X). A companion low-pass whole-genome sequencing (LP-WGS) is performed to monitor copy number variation (CNV).

In the current study of patients with MIBC, urine samples at baseline were used for personalized variant generation. Urine samples under neoadjuvant immunotherapy were collected and tested with the PredicineBEACON MRD assay (Fig 1).

Fig 1. Urine based PredicineBEACON MRD assay.

RESULTS

Analytic Performance of PredicineBEACON MRD assay:

Number of mutations traced for MRD detection		Tumor fraction 0.0025%	Tumor fraction 0.005%	Tumor fraction 0.01%	Tumor fraction 0.025%	Tumor fraction 0.05%
32	Average number of mutations detected	1.5	3.75	5.75	12.5	20
	Sensitivity	50%	100%	100%	100%	100%
16	Average number of mutations detected	0.75	1.88	2.88	6.25	10
	Sensitivity	0	50%	100%	100%	100%

Table 1. Sensitivity of PredicineBEACON MRD assay.
Plasma cfDNA from cancer patients was diluted in normal cfDNA background at five tumor fraction levels: 0.05%, 0.025%, 0.01%, 0.005%, 0.0025%. 32 and 16 somatic mutations were selected from baseline and used for MRD tracking. The assay reached 100% sensitivity with a tumor fraction greater than 0.005%.

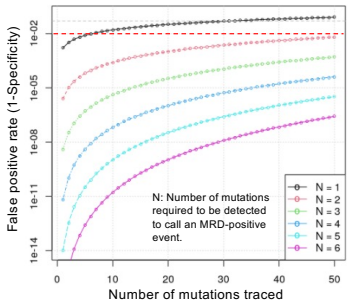
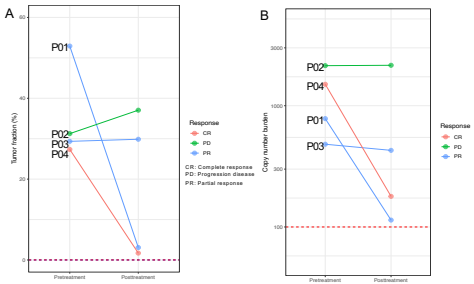


Fig 2. Specificity of PredicineBEACON MRD assay.
To evaluate the specificity of MRD calling, plasma samples from healthy donors were tested with the MRD assay. The assay reached >99% specificity when 2 or mutations were required to be detected to call an MRD-positive event by tracking up to 50 mutations.

Urine based MRD detection in MIBC cancer patients:

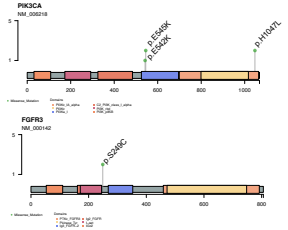
Four patients with MIBC under neoadjuvant immunotherapy were tested with PredicineBEACON assay. Urine samples were collected before and after neoadjuvant therapy (NAT). For MRD testing, urine samples collected before NAT were treated as the baseline and tested with WES, and fifty somatic mutations were selected for MRD tracking. LP-WGS sequencing was performed for the urine samples. Tumor fraction inferred from the MRD test (Fig 3. A) and copy number burden (CNB) inferred from the LP-WGS test (Fig 3. B) both correlate with the clinical response under NAT.

Fig 3. Changes of tumor fraction and CNB in urine before and after neoadjuvant therapy



A. Tumor fraction inferred from MRD test. All four patients were detected as MRD positive after four cycles of NAT. Tumor fraction was decreased in patient P04 (complete response) and P01 (partial response) and increased in patient P02 (progression disease) during NAT.
B. CNB detected from LP-WGS test. All four patients were detected with abnormal CNB. CNB was decreased in patient P04 (complete response) and P01 (partial response) and increased in patient P02 (progression disease) during NAT.

Fig 4. Actionable mutations detected from the MRD test.



PatientID	Urine sample collection	Gene	Mutation	MAF(%)
P01	Pretreatment	TERT	C250T	35.79
P01	Pretreatment	FGFR3	p.S249C	50.20
P01	Posttreatment	FGFR3	p.S249C	1.49
P01	Pretreatment	PIK3CA	p.E542K	9.30
P01	Posttreatment	PIK3CA	p.E542K	0.08
P01	Pretreatment	PIK3CA	p.E545K	36.09
P01	Posttreatment	PIK3CA	p.E545K	1.80
P02	Pretreatment	TERT	C228T	28.38
P02	Posttreatment	TERT	C228T	26.62
P02	Pretreatment	PIK3CA	p.H1047L	47.35
P02	Posttreatment	PIK3CA	p.H1047L	43.10
P03	Pretreatment	TERT	C228T	28.24
P03	Posttreatment	TERT	C228T	26.67
P04	Pretreatment	TERT	C228T	19.89

CONCLUSIONS

PredicineBEACON tumor-agnostic MRD assay provides an ultra-sensitive and actionable MRD detection with high sensitivity and specificity. Assessment of MRD using urine could potentially be leveraged, for example, to guide the treatment of early stage of genitourinary cancers such as non-metastatic prostate cancer, non-muscle-invasive bladder cancer and muscle-invasive bladder cancer.