



Longitudinal personalized urinary tumor DNA analysis in muscle invasive bladder cancer from the neoadjuvant immunotherapy trial RJBLC-I2N003.

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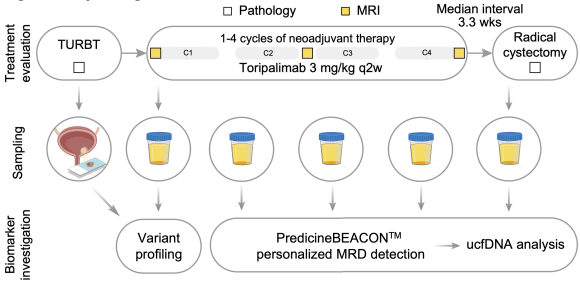
INTRODUCTION

RJBLC-I2N003 is an investigator-initiated study to evaluate the clinical activity and predictive biomarkers for neoadjuvant immunotherapy with toripalimab (anti-PD-1) in muscle invasive bladder cancer (MIBC). Here we present the evaluation of the PredicineBEACON™ personalized MRD assay to monitor response to therapy in this trial. Twenty patients were enrolled in this study. Sixteen patients (80%) completed all 4 cycles of neoadjuvant treatment. Grade 3-4 immune-related adverse events occurred in two patients (10%). Eight patients (40%) achieved a pathological complete response (pCR). Thirteen patients (65%) had no remaining invasive disease (pCR or pTisN0/pTaN0). On-treatment urinary tumor DNA (utDNA) clearance was associated with objective responses. Preliminary concordance was observed between molecular and pathological MRD status.

METHODS

Twenty patients with pathologically confirmed MIBC were enrolled and received toripalimab (3 mg/kg Q2W, 4 cycles) before radical cystectomy. The safety and efficacy of neoadjuvant toripalimab were assessed. Serial urinary cell-free DNA (ucfDNA) and blood cell-free DNA (bcfDNA) were obtained at baseline and after each cycle of toripalimab treatment. The PredicineBEACON™ personalized MRD assay was used for therapy monitoring, which includes whole exome sequencing of baseline urine and/or tissue samples collected before neoadjuvant therapy, followed by ultra-deep sequencing of urine samples to track 50 personalized mutations and 500 actionable genes, in combination with low-pass whole genome sequencing (LP-WGS) to assess copy number burden (CNB).

Fig 1. Study design



Clinical response of MIBC patients undergoing neoadjuvant immunotherapy with toripalimab (anti-PD-1).

- Three patients (15%) achieved complete response (CR), and eleven patients (55%) achieved partial response (PR). The overall response rate (ORR, CR or PR) was 70% (Fig 2 and Fig 3).
- Grade 3-4 immune-related adverse events occurred in two patients (10%) (Fig 2).
- Eight patients (40%) achieved a pathological complete response (pCR) (Fig 3).

Fig 2. Treatment and imaging evaluation of patients.

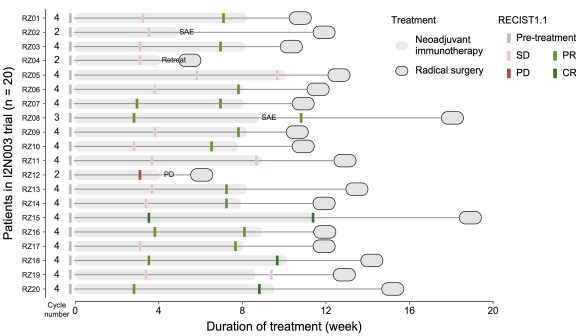
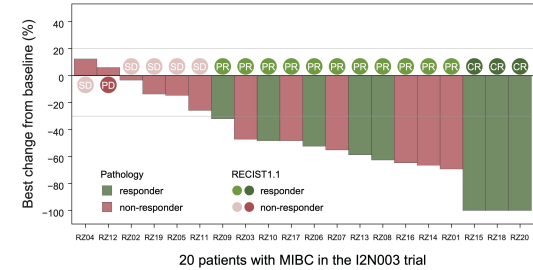


Fig 3. Best percentage change in tumor size from baseline. Green and red color in circles indicate responder and non-responder assessed by the RECIST 1.1. Green and red color in rectangles indicate responder and non-responder assessed by the pathology.



RESULTS

PredicineBEACON™ - personalized MRD assay for therapy monitoring.

Pre- and post-neoadjuvant immunotherapy urine samples were collected from eighteen patients and tested with the PredicineBEACON™, the personalized MRD assay. MRD positive was called when two or more mutations were detected or copy number burden (CNB) above defined threshold.

Fig 4. PredicineBEACON™ MRD-based urinary tumor DNA (utDNA) clearance was associated with objective responses. Cancer cell fraction (CCF) inferred from personalized mutation tracking was significantly lower in the responder group ($P < 0.036$) after neoadjuvant immunotherapy, and a similar trend was observed for CNB ($P < 0.060$).

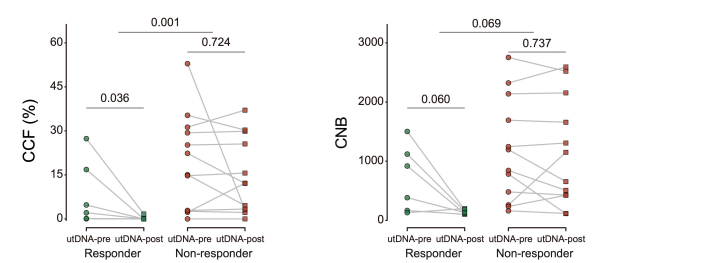


Fig 5. MRD status post-neoadjuvant immunotherapy correlates with clinical response. Fifteen MRD+ (83.3%) and three MRD- (16.7%) were detected from the urine samples collected from post-neoadjuvant immunotherapy. Under RECIST1.1 criteria, 2/3 (66.7%) patients in CR group were detected as MRD-, 8/9 (88.9%) patients in PR group were detected as MRD+, and all (100%) patients in SD and PD were detected as MRD+. Under pathology assessment criteria, 3/6 (50%) patients in CR group were detected as MRD-, and all (100%) patients in non-CR group were detected as MRD+.

RECIST1.1	R220	R218	R215	R208	R213	R206	R210	R209	R201	R214	R216	R207	R217	R203	R211	R205	R219	R202	R212	R204
Pathology	CR	CR	CR	CR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	SD	SD	SD	SD	SD	SD
MRD	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

NA: sample not available.

CONCLUSIONS

These findings suggest that neoadjuvant administration of PD-1 blockade followed by surgical resection represents a feasible and efficacious approach to treat MIBC. PredicineBEACON™-based urine MRD biomarker assessment identified MRD-positive patients that achieved pCR, demonstrating the potential clinical utility of longitudinal personalized utDNA analysis to complement existing trial endpoints. This study suggests that urine-based MRD test could be used to identify MRD-negative MIBC patients after neoadjuvant therapy who could potentially avoid radical cystectomy.