Predicting pathological response after ipilimumab plus nivolumab in stage III urothelial cancer by liquid biopsy assessment of plasma and urine ctDNA using the RaDaR assay

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Introduction

- Patients with stage III (cT3-4aN0M0 or cT1-4aN1-3M0) urothelial cancer (UC) have a poor prognosis.
- Currently, there are no good biomarkers to assess response before surgery, potentially leading to overtreatment and unnecessary surgical complications.
- The NABUCCO study is an open-label, phase Ib trial evaluating the effects of short-term neo-adjuvant (**NeoAdj**) treatment with two immune checkpoint inhibitors, ipilimumab (ipi) and nivolumab (nivo) in patients with high-risk resectable UC (**Figure 1**).
- Here, we investigated whether detection of circulating tumor DNA (ctDNA) in longitudinally-collected plasma and urine samples using Inivata's RaDaR™ personalized liquid biopsy assay was associated with treatment response and outcomes (**Figure 2**).

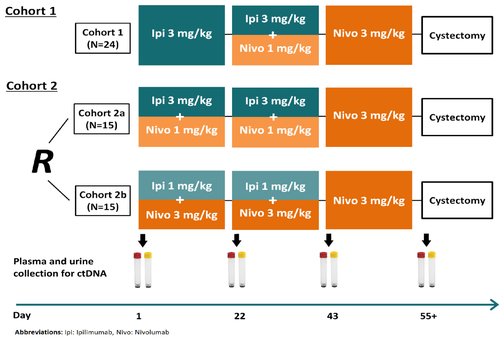

Figure 1. NABUCCO Cohort 1 and 2 study design. EDTA-plasma and urine samples were collected from patients with stage III urothelial cancer prior to initiation of each one of the three pre-operative treatment cycles with ipilimumab and/or nivolumab at day 1 (**Baseline/Pre-C1**), day 22 (**Pre-C2**) and day 43 (**Pre-C3**). Samples were also collected prior to surgery (**Pre-Surgery**) to investigate whether post-treatment ctDNA levels correlate with treatment response and outcome.

Methods

- A total of 24 NABUCCO Cohort 1 patients were retrospectively analyzed in this study (**Table 1**).
- 14/24 patients (58%) showed a pathological response (ypT0/Tis/Ta N0).
- Whole exome sequencing (WES) was performed on patients' formalin-fixed, paraffin-embedded (FFPE) tumor tissue obtained prior to systemic treatment and peripheral blood DNA to identify patient-specific somatic variants.
- Personalized, multiplex PCR-based NGS RaDaR panels were designed to target a median of 48 WES-derived somatic variants (range: 43-51).
- Plasma and urine ctDNA collected at the pre-defined timepoints (**Figure 1**) was analyzed using these panels to determine ctDNA detection and their estimated variant allele frequency (**eVAF**).

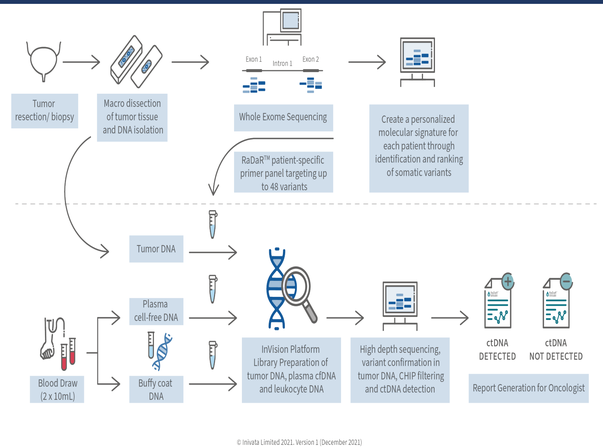

Figure 2. The RaDaR Workflow. Steps involved in the design of personalized RaDaR assays, from tumor tissue WES profiling to variant identification and selection for panel design and plasma analysis for monitoring response to treatment.

Results

Table 1. NABUCCO Study Cohort 1 Patient Characteristics

Patient Demographics	
Males	18/24 (75%)
Median age (years) at time of cystectomy (range)	65 (50-81)
Primary Tumor Location	
Lower tract (Bladder-only disease)	24/24 (100%)
Upper tract (Kidney and ureter disease)	1/24 (4%) [a]
Clinical tumor and nodal stage	
cT3-4aN0	14/24 (58%)
cT2-4aN1-3	10/24 (42%)
Number of treatment cycles	
2 [b]	6/24 (25%)
3	18/24 (75%)

[a] One patient had bladder and upper tract disease; all others had bladder-only disease.

[b] Six patients received the first two cycles of systemic therapy and were not eligible for the third cycle due to toxicity.

Overall Detection

- ctDNA was detected in 50/94 plasma samples (53%) and in 74/93 urine samples (80%).
- Detection levels were higher in urine with a median eVAF of 1.98% (range: 0.00057%-35.85%) vs. 0.049% (range: 0.00026%-18.94%) in plasma.

Changes in plasma ctDNA levels reflect clinical response

- ctDNA was detected in pre-NeoAdj baseline plasma samples in:
  - 10/14 (71%) patients with pathological response (**median eVAF:** 0.325%, **range:** 0.001-12%), and
  - 8/10 (80%) non-responders (**median eVAF:** 0.107%; **range:** 0.009-1.2%).
- Following NeoAdj treatment and prior to surgery, ctDNA was undetected in:
  - 13/14 (93%) patients with pathological response and in 4/10 (40%) of non-responders (***p*=0.0088**).
  - Of the 17 patients with undetected ctDNA following NeoAdj treatment and before surgery, 13 (76%) had pathological response and 16 (94%) remained recurrence-free after a median follow-up of 34 months.

Pre-surgery urine ctDNA levels are not associated with clinical response

- ctDNA was detected in pre-NeoAdj baseline urine samples in:
  - 12/14 (86%) patients with pathological response (**median eVAF:** 9.634%; **range:** 0.093-35.85%), and
  - 8/10 (80%) non-responders (**median eVAF:** 2%; **range:** 0.026-7.05%).
- Following NeoAdj treatment and prior to surgery, urine ctDNA was detected in:
  - 8/14 (57%) patients with pathological response (**median eVAF:** 0.87%, **range:** 0.0006-9.1%), and
  - 8/10 (80%) non-responders (**median eVAF:** 0.162%, **range:** 0.0009-10.97%).
- No significant association was observed between urine ctDNA detection and response (***p*=0.39**).

Longitudinal monitoring of ctDNA dynamics in response to treatment and association with outcomes

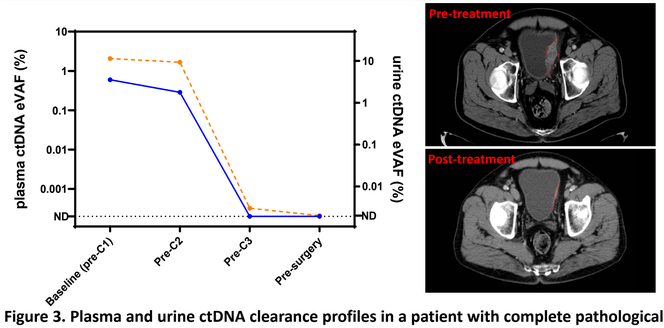

Figure 3. Plasma and urine ctDNA clearance profiles in a patient with complete pathological response. Presence of a cT3N1 tumor (pre-treatment scan) correlated with detection of plasma (blue solid line) and urine (orange dashed line) ctDNA at baseline (Pre-C1) at an estimated variant allele frequency (eVAF) of 0.597% and 11.398%, respectively. Plasma and urine ctDNA clearance prior to surgery was associated with complete pathological response (pCR, ypT0N0). No recurrence has been documented 39 months post-cystectomy.

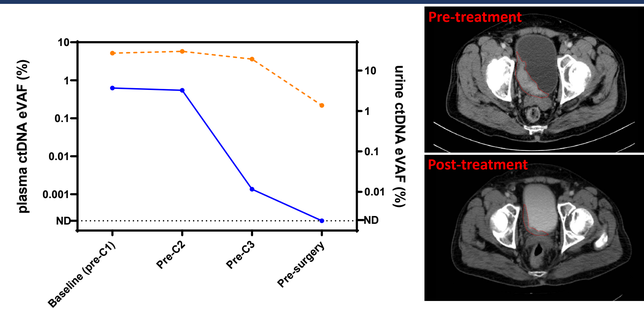

Figure 4. Plasma and urine ctDNA clearance profiles in a patient with complete pathological response. Baseline plasma (blue solid line, 0.622% eVAF) and urine (orange dashed line, 26.938% eVAF) ctDNA detection in a patient with a cT3N2 tumor. Following three pre-operative treatment cycles and prior to surgery, ctDNA was undetected in plasma and was associated with a confirmed complete pathological response (ypT0N0). In contrast, persistent ctDNA levels (1.369% eVAF) were still present in urine. The patient is still disease-free after a follow-up period of 41 months following cystectomy.

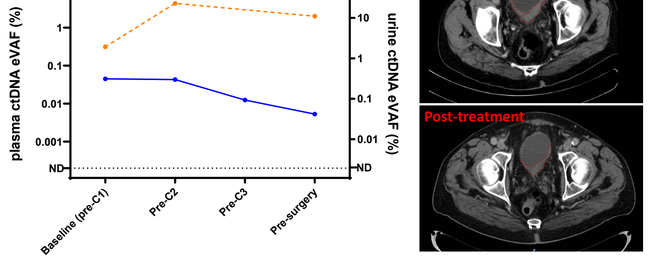

Figure 5. Plasma and urine ctDNA profiles in a patient with no pathological response. Baseline plasma (blue solid line; 0.045% eVAF) and urine (orange dashed line, 1.918% eVAF) ctDNA levels in a patient with a cT4aN1 tumor. Persistent detection of ctDNA just before surgery (eVAF plasma: 0.005% and urine: 10.966%) was associated with lack of treatment response (ypT2bN0). The patient eventually developed metastatic disease 8.5 months after cystectomy.

Discussion

Detection of plasma ctDNA by RaDaR after neoadjuvant treatment was associated with pathological response and clinical outcome. In contrast, ctDNA detection in urine was not associated with outcomes. Absence of plasma ctDNA pre-surgery may predict complete response to ipi plus nivo at surgery and may be helpful in guiding clinical decisions in stage III UC, in particular to select patients for bladder-sparing strategies. Further work involving patients included in NABUCCO Cohort 2 is currently underway.

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Disclosures

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- The presenting author has no conflicts of interest to declare.
- The authors were fully responsible for all content and editorial decisions, were involved in all stages of poster development and have approved the final version.