Sequencing of Plasma cfDNA from Patients with Locally Advanced Bladder Cancer for Surveillance and Therapeutic Efficacy Monitoring

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Introduction

- Studies on different cancer types have shown that circulating tumor DNA (ctDNA) levels can be efficiently used to monitor treatment response and/or detect disease recurrence earlier than clinical and radiological detection.[1-3]
- In bladder cancer, mutations in plasma have been previously used to monitor response during treatment and identify early signs of metastatic disease.[4-5]
- Recently, longitudinal ctDNA detection in patients with non-small cell lung cancer was described[6] and a personalized ctDNA detection assay was developed and made available for research use only (Signatera™ RUO).

Objectives

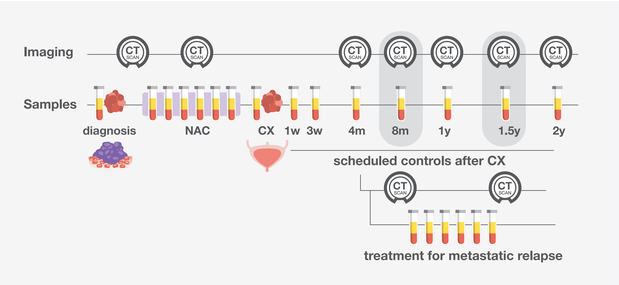
The aim of the study was to use patient-specific mutations identified in the primary tumor to detect metastatic relapse, evaluate prognosis, and monitor treatment response in cell-free DNA (cfDNA) from longitudinally-collected plasma samples.

Methods

Clinical Protocol

- Patients diagnosed with locally advanced muscle-invasive bladder cancer (MIBC) were prospectively recruited between 2013 and 2017.
- All patients were treated with neoadjuvant or first line chemotherapy before radical cystectomy (CX) and had up to 2 years follow-up (**Figure 1**); a small subset of patients who volunteered for additional follow-up visits were monitored for greater than 2 years.
  - Plasma samples were longitudinally collected before, during and after therapy at scheduled control visits.

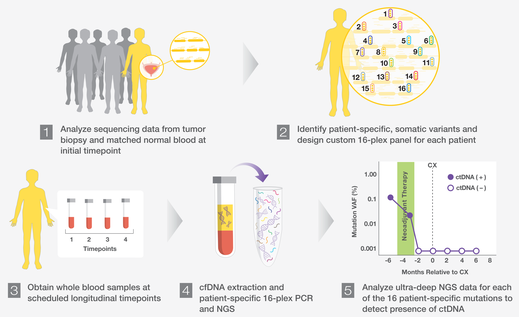
Figure 1. Clinical Protocol and Sampling



Molecular Protocol (Signatera RUO)

- The workflow of the molecular protocol is outlined in **Figure 2**.
- Patient-specific somatic mutations were identified by whole exome sequencing (WES) of tumor and matched normal blood.
- Personalized multiplex-PCR assays and NGS were used to detect patient-specific tumor DNA in plasma using cfDNA from longitudinally-collected plasma samples.
- For each patient, sequencing of 16 tumor-specific targets was performed and data were analyzed in a clinically-blinded fashion for the presence of ctDNA.
- Samples were considered ctDNA positive if at least two patient-specific targets were called that met the qualifying confidence score threshold.
- Clinical results (radiographic imaging and treatment response) were unblinded and were directly compared with the plasma call results.

Figure 2. Schematic of Molecular Protocol



Results

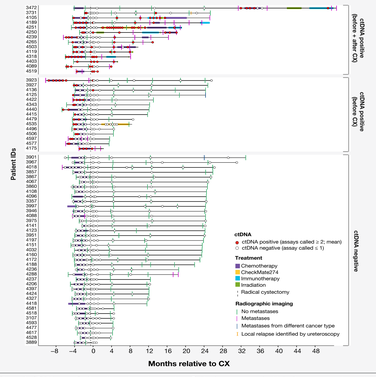
- A total of 68 patients were included in the study (**Table 1**).

Table 1. Patient Characteristics and Demographics

Patients, N = 68 Age[a] (years), median (range) = 65.2 (43–79)			
Gender, n (%)		**Tumor stage at CX,[b] n (%)**	
Female	12 (17.6)	T0/Cis/Ta	44 (64.7)
Male	56 (82.4)	T1/T2/T3/T4a	22 (32.3)
Tumor stage at TUR-B, n (%)		**Metastatic Relapse after CX,[c] n (%)**	
T1/T2	58 (85.3)	Local relapse (pelvis, rectum, urethra)	6 (17.6)
T4a/b	10 (14.7)	Distant metastases (bone, lung, liver, skin)	8 (11.8)
N stage before treatment, n (%)		**Clinical follow-up after CX, days (range)**	
N0	58 (85.3)	Disease free (n=52)	498 (103–991)
N1/N2	10 (14.7)	Clinical relapse (n=14)	321 (57–1119)
		CX unsuccessful (n=2)	-

[a]At sampling; [b]n=66; [c]n=14; CX, cystectomy

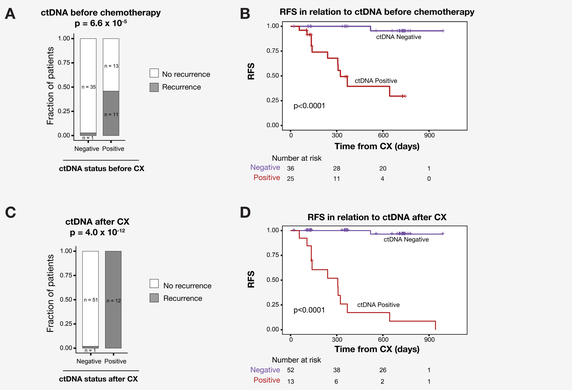
Figure 3. Summary of Individual ctDNA Analysis



Longitudinal representation of ctDNA results for all analyzed samples. Patients are separated into three groups based on ctDNA status. Horizontal lines represent patients and circles represent ctDNA status, red circles indicate samples with at least 2 positive assays. Treatment and imaging information is indicated for each patient. CX, cystectomy.

Prediction of Outcome

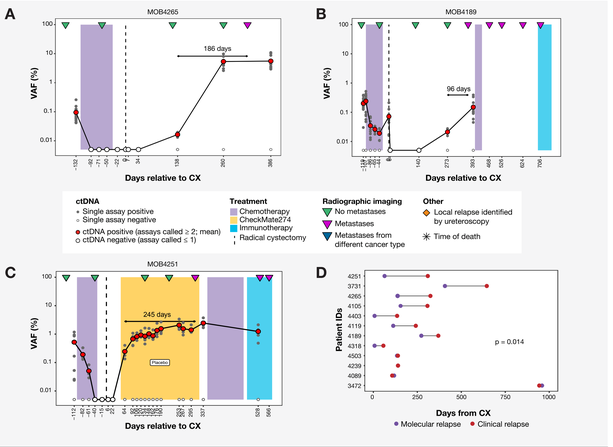
Figure 4. Recurrence-Free Survival and ctDNA Status Before Chemotherapy and After Cystectomy (CX)



Prediction of Outcome (A) and **(C)** Association between disease recurrence and ctDNA status before chemotherapy and after CX. **(B)** and **(D)** Kaplan-Meier survival analysis showing probability of recurrence-free survival (RFS) stratified by ctDNA status before chemotherapy and after CX.

Early Detection of Relapse

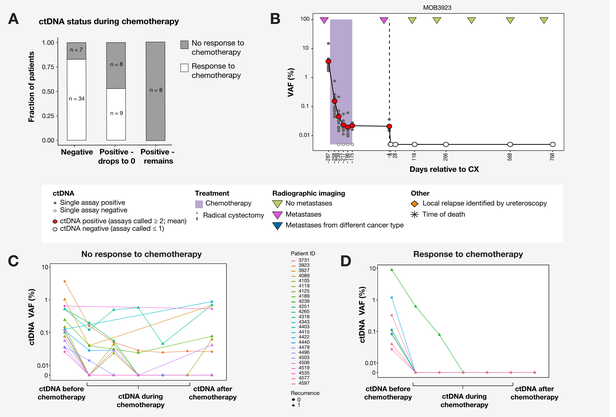
Figure 5. Early Relapse Detection



ctDNA-based relapse detection. (A-C) Representation of detailed disease courses, applied treatments and associated longitudinal ctDNA analyses from selected patients. ctDNA status, applied treatment and imaging results are presented according to the legend. Positive lead times for ctDNA based recurrence detection are indicated **(D)** Lead time between molecular recurrence (ctDNA positivity) and clinical recurrence (radiographic imaging positive). P-value was calculated using a paired Wilcoxon rank-sum test. CX, cystectomy; VAF, variant allele frequency.

Response to Therapy

Figure 6. Treatment Response



Response to Therapy (A) Association between ctDNA and response to chemotherapy for patients being ctDNA negative throughout the whole disease course, patients where ctDNA level drops down to zero and patients where ctDNA level remains to be positive. **(B)** Depiction of treatment response in representative patient. **(C)** and **(D)** ctDNA VAF (variant allele frequency) is shown for all patients with detectable ctDNA before, during and after chemotherapy. Patients are grouped by response to chemotherapy, and recurrence status is indicated.

Conclusions

- ctDNA analyses (eg, via Signatera) can help inform on treatment response and identify disease recurrences with an average lead time of 100 days compared to radiographic imaging.
- Survival analyses identified significantly shorter relapse-free survival for patients with ctDNA both at diagnosis and after CX.
- Ultimately, ctDNA analysis could be incorporated into routine follow-up for early detection of relapse and consequently earlier initiation of treatment.
- The benefit in overall survival gained by ctDNA relapse detection should be assessed in randomized clinical trials.

References

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