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

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Abstract Number 3653

Introduction

- Studies on different cancer types have shown that circulating tumor DNA (ctDNA) levels can be efficiently used to monitor treatment response to neoadjuvant therapy 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 circulating tumor DNA (ctDNA) deteciton assay was developed and made available for research use only (Signatera™ RUO).

Objective

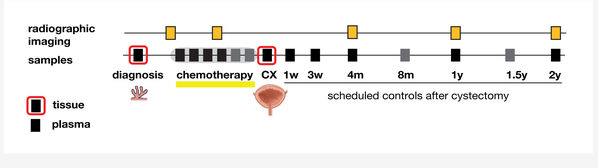
- The aim of the study was to use patient-specific mutations identified in the primary tumor to detect metastatic relapse, evaluate prognosis, and monitor treatment response in ctDNA from longitudinally-collected plasma samples.

Methods

Clinical Protocol

- Patients diagnosed with locally advanced muscle-invasive bladder cancer (MIBC) and scheduled for chemotherapy were prospectively recruited between 2013 and 2017.
- All patients were treated with neoadjuvant or first line chemotherapy before cystectomy (CX) and had up to 2 years follow-up (**Figure 1A**).
  - Plasma samples were longitudinally collected pre- and post-systemic therapy and at scheduled control visits after CX.

Figure 1. Schematic of Clinical Sample Collection



Molecular Protocol (Signatera RUO)

- Patient-specific somatic mutations were identified by whole exome sequencing (WES) of tumor and matched normal samples.
- Personalized multiplex-PCR assays 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 analayzed in a clinically-blinded fashion for the presence of ctDNA.
- Samples were considered ctDNA positive if and only if at least two positive patient-specific targets were called and met the qualifying confidence score threshold.
- Cinical results (radiographic imaging and treatment response) were unblinded and compared directly to the Signatera plasma call results.

Results

- A total of 50 patients were included in the study (**Table 1**).

Table 1. Patient Characteristics and Demographics (N=50)

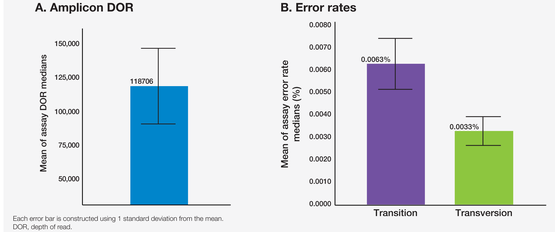
Age (years),[a] mean (range)	65.5 (43-77)		
Gender, n (%)		**Tumor stage at CX,[b] n (%)**	
Females	9 (18)	T0/Cis/Ta/T1	36 (72)
Male	41 (82)	T2/T3/T4a	12 (24)
Tumor stage at TUR-B, n (%)		**Metastasis,[c] n (%)**	
T1/T2	43 (86)	Local relapse (pelvis, rectum, urethra)	6 (12)
T4a/b	7 (14)	Distant metastases (bone, lung, liver, skin)	6 (12)
N stage before treatment, n (%)		**Clinical follow-up, days (range)**	
N0	43 (86)	Disease free (CX; n=38)	434 (119-778)
N1/N2	7 (14)	Clinical relapse (CX; n=10)	347 (65-973)
		Progression (CX unsuccessful; n=2)	280 (105-455)

[a]At sampling; [b]n=48; [c]n=12.
CX, cystectomy.

Plasma Sequencing QC

- In-line sequencing QC was performed across 5 HiSeq PE 2x50 runs; average per target depth-of-reads and background error rates are shown in **Figure 2**.

Figure 2. Plasma Sequencing QC



Each error bar is constructed using 1 standard deviation from the mean.
DOR, depth of read.

Early Detection of Relapse

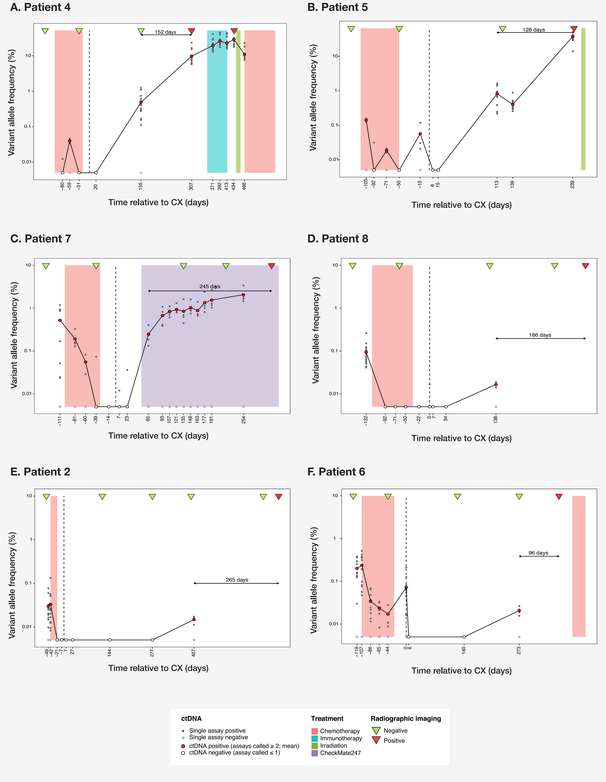
- Clinical relapse after CX was diagnosed for ten patients and ctDNA has been identified in plasma in nine of these patients with a median of 128 days prior to clinical relapse (**Table 2**). For one patient, the last control sample at 4 months post CX has not been analyzed yet.

Table 2. Molecular Versus Clinical Relapse (n=9)

Patient no.	Diagnosis stage	CX stage	ctDNA detection lead time (days)	Clinical relapse after CX (days)	Location of metastasis
1	T2, N0	T2, N0	0	973	bone
2	T2, N0	T0, N0	265	624	local (urethra)
3	T4a, N0	T4a, N0	0	119	local (pelvis)
4	T2, N0	T3, N0	152	298	LN, lung, bone, local (pelvis)
5	T2, N0	T2, N3	128	241	Liver, bone
6	T2, N0	T4a, N0	96	379	local (pelvis)
7	T2, N0	T1, N3	245	309	local
8	T4b, N0	T3, N0	186	324	local (rectum)
9	T4b, N0	T4a, N2	50	65	liver

CX, cystectomy.

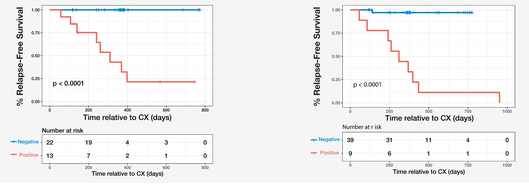
Figure 3. Early Relapse Detection



- **Figure 3** depicts 6 patients that had early relapse detection.
- ctDNA was detected at VAFs as low as 0.02% at the time of molecular recurrence, which had a lead time of up to 265 days prior to clinical recurrence (**Figures 3D, 3E and 3F**).

Prediction of Outcome

Figure 4. Relapse-Free Survival and ctDNA Status at Diagnosis and Post Cystectomy



Treatment Response

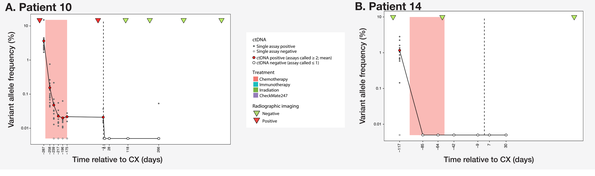
Table 3. Prediction of Treatment Response following Chemotherapy

Patient no.	Diagnosis stage	ctDNA VAF levels (%)	CX stage	ctDNA VAF levels (%)
10[a]	T4a	3.6	T2, N0	0.02
11	T2	0.1	T0, N0	ND
12[b,c]	T1	0.3	T4a, N0	0.05
13	T2	0.1	T0, N0	ND
14	T2	1.2	CIS, N0	ND
15[a]	T1	0.3	T2, N0	ND

[a]Received first line chemotherapy before CX. [b]Underwent TUR-P before TUR-B, T1 tumor from Urethra, pars prostatica. [c]Treatment response defined by advancment from inoperable to operable state after chemotherapy (despite clinical upstaging).
VAF, variant allele frequency; CX, cystectomy; ND, not detected.

- In most patients, response to chemotherapy corresponded to a decrease in ctDNA VAFs (**Table 3**).

Figure 5. Neoadjuvant Treatment Response



- **Figure 5** depicts two patients in which the treatment response was observed using ctDNA; ctDNA detected initially at diagnosis declined with neoadjuvant treatment and remained undetected after CX.

Conclusions

- These data demonstrate that ctDNA analyses (e.g., via Signatera) can help inform on treatment response and identify disease recurrences up to 265 days earlier than radiographic imaging.
- Survival analyses identified significantly lower relapse free survival for patients with ctDNA at diagnosis or after CX.
- Ultimately, ctDNA analysis could be incorporated into routine follow-up for early detection of relapse and consequently potentially earlier initiation of alternate treatment such as immunotherapy.
- The benefit in overall survival gained by ctDNA relapse detection should be assessed in randomized clinical trials.

References

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American Association for Cancer Research Annual Meeting 2018; Chicago, IL | April 14–18, 2018

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