Accuracy of genome-wide mutational analysis for tumor informed ctDNA guided MRD monitoring in bladder cancer.

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Introduction

- Circulating tumor DNA (ctDNA) levels can be used to monitor treatment response and detect Minimal Residual Disease (MRD) with a positive lead time over clinical and radiological detection[1-2].
- In bladder cancer, mutations in plasma have previously been used to monitor response during treatment and identify metastatic relapse at a very early timepoint[3].
- Recently, longitudinal ctDNA detection in patients with muscle invasive bladder cancer (MIBC) was described using ultra deep amplicon sequencing for early relapse detection[4].
- We applied a tumor informed whole genome sequencing (WGS) method to detect thousands of somatic mutations in cfDNA using only 1-2 mL of plasma.

Objective

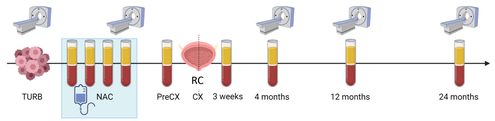
- To use a tumor informed WGS approach to analyze longitudinally-collected plasma samples for monitoring the response to neoadjuvant chemotherapy (NAC), and to detect MRD as well as metastatic relapse.
- To use small volume of plasma (~1mL) to facilitate usability.
- To improve the sensitivity of ctDNA detection by analyzing thousands of somatic mutations captured by WGS.

Methods

Clinical Protocol

- Patients, diagnosed with locally advanced MIBC, were prospectively recruited between 2014 and 2019, and followed until 2023.
- All patients received NAC before cystectomy (RC) and had up to 7.5 years of follow-up.
- Plasma samples were longitudinally collected pre- and post-systemic therapy as well as at scheduled control visits after RC (**Figure 1**).

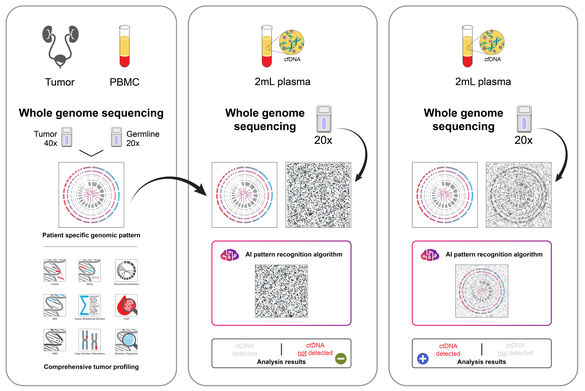
Figure 1. Schematic of Clinical Sample Collection



Molecular and data analysis protocol

- Genomic DNA from tumor/germline pairs (n=113) and cfDNA from 916 plasma samples were isolated.
- WGS of tumor/germline pairs followed by genome-wide integration of somatic mutations, enriched by signal processing and AI-based error suppression, were used to generate patient-specific tumor signatures (**Figure 2**).
- Patient-specific somatic variants were used for detecting ctDNA in WGS data based on about 15 ng cfDNA by the MRDetect[5] algorithm (**Figure 2**)
- Clinical data, such as pathological evaluation and radiographic imaging, were blinded until after analysis and compared directly to the MRDetect[5] plasma call results.

Figure 2. Schematic of workflow



Results

Table 1. Patient Characteristics and Demographics (n=113)

Patients, n = 113, median age = 67.0 years (range 42-82)

Sex, n (%)		**smoking status**	
Male	89 (78.8)	Current	49.3 (43.4)
Female	24 (21.2)	Former	45 (38.8)
		Never	18 (15.9)
Pre-therapeutic pathologic stage at TURB, n (%)		**Post-treatment pathologic stage at RC, n (%) (n=111)**	
pT1	8 (7.1)	pT0/pTIS/pTa	67 (59.8)
pT2	96 (85.0)	pT1	7 (6.3)
pT3	1 (0.9)	pT2	13 (11.6)
pT4a	4 (3.5)	pT3	17 (15.2)
pT4b	3 (2.7)	pT4a	7 (6.3)
		pT4b	1 (0.9)
Pre-therapy pathologic N stage, n (%)		**Post-treatment pathologic N stage at RC, n (%) (n=112)**	
N0	98 (87.5)	pN0	99 (89.2)
N1	11 (9.8)	pN1	4 (3.6)
N2	3 (2.7)	pN2	5 (4.5)
		pN3	3 (2.7)
Chemotherapy before RC		**Median Clinical follow-up, days (range)**	
Neoadjuvant chemotherapy (NAC)	111 (100)	Disease free (RC: n=84)	1914 (139-2810)
Pathologic downstaging (≤T1a,CIS,N0)	67 (60.4)	Clinical relapse (RC: n=27)	651 (65-2493)
No pathologic downstaging (≥T1 and/or ≥N1)	44 (39.6)		

Figure 3. Concordance between independent laboratories - United States vs. Denmark

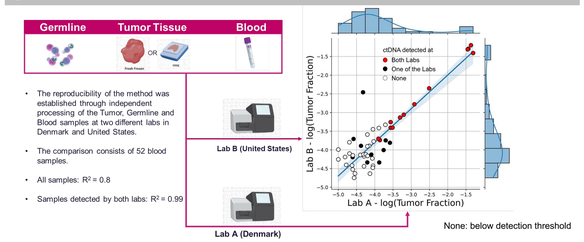


Figure 4. Disease courses and longitudinal ctDNA results for the 113 patient study cohort

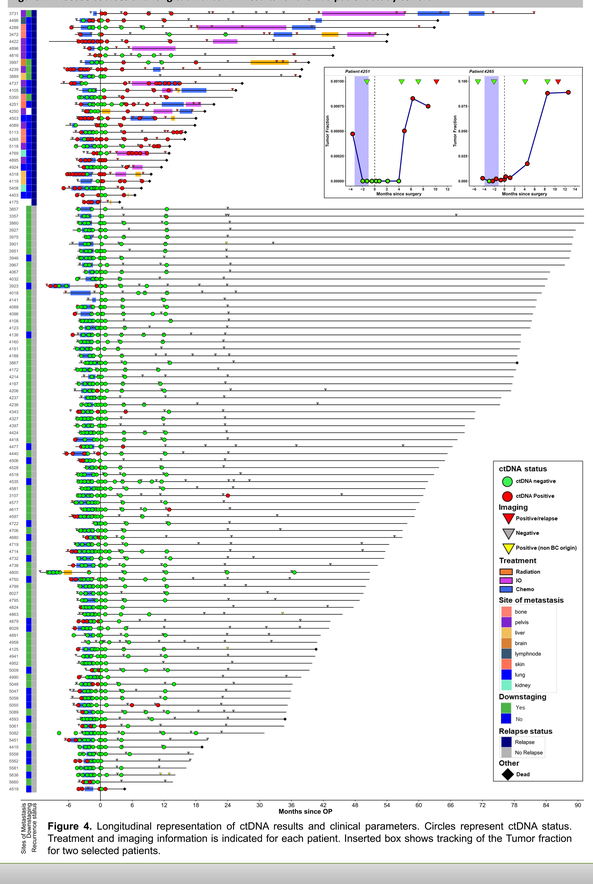


Figure 4. Longitudinal representation of ctDNA results and clinical parameters. Circles represent ctDNA status. Treatment and imaging information is indicated for each patient. Inserted box shows tracking of the Tumor fraction for two selected patients.

Results

Figure 5. Prognostic value of ctDNA

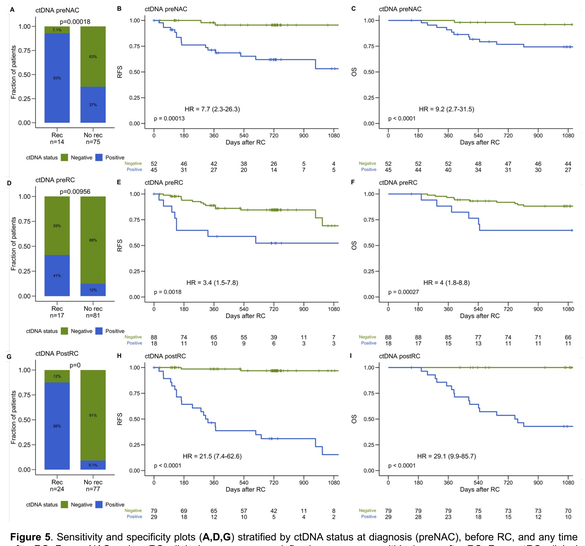


Figure 5. Sensitivity and specificity plots (**A,D,G**) stratified by ctDNA status at diagnosis (preNAC), before RC, and any time after RC. For preNAC and preRC, clinical recurrence was defined as recurrence within 1 year after RC. For postRC, clinical recurrence is defined as recurrence within 2 years after the last ctDNA test. Non-relapse patients have a minimum of two years follow up. Kaplan-Meier survival analysis of recurrence-free survival (RFS)(**B,E,H**) and overall survival (OS) (**C,F,I**).

Figure 6. ctDNA status/dynamics and pathological downstaging – dichotomized analysis.

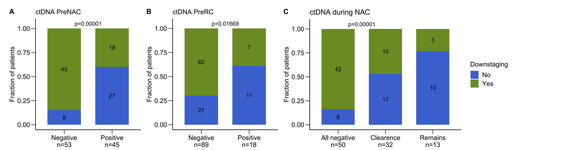


Figure 6. ctDNA status at diagnosis (preNAC) (**A**), before cystectomy (**B**) and dynamics during NAC (**C**).

Figure 7. RFS stratified by ctDNA dynamics vs pathological downstaging (NAC cycles >2)

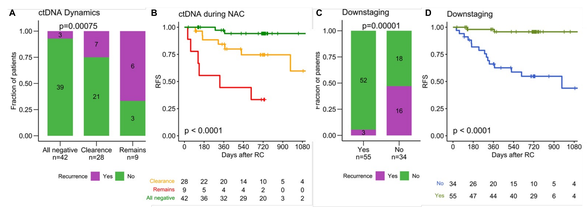


Figure 7. ctDNA dynamics vs pathological downstaging in predicting recurrence in patients being ctDNA positive before NAC. Only patients that completed at least three cycles of NAC were included in the analysis.

Figure 8. Lead time inferred from ctDNA-based recurrence detection.

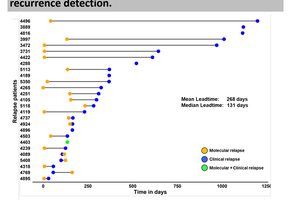


Figure 8. Lead time between first molecular relapse (ctDNA positive (orange dot) and clinical recurrence (radiographic imaging positive, blue dot). The green dot indicate that first molecular relapse and clinical recurrence are detected at the same time.

Figure 9. Lung metastasis release less ctDNA

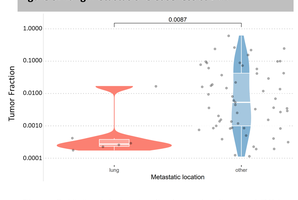


Figure 9. Violin plot showing the tumor fraction post RC for lung metastasis vs. all other metastasis. Only plasma samples having TF>0 after cystectomy are included. ctDNA shedding and thus probability for MRD detection is dependent on the metastatic site.

Results

Figure 10. Mutational characterization of tumors

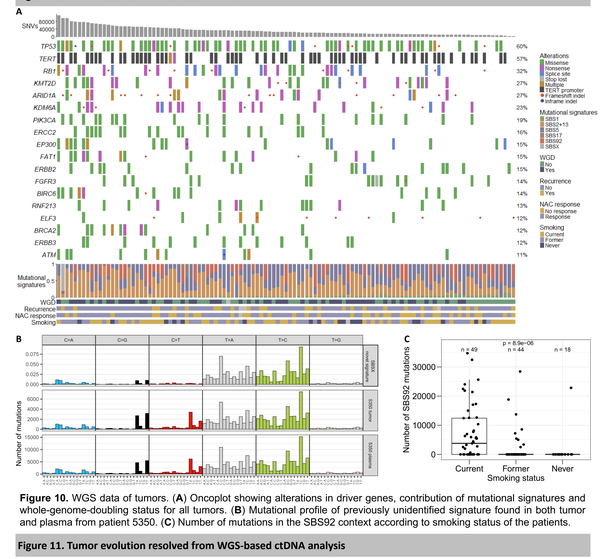


Figure 10. WGS data of tumors. (**A**) Oncoplot showing alterations in driver genes, contribution of mutational signatures and whole-genome-doubling status for all tumors. (**B**) Mutational profile of previously unidentified signature found in both tumor and plasma from patient 5350. (**C**) Number of mutations in the SBS92 context according to smoking status of the patients.

Figure 11. Tumor evolution resolved from WGS-based ctDNA analysis

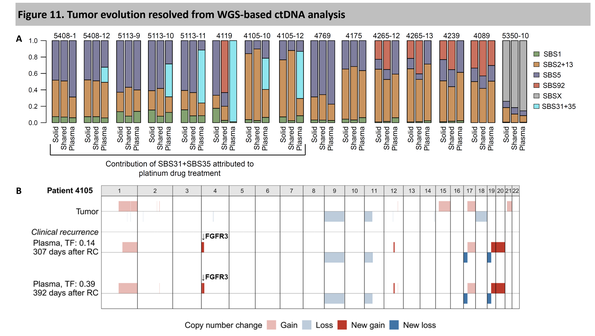


Figure 11. WGS data of plasma cfDNA for patients with a sufficient detected TF (cfDNA TF>10%). A) Contribution of mutational signatures when analyzing unique and shared mutations between primary tumors and plasma samples. Five plasma samples from three patients (5408, 5113 and 4105) showed contribution from the mutational signatures SBS31+SBS35, which are both attributed to platinum drug treatment. B) Evolution of copy number variations detected in post-treatment plasma samples compared to the primary tumor for patient 4105.

Conclusions

- The study demonstrates that WGS of ctDNA from a volume of only 1-2 mL of plasma can detect disease relapse in NAC treated MIBC 131 days (Median) before radiographic imaging.
- The recurrence free survival is significantly lower for patients that are ctDNA positive before neoadjuvant chemotherapy, before cystectomy and any time after cystectomy. CtDNA clearance during NAC is a predictor of PFS comparable to pathological downstaging.
- Lung metastasis release less ctDNA than other metastatic tumors.
- WGS of ctDNA from small volumes of plasma show clinical potential for personalized genome-wide mutation integration as an ultra-sensitive, non-invasive method for MRD detection and treatment response monitoring. This could aid in clinical management of patients with bladder cancer and guide clinical decisions.
- Mutation load in signature SBS92 attributed to tobacco smoking was significantly higher in tumors from current smokers compared to tumors from former- and never smokers.
- Chemotherapy-induced mutational signatures (platinum) was not present in any of the primary tumors, however emerge in post-treatment plasma samples.
- CNVs detected only in post-treatment plasma samples suggest that treatment resistant clones is a late event and is not present in the originally sequenced tumor.

References

1. Carpinetti P et al. Oncotarget. 2015. 6(35):38360–7. 2. Riva F et al. Clin Chem. 2017. 63(3):691–699, 3. Birkenkamp-Demtröder K et al. Eur Urol. 2018. 73(4):535–540,
4. Christensen E, Birkenkamp-Demtröder K et al J Clin Oncol. 2019 Jun 20;37(18):1547-1557, 5. Zviran et al., Nature Medicine. 2020

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