Genome-wide circulating tumor DNA for monitoring treatment response and metastatic relapse in bladder cancer

Iver Nordentoft[1], Karin Birkenkamp-Demtröder[1], Emil Christensen[1],Sunil Deochand[2] , Dillon Maloney[2], Danielle Afterman[2], Tomer Lauterman[2], Noah Friedman[2], Imane Bourzgui[2], Nidhi Ramaraj[2], Zohar Donenhirsh[2], Ronel Veksler[2], Sia Viborg Lindskrog[1], Mads Agerbæk[1], Jørgen Bjerggaard Jensen[1], Jonathan Rosenfeld[2], Ravi Kandasamy[2], Iman Tavassoly[2], Boris Oklander[2], Asaf Zviran[2], Lars Dyrskjøt[1]

[1]Department of Molecular Medicine, Aarhus University Hospital, Aarhus N, Denmark; [2]C2i Genomics, New York, NY 10014, USA

Aarhus University Hospital

Abstract Number: 540

Introduction

- Circulating tumor DNA (ctDNA) levels can be used to monitor treatment response and detect MRD with a positive lead time over clinical and radiological detection[1-2].
- In bladder cancer, mutations in plasma have been previously 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 tumor informed method to whole genome sequencing (WGS) in order to detect thousands of somatic mutations in cfDNA by using only 1-2 ml of plasma. The method increased MRD detection sensitivity as well as early relapse detection in MIBC patients.

Objective

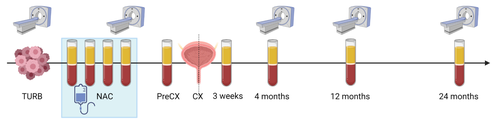
- Use tumor informed methods in WGS from longitudinally-collected plasma samples to monitor the response to neoadjuvant chemotherapy (NAC), and to detect MRD as well as metastatic relapse.
- Use small volume of plasma (~1 ml) to facilitate usability.
- To improve the sensitivity of ctDNA detection by analyzing thousands of somatic mutations captured by WGS.

Methods

Clinical Protocol

- Patients, diagnosed with locally advanced MIBC, were prospectively recruited between 2014 and 2017, and followed up until 2021.
- All patients received NAC before cystectomy (CX) and had up to 6 years of follow-up.
- Plasma samples were longitudinally collected pre- and post-systemic therapy as well as at scheduled control visits after CX (**Figure 1**).

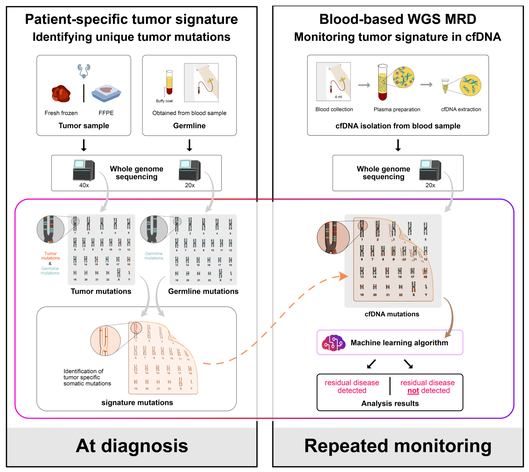
Figure 1. Schematic of Clinical Sample Collection



Molecular and data analysis Protocol

- Genomic DNA from tumor/germline pairs (n=67) and cfDNA from 510 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 signature (**Figure 2**).
- Patient-specific somatic variants were used for detecting ctDNA levels in about 15 ng cfDNA from plasma WGS data by the MRDetect[5] algorithm (**Figure 2**)
- Clinical data, such as pathological evaluation and radiographic imaging, were first unblinded 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=67)

Patients, n = 67, median age = 69.9 years (range 43-77)

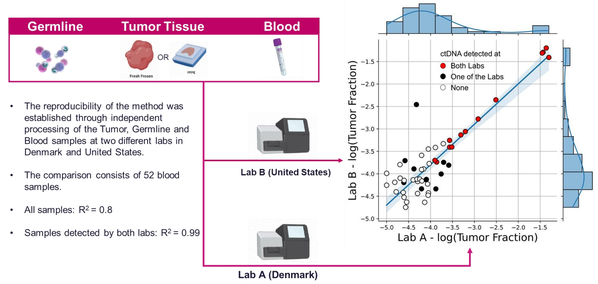
Gender, n (%)		**smoking status**	
Male	55 (82.1)	current	33 (49.3)
Female	12 (17.9)	former	25 (37.3)
		never	9 (13.4)
pre-therapeutic staging at TUR-B, n (%)		**post-therapeutic staging at CX, n (%) (n=65)**	
pT1	4 (6.0)	pT0/pTIS/pTa	44 (67.7)
pT2	54 (80.6)	pT1	2 (3.1)
pT3	1 (1.5)	pT2	6 (9.2)
pT4a	4 (6.0)	pT3	9 (13.8)
pT4b	5 (7.5)	pT4a	4 (6.2)
pre-therapeutic N stage, n (%)		**post-therapeutic N staging at CX, n (%) (n=65)**	
N0	57 (85.1)	pN0	58 (89.2)
N1	7 (10.4)	pN1	2 (3.1)
N2	3 (4.5)	pN2	3 (4.6)
		pN3	2 (3.1)
chemotherapy before CX		**Median Clinical follow-up, days (range)**	
neoadjuvant chemotherapy (NAC)	67 (100)	Disease free (CX; n=47)	1932 (583-2344)
patholog. downstaging (≤Ta,CIS,N0)	44 (65.7)	Clinical relapse (CX; n=17)	324 (65-1253)
no patholog. downstaging (≥T1 and/or ≥N1)	23 (34.3)	Progression (CX impossible; n=2, no post CX imaging; n=1)	
		Metastasis at relapse n (%)	
		Local relapse (pelvis, rectum)	6 (31.6)
		Distant metastases (bone, lung, liver, skin, brain)	13 (68.4)

CX, Cystectomy

Pilot study

- Longitudinal plasma samples from 19 MIBC patients were collected and the cfDNA was evenly split into two parts and processed at two independent laboratories to estimate the tumor fraction (TF).
- High concordance of TF between laboratory sites (**Figure 3**).

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



None: below detection threshold

Figure 4. Prognostic value of circulating tumor DNA (ctDNA)

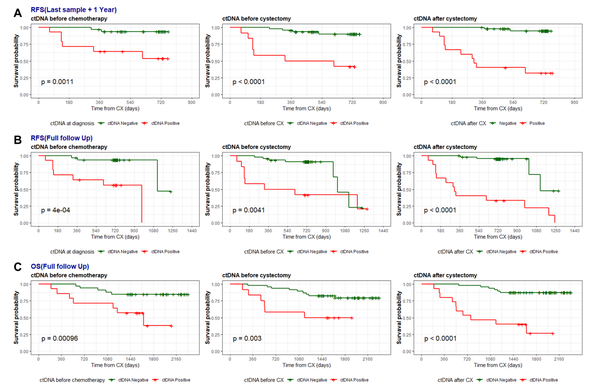


Figure 4. Kaplan-Meier survival analysis of recurrence-free survival (RFS)(A+B) and overall survival (OS) (C) stratified by ctDNA status before chemotherapy (or at diagnosis), before cystectomy, and any time after cystectomy. (A) The follow-up time set to one year after last plasma sample fore each patient if prior to relapse, last CT scan or death. (B+C) shows Kaplan-Meier analysis for the maximum follow-up time where patients without events were censored at end of follow-up.

Results

Figure 5. Disease courses and longitudinal ctDNA results for the 67 patient study cohort

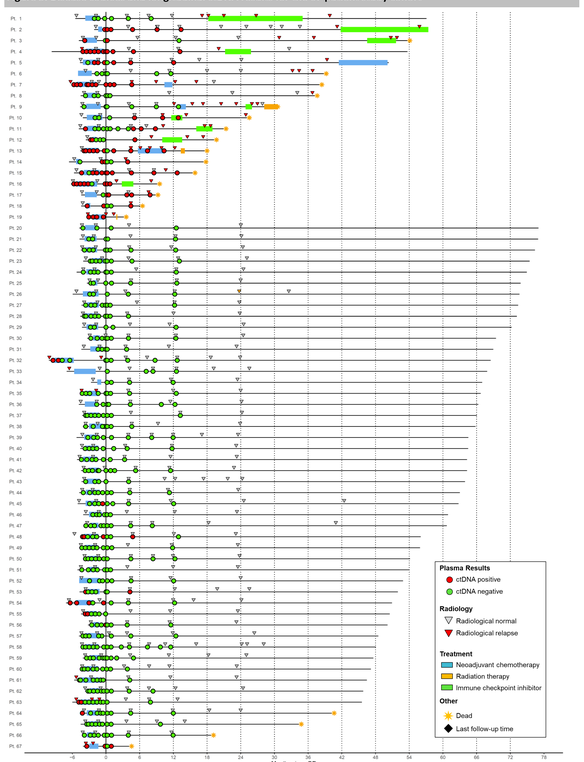


Figure 5. Longitudinal representation of ctDNA results and clinical parameters. Circles represent ctDNA status (see color code in the right box). Treatment and imaging information is indicated for each patient. Patients are separated into two groups on the basis of clinical relapse status. Metastatic relapse (Patient 1-19); Non Relapse (20-67).

Figure 6. Pathological downstaging vs. ctDNA status at diagnosis, pre-CX and post CX

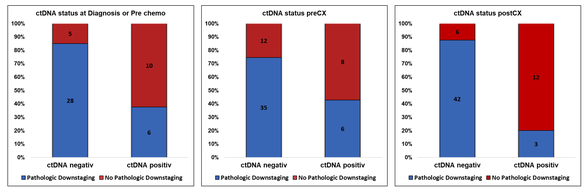


Figure 6. Association between pathological downstaging and ctDNA status before chemotherapy, before cystectomy, and any time after cystectomy.

Figure 7. Prognostic value of circulating tumor DNA (ctDNA) detection – dichotomized analysis.

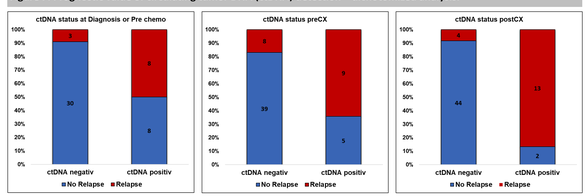


Figure 7. Association between disease recurrence and ctDNA status before chemotherapy, before cystectomy, and any time after cystectomy.

Results

Figure 8. Monitoring tumor fraction during patient disease courses.

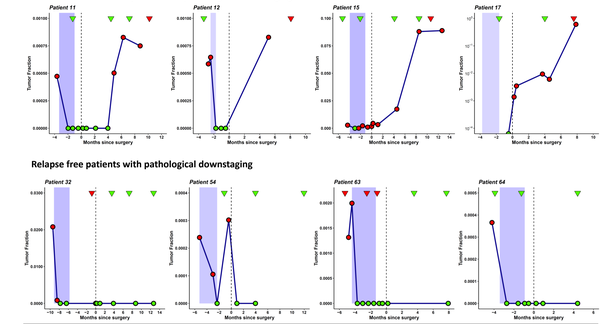


Figure 9. ctDNA dynamic during NAC as predictive markers of chemotherapy response

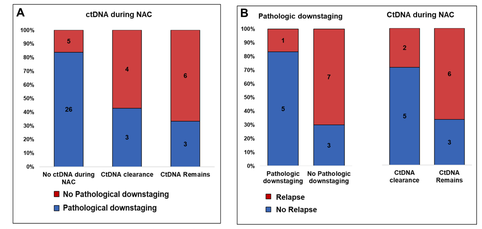


Figure 9. (**A**) Association between ctDNA dynamic during NAC and pathological downstaging for ctDNA-negative patients (before and after NAC), patients where ctDNA was cleared during NAC, and patients in whom ctDNA remained. (B) Association between recurrence and pathological downstaging (left) and ctDNA dynamics during NAC (right) for patients ctDNA-positive before NAC.

Figure 10. Lead time inferred from ctDNA-based recurrence detection

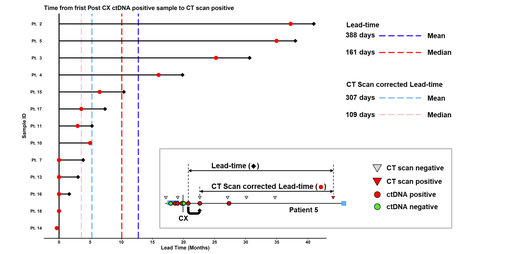


Figure 10. Lead time in days shown as the differences between first molecular relapse (ctDNA positive) and clinical recurrence (radiographic imaging positive). CT Scan corrected Lead time is calculated as the differences between the first CT scan after molecular relapse and clinical recurrence (see box in figure). The CT Scan corrected Lead time is more restrictive and included to provide a fair comparison to radiographic imaging.

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 161 days (Median) before radiographic imaging.
- The Relapse free survival is significantly lower for patients that are ctDNA positive before neoadjuvant chemotherapy, before cystectomy and any time after cystectomy
- WGS of ctDNA from small volumes of plasma show great clinical potential of 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. .

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

Contact: Iver Nordentoft – iver.Nordentoft@clin.au.dk | Lars Dyrskjøt – Lars@clin.au.dk

American Association for Cancer Research Annual Meeting 2022; New Orleans, Louisiana | April 8 – 18, 2022

C2i Genomics

AARHUS UNIVERSITET HEALTH