Ultra-sensitive detection of circulating tumor DNA by WGS of blood samples from locally advanced rectal cancer patients receiving neoadjuvant therapy and enrolled in watch-and-wait strategies for organ preservation

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Background and Study Design

Colorectal cancer is the third most common cancer in the United States and rectal cancer accounts for 1/3 of all new cases (>44,000 per year). Most rectal cancer patients are diagnosed with non-metastatic, locally advanced rectal cancer (**LARC**). Patients with a pathological complete response (**pCR**) - which can only be identified after radical resection - have significantly better outcomes. Accurate diagnosis of clinical complete response (**cCR**) is critical and may allow patients to avoid surgery and undergo non-operative management with frequent surveillance to ensure durability.

We analyzed data from 31 LARC patients treated with neoadjuvant therapy (**NAT**). Patients were randomized to receive either systemic chemotherapy (FOLFOX) followed by chemoradiation (**INCT-CRT**) or chemoradiation followed by systemic chemotherapy (**CRT-CNCT**). Patients with a cCR were enrolled in **watch-and-wait**, while the rest underwent surgical resection.

Goal:
To investigate the clinical utility of circulating tumor DNA (**ctDNA)** for accurate assessment of complete response and as a prognostic biomarker using **C2inform**, a whole genome minimal residual disease (**MRD**) test.

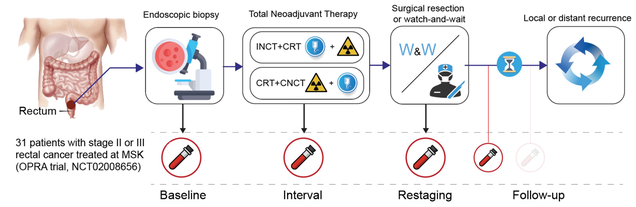


Figure 1. Study design

Characteristics	CR (n=16)	iCR (n=15)
Age, median (IQR)	61.2 (51.2–70.4)	51.9 (47.5–66.1)
Sex, No. (%)		
Male	10 (62.5)	11 (73.3)
Female	6 (37.5)	4 (26.7)
Clinical T, No. (%)		
cT1-2	3 (18.8)	1 (6.7)
cT3	13 (81.3)	11 (73.3)
cT4	0 (0)	3 (20)
Clinical N, No. (%)		
cN-negative	6 (37.5)	1 (6.7)
cN-positive	10 (62.5)	14 (93.3)
NAT Regimen, No. (%)		
INCT-CRT	6 (37.5)	9 (60.0)
CRT-CNCT	10 (62.5)	6 (40.0)

Table 1. Cohort characteristics. Complete response (CR) after NAT is defined as either pCR or a clinical complete response (cCR) sustained for ≥2 years. cCR is based on clinical examination and imaging. iCR stands for incomplete response.

Methods: C2inform Assay

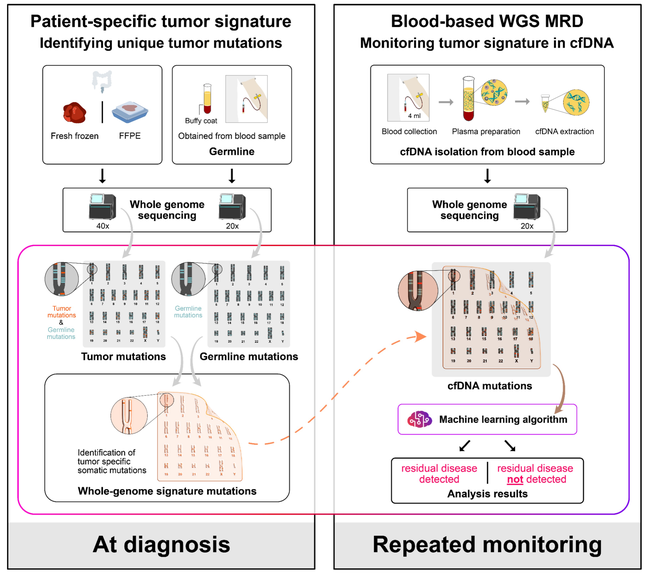


Figure 2. C2inform assay protocol.

C2inform ctDNA Detection Cohort Overview

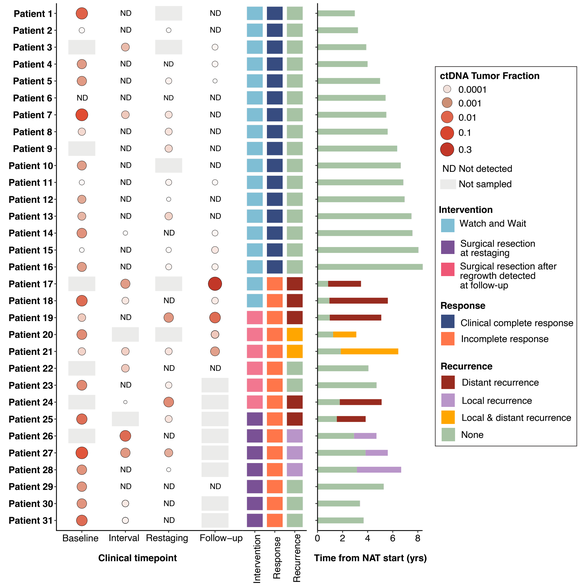


Figure 3. Tumor was detected in plasma samples from 24/25 patients at baseline (96% sensitivity). At first follow-up, ctDNA was detected in 5/5 patients who had a recurrence.

ctDNA Detection & Clinical Outcomes

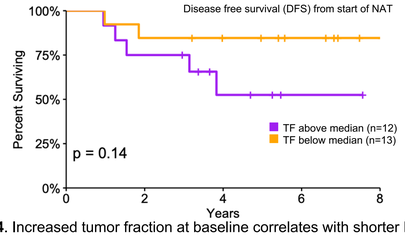


Figure 4. Increased tumor fraction at baseline correlates with shorter DFS after NAT.

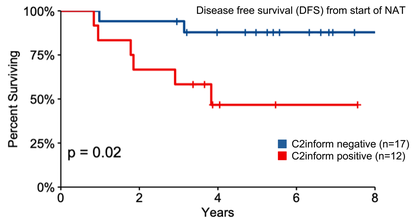


Figure 5. C2inform positive status at interval was associated with a lower rate of CR (25% vs. 75%, p=0.0095) and shorter time to recurrence (58.3% vs. 94.1% 3-year DFS, p=0.02).

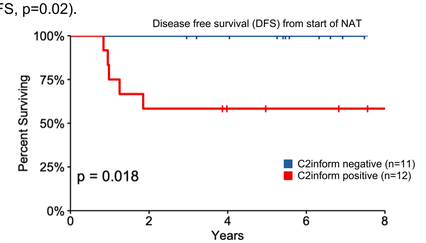


Figure 6. C2inform positive status at follow-up was associated with higher rate of recurrence (p=0.037). Tumor was detected for 5/5 patients who recurred.

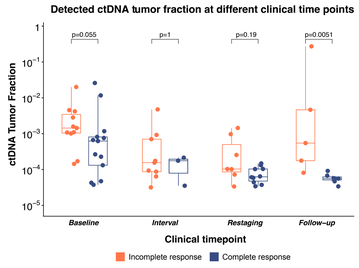
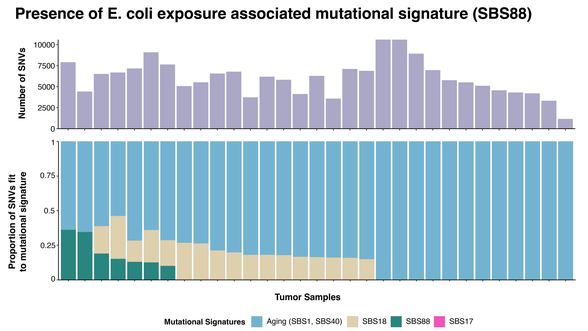


Figure 7. ctDNA tumor fraction at clinical timepoints. Patients with a clinical complete response (cCR) showed clearance of ctDNA throughout treatment, whereas tumor fraction increased or remained stable in patients with incomplete response.

Mutational Signatures & Tumor Evolution



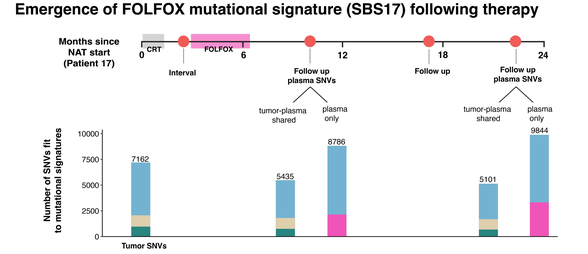


Figure 8. Analysis of tissue WGS data identified multiple patients with colibactin associated mutational signature (SBS88), which provides additional insights into their cancer etiology (Top). Plasma from some patients exhibited treatment related signatures emerging throughout therapy. Shown here, timeline for Patient 17 (Bottom).

Conclusions

- The C2inform platform exhibited very high sensitivity for detection at baseline.
- Tumor fraction across multiple time points separated responders from non-responders, suggesting potential value as a prognostic marker.
- Detection of ctDNA at follow-up for all patients who recurred is indicative of potential clinical utility for treatment de-escalation in the context of organ preservation strategies.
- WGS analysis also provides valuable insights about tumor etiology and tumor progression during and after treatment.

References

[1] Garcia-Aguilar J et al. Organ preservation in patients with rectal adenocarcinoma treated With total neoadjuvant therapy. *J Clin Oncol.* 2022, doi:10.1200/JCO.22.00032. PMID:35483010.

[2] Zviran A et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. *Nat Med.* 2020, doi: 10.1038/s41591-020-0915-3. PMID: 32483360.

[3] Alexandrov LB et al. The repertoire of mutational signatures in human cancer. *Nature.* 2020, doi: 10.1038/s41586-020-1943-3. PMID: 32025018.

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