Clinical Performance of a Blood-Only, Targeted Methylation Circulating Tumor DNA (ctDNA) Assay for Minimal Residual Disease (MRD) Detection in Colorectal Cancer (CRC)

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INTRODUCTION

- A substantial proportion of CRC patients recur after curative-intent treatment[1]
- Conventional surveillance with imaging or serum markers has limited sensitivity, specificity, and timeliness in detecting MRD[2]
- ctDNA can enable MRD detection through the identification of molecular relapse[3]; however, many ctDNA technologies are constrained by the need for tumor tissue
- Blood-only methylation approaches to ctDNA assessment offer a non-invasive alternative that does not require a tumor specimen
- This study evaluates a blood-only, targeted methylation ctDNA assay for MRD detection and recurrence risk stratification in resectable CRC

OBJECTIVES

- Evaluate the targeted methylation assay performance for MRD detection in resectable CRC at post-surgery and post-curative-intent landmark timepoints
- Correlate ctDNA status with 2-year radiologic recurrence, recurrence-free survival (RFS) and overall survival (OS)

METHODS

Cohort and Study Design

- The study represents a retrospective analysis conducted on biobanked plasma samples (N=231) obtained from 178 patients from ethically approved Walter and Eliza Hall Institute studies (**Table 1**)
  - Patients included were diagnosed with resectable CRC and had surgery and one or more plasma samples available for analysis; patients had either a recurrence event or at least two years of clinical follow-up after surgery
- MRD assay performance was assessed at two predefined landmark timepoints (**Figure 1**):
  - **Post-surgery landmark:** plasma collected after surgery and before any adjuvant treatment (if administered)
  - **Post-curative intent treatment landmark:**
    - For patients who received adjuvant therapy: plasma collected after completion of adjuvant treatment
    - For patients who did not receive adjuvant therapy: same as post-surgery landmark timepoint

Sample Processing and Data Analysis

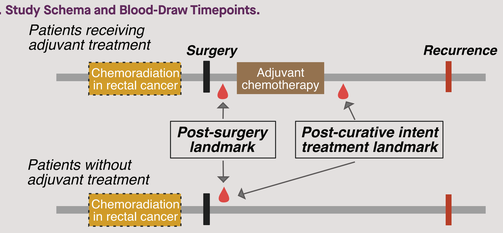
- Plasma samples were analyzed with GRAIL's blood-only, targeted methylation assay,[4] which analyzes >100,000 methylation regions with a locked machine-learning classifier to determine ctDNA status
- Tumor methylation fraction (TMeF)[5] was estimated based on tumor-associated differentially methylated regions (DMRs)
- Sensitivity and specificity for predicting radiographic recurrence were calculated at each landmark timepoint
- Associations between ctDNA status with RFS and OS were evaluated using Kaplan–Meier and Cox proportional hazard models

Table 1. Patient Demographics and Tumor Characteristics.

Characteristic	Overall Cohort No. (%) (N = 178)
Age, median (IQR)	61 (52, 69)
Sex	
Female	77 (43)
Male	101 (57)
Primary tumor site	
Colon	139 (78)
Rectum	39 (22)
Clinical or pathological stage	
I	1 (0.6)
II	4 (2.2)
III	173 (97)
T stage	
T0	8 (4.5)
T1	9 (5.1)
T2	32 (18)
T3	95 (53)
T4	34 (19)
N stage	
N0	22 (12)
N1	118 (66)
N2	38 (22)
Had adjuvant chemotherapy	161 (90)
Recurrence*	
Yes	37 (32)
No	135 (78)
Unknown (follow-up <2 yr and no recurrence up to last follow-up)	6

**Defined as recurrence events occurring within 2 years after surgery. IQR, interquartile range.*

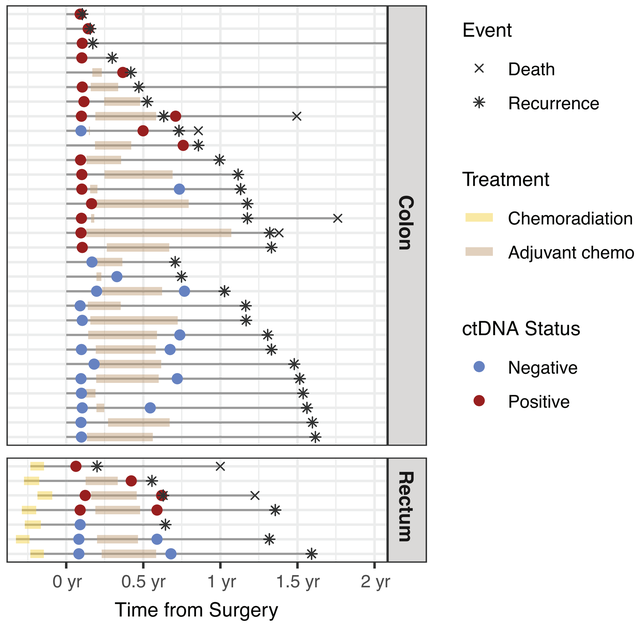
Figure 1. Study Schema and Blood-Draw Timepoints.



KEY RESULTS: ctDNA DETECTION WITH A BLOOD-ONLY METHYLATION ASSAY PRECEDES RADIOLOGIC RECURRENCE IN CRC.

ctDNA DETECTION STATUS PRIOR TO RECURRENCE IN CRC PATIENTS AND MRD ASSAY PERFORMANCE

Figure 2. Postoperative ctDNA Detection Precedes Radiologic Recurrence and Is Associated with Shorter Time to Recurrence. Swimmer plot shows ctDNA results, duration of treatment, and clinical outcomes in patients with recurrence events



- ctDNA was detected in ~50% of patients who had a recurrence event (**Figure 2, Table 2**):
  - Post-surgery samples:
    - 17 of 32 (53.1%) patients who recurred within 2 years (true positives [TP])
    - 10 of 119 (8.4%) patients who did not recur within 2 years (false positives [FP])
  - Post-curative intent treatment samples:
    - 12 of 22 (54.5%) patients who recurred within 2 years (TP)
    - 3 of 56 (5.4%) patients who did not recur within 2 years (FP)
- Median time to recurrence was significantly shorter in those with ctDNA detected:
  - 225 days (IQR 131–418 days) for ctDNA-positive patients vs 484 days (IQR 404–573 days) for ctDNA-negative patients (Wilcoxon test: *P* value <.001)

Table 2. MRD Assay Performance.

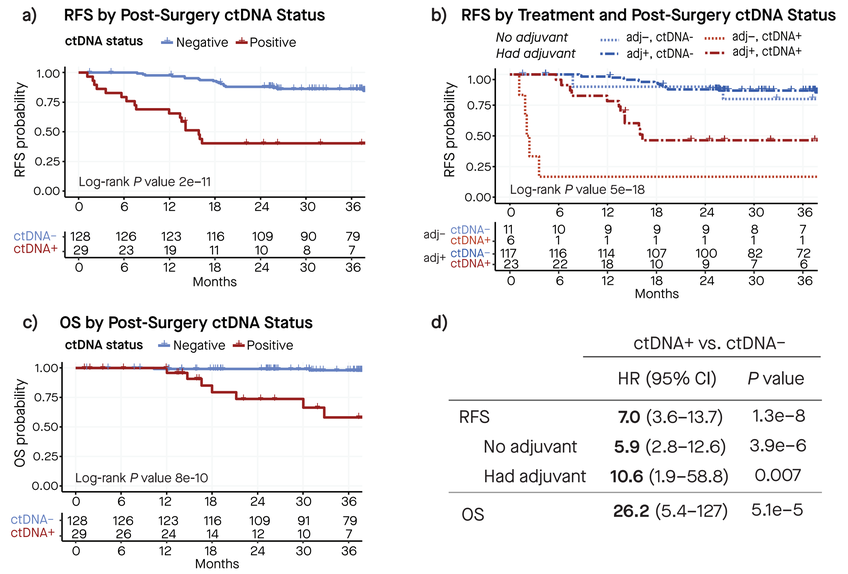
Timepoint	Sensitivity (TP/(TP+FN)) [95% CI]	Specificity (TN/(TN+FP)) [95% CI]
Post-surgery landmark	53% (17/32) [35%–70%]	92% (109/119) [85%–96%]
Post-curative intent treatment landmark	55% (12/22) [33%–75%]	95% (53/56) [84%–99%]

CI, confidence interval; FN, false negative; FP, false positive; MRD, minimum residual disease; TN, true negative; TP, true positive.

ctDNA DETECTION WAS ASSOCIATED WITH INFERIOR RFS AND OS

- RFS and OS were shorter among patients with detectable ctDNA postoperatively, regardless of whether they received adjuvant therapy (**Figure 3**)

Figure 3. Association Between Landmark ctDNA Detection Post-Surgery and Probability of Survival. a) Kaplan–Meier estimates for RFS stratified by ctDNA status over a median follow-up of 37 months (combined patients with or without adjuvant chemotherapy). b) Kaplan–Meier estimates for RFS stratified by ctDNA detection and adjuvant treatment status. c) Kaplan–Meier estimates for OS stratified by ctDNA status (combined patients with or without adjuvant chemotherapy). *P* values in the plot were calculated using the two-sided log-rank test d) Hazard ratio of RFS and OS from Cox proportional hazard model.



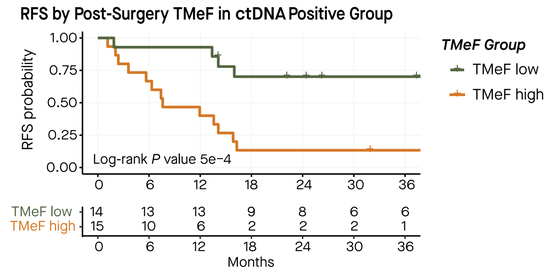
d)

	ctDNA+ vs. ctDNA−	
	HR (95% CI)	*P* value
RFS	**7.0** (3.6–13.7)	1.3e−8
No adjuvant	**5.9** (2.8–12.6)	3.9e−6
Had adjuvant	**10.6** (1.9–58.8)	0.007
OS	**26.2** (5.4–127)	5.1e−5

adj, adjuvant chemotherapy; CI, confidence interval; ctDNA, circulating tumor DNA; HR, hazard ratio; OS, overall survival; RFS, recurrence-free survival.

RISK STRATIFICATION USING TMeF WITHIN ctDNA-POSITIVE PATIENTS

Figure 4. Kaplan–Meier Estimates for RFS Stratified by TMeF Group in the ctDNA-Positive Group. TMeF high/low: above/below median TMeF (185 PPM).



ctDNA, circulating tumor DNA; PPM, parts per million; RFS, recurrence-free survival; TMeF, tumor methylation fraction.

- TMeF values from post-surgery plasma samples enabled further risk stratification within ctDNA-positive patients (TMeF high vs. TMeF low: HR = 5.5 [95% CI: 1.8–17, *P*=.003]) (**Figure 4**)

References: 1. Pita-Fernández, S. et al. *Ann. Oncol.* 26, 644 (2015). 2. Reinert, T. et al. *JAMA Oncol.* 5, 1124 (2019). 3. Peng, Y., Mei, W., Ma, K. & Zeng, C. *Front. Oncol.* 11, 763790 (2021). 4. Liu, M. C. et al. *Ann. Oncol.* 31, 745 (2020). 5. Melton, C. A. et al. *Cancers* 16, 82 (2024).

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CONCLUSIONS

- Landmark ctDNA detection was associated with significantly shorter RFS and OS
- TMeF values from post-surgery plasma samples enabled further risk stratification within ctDNA-positive patients
- GRAIL's blood-only, targeted methylation assay demonstrated promising performance for MRD detection in curatively treated CRC, potentially enabling tissue-independent recurrence prognostication post-treatment
- These data support future investigation of the assay for disease surveillance or risk-stratified therapeutic studies