Tumor-Informed Assessment of Molecular Residual Disease and its Incorporation into Practice for Patients with Early and Advanced Stage Colorectal Cancer (CRC-MRD Consortia)

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Poster #100

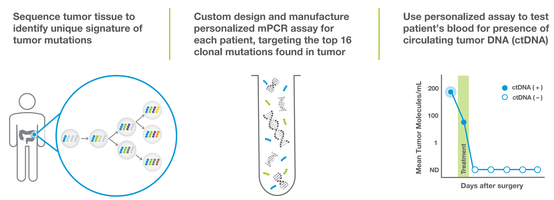
Background

- Colorectal cancer (CRC) cancer is the second leading cause of cancer-related mortality in the United States[1]
  - A great amount of variability exists in the 5-year relative survival when stratified by stage at diagnosis with 90% survival for localized cancers; declining to 71% for regional cancers; and 14% for metastatic cancers[1,2]
  - Although surgery is considered the preferred curative treatment for local or regionally advanced CRC, approximately 30-40% of CRC patients relapse after resection with ~80% of relapses occurring in the first two years[3,4]
- **Circulating tumor DNA (ctDNA)** testing can be used for the assessment of **minimal residual disease (MRD)** in patients with **early-stage or advanced CRC**
  - The clinical utility of ctDNA as a non-invasive biomarker has been well established in literature for MRD detection and for stratifying patients based on their risk of developing relapse[5,6]
  - Prospective evaluation of ctDNA-based testing in clinical practice has been limited to date

Methods

- A total of **715 plasma samples** were analyzed from **535 unique CRC patients** who underwent ctDNA MRD testing as part of an **early adopter program** across the spectrum of CRC management
  - This cohort included 432 patients with colon cancer, 77 with rectal cancer, 27 with lower gastrointestinal cancers (anal, appendiceal, small bowel). Majority of patients were male (57%, n=304) with an average age of 61 years
- **A personalized and tumor-informed multiplex PCR assay (Signatera™ bespoke, mPCR NGS assay)** was used for the detection and quantification of ctDNA for MRD assessment **(Figure 1)**
- The study evaluated the relationship between ctDNA status and clinical outcomes including radiologic imaging and multivariable analysis was performed with all clinical variables

Figure 1. Signatera™ residual disease test (MRD), a personalized and tumor-informed approach



MRD rates across early-stage and oligometastatic CRC patients reflect expected relapse rates and demonstrate treatment response dynamics in a clinically useful way.

Table 1. MRD rates and ctDNA quantity in patients with locoregionally advanced (stage I-III) CRC (n = 300)

Setting	MRD Rates	Quantity of ctDNA (MTM/mL)		
		Mean	Median	Range
Neoadjuvant setting	4/5 (80%)	21.04	11.69	0.24-60.55
Post-surgery MRD (Stage I)	2/15 (13%)	2.65	2.65	0.13-5.18
Post-surgery MRD (Stage II)	7/68 (10%)	78.5	1.33	0.31-543.77
Post-surgery MRD (Stage II, T3N0)	3/53 (5.6%)	1.63	1.74	1.33-1.84
Post-surgery MRD (Stage II, T4N0)	4/14 (28.6%)	136.24	0.44	0.31-543.77
Post-surgery MRD (Stage III)	19/71 (26.7%)	48.81	1.23	0.13-872.2
Post-surgery MRD (Stage III, low-risk: T1-3N1)	3/32 (9.3%)	2.43	0.40	0.23-6.67
Post-surgery MRD (Stage III, high-risk: T4, N1-2, T Any, N2)	15/38 (39.4%)	61.17	1.23	0.13-872.2
During Adjuvant Therapy	2/38 (5.2%)	1.37	1.37	0.27-2.47
Surveillance	4/103 (3.8%)	36.65	4.76	2.29-134.8

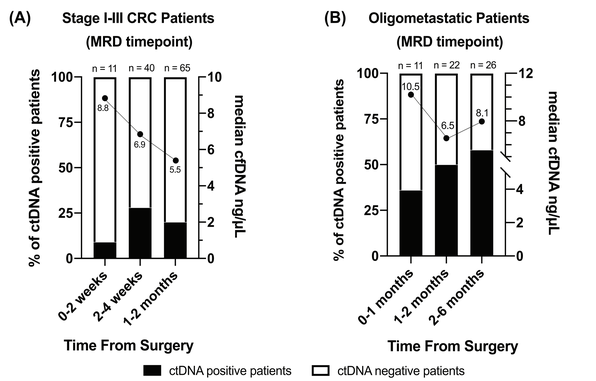
Table 2. MRD rates and ctDNA quantity in metastatic CRC patients on treatment monitoring (n = 41)

Setting	MRD Rates	Quantity of ctDNA (MTM/mL)		
		Mean	Median	Range
Progressive/Active Disease	16/16 (100%)	203.75	7.51	0.13-2149.96
Stable Disease/Partial Response	9/15 (60%)	7.06	1.23	0.17-51.03
No Evidence of Disease	4/12 (33%)	232.07	21.41	0.62-884.83

Table 3. MRD rates and ctDNA quantity in oligometastatic setting (n = 93)

Setting	MRD Rates	Quantity of ctDNA (MTM/mL)		
		Mean	Median	Range
Neoadjuvant (presurgical)	9/9 (100%)	3045.04	36.53	0.49-27,077.71
MRD (post-surgical)	26/53 (49%)	454.15	5.61	0.11-13274.05
During Adjuvant Treatment	5/15 (33%)	4.54	2.21	0.48-17.75

Figure 2. Percentage of MRD positive cases vs. timing from surgery in locoregionally advanced and oligometastatic CRC patients



Percentage of ctDNA positive patients are plotted based on the timing of the MRD drawn in relation to the definitive surgery in both locoregionally and oligometastatic patients. Overlayed is the cfDNA concentration in ng/µl.

Table 4. Multivariate Analysis

Covariates	Odds Ratio	95% CI for Odds Ratio		p-value
		Lower	Upper	
T4 vs T1-3	3.04	1.19	7.66	0.02
LN (+) vs (-)	1.8	0.74	4.8	0.18
MSS vs MSI-H	2.19	0.63	6.98	0.20
High-risk vs Low-risk	1.56	0.60	4.21	0.35

ctDNA as dependent variable and covariates as independent.
*High-risk: obstruction, perforation, LVI/PNI, undifferentiated/high grade histology, insufficient LN sampling, positive margins

Results

- MRD positivity rates and ctDNA quantification (mean tumor molecules/mL) for patients with local and regionally advanced (stage I-III) CRC, metastatic CRC and oligometastatic CRC are presented in **Table 1, 2, and 3,** respectively
- ctDNA detection was significantly associated with stage of disease ($p<0.0001$; Chi square: 50.94, df = 3) **(Table 1and 2).** In the multivariate analysis T4 on pathology in particular was a significant covariate associated with the ctDNA-positivity **(Table 4)**
- In patients with radiologically measurable active metastatic disease as demonstrated in **Table 2**, ctDNA detection rate was 100%. On the contrary, patients with advanced/ metastatic disease who had partial response to treatment or no evidence of disease (NED) showed 60% and 33% of ctDNA-positivity, respectively
- In neoadjuvant (presurgical/pretreatment setting), ctDNA detection was 100% in oligometastatic CRC patients, compared to patients with any definitive therapy; MRD postsurgical timepoint (49%), during adjuvant treatment (33%) and surveillance (46%) **(Table 3)**
- In patients with locoregionally advanced CRC, MRD detection rates were observed to be low within 2 weeks post-surgery, whereas the background cfDNA levels were found to be elevated. This may suggest that this timepoint is suboptimal for MRD detection due to the increased cfDNA load from surgery. In contrast, the timepoint 6 weeks after surgery would be appropriate for MRD detection, when the cfDNA levels have normalized close to the baseline. A similar trend was observed in the oligometastatic setting post-surgery as well **(Figure 2)**.

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Acknowledgments and Conflicts of Interest

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