Real-time measurements of circulating tumor DNA levels during FOLFOX treatment in patients with colorectal cancer

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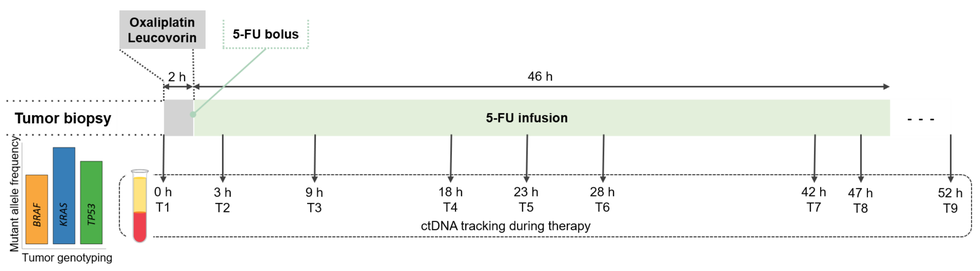
Background and Objectives

In recent years, various efforts have been made to identify biomarkers in metastatic colorectal cancer patients (mCRC), all with the goal of improving patients' outcomes, including maximizing therapeutic response and minimizing exposure to ineffective treatments. Nevertheless, there is still no valid biomarker for the early assessment of therapeutic efficacy in the patient management strategy. Here, we addressed a significant unknown in the field of circulating tumor DNA (ctDNA) analysis, i.e. how do ctDNA levels change during one of the most commonly administered drug regimens, the 48-hour application of FOLFOX (FOLinic acid (leucovorin), Fluorouracil (5-FU), Oxaliplatin) and whether these changes are informative about response to treatment.

Workflow and Patients

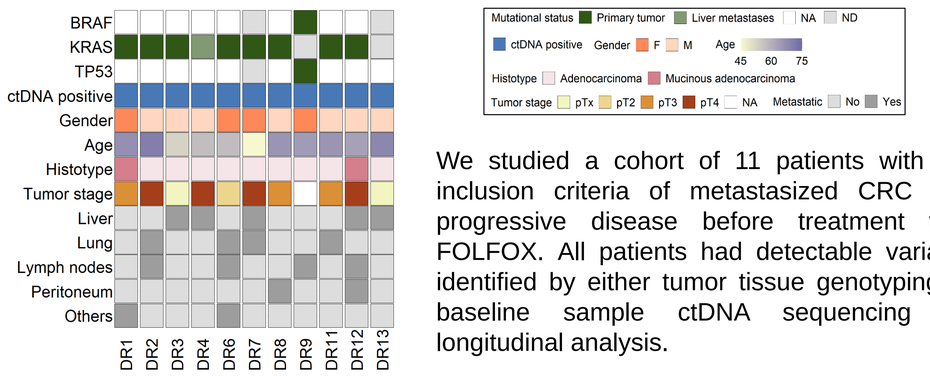
STUDY DESIGN



First, we established the *BRAF*, *KRAS* and *TP53* mutation status from tumor tissues. During the 48-hour FOLFOX cycle, we performed serial ctDNA analysis before treatment start (T1) and at seven further time points (T2-T8). The last blood sample was drawn after completion of treatment (T9). We assessed ctDNA mutant allele frequencies (mAFs), employing ultrasensitive mutation-based assays*. We additionally analyzed all plasma samples for somatic copy number alterations (SCNAs) and determined respective tumor fractions with the ichorCNA algorithm.

*mutation-based analysis: AVENIO ctDNA Targeted Panel, Simple, multiplexed, PCR-based barcoding of DNA for sensitive mutation detection using sequencing (SiMSen-seq), Deep sequencing

SUMMARY OF PATIENTS CHARACTERISTICS



We studied a cohort of 11 patients with the inclusion criteria of metastasized CRC and progressive disease before treatment with FOLFOX. All patients had detectable variants identified by either tumor tissue genotyping or baseline sample ctDNA sequencing for longitudinal analysis.

Key findings

- The fraction of mutant DNA fragments varied widely in advanced CRC patients.
- Surprisingly, we did not observe a spike in ctDNA as a sign of a responsive tumor.
- Instead, ctDNA levels initially decreased during the first 23 hours (T5) in all cases.
- In patients with SD or PR, ctDNA levels remained at decreased levels at the time of our last blood collection (T9).
- In patients with PD, ctDNA levels increased at the end of the treatment cycle.
- The plasma DNA fragmentation patterns under FOLFOX therapy did not differ from those generally observed in patients with cancer and are consistent with a preferential release from apoptotic cells.

Conclusion

Our study may contribute to defining optimal time points for measuring treatment efficacies. For patients under FOLFOX therapy, ctDNA levels at time points T1 (baseline) and T9 (~52 hours after treatment start), may be useful for measuring treatment response, but further studies are clearly required before the clinical utility can be evaluated. Hence, our work may initiate early dynamic therapy response modeling and the establishment of a real-time personalized treatment response assessment.

References

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Heitzer, E., et *al*. Cell-Free DNA and Apoptosis: How Dead Cells Inform About the Living. *Trends Mol Med.,* 2020

Tie, J., et *al*. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol.*, 2015

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Results

SUMMARY OF CTDNA DETECTION IN ALL MCRC PATIENTS

The median mAFs for each plasma DNA analysis and the timing of each blood draw are displayed.

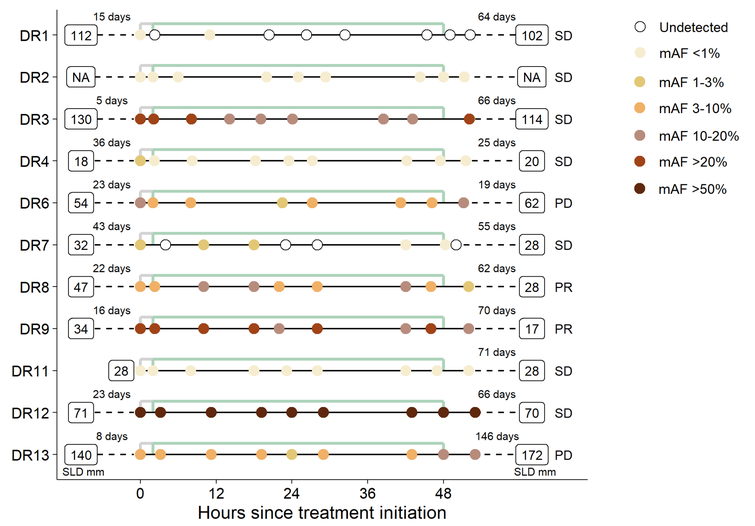

Figure 1: General plasma parameters are highly variable in mCRC patients.
In five patients, we observed low ctDNA mAFs of less than 1% in most samples. The other six cases had ctDNA mAFs of ≥10% in at least one plasma analysis.

The sum of longest diameters (SLD) as established by CT imaging for the selected target lesions according to RECIST are shown prior (left side) and after (right side) completion of the FOLFOX cycle.

CTDNA LEVELS IN PLASMA FROM THREE PATIENTS

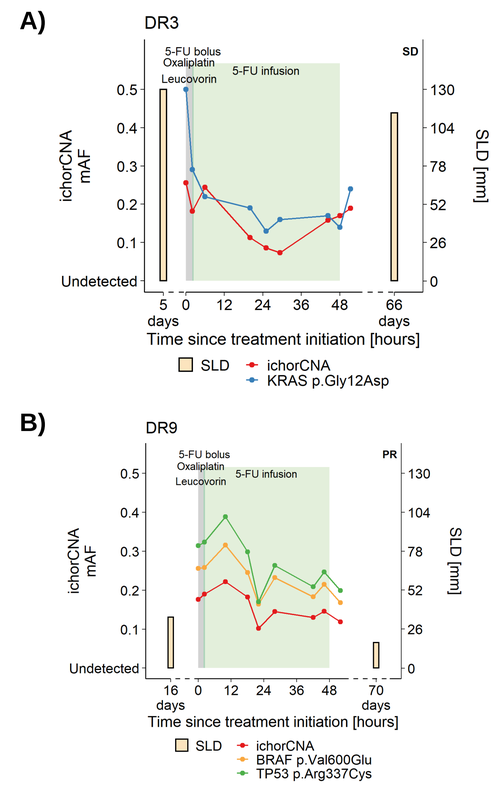

Figure 2: ctDNA mAF fluctuations under FOLFOX administration.
In each panel, the tumor fraction as established by ichorCNA and the respective mutations are displayed. Before and after the FOLFOX cycle, the SLD for the target lesions is displayed.
A) and **B)** We observed a decrease of the ctDNA mAF within the first 18-23 hours and after treatment (T9), mAFs were lower than at baseline (T1).
C) In the plasma of patient DR13 we observed the initial decrease of mAFs within the first 23 hours followed by an increase of mAFs higher at T9 than at T1. **A)**, **B)**, **C)** The mAFs closely paralleled the ichorCNA quantification.

MAF PATTERN ACROSS PATIENTS WITH SD, PR AND PD

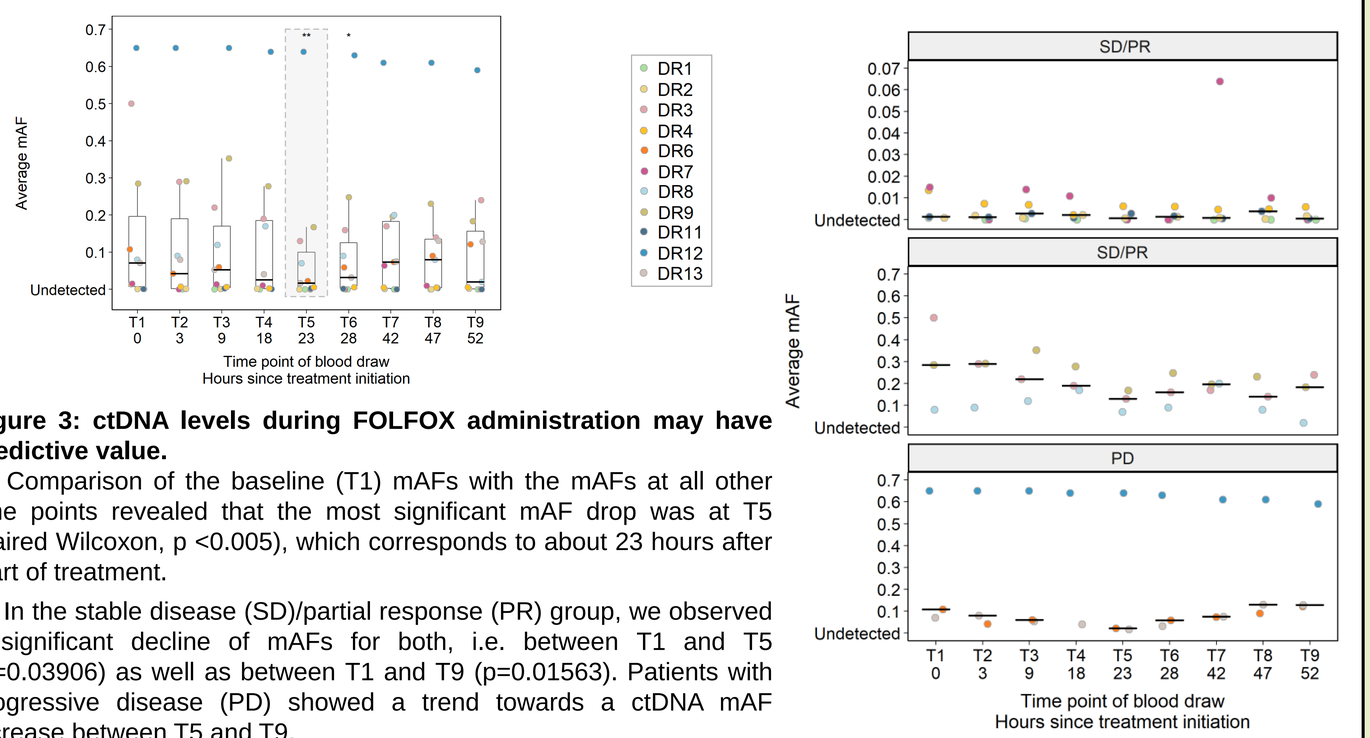

Figure 3: ctDNA levels during FOLFOX administration may have predictive value.
A) Comparison of the baseline (T1) mAFs with the mAFs at all other time points revealed that the most significant mAF drop was at T5 (paired Wilcoxon, p <0.005), which corresponds to about 23 hours after start of treatment.

B) In the stable disease (SD)/partial response (PR) group, we observed a significant decline of mAFs for both, i.e. between T1 and T5 (p=0.03906) as well as between T1 and T9 (p=0.01563). Patients with progressive disease (PD) showed a trend towards a ctDNA mAF increase between T5 and T9.

(SD/PR group: upper panel: patients with very low ctDNA levels, center panel: high ctDNA mAFs)

PLASMA DNA RELEASE

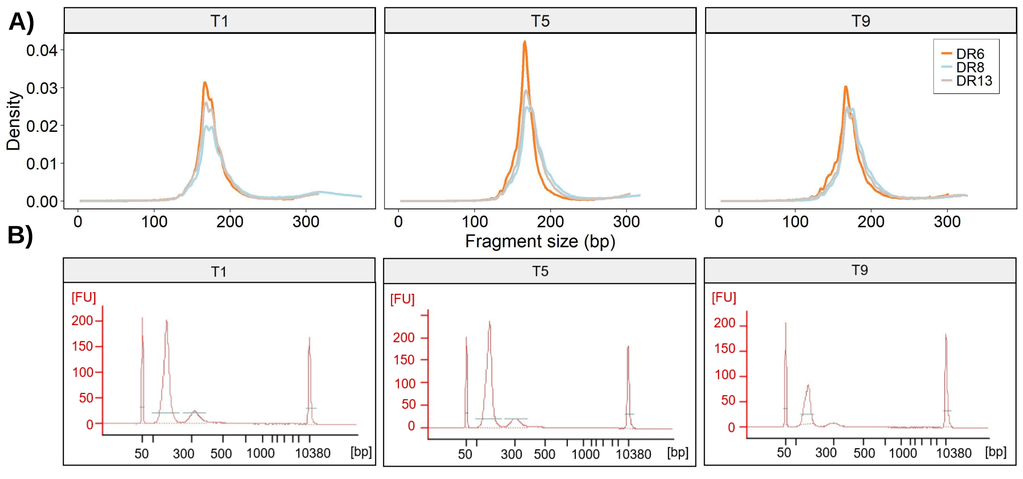

Figure 4: Plasma DNA fragmentation patterns during therapy.
A) cfDNA size profiles determined from paired-end sequencing data from patients DR6, DR8 and DR13 at time points T1, T5 and T9. Consistent with an apoptotic origin, we observed a modal value of DNA fragments lengths near 166 bp. This pattern did not change during therapy.
B) Via electrophoresis, we excluded the presence of high-molecular weight DNA, which has been associated with release from necrotic cells.