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Sensitive detection of circulating tumor DNA by whole genome sequencing

Validation of MRDetect using serial blood samples from stage III colorectal cancer patients

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Conclusions

1) Detection of circulating tumor DNA (ctDNA) through whole genome sequencing (WGS) of plasma is associated with high risk of recurrence
2) Our approch show great reproducebilty across independent laborarories
3) Detection of ctDNA immediately after surgery may be affected by high amounts of trauma-induced circulating free DNA (cfDNA)
4) Differences between tumor and plasma WGS suggest tumor heteroeneity

Study summary

Background

- Detection of circulating tumor DNA (ctDNA) is associated with poor prognosis.
- Sensitive detection of ctDNA is challenged by very low amounts of ctDNA, often only one mutated copy in 10 mL blood.
- We developed MRDetect; a whole genome sequencing approach, which detects ctDNA using a patient-specific signature generated from tens of thousands of genetic alternations throughout the genome.

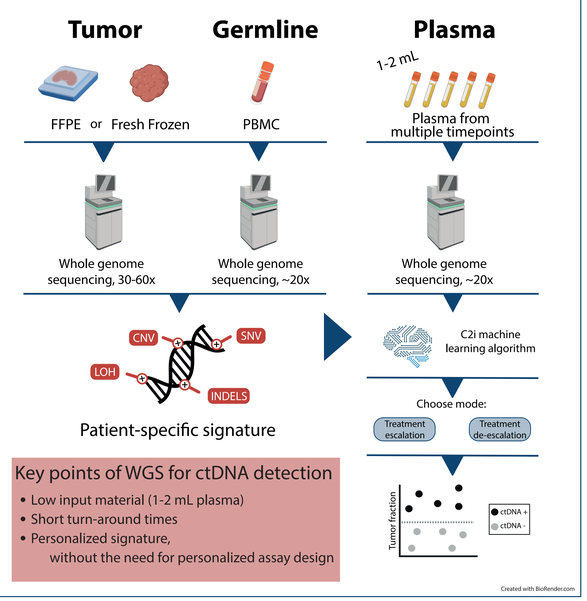
Aim

- Validation of MRDetect for sensitive detection of ctDNA to monitor disease in stage III colorectal cancer patient, including assessment of reproducibility.

Methods

- Reproducibility: Analysis of paired aliquots of 136 blood samples from 14 patients.
- Clinical utility of MRDetect: Whole genome sequencing (WGS) of tumor (n = 129), normal (n = 129) and serially collected plasma (n = 921) samples for ctDNA assessment using MRDetect.

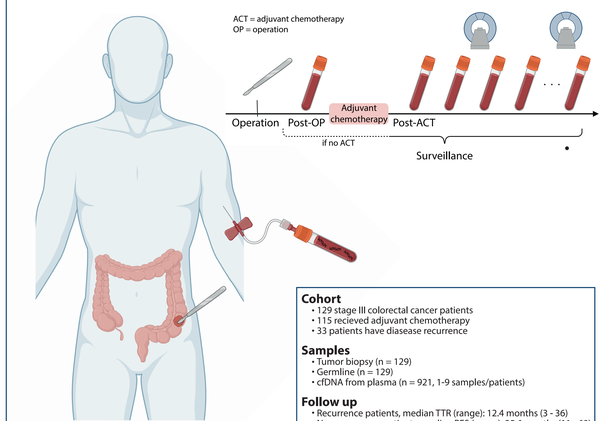
Workflow



Key points of WGS for ctDNA detection

- Low input material (1-2 mL plasma)
- Short turn-around times
- Personalized signature, without the need for personalized assay design

Study population



Cohort
- 129 stage III colorectal cancer patients
- 115 recieved adjuvant chemotherapy
- 33 patients have disease recurrence

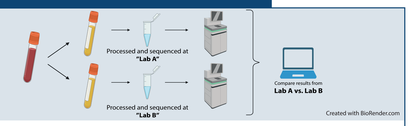
Samples
- Tumor biopsy (n = 129)
- Germline (n = 129)
- cfDNA from plasma (n = 921, 1-9 samples/patients)

Follow up
- Recurrence patients, median TTR (range): 12.4 months (3 - 36)
- Non-recurrence patients, median RFS (range): 35.6 months (11 - 60)

TTR = Time to recurrence, RFS = Recurrence free survival

Reproducebility

The reproducebility of MRDetect was tested in paired aliquots of 136 blood samples in two laboratories:
- "Lab A" in Denmark
- "Lab B" in the United States



Excellent agreement between the independet analyses, both in terms of ctDNA status and tumor fraction estimation

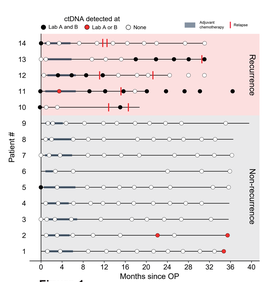


Figure 1
ctDNA status of paried aliquoted plasma are in agreement for 97% (132/136) of samples (cohens kappa: 0.9, McNemar test, p = 1).

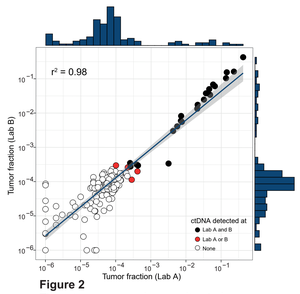
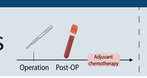


Figure 2
There is an excellent correlation between estimated tumor fractions ($r^2 = 0.98$) in plasma processed at Lab A and Lab B.

Results

Prognostic impact of post-OP ctDNA status



Timing of post-OP sampling affects detection of ctDNA

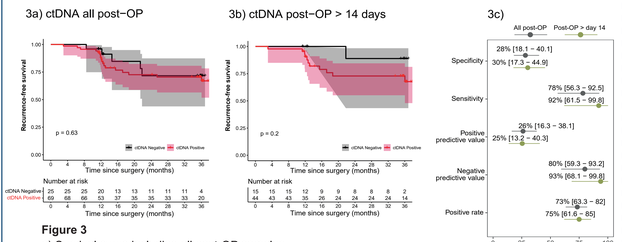


Figure 3
a) Survival curve including all post-OP samples
b) Survival curve including post-OP samples drawn > 14 days after surgery
c) Point estimates with 95%-CI

High cfDNA levels post-OP increase the risk of false negative ctDNA detection

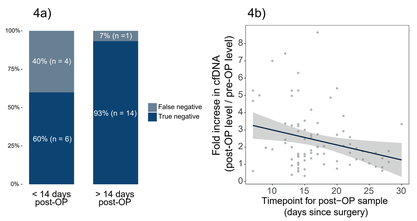


Figure 4
a) Samples drawn within two weeks after surgery has a higher false negative rate than samples drawn after day 14.
b) High false negative rate might be explained by high amount of trauma induced cfDNA in relation to surgery, which decrease within a few.

Post-ACT ctDNA status and prognosis

ctDNA assesment after adjuvant chemotherapy identifies patients with residual disease

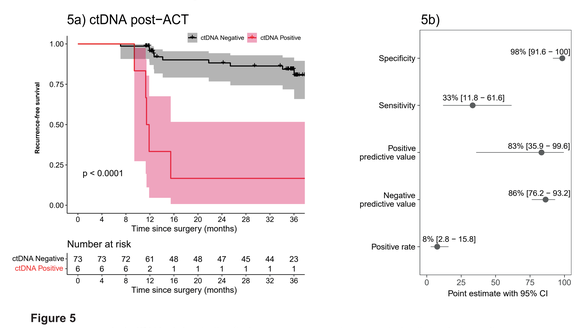
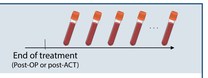


Figure 5
a+b) Assesment of ctDNA status in the first plasma sample drawn end of adjuvant chemotherapy (up to 3 months after end of therapy).

Disease surveillance using ctDNA



Serial assesment of ctDNA idenfies early recurrence

A total of 102 patients (16 recurrence, 86 non-recurrence) are included in the surveillance analysis

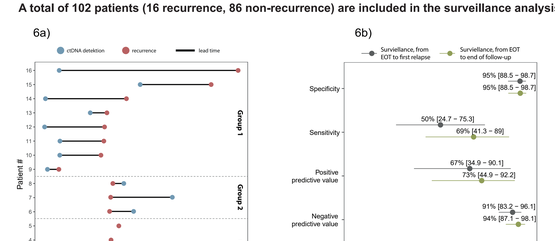


Figure 6
a) ctDNA is detected prior to recurrence in 8/16 patients (group 1) with a median lead time of 9.5 months (mean = 11.6 months). In 3 patients (group 2), the recurrence is detected prior of ctDNA and in 5 patients (group 3) there is no ctDNA detected at any timepoint.
b) Point estimates with 95% CI showing the performance of MRDetect when assessing ctDNA status in samples from end-of-treatment to first radiological relapse (grey) or to end of follow-up (green).

WGS of tumor and plasma reveals tumor heterogeneity

Chomosome 3 copy number change adjusting mutant SETD2 frequency

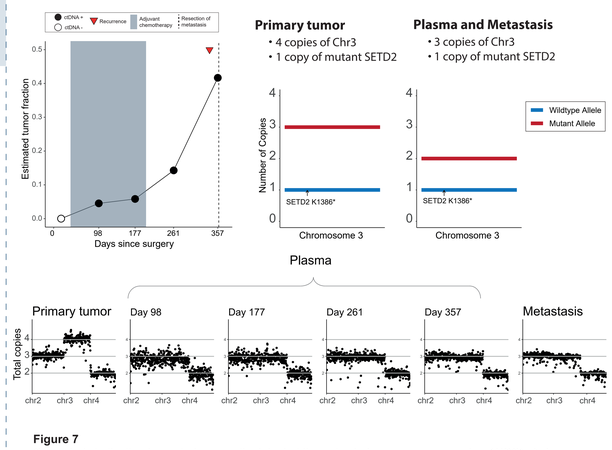


Figure 7
Case study indicating tumor heterogeneity detected through whole genome sequencing (WGS) of plasma samples. WGS of metasis confirms findings in plasma.