Abstract #3358

Prognostic value of post-surgery liquid biopsy cell-free circulating tumor DNA in stage III colon cancer patients - PLCRC-PROVENC3 study

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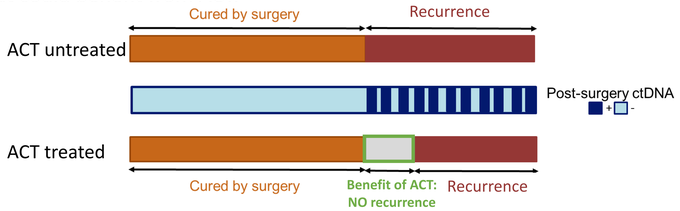
Background

- Current clinical guidelines in the Netherlands recommend adjuvant chemotherapy treatment (ACT) following resection of the primary tumor for all stage III colon cancer patients.
- Only **15-20%** of the patients benefit from ACT: around 55% of stage III colon cancer patients are cured by surgery alone and are being overtreated, and 30% will relapse despite ACT.
- **Prognostic biomarkers** may improve ACT decisions and reduce futile treatment in this group of patients by identifying the patients at a higher risk of recurrence that could benefit from ACT.



- Post-surgery circulating tumor DNA (**ctDNA**) detection indicates presence of minimal residual disease, and it is a strong prognostic factor in stage II and III colorectal cancer.

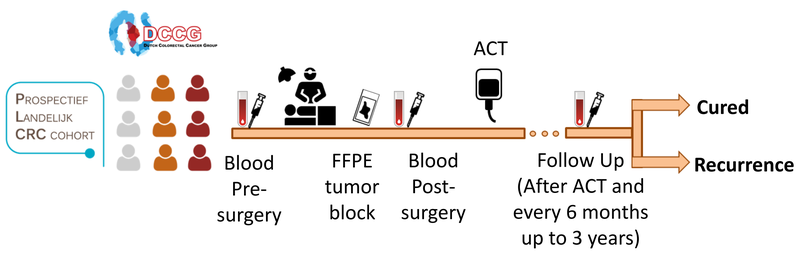
Goal of the study

Investigate the prognostic value of post-surgery ctDNA testing for disease recurrence in ACT treated stage III colon cancer patients

Experimental approach: PROVENC3 study

PROVENC3: (**PRO**gnostic **V**alue of **E**arly Notification by **C**tdna in Colon Cancer stage **3**).

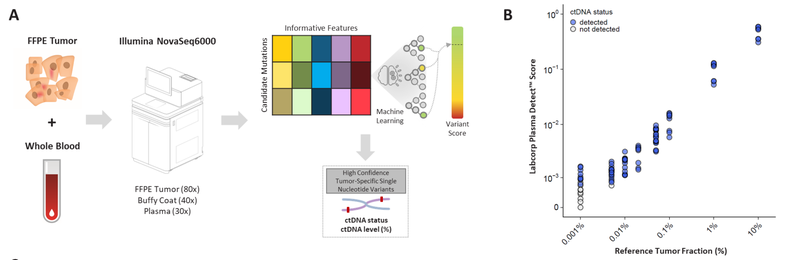
- PROVENC3 is an **observational** study within the Prospective Dutch Colorectal Cancer Cohort (PLCRC, https://plcrc.nl/for-international-visitors).
- 26 Participating hospitals in PROVENC3.
- **236 patients included** (Dec2016-Oct2021): Stage III, ACT treated, post-surgery blood available.



- One post-surgery blood sample is collected from day 3-65 after surgery (Median: 10 days, IQR: 14 days), before ACT.
- All biosamples are sent to a central laboratory (The Netherlands Cancer Institute).
- Clinical data is collected in the Netherlands Cancer registry by IKNL.

Experimental approach: ctDNA detection method

Tumor-informed detection of plasma ctDNA through integrated **whole genome sequencing (WGS)** analyses:



- A) Schematic of the Labcorp Plasma Detect™ assay workflow (adapted from Keefer et al., 2022 Nature Communications and Wood et al., 2018 Science Translational Medicine)
- B) Analytical studies demonstrated a limit of detection (95%) of 0.005% tumor content utilizing contrived reference models derived from commercially available cell lines (including lung cancer, breast cancer, and melanoma), with a specificity of 99.6% (2,015/2,023) observed across 119 noncancerous donor plasma specimens evaluated against 17 reference somatic mutation datasets. The observed tumor fraction was also highly correlated with the reference tumor fraction (Pearson correlation coefficient = 0.96, p<0.001).
- C) Analysis of an external contrived reference control sample demonstrated reproducible results across 24 independent runs evaluated for the PROVENC3 clinical study (CV = 7.2%)

Clinicopathological characteristics and ctDNA MRD results

Results for **114 patients** with post-surgery ctDNA analyzed and clinical follow up (FU) information about recurrence available. Median FU 31 months, IQR: 18 months.

		ctDNA positive 22 (18%)	ctDNA negative 92 (82%)	Total 114
Median age (years) (Range)		66 (43-83)	63 (34-79)	63
Gender				
	Male	17 (77%)	45 (49%)	62 (54%)
	Female	5 (23%)	47 (51%)	52 (46%)
Tumor location				
	Right	11 (50%)	34 (37%)	45 (39%)
	Left	11 (50%)	58 (63%)	69 (61%)
Differentiation grade				
	Well differenciated	0	0	0
	Moderately differenciated	17 (77%)	81 (88%)	98 (86%)
	Poorly differenciated	3 (14%)	7 (8%)	10 (9%)
	Undifferenciated	0	0	0
	UNK	2 (9%)	4 (4%)	6 (5%)
T				
	T1	0	2 (2%)	2 (2%)
	T2	1 (5%)	3 (3%)	4 (4%)
	T3	15 (68%)	67 (73%)	82 (72%)
	T4	6 (27%)	20 (22%)	26 (23%)
N				
	N1	13 (59%)	66 (72%)	79 (69%)
	N2	9 (41%)	26 (28%)	35 (31%)
Clinical risk				
	Low risk	12 (55%)	56 (61%)	68 (60%)
	High risk	10 (45%)	36 (39%)	46 (40%)
MR status				
	MSS	21 (95%)	79 (86%)	100 (88%)
	MSI	1 (5%)	10 (11%)	11 (10%)
	UNK	0	3 (3%)	3(3%)
Resection				
	Radical	20 (91%)	88 (96%)	108 (95%)
	Non radical	2 (9%)	1 (1%)	3 (3%)
	UNK	0	3 (3%)	3 (3%)
ACT				
	3 months CAPOX	15 (68%)	72 (78%)	87 (76%)
	6 months capecitabine	2 (9%)	3 (3%)	7 (6%)
	6 months CAPOX	3 (14%)	12 (16%)	16 (14%)
	3 months capecitabine	2 (9%)	2 (2%)	4 (4%)

41% of the patients that experienced a recurrence had detectable minimal residual disease (MRD) post-surgery, and 30% of the ctDNA+ patients were likely cured by ACT:

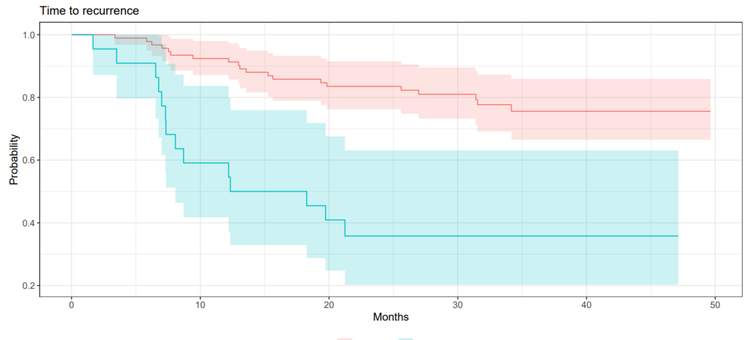
	ctDNA +	ctDNA -	Total
	22 (18%)	92 (82%)	114
Recurrence	14 (64%)	20 (22%)	34 (30%)
Non recurrence	8 (36%)	72 (78%)	80 (70%)

Clinical risk per ctDNA and recurrence status:

	Relapse 34	No relapse 80	Total 22
ctDNA+ patients			
Total	**14**	**8**	**22**
Low risk	6 (43%)	6 (75%)	12 (55%)
High risk	8 (57%)	2 (25%)	10 (45%)
ctDNA- patients			
Total	**20**	**72**	**92**
Low risk	6 (30%)	50 (70%)	56 (61%)
High risk	14 (70%)	22 (30%)	36 (39%)

Prognostic value of post-surgery ctDNA detection

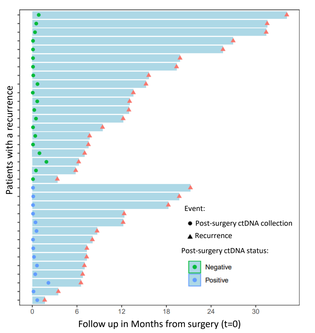
Time to recurrence (TTR) was evaluated for all **114 patients** based on **post-surgery ctDNA status**. Patients with a positive ctDNA status post-surgery are at a higher risk of experiencing a recurrence **(HR: 4,52; 95%CI: 2,27-9,01; p<0,001)**

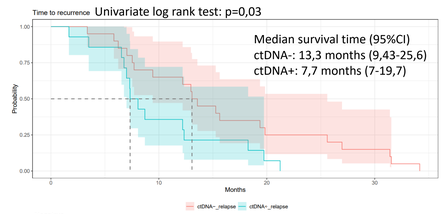


	Negative					
At risk	92	85	73	55	18	0
Events	0	7	15	17	20	20
	Positive					
At risk	22	13	9	6	2	0
Events	0	9	13	14	14	14

Time to relapse informed by post-surgery ctDNA results

Time to recurrence was evaluated for the **34 patients experiencing a recurrence** based on **post-surgery ctDNA status**. **CtDNA positive patients** experiencing a recurrence (ctDNA+_relapse) **show a shorter time to recurrence than ctDNA negative patients** experiencing a recurrence (ctDNA-_relapse).





Negative				
At risk	20	13	5	3
Events	0	7	15	17
Positive				
At risk	14	5	1	0
Events	0	9	13	14

Conclusions

- Post-surgery ctDNA testing improves the stratification of stage III colon cancer patients for disease recurrence on top of current clinicopathological risk factors
- Approximately one third of the ctDNA-positive patients seem to benefit from ACT.

Next steps

- Complete sample analysis and clinical data collection.
- Based on the results of this study, design of an interventional study towards implementation of ctDNA testing for stage III colon cancer patients.

FUNDED BY

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