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## Original Research

# Circulating tumor DNA is a prognostic marker of tumor recurrence in stage II and III colorectal cancer: multicentric, prospective cohort study (ALGECOLS)



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**KEYWORDS**

Circulating tumor DNA;  
Colorectal cancer;  
Picodroplet droplet-based digital PCR;  
Liquid biopsy;  
Methylated DNA;  
Prognostic marker

**Abstract Background:** In non-metastatic colorectal cancer (CRC), we evaluated prospectively the pertinence of longitudinal detection and quantification of circulating tumor DNA (ctDNA) as a prognostic marker of recurrence.

**Method:** The presence of ctDNA was assessed from plasma collected before and after surgery for 184 patients classified as stage II or III and at each visit during 3–4 years of follow-up. The ctDNA analysis was performed by droplet-based digital polymerase chain reaction, targeting mutation and methylation markers, blindly from the clinical outcomes. Multivariate analyses were adjusted on age, gender, stage, and adjuvant chemotherapy.

**Results:** Before surgery, 27.5% of patients were positive for ctDNA detection. The rate of recurrence was 32.7% and 11.6% in patients with or without detectable ctDNA respectively ( $P = 0.001$ ). Time to recurrence (TTR) was significantly shorter in patients with detectable ctDNA before (adjusted hazard ratio [HR] = 3.58, 95% confidence interval [CI] 1.71–7.47) or immediately after surgery (adjusted HR = 3.22, 95% CI 1.32–7.89). The TTR was significantly shorter in patients with detectable ctDNA during the early postoperative follow-up (1–6 months) (adjusted HR = 5, 95% CI 1.9–12.9). Beyond this period, ctDNA remained a prognostic marker with a median anticipated diagnosis of recurrence of 13.1 weeks (interquartile range 28 weeks) when compared to imaging follow-up. The rate of ctDNA+ might be underestimated knowing that consensus pre-analytical conditions were not described at initiation of the study.

**Conclusion:** This prospective study confirms the relevance of ctDNA as a recurrence risk factor in stage II and III CRC before surgery and as a marker of minimal residual disease after surgery that may predict recurrence several months before imaging techniques.

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## 1. Introduction

Colorectal cancer (CRC) is the third most frequent cancer worldwide [1]. Most non-metastatic patients will achieve remission but 20–40% of them will recur, the risk depending on initial tumor staging [2]. In patients with ‘histological high-risk’ stage II and III, surgery is followed by adjuvant chemotherapy (ACT) that decreases the risk of recurrence. However, the survival is largely contrasted. In stage II, the overall survival (OS) is 80% but decreases up to 58% in case of invasion of the nearby structures (stage IIc). Likewise, the 3-year disease-free survival (DFS) is superior to 80% for patients with early stage III tumors but inferior to 66% for T4/N2 patients [2]. Such heterogeneity suggests that other factors should be taken into consideration to sharpen algorithms for CRC prognostication.

In metastatic CRC (mCRC), the longitudinal detection of circulating tumor DNA (ctDNA) is strongly correlated with the course of disease. At diagnosis, ctDNA is detectable (ctDNA+) in 80–90% of the patients [3,4]. High ctDNA concentration has been associated with tumor burden [5], liver metastasis [6], shorter progression-free survival [7], and shorter OS [3,8]. During chemotherapy, variations in ctDNA detection could provide early indication of recurrence [3,9–11] and clonal resistance [12,13]. In operable patients with liver metastasis, ctDNA+ before surgery is associated with an increased risk of recurrence [4,14].

Some questions remain in the landscape of non-mCRC. Before treatment, the reported frequency of ctDNA+ patients comprises between 50% and 90%

[15–19]. The ctDNA clearance during treatment has rarely been evaluated. Within patients with preoperative ctDNA+ about 80% present no detectable DNA after surgery. Within patients with postoperative ctDNA+, 50% are cleared by ACT [16,21]. In locally advanced rectal cancer, Tie et al. [22] reported that 80% of ctDNA+ patients at baseline are cleared by radio-chemotherapy and surgery. After resection of the primary tumor, ctDNA+ could be associated with local and distant recurrence [16,20,23,24]. At the end of the ACT, ctDNA+ patients have shown an increased risk of relapse [16]. Finally, this marker may provide months’ lead-time on the detection of recurrence compared to conventional imaging [16]. Overall, patients with early stages of CRC should be cured and ctDNA tracking is essential to understand the crossroad between non-metastatic and micro-metastatic disease.

In addition to the limited number of studies at early cancer stages, the absence of standardization and the multiple detection technics might explain the discrepancies. The quantitative detection of ctDNA at early stages requires highly sensitive tools such as droplet-based digital polymerase chain reaction (ddPCR) and/or optimized next generation sequencing (NGS) [3,16]. The ctDNA monitoring by ddPCR targeting tumor-specific genetic and/or epigenetic alterations has been recently described as cost-effective and time-efficient [3,6,9].

In this study, we investigated the prognostic impact of the longitudinal ctDNA detection of patients with stage II or III CRC.

## 2. Materials and methods

### 2.1. Study design and patients

The prospective multicenter ALGECOLS (Presence of Circulating Tumor DNA in Colorectal Cancer) study (NCT01198743) received the ethical approval from ‘the committee Ile-de-France II’ and all patients provided written informed consent. Patients were eligible if they had a resectable CRC above the Douglas reflection (colon and high rectum) without previous history of cancer. In per-protocol, the inclusions were restricted to stage II and III cancer (see workflow in [Supplementary Fig. S1](#)).

Plasma samples were collected before surgery ( $D_0$ ), 5 d after surgery ( $D_5$ ), and every 3–6 months during 3–5 years of follow-up. The follow-up was performed according to national recommendations [25] and ended in May 2017. For the first 3 years, the follow-up included a clinical examination every 4 months and abdominal–pelvic ultrasound or abdominal–pelvic scan every 3–6 months. For the following 2 years of follow-up, the patients had clinical examination and abdominal–pelvic ultrasound or abdominal–pelvic scan every 6 months. All patients received annual chest scan. The follow-up was conducted blindly from ctDNA results.

### 2.2. Sample processing and data analysis

Method description has been performed in order to comply with digital dMIQE2020 guidelines ([Supplementary Fig. S2](#)) [26]. Samples preparation, storage, DNA extraction, ddPCR detection and analysis are described in [Supplementary Materials and methods](#). [Tables S1, S2, and S3](#) summarize the assay characteristics.

### 2.3. Statistical analysis

The risk of recurrence at 3 years in this cohort was estimated between 25% and 35%. Considering a mean of 5–10 events for each variable analyzed in a multivariate model (i.e. age, tumor-node-metastasis [TNM] stage, differentiation, number of invaded lymph nodes, total number of lymph nodes, ACT, preoperative carcinoembryonic antigen (CEA), ctDNA status) and an odds ratio of 2, the number of subjects required was 180 [27,28].

All analyses were carried out with a bilateral 5% alpha-type I error. Chi-square test was used to compare distributions of qualitative and ordinal variables. Time to recurrence (TTR) was defined as the time elapsed from surgery until the date of first recurrence or death from cancer. Surviving patients without recurrence were censored at the last follow-up date. Other causes of death from unknown reasons were censored.

Survival curves were drawn with the Kaplan–Meier method and compared with the log-rank test. The ctDNA

concentrations at  $D_0$ ,  $D_5$ , and at different time-points of follow-up were analyzed according to TTR. Cox regression models were used to estimate hazard ratio (HR) with 95% confidence intervals (CIs) and adjusted based on age, gender, stage, sidedness, and ACT. Analyses were performed using R survival package.

## 3. Results

### 3.1. Patients’ characteristics

Overall, 250 patients were included. Among them, 187 presented a stage II or III (per protocol) and were thus further studied ([Supplementary Fig. S1](#)). The mean age was 66.7 11.3 [30.1–86.1] and the gender ratio 1.37. The TNM classification showed 54.5% and 45.5% of stage II and III respectively and 52.4% received ACT ([Table 1](#)). Three patients were without plasma samples leading to 184 patients with both tumor tissues and serial plasma sample collected ([Supplementary Fig. S1](#)). Plasma samples were analyzed by ddPCR targeting tumor-specific mutation ( $N = 94$ ) or WNT inhibitory factor 1 (WIF1) or neuropeptide Y (NPY) methylation ( $N = 90$ ) (see Garlan et al. and Garrigou et al. [3,15] for studies that respectively compare these two methods of ctDNA detection and used them in combination for ctDNA analysis of mCRC patient follow-up).

### 3.2. Prognostic impact of circulating tumor DNA status before surgery ( $D_0$ )

At  $D_0$ , 181 plasma samples were available. Three samples led to non-interpretable results (low DNA content). Among the remaining 178 samples, 49 (27.5%) were ctDNA+ ([Table 1](#)). The method for ctDNA analysis did not impact its detection and we did not observe significant difference between the ctDNA positivity at  $D_0$  for samples analyzed by tumor-specific mutation (27.5%) or universal methylation markers (27.6%). No difference in the patients’ clinical characteristics for each type of markers (methylation or mutation markers) was observed ([Table 2](#)). No significant difference in age, gender, disease stage, sidedness, or administration of ACT was observed between samples with or without detectable ctDNA at  $D_0$  ([Table 1](#)).

The ctDNA+ patients at  $D_0$  showed more recurrences (32.7% versus 11.6%,  $P = 0.001$ ) and shorter TTR (log-rank  $P < 0.0001$ , adjusted HR = 3.58, 95% CI 1.71–7.47) ([Fig. 1A](#)) than patients without. We observed an inverse correlation between ctDNA concentration, taken as a continuous variable, and TTR at  $D_0$  even if it does not reach statistical significance (HR 1.41, 95% CI 0.92–2.16). When the concentration of ctDNA is divided in three groups based on the following cut points (0.01 and 0.1 ng/ml), the recurrence rate was 42.9%, 24%, and 12.1% for baseline ctDNA concentration superior to 0.1 ng/ml,

Table 1

Description of clinical variables according to the presence or absence of ctDNA at D0 (before surgery) or D5 (after surgery).

	ctDNA at D0		ctDNA at D5		Overall (N = 187)
	Present (N = 49)	Absent (N = 129)	Present (N = 18)	Absent (N = 153)	
Age					
Mean (SD)	65.3 (12.3)	67.1 (11.1)	63.7 (9.43)	67.3 (11.6)	66.7 (11.3)
Median [Min, Max]	65.8 [30.1, 86.1]	68.6 [38.0, 84.4]	64.8 [47.0, 80.0]	70.2 [30.1, 86.1]	67.8 [30.1, 86.1]
Gender					
Female	17 (34.7%)	58 (45.0%)	9 (50.0%)	64 (41.8%)	79 (42.2%)
Male	32 (65.3%)	71 (55.0%)	9 (50.0%)	89 (58.2%)	108 (57.8%)
Stage					
Stage II	25 (51.0%)	74 (57.4%)	6 (33.3%)	84 (54.9%)	102 (54.5%)
Stage III	24 (49.0%)	55 (42.6%)	12 (66.7%)	69 (45.1%)	85 (45.5%)
Sidedness					
Distal	28 (57.1%)	75 (58.1%)	12 (66.7%)	89 (58.2%)	109 (58.3%)
Proximal	21 (42.9%)	53 (41.1%)	6 (33.3%)	63 (41.2%)	77 (41.2%)
Missing	0 (0%)	1 (0.8%)	0 (0%)	1 (0.7%)	1 (0.5%)
Adjuvant chemotherapy					
No	20 (40.8%)	65 (50.4%)	6 (33.3%)	74 (48.4%)	89 (47.6%)
Yes	29 (59.2%)	64 (49.6%)	12 (66.7%)	79 (51.6%)	98 (52.4%)
Recurrence					
No	34 (69.4%)	114 (88.4%)	10 (55.6%)	132 (86.3%)	154 (82.4%)
Yes	15 (30.6%)	15 (11.6%)	8 (44.4%)	21 (13.7%)	33 (17.6%)

ctDNA, circulating tumor DNA; D0, plasma samples collected before surgery; D5, plasma samples collected 5 days after surgery; Max, maximum; Min, minimum; N, number of patients; SD, standard deviation.

comprised between 0.01 and 0.1 ng/ml and inferior or equal to 0.01 ng/ml respectively (Fig. 1B).

At D0, the sensitivity was 50%, the specificity was 77%, the positive predictive value was 30%, and the negative predictive value was 88%.

### 3.3. Prognostic impact of immediate postoperative circulating tumor DNA status (D<sub>5</sub>)

At D<sub>5</sub>, 18 of the 171 tested samples (10.5%) were ctDNA+ (95% CI 5.9–15.1) (Table 1). Among them, 11 had preoperative ctDNA+, 5 had non-detectable preoperative ctDNA (ctDNA–), and 2 were not preoperatively tested. Overall, among the 49 patients with

detectable ctDNA at D0, 34 patients became ctDNA– at D5 (75%). No correlation with age, gender, stage, sidedness of disease, or ACT was observed.

CtDNA+ after surgery was associated with a 44.4% (95% CI 21.5–67.3) rate of recurrence versus 13.7% in ctDNA– patients ( $P = 0.003$ ). The TTR of patients with ctDNA+ at D<sub>5</sub> was significantly shorter than those without (log-rank  $P = 0.00027$ , adjusted HR = 3.22, 95% CI 1.32–7.89) (Fig. 2A).

At D5, the sensitivity was 27%, the specificity was 93%, the positive predictive value was 44%, and the negative predictive value was 86%.

The recurrence rate was 58% for patients receiving ACT (95% CI 30–86) and 16.6% for those who did not receive chemotherapy (95% CI 0–46). The low number of patients who recurred and the large CI impair any robust conclusions. The odds ratio for the risk of recurrence for patients with positive ctDNA at D5 is 4.88 after stratification for use of ACT.

Overall, when pooling D<sub>0</sub> or D<sub>5</sub> plasma sample results, the TTR of the ctDNA+ patients at either D<sub>0</sub> or D<sub>5</sub> was significantly shorter than those without (log-rank  $P < 0.0001$ , adjusted HR = 3.34, 95% CI 1.56–7.16) (Fig. 2B).

When analyzing the sequence of perioperative ctDNA status, the recurrence rate for the 115 patients with double ctDNA– (before and after surgery) was 10.4%.

When compared to double ctDNA– patients, the ctDNA negativation after surgery (ctDNA+ at D0 and ctDNA– at D5) remained associated with a higher risk of recurrence (HR 2.74, 95% CI 1.12–6.73), the highest risk being for the preoperative and postoperative ctDNA+ patients (HR 6.37, 95% CI 2.58–15.74).

Table 2

Description of clinical variables associated with the patients for whom the ctDNA was detected by targeting methylation or mutation markers.

Characteristic	Assays		P-value <sup>b</sup>
	Methylation (N = 90) <sup>a</sup>	Mutation (N = 94) <sup>a</sup>	
Patient age	68 (60, 77)	68 (60, 75)	0.85
Gender			0.28
Female	34 (38%)	44 (47%)	
Male	56 (62%)	50 (53%)	
Stage			0.36
Stage II	53 (59%)	48 (51%)	
Stage III	37 (41%)	46 (49%)	
Recurrence	17 (19%)	15 (16%)	0.74

<sup>a</sup> Statistics presented as median (IQR) or  $n$  (%).

<sup>b</sup> Statistical tests performed: Wilcoxon rank-sum test or chi-squared test of independence.

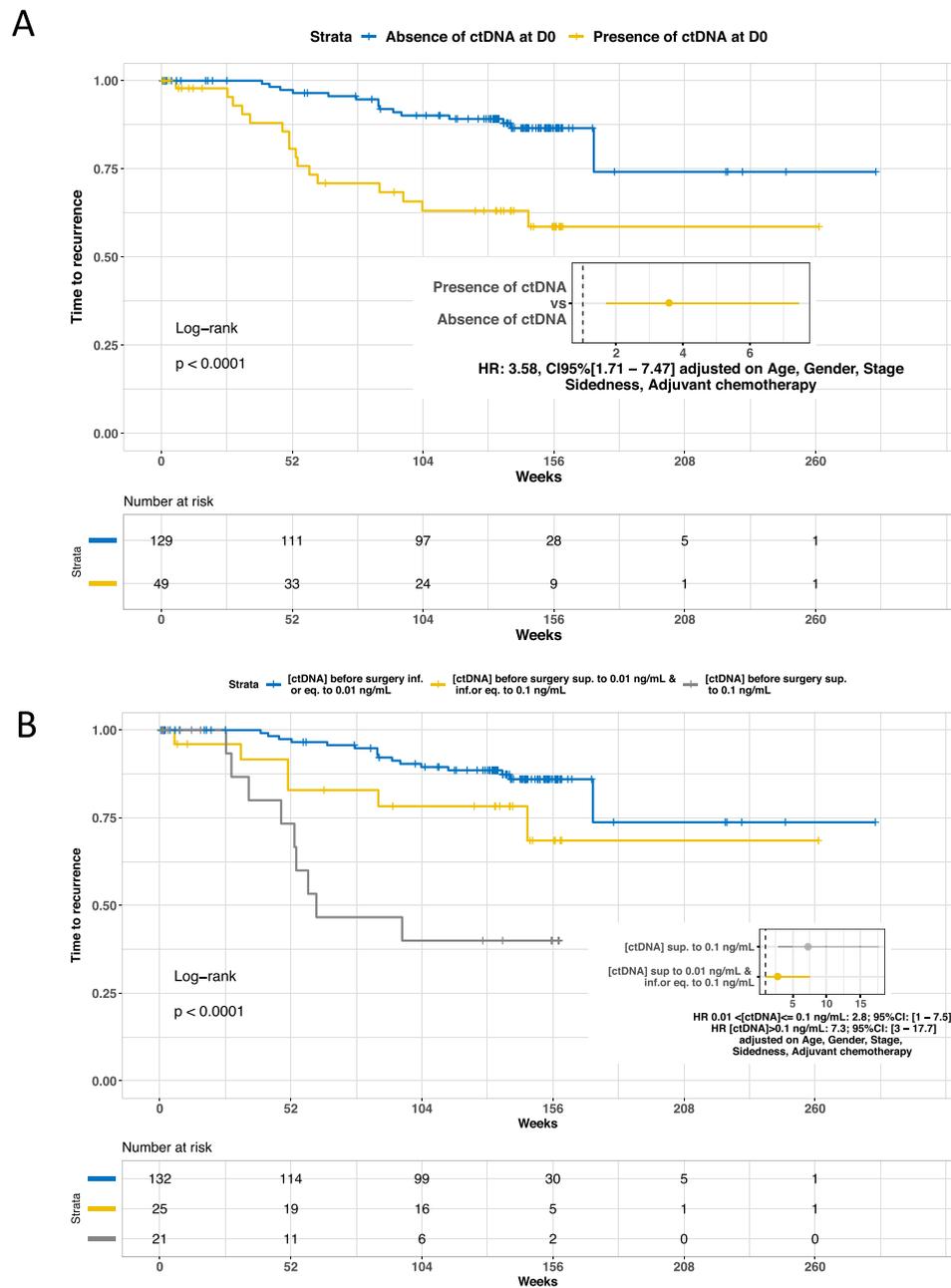


Fig. 1. TTR curves according to the presence or absence of ctDNA before surgery D0 (A) and to the ctDNA plasma concentration at D0 (B). CI, confidence interval; ctDNA, circulating tumor DNA; D0, plasma samples collected before surgery; D5, plasma samples collected 5 days after surgery; HR, hazard ratio; TTR, time to recurrence.

### 3.4. Prognostic impact of circulating tumor DNA detection within 6 months after surgery

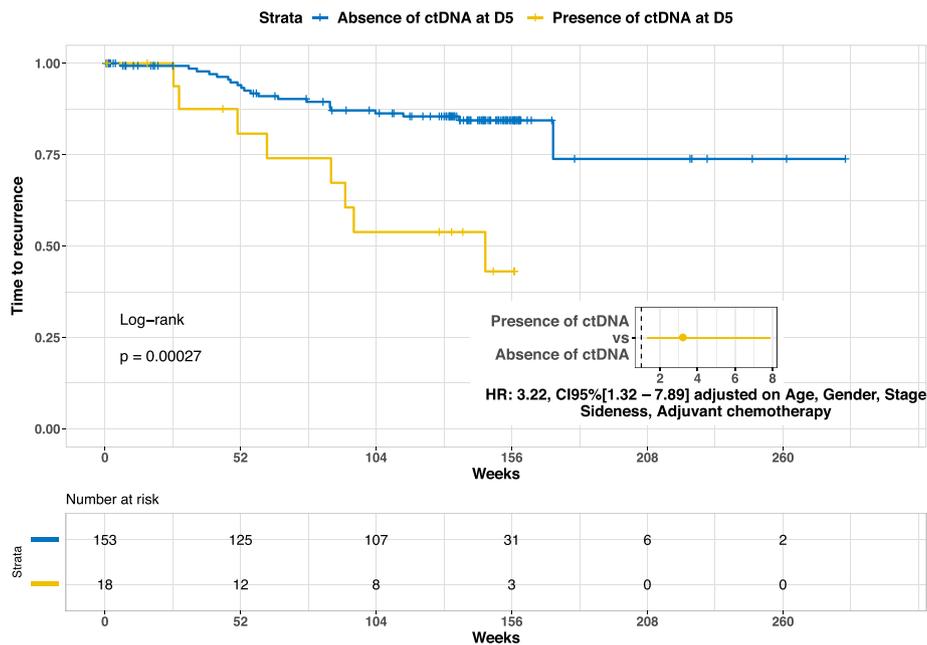
Samples collected between 1 and 6 months after surgery (30–180 d post-surgery) were analyzed. The TTR of ctDNA+ patients during this period was significantly shorter than those of patients with no detectable ctDNA ( $P < 0.0001$ , adjusted HR = 5, 95% CI 1.9–12.9) (Fig. 3). All patients with at least one ctDNA+ sample ( $N = 11$ ) during this period had shown ctDNA+ at D<sub>0</sub> and/or D<sub>5</sub> (Fig. 4). Seven patients recurred (63.6%) although 5 had received ACT. One patient died 1.4

weeks after the first evaluation post-surgery without evidence of recurrence. The remaining 3 patients received ACT and did not show recurrence during follow-up. The quantitative assessment did not bring additional information (Supplementary Fig. S3). ACT was not associated with survival in this cohort.

### 3.5. Prognostic impact of circulating tumor DNA detection during the long-term follow-up

Among the 139 patients for whom at least one sample was available during the follow-up, 21 were ctDNA+ at

A



B

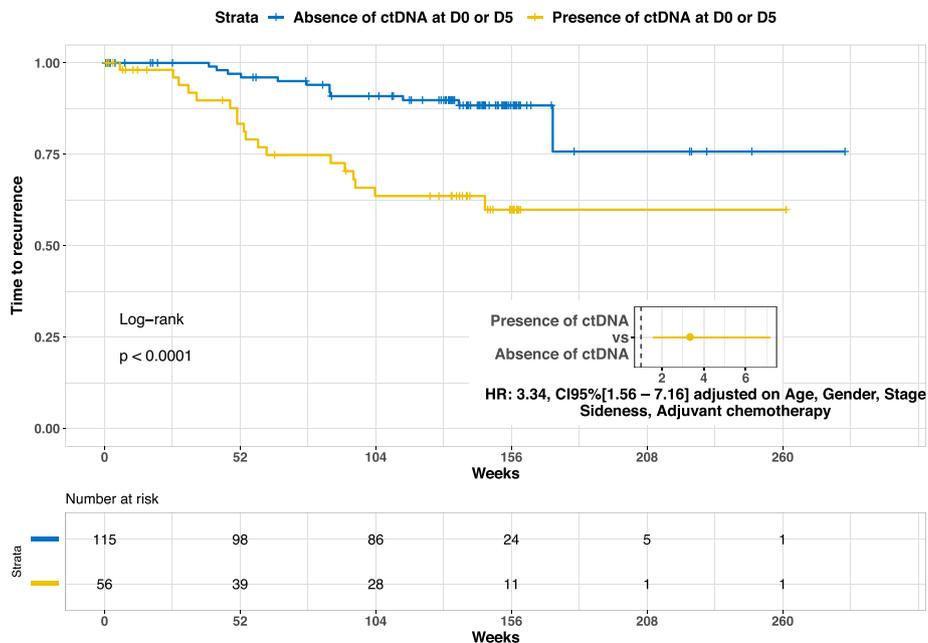


Fig. 2. TTR curves according to the presence or absence of ctDNA after surgery D5 (A) and to the presence or absence of ctDNA before and/or after surgery (B). CI, confidence interval; ctDNA, circulating tumor DNA; D0, plasma samples collected before surgery; D5, plasma samples collected 5 days after surgery; HR, hazard ratio; TTR, time to recurrence.

least one time (Fig. 4 and Fig. S4). When compared to imaging analysis, the ctDNA detection anticipated the recurrence with a median delay of 13.1 weeks (interquartile range 28 weeks). We compared TTR at various periods of sampling (Supplementary Fig. S4). The respective adjusted HRs for recurrence in ctDNA+ patients are: 4.1, 95% CI 1.39–12.2,  $P = 0.0005$  for the

period 30–120; 9.7, 95% CI 2.6–36.1,  $P < 0.0001$  for the period 120–240; 32.5, 95% CI 6.2–171,  $P < 0.0001$  for the period 240–360; and 9.4, 95% CI 1.9–47.7,  $P < 0.0001$  for the period 360–540 compared to the ctDNA– patients. CtDNA follow-up and clinical outcomes are illustrated using a survival–swimmer plot (Fig. 4).

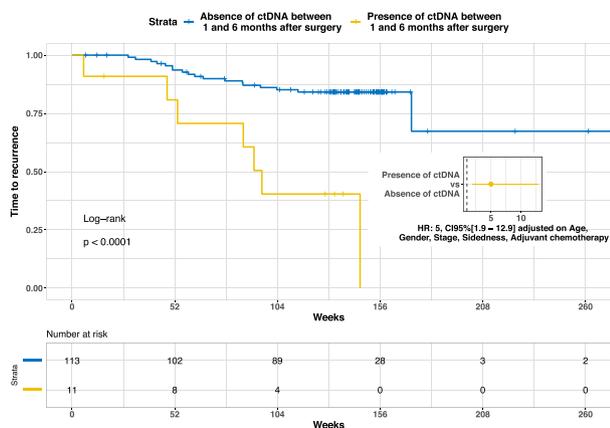


Fig. 3. TTR curves according to the presence or absence of ctDNA during the first 6 months after surgery. CI, confidence interval; ctDNA, circulating tumor DNA; HR, hazard ratio; TTR, time to recurrence

### 4. Discussion

Beyond histopathology, the molecular assessment of microsatellite status is the only prognostic factor used in daily practice for the decision of ACT.

In this work, ctDNA was detectable before surgery in 25% of stage II and 30% of stage III CRC. This rate is lower to what was observed in many other series [15–19]. Using multiplex assay PCR directed toward methylated genes, Symonds et al. [5] detected ctDNA+ preoperatively in 64% of stage II and 74% of stage III. The highest rate of pre-treatment detection reported by

Reinert et al. [16] was 92% of stage II and 90% of stage III using ultra-deep NGS sequencing. Since our study started years before the routine use of conservative tubes, our lower detection rate could be partly explained by the quality of sample preservation. The ethylene diamine tetra-acetic acid tubes were delivered at room temperature to the centralized laboratory within 24–48 h transport time, potentially resulting into DNA degradation [29].

The prognostic value of ctDNA detection before or after surgery is contested. In our series, ctDNA+ before surgery was associated with a three-fold higher recurrence risk. When using highly sensitive methods for ctDNA detection, most patients are found with preoperative ctDNA+ which has no impact on survival [16]. When using less sensitive methods only patients with high level of ctDNA are detected. The beaming assay used by Pazdirek et al. detected baseline ctDNA+ in 21% of patients with rectal cancer which was associated with shorter OS. These data suggest that the prognostic value could be associated with the ctDNA concentration rather than a ‘yes/no’ detection [30].

Interestingly, the sequence of the ctDNA detection was also important in our cohort. The rate of recurrence was 54% for patients with positive ctDNA both before and after surgery. Overall, for the patients who were ctDNA+ before but ctDNA– after surgery the risk of recurrence was 23.5%. Finally, the risk of recurrence was only 10.4% for patients with double ctDNA–. This observation reinforces the importance of multiple sampling.

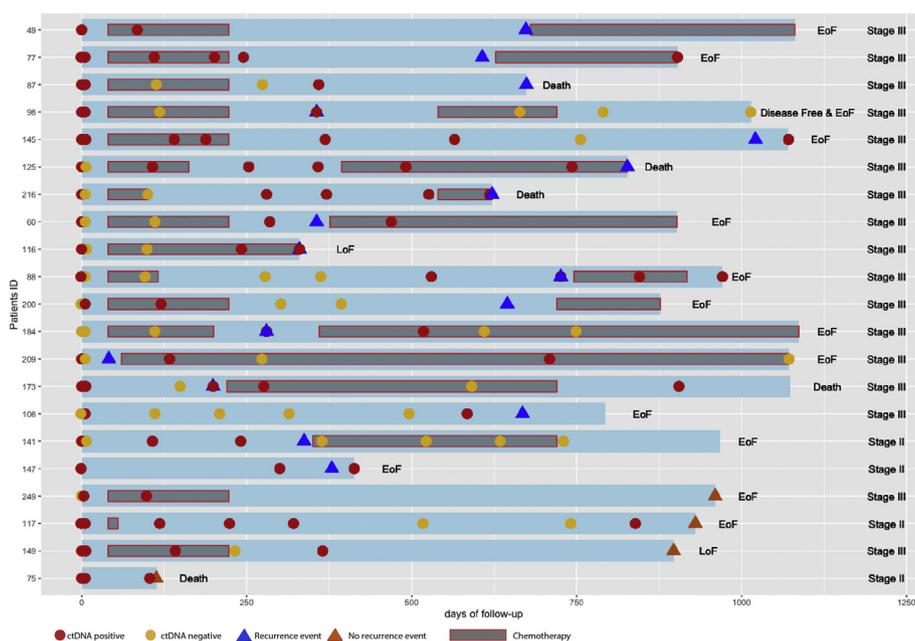


Fig. 4. ddPCR detection of ctDNA for early-stage colorectal cancer patients and relation to clinical outcomes. A summary of the treatments, status of serial plasma samples, and recurrence events observed each ctDNA+ patient during follow-up. ctDNA, circulating tumor DNA; ddPCR, droplet-based digital polymerase chain reaction; EoF, end of follow-up; LoF, lost to follow-up.

At D<sub>5</sub>, 10.5% (95% CI 5.9–15.1) of the patients had ctDNA+ (6.6% in stage II and 14.8% in stage III) which is concordant with other series [16,20,22,31,32]. It is yet important to notice that the optimal timing to draw blood after surgery is critical. In most series, the first sample was collected 30 d after surgery [16–21]. In the present series, the blood was first drawn at D<sub>5</sub> and the ctDNA+ was associated with 44.4% (95% CI 21.5–67.3) of recurrence. These results are comparable to what was observed by Tie et al. [33] who combined individual data from three independent cohorts of non-mCRC. In Tie study, the authors used the SafeSeqS method in 485 CRC patients and detected 12% ctDNA+ after surgery, which is close to the 10% that we observed in our series. The HR for DFS was 7.6 (95% CI 4.9–11.8) in their series and 5 (95% CI 1.9–12.9) in ours. In the clinical practice, the postoperative ctDNA status could be of major interest to drive the decision of adjuvant treatment. In the present study, we were able to deliver ctDNA results before week 4 (i.e. before ACT initiation). Indeed, it seems that after an immediate postoperative drop in ccfDNA concentration, the ccfDNA rises again from 24 h after surgery and emerged to discriminate patients with recurrence after 48 h [34]. This early blood collection (before week 4) may yet impact the sensitivity of ctDNA detection due to the release of wild-type DNA related to surgical trauma [35]. In the study by Schøler et al., the blood samples were collected at day 8, day 30, and every month. Interestingly, out of 26 operated patients, 2 were ctDNA+ 8 d after surgery but 2 others became ctDNA+ 1 month after surgery [21]. Overall, collecting blood early after surgery might be more relevant for immediate clinical application but has to be balanced with a higher rate of false negatives.

If we now consider a period comprised between 30 d and 6 months after surgery, ctDNA+ was observed in 11 patients. Among them, 7 recurred (63.6%) and 1 died postoperatively without recurrence. The 3 other patients did not recur possibly due to ACT. In the series of Tie et al. [31], ctDNA was cleared under chemotherapy in 50% of the cases which reduced the risk of recurrence. Remarkably, in our series the highest HR predicting recurrence was observed between 8 months and 1 year after surgery (HR = 32.5), which corresponds to the end of ACT (period 240–360). During this period ctDNA– led to a risk of recurrence around 14%. For the same period, the HR predicting recurrence was 17.5 (95% CI 3.8–79.9) for patients receiving ACT (Supplementary Fig. S4), close to that observed by Reinert et al. [16].

The ctDNA monitoring may allow performing less frequent imaging follow-up. In the present study, the first positive sample anticipated the imaging evidence of recurrence from 13.1 weeks. Although these results are too preliminary to translate in our daily practice it paves the way of new algorithms for the recurrence detection. Another critical point that requires clarification is that

ctDNA detection is not entirely synonymous of recurrence. In two series of Tie et al. [22,31], 33–50% of the ctDNA+ patients after treatment did not recur. In the ALGECOLS cohort, one ctDNA+ patient did not recur after ACT (period 360–540 and 540–900) (11%). Whether this could be explained by rare false positive and/or by a disease control managed by the immune system remains unclear.

The main limitations of our work mostly consist of the pre-analytical handling of our samples. As mentioned above, unwanted ccfDNA release in the sample could lead to contamination by wild-type DNA, thus leading to ctDNA dilution. The concentration of ccfDNA in our study (see Table S4) reached 102 ng/ml significantly higher than concentrations obtained with more recent studies (data not shown). However to circumvent this issue, we looked into the concentration of ctDNA (ng/ml) independently. Moreover, it is to note that the use of the Raindrop platform limits the impact of this potential dilution by using a large number of droplets. This technology permits that the droplet occupancy remains low enough to ensure that vast majority of non-empty droplets will contain at maximum one DNA molecule thus leading to accurate copy evaluation.

Finally, our detection method cumulates several advantages including good sensitivity, reduced technical costs, shorter turnaround time, and easy data interpretation.

In the future, ctDNA monitoring could refine the therapeutic strategies including treatment escalation or de-escalation [36].

## 5. Conclusion

Using a cost-effective and financially affordable method for a routine practice, this prospective study confirms the relevance of ctDNA as a recurrence risk factor in stage II and III CRC before surgery and as a marker of minimal residual disease after surgery that may predict recurrence several months before imaging techniques. It confirms the likelihood of ctDNA longitudinal sampling to become part of the decision process. More than a one-shot picture, it appears fundamental to determine ctDNA status at each step of the treatment to drive future decisions.

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### Conflict of interest statement

The authors of this work have conflicts of interest to declare:

Valerie Taly: Honoraria from Raindance Technologies and Boehringer Ingelheim; cofounder Emulseo; board Emulseo.

Pierre Laurent-Puig: honoraria and board: Amgen, Merck-Serono, Boehringer Ingelheim, Sanofi, Roche, Lilly.

Julien Taieb: Honoraria from Merck, Amgen, Roche, Pierre Fabre, MSD, Sanofi, Lilly, Servier, Astra-Zeneca.

AZ: consulting and/or advisory boards: Roche, Merck Serono, Amgen, Sanofi, and Lilly.

Olivier Bouché: honoraria and board: Merck, Amgen, Roche, Bayer, Servier, Pierre Fabre, MSD.

Delphine Le Corre: Bio-Rad Employee.

All remaining authors have declared no conflicts of interest.

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### Credit statement

The individual contributions for this work are:

L. Benhaim: Formal analysis; Investigation; Methodology; Resources; Data Curation; Writing - Original Draft; Writing - Review & Editing.

O. Bouché: Conceptualization; Investigation; Resources; Writing - Review & Editing.

C. Normand: Investigation; Methodology.

A. Didelot: Investigation; Data Curation.

C. Mulot: Resources; Data Curation; Project administration.

D. Le Corre: Investigation; Data Curation; Project administration.

S. Garrigou: Investigation; Methodology.

J. Djadi-Prat: Data Curation; Formal analysis.

S. Wang-Renault: Investigation.

K. Perez-Toralla: Investigation; Methodology.

D. Pekin: Investigation; Methodology.

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J. Taieb: Methodology; Resources; Writing - Review & Editing.

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V. Taly: Methodology; Software; Validation; Formal analysis; Investigation; Resources; Data Curation; Writing - Original Draft; Writing - Review & Editing; Visualization; Supervision; Funding acquisition.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2021.09.004>.

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