Prospective Cohort Study

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Personalized circulating tumor DNA monitoring improves recurrence surveillance and management after curative resection of colorectal liver metastases: a prospective cohort study

Yaqi Li, MD, PhD[a,e], Jing Xu, PhD[h], Xiang Hu, MD, PhD[a,e], Yikuan Chen, MD, MSc[a,e], Fangqi Liu, MD[a,e], Yun Chen, MSc[h], Xiaoji Ma, MD[a,e], Qiduo Dong, MSc[h], Lei Sun, PhD[f], Shaobo Mo, MD, PhD[a,e], Long Zhang, PhD[a,e,d], Xingfeng He, MSc[a,e], Shanyou Tong, MSc[a,e], Huizi Wu, PhD[h], Wenhua Li, MD, PhD[e,c], Sanjun Cai, MD, PhD[a,e], Shida Zhu, MSc[h,g,*], Qi Pan, MD, PhD[e,b,*], Junjie Peng, MD, PhD[a,e,*]

Background: Approximately 60% of patients with colorectal liver metastases (CRLM) experience relapse within 2 years after radical resection, previous studies have proven that repeat local treatment (LT) could prolong survival, however, it is difficult to seize the window for LT due to the lack of a high-sensitive surveillance method. In this study, the authors aim to examine the value of longitudinal circulating tumor DNA (ctDNA) in guiding adjuvant chemotherapy, optimizing clinical surveillance strategy, and thereby improving CRLM outcomes.

Materials and methods: The authors conducted a prospective clinical trial using a personalized, tumor-informed ctDNA assay to monitor 60 CRLM patients undergoing resection with curative intent. Formalin-fixed paraffin-embedded tumor samples were collected after surgery. Blood samples were collected before surgery, 30 days after surgery (post-OP), and every third month until relapse or up to 2 years.

Results: A total of 394 plasma samples from 60 eligible patients were analyzed, with a median follow-up time of 31.3 months. Landmark analyses revealed that detectable ctDNA at post-OP (HR, 4.8), postadjuvant chemotherapy (HR, 6.0), and end-of-treatment (HR, 5.6) were associated with higher recurrence risk ($P < 0.001$). Post-OP ctDNA positivity served as the only independent prognostic marker in the multivariant analysis (HR, 5.1; $P < 0.001$). Longitudinal ctDNA analysis identified relapsed patients at both sensitivity and specificity of 100%. Most (75%) patients were found with radiological relapse within 6 months after the first detectable ctDNA with a median lead time of 3.5 months. In relapsed patients, 73.2% had oligometastatic disease and 61% were liver-restricted, of which 72.0% received repeat LTs, and 60.0% achieved a secondary no evidence of disease status.

Conclusions: Longitudinal ctDNA monitoring assists in early prediction of relapse, and thereby improves survival of CRLM patients by increased secondary resection rate and secondary no evidence of disease rate.

Keywords: circulating tumor DNA, colorectal liver metastases, local treatment, molecular residual disease, no evidence of disease

[a]Department of Colorectal Surgery, [b]Department of Hepatic Surgery, [c]Department of Medical Oncology, [d]Cancer Institute, Fudan University Shanghai Cancer Center, [e]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, [f]Tianjin Medical Laboratory BGI, BGI-Tianjin, Tianjin, [g]Shenzhen Engineering Laboratory for Innovative Molecular Diagnostics and [h]BGI Genomics, BGI-Shenzhen, Shenzhen, People's Republic of China

Yaqi Li and Jing Xu contributed equally to this study.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

**Corresponding authors. Address: Department of Colorectal Surgery, Fudan University Shanghai Cancer Center and Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China. Tel.: +86 139 173 733 12. E-mail: pengjj@shca.org.cn (J. Peng); Department of Oncology, Shanghai Medical College, Fudan University and Department of Hepatic Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, People's Republic of China. Tel.: +86 181 212 997 77. E-mail: panqi20@163.com (Q. Pan); BGI Genomics, BGI-Shenzhen and Shenzhen Engineering Laboratory for Innovative Molecular Diagnostics, BGI-Shenzhen, Shenzhen 518083, People's Republic of China. Tel.:+86 158 140 038 23. E-mail: zhushida@genomics.cn (S. Zhu).*

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, and metastatic liver disease is the leading cause of death in CRC[1]. Surgical resection remains the mainstay for treating colorectal liver metastases (CRLMs), with a 5-year survival rate of 58%[2]. Over the past two decades, advances in chemotherapeutic regimens and local treatment (LT) modalities have boosted the resection and conversion rates, thereby improving patient outcomes. However, 40–75% of patients still relapse within 2 years after resection[3–5], with limited benefit from perioperative chemotherapy[6–9]. Previous studies proved that if the recurrent disease was amenable to further repeat LTs, it could still lead to a 5-year overall survival rate of 51–55%[10–12]. Therefore, early recurrence detection is of critical importance to prolong the therapeutic window for CRLM.

Circulating tumor DNA (ctDNA) is a noninvasive biomarker that reflects molecular residual disease (MRD) after radical resection. Numerous studies have reported that postoperative ctDNA can redefine patients' outcome groups[13–18] and guide postoperative treatment[18–20] in stage I–III CRCs, yet few studies have focused on its prognostic values in the CRLM population[21–27]. However, due to the shorter recurrence interval, a more accurate detection may be required for late-stage disease.

Here, we report results from a prospective, observational cohort study in CRLM patients undergoing resection with curative intent, aiming to assess the clinical value of personalized ctDNA monitoring in recurrence prediction and optimization of the postoperative surveillance strategy for CRLM.

HIGHLIGHTS

- The landmark circulating tumor DNA (ctDNA) monitoring was not sufficient for adjuvant chemotherapy counseling in colorectal liver metastases as it is known for stage II–III patients. Longitudinal ctDNA monitoring is needed. We identified relapsed patients at both sensitivity and specificity of 100%.
- Longitudinal ctDNA monitoring improves surveillance strategy: we propose that colorectal liver metastases patients should switch to an intensified computed tomography scan every 2 months when longitudinal ctDNA turns positive, while for those who continued to be negative in ctDNA, computed tomography scans could be performed less intensively.
- We observed a significantly improved postrecurrence survival in patients who received a secondary resection and achieved secondary no evidence of disease under ctDNA-guided intensified imaging.

Materials and methods

Study design and participants

This prospective cohort study recruited CRLM patients between 1 July 2020 and 31 July 2021. Key eligibility criteria included the following: 1) pathologically and radiologically diagnosed CRLM without synchronous extrahepatic metastases; 2) patients underwent R0 resections of both primary and metastatic lesions; and 3) radiologically confirmed no evidence of disease (NED) 1 month after surgery. Patients with a second malignancy within the previous 5 years were excluded. This work was reported in line with the strengthening the reporting of cohort, cross-sectional, and case–control studies in surgery (STROCSS) criteria[28] (Supplemental Digital Content 1, http://links.lww.com/JS9/C40).

The study design and details of blood collection timepoints are shown in Figure 1A. Tumor tissue was collected at the surgery. Blood samples for ctDNA and carcinoembryonic antigen (CEA) analysis were collected before surgery (pre-OP), 30 days after surgery (post-OP), and every third month until relapse or 24 months after surgery. The first sample taken after the completion of adjuvant chemotherapy (ACT) was termed post-ACT. The 'end-of-treatment' (EOT) samples referred to the post-OP samples for patients treated with surgery alone and the post-ACT samples for those who received ACT. Serum CEA level was measured by the diagnostic laboratory with CEA concentrations of less than 5 ng/ml considered within the reference range.

Postoperative surveillance

The routine postoperative surveillance of CRLM patients followed the NCCN recommendations for resected stage IV CRCs[29]. An intensified surveillance protocol was established for patients with detected ctDNA during surveillance: 1) radiological scanning and physical examination were performed every 2–3 months with ctDNA tested every 3 months; 2) 6 months was considered a surveillance cycle; 3) patients returned to routine surveillance upon negative radiological findings in the previous surveillance cycle; and 4) ctDNA sampling is still performed every 3 months during the period of intensified radiological surveillance. Oligometastatic disease (OMD) was defined according to ESMO guidelines[30]. Recurrent patients were assessed by the CRC multidisciplinary team for the possibility of LTs. Patients who achieved secondary NED after LTs returned to a 3-month ctDNA testing schedule. Baseline clinicopathological data and the analysis overview were summarized in Supplementary eTable 1 (Supplemental Digital Content 3, http://links.lww.com/JS9/C42) and Supplementary eTable 2 (Supplemental Digital Content 3, http://links.lww.com/JS9/C42).

Personalized tumor-informed MRD detection

Plasma ctDNA was evaluated by the Huajianwei bespoke MRD assay based on Signatera as previously described[13,31]. Briefly, 16 top-ranked SNVs were selected based on the tumor tissue whole exome sequencing (WES) data generated from a pan-cancer WES panel (Quanxi) on the MGISEQ-2000 platform (MGI Tech), and 16-plex specific primer pairs were used to amplify the universal cfDNA libraries. The products were then sequenced on the HiSeq2500 system (Illumina Inc). Detailed methods are described in the Supplemental Digital Content 2, http://links.lww.com/JS9/C41. To minimize the false positive rate, the plasma sample with at least two variants positive (out of 16 variants in total per person) was defined as ctDNA or MRD positive, and ctDNA was quantified in mean tumor molecule per milliliter (MTM/ml) plasma (Supplementary eTable 3, Supplemental Digital Content 3, http://links.lww.com/JS9/C42). The raw data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA)[32] of China National GeneBank DataBase (CNGBdb)[33] with accession number CNP0005016 (https://db.cngb.org/).

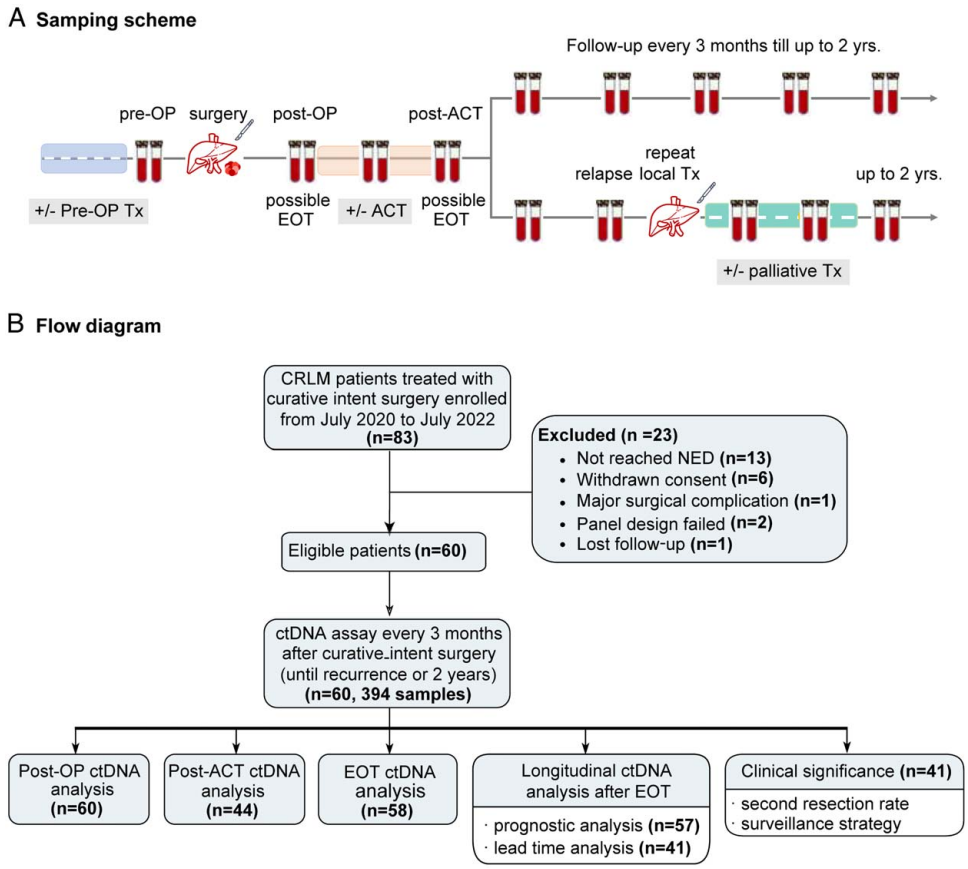

Figure 1. Study Design and Patient Enrollment. (A) Study design and overview of the blood collection timepoints. Blood samples were collected including preoperative and postoperative samples (30 days after surgery) and every 3rd month until relapse or up to 24 months after resection. Every recurrent CRLM patient was evaluated by the multidisciplinary team for possible repeat local treatment of recurrent lesions. Recurrent CRLM patients continue ctDNA surveillance after reaching the secondary status of NED. For patients treated with surgery alone, the first postoperative blood sample was termed post-OP and EOT. For patients administered ACT, the first blood sample collected after the end of therapy was termed post-ACT and EOT. (B) The study enrolled 83 patients of which 60 were included in the final analysis with a total of 394 blood samples. An overview of patients available for each ctDNA analysis is presented.

Statistical analysis

The primary endpoint was recurrence-free survival (RFS), defined as the date from initial hepatic resection to recurrence or death. Postrecurrence survival (PRS) was measured from the first relapse after initial hepatic resection until death or the last follow-up. The Kaplan–Meier (KM) method with the log-rank test was used to analyze the outcome. Hazard ratios (HRs) were estimated by univariate and multivariate Cox proportional hazards regression models. Two-sided $P < 0.05$ was considered significant. All statistical analyses were performed in R Statistical Software version 4.2.1[34].

Results

Clinicopathological characteristics and ctDNA detection

In total, 83 CRLM patients were enrolled; 23 patients were excluded due to failure to achieve NED status (n = 13), consent withdrawal (n = 6), major surgical complications (n = 1), failed panel design (n = 2), and loss to follow-up (n = 1), leaving 60 patients eligible for analysis (Fig. 1B). The baseline characteristics are detailed in Supplementary eTable 4 (Supplemental Digital Content 3, http://links.lww.com/JS9/C42). The median age was 59 years at enrollment, 70.0% were male, 76.7% had synchronous disease, 68.3% had multiple CRLM lesions, and 55.0% had initially resectable CRLM disease. Neoadjuvant chemotherapy was administered to 18.2% (6/33) of patients with resectable disease, and all patients with unresectable disease received conversion chemotherapy. A total of 76.7% (46/60) of patients received ACT. The median follow-up time of 31.3 months (range, 25.0–36.6). Within this period, 44 patients (73.3%) relapsed, and 17 patients (28.3%) died.

Somatic mutations of the tumors were profiled by WES, with *TP53* (83%) and *APC* (78%) being the most frequently mutated genes (Supplementary eFig. 1, Supplemental Digital Content 2, http://links.lww.com/JS9/C41). In total, 857 genes were selected for 60 patients by the personalized, tumor-informed MRD workflow, with more than 92% of genes being unique to each patient (Supplementary eFig. 2, Supplemental Digital Content 2, http://links.lww.com/JS9/C41). *TP53* and *APC* were selected in 30 and 13.3% of patients, respectively, demonstrating substantial diversity in mutations outside of the known hotspots in CRC, which may be missed by conventional fixed ctDNA panels. ctDNA was detectable in 96.7% (58/60) of patients at pre-OP, 58.3% (35/60) at post-OP, 40.9% (18/44) at post-ACT, 39.7% (23/58) at EOT, and 71.9% (41/57) in serial samples, while CEA had a lower detection rate at

all timepoints, with 78.0% (46/59) at pre-OP, 25.9% (14/54) at post-OP, 32.5% (13/40) at post-ACT, 28.9 (15/52) at EOT and 54.5% (30/55) in serial samples. For samples with detectable ctDNA, the median ctDNA content measured in mean tumor molecules per milliliter (MTM/ml) was 28.2 at pre-OP, 4.12 at post-OP samples, and 19.0 at post-ACT (Supplementary eFig. 3, Supplemental Digital Content 2, http://links.lww.com/JS9/C41).

Prognostic significance of ctDNA at landmark timepoints

We first assessed the prognostic value of ctDNA at post-OP, post-ACT, and EOT on RFS. In terms of post-OP ctDNA, the recurrence rate of the ctDNA-positive patients was significantly higher (94.3%, 33/35) compared to those with negative ctDNA (44.0%, 11/25). The presence of ctDNA in post-OP samples was associated with a significantly reduced RFS as compared to ctDNA-negative patients [HR 4.8 (95% CI: 2.4–9.9); $P < 0.001$; Supplemental Digital Content 3, http://links.lww.com/JS9/C42 and Supplementary eFig. 4 A–C, Supplemental Digital Content 2, http://links.lww.com/JS9/C41).

Clinicopathological characteristics and their association with post-OP ctDNA status are shown in Supplementary eTable 4 (Supplemental Digital Content 3, http://links.lww.com/JS9/C42). Post-OP ctDNA status was associated with clinicopathological variables including multiple CRLM lesions, bilobar distribution of liver metastases, synchronous liver metastases, high-risk clinical risk score[35], elevated post-OP CEA, RAS mutation, recurrence with non- OMD and extrahepatic relapse. Univariate analysis identified post-OP, post-ACT, and EOT ctDNA status, resectability of CRLM, clinical risk score, and preoperative chemotherapy as significant prognostic factors for RFS (Table 1). However, in the multivariate Cox regression model, including only the significant prognostic factors that are not correlated with others, post-OP ctDNA status remained the only significant prognostic factor for RFS [HR 5.1 (95% CI: 2.3–11.1); $P < 0.001$; Table 1].

Prognostic significance of longitudinal ctDNA analysis after EOT

Samples collected longitudinally from EOT were available for 57 patients. Three patients were not included in the analysis due to diagnosis with recurrence before ACT (n = 1) and lack of EOT plasma (n = 2, both diagnosed with recurrence afterwards). Longitudinal ctDNA analysis after EOT detected ctDNA in 41 patients, 95.1% (39/41) of whom recurred within 2 years, with the other two patients recurring at 25.8 months and 32.4 months. None of the patients with consistently negative ctDNA recurred till the last follow-up ($P < 0.001$; Fig. 2D). Both of sensitivity and specificity of longitudinal ctDNA detection reached 100%. None of the patients with persistently negative ctDNA relapsed. In contrast, longitudinal CEA analysis revealed a sensitivity of 69.2% with a specificity of 81.3% (Supplementary eTable 3, Supplemental Digital Content 3, http://links.lww.com/JS9/C42 and Supplementary eFig. 4D, Supplemental Digital Content 2, http://links.lww.com/JS9/C41).

ctDNA clearance with ACT and clinical outcomes

Among 47 patients who received ACT after surgery, 44 patients had both post-OP and post-ACT ctDNA samples available for analysis. ctDNA clearance was observed in 44.8% of post-OP ctDNA-positive (13/29) patients (indicated as 'pos-neg' in for all 44 patients. The recurrence rate of these patients in each ctDNA dynamic change group was compared in Figure 3C. A significant increase in recurrence rates ($P < 0.001$) can be observed in neg-neg (15.4%), neg-pos (50.0%), pos-neg (84.6%), and pos-pos (100%) groups. Two patients with initial negative post-OP ctDNA turned positive after completion of ACT (indicated as 'neg-pos') both recurred. Three patients (3/13, 23.1%) with negative ctDNA before and after ACT relapsed.

Table 1

Univariate and multivariate Cox analysis of RFS by clinicopathological variables and ctDNA status.

			Multivariate	
Variables	**Univariate HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[a]**
Age (> 60.0 /≤ 60.0)	0.9 (0.5–1.6)	0.668		
Sex (male / female)	1.3 (0.7–2.5)	0.487		
Tumor location (left-sided/right-sided)	0.7 (0.4–1.4)	0.368		
Pre-OP CEA (> 5.0 ng/ml /≤ 5.0)	1.1 (0.5–2.4)	0.783		
N stage (N1-2/N0)	1.9 (0.9–4.4)	0.109		
Primary tumor differentiation (Poor/well - moderate)	1.4 (0.7–2.5)	0.332		
Resectability (Unresectable/resectable)	3.6 (2.0–6.7)	**< 0.001**	3.0 (0.9–9.5)	0.067
Time interval from diagnosis of primary tumor to liver metastases (synchronous/metachronous)	1.4 (0.7–2.8)	0.414		
CRS (high-risk/low-risk)	3.3 (1.5–7.1)	**0.002**	1.3 (0.5–3.0)	0.624
Concomitant ablation (yes/no)	1.9 (0.8–4.7)	0.140		
Post-OP CEA (> 5.0 ng/ml /≤ 5.0)	1.6 (0.8–3.1)	0.182		
RAS mutation status (mutated/wildtype)	1.2 (0.6–2.1)	0.662		
Pre-OP chemotherapy (yes/no)	3.0 (1.6–5.7)	**< 0.001**	1.3 (0.4–4.1)	0.640
Adjuvant chemotherapy (yes/no)	0.9 (0.4–1.8)	0.665		
Post-OP ctDNA (positive/negative)	4.9 (2.4–9.9)	**< 0.001**	5.1 (2.3–11.1)	**< 0.001**
Post-ACT ctDNA (positive/negative)	6.0 (2.9–12.8)	**< 0.001**		
EOT ctDNA (positive/negative)	5.6 (2.9–10.8)	**< 0.001**		

[a] *P*-value in bold denotes statistically significant.

ACT, adjuvant chemotherapy; CEA, carcinoembryonic antigen; CRS, clinical risk score; ctDNA, circulating tumor DNA; EOT, end of treatment; post-OP, post-operation; pre-OP, pre-operation; RFS, recurrence-free survival.

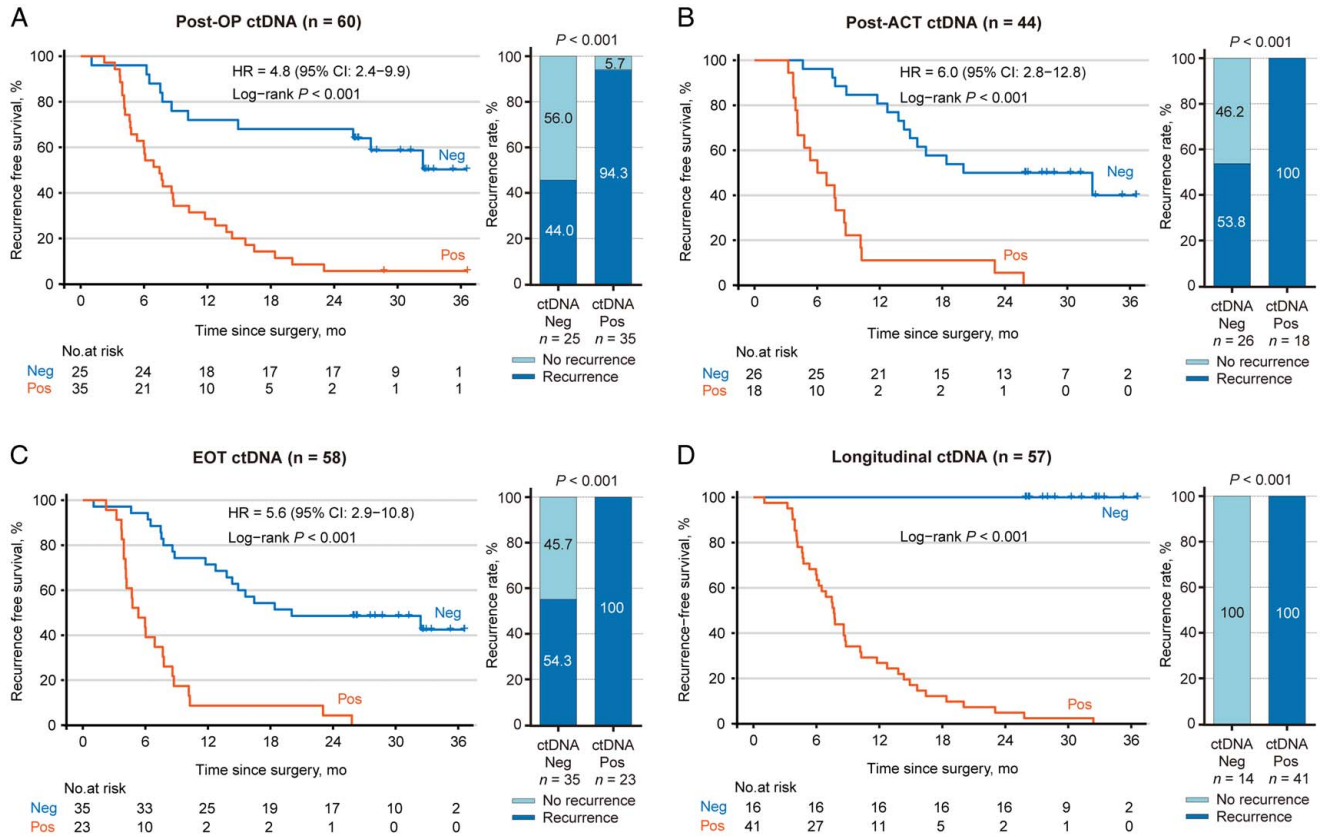

Figure 2. Circulating Tumor DNA (ctDNA) Status and Recurrence-Free Survival (RFS). Kaplan-Meier estimates of RFS at different monitoring timepoints of (A) post-OP (n = 60); (B) post-ACT (n = 44); (C) EOT (n = 58); and (D) longitudinal ctDNA analysis (n = 57).

Association of longitudinal ctDNA monitoring with early detection of recurrence and surveillance strategy

Longitudinal ctDNA analysis was conducted in 57 patients, including a median of seven samples per patient (range, 2–11 samples). The courses of disease for 41 recurrent patients and 16 nonrecurrent patients are presented in Figure 4A, B. A total of 97.6% (40/41) of recurrent patients were ctDNA-positive before clinical relapse, and only one patient with recurrence in the lungs had ctDNA detected after clinical relapse. The median time to relapse detection was 3.9 months (range, 0.8–24.9 months) for ctDNA, and 7.7 months (range, 2.9–32.4 months) for radiological imaging. Longitudinal ctDNA analysis revealed MRD up to 22.0 months before clinical relapse, with a median lead time of 3.5 months (Wilcoxon test; $P < 0.001$; Fig. 5A). In contrast, CEA was significantly inferior to ctDNA in detecting recurrence. Among the 41 recurrence patients, except for one with missing CEA data, 10 (25%) had persistently negative CEA results after surgery. Among 30 patients with positive CEA, 8 had positive CEA results after radiological recurrence (Supplementary eFig.5, Supplemental Digital Content 2, http://links.lww.com/JS9/C41). A total of 22 (55.0%) patients were found to be CEA positive before or on the day of radiological recurrence (19 and 3, respectively), and the median time to relapse detection was 4.5 months (range, 0.9–25.7 months) for CEA, with a median lead time of one month (Supplementary eFig.5, Supplemental Digital Content 2, http://links.lww.com/JS9/C41).

Under longitudinal ctDNA monitoring, the median frequency of imaging surveillance for all patients was 3.2 months (range, 0.6–9.6 months). The accumulative incidence of radiological relapses diagnosed after positive ctDNA is shown in Figure 5B. After the first positive finding in postoperative ctDNA, 75.0% of patients relapsed within 6 months, with a sharply increased number of patients (47.5%) relapsed between 1.5 and 4 months.

Repeat LTs and PRS

The clinicopathological characteristics of 41 recurrent patients are detailed in Supplementary eTable 5 (Supplemental Digital Content 3, http://links.lww.com/JS9/C42). The liver is the most prevalent recurrence location at initial recurrence, accounting for 61.0% (25/41) of all sites of recurrence. Synchronous relapses in both the liver and lungs account for 12.2% (5/41), and relapses in the liver and other extrahepatic sites account for 7.3% (3/41). Extrahepatic-only relapses are mainly attributed to the lungs (14.6%, 6/41) (Supplementary eTable 6, Supplemental Digital Content 3, http://links.lww.com/JS9/C42).

OMD accounted for 73.2% of all clinical relapses. For recurrence of all sites, 58.5% (24/41) of patients received repeat LTs, and 43.9% (18/41) reached a secondary NED status. For recurrence restricted to the liver, 72.0% (18/25) of patients received repeat LTs, and 60.0% (15/25) reached a secondary NED status (Supplementary eTable 7, Supplemental Digital Content 3, http://links.lww.com/JS9/C42] had a significantly increased PRS

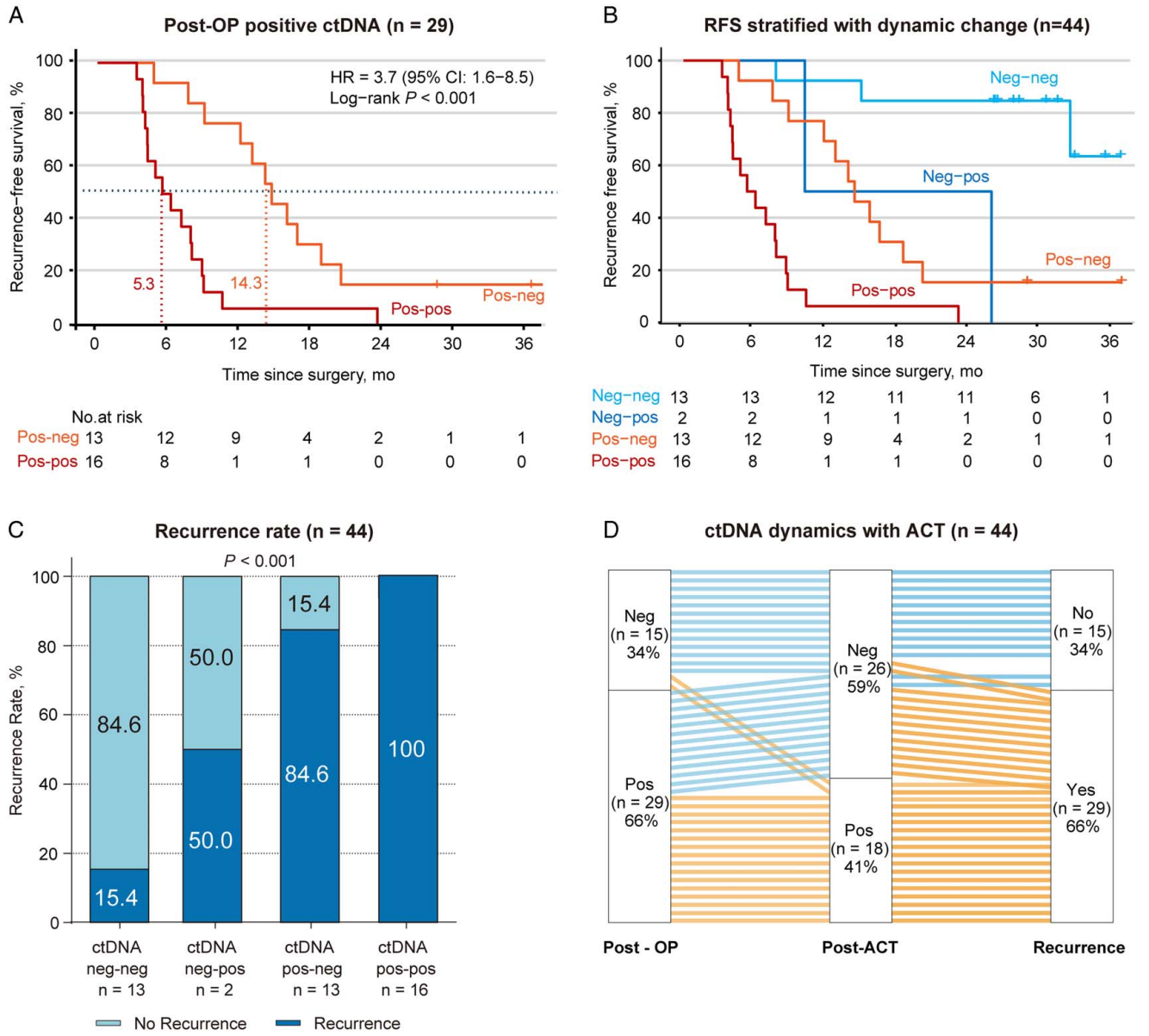

Figure 3. ctDNA Dynamics with Adjuvant Chemotherapy and Recurrence. (A) Kaplan-Meier estimates of RFS according to ctDNA clearance after the completion of ACT for the patient with positive post-OP ctDNA (n = 29). Dashed lines indicate the median RFS time for patients with and without ctDNA clearance after ACT, which is 14.3 months and 5.3 months, respectively. (B) Kaplan-Meier estimates of RFS in 44 patients treated with ACT stratified by ctDNA dynamic change groups before and after ACT. (C) Recurrence rate comparison in each ctDNA dynamic change group. (D) Individual changes of ctDNA for 44 patients treated with ACT. The blue lines indicate negative ctDNA results at the latter point regardless of the previous test result, while the orange lines are indicative of positive ctDNA results.

compared to those who did not. Dynamic ctDNA changes of representative patients who reached secondary NED were shown in Figure 6.

Discussion

Postoperative ctDNA has proven to be a strong predictor of recurrence in localized CRC and CRLM patients[18,23,25–27]. Here, we showed the value of longitudinal, personalized ctDNA tests in prognosis prediction, ACT counseling, and surveillance strategy optimization, which paved the way for future ctDNA-guided postoperative management of CRLM.

In the preoperative setting, we found the ctDNA detection rate at 96.7% (58/60), even though 55% of enrolled patients were administered with conversion chemotherapy, which was higher than ~85% as reported previously with conventional ctDNA tests[23,24], suggesting that the personalized, tumor-informed ctDNA assay is more sensitive than the fixed-panel strategy or tumor-informed single marker ctDNA assay. Selecting a method at a time point with a high positive prediction value (PPV) is critical for getting more intensive monitoring. The PPV of ctDNA is prominent at all landmark timepoints in the present study with 94.3% at post-OP, 100.0% at post-ACT, and 100.0% at EOT, suggesting this assay is reliable enough to be used along the disease course of CRLM.

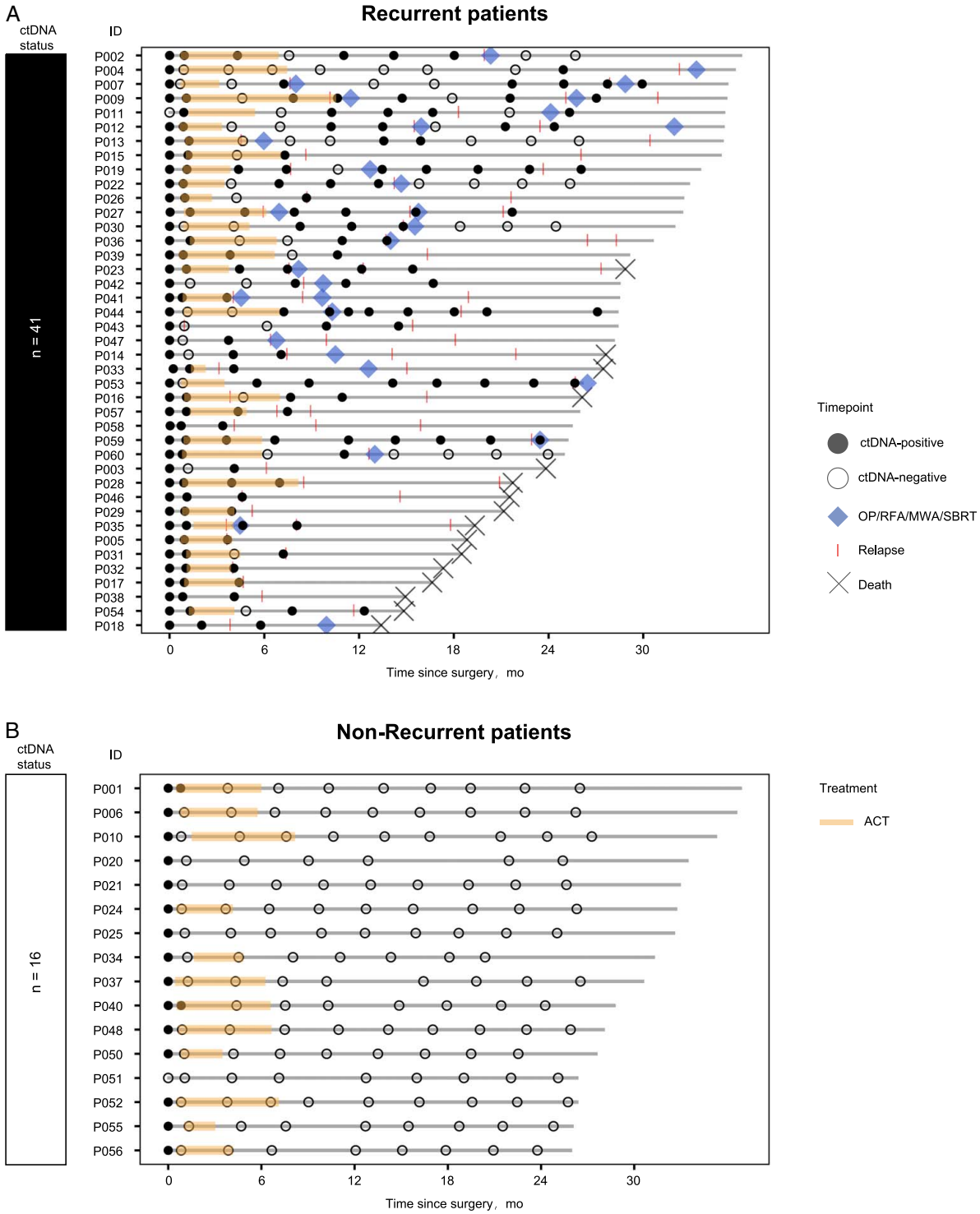

Figure 4. Circulating Tumor DNA (ctDNA) Profiling Results. An overview of ctDNA results and disease course for (A) 41 recurrent patients; and (B) 16 non-recurrent patients included in the longitudinal ctDNA analysis. Abbreviations: OP: operation, RFA: radiofrequency ablation, MWA: microwave ablation, SBRT: stereotactic body radiation therapy.

Consistent with prior studies[23,25–27], post-OP ctDNA detection identified 75.0% (33/44) of patients who experienced recurrence later, while those with negative post-OP ctDNA had a significantly reduced recurrence risk of 44.0%. Nine patients who were ctDNA-negative at post-OP later became positive during follow-up, and all experienced clinical relapse within 2 years, indicating the need for longitudinal monitoring. On the other hand, two patients who were post-OP ctDNA-positive and

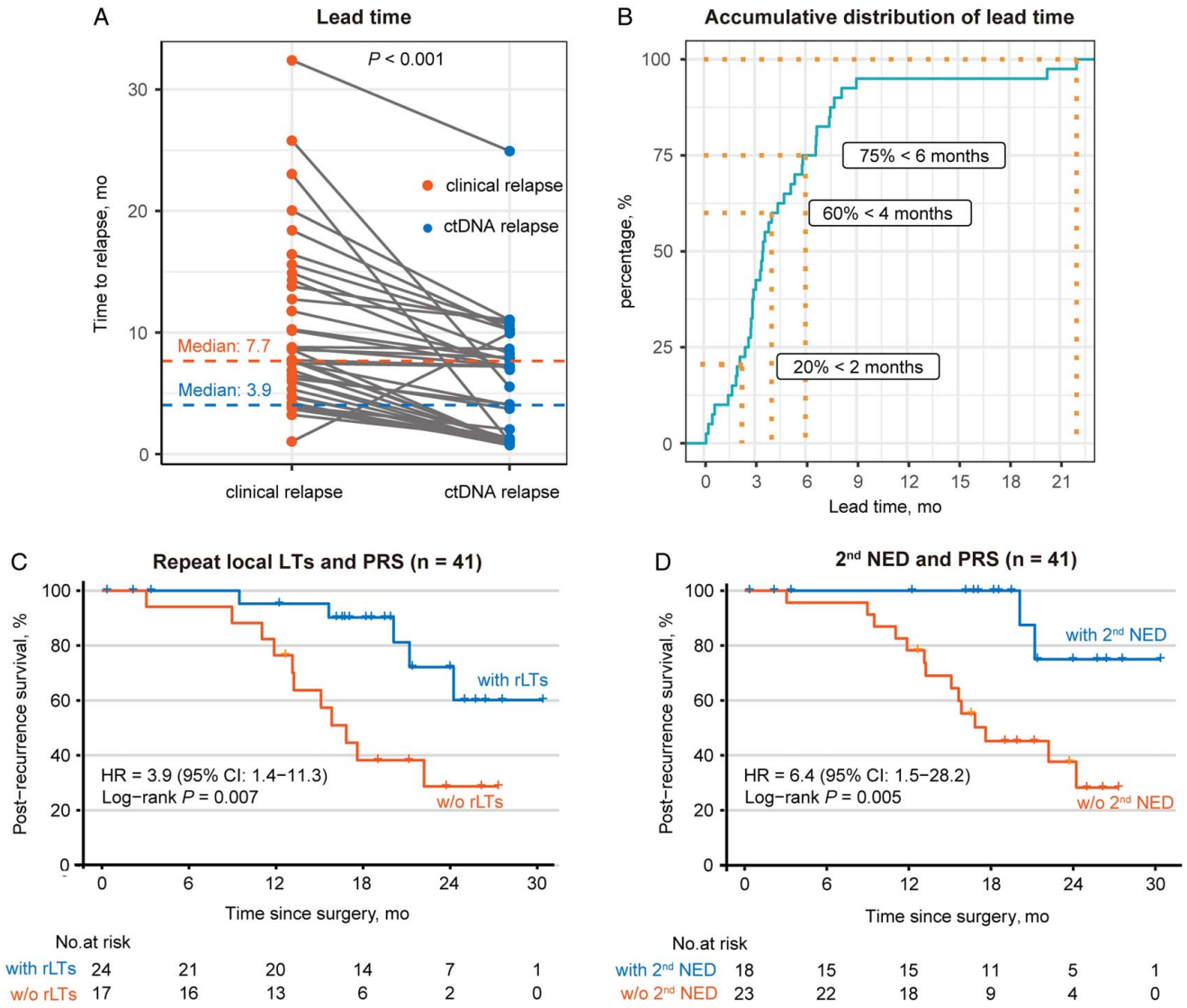

Figure 5. Association of Longitudinal ctDNA Analysis with Early Detection of Recurrence, Repeat LTs and Surveillance Strategy. (A) Comparison of time to relapse by ctDNA and radiological imaging. Dashed lines indicate the median time in months of recurrence based on CT and ctDNA, which was 7.7 months (range, 2.9-32.4 months) for radiological imaging and 3.9 months (range, 0.8-24.9 months) for ctDNA. (B) The accumulative incidence of radiological relapse after positive findings of longitudinal ctDNA analysis. 75.0% of relapsed patients were identified within 6 months after the first positive finding in postoperative ctDNA, and 47.5% of relapsed patients were diagnosed between 1.5-4 months. (C) Kaplan-Meier estimates of PRS for 41 recurrent patients stratified by repeat LTs (rLTs) status. (D) Kaplan-Meier estimates of PRS for 41 recurrent patients stratified by 2nd NED status

received ACT did not recur. The ctDNA of both was cleared during ACT and remained negative in subsequent samples, indicating ACT may improve their survival by eliminating or suppressing the residual disease, similar to prior reports on a small subset of patients[23,24]. Interestingly, a high ctDNA clearance rate of 44.8% was observed in 29 patients. Despite the ctDNA clearance prolonged the median RFS significantly (14.3 months vs. 5.3 months, $P < 0.001$), the 2-year recurrence rate remained high in these patients (84.6 vs. 100%). Thus, landmark timepoints of post-OP or post-ACT ctDNA may not be sufficient to guide ACT administration as it is for stage II–III patients. For patients with positive post-ACT ctDNA, prolonged treatment duration or a shift to a second-line regimen may improve survival. For all nine patients with ctDNA clearance who recurred within 2 years, a positive ctDNA was detected before recurrence in the longitudinal ctDNA analysis. This subgroup may benefit from maintenance chemotherapy or the timely restart of chemotherapy. This re-emphasizes that the longitudinal ctDNA tests could potentially be used as a real-time biomarker for modification of ACT in CRLM.

In longitudinal ctDNA analysis, both the sensitivity and specificity reached 100%. The median lead time of personalized ctDNA was 3.5 months compared to radiological imaging – a reduced lead time compared to that of localized CRC patients, which is consistent with previous studies[13,15,26,27,36,37]. Only one patient with lung metastases had ctDNA detected after radiological relapse. Multiple studies showed that the ctDNA detection rate can be lower in the lung than in the digestive

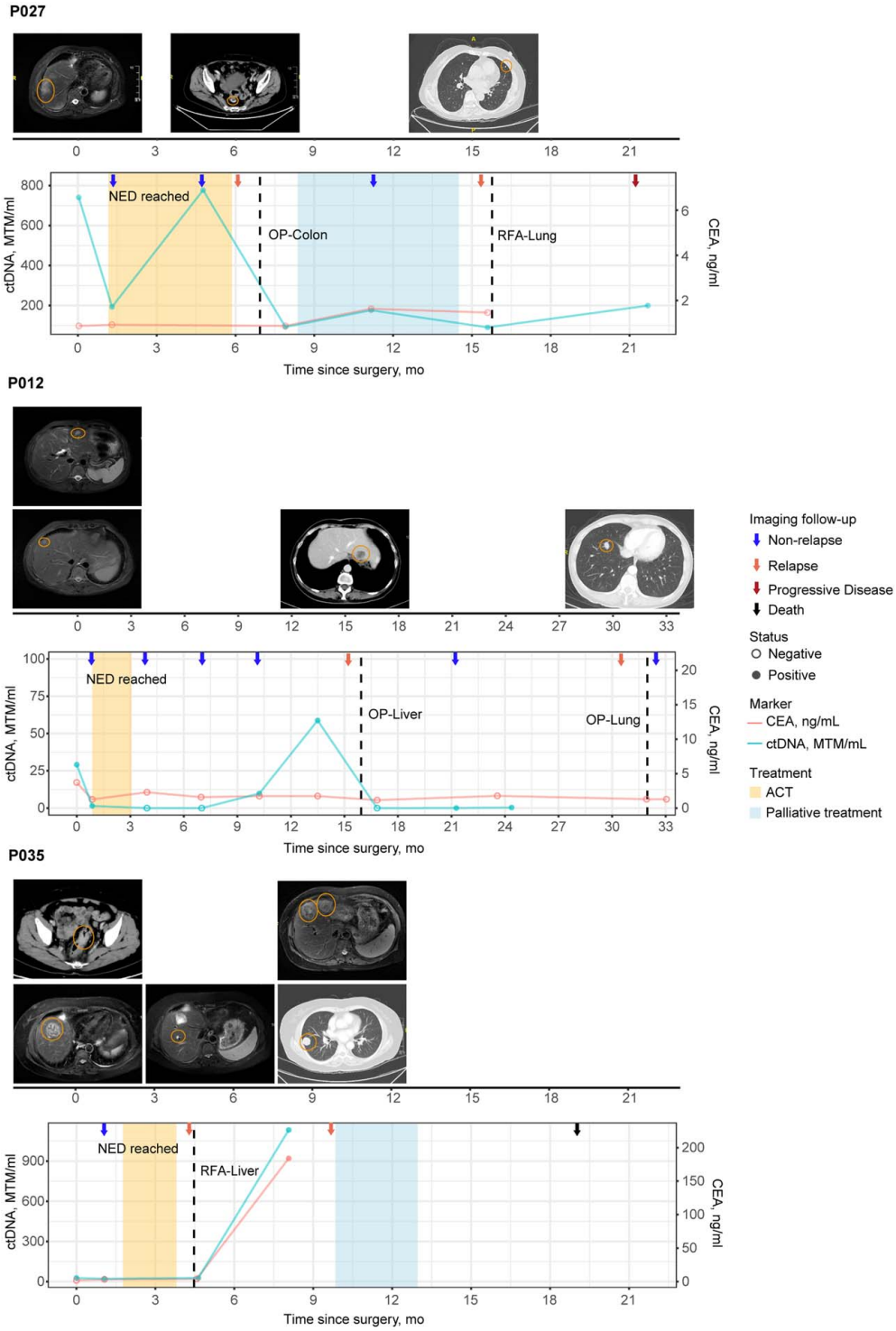

Figure 6. Representative Patients with Secondary NED Surgery with Detailed ctDNA Change and Disease Course Information. The courses of disease including applied treatment, longitudinal CT scan, ctDNA, and CEA test results are shown.

organs, which is also true for lung metastases[24,26,27]. This discrepancy may be attributed to the high radiographic resolution in the lungs, which allows small lung lesions (less than 10 mm) to be spotted. However, the lesions may potentially be too small to shed enough ctDNA into the blood[38,39].

During ctDNA-guided surveillance, OMD accounted for up to 73.2% of all radiological relapses in the present study. The liver is the most common recurrence site, accounting for 61% (25/41) of all relapses. The liver-restricted recurrence rate found by ctDNA-guided surveillance was much higher than that found by

standard-of-care radiological imaging, which was 43.2% reported in a large multicenter cohort ($n = 1669$) of CRLM patients after curative-intent surgery[40]. The high rate of OMD detected and early detection of liver-restricted recurrence guided by ctDNA analysis contributed to the possibility of repeat LTs. A total of 72.0% of CRLM patients with liver-restricted recurrence were able to undergo repeat LTs, which is much higher than ~57.7% as published[41], and 58.3% achieved a secondary NED status, which was proven to bring a better survival compared to earlier studies[10–12,41]. Thus, it is imperative to bring longitudinal ctDNA analysis into the surveillance strategy for CRLM patients after curative surgery.

In clinical practice, how to combine ctDNA analysis and radiological imaging in surveillance remains unclear. In the present study, 75.0% of patients after the first positive ctDNA findings recurred within the first surveillance cycle of 6 months, especially within 1.5 to 4 months, suggesting an intensified monitoring with a 2-month interval may be recommended in case of positive ctDNA detection. By contrast, none of the patients who continued to be ctDNA-negative after EOT recurred, which is suggestive of less intensive CT monitoring (every 6 months) for patients with negative ctDNA. Future rigorous clinical trials, such as the BESPOKE study[42], are required to determine the potential benefit of applying personalized ctDNA to improve CRLM surveillance strategy in the balance of efficiency, cost, and throughput.

There are some potential limitations in this study. First, the relatively small sample size may reduce the statistical power of subgroup analyses. Moreover, due to the observational nature of the study, we cannot conclude a definitive surveillance strategy for CRLM patients after curative-intent surgery, but we put forward a feasible optimization consisting of a 3-month longitudinal ctDNA analysis and ctDNA-guided CT scans.

Conclusion

In conclusion, our study adds to the growing evidence of the prognostic significance of ctDNA for CRLM patients after curative-intent surgery, both at defined timepoints and in longitudinal analyses during surveillance. We demonstrated, for the first time, an improved secondary resection rate and secondary NED rate under ctDNA-guided intensified imaging surveillance. Furthermore, our study provides a framework for future randomized trials to explore the clinical benefits of personalized ctDNA-guided surveillance strategies.

Ethical approval and consent to participate

The study was approved by the Ethics Committees of Fudan University Shanghai Cancer Center (2007221-18) and BGI Genomics (BGI-IRB 22082). All patients provided written informed consent. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. The study was registered at the Chinese Clinical Trial Registry (https://www.chictr.org.cn/), with the registry ID of ChiCTR2000035677.

Consent

We confirmed that all patients included in our study provided written informed consent. And we have a statement in the part of 'Ethics approval and consent to participate' in title page.

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Author contribution

Y.L.: conceptualization, data curation, formal analysis, funding acquisition, project administration, visualization, and writing – original draft; J.X.: data curation, formal analysis, project administration, visualization, and writing – original draft; X.H.: data curation, funding acquisition, and resources; YK. C.: data curation; F.L.: data curation and resources; Y.C.: data curation, formal analysis, and visualization; X.M.: data curation; Q.D.: data curation, formal analysis, and visualization; L.S.: project administration; S.M.: data curation and funding acquisition; L.Z.: data curation; XF. H.: data curation; S.T.: data curation; H.W.: conceptualization and project administration; W.L.: data curation; S.C.: funding acquisition, project administration, supervision, and writing – review and editing; S.Z.: project administration, supervision, and writing – review and editing; Q.P.: conceptualization, data curation, project administration, resources, and writing – review and editing; J.P.: conceptualization, funding acquisition, resources, supervision, and writing – review & editing.

Conflicts of interest disclosure

J.X., Y.C., Q.D.D., L.S., H.Z.W., and S.D.Z. are employees of BGI Genomics, where all lab work, sequencing and sequencing data analyses were conducted. All other authors declare no competing financial interest.

Research registration unique identifying number (UIN)

The study was registered at the Chinese Clinical Trial Registry (https://www.chictr.org.cn/), with the registry ID of ChiCTR2000035677

Guarantor

Junjie Peng.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Provenance and peer review

Our paper was not invited.

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