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ARTICLE

Circulating Tumor DNA Predicts Conversion Therapy Response and Prognosis in Initially Unresectable Colorectal Liver-Limited Metastases: A Retrospective Study

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Abstract

Aims/Background Effective molecular biomarkers for predicting prognosis and guiding treatment in patients with initially unresectable colorectal liver metastases (CRLMs) undergoing conversion therapy are currently lacking. This study investigated the predictive value of circulating tumor DNA (ctDNA) conversion therapy outcomes in initially unresectable CRLMs.

Methods A retrospective analysis was conducted on 81 patients with CRLMs treated at the Sixth Affiliated Hospital, Sun Yat-sen University from January 2017 to April 2021. The relationships between baseline and treatment ctDNA levels and clinical responses were evaluated using group comparisons based on data type. The impact of ctDNA on survival outcomes was analyzed through Cox regression survival analysis.

Results Analysis of 81 patients with ctDNA-positive at baseline showed that patients in the ctDNA low-level group had a significantly longer median progression-free survival (mPFS) ($p = 0.039$). Among 45 patients who underwent ctDNA testing during systemic therapy, the proportion of patients in the ctDNA-negative group receiving local ablative treatment (LAT) was significantly higher (70.0% *vs* 26.7%, $p = 0.006$). Furthermore, 50% of patients in the ctDNA-negative group achieved no evidence of disease (NED) status, compared to 6.7% in the ctDNA-positive group ($p = 0.004$). Both mPFS and median overall survival (mOS) were significantly longer in ctDNA-negative patients compared to ctDNA-positive patients ($p < 0.05$). Of the 61 patients who underwent LAT, 37 received ctDNA testing at the same time as imaging assessment for NED. The proportion of patients with ctDNA clearance who achieved NED status was markedly higher than that of patients with ctDNA non-clearance (78.6% *vs* 33.3%, $p = 0.036$). Patients with ctDNA clearance demonstrated significantly improved mOS compared to those with ctDNA non-clearance (not reached *vs* 30.1 months, $p = 0.036$).

Conclusion Dynamic changes in ctDNA levels can predict both long-term survival and the effectiveness of conversion therapy in patients with initially unresectable CRLMs.

Key words: circulating tumor DNA; treatment outcome; colorectal cancer; liver neoplasms; prediction

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related mortality (Sung et al, 2021). The liver

remains the predominant site of CRC distal metastasis. Conversion therapy has significantly improved the 5-year survival rate for patients with unresectable liver metastases, increasing it from 0% to a range of 30% to 60% (Fujimoto et al, 2009). However, Takahashi et al (2013) reported that over one-third of patients with colorectal liver metastases (CRLMs) experienced early recurrence within 6 months following after conversion therapy, with a 5-year survival rate of 0%. For patients with initially unresectable CRLMs undergoing conversion therapy, there is a lack of effective molecular markers to predict prognosis and guide treatment.

Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for prognosis due to its non-invasive nature, real-time monitoring capability, and rapid turnaround (Ignatiadis et al, 2021). Increasing evidence suggests that ctDNA can dynamically monitor minimal residual disease (MRD) in colorectal cancer, enabling the early identification of individuals at high risk of recurrence and predicting tumor recurrence and treatment efficacy (Parikh et al, 2020; Tie et al, 2019). A prospective ctDNA analysis conducted by Professor Ruihua Xu's team in China revealed the temporal heterogeneity of somatic mutations associated with metastatic colorectal cancer, demonstrating that changes in gene mutation status could significantly impact patient survival outcomes (Wang et al, 2022). Next-generation sequencing (NGS)-based ctDNA testing has demonstrated considerable potential in prognosis prediction, genomic analysis, and recurrence monitoring in colorectal cancer.

While numerous studies have reported on ctDNA applications in colorectal cancer, research on CRLMs is limited, especially in the context of patients initially deemed unresectable and undergoing conversion therapy. Therefore, we retrospectively collected clinical information on patients with initially unresectable CRLMs, analyzed plasma ctDNA test results, and aimed to explore the predictive value of ctDNA analysis for the efficacy of conversion therapy in these patients, providing data reference for clinical applications.

Methods

Study Design and Patient Enrollment

This retrospective cohort study included 81 patients with CRLMs who were continuously diagnosed and treated by a multidisciplinary team (MDT) in the Sixth Affiliated Hospital, Sun Yat-sen University between January 2017 and April 2021. The study population was selected from an initial cohort of 545 patients with CRLMs. The criteria for initially technically unresectable CRLMs were defined as: (1) inability to completely remove all liver metastases visually; (2) residual functional volume $<30\%$ of standard liver volume; (3) involvement of three hepatic veins, bilateral hepatic arteries, or bilateral portal vein branches (Chinese College of Surgeons et al, 2023).

The inclusion criteria were: (1) age 18–80 years; (2) colorectal adenocarcinoma diagnosed by pathology; (3) liver metastases diagnosed by chest-abdominal-pelvic contrast-enhanced computed tomography or liver-enhanced magnetic resonance imaging; (4) absence of extrahepatic metastases; (5) initially technically unresectable liver metastases as assessed by a local MDT; (6) resectable primary tumor;

(7) continuously received at least 2 cycles of systemic therapy; (8) at least one tumor response assessment during systemic therapy; (9) baseline ctDNA-positive patients before treatment; (10) plasma ctDNA testing performed during conversion therapy (from the initiation of systemic therapy to the evidence of disease was assessed).

Exclusion criteria included: (1) other primary malignancies other than colorectal cancer; (2) metastases to organs other than the liver; (3) voluntary treatment discontinuation or transfer to other hospitals resulting in incomplete clinical information. A total of 81 patients with baseline ctDNA-positive who were eligible for the study were included in the study analysis (details shown in Fig. 1).

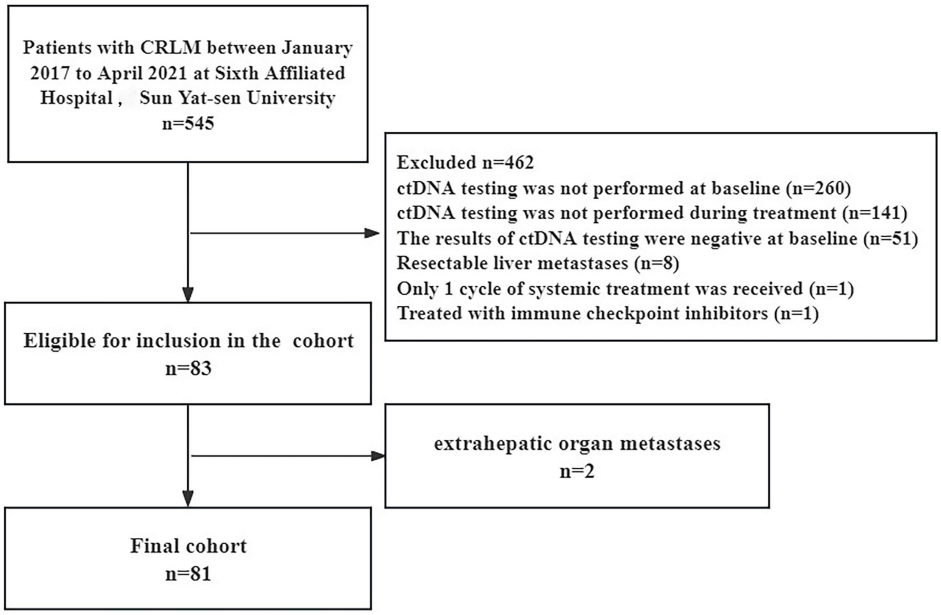

Fig. 1. Flowchart showing patient selection criteria. CRLM, colorectal liver metastase; ctDNA circulating tumor DNA.

The study protocol was approved by the Ethics Committee of the Sixth Affiliated Hospital, Sun Yat-sen University (Approval ID: 2023ZSLYEC-686) and conducted in accordance with the Declaration of Helsinki. The Ethics Committee granted a waiver of informed consent for this retrospective study involving anonymized data without direct patient intervention.

Treatment and Follow-up Procedures

Patients were managed according to MDT recommendations, with conversion therapy administered in 2-week cycles. First-line systemic chemotherapy regimens included modified FOLFOX6 (5-fluorouracil, leucovorin and oxaliplatin, mFOLFOX6) and modified FOLFOXIRI (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan, mFOLFOXIRI), combined with anti-vascular endothelial growth factor (anti-*VEGF*, bevacizumab) or anti-epidermal growth factor receptor (anti-*EGFR*, cetuximab) targeted therapy based on rat sarcoma virus (*RAS*) and B-Raf proto-oncogene (*BRAF*) gene mutation status. Conversion therapy was limited to a maximum of 12 cycles.

Local ablative treatment (LAT) decisions were made by an MDT consisting of colorectal surgeons, oncologists, liver surgeons, and radiologists, considering institutional experience, characteristics of the tumor, and patient preferences. LAT options included: (1) R0/R1 surgical resection; (2) ultrasound-guided microwave ablation; (3) hepatic arterial infusion chemotherapy (HAIC); (4) transarterial chemotherapy embolization (TACE); and (5) stereotactic body radiation therapy (SBRT).

Patient follow-up data were obtained through outpatient reviews, inpatient cases, or telephone follow-up until 31 December 2021. The median follow-up duration of 24.2 months, with an average follow-up interval of two months.

Conversion Therapy Response Evaluation

Treatment efficacy was assessed by a senior radiologist and oncologist based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Conversion therapy outcomes were classified as either success or failure. Conversion success was defined as patients achieving complete response (CR) or no evidence of disease (NED) status following LAT after first-line systemic therapy. Conversion failure was defined as the inability to perform LAT or achieve NED status after first-line systemic therapy. NED status was defined as CR or the absence of residual tumor detected by contrast-enhanced computed tomography (CT) of the chest, abdomen, and pelvis or contrast-enhanced magnetic resonance imaging (MRI) of the liver 4 weeks after LAT.

ctDNA Measurement and Cutoff Point for ctDNA Change

A total of 81 eligible patients underwent plasma ctDNA testing before systemic therapy (baseline) and during conversion therapy (at the same time as NED assessment during systemic therapy or after LAT). In this study, a next-generation sequencing (NGS) 88-gene panel was used to detect single nucleotide variations, indels, and copy number variations in exons of 22 genes relevant to colorectal cancer and lung cancer (epidermal growth factor receptor (*EGFR*), erb-B2 receptor tyrosine kinase 2 (*ERBB2*), fibroblast growth factor receptor 1 (*FGFR1*), fibroblast growth factor receptor 2 (*FGFR2)*, fibroblast growth factor receptor 3 (*FGFR3)*, mesenchymal epithelial transition factor (*MET*), phosphoinositide-3-kinase catalytic subunit alpha (*PIK3CA*), v-akt murine thymoma viral oncogene homolog 1 (*AKT1)*, anaplastic lymphoma kinase (*ALK*), *BRAF*, catenin beta 1 (*CTNNB1*), discoidin domain receptor tyrosine kinase 2 (*DDR2*), erb-B2 receptor tyrosine kinase 4 (*ERBB4*), F-Box and WD repeat domain containing 7 (*FBXW7)*, Kirsten rat sarcoma viral oncogene homolog (*KRAS*), mitogen-activated protein kinase kinase 1 (*MAP2K1*), neurogenic locus notch homolog protein 1 (*NOTCH1)*, neuroblastoma RAS viral oncogene homolog (*NRAS)*, phosphatase and tensin homolog (*PTEN*), SMAD family member 4 (*SMAD4)*, serine/threonine kinase 11(*STK11*), and tumor protein P53 (*TP53*)). The NGS 88-gene panel had an effective sequencing depth of 10,000× and a sensitivity of 0.25%.

The main workflow for ctDNA analysis comprised: (1) detection of key genetic mutations using the Illumina platform; (2) collection of peripheral blood samples and extraction of circulating free DNA from plasma; (3) library construction

and purification; (4) quality control, hybridization, and capture of libraries; (5) amplification and purification of captured products; (6) high-throughput sequencing. **Supplementary Material 1** provide detailed information on the reagents, equipment, and consumables used for ctDNA detection, as well as the specific quality control procedures implemented throughout the process.

ctDNA-positive was defined as detection of at least one somatic variant. The ctDNA fraction was quantified using the maximal somatic variant allelic frequency (maxVAF) in each sample, with ctDNA-negative samples having a maxVAF of 0. Germline mutations (50 to 100% consistent for VAF) were not analyzed. The median baseline maxVAF was 31.0% (11.8%–54.6%). According to the median value of baseline maxVAF, all patients were classified into two groups: a high-level group (maxVAF $>$31.0%, n = 40) and a low-level group (maxVAF $\leq$31.0%, n = 41). For the 45 patients who underwent plasma ctDNA testing during systemic therapy, 15 were classified as sustained ctDNA-positive group and 30 as ctDNA-negative group. Among the 61 patients who received LAT treatment, 37 underwent both NED assessment and plasma ctDNA testing. These patients were further categorized into ctDNA clearance (n = 28) and non-clearance (n = 9) groups. At progressive disease (PD), 34 patients underwent ctDNA testing, with 30 patients being ctDNA-positive and 4 remaining ctDNA-negative.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 (International Business Machines Corporation, Armonk, NY, USA), R-4.1.3 (R Foundation for Statistical Computing, Vienna, Austria), and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). The Kolmogorov-Smirnov test was employed to assess data normality. Normally distributed data are presented as mean $\pm$ standard deviation (SD), with group comparisons performed using Student's *t*-test. For continuous variables in non-normally distribution, median and interquartile range are reported, and the Wilcoxon rank-sum test was utilized for group comparisons. Categorical variables are expressed as counts and percentages, with group comparisons conducted using the chi-square test, chi-square correction test, or Fisher's exact test, depending on the expected frequencies. Survival times were calculated and survival curves plotted using the Kaplan-Meier method, with the Log-rank test applied to compare differences between curves. The impact of ctDNA levels on progression-free survival (PFS) and overall survival (OS) was evaluated using multivariate Cox regression analysis. Variables with $p < 0.05$ in the univariate Cox regression model were included in the multivariate Cox regression model using the "Enter" method. PFS was defined as the time from treatment initiation or study enrollment to disease progression or death from any cause, whichever occurred first. Median OS and PFS with corresponding 95% confidence intervals (CI) were reported. Statistical significance was set at $p < 0.05$.

Results

The Baseline Level of ctDNA and Patient Characteristics

The baseline level of ctDNA and patient characteristics were analyzed in a cohort of 81 patients (Table 1). Comparative analysis revealed significant differences between the high-level and low-level ctDNA groups. Patients with high ctDNA levels exhibited a greater number of liver metastases (>10), larger (≥5 cm) maximum diameter, and increased incidence of portal vein and hepatic vein involvement. Additionally, elevated baseline carcinoembryonic antigen (CEA) levels were observed in the high ctDNA group ($p < 0.05$) (Table 1). **Supplementary Fig. 1** illustrates the relationship between baseline plasma ctDNA characteristics (mutated gene types, quantities, maxVAF, serum CEA levels, and characteristics of liver metastatic tumors) in 81 patients. The heatmap demonstrates an association between ctDNA maxVAF and the number and maximum diameter of liver metastatic tumors, as well as the presence of portal vein and hepatic vein involvement.

Supplementary Table 1 displays the relationship between baseline ctDNA levels and the therapeutic efficacy of conversion treatment. In the low-level ctDNA group, 63.4% of patients achieved NED status, which was higher than the 42.5% in the high-level ctDNA group, although this difference did not reach statistical significance ($p = 0.059$). No significant differences were observed between the two groups in terms of recent efficacy, including objective response rate (ORR) ($p = 0.353$) and depth of tumor response (DpR) ($p = 0.502$). Regarding long-term survival, patients in the low-level ctDNA group demonstrated a significantly longer median PFS compared to the high-level group (19.8 months *vs* 15.6 months, $p = 0.039$, hazard ratio (HR) 0.569, 95% CI 0.333–0.971). However, Cox regression analysis did not identify baseline ctDNA levels as an independent prognostic factor for PFS ($p = 0.204$, HR 0.679, 95% CI 0.373–1.235) (**Supplementary Table 2, Supplementary Fig. 2**).

ctDNA Levels During Systemic Treatment and Patient Characteristics

In this cohort, 45 patients underwent plasma ctDNA testing during systemic treatment. Among them, 30 patients were ctDNA negative (66.7%), while 15 patients were ctDNA positive (33.3%). The median number of cycles of systemic therapy for the two groups were 7 (range: 6–8) and 8 (range: 5.5–8), respectively, with no statistically significant difference observed ($p = 0.775$) (**Supplementary Fig. 3A**). **Supplementary Fig. 3B** presents the swimmer plot illustrating the timing of actual ctDNA testing during systemic treatment for patients. Patient demographics and tumor characteristics were comparable between the ctDNA-positive and ctDNA-negative groups. However, the ctDNA-positive group had a significantly higher proportion of patients with more than 10 liver metastases ($p = 0.027$). Notably, the ctDNA-negative group had a significantly higher percentage of patients undergoing LAT after systemic treatment (70.0% *vs* 26.7%, $p = 0.006$) (**Supplementary Table 3**).

Table 1. Relationship between baseline ctDNA levels and patient clinical characteristics.

Patient characteristics	ctDNA high-level (n = 40)	ctDNA low-level (n = 41)	*p*	Statistic
Age (years)			0.441	$\chi^2 = 0.593$
≤55	21 (52.5)	25 (61.0)		
>55	19 (47.5)	16 (39.0)		
Gender			0.829	$\chi^2 = 0.046$
Male	32 (80.0)	32 (78.0)		
Female	8 (20.0)	9 (22.0)		
Primary site†			0.704	$\chi^2 = 0.144$
Right	6 (15.0)	4 (9.8)		
Left	34 (85.0)	37 (90.2)		
Degree of tumor tissue differentiation			0.973	$\chi^2 = 0.001$
Well-differentiated/moderately-differentiated	37 (92.5)	37 (90.2)		
Poorly-differentiated	3 (7.5)	4 (9.8)		
Occurrence time of liver metastases‡			0.590	$\chi^2 = 0.290$
Simultaneity	37 (92.5)	40 (97.6)		
Metachronism	3 (7.5)	1 (2.4)		
Distribution of liver metastases			0.651	$\chi^2 = 0.204$
Single lobe	9 (22.5)	11 (26.8)		
Bilateral lobes	31 (77.5)	30 (73.2)		
Number of liver metastases			0.008	$\chi^2 = 9.554$
<5	12 (30.0)	8 (19.8)		
5–10	10 (25.0)	24 (58.5)		
>10	18 (45.0)	9 (22.0)		
Maximum diameter of liver metastases (cm)			<0.001	$\chi^2 = 19.263$
<5	14 (35.0)	34 (82.9)		
≥5	26 (65.0)	7 (17.1)		
Portal vein involvement			0.028	$\chi^2 = 4.830$
Yes	13 (32.5)	5 (12.2)		
No	27 (67.5)	36 (87.8)		

Table 1. Continued.

Patient characteristics	ctDNA high-level (n = 40)	ctDNA low-level (n = 41)	*p*	Statistic
Hepatic vein involvement			0.043	$\chi^2 = 4.076$
Yes	16 (40.0)	8 (19.5)		
No	24 (60.0)	33 (80.5)		
Chemotherapy			0.969	$\chi^2 = 0.002$
Two-drug	4 (10.0)	5 (12.2)		
Three-drug	36 (90.0)	36 (87.8)		
Combined with targeted therapy			0.626	$\chi^2 = 0.238$
Yes	39 (97.5)	38 (92.7)		
No	1 (2.5)	3 (7.3)		
Local ablative treatment			0.274	$\chi^2 = 1.198$
Yes	28 (70.0)	33 (80.5)		
No	12 (30.0)	8 (19.5)		
Baseline CEA[§]			<0.001	$\chi^2 = 13.438$
Low-level	12 (30.0)	29 (70.7)		
High-level	28 (70.0)	12 (29.3)		

[†], The right colon included tumors from cecum to transverse colon, and the left colon included tumors from splenic flexure of colon to rectum; [‡], Simultaneity was defined as liver metastases detected before or at the time of colorectal cancer diagnosis, while metachronism was defined as liver metastases detected after radical resection of colorectal cancer; [§], According to the median baseline serum carcinoembryonic antigen (CEA) level of 65.74 ng/mL before systemic treatment, the patients were divided into high level group (CEA >65.74 ng/mL) and low level group (CEA ≤65.74 ng/mL). ctDNA, circulating tumor DNA; χ^2: Chi-square test.

Table 2. Relationship between ctDNA levels during systemic treatment and clinical outcomes (n = 45).

Variables	ctDNA-negative (n = 30)	ctDNA-positive (n = 15)	*p*	Statstic
NED, n (%)	15 (50.0)*	1 (6.7)	0.004	$\chi^2 = 8.195$
ORR†, n (%)	27 (90.0)	12 (80.0)	0.642	$\chi^2 = 0.216$
DpR†, Median (IQR), %	51.6 (41.0–59.4)	45.0 (38.2–59.7)	0.386	Z = –0.956
PFS, Median (95% CI), month	18.7 (17.4–20.0)	7.3 (6.3–8.2)	<0.001	Z = –3.826
OS, Median (95% CI), month	NR	22.1 (13.2–31.1)	0.003	Z = –2.961

*, One patient achieved complete response; †, the best radiological response from the start of systemic treatment to the time of ctDNA detection; ctDNA, circulating tumor DNA; CI, confidence intervals; IQR, interquartile range; DpR, depth of tumor response; NED, no evidence of disease; NR, not reached; PFS, progression-free survival; OS, overall survival; ORR, objective response rate. χ^2: Chi-square test. Z, Wilcoxon rank-sum test. The Z values for OS and PFS were calculated based on the Cox regression analysis.

The percentage of patients achieving NED status was significantly higher in the ctDNA-negative group compared to the ctDNA-positive group (50.0% *vs* 6.7%, $p = 0.004$) (Table 2). Regarding long-term survival, ctDNA-negative patients demonstrated significantly longer median PFS (18.7 months *vs* 7.3 months, $p < 0.001$, HR 0.254, 95% CI 0.126–0.514). Cox regression analysis identified ctDNA level during systemic treatment as an independent factor influencing PFS ($p = 0.001$, HR 0.264, 95% CI 0.120–0.570) (**Supplementary Table 4**). Compared to ctDNA-positive patients, ctDNA-negative patients had a 74% reduction in the risk of disease progression or death. Additionally, ctDNA-negative patients exhibited a significantly longer median overall survival (NR *vs* 22.1 months, $p = 0.003$, HR 0.178, 95% CI 0.057–0.558) compared to ctDNA-positive patients (**Supplementary Table 5**, **Supplementary Fig. 4**).

ctDNA Level and Clinical Efficacy after LAT

Among the 61 patients who underwent LAT, 37 underwent plasma ctDNA testing simultaneously with imaging evaluation for NED. The results showed that 28 patients (75.7%) achieved ctDNA clearance, while 9 patients (24.3%) did not. A significantly higher proportion of patients in the ctDNA clearance group attained NED status compared to the non-clearance group (78.6% *vs* 33.3%, $p = 0.036$). Regarding DpR and ORR, both were higher in the ctDNA clearance group compared to the non-clearance group, although the differences were not statistically significant (Table 3). Fig. 2 illustrates the optimal size changes in liver target lesions before LAT in patients who achieved ctDNA clearance and those who did not. Regarding long-term survival, patients with ctDNA clearance had a longer median progression-free survival (mPFS) compared to those without clearance (21.3 months *vs* 13.9 months), though this difference did not reach statistical significance ($p = 0.153$). Furthermore, patients with ctDNA clearance demonstrated significantly improved median overall survival (mOS) compared to those without clearance (NR *vs* 30.1 months, $p = 0.036$) (**Supplementary Fig. 5**).

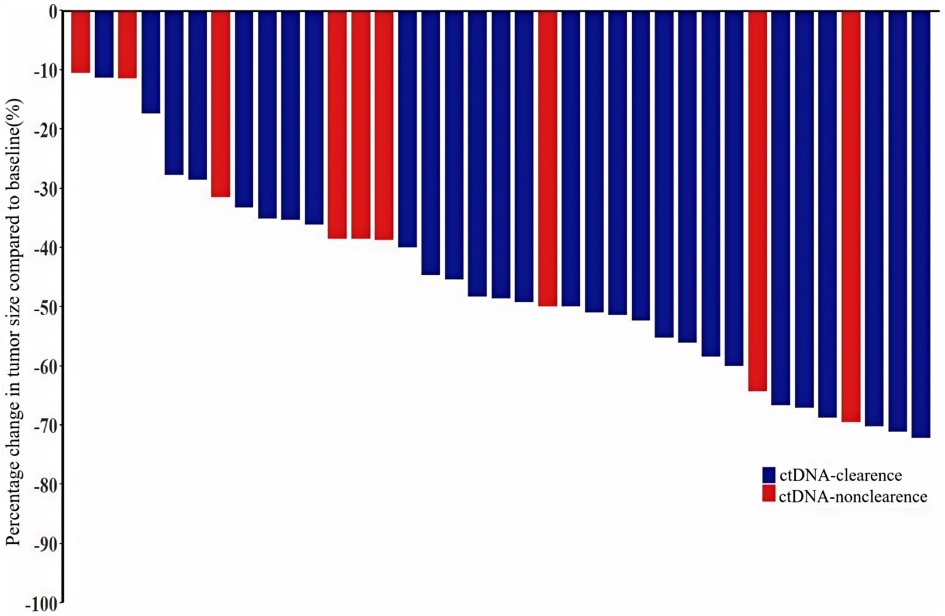

Fig. 2. Waterfall plots show the largest percentage change in tumor size from baseline for ctDNA clearance and ctDNA without clearance. ctDNA, circulating tumor DNA.

Table 3. Relationship between ctDNA levels after local ablative treatment (LAT) and clinical outcomes (n = 37).

Variables	ctDNA clearance (n = 28)	ctDNA non-clearance (n = 9)	*p*	Statistic
NED, n (%)	22 (78.6)	3 (33.3)	0.036	$\chi^2 = 4.464$
ORR, n (%)	24 (85.7)	7 (77.8)	0.966	$\chi^2 = 0.002$
DpR, Median (IQR), %	49.6 (35.6–59.6)	38.6 (21.5–57.1)	0.213	Z = –1.216
PFS, Median (95% CI), month	21.3 (16.0–26.7)	13.9 (3.9–23.8)	0.153	Z = –1.418
OS, Median (95% CI), month	NR	30.1 (13.9–NA)	0.036	Z = –2.137

ctDNA, circulating tumor DNA; NED, no evidence of disease; NR, not reached; PFS, progression-free survival; OS, overall survival; ORR, objective response rate; χ^2: Chi-square test. Z, Wilcoxon rank-sum test. The Z values for OS and PFS were calculated based on the Cox regression analysis.

ctDNA Analysis when PD

Plasma samples from 34 patients were analyzed at PD, with 30 patients exhibiting ctDNA-positive results and 4 patients showing ctDNA-negative results. The median maxVAF of ctDNA at PD (3.9%, range 0.8–28.1%) was significantly elevated compared to the median maxVAF during systemic therapy (0%, range 0–1.12%) ($p < 0.001$). While the median maxVAF of ctDNA at PD was significantly higher than after LAT ($p < 0.001$), it was notably lower than the baseline ctDNA median maxVAF of 31.0% (range 11.8%–54.6%) (Fig. 3A). Overall survival (OS) analysis indicated a trend towards improved outcomes in ctDNA-negative patients, although the difference did not reach statistical significance ($p = 0.181$) (Fig. 3B). Additionally, in three patients with wild-type KRAS who received conversion therapy comprising chemotherapy and cetuximab, ctDNA testing at PD identified novel mutations in *KRAS*, *BRAF*, and *PIK3CA* genes (Fig. 3C).

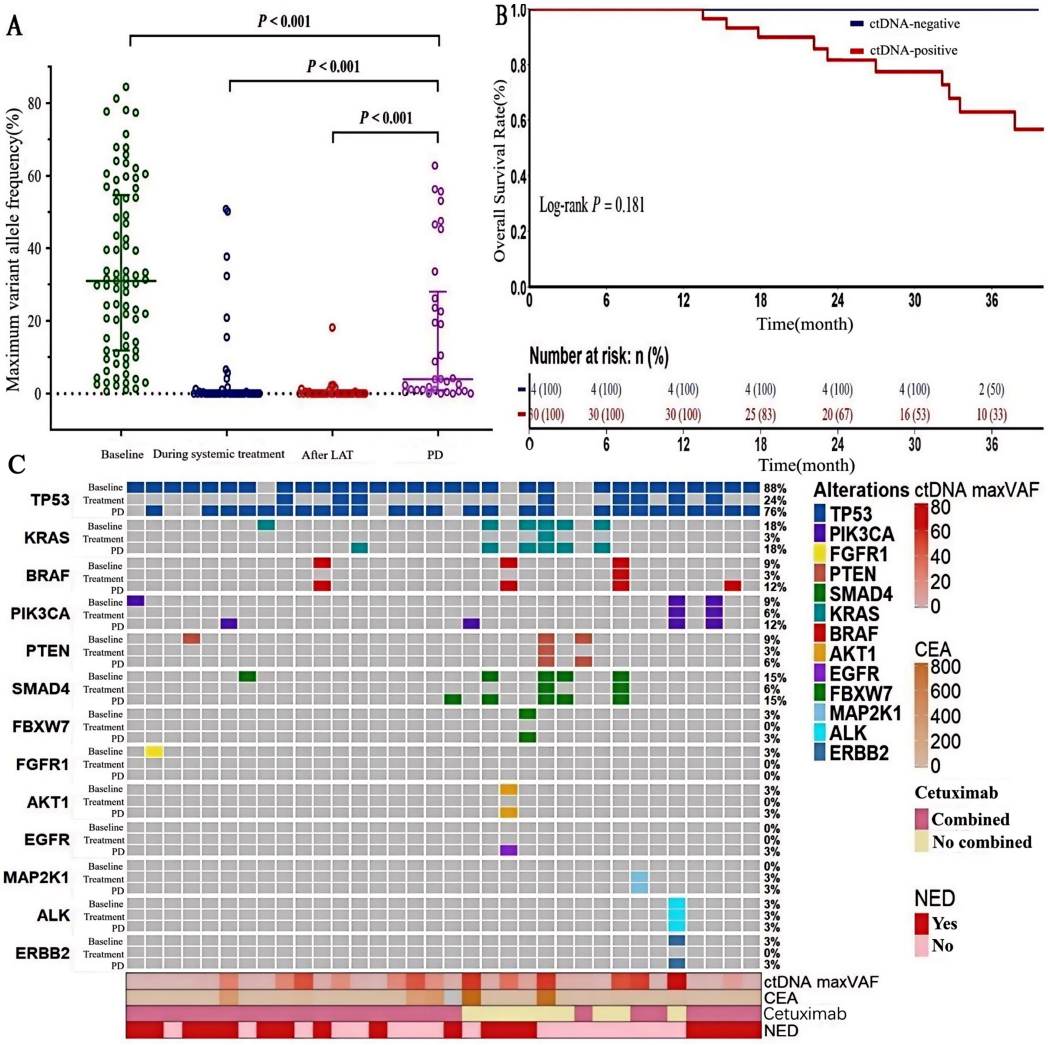

Fig. 3. ctDNA analysis at PD. (A) Comparison of ctDNA maxVAF in PD to baseline during systemic treatment and after LAT. (B) OS curves of ctDNA-negative and ctDNA-positive patients at PD. (C) Heatmaps showing ctDNA gene variation sites and cetuximab treatment in the same patient at baseline, during treatment, and PD. ctDNA, circulating tumor DNA; LAT, local ablative treatment; PD, progressive disease; NED, no evidence of disease; CEA, carcinoembryonic antigen; maxVAF, the maximal somatic variant allelic frequency; *TP53*, tumor protein P53; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *BRAF*, B-Raf proto-oncogene; *PIK3CA*, phosphoinositide-3-kinase catalytic subunit alpha; *PTEN,* phosphatase and tensin homolog; *SMAD4,* SMAD family member 4; *FBXW7,* F-Box and WD repeat domain containing 7; *FGFR1*, fibroblast growth factor receptor 1; *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *EGFR*, epidermal growth factor receptor; *MAP2K1*, mitogen-activated protein kinase kinase 1; *ALK*, anaplastic lymphoma kinase; *ERBB2*, erb-B2 receptor tyrosine kinase 2.

Discussion

Our study demonstrates that ctDNA levels at various time points—baseline, during systemic treatment, post-LAT, and at PD—play crucial roles in disease assessment, treatment efficacy, and prognostic prediction in CRLMs.

Tumor cell apoptosis, necrosis, and the release of ctDNA into the bloodstream can be detected using NGS technology that enables the identification of specific

gene mutations as either 'positive' or 'negative' and allows for the reporting of ctDNA levels via VAF, such assessments are valuable for the repeated evaluation of treatment efficacy, tumor burden, and genomic profiling (Dasari et al, 2020). ctDNA has wide applications in colorectal cancer, with studies indicating its potential value for treatment stratification in I-III stage colorectal cancer during adjuvant therapy (Tie et al, 2022). In advanced colorectal cancer, ctDNA not only predicts prognosis but also identifies resistance to targeted therapies, helping to determine the timing for treatment rechallenge (Cohen et al, 2023; Loft et al, 2023).

Some studies on other types of tumors report that the VAF of ctDNA is linearly related to tumor volume, suggesting that ctDNA can provide information about tumor size (Abbosh et al, 2017; Newman et al, 2014; Parkinson et al, 2016). Our research findings indicate that patients with high baseline ctDNA levels exhibit a significantly higher proportion of liver metastases with a maximum diameter of $\geq$5 cm and diffuse liver metastases compared to those with low baseline levels. Consistent with the viewpoint of Jia et al (2020), we believe that plasma ctDNA levels are positively correlated with the tumor burden of liver metastases. For patients with high baseline ctDNA maxVAF in CRLMs, effective systemic treatment is needed to rapidly reduce the tumor burden. We observed a higher incidence of portal vein invasion in the high-level baseline ctDNA group, consistent with the increased proportion of diffuse liver metastases. Hayashi et al (2010) suggested that portal vein invasion reflects the degree of tumor invasiveness or the likelihood of intrahepatic micro-metastases. Our data suggest that portal vein invasion by liver tumors may lead to the spread of liver metastases through the portal venous system, resulting in diffuse liver metastases. In addition, literature has reported that the number of liver metastases is an adverse prognostic factor for patients with CRLMs (Zhang et al, 2022). In this study, the analysis was conducted using a cutoff value of 8, referring to the classification of the number of liver metastases from the CELIM study published in The Lancet Oncology (Folprecht et al, 2010). Based on our findings of significant differences in the baseline high-level and low-level ctDNA groups and during systemic chemotherapy, as well as in the ctDNA-positive and ctDNA-negative groups regarding liver metastases $>$10, we chose 10 as the cutoff value for analysis. We discovered that having more than 10 liver metastases is an independent risk factor for both PFS and OS. Our previous research also indicated that more than 10 liver metastases significantly reduced the likelihood of achieving a NED status in patients (Shen et al, 2022). Therefore, for patients with initially diffuse liver metastases, it is crucial to explore more effective conversion therapy strategies to improve prognosis.

Our study demonstrates a strong correlation between ctDNA levels during systemic treatment and conversion therapy outcomes. However, no significant association was observed between baseline ctDNA levels and the outcomes of conversion therapy. Despite the absence of a uniform time point for ctDNA testing during chemotherapy, all patients were tested at a corresponding time point after a median of 8 cycles of conversion therapy. This aligns with findings from the randomized controlled CELIM study conducted by Folprecht et al (2010), which reported an average of 8 cycles of conversion therapy administered from initiation of conver-

sion therapy to achieving curative surgery. We propose that for patients unable to undergo curative surgery after initial imaging evaluation following systemic treatment initiation, conducting imaging examinations concurrently with ctDNA testing after 8 treatment cycles can aid clinicians in more accurately determining optimal timing for local ablative therapy and assessing the overall success of conversion therapy.

In this study, some patients underwent plasma ctDNA testing concurrently with imaging assessment of NED. A significant association was observed between ctDNA clearance and longer mOS in patients undergoing plasma ctDNA testing concurrently with imaging assessment of NED. This finding corroborates research results reported by Wang et al (2022) on the prognostic impact of ctDNA level changes in patients with metastatic colorectal cancer. Among 25 patients achieving NED, 3 remained ctDNA-positive. The median NED duration in ctDNA-negative patients was longer than in ctDNA-positive patients (15.7 months *vs* 9.0 months), although not statistically significant ($p = 0.440$). Combining routine imaging assessment of NED with ctDNA analysis may enhance prognostic prediction. For patients achieving NED but remaining ctDNA-positive, microscopic residual lesions may be present, necessitating continued systemic treatment to prolong NED duration.

In 34 patients undergoing ctDNA testing at disease progression, the maxVAF of ctDNA significantly increased compared to levels during systemic treatment and after local ablative therapy. This suggests that dynamic ctDNA changes reflect disease status, consistent with findings by Chang et al (2016). In four patients with imaging-determined progressive disease, ctDNA remained negative, possibly due to delayed release from tumor. We propose that simultaneous ctDNA testing during imaging assessment of disease progression may assist clinicians in predicting patient prognosis.

ctDNA demonstrates significant value as a non-invasive and cost-effective method for tumor biopsy when repeated assessment of tumor molecular characteristics is required for treatment optimization. van Helden et al (2019) reported that the majority of patients initially benefiting from cetuximab treatment showed acquired mutations in *RAS* and *BRAF* genes in ctDNA testing upon tumor progression. Although our study had limited cases with simultaneous ctDNA testing during imaging assessment of disease progression, we observed the emergence of secondary mutations in *RAS*, *BRAF*, and *PIK3CA* genes in plasma ctDNA samples from patients undergoing combination cetuximab conversion therapy. Based on the ctDNA testing results, cetuximab was discontinued in the second-line treatment plan, and a switch to bevacizumab treatment yielded favorable therapeutic effects.

This study has several limitations. First, it is a retrospective observational study with a relatively small sample size. Second, although we used a 22-gene panel covering common genetic mutation sites in colorectal cancer, the overall reflection of tumor genetic information is limited, potentially overlooking other prognostic and treatment-guiding variant genes. Third, despite using NGS-based detection, the possibility of false negatives in ctDNA results during treatment and disease progression cannot be ruled out due to tumor ctDNA dropout rates. Future prospective

studies with larger sample sizes, expanded gene panels, and advanced detection technologies are needed to provide stronger evidence for predicting the efficacy of conversion therapy and guiding personalized treatment in patients with initially unresectable CRLMs.

Conclusion

Routine radiological imaging combined with ctDNA analysis can help clinicians better understand the tumor burden in patients with CRLMs before conversion therapy. Dynamic changes in ctDNA levels predict not only long-term survival but also the effectiveness of conversion therapy, providing valuable clinical information for personalized treatment of this patient population.

Key Points

- Pre-treatment ctDNA assessment improves clinicians' understanding of liver metastatic burden in patients with initially unresectable CRLMs.
- Monitoring ctDNA levels during systemic therapy and after LAT aids in evaluating conversion therapy outcomes and predicting long-term survival.
- Concurrent ctDNA testing during disease progression assessments refines patient prognostication.
- Dynamic changes in ctDNA levels serve as a potential biomarker for predicting conversion therapy effectiveness in patients with technically unresectable CRLMs, providing valuable clinical insights for personalized treatment.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

BH and CS designed the research. JZ, HH and YD were involved in its execution. BH, CS, XX, QM handled data collection and analysis. BH contributed to drafting the manuscript, and all authors engaged in the critical revision of the manuscript. All authors approved the final manuscript and took responsibility for the integrity of the work, ensuring that any concerns about accuracy or completeness are addressed.

Ethics Approval and Consent to Participate

The Ethics Committee of the Sixth Affiliated Hospital, Sun Yat-sen University approved the protocols (Approval ID: 2023ZSLYEC-686), which complied with the principles of the Declaration of Helsinki. The Ethics Committee granted a

waiver of informed consent for this retrospective study involving anonymized data without direct patient intervention.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.magonlinelibrary.com/doi/suppl/10.12968/hmed.2024.0695.

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