Circulating tumour DNA in predicting and monitoring survival of patients with locally advanced rectal cancer undergoing multimodal treatment: long-term results from a prospective multicenter study

Jiaolin Zhou,[a,l] Lifeng Li,[b,l] Yuxin Liu,[a,l] Wenzhuo Jia,[c] Qian Liu,[d] Xuan Gao,[b] Aiwen Wu,[e] Bin Wu,[a] Zhanlong Shen,[f] Zhenjun Wang,[g] Jiagang Han,[g] Beizhan Niu,[a] Yuhua Gong,[b] Yanfang Guan,[b] Jianfeng Zhou,[h] Huadan Xue,[i] Weixun Zhou,[j] Ke Hu,[k] Junyang Lu,[a] Lai Xu,[a] Xuefeng Xia,[b] Xin Yi,[b] Ling Yang,[b,l,**] and Guole Lin[a,l,*]

[a]Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[b]Geneplus-Beijing, Beijing 102206, China
[c]Department of General Surgery, Beijing Hospital, National Center of Gerontology, Beijing 100730, China
[d]National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China
[e]State Key Laboratory of Holistic Integrative Management of Gastrointestinal Cancers, Beijing Key Laboratory of Carcinogenesis and Translational Research, Unit III, Gastrointestinal Cancer Center, Peking University Cancer Hospital & Institute, Beijing 100142, China
[f]Department of Gastroenterological Surgery, Peking University People's Hospital, Beijing 100871, China
[g]Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China
[h]Department of Medical Oncology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[i]Department of Radiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[j]Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[k]Department of Radiotherapy, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China

eBioMedicine
2025;112: 105548
Published Online xxx
https://doi.org/10.1016/j.ebiom.2024.105548

Summary

Background Neoadjuvant chemoradiotherapy (nCRT) is the standard for locally advanced rectal cancer (LARC). However, distant metastasis remains the primary cause of treatment failure. Early identification of high-risk individuals for personalized treatment may offer a solution. Circulating tumour DNA (ctDNA) could assist in this process.

Methods From September 2017 to June 2019, the study prospectively recruited 113 patients with LARC (cT3-4N0M0 or cTanyN + M0) who underwent nCRT followed by radical surgery across 8 tertiary centers. ctDNA was analysed using large-panel targeted sequencing at baseline, during nCRT, pre-surgery, post-surgery, post-adjuvant chemotherapy (ACT), and during annual follow-ups for 3 years.

Findings We analysed 103 tissue and 669 plasma samples from 103 patients. With a median 53-month follow-up, significantly worse progression-free survival (PFS) and overall survival (OS) were observed if median variant allele frequency (mVAF) of baseline ctDNA per patient was ≥0.5% (PFS, HR 4.39, $p < 0.001$; OS, HR 5.61, $p = 0.004$) or ctDNA was still detectable two weeks into nCRT (PFS, HR 7.63, $p < 0.001$; OS, HR 5.08, $p < 0.001$). Furthermore, when compared to the low-risk (C1) group (characterized by "ctDNA undetected during nCRT with baseline mVAF <0.5%" or "ctDNA undetected during nCRT with TMB (tumour mutational burden) ≥20/Mb"), the high-risk (C2) group (characterized by "ctDNA detected during nCRT" or "baseline mVAF ≥0.5% with TMB <20/Mb") showed significantly worse long-term outcomes (3 y-PFS, 55.9% vs. 94.2%; 3 y-OS, 79.4% vs. 100%). The ctDNA clearance during nCRT, baseline mVAF, and TMB may be effective prognostic indicators.

*Corresponding author. Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 1, Shuai Fu Yuan, Dong Cheng District, Beijing, 100730, China.
**Corresponding author. Geneplus-Beijing, Peking University Medical Industrial Park, Zhongguancun Life Science Park, 9th Floor, No.6 Building, Beijing 102206, China.
E-mail addresses: linguole@126.com (G. Lin), yangl@geneplus.org.cn (L. Yang).
[l]These authors contributed equally to this work.

Interpretation Our findings reaffirm the clinical monitoring value of ctDNA and demonstrate the strong prognostic value of baseline ctDNA and its early clearance status in patients with LARC undergoing nCRT. This highlights the potential of dynamic ctDNA monitoring as actionable stratified indicators to guide personalized neoadjuvant treatment strategies.

Funding This work was supported by the Major Grants Program of Beijing Science and Technology Commission (No. D171100002617003) and the National High Level Hospital Clinical Research Funding (2022-PUMCH-C-005).

Keywords: Circulating tumour DNA; Next-generation sequencing; Locally advanced rectal cancer; Neoadjuvant chemoradiotherapy; Prognostic factor

Research in context

Evidence before this study

ctDNA serves as a dynamic biomarker for real-time tumour burden and is widely applied in postoperative monitoring of solid tumours. The DYNAMIC study highlights its utility in risk stratification for stage II colorectal cancer, enabling personalized guidance for adjuvant chemotherapy. However, in locally advanced rectal cancer (LARC), research on ctDNA dynamics at baseline and during neoadjuvant chemoradiotherapy (nCRT) and its clinical value in predicting long-term outcomes remains limited.

Added value of this study

This study provides long-term data from a prospective cohort of patients with LARC undergoing nCRT with dynamic ctDNA monitoring, contributing valuable evidence to the field. Specifically, our findings demonstrate that early molecular indicators, including baseline ctDNA abundance, early ctDNA clearance, and tumour mutational burden (TMB), are significantly associated with long-term prognosis.

Implications of all the available evidence

These findings suggest that a risk stratification method based on early ctDNA status may guide personalized neoadjuvant treatment strategies for LARC, optimizing therapy intensity, duration, and sequencing to improve therapeutic outcomes and minimize unnecessary interventions.

Introduction

Colorectal cancer (CRC) currently is the second leading cause of cancer death worldwide.[1] For locally advanced rectal cancer (LARC),[2] preoperative chemoradiotherapy followed by surgery is the standard treatment to reduce local recurrence but with limited benefits for systemic disease control.[3] Recently, total neoadjuvant therapy (TNT) has been recommended as the preferred treatment for LARC, which involves administering additional chemotherapy during the preoperative chemoradiotherapy phase and has shown potential for improving long-term survival.[4–6] However, not all patients can benefit from TNT, especially considering its disadvantages such as increased toxicity and economic burden. Therefore, selecting suitable patients for TNT through risk stratification has become an important topic.

Circulating tumour DNA (ctDNA), which harbours tumour-specific mutations, is detectable within the cell-free DNA (cfDNA) fraction of peripheral blood in patients with solid tumours.[7,8] Its presence in plasma provides a real-time and dynamic indicator of tumour burden, often indicating tumour progression earlier than radiological imaging.[9–11] Molecular residual disease (MRD), typically refers to the detection of ctDNA following definitive treatments like surgery, has proven to be a robust predictor of tumour recurrence. Additionally, ctDNA offers a non-invasive approach to overcome intratumoural heterogeneity by capturing a broad range of cancer genome alterations and tracking clonal evolution, though it faces challenges, such as false negatives in tumours with limited ctDNA release. Current evidence underscores the broad utility of ctDNA as a biomarker for diagnosis, prognosis, and postoperative treatment guidance in CRC.[12,13] However, for LARC, the unique context of nCRT calls for further investigation to determine whether early ctDNA status and genetic features can predict treatment response and prognosis, thereby guiding neoadjuvant strategies. Research in this area remains limited.

We conducted a prospective, multicentre, observational study to investigate the value of ctDNA in predicting treatment response and prognosis, as well as in monitoring long-term outcomes in patients with LARC undergoing multimodal treatment. In addition to the four ctDNA monitoring timepoints included in our previous mid-term results report,[14] this study incorporated four additional follow-up timepoints and analysed long-term survival outcomes. We focused on baseline ctDNA concentration and its early changes during

neoadjuvant chemoradiation in predicting metastasis and long-term survival. Furthermore, our findings suggest distinct molecular characteristics between liver and lung metastases.

Methods

Study design and patient characteristics

This prospective observational study (Study D) is one of the four sub-studies (Study A to D) of the large-scale prospective multicentre clinical trial, the MONT-R study, which recruited patients with LARC treated with multimodality therapy at 8 Chinese tertiary referral hospitals. Key eligibility criteria included: patients aged 18–75 years with pathologically confirmed rectal adenocarcinoma; tumour located within 12 cm above anal verge, with clinical staging of cT3-4N0 or cTanyN1-2; baseline chest/abdominal/pelvic enhanced CT confirmed the absence of distant metastasis; and American Society of Anesthesiologists (ASA) scores of I to III. This study did not restrict participant enrolment based on sex, which was self-reported by the participants.

The multimodality treatment involves long-term radiotherapy (50Gy/25 fractions) combined with three cycles of concurrent single-agent capecitabine/XELOX chemotherapy, followed by radical surgeries 8–10 weeks after the completion of radiotherapy, and then adjuvant chemotherapy (ACT). Pathology examination of surgical specimens were performed to assess the histological type and grade of the tumour, pathologic Tumour-Node-Metastasis (TNM) staging, and the pathologic tumour regression grade (pTRG) (following the College of American Pathologists (CAP) grading system).[15] Therapeutic regimens for routine ACT and second-line treatment for metastatic disease were determined at the physicians' discretion. The primary end point is progression-free survival (PFS), defined as the time from the start of therapy to the earliest occurrence of any of the following events: identification of distant metastasis or progression to unresectable disease before surgery, non-radical resection of the primary tumour, local recurrence or distant metastasis after radical surgery. Incidence of second non-colorectal malignancies was not included in the PFS analysis. The secondary end point is overall survival (OS), defined as the time from the start of therapy to death from any cause. Patients without an end point (progression or death events) were censored at the date of the last follow-up.

Sample collection

Peripheral blood samples were collected from all participants at different time points: prior to chemoradiotherapy (baseline), two weeks after the initiation of nCRT (in process nCRT), within 7 days before surgery (pre-op), within 7–14 days after surgery (post-op), and annually for three years post-surgery. Tissue samples were obtained through endoscopic biopsy before treatment. Patients with paired baseline tissue and plasma samples were included in this study.

Targeted capture sequencing and genomic data analysis

Blood samples collected in EDTA Vacutainer Tubes (BD Diagnostics) were subjected to laboratory process within 3 h. Peripheral blood lymphocytes (PBLs) and plasma were separated with sequential centrifugation (2500 g, 10 min and 16,000 g, 10 min). Genomic DNA from tissues and cfDNA from plasma was extracted using DNeasy Blood & Tissue Kit (Qiagen) and QIAamp Circulating Nucleic Acid Kit (Qiagen), respectively. Sequencing libraries were constructed using the KAPA DNA Library Preparation Kit (Kapa Biosystems) as per the manufacturer's instruction. Barcoded libraries were hybridized to a customized panel of 1021 genes for both gDNA and ctDNA samples. Paired-end sequencing was performed using NBSEQ-T7RS sequencing platform (BGI, Shenzhen, China).

Targeted capture sequencing yielded a median depth of 742 × in gDNA and 1713 × in cfDNA. Single-nucleotide variants and small insertions and deletions were called by MuTect (ref.[16]; version 1.1.4). For quality control, somatic mutations in tissue samples were identified only when (i) present in <1% of the population in the 1000 Genomes Project (https://www.internationalgenome.org/), the Exome Aggregation Consortium, and the Genome Aggregation Database (https://gnomad.broadinstitute.org), (ii) not present in paired germline DNA from PBLs, and (iii) detected in at least five high quality reads containing the particular base, where high-quality reads were defined with Phred score ≥30, mapping quality ≥30, and without paired-end reads bias. Baseline ctDNA positivity was defined as when at least one mutation in a tissue sample had also been detected in the matched ctDNA. In particular, high frequency mutations in ctDNA which were persistently detected across time points were utilized to additionally retrieve several somatic mutations with relatively low detected frequencies. We re-defined the median VAF by calculating the median value of VAF for each patient, rather than ranking all patient VAFs and taking the median.

NGS assay panel design

We performed comprehensive reviews of the literature and genetic database, including COSMIC (https://doi.org/10.1093/nar/gku1075) and My Cancer Genome (https://doi.org/10.1016/j.jmoldx.2015.05.002) (http://www.mycancergenome.org, last accessed January 20, 2017), to identify genes biologically and clinically relevant to breast cancer, including the following: i) genes that harbour mutations and are currently clinically actionable, by providing information about sensitivity or resistance to specific targeted therapies or prognostic information related to patient outcomes; ii) genes

expected to be actionable in the near future using targeted therapies currently under active development in clinical trials; and iii) genes known to be recurrently mutated in cancer, but currently lacking robust prognostic information or targeted therapies.

LOD analysis

To assess analytical sensitivity and define limit of detection (LOD), a series of dilutions containing artificial mutation fragments spiked into a normal genomic DNA sample (NA12878 cells) were generated. PCR primers for each artificial mutation sequence used in our study were designed and synthesized. Overlap-extension PCR for site-directed mutagenesis was performed using genomic DNA as the template to obtain six mutation sequences that are approximately 2 Kb in length. Briefly, genomic DNA was extracted from the lymphoblastoid NA12878 cells from the International HapMap Project, which were purchased from Coriell Cell Repositories (Camden, NJ). For SNVs with an average coverage of targeted positions of 1774.58X ± 408.2X, we found the VAFs 0.1%, 0.3%, 0.5%, and 1% have a sensitivity of detection of 75.93% (95% CI, 71.56%–79.83%), 92.01% (95% CI, 88.10%–94.76%), 96.30% (95% CI, 92.57%–98.27%), and 98.61% (95% CI, 94.56%–99.76%), respectively.

Tumour mutation burden

For the calculation of the TMB, we applied three criteria for competent mutations: (1) somatic but not germline mutation; (2) located in coding region, nonsynonymous SNVs/Indels, including ±2 splice; and (3) a mutation allele frequency ≥1%. The TMB was calculated as the number of competent mutations divided by the length of the panel-covered genomic region (1.09 Mb).

Statistics

Spearman correlation test was performed to determine the association of mutational spectrum between plasma and tissue. Fisher's exact test or the χ^2 test was employed to compare differences in categorical clinicopathologic parameters, such as Metastasis/Non-metastasis, between the ctDNA detectable vs. ctDNA undetectable group across time. Wilcoxon Mann–Whitney test was used for comparing continuous clinicopathologic parameters. The association between variables and PFS were calculated using the Kaplan–Meier method and compared using the log-rank test. A univariate/multivariate Cox proportional hazards regression was used to estimate hazard ratios and 95% confidence intervals (CIs) to identify prognostic factors based on variables with p values under 0.05. Time to progression was calculated from the date of nCRT initiation until disease progression. All data analyses were performed using R software (version 4.1.2). Statistical significance was defined as $p < 0.05$.

Ethics statement

The study was designed and conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Peking Union Medical College Hospital (PUMCH) (approval number:JS-1296) (NCT03042000). All patients provided written informed consent prior to their participation. During the courses of multimodality treatment, all the clinicians were blinded to the ctDNA analysis results.

Role of funders

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of this report.

Results

Patient clinicopathological characteristics, genomic and ctDNA features at baseline

The study was designed to explore the potential of ctDNA in predicting and monitoring long-term survival in patients with LARC undergoing nCRT. The flowchart and sampling time point information of the study are presented in Figs. 1 and 2a. In short, we enrolled 113 patients with LARC from September 2017 to June 2019. 10 patients were excluded due to non-collection of plasma samples after baseline or withdrawal of informed consent. The median age of the 103 patients was 59 years old (range 26–75), with a higher proportion of males (n, 67, 65.0%). The baseline clinical tumour-node-metastasis (TNM) stage of patients was classified into 98 patients (95.1%) with stage cIII and 5 patients (4.9%) with stage cII. Patients were randomly assigned to receive chemotherapy of single-agent capecitabine (n, 47, 45.6%) or the XELOX regimen (n, 56, 54.4%) during nCRT (Table 1). The median follow-up was 53 months (range, 5–64), disease progression of metastasis occurred in 20 patients (19.4%). The 3-year PFS (3 y-PFS) and 3-year OS (3 y-OS) was 81.6% and 93.2%, respectively. Baseline CA19-9 value, post-surgery pTRG score, and pCR status were significantly associated with eventual tumour metastasis (Figs. 2b and 3a). We obtained 1085 and 491 somatic variants (nonsynonymous single-nucleotide variants and indels) in baseline tissue and plasma samples, respectively. The median TMB was 7 per Mb (mean, 10.5 per Mb) and median blood TMB (bTMB) was 3 per Mb (mean, 4.8 per Mb). Patients without distant metastasis had higher TMB values than those with metastasis (Fig. 2b). The most recurrent mutant genes in tissue included *TP53* (79.6%), *APC* (76.7%), *KRAS* (37.9%), *FBXW7* (19.4%), *PIK3CA* (18.4%) and *SMAD4* (17.5%). High-frequency mutations (frequency more than 5% in tissue) in baseline tissue and plasma were shown in Fig. 2b. We found that higher *SMAD4* and *TCF7L2* frequency in tissue and plasma, respectively, were significantly associated with eventual tumour metastasis. *SMAD4* and *TCF7L2* gene

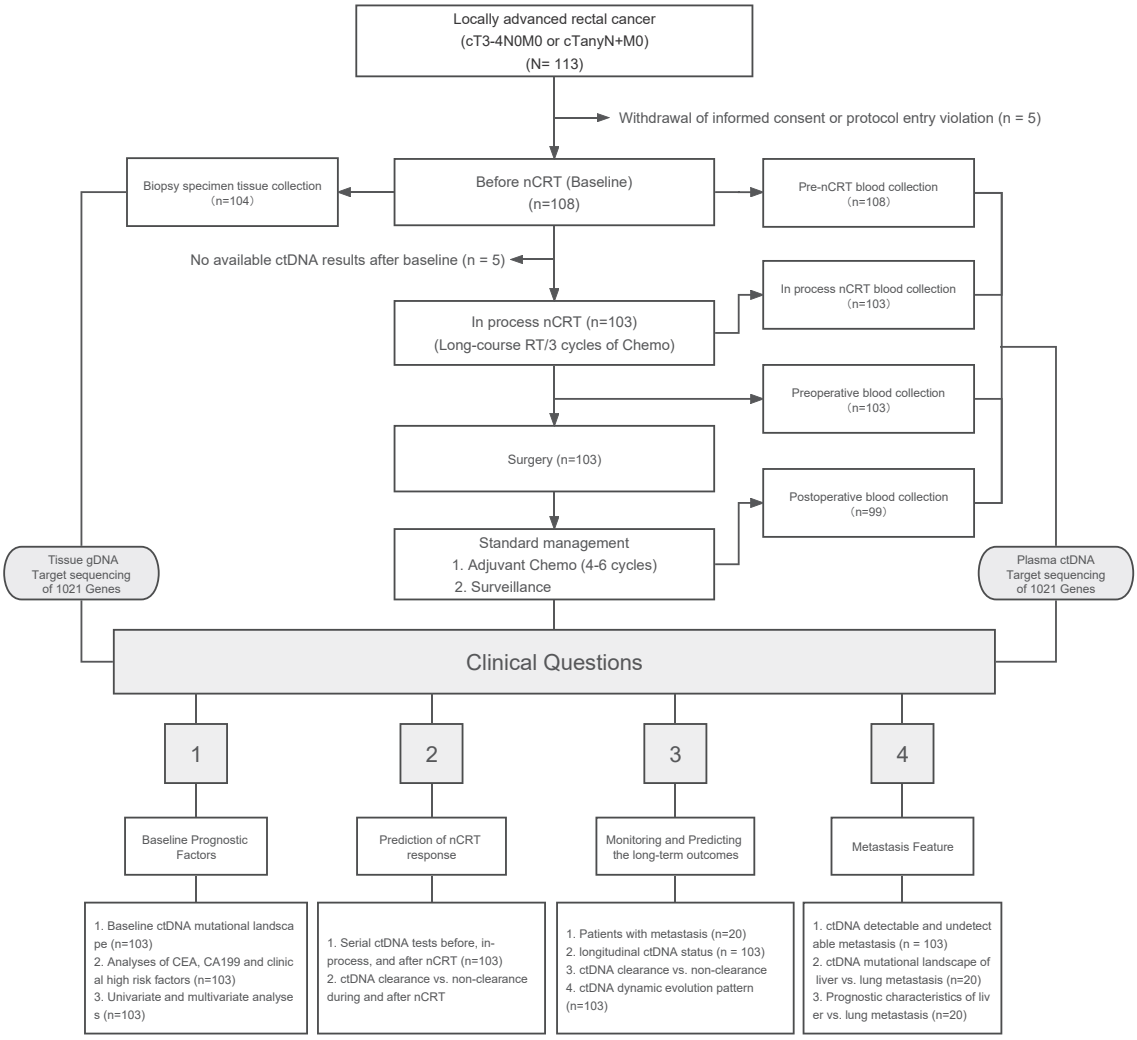

Fig. 1: Flow diagram of patient inclusion in subanalyses with clinical questions answered by each analysis denoted. nCRT, Neoadjuvant chemoradiotherapy; RT, radiotherapy; chemo, chemotherapy.

variations were also independent prognostic factors for PFS. In contrast, mutations in *FBXW7*, *LRP1B*, and *FAT2* were exclusively detected in tissue samples from patients without metastasis (Fig. 2b and Supplementary Fig. S1a and b). These results suggest that patients with different gene variants may respond differently to standard treatment. We also compared the consistency of somatic variants between ctDNA samples and corresponding tissue samples and found ctDNA was positively correlated with that observed in tumour tissues (R^2 = 0.96; Spearman's rank correlation $p < 0.001$; Fig. 2c).

Long-term prognostic value of baseline clinicopathological and ctDNA features

To further identify risk factors affecting the long-term prognosis of LARC, we preformed univariate Cox regression analysis. We found that an increase in baseline CA19-9 level (HR = 4.10, 95% CI: 1.49–11.31, uni-cox $p = 0.006$) and high median variant allele frequency (mVAF) of baseline ctDNA (HR = 1.16, 95% CI: 1.06–1.26, uni-cox $p < 0.001$) were significant risk factors for PFS. However, in multivariate Cox regression, only the mVAF of baseline ctDNA (HR = 1.17, 95% CI: 1.042–1.31, multi-cox $p = 0.0078$) showed significant prognostic value (Fig. 3a). In addition, the predictive performance AUC (area under the curve) for the baseline mVAF at 1, 2 and 3 years is 86%, 76%, and 73%, respectively (Fig. 3b). This implies the superiority of baseline mVAF in terms of metastasis/non-metastasis discrimination.

To optimize the clinical utility of mVAF, we utilized the Youden index to determine the optimal threshold on the ROC curve, which was set at 0.5% (Fig. 3c). Among

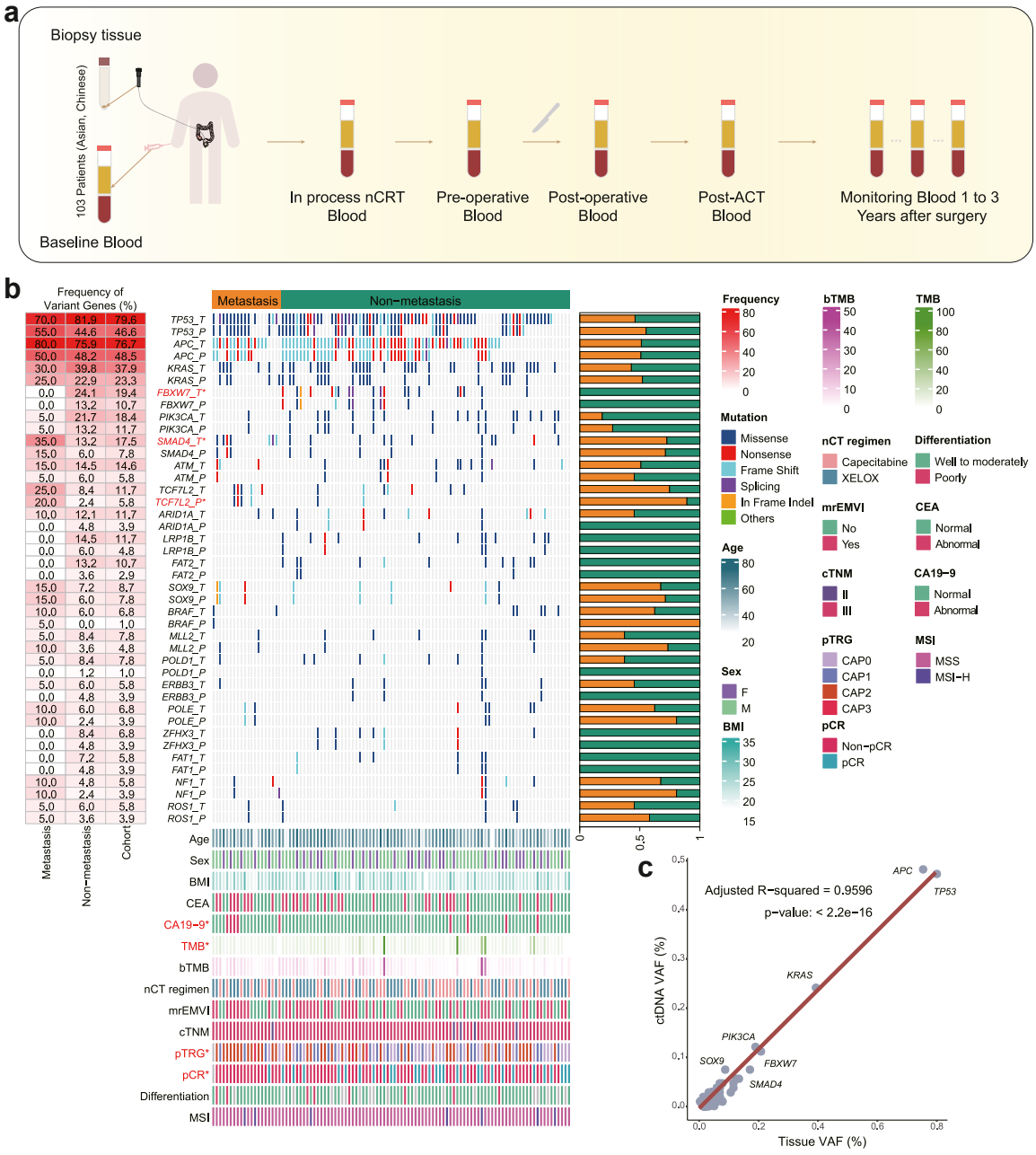

Fig. 2: Study schematic and baseline characteristics. (a) Study flowchart. Patients with locally advanced rectal cancer receive multidisciplinary treatment. Peripheral blood samples were collected before, during, and after multi-modality treatment with neoadjuvant chemoradiation, radical surgery, and adjuvant chemotherapy. (b) Genomic profiling of high-frequency mutations between baseline tissue and plasma samples (nonsynonymous single-nucleotide variants and indels). The top bar represents the group label for patients with metastasis and patients without metastasis; The left heatmap represents the frequency of patients with a certain mutation in each group and in the entire cohort; Fisher's exact test was used to compare gene detection in the metastasis and non-metastasis groups. Significant genes are marked with a red asterisk; The right-hand bar represents the ratio between the metastatic and non-metastatic groups. (c) Correlation analysis between mutation frequencies of 1021 genes from the NGS panel in 103 ctDNA samples vs. tissue samples (Spearman's rank correlation). The dot indicates the gene, the y-axis indicates the VAF of the gene in the tissue and the x-axis indicates the VAF of the gene in plasma. ACT, Adjuvant chemotherapy.

Characteristics	All patients (n = 103, Asian, Chinese)	Baseline ctDNA (n = 103)		p Fisher's exact/χ2 test	Baseline VAF (n = 103)		p Fisher's exact/χ2 test	In-process nCRT ctDNA (n = 103)		p Fisher's exact/χ2 test	Pre-operative ctDNA (n = 103)		p Fisher's exact/χ2 test	Post-operative ctDNA (n = 96)		p Fisher's exact/χ2 test
		Detected (n = 90)	Undetacted (n = 13)		≥0.5% (n = 35)	<0.5% (n = 68)		Detected (n = 17)	Undetacted (n = 86)		Detected (n = 12)	Undetacted (n = 91)		Detected (n = 12)	Undetacted (n = 84)	
Age (years)																
Median	59	60	55		61	58.5		63	59		62	59		62	59	
Range	26–75	26–75	42–73		30–75	26–73		30–74	26–75		45–75	26–74		45–73	26–75	
Sex, n (%)																
Male	67	56	11	0.1335	26	41	0.1936	12	55	0.7822	9	58	0.5343	9	53	0.5299
Female	36	34	2		9	27		5	31		3	33		3	31	
CEA, n (%)																
Positive	32	29	3	0.7815	17	15	**0.0126**	10	22	**0.0152**	4	28	1.0000	7	22	0.0737
Negative	70	60	10		18	52		7	63		8	62		5	61	
Unknown	1	1	0		0	1		0	1		0	1		0	1	
CA19–9, n(%)																
Positive	10	8	2	0.6610	6	4	0.1119	2	8	0.7256	3	7	0.1990	2	5	0.3126
Negative	92	81	11		29	63		15	77		9	83		10	78	
Unknown	1	1	0		0	1		0	1		0	1		0	1	
cT stage, n (%)																
cT2	2	2	0	0.6241	0	2	0.6122	0	2	1.0000	0	2	0.6120	0	2	1.0000
cT3	73	62	11		24	49		12	61		10	63		9	60	
cT4	28	26	2		11	17		5	23		2	26		3	22	
cN stage, n (%)																
cN0	6	4	2	0.2817	0	6	0.2476	1	5	0.5404	0	6	0.8831	1	5	0.6764
cN1	30	27	3		11	19		3	27		3	27		4	24	
cN2	67	59	8		24	43		13	54		9	58		7	55	
cTNM stage, n (%)																
II	5	3	2	0.1185	0	5	0.1635	1	4	1.0000	0	5	1.0000	1	4	0.4949
III	98	87	11		35	63		16	82		12	86		11	80	
Distance from anal verge (cm), n (%)																
0–5	40	34	6	0.5598	10	30	0.1408	6	34	0.7927	2	38	0.1213	4	34	0.7584
>5	63	56	7		25	38		11	52		10	53		8	50	
Tumour vertical diameter(cm), n (%)																
0–5	78	68	10	1.0000	20	58	**0.0030**	8	70	**0.0050**	9	69	1.0000	9	66	0.7213
>5	25	22	3		15	10		9	16		3	22		3	18	
mrPCI, n(%)																
<50	7	5	2	0.2144	1	6	0.4179	0	7	0.5966	0	7	1.0000	1	6	1.0000
50–100	96	85	11		34	62		17	79		12	84		11	78	
mrMRF, n(%)																
Yes	52	43	9	0.2350	20	32	0.4067	8	44	0.7961	6	46	1.0000	3	44	0.1213
No	51	47	4		15	36		9	42		6	45		9	40	
mrEMVI, n(%)																
Yes	52	48	4	0.1491	23	29	**0.0372**	11	41	0.2888	8	44	0.3579	5	41	0.7617
No	51	42	9		12	39		6	45		4	47		7	43	

(Table 1 continues on next page)

Characteristics	All patients (n = 103, Asian, Chinese)	Baseline ctDNA (n = 103)		p Fisher's exact/χ2 test	Baseline VAF (n = 103)		p Fisher's exact/χ2 test	In-process nCRT ctDNA (n = 103)		p Fisher's exact/χ2 test	Pre-operative ctDNA (n = 103)		p Fisher's exact/χ2 test	Post-operative ctDNA (n = 96)		p Fisher's exact/χ2 test
		Detected (n = 90)	Undetacted (n = 13)		≥0.5% (n = 35)	<0.5% (n = 68)		Detected (n = 17)	Undetacted (n = 86)		Detected (n = 12)	Undetacted (n = 91)		Detected (n = 12)	Undetacted (n = 84)	
(Continued from previous page)																
mrLAI, n(%)																
Yes	15	13	2	1.0000	3	12	0.4271	2	13	1.0000	0	15	0.2980	1	13	0.7264
No	87	76	11		32	55		15	72		12	75		11	70	
Unknown	1	1	0		0	1		0	1		0	1		0	1	
MSI, n(%)																
MSI-H	7	7	0	0.5915	3	4	0.6870	1	6	1.0000	0	7	1.0000	1	6	1.0000
MSS	96	83	13		32	64		16	80		12	84		11	78	
Differentiation, n (%)																
Well/ Moderate	77	67	10	0.5908	24	53	0.4873	15	62	0.3810	9	68	0.3443	11	61	0.4459
Poor	8	8	0		4	4		1	7		2	6		0	6	
Others	18	15	3		7	11		1	17		1	17		1	17	
ypT stage, n (%)																
ypT0-2	60	51	9	0.8800	14	46	**0.0175**	4	56	**0.0039**	4	56	0.1233	6	52	0.5325
ypT3-4	38	34	4		18	20		11	27		7	31		6	28	
Unknown	5	5	0		3	2		2	3		1	4		0	4	
ypN stage, n (%)																
ypN0	84	75	9	0.3340	27	57	0.3136	13	71	0.2265	8	76	0.0528	10	70	1.0000
ypN1-2	18	14	4		7	11		3	15		3	15		2	14	
Unknown	1	1	0		1	0		1	0		1	0		0	0	
ypTNM stage, n (%)																
ypTNM 0–2	81	72	9	0.5287	25	56	0.1880	10	71	**0.0207**	7	74	**0.0406**	10	70	1.0000
ypTNM 3–4	21	17	4		9	12		6	15		4	17		2	14	
Unknown	1	1	0		1	0		1	0		1	0		0	0	
ypCR, n (%)																
Yes	30	28	2	0.4194	9	21	0.4466	3	27	0.0948	2	28	0.0916	1	29	0.0965
No	72	61	11		25	47		13	59		9	63		11	55	
Unknown	1	1	0		1	0		1	0		1	0		0	0	
ypTRG score, n (%)																
0–1	57	50	7	1.0000	17	40	0.3134	7	50	0.0930	3	54	**0.0108**	4	52	0.1145
2–3	45	39	6		17	48		9	36		8	37		8	32	
Unknown	1	1	0		1	0		1	0		1	0		0	0	
ymrTRG score, n(%)																
0–1	51	45	6	0.8276	16	35	0.8316	8	43	1.0000	6	45	1.0000	8	41	0.2031
2–3	41	36	5		15	26		7	34		5	36		4	33	
Unknown	11	9	2		4	7		2	9		1	10		0	10	
nCT regimen, n (%)																
Capecitabine	47	41	6	1.0000	14	33	0.5314	8	39	1.0000	8	39	0.1362	4	39	0.5384
XELOX	56	49	7		21	35		9	47		4	52		8	45	

p-values less than 0.05 are highlighted in bold.

Table 1: **Clinical characteristics of the 103 enrolled patients.**

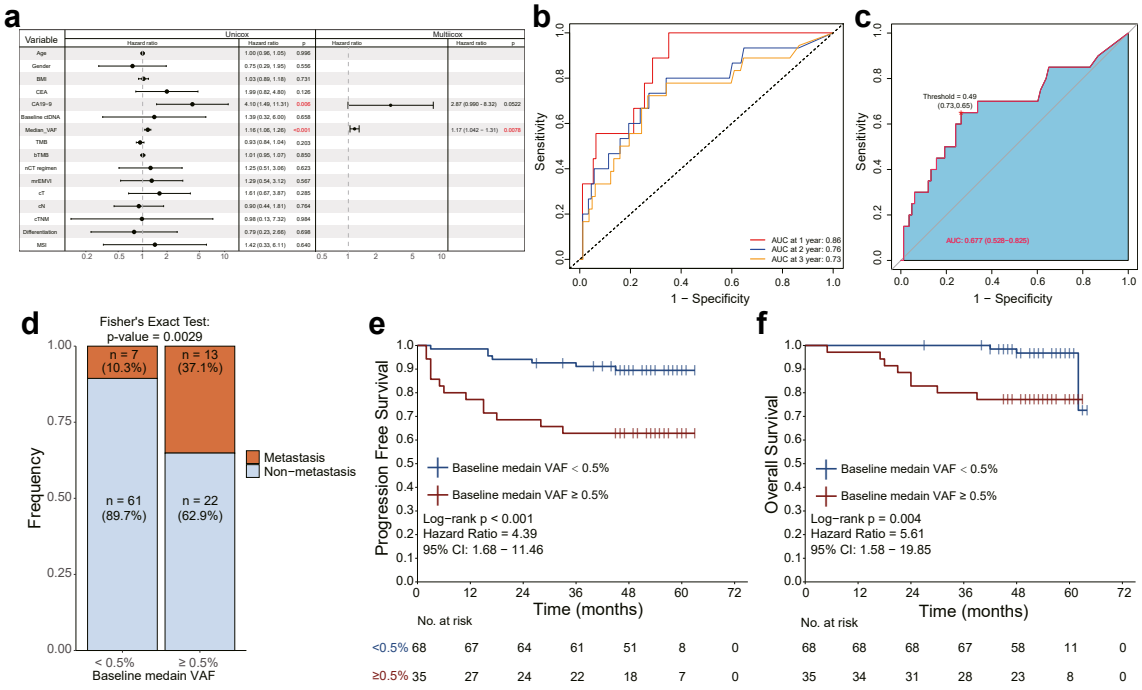

Fig. 3: Baseline clinical characteristics and ctDNA stratification efficacy. (a) Univariate and multivariate logistic regression models for baseline clinical indicators and ctDNA VAF. (b) Baseline median VAF timeROC performance analysis. (c) Stratified baseline median VAF based on the ROC curve Yoden index threshold. (d) Metastasis rates of patients stratified by baseline median VAF. Kaplan–Meier analysis of PFS (e) and OS (f) stratified by baseline median VAF. VAF, Variant Allele Frequency.

the patients, 35 (34.0%) had baseline mVAF ≥0.5%, of which 13 (37.1%) experienced tumour metastasis. In contrast, among the 68 patients (66.0%) with baseline mVAF <0.5%, only 7 (10.3%) eventually developed metastasis (Fisher's exact test $p = 0.0029$; Fig. 3d). A baseline mVAF of ≥0.5% is a significant predictor of poor prognosis in both PFS and OS (PFS, HR = 4.39, 95% CI: 1.68–11.46, Log-rank $p < 0.001$; OS, HR = 5.61, 95% CI: 1.58–19.85, Log-rank $p = 0.004$) (Fig. 3e and f). For patients with the baseline mVAF ≥0.5%, a larger tumour vertical diameter (χ^2 test $p = 0.003$), a higher rate of abnormal baseline CEA (χ^2 test $p = 0.0126$), and a higher incidence of positive extramural vascular invasion in magnetic resonance imaging (mrEMVI) (χ^2 test $p = 0.0372$) were observed. Additionally, a significant higher pT stage (χ^2 test $p = 0.0175$) was observed after radical surgery compared to patients with baseline mVAF ≥0.5% (Table 1).

CtDNA features and dynamic changes in response to nCRT

After establishing the long-term prognostic value of baseline mVAF, we further explored the dynamic changes of ctDNA in response to nCRT. We found that ctDNA mVAF fluctuated considerably after only 2 weeks of neoadjuvant chemoradiation (Fig. 4a). In patients with metastasis, both in process and preoperative mVAF showed no significant change compared to baseline mVAF. In contrast, in patients without metastasis, both in process and preoperative mVAF were significantly lower than baseline mVAF (Fig. 4b). This suggests that early ctDNA changes may be effective indicators of nCRT treatment efficacy.

Prognostically, we observed that at the in process nCRT time point, patients with persistent ctDNA detection showed significantly shorter PFS and OS compared to the clearance individuals (PFS, HR = 7.81, 95% CI: 2.05–29.82, $p < 0.001$; OS, HR = 6.22, 95% CI: 1.08–35.8, Log-rank $p = 0.002$; Fig. 4c). This trend was also evident at the preoperative time point (PFS, HR = 8.68, 95% CI: 1.05–45.78, Log-rank $p < 0.001$; OS, HR = 15.65, 95% CI: 1.99–122.96, $p < 0.001$; Fig. 4d). We compared the C-index values of mVAF at the two time points to evaluate their prognostic performance and found that the in process nCRT mVAF was slightly better, but the difference was not significant (Fig. 4e). This suggests that early changes in ctDNA during nCRT may be a key factor in predicting long-term prognosis.

Regarding genetic features, we investigated the correlation between gene variations and the ctDNA clearance status at both the in process nCRT and preoperative time points, respectively. At the in-process nCRT time point, we found no significant correlation between genetic mutations and the ctDNA clearance

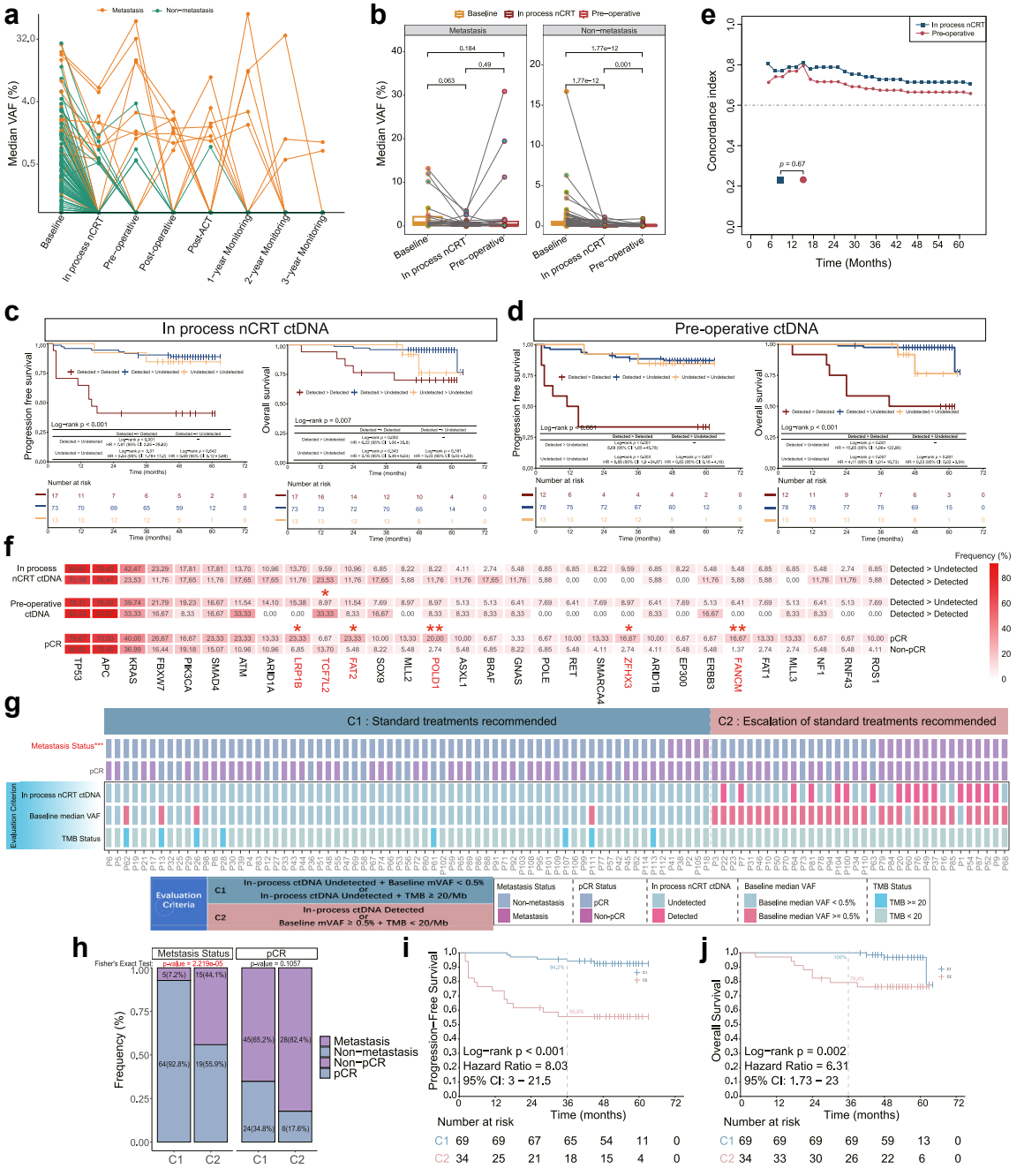

Fig. 4: Evaluation of the efficacy of ctDNA status prediction for long-term outcomes. (a) ctDNA median VAF dynamics change at baseline, in process nCRT, pre-operative, post-operative, post-ACT, 1–3 year monitoring time points. (b) Statistical analysis of the change in median VAF dynamics between baseline, in process nCRT and pre-operative, paired Wilcox test, Bonferroni correction. (c) Kaplan–Meier analysis of the PFS and OS stratified by ctDNA detectable status at in process nCRT time point. (d) Kaplan–Meier analysis of the PFS and OS stratified by ctDNA detectable status at pre-operative time point. (e) Depiction of C-index estimates over time according to ctDNA detectable status of in process nCRT and pre-operative time points. Significance was calculated using R packages pec: cindex and compareC. (f) The heatmap shows the mean VAF values of the high-frequency genes in each group, stratified by in process nCRT ctDNA detectable status, pre-operative ctDNA detectable status and pCR status. The statistical tests were performed by calculating VAF values of each sample between the groups by the Wilcox test. *, $p < 0.05$; **, $p < 0.01$; fisher exact test. (g) Patients profiling of the binary stratification feasible combination. ***, $p < 0.001$; fisher exact test. (h) Metastasis and pCR rates of patients stratified by binary stratification feasible combination. Kaplan–Meier analysis of PFS (i) and OS (j) stratified by binary stratification feasible combination.

status. However, at the pre-operative time point, the *TCF7L2* mutation was significantly more frequently observed in the ctDNA persistence group. Additionally, the *POLD1*, *FANCM*, *LRP1B*, *FAT2*, and *ZFHX3* mutations were significantly more common in patients achieving pCR postoperatively compared to those in the non-pCR individuals (Fig. 4f).

Given the significant prognostic prediction role of early changes in ctDNA, clear stratification criteria can help identify patients with a high risk of metastasis at the very early stages of routine nCRT, guiding them to a timely transition to more effective therapies. Therefore, we constructed a binary stratification feasible combination, classifying 69 patients (67.0%) into the low-risk (C1) group (characterized by "ctDNA undetected during nCRT with baseline mVAF <0.5%" or "ctDNA undetected during nCRT with TMB ≥20/Mb"). The remaining 34 patients (33%) were categorized into the high-risk (C2) group (characterized by "ctDNA detected during nCRT" or "baseline mVAF ≥0.5% with TMB <20/Mb") (Fig. 4g). With a median follow-up of 53 months, only 5 patients (7.2%) in Group C1 eventually developed distant metastases, while 15 patients (44.1%) in Group C2 did (fisher exact test $p < 0.001$). In addition, we observed that 34.8% of patients in Group C1 achieved pCR, while the pCR rate in Group C2 was only 17.6% (Fisher's exact test $p = 0.1057$, Fig. 4h). Furthermore, the long-term prognosis was significantly worse in Group C2 (PFS, HR = 8.03, 95% CI: 3–21.5, Log-rank $p < 0.001$; OS, HR = 6.31, 95% CI: 1.73–23, Log-rank $p = 0.002$) and the 3 y-PFS and 3 y-OS rates in Group C2 were significantly lower than those in Group C1 (3 y-PFS, 55.9% vs. 94.2%; 3 y-OS, 79.4% vs. 100%, Fig. 4i and j). This suggests that a combination of the three clinical indicators may be able to timely identify patients with high-risk in the very early stages of nCRT.

Postoperative ctDNA and longitudinal ctDNA monitoring

We further explored the monitoring value of ctDNA after surgery. To validate that postoperative ctDNA monitoring serves as a clear subclinical indicator for tumour metastasis, we defined postoperative longitudinal monitoring positivity as the detection of ctDNA at least once during postoperative (PFS: HR = 33.67, Log-rank $p < 0.001$, 95% CI: 0.28–4068.09; OS: HR = 19.09, Log-rank $p < 0.001$, 95% CI: 0.12–3050.06; Fig. 5a), post-ACT (PFS: HR = 19.15, Log-rank $p < 0.001$, 95% CI: 1.31–280.58; OS: HR = 18.55, Log-rank $p < 0.001$, 95% CI: 0.49–701.39; Figs. 5a), and 1–3 years of follow-up timepoints (PFS: HR = 69.89, Log-rank $p < 0.001$, 95% CI: 8.64–565.24; OS: HR = 36.88, Log-rank $p < 0.001$, 95% CI: 3.13–434.96; Fig. 5a). The results indicate that ctDNA detection during postoperative longitudinal monitoring may associated with shorter PFS and OS. Furthermore, in the longitudinal ctDNA monitoring of the 20 patients with metastasis, ctDNA was detectable at least once in all cases (Fig. 5b). The median time to first detectable ctDNA after treatment was 3.8 months, compared to 11.0 months for positive CT findings (Fig. 5c), with a median lead time of 2.5 months (Paired-Samples T test $p = 0.0302$; Fig. 5d). These findings suggest that in monitoring disease progression, ctDNA outperforms routine clinical methods.

Molecular and clinical signatures of liver and lung metastasis

The metastatic sites of this cohort with LARC included the liver, lung, peritoneum, bone, brain, adrenal gland, and kidney. Among these, 11 patients had the liver as the initial site of metastasis, while 9 patients had lung metastasis first (Fig. 6a). In comparison to lung metastasis, patients with liver metastasis demonstrated a significantly higher mVAF of baseline ctDNA (Mann–Whitney U test $p = 0.0049$; Fig. 6b). Interestingly, we observed a higher detectable rate of ctDNA *APC* and *TP53* mutations in liver metastatic cases, and a higher detectable rate of genomic DNA (gDNA) *SMAD4* and *NF1* mutations in lung metastatic cases (Fig. 6c). In addition, patients with lung metastasis exhibited a significantly longer PFS compared to those with liver metastasis (HR = 0.27, Log-rank $p < 0.001$) (Fig. 6d). Although there was no significant difference in OS between patients with liver and lung metastasis, we observed that the median OS of lung metastasis was longer than that of liver metastasis (62 months in lung metastasis vs. 24 months in liver metastasis). These results showed that patients with liver or lung metastases exhibit distinct clinical and molecular characteristics, suggesting a potential need for different clinical management strategies.

Discussion

Neoadjuvant therapy for LARC has evolved over the years, yet there remains a pressing need to establish more personalized treatment strategies. ctDNA, a robust biomarker that quantitatively reflects tumour burden and captures tumour-specific molecular features, holds significant promise in guiding risk stratification and informing neoadjuvant treatment decisions.

In line with previous literature,[17–21] this study confirms that ctDNA is a significant biomarker that can dynamically reflect tumour burden and predict treatment responses and oncological outcomes in patients with LARC.

Our findings revealed a significant association between baseline ctDNA abundance and tumour size, vascular invasion, as well as the ypT stage after surgery. This suggests that baseline ctDNA can reflect both tumour size and the extent of its invasion into surrounding vasculature. In a study of metastatic colorectal cancer (mCRC), Bando et al. also proposed that tumour size is the most important factor for ctDNA shedding.[22]

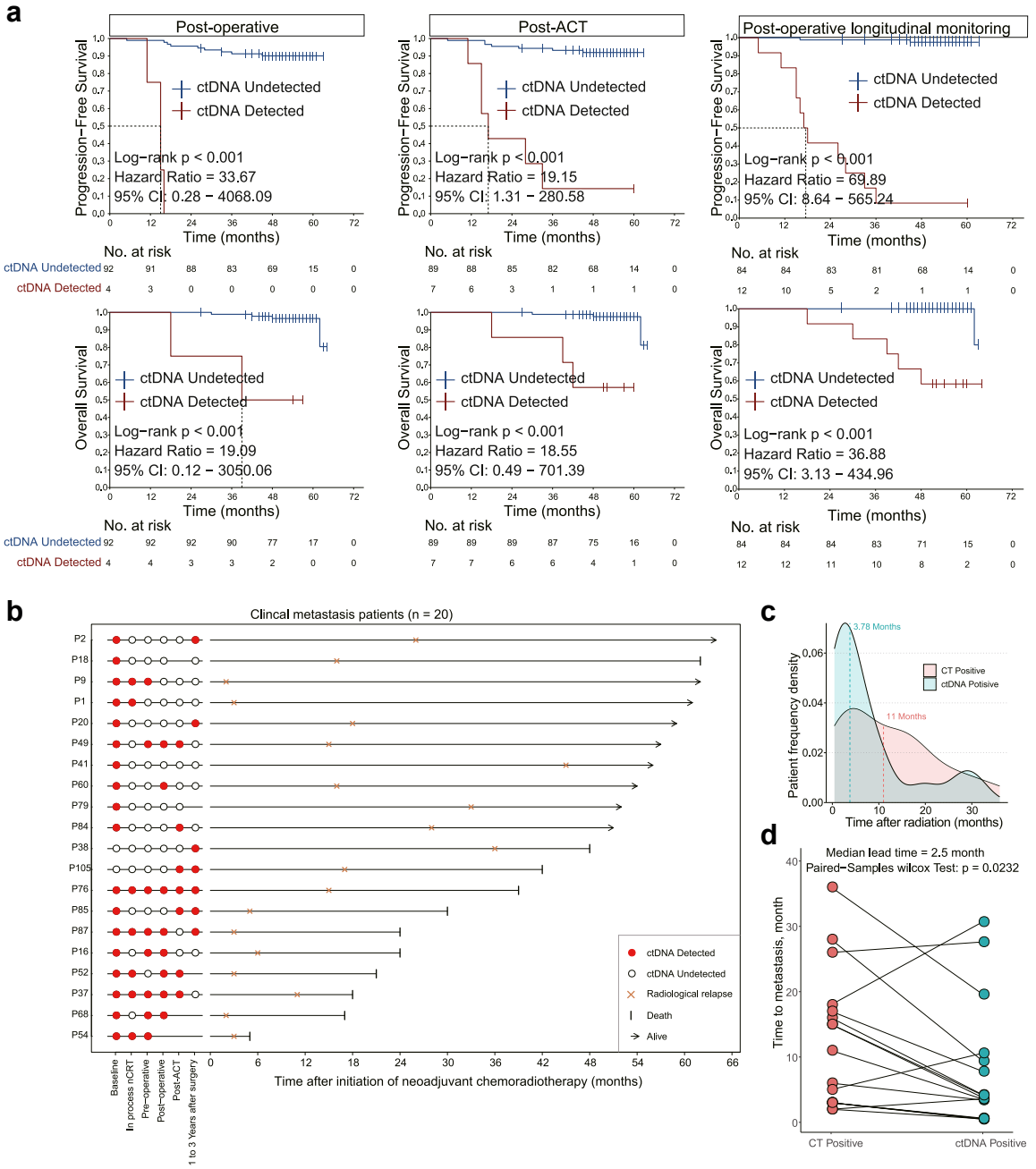

Fig. 5: Postoperative ctDNA monitoring. (a) Kaplan–Meier analysis of PFS and OS stratified by post-operative, post-ACT and combined post-operative ctDNA detectable status at 5 time points, respectively. An overview of ctDNA dynamic monitoring in patients undergoing multi-modality treatment. (b) The Swimmer plot depicts the length of follow-up and clinical events in patients with metastasis. (c) Density distribution of metastasis time of ctDNA positive and computed tomography (CT) positive in patients. (d) Comparison of ctDNA and standard-of-care CT for time to metastasis.

Moreover, our results showed a significant association between preoperative ctDNA status and pathological outcomes, including ypTNM staging and pathological TRG. This reaffirms the ability of ctDNA to precisely portray the real-time tumour burden.

In continuation of our previously reported mid-term results,[14] the current data further confirm a significant correlation between baseline ctDNA abundance and long-term prognosis. In the mid-term follow-up study, we defined mVAF by ranking all patients' VAFs and

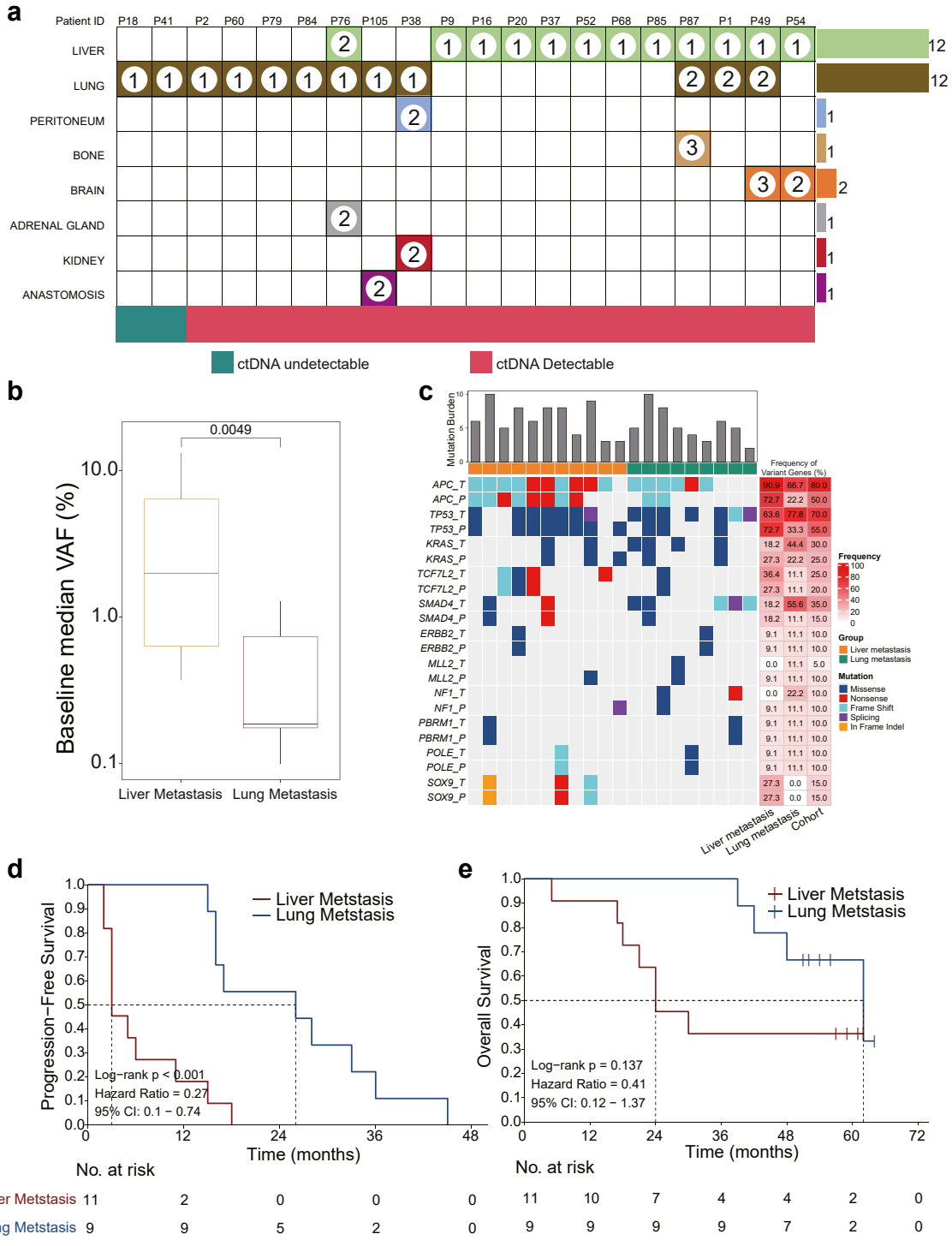

Fig. 6: Molecular and clinical prognostic features of liver and lung metastasis. (a) The grid fillings show patient-specific metastases, and the numbers represent the sequence of metastases. (b) Comparison of baseline median VAF between liver metastasis and lung metastasis, Mann–Whitney U test. (c) Molecular profiles of liver and lung metastasis. Kaplan–Meier analysis of PFS (d) and OS (e) stratified by liver metastasis and lung metastasis.

taking the median, finding that patients with a baseline VAF greater than 1.0% had significantly worse outcomes. Considering that the 1.0% threshold may vary across study cohorts, we calculated the baseline ctDNA mVAF for each patient in this study. The results showed that patients with an mVAF greater than 0.5% had poorer prognoses. Multivariate Cox analysis demonstrated that, after adjusting for conventional prognostic factors such as CEA, CA19-9, tumour grade, lymph node metastasis, and neurovascular invasion, the baseline ctDNA mVAF remained the sole independent predictor of PFS and OS.

Apart from baseline ctDNA, our study also demonstrated that patients who achieved ctDNA clearance during or after nCRT had significantly longer PFS and OS compared to those without ctDNA clearance. Their survival outcomes resembled those of patients with persistently undetectable ctDNA. Interestingly, we found that rapid ctDNA clearance as early as two weeks into treatment was already indicative of a significantly improved long-term survival. Previous studies in mCRC have revealed that early changes in ctDNA levels, even prior to the second cycle of chemotherapy, exhibit a significant correlation with prognostic outcomes.[23–25] This study demonstrate that early ctDNA clearance during nCRT in LARC is valuable for evaluating treatment efficacy and predicting prognosis, a feature distinctly different from traditional biomarkers such as CEA and CA19-9.[25,26]

Recent studies have highlighted the potential of ctDNA in risk stratification for various cancer types, identifying patients who are most likely to benefit from adjuvant therapy or safely avoid unnecessary treatment.[13,27–29] In guiding the neoadjuvant treatment strategy for LARC, our study demonstrates that baseline ctDNA and early ctDNA clearance status, combined with TMB, can effectively reflect tumour burden and oncological biology, and are significantly associated with treatment response and survival outcomes. A comprehensive analysis of these three indicators revealed that approximately two-thirds of patients were at low risk for recurrence and metastasis, achieving significantly favourable long-term outcomes through conventional nCRT. The remaining one-third of patients were at high risk, with nearly half experiencing disease progression. These three molecular indicators can be obtained at the early stage of neoadjuvant treatment, providing the feasibility of timely adjustments to treatment plans, and may assist in optimizing the implementation of the TNT strategy.

It is important to note that certain types of tumours exhibit lower ctDNA detectable rates, posing challenges for the ctDNA-based strategy. Previous studies have indicated that ctDNA is less detectable in patients with lung-only or peritoneum-only mCRC than in those with liver metastases,[22] and our study yielded similar results.

Genomic features significantly impact tumour biology. In this study, somatic variations in *TCF7L2* and *SMAD4* were significantly correlated with tumour metastasis, consistent with previous reports.[30,31] Interestingly, *TCF7L2* mutations appeared more prevalent in patients with liver metastases, while *SMAD4* variations were more frequent in lung metastatic cases. In contrast, mutations in *FBXW7*, *LRP1B*, or *FAT2* were associated with notably favourable prognoses. Literature suggests that these three genes are linked to higher TMB and better prognosis in gastrointestinal cancers.[32–34] Therefore, clinical risk stratification for LARC may also incorporate genomic factors to enhance its predictive efficacy.

Before surgery, accurate determination of tumour residuals is critical for treatment planning. Although we have observed a significant correlation between preoperative ctDNA detection and pathological TNM staging as well as pathological tumour response, it remains insufficient to precisely determine whether a complete tumour response (CR) has been achieved. Therefore, relying solely on ctDNA analysis to confidently implement a watch-and-wait (W&W) strategy is impractical at this stage. In the future, the development of higher-sensitivity ctDNA detection technologies, such as incorporating genetic/epigenetic features and tracking tumour clone evolution, along with the integration of tumour microenvironment, transcriptomics, and radiomics approaches, is expected to improve the accuracy of CR assessment and facilitate the safe implementation of organ-preservation strategies. Meanwhile, preoperative detection of ctDNA and high-risk gene mutations can help effectively identify patients unsuitable for W&W strategies, aligning with current literature consensus.[26]

Consistent with previous literature,[19–21] our results indicated that ctDNA positivity at all postoperative time points was associated with poor long-term prognosis. Postoperative ctDNA detection demonstrated notable predictive ability, and the longitudinal ctDNA status added additional predictive value, highlighting the importance of multi-time point monitoring. In this study, the median 2.5-month lead time for ctDNA detection prior to radiological recurrence is comparable to the 1.7–11.5 months reported in the literature,[26] further supporting the superiority of ctDNA over conventional clinical methods for monitoring disease progression. The clinical significance of this early warning, such as the rationale for early therapeutic intervention, warrants further investigation.

We must acknowledge certain limitations of this study. The sample size is relatively small, but the research was meticulously conducted per protocol, with close patient monitoring and comprehensive data collection, which partially compensates for this limitation. The uniform treatment approach required by the study design restricted our ability to explore different

therapeutic regimens for patients with varying ctDNA statuses.

In summary, our study highlights the predictive and monitoring value of ctDNA in patients with LARC undergoing nCRT. Early indicators demonstrate significant prognostic value, positioning ctDNA as a promising tool for guiding personalized neoadjuvant treatment strategies. To validate these findings, we are conducting a multicentre randomized trial (NCT05601505). This trial employs risk stratification based on ctDNA clearance during nCRT, baseline mVAF, and TMB to guide decisions between standard and escalated neoadjuvant treatment strategies, ultimately aiming to optimize LARC management.

Contributors

Study Design: Jiaolin Zhou, Guole Lin.

Research Operations and Support: All authors.

Drafting of the manuscript: Jiaolin Zhou, Lifeng Li, Yuxin Liu.

Statistical analysis: Jiaolin Zhou, Lifeng Li, Yuxin Liu.

Administrative, technical, or material support: Guole Lin.

Study supervision: Guole Lin, Ling Yang.

Jiaolin Zhou, Lifeng Li and Yuxin Liu had access to data for verification; all authors read and approved the final version of this manuscript for submission.

Data sharing statement

Upon request and subject to review, we are willing to provide access to the data that support the findings of this study. Data can be accessed by submitting a reasonable request to GLL (linguole@126.com). Access will be strictly limited to non-commercial research purposes, and all patient-sensitive information will be removed to maintain confidentiality.

Declaration of interests

Authors LFL, XG, YHG, YFG, XFX, XY, and LY are employed by Geneplus-Beijing. The remaining authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This work was supported by the Major Grants Program of Beijing Science and Technology Commission (No. D171100002617003) and the National High Level Hospital Clinical Research Funding (2022-PUMCH-C-005).

The authors would like to thank all coinvestigators from the participating medical centers of this trial. We thank Dr. Weijie Chen, Dr. Weikun Shi, and our graduate students Xiaoyuan Qiu, Ganbin Li, Yang An, Xiao Zhang, Chentong Wang, Han Chen, as well as our research assistants Danyang Zhu and Yue Wang for their assistance in data collection.

During the preparation of this work the authors used ChatGPT-4.0 in order to improve the quality of writing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105548.

References

1 Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249.
2 Li Y, Wang J, Ma X, et al. A review of neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Int J Biol Sci*. 2016;12(8):1022–1031.
3 Saraf A, Roberts HJ, Wo JY, Parikh AR. Optimal neoadjuvant strategies for locally advanced rectal cancer by risk assessment and TumorLocation. *J Natl Compr Canc Netw*. 2022;20(10):1177–1184.
4 Benson AB, Venook AP, Al-Hawary MM, et al. Rectal cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2022;20(10):1139–1167.
5 Conroy T, Bosset JF, Etienne PL, et al. Neoadjuvant chemotherapy with FOLFIRINOX and preoperative chemoradiotherapy for patients with locally advanced rectal cancer (UNICANCER-PRODIGE 23): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol*. 2021;22(5):702–715.
6 Bahadoer RR, Dijkstra EA, van Etten B, et al. Short-course radiotherapy followed by chemotherapy before total mesorectal excision (TME) versus preoperative chemoradiotherapy, TME, and optional adjuvant chemotherapy in locally advanced rectal cancer (RAPIDO): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2021;22(1):29–42.
7 Dasari A, Morris VK, Allegra CJ, et al. ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal-Anal Task Forces whitepaper. *Nat Rev Clin Oncol*. 2020;17(12):757–770.
8 Raza A, Khan AQ, Inchakalody VP, et al. Dynamic liquid biopsy components as predictive and prognostic biomarkers in colorectal cancer. *J Exp Clin Cancer Res*. 2022;41(1):99.
9 Henriksen TV, Tarazona N, Frydendahl A, et al. Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, toward assessment of adjuvant therapy efficacy and clinical behavior of recurrences. *Clin Cancer Res*. 2022;28(3):507–517.
10 Parikh AR, Van Seventer EE, Siravegna G, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. *Clin Cancer Res*. 2021;27(20):5586–5594.
11 Chen G, Peng J, Xiao Q, et al. Postoperative circulating tumor DNA as markers of recurrence risk in stages II to III colorectal cancer. *J Hematol Oncol*. 2021;14(1):80.
12 Malla M, Loree JM, Kasi PM, Parikh AR. Using circulating tumor DNA in colorectal cancer: current and evolving practices. *J Clin Oncol*. 2022;40(24):2846–2857.
13 Tie J, Cohen JD, Lahouel K, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. *N Engl J Med*. 2022;386(24):2261–2272.
14 Zhou J, Wang C, Lin G, et al. Serial circulating tumor DNA in predicting and monitoring the effect of neoadjuvant chemoradiotherapy in patients with rectal cancer: a prospective multicenter study. *Clin Cancer Res*. 2021;27(1):301–310.
15 Benson AB, Venook AP, Al-Hawary MM, et al. Rectal cancer, version 2.2018, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2018;16(7):874–901.
16 Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351(17):1731–1740.
17 Khakoo S, Carter PD, Brown G, et al. MRI tumor regression grade and circulating tumor DNA as complementary tools to assess response and guide therapy adaptation in rectal cancer. *Clin Cancer Res*. 2020;26(1):183–192.
18 Vidal J, Casadevall D, Bellosillo B, et al. Clinical impact of presurgery circulating tumor DNA after total neoadjuvant treatment in locally advanced rectal cancer: a biomarker study from the GEMCAD 1402 trial. *Clin Cancer Res*. 2021;27(10):2890–2898.
19 Tie J, Cohen JD, Wang Y, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. *Gut*. 2019;68(4):663–671.
20 Wang Y, Yang L, Bao H, et al. Utility of ctDNA in predicting response to neoadjuvant chemoradiotherapy and prognosis assessment in locally advanced rectal cancer: a prospective cohort study. *PLoS Med*. 2021;18(8):e1003741.
21 Schou JV, Larsen FO, Sørensen BS, et al. Circulating cell-free DNA as predictor of treatment failure after neoadjuvant chemoradiotherapy before surgery in patients with locally advanced rectal cancer. *Ann Oncol*. 2018;29(3):610–615.
22 Bando H, Nakamura Y, Taniguchi H, et al. Effects of metastatic sites on circulating tumor DNA in patients with metastatic colorectal cancer. *JCO Precis Oncol*. 2022;6:e2100535.
23 Garlan F, Laurent-Puig P, Sefrioui D, et al. Early evaluation of circulating tumor DNA as marker of therapeutic efficacy in metastatic colorectal cancer patients (PLACOL study). *Clin Cancer Res*. 2017;23(18):5416–5425.

24 Osumi H, Shinozaki E, Yamaguchi K, Zembutsu H. Early change in circulating tumor DNA as a potential predictor of response to chemotherapy in patients with metastatic colorectal cancer. *Sci Rep*. 2019;9(1):17358.
25 Tie J, Kinde I, Wang Y, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. *Ann Oncol*. 2015;26(8):1715–1722.
26 Loft M, To YH, Gibbs P, Tie J. Clinical application of circulating tumour DNA in colorectal cancer. *Lancet Gastroenterol Hepatol*. 2023;8(9):837–852.
27 Gale D, Heider K, Ruiz-Valdepenas A, et al. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. *Ann Oncol*. 2022;33(5):500–510.
28 Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019;37(18):1547–1557.
29 Lipsyc-Sharf M, de Bruin EC, Santos K, et al. Circulating tumor DNA and late recurrence in high-risk hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer. *J Clin Oncol*. 2022;40(22):2408–2419.
30 Wenzel J, Rose K, Haghighi EB, et al. Loss of the nuclear Wnt pathway effector TCF7L2 promotes migration and invasion of human colorectal cancer cells. *Oncogene*. 2020;39(19):3893–3909.
31 Yamamoto T, Kawada K, Itatani Y, et al. Loss of SMAD4 promotes lung metastasis of colorectal cancer by accumulation of CCR1+ tumor-associated neutrophils through CCL15-CCR1 Axis. *Clin Cancer Res*. 2017;23(3):833–844.
32 Liu Y, Chen H, Bao H, Zhang J, Wu R, Zhu L. Comprehensive characterization of FBXW7 mutational and clinicopathological profiles in human colorectal cancers. *Front Oncol*. 2023;13:1154432.
33 Brown LC, Tucker MD, Sedhom R, et al. LRP1B mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types. *J Immunother Cancer*. 2021;9(3).
34 Wang LL, Zheng W, Liu XL, Yin F. Somatic mutations in FAT cadherin family members constitute an underrecognized subtype of colorectal adenocarcinoma with unique clinicopathologic features. *World J Clin Oncol*. 2022;13(10):779–788.