



Genomic and Clinical Predictors of Conversion in Initially Unresectable Colorectal Cancer Liver Metastases

Daocheng Zuo, BS^{1,2}, Lu Wang, PhD^{1,2}, Kangpeng Jin, PhD^{1,2}, Yue Zhang, PhD^{1,2}, Yaping Wang, MD³, Yueming Sun, MD^{1,2}, and Junwei Tang, MD^{1,2}

¹The First Clinical Medical College, Nanjing Medical University, Nanjing, Jiangsu, China; ²Department of General Surgery, Colorectal Institute of Nanjing Medical University, The First Affiliated Hospital of Nanjing Medical University, Nanjing Medical University, Nanjing, Jiangsu, China; ³Department of Hematology and Oncology, Children's Hospital of Nanjing Medical University, Nanjing Medical University, Nanjing, Jiangsu, China

ABSTRACT

Background. Colorectal cancer liver metastases (CRLM) present significant treatment challenges, requiring multimodal conversion therapies. Identifying factors that influence treatment outcomes is crucial for improving clinical management.

Patients and Methods. This retrospective cohort study included 286 patients with synchronous CRLM who underwent conversion therapies on the basis of sequencing results. Patients were categorized into successful conversion therapy group (SCTG) and failed conversion therapy group (FCTG). Clinical factors and genomic mutations were analyzed for associations with therapy outcomes and survival.

Results. Among the patients, 106 (37.1%) achieved successful conversion (SCTG), while 180 (62.9%) failed (FCTG). Compared with SCTG, patients in the FCTG had significantly larger metastatic lesions, higher preoperative mesenteric lymph node positivity, and elevated carcinoembryonic

antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels. Six genes (*FAT*, *BRAF*, *SERPINA3*, *GRIN2A*, *ERBB2*, and *ALK*) showed the highest mutation frequency differences in FCTG, correlating with worse outcomes. Any of these mutations was associated with shorter overall survival compared with wild-type patients. A nomogram model using tumor mutation status, CEA, CA19-9, lesion diameter ≥ 5 cm, and positive lymph nodes at diagnosis predicted conversion efficacy (area under the curve = 89.6, 95% confidence interval 22.6–92.4). An extended conversion-related clinical risk score scoring system incorporating these factors effectively stratified poor prognosis populations, serving as a prognostic tool for patients with unresectable CRLM.

Conclusions. Genomic profiling improves precision management of CRLM, facilitating tailored conversion strategies and better prognostic prediction. Future studies should validate these findings in prospective cohorts to refine personalized treatment for patients with initially unresectable CRLM.

Keywords Colorectal cancer liver metastases · Conversion therapy · Next-generation sequencing · Clinical risk score · Biomarker

Daocheng Zuo, Lu Wang, Kangpeng Jin and Yue Zhang have contributed equally to this work.

© Society of Surgical Oncology 2025

First Received: 15 April 2025
Accepted: 25 June 2025

Y. Wang, MD
e-mail: wyp_0919@163.com

Y. Sun, MD
e-mail: sunyueming@njmu.edu.cn

J. Tang, MD
e-mail: pepsitjw@njmu.edu.cn

Colorectal cancer liver metastases (CRLM) represent a significant clinical challenge, accounting for a substantial proportion of advanced colorectal cancer cases. Epidemiologically, it is estimated that up to 25% of patients with colorectal cancer present with synchronous liver metastases, while an additional 20–50% develop metachronous metastases during their disease course.¹ Management of CRLM often necessitates a multidisciplinary approach, incorporating surgical, medical, and interventional oncology.² Surgical

resection remains the gold standard for eligible patients, with the goal of achieving a margin-negative (R0) resection. However, only a minority of patients are candidates for upfront surgery owing to factors such as tumor burden, location, and patient fitness.³ In this context, neoadjuvant and conversion therapies have emerged as pivotal strategies to downstage initially unresectable tumors, rendering them operable.⁴ The clinical risk score (CRS) is a prognostic scoring system that aims to stratify patients with CRLM according to their risk of postoperative complications and survival outcomes.^{5,6} Initially introduced by Fong et al., the CRS incorporates various clinical parameters to predict surgical outcomes and guide decision-making in the management of CRLM.⁷ Patients with low CRS scores are considered better candidates for resection owing to their higher likelihood of prolonged survival and lower risk of perioperative complications.⁸

In recent years, advancements in systemic therapies and precision medicine, such as biomarker-driven approaches and immunotherapy, have expanded the therapeutic landscape. For instance, understanding the molecular profile of tumors, including RAS and BRAF mutation status, can guide treatment decisions and predict response to targeted therapies.⁹ The causes of failure in neoadjuvant/conversion therapy were associated with the following factors: (1) Intrinsic tumor resistance, driven by genetic alterations such as RAS or BRAF mutations, can limit treatment response.¹⁰ (2) Even with treatment, some tumors do not sufficiently shrink or may exhibit heterogeneous responses, with viable tumor cells persisting amidst necrotic tissue.¹¹ (3) Severe side effects can lead to dose reductions or discontinuation of therapy, impacting treatment efficacy. (4) A subset of patients experiences disease progression despite neoadjuvant efforts, possibly indicating aggressive biology or acquired resistance.¹² (5) Currently, there is no universally accepted biomarker to reliably predict response to specific neoadjuvant regimens, leading to a trial-and-error approach.

Ongoing research is focused on identifying predictive and prognostic biomarkers to optimize patient selection and tailor therapies. Efforts include evaluating circulating tumor DNA (ctDNA), microsatellite instability (MSI) status, tumor mutational burden (TMB), and expression profiles of genes involved in drug metabolism or signaling pathways.¹³ However, the translation of these biomarkers into routine clinical practice remains challenging owing to inconsistent findings across studies, lack of standardized testing methodologies, and the complexity of tumor heterogeneity. The advantages of conversion therapy for colorectal cancer with liver metastases are well established. However, researchers are increasingly scrutinizing factors that may impede its effectiveness. Building on current research in this area, we contemplate whether genomic alterations could be pivotal in shaping treatment outcomes and predicting survival benefits

for patients. Given that clinical decision-makers currently rely on patient-specific next-generation sequencing results to tailor treatment strategies, we have embarked on a retrospective study within our patient cohort. Our goal is to identify genomic mutations closely linked to the efficacy of conversion therapy and patient clinical profiles. Through integrated analysis, we aim to develop predictive models that can guide optimal treatment strategies for these patients.

PATIENTS AND METHODS

Study Design

All procedures involving human participants in this study adhered to ethical standards set by institutional and/or national research committees, as well as the 1964 Helsinki Declaration and its later amendments or similar ethical standards. This cohort study has been reported in line with the Strengthening The Reporting Of Cohort Studies in Surgery (STROCSS) criteria. All patients were enrolled in a retrospective study database, and this research has also been registered on ClinicalTrials.gov (NCT06477718). Our study has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

Patients' Information

This study enrolled 286 patients initially diagnosed with colorectal cancer concomitant with synchronous liver metastases. All patients received comprehensive treatment and follow-up at the Department of Colorectal Surgery, First Affiliated Hospital of Nanjing Medical University between 2016 and 2018. At diagnosis, all patients met the criteria for preoperative conversion therapy, with a CRS of ≥ 3 . Patients were excluded if they: (1) could not tolerate a full course of systemic therapy, (2) had a history of other malignancies, (3) had previously undergone cancer treatment, or (4) patients who were not rendered disease-free at time of hepatic resection (i.e., primary intact, unresected extrahepatic disease, or gross [R2] residual hepatic disease). Next-generation sequencing was conducted on biopsy tissues obtained via colonoscopy prior to treatment initiation, and conversion therapies were subsequently administered. Systemic treatment regimens, based on next-generation sequencing (NGS) results, included FOLFOX/FOLFIRI or CAPOX combined with anti-EGFR or anti-VEGF agents, excluding those who received selective internal radiation therapy (SIRT) or stereotactic body radiation therapy (SBRT). All patients were microsatellite stable (MSS) and did not receive any immune checkpoint therapies such as PD-1 inhibitors. Patients with locally advanced rectal cancer received additional neoadjuvant radiotherapy to the rectal area. Treatment response was assessed every two cycles, and resectability of the primary

tumor and metastases were evaluated post-treatment using abdominal contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) (Supplementary Fig. 1).

Failed conversion therapy (FCTG) was defined as persistent unresectability after first-line systemic therapy, confirmed by multidisciplinary team (MDT) consensus on the basis of anatomical/oncological criteria: (1) inadequate future liver remnant (< 30%); (2) bilobar multifocal disease unamenable to resection/ablation; (3) radiological progression (RECIST version 1.1); or (4) insufficient downstaging for R0 resection. Patients excluded for non-oncologic reasons (e.g., comorbidities or refusal; $n = 19$) were not classified as FCTG and were removed per exclusion criterion.

Genomic Detection

Formalin-fixed paraffin-embedded (FFPE) tumor samples were procured via colonoscopic biopsies. Genomic DNA was extracted from FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, USA), while genomic DNA from whole blood controls was isolated with the DNeasy Blood and Tissue Kit (Qiagen, USA). Qubit 3.0 Fluorometer (Thermo Fisher Scientific) and Nanodrop 2000 were employed to assess the quantity and quality of the extracted DNA, respectively. Libraries were prepared following the manufacturer's protocol with the KAPA Hyper Prep Kit (KAPA Biosystems). Hybridization-based target enrichment was executed using the GeneseeqPrime® pan-cancer gene panel along with xGen Lockdown Hybridization and Wash Reagents Kit (Integrated DNA Technologies). Captured libraries were amplified in KAPA HiFi HotStart ReadyMix (KAPA Biosystems) and quantitated by quantitative polymerase chain reaction (qPCR) utilizing the KAPA Library Quantification Kit (KAPA Biosystems). The enriched libraries were then sequenced on the Illumina HiSeq4000 NGS platform adhering to the manufacturer's instructions.

c-CRS Score Analysis

Furthermore, we utilized multivariable Cox proportional hazards regression to evaluate the relationship between mutations and overall survival (OS), adjusting for clinical covariates. Considering the number of events, we controlled for four clinically significant factors (with unadjusted p -values < 0.05) in the univariate analysis: pre-treatment carcinoembryonic antigen (CEA) levels, pre-treatment carbohydrate antigen 19-9 (CA19-9) levels, the largest diameter of CRLM, and lymph node status at initial diagnosis. Both univariate and multivariable analyses were conducted to examine associations with survival, with a two-sided adjusted p -value < 0.05 deemed statistically significant.

In subsequent analyses, variables significantly associated with conversion success or failure (univariate $p < 0.05$) were included in a multivariable analysis using logistic regression. Briefly, a nomogram was constructed on the basis of the final regression analysis results, integrating predictors of conversion therapy success or failure. The total score was calculated from baseline CEA/CA19-9 levels, the maximum diameter of liver metastases, and primary tumor lymph node status at diagnosis. Each variable was assigned a score on the nomogram's scale. By summing these individual scores and projecting the total onto the nomogram's total points scale, the probability of a successful conversion therapy event could be estimated. The nomogram's performance was assessed through discrimination and calibration; the former via the area under the receiver operating characteristic (ROC) curve ranging from 0.5 (no discrimination) to 1 (perfect discrimination), and the latter visually through a calibration plot contrasting predicted probabilities of conversion therapy success against actual outcomes.

Given current risk factors, each was assigned a value of 1, and Kaplan–Meier curves were plotted for patients with scores ranging from 0 to 5, with log-rank tests used to compare survival rates according to mutation status. We defined scores of 0–1 as low risk, 2–3 as medium risk, and 4–5 as high risk. All statistical analyses were conducted within the R statistical computing environment. Parameters showing significance in the univariate analysis were incorporated into the multivariable Cox regression. A p -value < 0.05 was considered statistically significant.

CRS Definition

The five parameters of the cancer recurrence score include primary tumor lymph node status, disease-free survival time, presence of more than one hepatic metastasis, preoperative CEA levels above 200 ng/mL, and a maximum diameter of metastatic tumors exceeding 5 cm, with each criterion assigned 1 point. Higher CRS scores indicate greater benefit from perioperative chemotherapy, especially when there are more than five liver metastases.

Statistical Analysis

We employed Fisher's exact test and Wilcoxon rank-sum test to compare the clinical characteristics between the successful conversion therapy group (SCTG) and failed conversion therapy group (FCTG), encompassing general patient information, clinical staging, laboratory findings, and administered treatment regimens. In addition, on the basis of sequencing results, we selected mutations present in at least 5% of patients for survival staging, ensuring a minimum presence in ten patients per group to mitigate bias and statistical uncertainty by guaranteeing sufficient events

in each cohort. For survival analysis, Kaplan–Meier curves depicted survival outcomes, with log-rank tests comparing survival rates by mutation status post adjustment for multiple comparisons. Overall survival (OS) was measured from the end of systemic therapy until patient death.

RESULTS

Demographic and Clinical Characteristics of Patients with CRLM

In this study, after excluding patients who could not complete the entire treatment regimen or did not meet the inclusion criteria, a total of 286 patients were initially diagnosed with colorectal cancer with synchronous liver metastases, all with an initial treatment goal of no evidence of disease (NED). Preoperative conversion therapy was administered on the basis of NGS results. Eventually, 106 patients achieved successful conversion and underwent resection or ablation of the primary and metastatic lesions, attaining NED status. Meanwhile, 180 patients, after first-line treatment, were confirmed by MDT to have unresectable metastases and were classified as the failed conversion therapy group.

Baseline data assessment revealed significant differences between FCTG and SCTG in four indicators: maximum metastatic lesion diameter (greater than 5 cm, 33.0% vs. 22.2%, $p = 0.045$), preoperative mesenteric lymph node positivity rate (74.5% vs. 61.7%, $p = 0.026$), CEA levels (median, 11.2 ng/mL vs. 5.2 ng/mL, $p = 0.021$), and CA19-9 levels (median, 63.2 U/mL vs. 49.2 U/mL, $p = 0.016$). However, no significant differences were observed in age (median, 51.2 vs. 50.3 years), gender (male/female, 61.3%/38.7% vs. 60.0%/40.0%), tumor location (right/left/rectum, 30.2%/33.0%/36.8% vs. 29.4%/32.2%/38.4%), number of metastatic lesions (≤ 4 / > 4 , 60.4%/39.6% vs. 55.0%/45.0%), distribution of metastatic lesions in the liver lobes (unilobar/bilobar, 34.0%/66.0% vs. 38.3%/61.7%), T-stage distribution (T1–2/T3/T4, 17.9%/38.7%/43.4% vs. 28.9%/36.1%/35.0%), and CRS score (3/4/5, 31.1%/52.8%/16.1% vs. 33.9%/51.7%/14.4%).

Treatment strategies showed no significant differences in the proportion of hepatic arterial infusion chemotherapy (30.2% vs. 32.8%) and transarterial chemoembolization (29.2% vs. 33.3%). There were also no differences in the use of targeted therapies, with similar rates of anti-EGFR (43.4% vs. 41.7%) and anti-VEGF (56.6% vs. 58.3%) between the groups (Table 1).

Genomic Mutation Spectrum Changes Related to Conversion Therapy in CRLM

In the overall cohort, analysis of detected mutations revealed that transition (Ti) mutations predominated in

both groups without significant differences (Supplementary Fig. 2A and B), indicating comparability between the groups. Regardless of whether the CRLM was convertible or non-convertible, the three most common mutations were TP53 (76% vs. 79%), APC (69% vs. 78%), and KRAS (50% vs. 56%) (Supplementary Fig. 3A and B). Pathway enrichment analysis identified the most commonly enriched pathways in the overall population as RTK/RTS, TP53, and WNT signaling pathways (Supplementary Fig. 3C and D).

We further compared the different mutation frequencies in FCTG and SCTG. To ensure subsequent verification, we focused on genes with a mutation frequency above 5% and those occurring in at least ten individuals. This screening identified six genes with the greatest differences in mutation frequency: FAT (20%), BRAF (19%), SERPINA3 (16%), GRIN2A (15%), ERBB2 (13%), and ALK (12%) (Supplementary Fig. 4A and B).

Association of Specific Mutations with Survival in Patients with CRLM

We further explored the association between gene mutations and survival in this group of patients with initially unresectable CRLM. Survival analysis of the overall population, consistent with current treatment status, showed that the 180 patients who achieved successful conversion had significantly prolonged survival compared with those who did not (median OS 41.1 months vs. 26.1 months, adjusted p -value < 0.001) (Fig. 1A).

Owing to the insufficient number of patients with the abovementioned six gene mutations in the successful conversion cohort for comparative analysis, we divided the non-conversion cohort into a mutation group (patients with any of the six mutations, $n = 65$) and a wild-type group (no mutations detected in the six genes, $n = 41$). Comparison revealed that patients with any of the six gene mutations had significantly shorter overall survival than wild-type patients (median OS 14.5 months vs. 35.3 months, adjusted p -value < 0.001) (Fig. 1B).

Further analysis at the single-gene level showed that patients with FAT1 mutations had significantly reduced overall survival compared with wild-type patients (median OS 13.5 months vs. 29.3 months, adjusted p -value = 0.001). Similarly, patients with mutated BRAF (class I/II) had the poorest prognosis, with all patients dying within 2 years (median OS 11.9 months vs. 29.1 months, adjusted p -value = 0.001). Mutations in SERPINA3, GRIN2A, ERBB2, and ALK were also associated with varying degrees of decreased overall survival (Fig. 2), suggesting that these important gene mutations may limit the effectiveness of conversion therapy and overall survival in CRLM.

TABLE 1 Clinical and demographic characteristics of patients with CRLM enrolled in this cohort

Variable	CRLM (<i>n</i> = 286)	CRLM FCTG (<i>n</i> = 106)	CRLM SCTG (<i>n</i> = 180)	<i>p</i> value
Age (years)	50.9 (24.9–74.7)	51.2 (25.3–74.4)	50.3 (24.3–74.9)	0.161
Sex				0.825
Male	173 (60.5%)	65 (61.3%)	108 (60.0%)	
Female	113 (39.5%)	41 (38.7%)	72 (40.0%)	
Location				0.967
Right colon	85 (29.7%)	32 (30.2%)	53 (29.4%)	
Left colon	93 (32.5%)	35 (33.0%)	58 (32.2%)	
Rectum	108 (37.8%)	39 (36.8%)	69 (38.3%)	
Metastatic node size				0.045
> 5 cm	75 (26.2%)	35 (33.0%)	40 (22.2%)	
≤ 5 cm	211 (73.8%)	71 (67.0%)	140 (77.8%)	
Number of CRLM				0.375
≤ 4	149 (52.1%)	64 (60.4%)	99 (55.0%)	
> 4	137 (37.9%)	42 (39.6%)	81 (45.0%)	
Lobar				0.459
Unilobar	105 (36.7%)	36 (34.0%)	69 (38.3%)	
Bilobar	181 (63.3%)	70 (66.0%)	111 (61.7%)	
T stage				0.101
T1–2	71 (24.8%)	19 (17.9%)	52 (28.9%)	
T3	106 (37.1%)	41 (38.7%)	65 (36.1%)	
T4	109 (38.1)	46 (43.4%)	63 (35.0%)	
N stage				0.026
Positive	210 (73.4%)	79 (74.5%)	111 (61.7%)	
Negative	76 (26.6%)	27 (25.5%)	69 (38.3%)	
CEA (ng/mL)	7.2 (0.6–872.3)	11.2 (1.1–872.3)	5.2 (0.6–714.3)	0.021
CA19-9 (U/mL)	55.3 (9.2–991.8)	63.2 (10.1–991.8)	49.2 (9.2–811.4)	0.016
CRS				0.868
3	94 (32.9%)	33 (31.1%)	61 (38.9%)	
4	149 (52.1%)	56 (52.8%)	93 (51.7%)	
5	43 (15.0%)	17 (16.1%)	26 (14.4%)	
Received HAI				0.650
Yes	91 (31.8%)	32 (30.2%)	59 (32.8%)	
Received TACE				0.473
Yes	91 (31.8%)	31 (29.2%)	60 (33.3%)	
Anti-EGFR				0.775
Yes	121 (42.3%)	46 (43.4%)	75 (41.7%)	
Anti-VEGF				0.775
Yes	165 (57.7%)	60 (56.6%)	105 (58.3%)	

Association of Mutation-Related Scores with Conversion Therapy Success Rates in CRLM

To further determine whether the mutations associated with survival also affect the efficacy of conversion therapy, we expanded our analysis to the entire cohort of patients with CRLM undergoing conversion therapy. Given the limitation of mutation frequencies, we could not assess the survival benefit in the converted cohort. However, specific mutations present in the non-converted cohort may be factors limiting conversion success.

In clinical practice, the ability to achieve NED status through conversion therapy for patients with colorectal cancer with synchronous unresectable liver metastases is a challenge. Therefore, identifying relevant risk factors to predict subsequent conversion success is crucial. We constructed a nomogram combining clinical baseline data and the abovementioned mutations to predict conversion success probabilities. Prior to nomogram model computation, clinical characteristics were analyzed using multivariable and univariable regression models. As shown in Supplementary Table 1, all variables except mutation factors exhibited

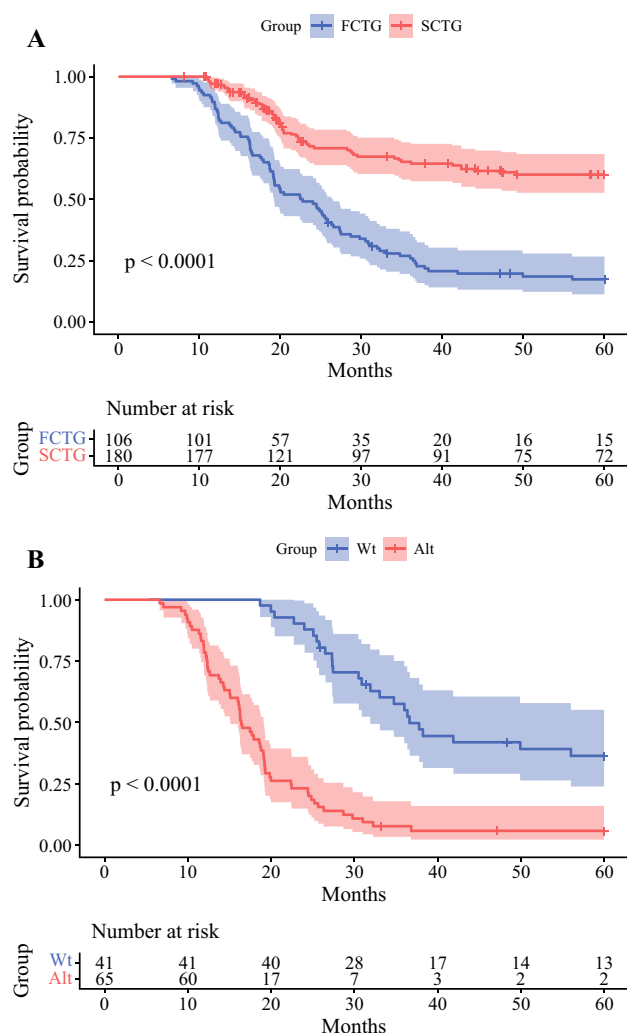


FIG. 1 Landmark analysis of overall survival was conducted in patients initially deemed unresectable due to colorectal cancer liver metastases, who received conversion therapy; **A** the cohort was stratified into those who achieved conversion to resection (SCTG; $n = 180$) and those who remained persistently unresectable (FCTG; $n = 106$); the number at risk at each time point is indicated in the accompanying risk table; **B** the patients in the FCTG were stratified into those who were detected with mutation in either of the six genes, including FAT, BRAF, SERPINA3, GRIN2A, ERBB2, and ALK (WT; $n = 41$), and those who remained persistently unresectable (Alt; $n = 65$)

significant differences in both univariate and multivariable regression analyses. Next, these risk factors were scored on the basis of their impact on probability estimation. The total score from all risk factors corresponded to the probability of conversion success (Supplementary Fig. 5A).

In the study cohort, risk stratification scores using single and combined indicators were evaluated for sensitivity and specificity via ROC curves (Supplementary Fig. 5B). Tumor mutation status (positive defined as at least one mutation), CEA level (> 200 ng/mL), and CA199 level (> 39 U/mL)

had the highest area under the curve (AUC) scores, at 80.1, 67.7, and 64.4, respectively. Tumor metastases diameter greater than 5 cm and initial mesenteric lymph node positivity had AUCs of 61.2 and 60.3, indicating some predictive capability. However, the risk stratification score as a combined indicator exhibited the strongest predictive ability (AUC 89.6, 95% CI 22.6–92.4).

Prognostic Model for Conversion-Related Risk Scores

The intriguing results prompted us to consider whether a conversion score could predict treatment efficacy or prognosis for patients with specific risk factors, similar to the CRS score for synchronous liver metastases in colorectal cancer. We developed a conversion-related clinical risk score (c-CRS) on the basis of five factors: metastases size (> 5 cm, 1 point), lymph node status (positive, 1 point), CEA level (> 200 ng/mL, 1 point), CA199 level (> 39 U/mL, 1 point), and conversion-related mutations (1 point). Cox regression models confirmed the prognostic significance of these factors for patients with CRLM (Supplementary Fig. 6).

Finally, we applied the c-CRS to our cohort and assessed its prognostic ability. Analysis of both the conversion failure patients (Fig. 3A) and the entire population (Fig. 3B) demonstrated that this scoring system could effectively stratify patients with relatively poor prognosis and shorter survival times, serving as an effective prognostic model for patients with initially unresectable CRLM (Table 2).

DISCUSSION

Initial unresectable CRLM represents a highly heterogeneous and complex disease. Treatment strategies include systemic chemotherapy, targeted therapy, and locoregional treatments. Common systemic chemotherapy regimens, such as FOLFOX (oxaliplatin, 5-FU, and leucovorin) and FOLFIRI (irinotecan, 5-FU, and leucovorin), are often combined with targeted agents such as bevacizumab or cetuximab to reduce tumor burden and increase the likelihood of surgical resection.¹⁴ Advancements in treatment modalities face challenges, including heterogeneous treatment responses, inaccurate prognostic predictions, and the absence of unified standards to evaluate the success of conversion therapies. Molecular-level heterogeneity and resistance mechanisms further limit treatment efficacy.¹⁵

This study included 286 patients initially diagnosed with colorectal cancer with synchronous liver metastases. All patients underwent individualized conversion therapies guided by next-generation sequencing. Results showed that after systemic treatment, 106 patients successfully achieved NED, while 180 did not. Significant differences were observed between the successful and unsuccessful conversion groups in terms of maximum metastatic lesion diameter,

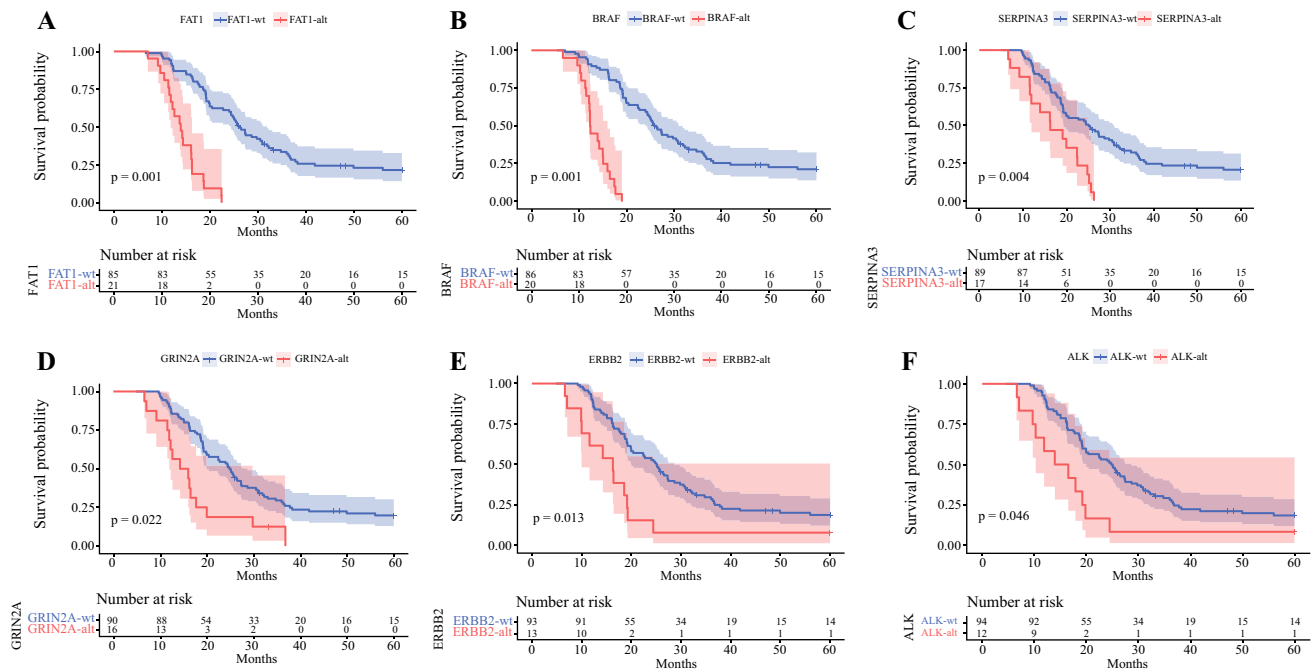


FIG. 2 Stratified overall survival analysis was conducted in patients with initially unresectable colorectal liver metastases ($n = 106$) who failed conversion therapy; patients were stratified on the basis of the

presence or absence of alterations in the following genes: **A** FAT1; **B** BRAF; **C** SERPINA3; **D** GRIN2A; **E** ERBB2; **F** ALK; the number at risk at each time point is indicated in the adjacent risk table

preoperative lymph node positivity in the mesentery, CEA levels, and CA19-9 levels. In addition, the study identified multiple gene mutations significantly associated with conversion treatment outcomes and overall survival, including FAT1, BRAF, SERPINA3, GRIN2A, ERBB2, and ALK. We developed the c-CRS model integrating patient clinical and molecular characteristics, including CEA and CA19-9 levels, tumor size, preoperative lymph node positivity, and various gene mutations. The c-CRS model effectively predicted the success of conversion therapy and patient prognosis.

Factors limiting the success rate of conversion in colorectal cancer liver metastases have garnered considerable attention. Previous studies indicate that molecular characteristics such as KRAS and NRAS mutations can hinder conversion success, while MSI-H colorectal cancer may be more responsive to immunotherapy.^{13,16} BRAF mutations suggest a relatively poor overall prognosis.¹⁷ Recent molecular marker studies continue to explore markers such as ctDNA for dynamic disease monitoring,¹⁸ DNA methylation changes in treatment response, and glucose metabolism imaging for predicting treatment responses.¹⁹ However, these studies face challenges related to standardization, the complexity of result interpretation, and clinical application validation.

The increasing adoption of NGS testing allows clinicians access to mutation profiles, aiding treatment decisions. Retrospective analyses have linked genetic alterations such as

KRAS mutations, CCND1 polymorphisms, and MTHFR mutations with pathological complete response (pCR) post neoadjuvant chemoradiotherapy in locally advanced rectal cancer.²⁰ Our study validates the adverse prognostic significance of the BRAF gene family in patients with initially unresectable CRLM. Unlike previous studies, our focus is on patients who undergo first-line conversion therapy, comparing those who achieve surgical opportunities versus those who do not or experience local progression, ensuring consistency in early treatment application and enhancing late-stage applicability for this patient group.²¹ In addition, CRS scores play a critical role in treatment selection for CRLM, providing substantial prognostic value, yet are currently unable to effectively predict patient benefit from subsequent neoadjuvant or conversion therapies. Our study integrates patient clinical data and related mutations into c-CRS scoring, offering a preliminary estimate for converting patients with CRLM and predicting the prognosis of patients with unresectable CRLM at initial diagnosis, providing a reference basis for overall treatment strategies and considering more suitable treatment options.

Many mutations closely associated with prognosis are located in genes intricately linked to colorectal cancer development. For instance, FAT1, a tumor suppressor gene, drives chromosome 4q35 deletion, a common deletion region in cancers. FAT1 mutations deactivate it, promoting Wnt pathway activation and tumorigenesis, affecting

FIG. 3 Overall survival according to conversion CRS model (point 0–5); **A** overall survival according to the c-CRS in FCTG group ($n = 106$); **B** overall survival according to the c-CRS in all patients of the cohort ($n = 286$); the low-risk group refers to those with scores of 0–1, the medium-risk group refers to those with scores of 2–3, and the high-risk group refers to those with scores of 4–5

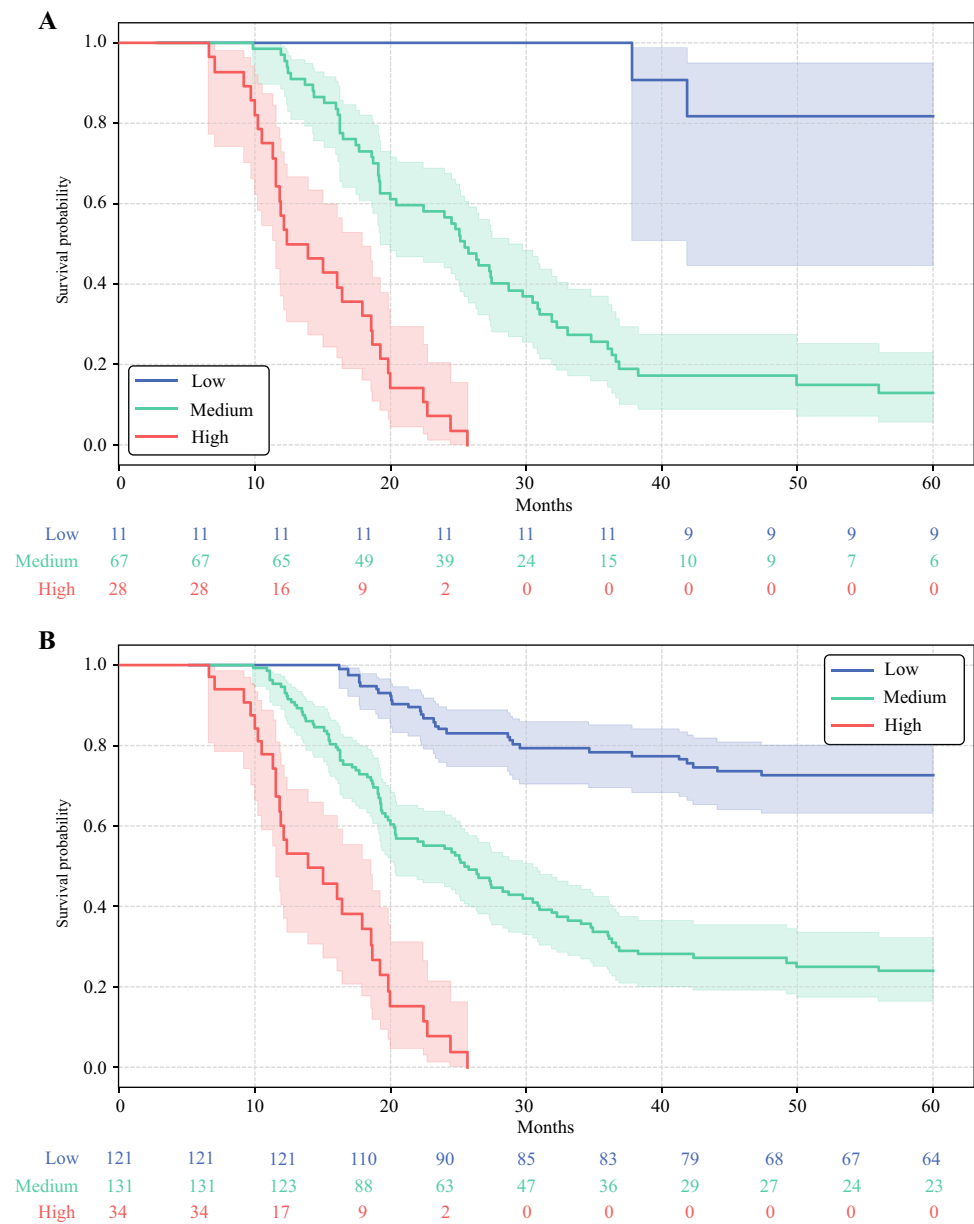


TABLE 2 Multivariable cox regression analysis and overall survival based on the conversion score

Cohort	Risk	No. (%)	Hazard ratio (95% CI) versus low	p-value	Hazard ratio (95% CI) versus prior	p-value
CRLM cohort	Low	121 (42.3%)	1	1	–	–
	Medium	131 (45.8%)	4.53 (2.98–6.88)	1.34e–12	4.53 (2.98–6.88)	1.34e–12
	High	34 (11.9%)	20.36 (10.91–38.00)	2.90e–21	4.37 (2.76–6.93)	3.14e–10

patient survival.²² In colorectal cancer prognosis analysis based on TCGA data, SERPINA3 and other molecules are observed to correlate with poor outcomes.²³ GRIN2A mutations affect tumor progression by influencing intracellular calcium ion homeostasis and signaling pathways, correlating

with poor prognosis in various cancers, including colorectal cancer.^{24,25} ERBB2 mutations, increasingly studied in colorectal cancer, occur in approximately 4.8–7% of cases and promote tumor cell proliferation through MAPK and AKT pathway activation.^{26,27} ALK gene fusion mutations,

although more common in lung cancer, are also significant in advanced colorectal cancer, with potential therapeutic benefits from ALK inhibitors.^{28,29}

This study has several limitations that should be acknowledged. First, the retrospective design introduces potential selection bias and unmeasured confounding factors, while the small sample size in high-risk mutation subgroups limits statistical power for detailed analysis. Second, treatment heterogeneity—such as the inclusion of patients receiving hepatic arterial infusion (HAI) or transarterial chemoembolization (TACE)—may obscure the interpretation of genetic associations, despite no significant survival differences between subgroups. Third, the heterogeneity of gene mutations across patients affects reproducibility and generalizability, underscoring the need for broader genetic testing to capture all relevant variants. Fourth, the absence of external validation restricts the applicability of our findings, emphasizing the necessity of multi-center studies to confirm reliability. Finally, as a single-center study, results may reflect local patient characteristics and treatment protocols, limiting broader clinical relevance. Future research should prioritize objective criteria for evaluating conversion therapy success, prospective trials to validate biomarkers, and exploration of novel predictive tools. These efforts aim to refine treatment strategies, reduce unnecessary interventions, and advance personalized care within precision oncology.

CONCLUSIONS

This study demonstrates the potential of existing biomarkers in molecular-guided therapies. We believe that once expanded, molecular spectrum analysis of cancer-related genes will become a part of routine clinical practice, it will lead to new diagnostic methods and further refine treatment strategies for colorectal cancer liver metastases (CRLM). Through iterative development and clinical application, these biomarkers are expected to maximize treatment responses in prospective analyses and minimize treatment complications, particularly in subgroups least likely to respond to treatment.

SUPPLEMENTARY INFORMATION The online version contains supplementary material available at <https://doi.org/10.1245/s10434-025-17809-5>.

ACKNOWLEDGEMENT We are grateful to Nanjing GENESEEQ Co. for assistance with sequencing and/or bioinformatics analysis.

AUTHOR CONTRIBUTIONS JT: conceptualization, methodology, writing—original draft, formal analysis, and data curation. YS: conceptualization, supervision, writing—review and editing, validation, formal analysis, writing—original draft, and data curation. YW: validation, writing—review and editing, supervision, writing—original draft, and data curation. DZ: validation and writing—review and editing, supervision, writing—original draft, and data curation. LW: validation,

writing—review and editing, supervision, and writing—original draft. KJ: writing—review and editing. YZ: writing—review and editing.

FUNDING This work was supported by the National Natural Science Foundation (82473049, 82273406, 82304221), Basic Research Program of Jiangsu Province (BK20221415, BK20230730), Jiangsu Key Medical: Discipline (General Surgery; ZDXK202222), and China Postdoctoral Science Foundation (2022M721679).

DATA AVAILABILITY The data that support the findings of this study are available on request from the corresponding author (sunyue-ming@njmu.edu.cn) upon reasonable request.

DISCLOSURE The authors declare that they have no competing interests.

REFERENCES

1. Kawazoe A, et al. Lenvatinib plus pembrolizumab versus standard of care for previously treated metastatic colorectal cancer: final analysis of the randomized, open-label, phase III LEAP-017 study. *J Clin Oncol*. 2024. <https://doi.org/10.1200/JCO.23.02736>.
2. Bertocchi A, et al. Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell*. 2021;39:708–24. <https://doi.org/10.1016/j.ccell.2021.03.004>.
3. Wu Y, et al. Spatiotemporal immune landscape of colorectal cancer liver metastasis at single-cell level. *Cancer Discov*. 2022;12:134–53. <https://doi.org/10.1158/2159-8290.CD-21-0316>.
4. Margonis GA, et al. Impact of surgical margin width on recurrence and overall survival following R0 hepatic resection of colorectal metastases: a systematic review and meta-analysis. *Ann Surg*. 2018;267:1047–55. <https://doi.org/10.1097/SLA.0000000000002552>.
5. Chen Q, et al. Personalized prediction of postoperative complication and survival among colorectal liver metastases patients receiving simultaneous resection using machine learning approaches: a multi-center study. *Cancer Lett*. 2024;593:216967. <https://doi.org/10.1016/j.canlet.2024.216967>.
6. Wankhede D, et al. Clinical significance of combined tumour-infiltrating lymphocytes and microsatellite instability status in colorectal cancer: a systematic review and network meta-analysis. *Lancet Gastroenterol Hepatol*. 2024;9:609–19. [https://doi.org/10.1016/S2468-1253\(24\)00091-8](https://doi.org/10.1016/S2468-1253(24)00091-8).
7. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg*. 1999;230:309–18. <https://doi.org/10.1097/0000658-199909000-00004>.
8. Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Ann Surg*. 2008;247:125–35. <https://doi.org/10.1097/SLA.0b013e31815aa2c2>.
9. Morris VK, et al. Treatment of metastatic colorectal cancer: ASCO guideline. *J Clin Oncol*. 2023;41:678–700. <https://doi.org/10.1200/JCO.22.01690>.
10. Margonis GA, et al. Association between specific mutations in KRAS codon 12 and colorectal liver metastasis. *JAMA Surg*. 2015;150:722–9. <https://doi.org/10.1001/jamasurg.2015.0313>.
11. Kopetz S, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved

- chemotherapy. *J Clin Oncol*. 2009;27:3677–83. <https://doi.org/10.1200/JCO.2008.20.5278>.
12. Loupakis F, et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med*. 2014;371:1609–18. <https://doi.org/10.1056/NEJMoa1403108>.
 13. Schrock AB, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Ann Oncol*. 2019;30:1096–103. <https://doi.org/10.1093/annonc/mdz134>.
 14. Johnston FM, Mavros MN, Herman JM, Pawlik TM. Local therapies for hepatic metastases. *J Natl Compr Cancer Netw*. 2013;11:153–60. <https://doi.org/10.6004/jnccn.2013.0023>.
 15. Schadde E, Grunhagen DJ, Verhoef C, Krzywon L, Metrakos P. Limitations in resectability of colorectal liver metastases 2020: a systematic approach for clinicians and patients. *Semin Cancer Biol*. 2021;71:10–20. <https://doi.org/10.1016/j.semcancer.2020.09.008>.
 16. Bridgewater JA, et al. Systemic chemotherapy with or without cetuximab in patients with resectable colorectal liver metastasis (new EPOC): long-term results of a multicentre, randomised, controlled, phase 3 trial. *Lancet Oncol*. 2020;21:398–411. [https://doi.org/10.1016/S1470-2045\(19\)30798-3](https://doi.org/10.1016/S1470-2045(19)30798-3).
 17. Ros J, et al. Plasmatic BRAF-V600E allele fraction as a prognostic factor in metastatic colorectal cancer treated with BRAF combinatorial treatments. *Ann Oncol*. 2023;34:543–52. <https://doi.org/10.1016/j.annonc.2023.02.016>.
 18. Bachet JB, et al. RAS mutation analysis in circulating tumor DNA from patients with metastatic colorectal cancer: the AGE0 RASANC prospective multicenter study. *Ann Oncol*. 2018;29:1211–9. <https://doi.org/10.1093/annonc/mdy061>.
 19. Nasti G, Ottaiano A, Rosario Iaffaioli V, Berretta M, Delrio P. Trials on preoperative chemotherapy in resectable colorectal liver metastases need prospective evaluation of predictive factors of response. *J Clin Oncol*. 2008;26:3812–3. <https://doi.org/10.1200/JCO.2008.17.8301>.
 20. Garcia-Aguilar J, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg*. 2011;254:486–92. <https://doi.org/10.1097/SLA.0b013e31822b8cfa>.
 21. Margonis GA, et al. Association of BRAF mutations with survival and recurrence in surgically treated patients with metastatic colorectal liver cancer. *JAMA Surg*. 2018;153:e180996. <https://doi.org/10.1001/jamasurg.2018.0996>.
 22. Morris LG, et al. Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. *Nat Genet*. 2013;45:253–61. <https://doi.org/10.1038/ng.2538>.
 23. Zhou L, et al. Comprehensive analysis of CXCL14 uncovers its role during liver metastasis in colon cancer. *BMC Gastroenterol*. 2023;23:273. <https://doi.org/10.1186/s12876-023-02896-z>.
 24. Carvill GL, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. *Nat Genet*. 2013;45:1073–6. <https://doi.org/10.1038/ng.2727>.
 25. Palaniappan A, Ramar K, Ramalingam S. Computational identification of novel stage-specific biomarkers in colorectal cancer progression. *PLoS ONE*. 2016;11:e0156665. <https://doi.org/10.1371/journal.pone.0156665>.
 26. Nakamura Y, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. *Nat Med*. 2021;27:1899–903. <https://doi.org/10.1038/s41591-021-01553-w>.
 27. Siena S, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2021;22:779–89. [https://doi.org/10.1016/S1470-2045\(21\)00086-3](https://doi.org/10.1016/S1470-2045(21)00086-3).
 28. Noe J, et al. ALK mutation status before and after alectinib treatment in locally advanced or metastatic ALK-positive NSCLC: pooled analysis of two prospective trials. *J Thorac Oncol*. 2020;15:601–8. <https://doi.org/10.1016/j.jtho.2019.10.015>.
 29. Siravegna G, et al. Tracking a CAD-ALK gene rearrangement in urine and blood of a colorectal cancer patient treated with an ALK inhibitor. *Ann Oncol*. 2017;28:1302–8. <https://doi.org/10.1093/annonc/mdx095>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.