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Multimodal treatment of recurrence and distant metastases of colorectal cancer,
2nd edition

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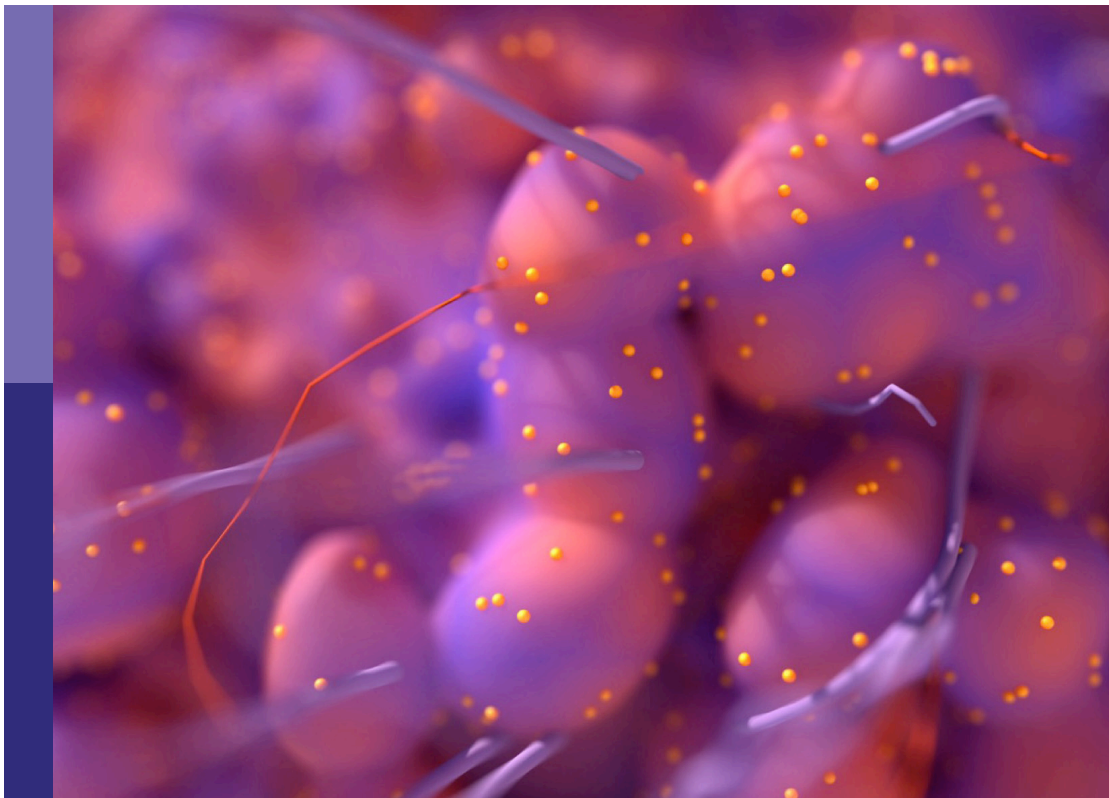

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Multimodal treatment of recurrence and distant metastases of colorectal cancer, 2nd edition

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Editorial: Multimodal treatment of recurrence and distant metastases of colorectal cancer

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KEYWORDS

rectal cancer (RC), chemotherapy, radiotherapy, local recurrence, distant metastases

Editorial on the Research Topic
Multimodal treatment of recurrence and distant metastases of colorectal cancer

For the treatment of rectal cancer and recurrence or distant metastases several critical improvements have been developed in the last decades. Since the first milestone intervention proposed by Miles more than one hundred years ago, for the treatment of low rectal tumors, surgery has made great progress with the introduction of low anterior resection with Knight and Griffits anastomosis, colonic pouches, intersphincteric resection, the introduction of the minimally invasive approach by laparoscopy or by robot, the use of transanal device, or the use of fluorescence angiography and lymphangiography, up to the use of artificial intelligence intraoperatively (1–3). However, we must not forget maybe the most important advance in the treatment of rectal cancer such as the description by Heald in the 80's of the concept of total mesorectal excision (TME) (4). In fact the introduction of this new paradigm was a sliding door for the oncological results allowing to reduce drastically the recurrence rate after surgery (4). Similarly the introduction of the neoadjuvant chemo-radiotherapy (n-CRT) contributed to reduce dramatically the recurrence rate (4).

Anyway, rectal cancer, apart from local recurrence, is also responsible for distant metastases, especially liver metastases, and in the last years a great effort has been made to investigate about the tumor biology, in order to improve survival and disease free survival of these patients. I want to thank to Frontiers in Oncology to have the opportunity to serve as Editor of this monographic issue about the multimodal treatment of recurrence and distant metastases of colorectal cancer, and I want to thank all authors involved.

Important findings are reported in this Research Topic by using nomograms and machine learning, about the prediction of the survival outcomes for patients affected by

young-onset colorectal cancer with the aim to assist in developing clinical treatment strategies for these patients (Li et al.), and about the prediction of distant metastatic sites and risk facilitating the clinical decision-making process (He et al. and Qiu et al., respectively).

On the other hand, Dai et al., He et al., Gao et al., and Zhou et al. focused their findings on the use of new protocol of immunotherapy, chemotherapy and radiotherapy for the treatment of metastatic colorectal cancer with encouraging results.

Li et al. reported an interesting literature review about acupuncture showing its use for the treatment of colorectal cancer symptoms, while Xu et al. reported as positive clinical circumferential resection margin is an independent risk factor for recurrence after TME. Finally, Baba et al. reported as irinotecan induced interstitial lung disease even in a patients underwent bone marrow transplantation for aplastic anemia decades before.

Treatment of rectal cancer, especially in case of local recurrence or distant metastases is still a debated topic and further topics will be of interest in the future, however, we considered that the present issue includes high-quality studies. We hope that this monographic issue will be of interest for the reader, helping to update the most advanced knowledge on rectal cancer treatment.

Author contributions

AB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DCor: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DCol: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SDS: Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis. SM-C: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Development and validation of a clinical survival model for young-onset colorectal cancer with synchronous liver-only metastases: a SEER population-based study and external validation

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Background: The morbidity and mortality of young-onset colorectal cancer (YO-CRC) patients have been increasing in recent years. Moreover, YO-CRC patients with synchronous liver-only metastases (YO-CRCSLM) have various survival outcomes. Therefore, the purpose of this study was to construct and validate a prognostic nomogram for patients with YO-CRCSLM.

Methods: The YO-CRCSLM patients were rigorously screened from the Surveillance, Epidemiology, and End Results (SEER) database in January 2010 and December 2018 and then assigned to a training and validation cohort randomly (1488 and 639 patients, respectively). Moreover, the 122 YO-CRCSLM patients who were enrolled in The First Affiliated Hospital of Nanchang University were served as a testing cohort. The variables were selected using the multivariable Cox model based on the training cohort and then developed a nomogram. The validation and testing cohort were used to validate the model's predictive accuracy. The calibration plots were used to determine the Nomogram's discriminative capabilities and precision, and the decision analysis (DCA) was performed to evaluate the Nomogram's net benefit. Finally, the Kaplan-Meier survival analyses were performed for the stratified patients based on total nomogram scores classified by the X-tile software.

Results: The Nomogram was constructed including ten variables: marital status, primary site, grade, metastatic lymph nodes ratio (LNR), T stage, N stage, carcinoembryonic antigen (CEA), Surgery, and chemotherapy. The Nomogram performed admirably in the validation and testing group according to the calibration curves. The DCA analyses showed good clinical utility values. Low-risk patients (score<234) had significantly better survival outcomes than middle-risk (234–318) and high-risk (>318) patients ($P < 0.001$).

Conclusion: A nomogram predicting the survival outcomes for patients with YO-CRCSLM was developed. In addition to facilitating personalized survival prediction, this nomogram may assist in developing clinical treatment strategies for patients with YO-CRCSLM who are undergoing treatment.

KEYWORDS

survival model, YO-CRCSLM, survival, nomogram, SEER

Introduction

Colorectal cancer (CRC) is a prevalent and aggressive malignancy of the gastrointestinal tract, ranking third in morbidity and second in mortality among malignant tumors globally (1). CRC patients aged 50 years and older have experienced a reduction in the incidence and mortality rates due to the general screening by colonoscopy (2), while the morbidity of young-onset CRC (YO-CRC) patients age younger than 50 years has been growing (3), with an increasing speeding at annually 3.2% from 1974-2013. The average age of diagnosis for YO-CRC is 40 years, with a comparable incidence in men and women (4). In 2010, the proportion of young-onset colon and rectal cancers was 4.8% and 9.5%, respectively, and is expected to increase to 10.9% and 22.5% by 2030 (5).

YO-CRC is characterized by the presence of microsatellite instability-high (MSI-H, 10%-30%), poorly differentiated tumor cells, and an abundance of signet-ring cell components (6). Especially, YO-CRC had a higher rate of synchronous liver metastasis than later-onset colorectal cancer (LO-CRC) patients, possibly due to diagnostic delays (7). A retrospective study by Cheng et al. demonstrated that 12.2% of YO-CRC patients who were under surgical resection developed liver metastases (8). James et al. showed that the 5-year survival rate of young colorectal patients with synchronous liver-only metastases was only 18% (9). According to another study, the median survival time for individuals with young-onset colorectal live metastases who received both primary and metastatic resection was 35 months. However, the median survival rate dropped to 18% for those patients who did not have any surgery. The 5-year survival rate of colorectal cancer with liver metastasis was only 28% (10). Radical excision of the primary lesion and metastasis is the only method for patients with liver metastases of colorectal cancer to achieve long-term survival (11). Therefore, investigating the prognosis factor affecting those patients is valuable. Previous research has investigated factors affecting the prognosis of young colorectal cancer with liver-only metastases. A retrospective study by Ding et al. showed the 5-year cancer-specific survival rate was influenced by some independent factors, such as primary tumor location, chemotherapy, and histopathological grade (12). Another indicated that the excision of the original tumor and liver metastases was substantially linked with the OS for YO-CRCSLM (9). However, a more effective model for long-term prognostic factors regarding YO-CRC with synchronous Liver-Only metastasis (YO-CRCSLM) needed to be explored.

Therefore, this study aimed to develop and evaluate a more effective model that incorporates clinicopathological factors and blood indicators to predict survival in YO-CRCSLM. Our findings may offer clinicians a more individualized and thorough outlook for YO-CRCSLM receiving treatment.

Methods

Study patients

YO-CRCSLM patients between January 2010 and December 2018 were retrospectively extracted from the SEER database. The inclusion criteria include (1): CRC was the only primary tumor (2), patients aged 18 to 49 at the time of diagnosis, and (3) complete prognostic information. Patients who lacked or had insufficient clinicopathological data of interest, such as age, gender, histological differentiation, primary site, tumor size, and treatment, were excluded. According to the above inclusion and exclusion criteria, 2127 pathologically proven YO-CRCSLM patients were ultimately identified for model construction and were then randomly assigned to the training cohort (approximately 70%, n = 1488) to create the prediction model and the validation cohort (remaining 30%; n = 639). Moreover, 122 YO-CRCSLM patients recruited from the First Affiliated Hospital of Nanchang University during January 2012 to December 2020 were finally selected as a testing cohort. The First Affiliated Hospital of Nanchang University Ethics Committee approved this observational retrospective investigation, and patients who participated in the study signed informed consent forms (2022)CDYFYYLK(12-003).

Variables and outcomes

We collected the following sixteen demographic and clinicopathologic variables: gender, age, marital status, tumor size, primary site, histological type, grade, metastatic lymph nodes ratio (LNR), perineural invasion, T stage, N stage, carcinoembryonic antigen (CEA), Surgery, radiotherapy, and chemotherapy. The patients were divided into three groups based on their primary site: the right-side colon (cecum, ascending colon, hepatic flexure of

the colon, transverse colon), the left-side colon (splenic flexure of the colon, descending colon, sigmoid colon, rectosigmoid), and the rectum. The variable of Surgery included primary site surgery (Surg Prim Site), distant metastasis surgery (Surg Dis Site), primary and distant metastasis site combined surgery Surg Com Site and no surgery. Specifically, the information about R0 (Microscopically negative margins) or R1(Microscopically positive margins) resection performed on primary or metastasis sites is unavailable. The LNR was defined as the ratio of the number of lymph nodes with pathologically confirmed tumor infiltration to the total number of lymph nodes cleared. The primary outcome was overall survival (OS), defined as the time from diagnosis of YO-CRCSLM to death from any cause or the last follow-up.

Develop and validate the prognostic model

Univariate Cox proportional hazards regression was used to identify potential risk factors, and the statistically significant variables were found as independent prognostic factors in a multivariate Cox regression analysis, and a nomogram was developed to predict the OS of patients with YO-CRCSLM.

The Nomogram's performance was evaluated using discriminative ability and calibration (13). The calibration was used to evaluate the predictive performance of the Nomogram. The receiver operating characteristic (ROC) curve was used to evaluate the discriminatory ability of the Nomogram. Kaplan-Meier curves were drawn for additional examination based on the nomogram-predicted score categorized by the X-tile software. Finally, the decision curve analysis (DCA) was then utilized to evaluate the model's net benefit.

Statistical analysis

The Chi-square test was utilized to compare categorical data represented as numbers and percentages. A COX proportional risk model was used to analyze the prognosis of YO-CRCSLM patients, and a nomogram map was drawn using the R package "rms". Calibration curves were plotted using a bootstrap of 1000 samples to evaluate the nomogram fit. Calibration plots were assessed using the R package "rms". The R package "DCA" was used for the net benefits analysis of the model, and time points of 1, 2, and 3 years were selected, respectively. R statistical software was used for statistical analysis of the data, X-tile software was used for risk stratification according to the Nomogram prediction score, and survival curves were drawn to compare the prognosis of patients at different risks. All tests were bilateral, and $P < 0.05$ was considered statistically significant.

Results

Clinicopathological characteristics of patients

Following the inclusion criteria, the SEER database was eventually used to acquire 2127 eligible YO-CRCSLM patients, who were then assigned into a training group (n=1488) and a validation cohort (n=639) by the stratified random sampling method at a 7:3 ratio. Moreover, a cohort of 122 CRCSLM patients from the First Affiliated Hospital of Nanchang University was defined as the testing cohort, among whom 70 patients died at

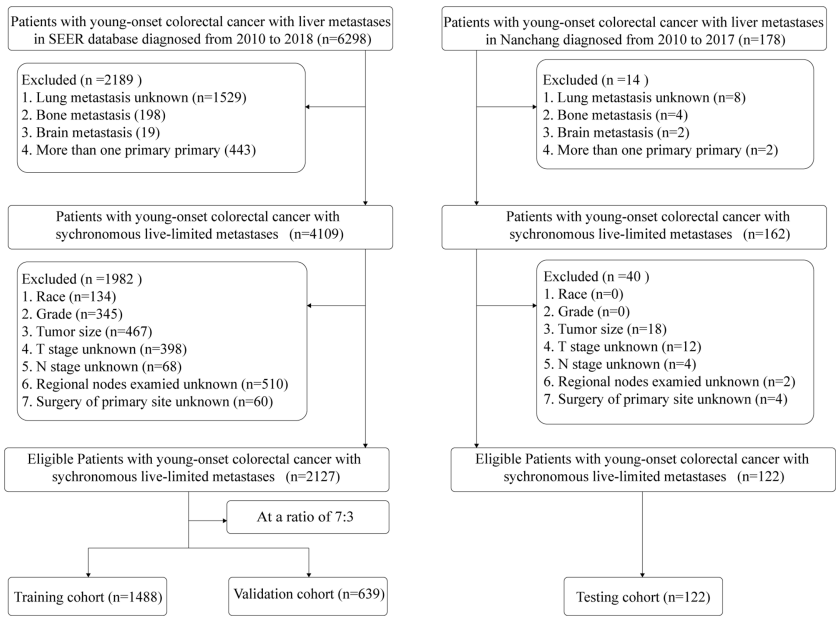

FIGURE 1
Flow chart for the selection of patients.

the follow-up. Figure 1 shows a detailed flow chart of standard procedures for the patient screening process.

Table 1 shows the baseline clinicopathological features of the training and validation sets. Among all patients, the median follow-up was 38 months (3-57 months). The vast majority of the age at diagnosis was 40-49(74.89%), followed by 30-39(20.87%) and 18-29 (4.23%). The male patients were more than females (55.10% vs. 44.90%). The most common tumor location was the left-side colon, which accounted for more than the right-sided colon and rectum combined (50.68% vs. 49.32%). Most patients were colorectal adenocarcinomas (89.89% vs 10.11%). Surgical resection of the primary lesion is performed in most patients (85.00% vs 15.00%). In contrast, Surgery for Liver-Only metastases is rare (19.00% vs 81.00%). Most patients received chemotherapy (88.34% vs 11.66%), but fewer received radiation therapy (13.35% vs 86.65%). There was no significant difference between the training and validation cohorts in the baseline demographic and clinicopathological characteristics, so the random of these two cohorts was comparable (Table 1). The mortality was 69.34% and 57.38% for all YO-CRCSLM patients in the SEER and testing cohorts, respectively (Supplemental Table 1).

Analysis of prognostic factors

Moreover, we included sixteen clinicopathological factors to explore their association with overall survival in the training cohort (Table 2). Finally, we obtained nine prognostic factors with significant differences by multivariate Cox regression analysis. The results showed poorly differentiated grade (HR = 2.14, 95% CI = 1.52 – 3.01, $P < 0.001$), higher LNR (>0.6: HR = 1.92, 95%CI = 1.50 – 2.45, $P < 0.001$), higher N stage (N2: HR = 1.43, 95%CI = 1.12 – 1.82, $P < 0.001$) and positive CEA (HR = 1.26, 95%CI = 1.04 – 1.52, $P = 0.019$) were related to the worse prognosis. In addition, the primary site (left-side colon: HR = 0.63, 95%CI = 0.54 – 0.72, $P < 0.001$; rectum: HR = 0.52, 95%CI = 0.41 – 0.65, $P < 0.001$), Surgery (Surg Prim Site: HR = 0.46, 95%CI = 0.31 – 0.69, $P < 0.001$; Surg Dis Site: HR = 0.54, 95%CI = 0.28 – 0.89, $P < 0.001$; Surg Com Site: HR = 0.29, 95%CI = 0.19 – 0.45, $P < 0.001$) and chemotherapy (HR = 0.49, 95%CI = 0.40 – 0.60, $P < 0.001$) were significantly associated with better survival outcome. Interestingly, married status was also a protective factor for these patients' prognosis.

Construction and validation of the nomogram

Then a predictive nomogram model was established based on the factors identified above (Figure 2). The risk score of each variable was obtained according to this Nomogram and then added to get the total score to predict the OS of each patient at 1, 2, and 3 years (Figure 2).

In this study, we evaluated the discriminatory ability of the Nomogram by the ROC curve. In the training cohort, the AUC values of the Nomogram for the probability of survival at 1- (Figure 3A), 2- (Figure 3B), and 3- (Figure 3C) years had excellent discriminatory power. Meanwhile, the AUC values of the monogram 0.778, 0.776, and 0.744 (Figures 3D–F) and 0.755, 0.885, and 0.908 in the validation and testing cohort, respectively (Figures 3G–I), suggesting the model's discriminatory ability. Then we evaluated our Nomogram's calibration using the calibration plots, and the result indicated a good agreement between the actual observation and the nomogram prediction in both the training and validation cohorts (Figures 4A–I). Moreover, the DCA analyzed the Nomogram's clinical usefulness in the training, validation, and testing cohort, indicating excellent positive net benefits (Figures 5A–I).

The model's risk scores and survival curves based on risk stratification

The training cohort was analyzed using the R language to calculate nomogram scores. Then, our nomogram scores for clinicopathological variables are displayed in Table 3. Based on the risk scores of patients in the Nomogram model, we divided patients into a low-risk group (defined as a total score less than 234), a medium-risk group (defined as a total score from 234 to 318), and a high-risk group (Defined as a total score more than 318) by x-tile software (Supplemental Figure 1). As expected, based on risk stratification, we performed a Kaplan-Meier survival analysis for the three groups. As expected, the result showed that the low-risk cohort had better survival outcomes compared to the middle-risk and high-risk groups in the training cohort (Figure 6A), testing cohort (Figure 6B) and validation cohort (Figure 6C).

Discussion

The number of individuals diagnosed with EO-CRC has been on the rise, in contrast to the declining trend seen in the elderly since the middle of the 1990s. In addition, colorectal cancer metastases occur most frequently in the liver, which is also a common cause of cancer-related death (14). Therefore, to determine patients' prognoses and make individual treatment decisions, it is essential to arrive at an accurate survival prediction for YO-CRCSLM patients. To the best of our knowledge, this is the first study to evaluate the prognosis of YO-CRCSLM patients and develop an OS prediction model.

In this study, we evaluated the independent predictive factors influencing survival in 2,127 YO-CRCSLM patients based on the SEER database who were diagnosed between 2010 and 2018 and then constructed a nomogram, including marital status, primary site, grade, LNR, T and N stage, CEA, Surgery and chemotherapy. In addition, 122 YO-CRCSLM hospitalized patients from our hospital were collected and analyzed as external validation cohorts to validate the established nomogram. Moreover, this nomogram can be used as a practical and reliable predictive model in clinical practice to assist doctors in decision-making.

Previous studies have predicted the survival outcomes in CRC patients with liver metastasis (15). however, there is a vital problem that has not received attention, which is that patients with liver metastasis from CRC are susceptible to complicated metastasis

TABLE 1 Clinicopathological characteristics of Young-onset colorectal cancer with synchronous liver metastasis patients from 2010 to 2018.

	All patients (n=2127) NO. (%)	training cohort (n=1488) NO. (%)	validation cohort (n=639) NO. (%)	*p*
Age				
18-29	90 (4.23)	56 (3.76)	34 (5.32)	0.172
30-39	444 (20.87)	320 (21.51)	124 (19.41)	
40-49	1593 (74.89)	1112 (74.73)	481 (75.27)	
Gender				
Male	1172 (55.10)	828 (55.65)	344 (53.83)	0.470
Female	955 (44.90)	660 (44.35)	295 (46.17)	
Marital status				
Single	623 (29.29)	444 (29.84)	179 (28.01)	0.688
Married	1194 (56.14)	830 (55.78)	364 (56.96)	
Unknown	310 (14.57)	214 (14.38)	96 (15.02)	
Primary site				
Right-side colon	650 (30.56)	440 (29.57)	210 (32.86)	0.167
Left-side colon	1078 (50.68)	756 (50.81)	322 (50.39)	
Rectum	399 (18.76)	292 (19.62)	107 (16.74)	
Tumor size				
≤5	1020 (47.95)	710 (47.72)	310 (48.51)	0.758
>5	871 (40.95)	608 (40.86)	263 (41.16)	
Unknown	236 (11.10)	170 (11.42)	66 (10.33)	
Histological type				
Adenocarcinoma	1912 (89.89)	1344 (90.32)	568 (88.89)	0.354
Non-adenocarcinoma	215 (10.11)	144 (9.68)	71 (11.11)	
Grade				
Well	91 (4.28)	69 (4.64)	22 (3.44)	0.659
Moderately	1488 (69.96)	1038 (69.76)	450 (70.42)	
Poorly	447 (21.02)	310 (20.83)	137 (21.44)	
Undifferentiated	101 (4.75)	71 (4.77)	30 (4.69)	
LNR				
≤0.2	902 (42.41)	641 (43.08)	261 (40.85)	0.424
0.2-0.6	614 (28.87)	422 (28.36)	192 (30.05)	
>0.6	245 (11.52)	163 (10.95)	82 (12.83)	
Unknown	366 (17.21)	262 (17.61)	104 (16.28)	
Perineural invasion				
No	1110 (52.19)	789 (53.02)	321 (50.23)	0.129
Yes	607 (28.54)	429 (28.83)	178 (27.86)	
Unknown	410 (19.28)	270 (18.15)	140 (21.91)	

(Continued)

TABLE 1 Continued

	All patients (n=2127) NO. (%)	training cohort (n=1488) NO. (%)	validation cohort (n=639) NO. (%)	*p*
T stage				
T1	144 (6.77)	109 (7.33)	35 (5.48)	0.469
T2	80 (3.76)	57 (3.83)	23 (3.60)	
T3	1188 (55.85)	825 (55.44)	363 (56.81)	
T4	715 (33.62)	497 (33.40)	218 (34.12)	
N stage				
N0	375 (17.63)	274 (18.41)	101 (15.81)	0.277
N1	865 (40.67)	606 (40.73)	259 (40.53)	
N2	887 (41.70)	608 (40.86)	279 (43.66)	
CEA				
Negative	333 (15.66)	224 (15.05)	109 (17.06)	0.393
Positive	1258 (59.14)	880 (59.14)	378 (59.15)	
Unknown	536 (25.20)	384 (25.81)	152 (23.79)	
Surgery				
No	300 (14.10)	216 (14.52)	84 (13.15)	0.437
Surg Prim Site	1285 (60.41)	882 (59.27)	403 (63.07)	
Surg Dis Site	19 (0.89)	14 (0.94)	5 (0.78)	
Surg Com Site	523 (24.59)	376 (25.27)	147 (23.00)	
Radiotherapy				
No/Unknown	1843 (86.65)	1284 (86.29)	559 (87.48)	0.503
Yes	284 (13.35)	204 (13.71)	80 (12.52)	
Chemotherapy				
No/Unknown	248 (11.66)	178 (11.96)	70 (10.95)	0.555
Yes	1879 (88.34)	1310 (88.04)	569 (89.05)	

CEA, carcinoembryonic antigen; Surg Prim Site, primary site surgery; Surg Dis Site, distant metastasis site surgery; Surg Com Site, primary and distant metastasis site combined surgery.

TABLE 2 Univariate and multivariate cox regression analysis of overall survival in the training cohort.

Characteristics	Univariate analysis Hazard ratio (95% CI)	*P*	Multivariate analysis Hazard ratio (95% CI)	*P*
Age				
18-29	Reference			
30-39	0.78(0.57-1.08)	0.141		
40-49	0.79(0.59-1.08)	0.137		
Gender				
Male	Reference			

(Continued)

TABLE 2 Continued

Characteristics	Univariate analysis Hazard ratio (95% CI)	*P*	Multivariate analysis Hazard ratio (95% CI)	*P*
Female	0.95(0.84-1.08)	0.434		
Marital status				
Single	Reference			
Married	0.70(0.61-0.80)	<0.001	0.79(0.69-0.91)	**0.002**
Unknown	0.90(0.75-1.09)	0.299	0.94(0.78-1.15)	0.561
Primary site				
Right-side colon	Reference			
Left-side colon	0.60(0.52-0.69)	<0.001	0.63(0.54-0.72)	**<0.001**
Rectum	0.56(0.47-0.66)	<0.001	0.52(0.41-0.65)	**<0.001**
Tumor size				
≤5	Reference			
>5	1.20(1.06-1.37)	0.006	1.05(0.91-1.20)	0.528
Unknown	1.56(1.29-1.89)	<0.001	1.12(0.90-1.39)	0.314
Histological type				
Adenocarcinoma	Reference			
Other	1.42(1.16-1.73)	0.001	0.97(0.77-1.22)	0.795
Grade				
Well	Reference			
Moderately	1.04(0.77-1.40)	0.812	1.35(0.98-1.86)	0.068
Poorly	2.04(1.49-2.79)	<0.001	2.14(1.52-3.01)	**<0.001**
Undifferentiated	2.40(1.63-3.53)	<0.001	2.94(1.95-4.42)	**<0.001**
LNR				
≤0.2	Reference			
0.2-0.6	1.66(1.43-1.94)	<0.001	1.30(1.06-1.60)	**0.012**
>0.6	2.54(2.08-3.09)	<0.001	1.92(1.50-2.45)	**<0.001**
Unknown	2.25(1.90-2.66)	<0.001	1.21(0.81-1.82)	0.348
Perineural invasion				
No	Reference			
Yes	1.29(1.12-1.48)	0.001	1.14(0.98-1.33)	0.082
Unknown	1.46(1.24-1.71)	<0.001	0.99(0.81-1.20)	0.887
T stage				
T1	Reference			
T2	0.41(0.28-0.61)	<0.001	0.50(0.33-0.76)	0.001
T3	0.49(0.39-0.61)	<0.001	0.67(0.51-0.87)	0.003
T4	0.80(0.64-1.01)	0.056	0.86(0.65-1.12)	0.262
N stage				
N0	Reference			

(Continued)

TABLE 2 Continued

Characteristics	Univariate analysis Hazard ratio (95% CI)	*P*	Multivariate analysis Hazard ratio (95% CI)	*P*
N1	1.25(1.05-1.50)	0.013	1.39(1.15-1.68)	**0.001**
N2	1.54(1.29-1.84)	<0.001	1.43(1.12-1.82)	**0.004**
CEA				
Negative	Reference			
Positive	1.37(1.14-1.66)	<0.001	1.26(1.04-1.52)	**0.019**
Unknown	1.48(1.20-1.82)	<0.001	1.17(0.94-1.45)	0.155
Surgery				
No	Reference			
Surg Prim Site	0.60(0.51-0.70)	<0.001	0.46(0.31-0.69)	**<0.001**
Surg Dis Site	0.49(0.26-0.73)	0.028	0.54(0.28-0.89)	**0.041**
Surg Com Site	0.34(0.28-0.42)	<0.001	0.29(0.19-0.45)	**<0.001**
Radiotherapy				
No/Unknown	Reference			
Yes	0.68(0.56-0.81)	<0.001	1.01(0.80-1.27)	0.943
Chemotherapy				
No/Unknown	Reference			
Yes	0.51(0.42-0.61)	<0.001	0.49(0.40-0.60)	**<0.001**

CEA, carcinoembryonic antigen; Surg Prim Site, primary site surgery; Surg Dis Site, distant metastasis site surgery; Surg Com Site, primary and distant metastasis site combined surgery. P values in bold indicate $p < 0.05$.

from other sites, including the lung, brain, and bone. If only colorectal cancer liver metastasis is analyzed without eliminating the combined metastasis of other organs, this will impact the prognosis of survival for individuals with colorectal cancer liver metastasis. To further investigate the factors independently determining the prognosis of patients with liver metastasis, we analyzed 2,127 individuals with only liver metastasis to fill this gap.

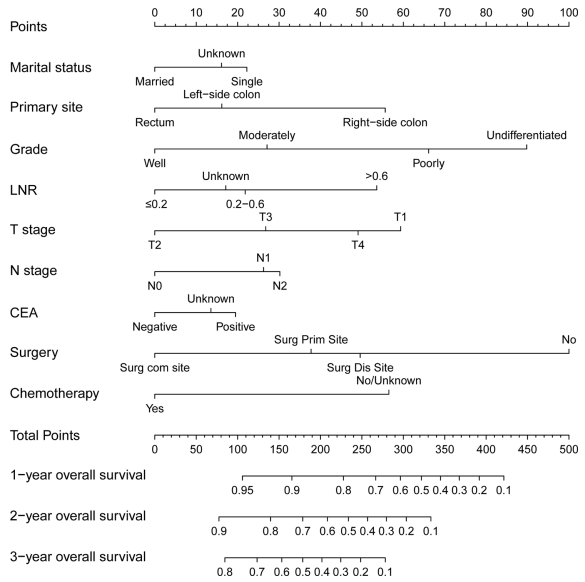

FIGURE 2
A nomogram for predicting 1-, 2- and 3-year overall survival of patients with young-onset colorectal cancer with synchronous liver-only metastases.

Nine parameters were considered in our model and assigned to various risk scores, which might reflect their influence on the decision. The current findings confirmed our hypothesis and made several important discoveries. Our Nomogram shared some variables with earlier research on predicting the survival of CRC with Liver-Only metastases. In our model, some characteristics, such as grade, T stage, N stage, primary tumor location, and chemotherapy, were assigned a high-risk score, which was also acknowledged mainly in other research (15, 16).

In our Nomogram, marital status was revealed as a significant prognostic factor, and the prognosis of married patients is better than that of unmarried patients, which was similar to several cancers in the previous study (17–19). The reason may be that unmarried cancer patients exhibit more remarkable anguish, sadness, and anxiety than their married counterparts (20), and married patients are more likely to adhere to therapy, which may improve cancer management (21). The T1 stage had the highest risk ratings, indicating those patients had the poorest survival prognosis. It is evident that this phenomenon is contrary to our common sense. However, a study by Lupo Wu et al. also linked this occurrence to the different genetic makeup of T1-stage tumors (22). The results demonstrated the need for increased surveillance and screening of YO-CRCSLM with an early T stage. Moreover, a higher N stage and a poorer tumor grade predicted worse survival,

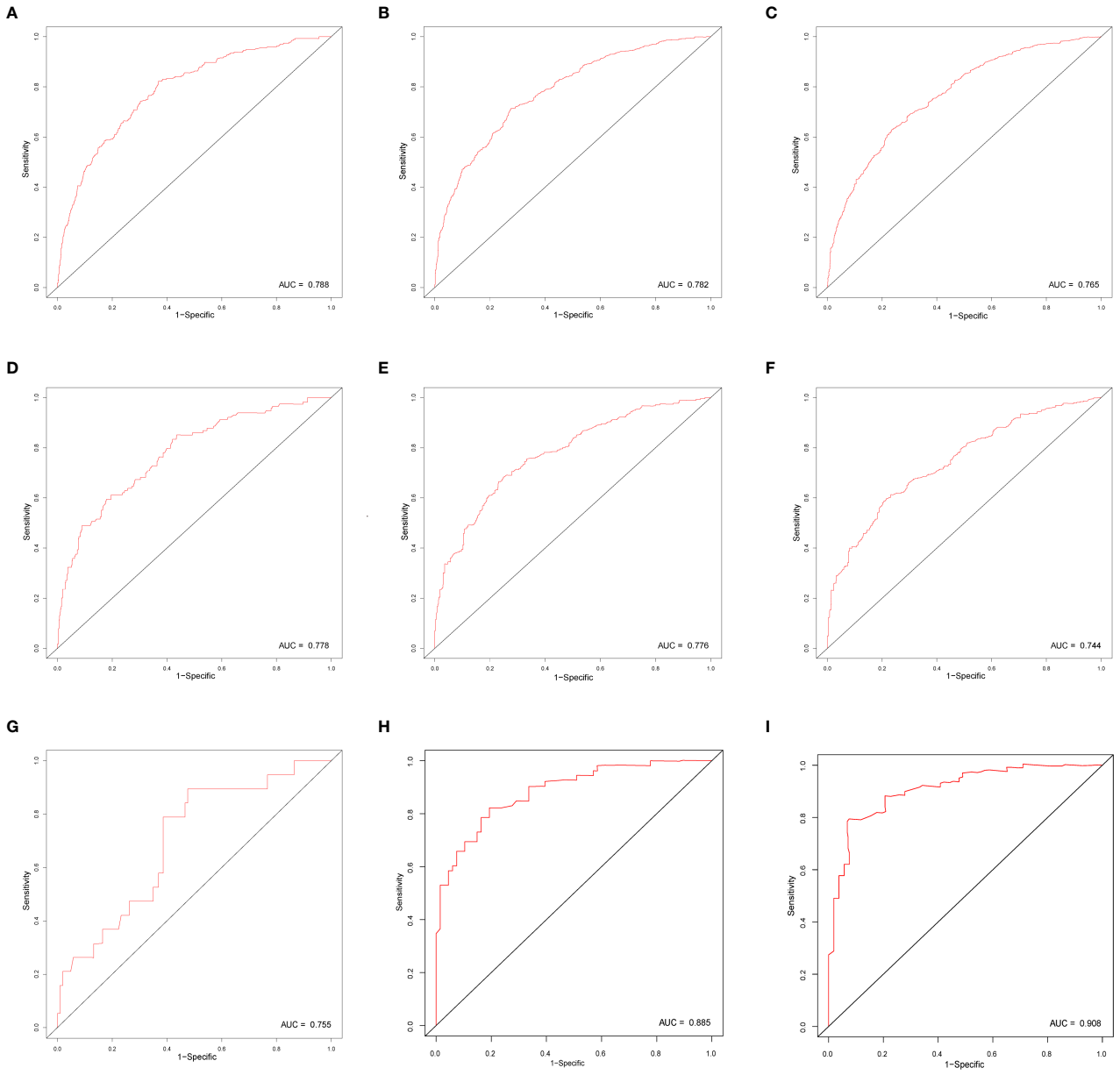

FIGURE 3
The ROC curve of nomograms to predict 1-, 2-, and 3-year overall survival in the training cohort **(A–C)**, validation cohort **(D–F)**, and testing cohort **(G–I)**.

which was similar to the previous study (23). The primary tumor locations served were a significant risk factor that might impact survival prognosis in this models, and this observation has also been confirmed by other studies (24–26). One research showed that patients with right-sided disease had worse survival outcomes than those with left -sided disease (12). Moreover, according to Shida et al.'s national multicenter retrospective study, right-sided CRC (RCRC) patients had a significantly lower OS than left-sided CRC (LCRC) patients (27). Several studies showed that this phenomenon was influenced considerably by histology and molecular traits because RCRC and LCRC have entirely different gene profiles (27–29). RCRC tends to exhibit an advanced clinical behavior than LCRC due to it has more mucinous histopathology, microsatellite instability, CpG island methylation, and BRAF mutations. In contrast, LCRC features many p53 and KRAS alterations (28, 30). Some previous studies demonstrated that preoperative serum CEA significantly affected the prognosis of CRC patients, which was consistent with our result (31, 32). Therefore, CEA might be crucial in the prognosis of CRCSLM, but more research was required to confirm the findings. This study concurs with previous findings that a high lymph node ratio (LNR) is strongly associated with poor overall and disease-free survival in metastatic colorectal cancer (33, 34). Surgery is crucial to the prognosis of cancer patients undergoing treatment. The

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TABLE 3 Score of each clinicopathological variable in our nomogram.

	Nomogram score of liver metastasis
Marital Status	
Single	22
Married	0
Unknown	16
Primary Site	
Right-side colon	56
Left-side colon	16
Rectum	0
Grade	
I	0
II	27
III	66
IV	99
LNR	
x<=0.2	0
0.2-0.6	22
x>0.6	54
Unknown	17
T stage	
T1	59
T2	0
T3	27
T4	49
N stage	
N0	0
N1	26
N2	30
CEA	
Negative	0
Positive	19
Unknown	14
Surgery	
No	100
Sur prim Site	38
Sur Dis Site	50
Sur Com Site	0
Chemotherapy	
No/Unknown	63
Yes	0

CEA, carcinoembryonic antigen; Surg Prim Site, primary site surgery; Surg Dis Site, distant metastasis site surgery; Surg Com Site, primary and distant metastasis site combined surgery.

advantages of primary tumor resection in CRCLM are still up for debate. In fact, primary tumor surgery has been performed in more than two-thirds of older individuals with stage IV CRC (35). This is because primary tumors may stimulate the development of metastasis and have severe consequences, such as obstruction, perforation, and bleeding, that can dramatically diminish patients' survival rates (36, 37). In addition, the CRC patients' autoimmunity may be enhanced through primary tumor resection (36). Previous studies had demonstrated the benefit of removing the primary tumor (10, 38, 39), while others had shown no clinical advantages for primary tumor resection (40, 41). In our study, initial tumor excision resulted in notable patient OS increases, which may enhance the survival and quality of life of YO-CRCSLM patients. Especially, our study suggests that performing surgery to remove both the primary tumor and synchronous liver metastasis may provide a substantial improvement in OS for YO-CRCSLM patients, and Chua et al. demonstrated that there were no significant statistical differences between simultaneous liver resection and staged liver resection in terms of overall survival in patients with synchronous liver metastasis from colorectal cancer (42), which indicates that simultaneous resection of primary colorectal cancer and liver metastases as a treatment strategy for YO-CRCSLM is safe and effective. In addition, chemotherapy is crucial in treating CRC patients and is one of the most prevalent techniques for treating colorectal cancer metastases. A retrospective study by Liu et al. indicated that CRCLM patients with chemotherapy had a better prognosis than those not (43). Similarly, in the current study, chemotherapy was demonstrated beneficial to OS.

This study has several advantages over prior research. On the one hand, our data underwent external validation in addition to internal validation, which increased the model's reliability. Moreover, our Nomogram included distinct factors, such as LNR, which were also found to be an important prognostic factor. However, our current study still remained several limitations, including its inevitable selection bias as a retrospective study. First, some critical information, such as chemotherapy medications and surgical procedures, was missing from the predictive model, which could impact its accuracy. Second, we developed the Nomogram from the extensive SEER database in the training cohort, whereas the testing cohort remained relatively small. Thus, large populations are needed to confirm the Nomogram's prediction capabilities. Third, it should be noted that a significant number of variables require a high level of information integrity in the constructed Nomogram, which may compromise its usefulness.

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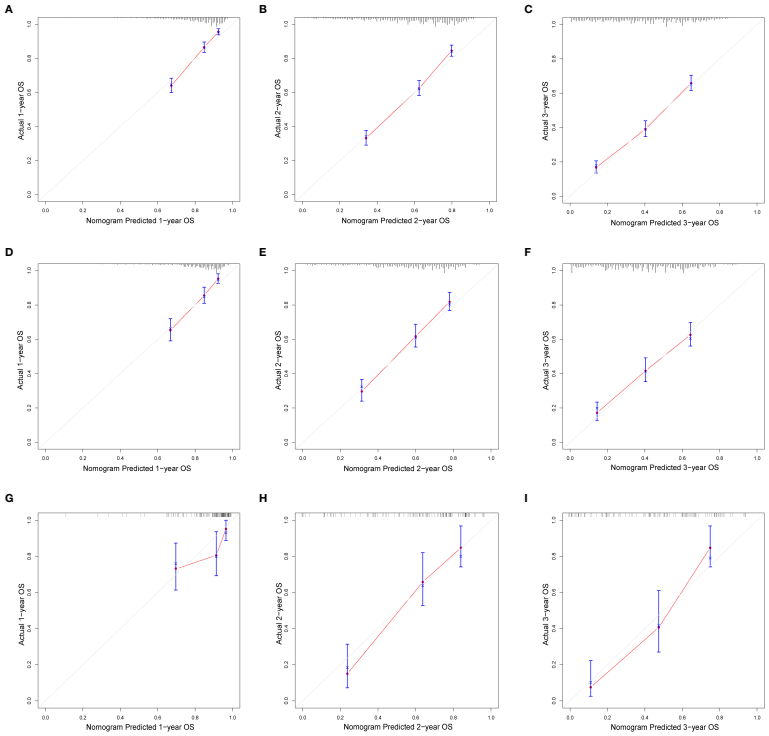

FIGURE 4
The calibration of nomograms to predict 1-, 2-, and 3-year overall survival in the training cohort **(A–C)**, validation cohort **(D–F)**, and testing cohort **(G–I)**.

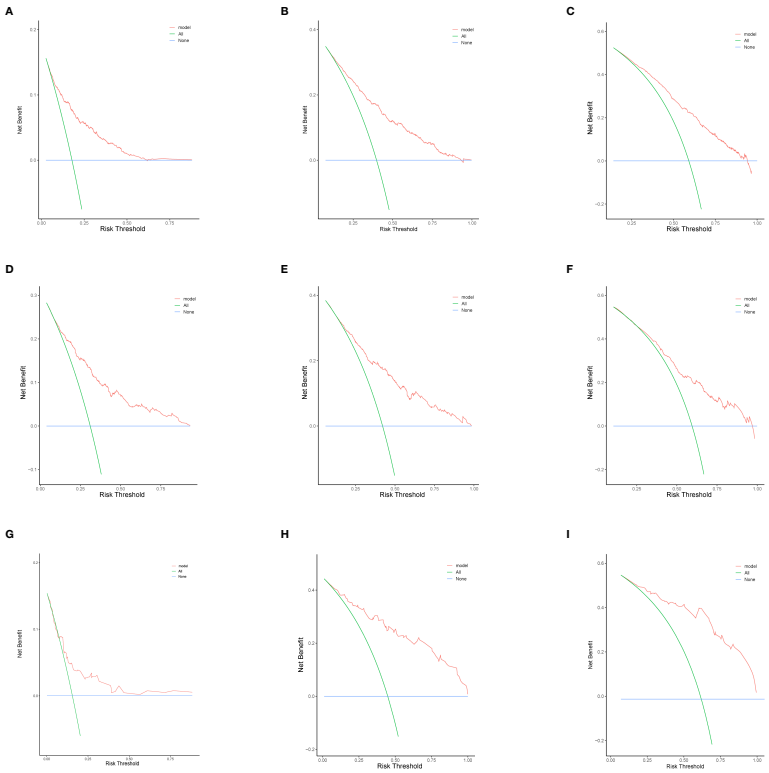

FIGURE 5
The decision curve analysis of nomograms to predict 1-, 2-, and 3-year overall survival in the training cohort **(A-C)** validation cohort **(D–F)**, and testing cohort **(G–I)**.

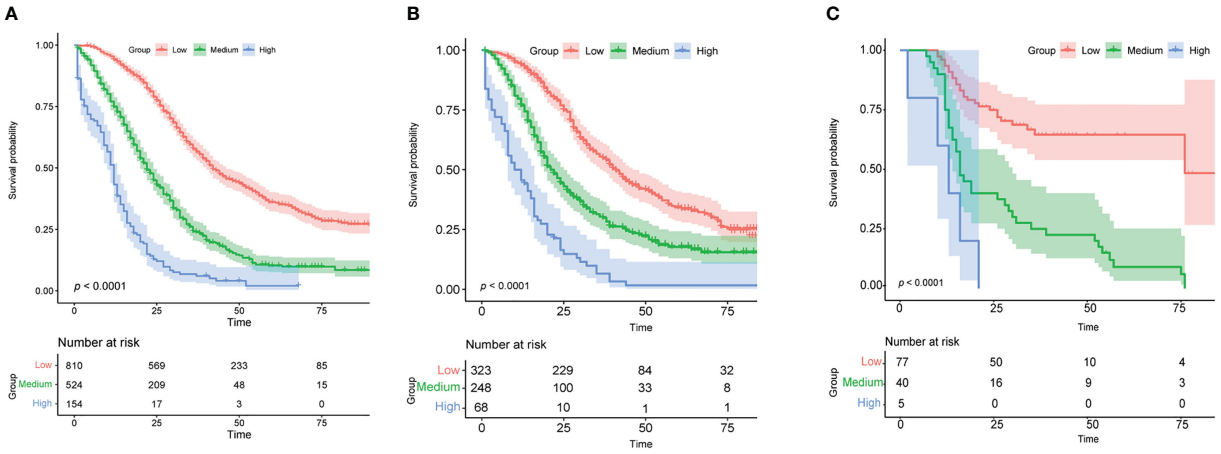

FIGURE 6
Analysis of survival based on risk stratification. Kaplan-Meier for patients categorized as low-risk, medium-risk, or high-risk in the training cohort **(A)**, validation cohort **(B)**, and testing cohort **(C)**.

In summary, a prognostic nomogram based on nine variables was constructed to predict overall survival in YO-CRCSLM patients, which could be a valuable tool for clinicians' decision-making. Finally, further research is needed in order to determine whether it is applicable to other patient groups.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the First Affiliated Hospital of Nanchang University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TYL and XL conceived and designed the study, acquisition and interpretation of data. TL was involved in interpretation of data and drafting of the manuscript; YL, ZZ and HS were involved in analysis, acquisition and interpretation of data; DW, ML and HL were involved in acquisition of data; TYL and XL were involved in critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1161742/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The cut-off values were determined using X-tile and the total patient scores from the training set. Patients in the training set were classified as low-risk (score < 234), moderate-risk (234≤score< 318), or high-risk (score≥318) according to the Nomogram for OS.

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A nomogram model for predicting distant metastasis of newly diagnosed colorectal cancer based on clinical features

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Objective: Colorectal cancer is one of the most common primary malignancies and the third most common cause of cancer death in both men and women in the United States. Among people diagnosed with initial colorectal cancer, 22% had metastatic colorectal cancer, while the 5-year survival rate was less than 20%. The purpose of this study is to develop a nomogram for predicting distant metastasis in newly diagnosed colorectal cancer patients and to identify high-risk groups.

Methods: We retrospectively reviewed the data of patients who were diagnosed with colorectal cancer at Zhong nan Hospital of Wuhan University and People's Hospital of Gansu Province between January 2016 and December 2021. Risk predictors for distant metastasis from colorectal patients were determined by the univariate and multivariate logistic regression analyses. Nomograms were developed to predict the probabilities of distant metastatic sites of colorectal cancer patients and evaluated by calibration curves, receiver operating characteristic curves, and decision curve analysis (DCA).

Results: A total of 327 cases were included in this study: 224 colorectal cancer patients from Zhong nan Hospital of Wuhan University were incorporated into the training set, and 103 colorectal cancer patients from Gansu Provincial People's Hospital were incorporated into the testing set. By univariate logistic regression analysis, platelet (PLT) level (p = 0.009), carcinoembryonic antigen (CEA) level (p = 0.032), histological grade (p < 0.001), colorectal cancer tumor markers (p < 0.001), N stage (p < 0.001), and tumor site (p = 0.005) were associated with distant metastasis in colorectal cancer patients. Multivariate logistic regression analysis showed that N stage (p < 0.001), histological grade (p = 0.026), and colorectal cancer markers (p < 0.001) were independent predictors of distant metastasis in patients initially diagnosed with colorectal cancer. The above six risk factors were used to predict distant metastasis of newly diagnosed colorectal cancer. The C-indexes for the prediction of the nomogram were 0.902 (95% confidence interval (CI), 0.857–0.948).

Conclusion: The nomogram showed excellent accuracy in predicting distant metastatic sites, and clinical utility may facilitate clinical decision-making.

KEYWORDS

colorectal cancer, distant metastasis, prognosis, nomogram, platelet count

Introduction

Colorectal cancer (CRC) is one of the most common primary malignant tumors and the third most common cause of cancer death in both men and women in the United States. In 2021, an estimated 1479,500 new cases and 52,980 deaths were projected in the United States (1). Among people diagnosed with initial colorectal cancer, 22% have metastatic CRC. Over the past 30 years, the incidence and overall survival (OS) rate of CRC have seen a significant improvement. The 5-year relative survival rate of CRC patients was approximately 65.1%. Although the prognosis of metastatic CRC is poor, with a 5-year survival rate of less than 20% (2, Accessed July 31, 2022), the survival rate has greatly improved because of the development of diagnosis and treatment schemes.

Metastatic CRC is defined as a metastatic disease or cancer that has spread beyond the original colorectal mass. The most common sites of distant metastasis include the liver, lung, and peritoneum (3). Many large sample studies (4–6) reported the cumulative metastatic rates of colorectal cancer in the liver (40% –50%), lung (10% –20%), and peritoneum (4%). Headways in the treatment of metastatic diseases, including improved surgical techniques, increased cancer-directed surgery, advances in the treatment of liver metastases, and the development of targeted therapies, are evident in survival gains for these patients in recent decades (3). It is clinically significant to detect distant metastasis (DM) in newly diagnosed CRC patients because early identification can help optimize treatment and management to increase the 5-year relative survival rate and quality of life.

In clinical practice, computed tomography (CT) is the most commonly used imaging examination to evaluate distant metastases of colorectal cancer patients. However, studies have reported that CT has a sensitivity of 65%–95% for colorectal cancer liver metastases with a diameter ≥ 1 cm, while it has a sensitivity of only 31%–38% for lesions with a diameter <1 cm, and the sensitivity further decreases if the patient has fatty liver (7). Recently, machine learning algorithms have played an important role in evaluating the metastasis and prognosis of malignant tumors. In gastric cancer, the literature reported seven machine learning algorithms to predict distant metastasis models, including logistic regression, random forest (RF), least absolute shrinkage and selection operator (LASSO) regression, support vector machine, k- nearest neighbor, naive Bayes model, and artificial neural network (8). David's research used 11 machine learning algorithms to predict the short- and long-term survival probability of CRC patients (9).

In the previous studies, many risk factors and prognostic variables were identified, including tumor markers, histological type, tumor location, platelet count (10), and tumor–node–metastasis (TNM) staging system. These factors are related to the prognosis of colorectal tumors (11). The prognosis of CRC patients varies in different clinicopathological factors, especially for colorectal cancer patients with distant metastasis. However, there is currently no predictive model for newly diagnosed Chinese colorectal cancer patients with distant metastasis, which means that the probability of outcome cannot be quantified.

Nomogram is a simple, multivariate visualization tool in which certain risk factors work together to predict and quantify the rate of the outcome of an individual patient (12). Therefore, in this study, we investigated clinicopathological factors in patients with colorectal cancer and aimed to develop a nomogram for predicting DM in newly diagnosed CRC patients. The results of this study will help to identify the high-risk groups of newly diagnosed colorectal cancer patients with DM according to the nomogram and help clinicians identify these patients early and choose appropriate treatment options, thereby improving prognosis and survival.

Materials and methods

Patients

The data included in the present study were obtained by two researchers at Zhong nan Hospital of Wuhan University and People's Hospital of Gansu Province from January 2016 to December 2021. The inclusion criteria were as follows: 1) patients diagnosed with colorectal cancer for the first time from 2016 to 2021; 2) demographic variables, including age, sex, and body mass index (BMI), were available; 3) hematology test indicators, including hemoglobin, platelet count, and colorectal cancer tumor markers (including carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), and carbohydrate antigen 19-9 (CA 19-9)); 4) all newly diagnosed patients with colorectal cancer underwent colorectal tumor resection at first hospitalization, or patients with distant metastases underwent primary resection at least. Detailed pathological data (including tumor size, diameter, TNM stage, and histological grade) were obtained. 5) Newly diagnosed colorectal cancers diagnosed with distant metastasis should be confirmed by at least two imaging examinations or histopathological diagnoses. The exclusion criteria were as follows: 1) incomplete information, including demographic variables and hematology test indicators; 2) absence of important clinicopathological factors, such as grade, histological type, T stage, N stage, and M stage; 3) before obtaining pathological information, the patients underwent adjuvant therapy such as radiotherapy and chemotherapy; 4) patients with other malignant tumors (such as lung malignancies, hematological malignancies, and primary liver cancer). Finally, 327 patients were included to study the diagnostic risk factors of CRC patients with DM. Among them, 224 colorectal cancer patients from Zhong nan Hospital of Wuhan University were incorporated into the training set, and 103 colorectal cancer patients from Gansu Provincial People's Hospital were incorporated into the testing set. In the present study, patients in the training set were used to develop the nomogram, and patients in the testing set were used to validate it. This study is a retrospective study and was conducted with the consent of the Ethics Committee of Zhong nan Hospital of Wuhan University. The ethics number is 2023019K.

Statistical analysis

All statistical analyses in our present study were conducted with SPSS 26.0 and R software (version 4.2.0). Mean ± standard deviation (SD) was used to describe the quantitative data; number and percentage (N, %) were used to describe these categorical data. Student's *t*- test was used to compare differences in continuous variables between groups if the variables followed a normal distribution. The χ^2 test or Fisher's exact test was used for categorical variables. In the present study, a *p*-value < 0.05 (two-sided) was considered statistically significant. Univariate logistic analysis was applied to identify DM-related factors. The variables with *p*- value < 0.05 in the univariate logistic analysis were included in the multivariate binary logistic regression analysis to determine independent risk factors of DM in initially diagnosed CRC patients. There are some indicators (including CEA level and platelet count), although the *p*- value >0.05 in multivariate analysis; they have important significance for the prognosis of colorectal cancer, which is also included to develop the nomogram. The predictive nomogram was developed by the "rms" package in R software, the "ROCR" package calculated the C-index, the "pROC" package calculated and plotted the receiver operating characteristic (ROC) curve, and the "rmda" package drew the calibration curve (CC)), decision curve analysis (DCA), and clinical impact curve. The ROC curve (13), C-index, and calibration curve were used to evaluate their performance. Moreover, DCA and clinical impact curve were also used to evaluate the stability of the model (14).

Results and discussion

Results

Clinical characteristics of the patients

According to inclusion and exclusion criteria, a total of 327 patients were included in this research: 224 colorectal cancer patients from Zhong nan Hospital of Wuhan University were incorporated into the training set, and 103 colorectal cancer patients from Gansu Provincial People's Hospital were incorporated into the testing set. The clinical characteristics of 327 patients are shown in Table 1.

Risk factors of distant metastasis in CRC patients and construction of predictive nomogram

The training set comprised 224 patients: 64 cases (28.6%) with DM at initial diagnosis and 160 cases (71.4%) without it (Table 2). The most common distant metastatic sites were the liver, lung, and peritoneum; some patients showed multiple- organ metastasis. For example, there were 37 patients with liver metastasis in the training set, accounting for 16.52% of the total population and 57.81% of the metastatic population. Through statistical analysis, the results showed that there were no significant differences in age (p = 0.662), sex (p = 0.096), body mass index (p = 0.590), hemoglobin level (p = 0.235), tumor size (p = 0.089), and T stage (p = 0.986) between non-metastatic colorectal cancer and metastatic colorectal cancer. Platelet (PLT) count, CEA, tumor markers, tumor site, lymph node stage, and histological grade (Grade) were statistically significant: PLT (p = 0.007), carcinoembryonic antigen (p = 0.028), tumor markers (p < 0.001), tumor site (p = 0.012), N stage (p < 0.001), and histological grade (p < 0.001). Notably, there was a statistically significant difference (p = 0.011) between the non-distant metastasis group and the distant metastasis group in the diagnosis year based on the COVID-19 epidemic. However, since the medical order and public life have gradually returned to normal, there is a bias in the variable of the year of diagnosis. To facilitate the subsequent use of the model, we did not include this variable in the formulation of the nomogram.

To identify DM-related variables in CRC patients, 11 predictors were analyzed using univariate logistic analysis. The results revealed six predictors that were associated with DM in CRC patients, including PLT level (p = 0.009), CEA level (p = 0.032), histological grade (p < 0.001), colorectal cancer tumor markers (p < 0.001), N stage (p < 0.001), and tumor site (p = 0.005). Moreover, multivariate logistic analysis was performed on these six factors and showed that N stage (p < 0.001), histological grade (p = 0.038), and colorectal tumor markers (p < 0.001) were independent predictors for distant metastasis of colorectal cancer in newly diagnosed CRC patients (Table 3). Through the above three predictive factors (N stage, histological grade and colorectal tumor marker) and six predictive factors (PLT level, CEA level, tumor site, N stage, histological grade and colorectal tumor marker), the prediction models were established respectively, and it was found that there was no significant difference in C- index between the two models. Ultimately, based on the six DM-related variables, a diagnostic nomogram was developed for the risk assessment of DM in newly diagnosed CRC patients (Figure 1).

Validation of training set for predictive nomogram

In the training set, we used ROC curves and C-index values to appraise the discrimination abilities of the nomogram. The C-index of the training set for predicting distant metastases was 0.902. The ROC curve of the training set was established, and the area under the curve (AUC) of the training set nomogram was 0.902 (95% CI, 0.857–0.948) (Figure 2). Furthermore, we also used a calibration curve, which is a novel method for appraising alternative prognostic instruments, and the DCA curve indicated that this nomogram can serve as an excellent diagnostic tool for DM in newly diagnosed CRC patients (Figure 2).

Validation of testing set for predictive nomogram

The testing set comprised 103 patients: 33 cases (32%) with DM at initial diagnosis and 70 cases (68%) without it. Similarly, univariate logistic analysis and multivariate logistic analysis were performed for six factors: platelet count, tumor markers, CEA, N stage, tumor site, and histological grade. The results showed that the platelet count (p = 0.010), tumor markers (p < 0.001), N stage (p < 0.001), tumor site (p < 0.001), and histological grade (p = 0.003) were statistically significant. Tumor markers (p = 0.001), N stage (p

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TABLE 1 Clinical and pathological features of patients diagnosed with CRC.

	Training set (224)	Validation set (103)
Age	56.99 ± 12.95	47.91 ± 12.05
Sex		
Female	96 (42.9%)	40 (38.8%)
Male	128 (57.1%)	63 (61.2%)
Year		
Before the COVID-19 pandemic	70 (31.25%)	85 (82.5%)
During the COVID-19 pandemic	154 (68.75%)	18 (17.5%)
BMI (kg/m^2)	23.46 ± 3.16	22.23 ± 2.99
Hypertension		
Yes	64 (28.6%)	11 (10.7%)
No	160 (71.4%)	92 (89.3%)
Diabetes		
Yes	27 (12.1%)	4 (3.9%)
No	197 (87.9%)	99 (96.1%)
Smoking		
Yes	51 (22.8%)	15 (14.6%)
No	173 (77.2%)	88 (85.4%)
Drinking		
Yes	28 (12.5%)	15 (14.6%)
No	196 (87.5%)	88 (85.4%)
HGB (g/L)	115.90 ± 24.31	121.19 ± 27.59
PLT (10^9/L)	246.56 ± 86.78	250.50 ± 110.84
Tumor markers*		
Positive	101 (45.1%)	56 (54.4%)
Negative	123 (54.9%)	47 (45.6%)
CEA (ng/ml)	51.32 ± 248.31	39.59 ± 140.83
Site		
Right	45 (20.1%)	20 (19.4%)
Left	50 (22.3%)	12 (11.7%)
Rectum	129 (57.6%)	71 (68.9%)
Size (cm)		
<5 cm	125 (55.8%)	59 (57.3%)
≥5 cm	99 (44.2%)	44 (42.7%)
Grade		
I	17 (7.6%)	4 (3.9%)
II	166 (74.1%)	73 (70.9%)
III	41 (18.3%)	26 (25.2%)

(Continued)

TABLE 1 Continued

	Training set (224)	Validation set (103)
T stage		
T1–T2	40 (17.9%)	17 (16.5%)
T3–T4	184 (82.1%)	86 (83.5%)
N stage		
N0	102 (45.5%)	51 (49.5%)
N1–2	120 (53.57%)	52 (50.5%)
M stage		
M0	160 (71.4%)	70 (68%)
M1	64 (28.6%)	33 (32%)
Metastatic sites		
Liver	37 (16.52%)	19 (18.45%)
Lung	16 (7.14%)	7 (6.80%)
Peritoneum	10 (4.46%)	6 (5.82%)
Pelvic cavity	6 (2.68%)	6 (5.82%)
Other distant diseases	3 (1.34%)	2 (1.94)

CRC, colorectal cancer; BMI, body mass index; HGB, hemoglobin; PLT, platelet; CEA, carcinoembryonic antigen; CA 125, cancer antigen 125; CA 19-9, carbohydrate antigen 19-9.
*Tumor marker positive means hematologic CEA > 5 ng/ml, or CA 125 > 35 U/ml, or CA 19-9 > 37 U/ml.

= 0.006), and tumor site ($p < 0.001$) were independent risk factors for distant metastasis of newly diagnosed colorectal cancer (Table 4). The statistical analysis results of the testing set were basically consistent with the results of the training set, which indicated that the six risk factors included in our study had good stability and universality, and the distant metastasis prediction model developed had high clinical practicability.

According to the data of the testing set, we also established the ROC curve, calibration curve, and DCA curve. The AUC of the testing set nomogram was 0.916 (95% CI, 0.836–0.973). The calibration curve indicated good stability, and the DCA curve showed high net benefits of the diagnostic nomogram (Figure 3).

ROC curves for each risk factor in training set and testing set

More importantly, the ROC curves of each predictor were also generated in both the training set and the testing set (Figure 4). In the testing set, the AUC was as follows: PLT count (AUC = 0.566), CEA (AUC = 0.775), histological grade (AUC = 0.623), colorectal cancer tumor markers (AUC = 0.764), N stage (AUC = 0.764), and tumor site (AUC = 0.624). In the testing set, the AUC was as follows: PLT count (AUC = 0.588), CEA (AUC = 0.728), histological grade (AUC = 0.641), colorectal cancer tumor markers (AUC = 0.753), N stage (AUC = 0.760), and tumor site (AUC = 0.753). The results showed that the AUC of all predictors alone was lower than the AUC of the nomogram, regardless of the training set or the testing set. In conclusion, the predictive diagnostic model can identify patients with a high risk of distant metastasis from newly diagnosed CRC patients.

Discussion

This study retrospectively analyzed the clinical data of 327 patients with colorectal cancer (224 patients in the training set and 103 patients in the testing set), and the results showed that 98 patients (29.97%) (64 patients in the training set and 34 patients in the testing set) had developed distant metastases at the first visit, with an average age of 55 years. We found that platelet counts greater than 350 ($10 * 10^9$/L), positive tumor markers, lymph node stage (N stage N1–N2), tumor histological grade (grade III), tumor location in the right colon, and high carcinoembryonic antigen concentration were associated with distant metastasis of colorectal cancer. Among these variables, tumor markers, lymph node stage, and histological grade were independent risk factors for distant metastasis.

In this study, we also found that patients with colorectal cancer who were first diagnosed during the COVID-19 epidemic had a higher risk of distant metastasis, which may be related to the delay in screening and diagnosis. The COVID-19 pandemic era impacted medical institutions/systems in various countries. The enormous diversion of medical resources toward SARS-CoV-2-dedicated wards dominated the clinical scenarios, with almost all planned public healthcare activities, including cancer screening, being suspended. A study in the United Kingdom (15) pointed out the detrimental effects on mortality of delaying diagnosis in symptomatic patients with CRC because of the SARS-CoV-2 pandemic. Recent data from Italy (16) also indicated that due to the impact of COVID-19, screening delays beyond 4–6 months would significantly increase advanced CRC cases and also mortality if lasting beyond 12 months. A large retrospective study from the

TABLE 2 Clinical and pathological features between distant and non-distant metastases of the training set.

	CRC without DM (N = 160)	CRC with DM (N = 64)	p
Age	57.23 ± 12.77	56.39 ± 13.47	0.662
Sex			0.096
Female	63 (39.4%)	33 (51.6%)	
Male	97 (60.6%)	31 (48.4%)	
Year			0.011
Before the COVID-19	58 (36.2%)	12 (18.8%)	
During the COVID-19	102 (63.7%)	52 (81.2%)	
BMI (kg/m^2)	23.39 ± 3.24	23.64 ± 2.96	0.590
HGB (g/L)	117.12 ± 23.87	112.85 ± 25.33	0.235
PLT (10^9/L)			0.007
<350	146 (91.2%)	50 (78.1%)	
≥350	14 (8.8%)	14 (21.9%)	
CEA	16.70 ± 101.50	137.86 ± 426.15	0.028
Tumor markers			<0.001
Negative	112 (70.0%)	11 (17.2%)	
Positive	48 (30%)	53 (82.8%)	
Tumor size (cm)			0.089
<5 cm	95 (59.4%)	30 (46.9%)	
≥5 cm	65 (40.6%)	34 (53.1%)	
Tumor site			0.004
Right	24 (15%)	21 (32.8%)	
Left	34 (21.3%)	16 (25%)	
Rectum	102 (63.7%)	27 (42.2%)	
T stage			0.986
T1–2	40 (25%)	0 (0%)	
T3–4	120 (75%)	64 (100%)	
N stage			<0.001
N0	97 (60.6%)	5 (7.8%)	
N1–2	63 (39.4%)	59 (92.2%)	
Grade			<0.001
Grade 1	16 (10%)	1 (1.6%)	
Grade 2	126 (78.8%)	40 (62.5%)	
Grade 3	18 (11.2%)	23 (35.9%)	

CRC, colorectal cancer; DM, distant metastasis; BMI, body mass index; HGB, hemoglobin; PLT, platelet.

Journal of the American Medical Association (*JAMA*) (17) also compared patients with colorectal cancer during the pandemic period and the prepandemic period, and the results showed that the SARS-CoV-2 pandemic was significantly associated with an increased rate of advanced-stage colorectal cancer.

Colorectal cancer is a common invasive tumor of the digestive system that is prone to distant metastasis. Metastases are a major driver of CRC-related mortality, with the liver and lung being the most frequently affected organs (18). Approximately 22% of colorectal cancer patients have distant metastases on their first visit to the hospital; meanwhile, the 5-year survival rate of these patients is less than 20%. For patients with resectable metastatic CRC, surgical resection of metastases is the only curative treatment option. For patients with unresectable metastatic CRC (3), the

TABLE 3 Logistic analysis of risk factors of DM in training set CRC patients.

	Univariate (*p*)	OR	95% CI	Multivariate (*p*)	OR	95% CI
Age	0.660	0.99	0.97–1.11	–	–	–
Sex (male)	0.097	0.61	0.34–1.09	–	–	–
PLT (<350) (10^9/L)	0.009	2.92	1.30–6.55	0.125	2.44	0.77–7.72
HGB (g/L)	0.235	0.99	0.98–1.01	–	–	–
Tumor markers (negative)	<0.001	11.24	5.41–23.38	<0.001	7.52	3.18–17.77
CEA (ng/ml)	0.032	1.00	1.00–1.01	0.406	1.01	0.99–1.00
N stage (N0)	<0.001	18.17	6.91–47.75	<0.001	15.48	5.07–47.32
Site (right)	0.005			0.059		
Left	0.145	0.54	0.23–1.24	0.048	0.30	0.09–0.99
Rectum	0.001	0.30	0.15–0.62	0.027	0.31	0.11–0.88
BMI (kg/m^2)	0.589	1.03	0.94–1.13	–	–	–
Grade (I–II)	<0.001	4.43	2.18–8.98	0.038	2.55	1.05–6.21
Size (cm)	0.090	1.66	0.92–2.97	–	–	–

DM, distant metastasis; CRC, colorectal cancer; PLT, platelet; HGB, hemoglobin; CEA, carcinoembryonic antigen; BMI, body mass index.

primary treatment is systemic therapy (including cytotoxic chemotherapy, biologic therapy such as antibodies to cellular growth factors, immunotherapy, and their combinations). Early treatment of patients with distant metastases can improve their survival rate. In some practical clinical features, the National Comprehensive Cancer Network (NCCN) guidelines said that TNM stage, age, tumor differentiation grade, vessel invasion, performance status, and tumor markers are important prognostic factors (19). Therefore, in this study, we established a nomogram based on clinical data and pathological features to predict the risk of distant metastasis in newly diagnosed CRC patients. The total score can be calculated by obtaining data on several easily accessible

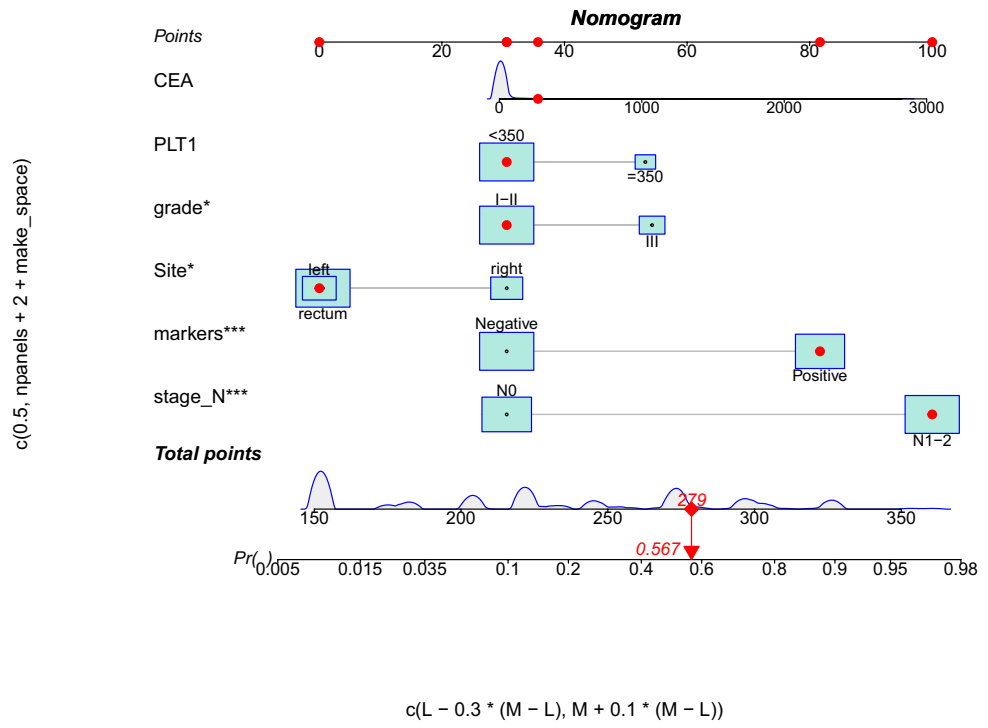

FIGURE 1
Nomogram for predicting DM from CRC patients. DM, distant metastasis; CRC, colorectal cancer.

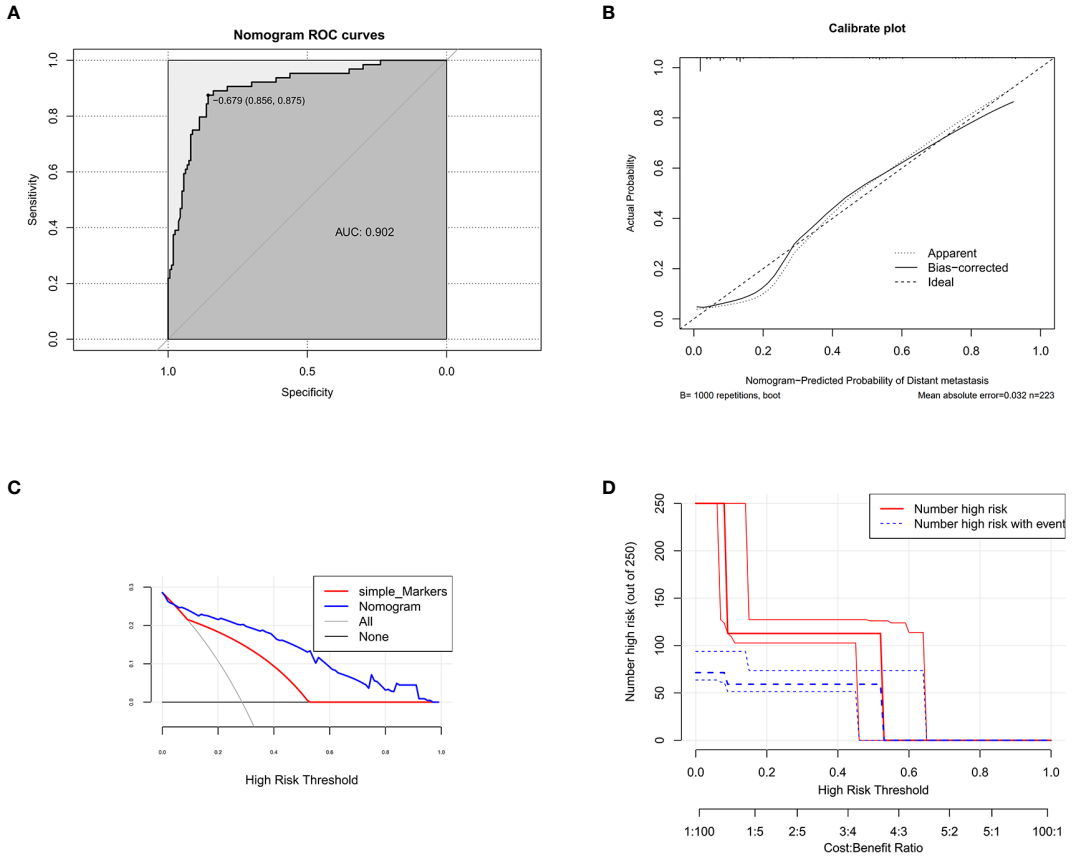

FIGURE 2
The receiver operating characteristic curve **(A)**, calibration curve **(B)**, decision curve analysis **(C)** (nomogram compared with tumor markers), and clinical impact curve **(D)** of the training set.

variables on the nomogram for each CRC patient. The risk of DM can then be easily identified on the nomogram, which will make the individualized clinical decision and clinical management more accurate.

The stratification theory of the left and right colon was proposed by American oncologist Bufill et al. in 1990 from the perspective of molecular genetics (20). Guideline (21) points out that the right side of the colon (cecum, ascending colon, and hepatic flexure) versus the left side of the colon (splenic flexure, descending colon, sigmoid, and rectosigmoid) and rectum represent a continuum of changes secondary to different embryological origins. Colorectal cancer is a heterogeneous malignant tumor with unique pathophysiological, anatomical, and clinical features. The location of tumor growth is an important factor affecting the progression, choice of treatment, and survival prognosis of colorectal cancer. Compared with that of the left colorectal tumor, the energy metabolism of the right colon tumor is mainly aerobic glycolysis of glucose, and tumor cells take advantage of aerobic glycolysis to decompose glucose and obtain

TABLE 4 Logistic analysis of risk factors of DM in the testing set CRC patients.

	Univariate (*p*)	OR	95% CI	Multivariate (*p*)	OR	95% CI
PLT (<350) (10^9/L)	0.010	8.96	1.7–44.49	0.35	3.36	0.26–42.7
Tumor markers (negative)	<0.001	12.4	3.92–39.22	0.001	15.65	3.06–79.97
CEA (ng/ml)	0.225	1.002	1.00–1.01	0.351	0.999	0.994–1.00
N stage (N0)	<0.001	11.6	3.97–33.92	0.006	7.17	1.76–29.22
Site (right)	<0.001			<0.001		
Left	0.844	0.86	0.18–3.98	0.769	1.49	0.1–21.51
Rectum	<0.001	0.09	0.03–0.27	0.003	0.08	0.02–0.44
Grade (I–II)	0.003	4.16	1.63–10.6	0.119	3.36	0.73–15.43

DM, distant metastasis; CRC, colorectal cancer; PLT, platelet; CEA, carcinoembryonic antigen.

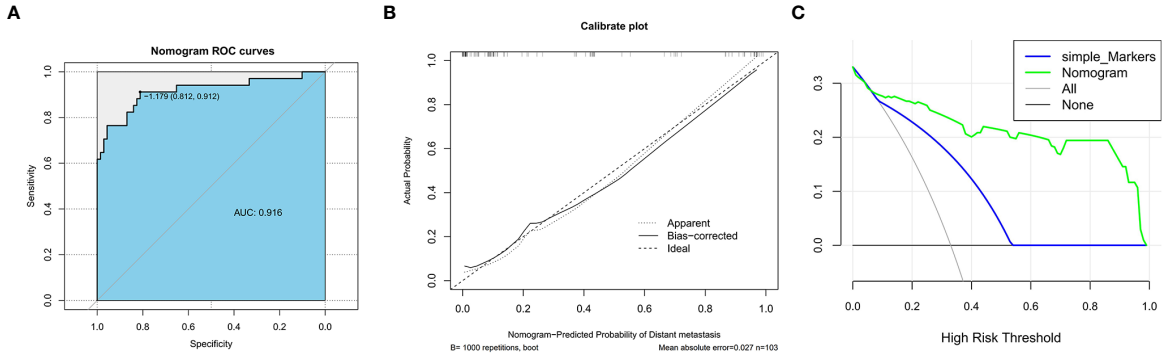

FIGURE 3
The receiver operating characteristic curve **(A)**, calibration curve **(B)**, and decision curve analysis **(C)** (nomogram compared with tumor markers) of the testing set.

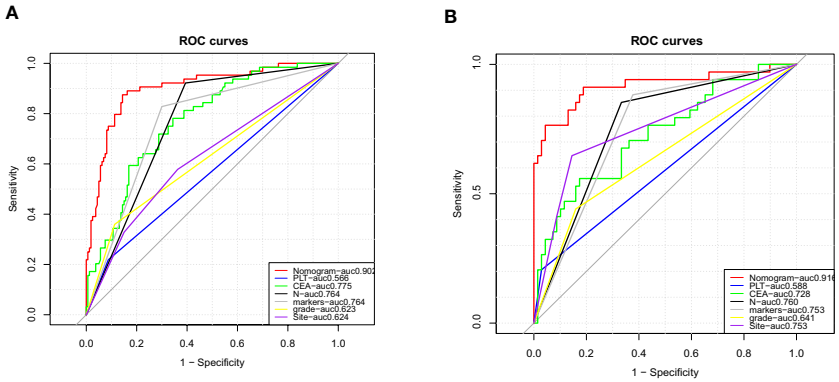

FIGURE 4
Comparison of area under the receiver operating characteristic curve between nomogram and each independent predictor in the training set **(A)** and the testing set **(B)**.

energy (22). In terms of tumor histopathology, mucinous carcinoma, undifferentiated carcinoma, and sigmoidal ring cell carcinoma were the most common tumors on the right side of the colon, with high histological grade and low differentiation, while the left side of the colon was dominated by adenocarcinoma with medium and high differentiation (23). In molecular biology, BRAF, PI3KCA, and TGFBR2 gene mutations and heat shock protein regulation disorders are common in right colon tumors. Conversely, left colon tumors are often rich in KRAS gene mutations, HGFR/HER2 amplification, and high expression of amphiregulin and epithelial regulatory proteins (24). A systematic review in *JAMA* (25) also indicated that the side of the origin of CC (left *vs.* right) should be acknowledged as a criterion for establishing prognosis in both earlier and advanced stages of the disease. These show that right colon tumors are more invasive than left colon tumors.

Serological tumor markers are non-invasive and cost-effective indicators for the diagnosis, treatment, and prognosis of colorectal cancer. CEA and CA 199 are the two most common tumor markers used in colorectal cancer (26). The American Society of Clinical Oncology (ASCO) and the European Panel on Tumor Markers (EGTM) recommend CEA levels as a marker for follow-up after curative surgical resection of colorectal cancer. Rising levels indicate tumor recurrence after surgery or the development of metastatic disease (26). Some studies have also shown that an elevated preoperative CEA level is associated with a poorer prognosis and an increased risk of malignant tumor recurrence (27). Several other serological tumor markers, including CA 125, cancer antigen 72-4 (CA 72-4), and combined serum tumor biomarker levels, were positively correlated with tumor stage (28).

In this study, we found that three patients did not show definite metastases on preoperative imaging examination but were found to have metastases on imaging reexamination less than 10 days after surgery. Therefore, the risk factors selected by logistic regression analysis and the developed model can be used to quantitatively score whether each newly diagnosed colorectal cancer patient is at risk of distant metastasis and identify high-risk groups. 1) For high-risk patients without metastasis detected by the first imaging examination, clinicians need to further improve the evaluation of MRI (or PET-CT) and other imaging examinations, shorten the follow-up time of high-risk patients, and emphasize the importance of follow-up. 2)

Clinicians should recommend molecular pathological testing for high-risk patients as early as possible. 3) For low-risk patients, the follow-up time can be appropriately extended to achieve individualized management for different patients.

However, several limitations to our study should be noted. First, this study is a retrospective study, which inevitably suffers from selection bias. Second, a limited number of patients (N = 327) included in this study may lead to possible errors. Therefore, follow-up studies need more prospective studies involving patients.

Conclusions

Our study showed that N stage, grade, tumor markers, tumor site, preoperative CEA level, and platelet level were the risk factors for DM from CRC. N stage, grade, and tumor markers were the independent predictors. The nomogram we created may be a personalized, convenient, and more intuitive visualization tool for DM risk assessment in CRC.

Preprint

A preprint has previously been published (https://doi.org/10.21203/rs.3.rs-2118512/v1) [17].

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study is a retrospective study and was conducted with the consent of the Ethics Committee of Zhong nan Hospital of Wuhan University. The ethics number is 2023019K.

Author contributions

J-HH collected the data, wrote the article, and analyzed the statistics. CC collected the data. YD, YY, Y-QL, and CW revised the article. YC provided fund support and revised the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Case report: Irinotecan-induced interstitial lung disease in an advanced colorectal cancer patient resurfacing decades after allogeneic bone marrow transplantation for aplastic anemia; a case report and narrative review of literature

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Two mechanisms of drug-induced interstitial lung disease (DILD) have been reported: 1) direct injury of lung epithelial cells and/or endothelial cells in lung capillaries by the drug and/or its metabolites and 2) hypersensitivity reactions. In both mechanisms, immune reactions such as cytokine and T cell activation are involved in DILD. While past and present lung diseases and accumulative lung damage due to smoking and radiation are risk factors for DILD, the association between the immune status of the host and DILD is not well known. Herein, we report a case of advanced colorectal cancer with a history of allogeneic bone marrow transplantation for aplastic anemia more than 30 years prior, in which DILD occurred early after irinotecan-containing chemotherapy. Bone marrow transplantation might be a potential risk factor for DILD.

KEYWORDS

drug-induced interstitial lung disease (DILD), bone marrow transplantation (BMT), irinotecan, colorectal cancer, graft-versus-host disease (GVHD), late-onset non-infectious pulmonary complication (LONIPC)

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1 Introduction

Bone marrow transplantation (BMT) is an important treatment option for patients with hematologic malignancies and severe hematopoietic and immune system disorders (1, 2). While long-term survival can be obtained after BMT, recipients are also reported to be at an increased risk of secondary cancers (3–5). As the probability of secondary cancer increases with prolonged survival after BMT, the number of BMT recipients receiving chemotherapy for secondary cancers has recently been increasing (6–8). However, there are few reports on the efficacy and side effects of chemotherapy for secondary cancers in recipients of BMT. Here, we report a case of incurable interstitial lung disease induced by irinotecan-containing chemotherapy for advanced colorectal cancer more than 30 years after allogeneic BMT.

2 Case description

A 52-year-old male was diagnosed with advanced colorectal cancer, with multiple metastases to the lungs, liver, and peritoneum. Histology revealed well-to-moderately differentiated adenocarcinoma, in which the *KRAS* and *NRAS* mutation tests were negative and microsatellite instability was stable, but the $BRAF^{V600E}$ mutation was positive. In his medical history, he had received an allogeneic BMT from his elder sister for severe aplastic anemia 33 years ago. As preparative therapy prior to BMT, the patient received total lymph node irradiation (7.5 Gy) and cyclosporine at 50 mg/kg for four days. Only the bilateral lung apices were involved in the irradiated field, but no pulmonary changes suggestive of radiation pneumonitis were noted thereafter. He experienced a grade 1 skin rash as an acute graft-versus-host disease (GVHD) symptom after BMT, which disappeared clinically without treatment; however, a skin biopsy performed several months later showed pathological findings suspicious of chronic GVHD. During follow-up, there was no worsening of GVHD at the skin or other sites, including interstitial pneumonia, and the patient received no immunosuppressive agents at the time of diagnosis of colon cancer. He had no history of smoking or known allergies. The patient had received two doses of COVID-19 vaccine manufactured by Moderna 9 and 10 months prior to the diagnosis of colorectal cancer and polymerase chain reaction (PCR) test for COVID-19 was negative multiple times during the DILD treatment. The vaccination history other than COVID-19 was unknown. He had type 2 diabetes and used long-acting insulin. Because the sigmoid colon tumor invaded the left ureter and lower gastrointestinal endoscope could not pass through the primary site, the patient underwent transverse colostomy for colon obstruction due to the tumor. Before receiving chemotherapy, the patient experienced a severe cough due to lung metastasis but had no respiratory distress, with a pulse oxygen saturation (SpO2) of 95% in room air. Chest computed tomography (CT) showed multiple pulmonary nodules, but no abnormal findings suggesting accumulative lung damage such as fibrosis (Figure 1). All screening tests including blood cultures for bacterial infections and serological examinations for viral infections were negative. One month after surgery, 1st-line chemotherapy with FOLFIRI (5-fluorouracil [FU] bolus 400 mg/m^2 + leucovorin 400 mg/m^2 + irinotecan 150 mg/m^2 followed by 5-FU 2400 mg/m^2 in a 46-hour infusion) + bevacizumab (5 mg/kg) with palonosetron hydrochloride and dexamethasone as prophylactic anti-emetic therapy was started. He developed a fever without myelosuppression on day 6; however, the bacterial culture was negative, and there was no new lung infiltration on chest radiography. Naproxen (600 mg/day) was administered for the noninfectious fever, which quickly resolved. He was discharged from the hospital in a good general condition and received a second course of chemotherapy at our outpatient clinic with no severe adverse events. On day 15 of the second course of chemotherapy, the patient had no dyspnea. However, because his SpO2 was 92% in room air, the patient was admitted for oxygen demand. A CT scan showed no apparent tumor growth, but there were slight bilateral ground-glass shadows (Figure 2). A bronchoscopy was performed on the same day. There were no abnormalities in the endotracheal lumen such as apparent redness, edema, or neoplastic lesions; bronchoalveolar lavage (BAL) was performed in the middle lobe, and a biopsy of the peripheral lung was performed in the lower lobe of the right lung. The pathology showed that there was only fibrosis around the tumor as a tumor environment, and there was no obvious fibrosis in the background lungs consistent with the CT findings (Supplementary Figure 1). Bacterial and fungal cultures and PCR tests for *pneumocystis jirovecii* DNA and cytomegalovirus (CMV) DNA of the BAL fluid were negative. Pathology of the lung biopsy showed pulmonary metastasis of the colon cancer and no

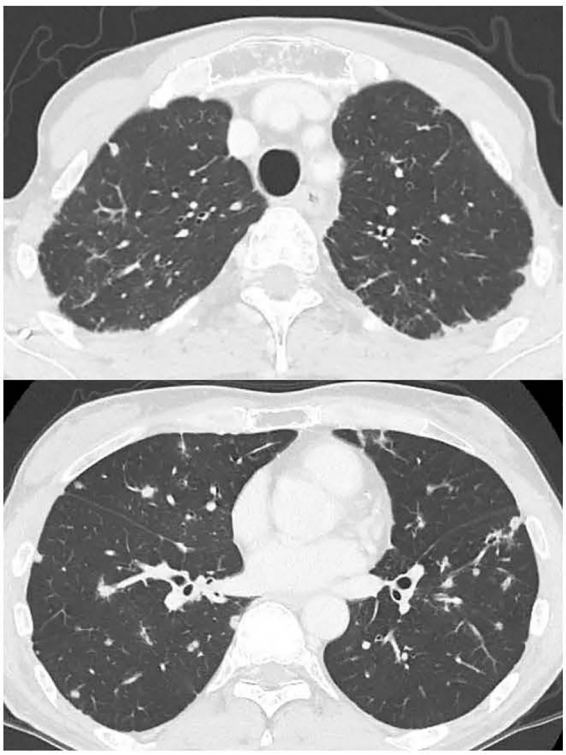

FIGURE 1
Chest CT showing multiple lung involvements at initial diagnosis.

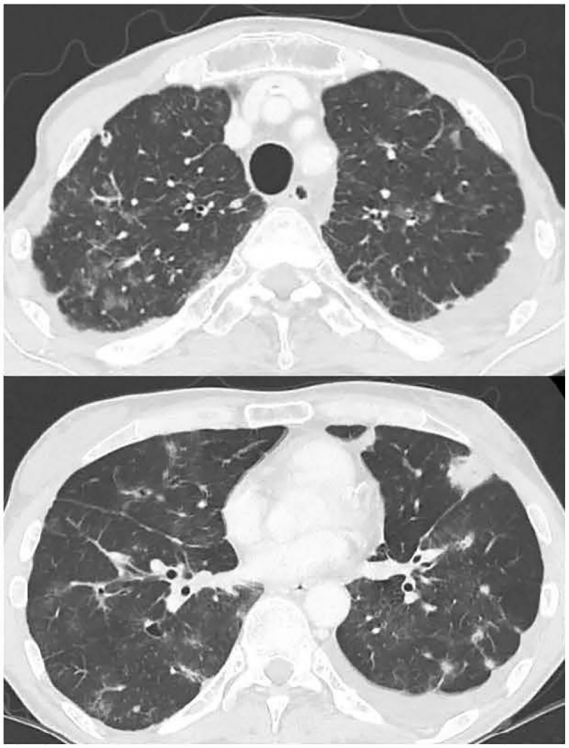

FIGURE 2
Chest CT showing the bilateral lung interstitial infiltrations.

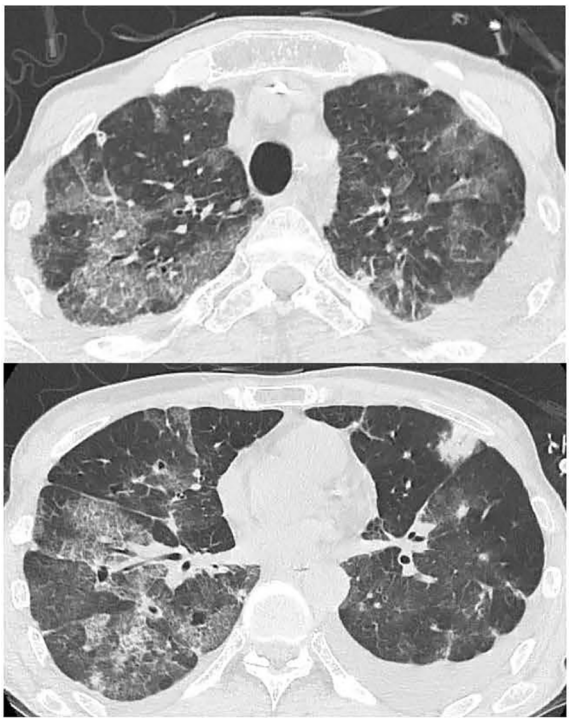

FIGURE 3
Chest CT showing exacerbation of bilateral lung interstitial infiltrate shadows on day 6 after admission.

apparent findings of inflammation. Serum lactate dehydrogenase (LDH) and KL-6 levels were within the normal ranges, and the βD-glucan test was negative. The white blood cell count was 2,740/μL, neutrophil count 1,140/μL, and lymphocyte count 880/μL. The interferon-gamma release assay for tuberculosis was negative. Differential diagnoses were interstitial pneumonitis or atypical pneumonia. The clinical course after onset of DILD is summarized in the Supplementary Figure 2. There were no apparent clinical manifestations of chronic GVHD around the time of DILD. As his respiratory status was stable, the antimicrobial agent levofloxacin was initiated. Four days after admission, the patient's respiratory status was stable. However, at night on day 5, the cough and dyspnea worsened and the oxygen demand increased. CT examination revealed expanded bilateral interstitial infiltrations (Figure 3). The white blood cell count was 6,260/μL, neutrophil count 5,440/μL, and lymphocyte count 720/μL, and the KL-6 level was mildly elevated (531 U/ml). On day 6, methylprednisolone (1 g/day) and piperacillin-tazobactam (4.5 g q6hr) were administered. Thereafter, the patient's respiratory condition improved rapidly, and the shadows in the bilateral lung fields disappeared on day 21 after starting steroid therapy (Figure 4). The steroid dose was gradually decreased and steroid administration was switched to oral prednisolone 30 mg/day after day 22. The prednisolone dose was reduced to 20 mg/day on day 33 after starting steroid treatment, and the patient was scheduled to be discharged. Immediately before discharge on day 39, the patient complained of abdominal pain. Abdominal CT revealed a retroperitoneal hematoma and pseudoaneurysm in the anterior

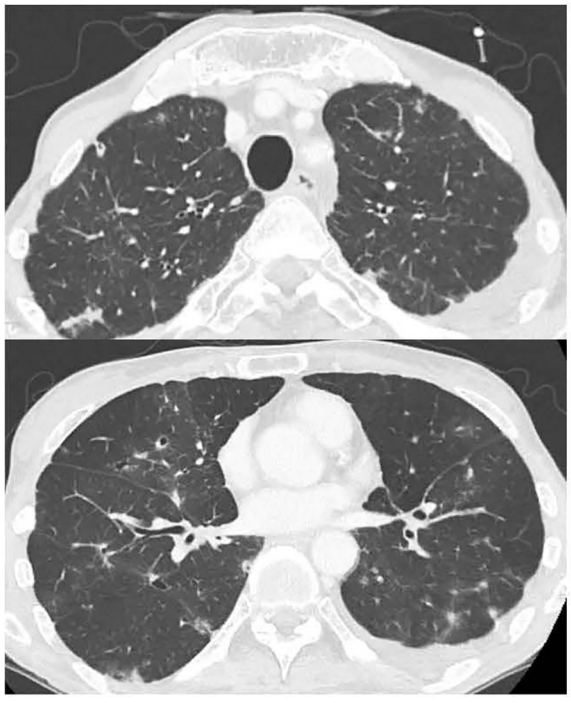

FIGURE 4
Chest CT showing the improvement in bilateral lung interstitial infiltrate shadows on day 21 of steroid treatment.

superior pancreatic duodenal artery. Because bleeding from the metastasis near the pancreatic head was suspected, the patient was transferred to a tertiary hospital, where intra-arterial embolization was performed. During the intervention, oral prednisolone was maintained at the same dose, and the patient returned to our hospital immediately after the intervention. On day 43, the lung infiltration reappeared. On day 44, steroid pulse therapy with methylprednisolone (500 mg/day) and piperacillin-tazobactam (4.5 g q6hr) was resumed. The methylprednisolone dose was tapered to 125 mg/day on day 47. On day 49, nasal high-flow therapy was initiated because oxygen demand increased. On the same day, intravenous cyclosporine (150 mg/day) was started after consultation with a hematologist, and methylprednisolone (500 mg/day) was retried. On day 51, laboratory tests reported the levels of aspartate aminotransferase (32 U/L), alanine transaminase (53 U/L), alkaline phosphatase (351 U/L), gamma-glutamyl transpeptidase (236 U/L), and total bilirubin (3.0 mg/dL). Considering the possibility of exacerbation of sepsis, an antifungal agent, micafungin (100 mg/day), was administered to treat all infectious diseases. On day 53, because serum CMV antigenemia tested on day 51 was positive (Cytomegalovirus antigen positive cell count was 200-206 positive cells per two slides, CMV IgG was > 250 AU/mL, CMV IgM INDEX was 1.04, absolute lymphocyte count was 280/μL), ganciclovir (500 mg/day) was added in combination with other antibiotics. The measurements of the same blood sample showed that the white blood cell count was 22,740/μL, neutrophil count 22,060/μL, lymphocyte count 280/μL, LDH 654 U/L, creatinine kinase 22 U/L, CRP 3.89 mg/dL, and βD-glucan test was negative. Unfortunately, the patient eventually died of respiratory and hepatic failure 56 days after starting the steroid therapy.

3 Discussion

The patient died of drug-induced interstitial lung disease (DILD) that developed shortly after irinotecan-containing chemotherapy for advanced colorectal cancer more than 30 years after allogeneic BMT. Two mechanisms of DILD have been reported: 1) direct injury of lung epithelial cells and/or endothelial cells in lung capillaries by the drug and/or its metabolites (9) and 2) hypersensitivity reactions (10). Although immune reactions such as cytokines and T cell activation are involved, and BMT may alter the immune status of the host, the association between BMT and DILD is not well known.

Past and present lung disease and cumulative lung damage due to exposure to toxic agents such as smoking and radiation are well-known risk factors for DILD, and BMT also causes cumulative lung damage as complications such as GVHD (11). In addition to acute GVHD, which is one of the most common non-infectious complications after BMT (12), long-term pulmonary injury after BMT is known as chronic GVHD. Lung-related late toxicity after allogeneic stem cell transplantation is called a late-onset non-infectious pulmonary complication (LONIPC) (13–15). Lung function in patients with LONIPC declines over time after hematopoietic stem cell transplantation, and this decline has a significant impact on survival (16). Lung damage after BMT presents in various nonspecific forms (17), and bronchiolitis obliterans (BO) is a well-known pulmonary complication of chronic GVHD. Interstitial lung disease (ILD) has recently attracted attention as a pulmonary complication of chronic GVHD (18, 19). A large French retrospective observational cohort study including 79 ILD patients and 159 BO patients diagnosed after allogeneic BMT reported that 56% of ILD patients had experienced acute GVHD and 75% had developed chronic GVHD and that significantly fewer ILD patients had a history of steroid treatment for chronic GVHD compared to BO patients (62% vs 86%, $p < 0.0001$) (20). However, this large cohort study did not specifically address DILD. In the medical history of the present case, grade 1 acute GVHD, and non-symptomatic chronic GVHD without steroid treatment after BMT were consistent with the French retrospective study. Because no structural changes, such as fibrosis, were evident on initial lung imaging in the present case and his symptoms (cough) were judged to be caused by lung metastasis, it was difficult to recognize that he had risk factors for DILD other than a history of BMT before initiating chemotherapy.

Irinotecan has been associated with the risk of drug-induced lung injury in multiple case reports (21, 22). Lung and colorectal cancer are the two most common solid tumors in which irinotecan-related lung injury occurs (23), but its incidence is so low that it was not reported in large phase III clinical trials of irinotecan-containing chemotherapy for advanced colorectal cancer (24, 25). Similarly, in a clinical trial limited to the Japanese population, ILD incidence in patients receiving irinotecan-based and oxaliplatin-based chemotherapy were less than 1% (26). However, post-marketing surveillance has reported that the mortality rate of severe ILD after irinotecan administration was >20%. Therefore, it is important to monitor ILDs during chemotherapy with irinotecan (27). Although triplet chemotherapy, 5-fluorouracil/leucovorin + oxaliplatin + irinotecan (FOLFOXIRI) with bevacizumab, is an option for *BRAF*-mutated advanced colon cancer, a meta-analysis did not show the significant superiority of triplet chemotherapy (28). Another treatment option for *BRAF*-mutated colorectal cancer is doublet chemotherapy with a BRAF inhibitor and cetuximab (anti-epidermal growth factor receptor antibody). However, this doublet chemotherapy has no indication as first-line chemotherapy in Japan, and cetuximab treatment carries a risk of interstitial pneumonitis similarly to irinotecan and oxaliplatin. Doublet chemotherapy plus bevacizumab was considered optimal in the present case with a history of BMT. FOLFIRI and FOLFOX (5-fluorouracil/leucovorin + oxaliplatin) in combination with bevacizumab showed equivalent efficacy and safety, including for ILD (26). FOLFIRI plus bevacizumab was preferred in the present case after explaining the use of both FOLFIRI and FOLFOX.

Considering that ILD is a well-known adverse event caused by immune checkpoint inhibitors, another possible risk factor for DILD may be changes in pulmonary immunity caused by BMT. Mature T cells in the graft encounter and respond not only to tumor-associated and tumor-specific antigens, but also to host alloantigens, such as incompatible large histocompatibility leukocyte antigens, which cause GVHD, as well as graft-versus-

tumor effects. Important advances have been made in our understanding of the role of regulatory T cells (Tregs) in immunomodulation; Tregs are involved in T cell self-regulation and to preferentially suppress alloreactive T cells (29). and act in a suppressive manner against tumor immunity (30). The T cell repertoire generated by thymus-independent mechanisms lacks diversity and is therefore biased. In the present case, it is possible that the immune editing mechanisms altered by BMT caused organ-specific autoimmune reactions (31). In the present case, the cause of DILD could not be identified because no specific findings were obtained in the BAL and in the biopsy by bronchoscopy. Moreover, because there were no apparent clinical manifestations of chronic GVHD, such as oral ulcers, keratoconjunctivitis, multiple sclerosis, esophagitis/stenosis, vaginal ulceration/stenosis, fasciitis, and myositis, it is unclear whether GVHD more than 30 years after BMT might be related to the respiratory failure. However, it cannot be ruled out that immunological changes, which could be a background factor activated by irinotecan, might finally cause the DILD. Future studies are warranted to clarify the immunological mechanism of DILD, and it would be meaningful to perform bronchoscopy for patients who develop DILD repeatedly, which can provide samples for translational research on the immunological environment.

Drug management during high-dose steroid therapy is important. Interstitial pneumonia encompasses a variety of diseases, and its clinical course and response to treatment differ depending on the cause and histopathological patterns. Idiopathic interstitial pneumonia, including idiopathic pulmonary fibrosis, is diagnosed when all identifiable pathogens are ruled out. In this case, the ground-glass shadows occurred shortly after starting chemotherapy and there was no obvious pathogen or exposure history; therefore, drug-induced lung injury was the most likely cause. The main therapeutic strategy includes discontinuation of the culprit drug and the use of steroid treatment for varying durations, guided by the clinical response. The reported efficacy of steroid treatment in DILD varied widely, the case of DILD accompanied by malignancies is often refractory to steroid therapy. Although there is no consensus or standard guidelines for the diagnosis and optimal treatment of DILD in cancer patients, administration of immunosuppressive agents, including biologic agents from an earlier stage is also suggested for treatment of steroid resistant DILD (32). In a study of 75 cancer patients with irinotecan-induced DILD treated with steroid treatment, over 60% of the patients recovered and 29% died (27). Other reports of drug-induced lung injury suggest that 50–100% of patients recover after drug discontinuation and steroid administration (10). Diffuse alveolar damage (DAD) patterns are less responsive to steroids and have a poor prognosis. In one study, none of the patients with DAD improved without steroid treatment, and the overall mortality rate was 37.5% (33). In the present case, the DAD pattern was not initially observed by chest CT or lung biopsy, and DILD improved rapidly after starting steroid therapy.

However, DILD flares in association with fibrosis and bronchiectasis after an episode of abdominal bleeding. The autoimmune disease field determined that steroid coverage should be considered for invasive interventions such as surgery in patients receiving prednisolone doses of 5 mg/kg/day or more for three weeks or longer (34). In retrospection, since the present case received arterial embolization for retroperitoneal hematoma and anterior superior pancreatic duodenal artery pseudoaneurysm after steroid use for more than three weeks, steroid coverage with the addition of hydrocortisone 100–150 mg/body/day or methylprednisolone 20–30 mg/body/day could have been administered.

CMV infection associated with liver dysfunction was detected near the end of the clinical course, and CMV-induced pneumonitis could not be ruled out. It has also been argued that patients receiving systemic steroids are more likely to require pre-emptive CMV treatment (35). Although, in this case, PCR for CMV DNA at the start of steroid treatment was negative and there were no pathologically positive cells, CMV antigenemia or PCR monitoring should be repeated, and preemptive CMV treatment should be considered during the long-term use of high-dose steroids.

Long-term survivors of BMT are at risk for secondary cancer, which might require specific treatment and care. In practice, however, treatment for patients who develop secondary cancers after BMT are scattered over long follow-up periods, resulting in few reports on chemotherapy for secondary cancers of BMT recipients. Therefore, large, retrospective, cohort and multicenter, prospective, observational studies are necessary to clarify whether BMT is a risk factor for DILD and other specific adverse events after chemotherapy.

4 Conclusion

This case suggests that BMT may be a risk factor for DILD.

5 Patient perspective

Even if a patient has had no specific problems in the long course of life after bone marrow transplantation, it is necessary to consider the possibility of suffering pulmonary damage and immune adverse events when suffering from a malignancy and receiving treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement

Written informed consent was obtained from the patient's family for the publication of this case report.

Author contributions

KB and NB contributed substantially to the conception or design of the work or to the acquisition, analysis, and interpretation of data for the work. All authors agree to be responsible for all aspects of the work in ensuring that any questions relating to the accuracy or completeness of any part of the work are properly investigated and resolved. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1215789/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Hematoxylin and eosin staining of transbronchial lung biopsy of the right lung. Fibrosis is not evident on the left side, and fibrosis associated with cancer and carcinoma is present from the middle to the right, representing fibrotic changes in the tumor environment.

SUPPLEMENTARY FIGURE 2
The clinical course after onset of DILD.

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A machine learning-based model for predicting distant metastasis in patients with rectal cancer

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Background: Distant metastasis from rectal cancer usually results in poorer survival and quality of life, so early identification of patients at high risk of distant metastasis from rectal cancer is essential.

Method: The study used eight machine-learning algorithms to construct a machine-learning model for the risk of distant metastasis from rectal cancer. We developed the models using 23867 patients with rectal cancer from the Surveillance, Epidemiology, and End Results (SEER) database between 2010 and 2017. Meanwhile, 1178 rectal cancer patients from Chinese hospitals were selected to validate the model performance and extrapolation. We tuned the hyperparameters by random search and tenfold cross-validation to construct the machine-learning models. We evaluated the models using the area under the receiver operating characteristic curves (AUC), the area under the precision-recall curve (AUPRC), decision curve analysis, calibration curves, and the precision and accuracy of the internal test set and external validation cohorts. In addition, Shapley's Additive explanations (SHAP) were used to interpret the machine-learning models. Finally, the best model was applied to develop a web calculator for predicting the risk of distant metastasis in rectal cancer.

Result: The study included 23,867 rectal cancer patients and 2,840 patients with distant metastasis. Multiple logistic regression analysis showed that age, differentiation grade, T-stage, N-stage, preoperative carcinoembryonic antigen (CEA), tumor deposits, perineural invasion, tumor size, radiation, and chemotherapy were-independent risk factors for distant metastasis in rectal cancer. The mean AUC value of the extreme gradient boosting (XGB) model in ten-fold cross-validation in the training set was 0.859. The XGB model performed best in the internal test set and external validation set. The XGB model in the internal test set had an AUC was 0.855, AUPRC was 0.510, accuracy was 0.900, and precision was 0.880. The metric AUC for the external validation set of the XGB model was 0.814, AUPRC was 0.609, accuracy was 0.800, and precision was 0.810. Finally, we constructed a web calculator using the XGB model for distant metastasis of rectal cancer.

Conclusion: The study developed and validated an XGB model based on clinicopathological information for predicting the risk of distant metastasis in

patients with rectal cancer, which may help physicians make clinical decisions. rectal cancer, distant metastasis, web calculator, machine learning algorithm, external validation

KEYWORDS

rectal cancer, distant metastasis, web calculator, machine learning algorithm, external validation

Introduction

Colorectal cancer is the third most common cancer worldwide and the second leading cause of cancer-related deaths (1, 2). The World Health Organization (WHO) estimates it kills more than 930,000 people yearly (3). It is estimated that people in Western and East Asian countries have a 5% and 1% lifetime risk of developing colorectal cancer (4). With increased health awareness and improved medical care, the prognosis for colorectal cancer has improved over the years. However, patients with early and advanced colorectal cancer show significant differences in prognosis. The five-year survival rate for patients with stage I-II colorectal cancer is 88-95%, while patients with metastatic colorectal cancer have a survival period of 3 months to 5 years, and approximately 60% of patients with metastatic colorectal cancer will die within 1-2 years (5). Rectal cancer is an essential subtype of colorectal cancer, accounting for over 40% of colorectal cancer patients in the United States (US) (6). Early assessment and screening of patients at high risk for distant metastasis from rectal cancer is beneficial in improving prognostic outcomes for patients with rectal cancer and helps to reduce the potential risks associated with aggressive multimodal therapy (7). The proportions of the most common sites of metastasis in rectal cancer were 45.2% liver, 15% lung, 10% bone, and 8% brain (8–11). This study focuses on distant metastasis from rectal cancer rather than primary tumors, as they account for 90% of all cancer deaths (12).

Artificial intelligence (AI) is the field of computer science dedicated to building intelligent machines that can perform intelligence that requires human-level intelligence (13). AI is generally divided into machine learning and deep learning. Machine learning is an essential branch of AI and can usually be classified as supervised, unsupervised, and reinforcement learning (14). Machine learning has successfully penetrated the medical field with great success, such as in developing patronymics and imaging histology. While traditional regression approaches are susceptible to narrow variables, machine learning allows for more detail to be mined from the data, allowing for the development of better diagnostic and prognostic tools than traditional approaches (15). Classical statistical methods focus primarily on inference, including model parameter estimation and hypothesis testing. Such techniques produce relatively simple models, emphasize interpretability over predictive accuracy, and are less suited to dealing with data with many relevant interacting factors (16). The emergence of machine learning shows promise in addressing many of the problems inherent in previous approaches. Machine learning is ideally suited to take advantage of emerging big data and increasing computer processing power, making it feasible and easier to run large-scale analyses (17).

In this study, we constructed eight machine-learning prediction models using common clinicopathological factors while exploring the factors influencing distant metastasis in rectal cancer. We evaluated model performance based on multiple metrics while analyzing the interpretability of the different influences on the models. The best-performing model was then applied to clinical assessments to facilitate the screening of patients at high risk of distant rectal metastasis, which should provide a more accurate diagnosis of distant rectal metastasis and can help develop treatment guidelines and standard of care for distant rectal metastasis.

Abbreviations: WHO (The World Health Organization); SEER (The Surveillance, Epidemiology, and End Results); ROC (Receiver operating characteristic curve); AUC (Area under the curve); AUPRC (Area under the precis ion-recall curve); PR (Precision-recall); DCA (Decision curve analysis); XGB (The extreme gradient boosting machine); RF (Random Forest); MLP (Multilayer perce ptron); SVM (Support vector machine); DT (Decision tree); KNN (K-nearest neighbor); LR (Logistic regression); BNB (Plain Bayes); CI (Confidence Interval); OR (Odds Ratio); SD (Standard Deviation); CEA (Carcinoembryonic antigen); SHAP (Shapley's Additive explanations); PI (perineural invasion).

Materials and methods

Patient cohort

The Surveillance, Epidemiology, and End Results (SEER) database is a US population-based cancer database created by the National Cancer Institute in 1973, representing approximately 28% of the US population and providing us with a wealth of data for cancer-related research (18). With access to the SEER database, we constructed an open-access rectal cancer patient cohort using the rectal cancer patient data. Details of the SEER database are available at the following website (http://seer.cancer.gov/about/). The SEER database has started collecting information on patients' distant metastasis since 2010. Therefore, the years of rectal cancer patients included in this study were 2010-2017. For the cohort of rectal cancer patients obtained from SEER, the following inclusion

criteria were established: 1. the patient was diagnosed with rectal cancer (pathological diagnosis of rectal cancer) according to ICD-O-3/WHO 2008; 2. the diagnosis was made between 2010 and 2017; 3. the rectal cancer was a primary tumor; 4. patients have complete clinicopathological information, including age, sex, race, marriage, T-stage, N-stage, M-stage, pathological grade, carcinoembryonic antigen (CEA), perineural invasion(PI), tumor size, tumor deposits, and primary site. The SEER database contains no sensitive content or patient identifiers; these data can be used without ethics committee approval. External validation data were used from 1,178 patients diagnosed with rectal cancer at the First Hospital of Jilin University from 2010-2017. The study was approved by the Ethical Review Committee of the First Hospital of Jilin University and was conducted by the guidelines of the Declaration of Helsinki. Specific information on SEER and the external validation rectal cancer cohort are shown in Table 1. The study flow for this paper is shown in Figure 1.

Data collection and processing

The SEER * STAT (8.4.0) software extracted data from SEER Research Plus Data, 18 Registries + Hurricane Katrina Impacted Louisiana Cases + Hispanic Ethnicity, Nov 2020 Sub (2000-2018) from the rectal cancer patient data. Baseline clinicopathological data from patients with rectal cancer from an external validation set were processed using the SEER classification criteria (Supplement Table 1). All pathological indicators in this study were processed using the 7th edition AJCC TNM staging and SEER-related guidelines (Supplement Table 1). We coded the categorical variables to facilitate data analysis and further application in model building (Supplement Table 2). We provide the code for Machine Learning in this paper in Supplementary Table 3.

Model construction and evaluation

In this study, we constructed models using eight machine learning algorithms, including extreme gradient boosting (XGB) (19), random forest (RF) (20), decision tree (DT) (21), logistic regression (LR) (22), K-nearest neighbor (KNN) (23), support vector machine (SVM) (24), naive Bayes (NBC) (25) and multilayer perceptron (MLP) (26). Machine learning models can obtain complex correlations between data from extensive data. So, we chose the SEER database data, which has a large sample size, to develop the models. We randomly divided the SEER data into a training set and an internal test set in a ratio of 7:3. We trained eight models using the training set. We used random hyperparameters to search for the optimal model parameters while calculating the average AUC value for each algorithm under 10-fold cross-validation. The AUC value is the area under the receiver operating characteristic curves (ROC) value, with values close to 1 indicating reliable predictive power and values close to 0.5 implying poor prognostic power. When the data is an unbalanced data set, the AUC is less effective for assessing the model than the area under the precision-recall curve (AUPRC), so we plotted the precision-recall curve and calculated the AUPRC, which was used to validate and complement the AUC values (27). We plotted decision curves to assess the models' clinical decision-making ability. To compare the predictive effectiveness of the models, calibration curves were plotted. The models were accurate if the calibration curves were close to the diagonal. We determined the best model by combining multiple metrics. To assess the generalization and extrapolation performance of the models, we applied the eight models trained to the internal test set and external validation set. We plotted the ROCs, precision-recall curves, and calibration curves. We identify the best model by combining the performance of the machine learning models on the training set, the internal test set, and the external validation set. Shapley's Additive explanations (SHAP) is a cooperative game-theoretic-based model agnostic technique used to explain predictions filtered through the best-integrated machine learning model (28). We use the interpretable model SHAP to calculate the importance of each variable of the optimal model. Finally, we create a web calculator to facilitate the clinical dissemination and use of the model.

Statistical analysis

We performed the statistical analysis and model building of clinicopathological information using R (version 4.2.3, http://www.r-project.org) and Python (version 3.8, Python Software Foundation, http://www.python.org). Categorical variables were expressed as frequency (percentage, %) and compared using the chi-square or Fisher's exact test. We used univariate logistic regression analysis to determine the factors associated with distant metastasis in rectal cancer. The multiple logistic regression analysis included elements with $P<0.05$ in the univariate logistic regression analysis. We identified the factors with $P<0.05$ in the multiple logistic regression as independent risk factors for distant metastasis of rectal cancer. We calculated each factor's odds ratio (OR) and confidence interval (CI). The independent risk factors identified by multiple logistic regression were incorporated into constructing subsequent machine-learning models. Bilateral $P<0.05$ we considered to be statistically different.

Result

Baseline population characteristics

The study included 23,867 rectal cancer patients from the SEER database. Among them, 2840 (11.90%) developed distant metastasis, and 21027 (88.10%) did not develop distant metastasis. The demographic and clinicopathological characteristics of all these patients are shown in Table 2. The SEER database patients were randomly divided into the training set (n = 16706) and the internal test set (n = 7161) in a ratio of 7:3. The external validation was performed using data from 1178 rectal cancer patients from the First Hospital of Jilin University (Table 3). Details of the training, testing, and validation sets are shown in Table 1.

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TABLE 1 Clinical and pathological characteristics of the training, testing, and validation sets.

Variables	SEER database (N=23867)		External validation (N=1178)	*P* Value
	Training (N=16706)	Testing (N=7161)		
Age, n (%)				
≤50	2931 (17.5)	1284 (17.9)	242 (20.5)	*P<0.001*
>50	13775 (82.5)	5877 (82.1)	936 (79.5)	
Sex, n (%)				
Male	9986 (59.8)	4311 (60.2)	693 (58.8)	*P=0.747*
Female	6720 (40.2)	2850 (39.8)	485 (41.2)	
Race, n (%)				
White	15079 (79.3)	6487 (79.6)	0	*P<0.001*
Black	1893 (9.9)	776 (9.5)	0	
Asian or Pacific Islander	889 (9.9)	809 (9.9)	1178 (100.0)	
American Indian/Alaska Native	165 (0.9)	82 (1.0)	0	
Marital status, n (%)				
Married (including common law)	10665 (56.1)	4600 (56.4)	940 (79.8)	*P<0.001*
Single (never married)	3177 (16.7)	1320 (16.2)	0	
Widowed	1998 (10.5)	866 (10.6)	0	
Divorced	1930 (10.1)	813 (10.0)	0	
Separated	208 (1.1)	85 (1.0)	0	
Unmarried or Domestic Partner	52 (0.3)	26 (0.3)	238 (20.2)	
T stage, n (%)				
T1	3009 (18.0)	1341 (18.7)	219 (18.6)	*P=0.272*
T2	2914 (17.4)	1235 (17.2)	192 (16.3)	
T3	9133 (54.7)	3905 (54.5)	657 (55.8)	
T4	1650 (9.9)	680 (9.5)	110 (9.3)	
N stage, n (%)				
N0	9313 (55.7)	3980 (55.6)	644 (54.7)	*P=0.517*
N1	5424 (32.5)	2374 (33.2)	395 (33.5)	
N2	1969 (11.8)	807 (11.3)	139 (11.8)	
Grade, n (%)				
Grade I	1396 (8.4)	613 (8.6)	79 (6.7)	*P=0.361*
Grade II	12904 (77.2)	5565 (77.7)	941 (79.9)	
Grade III	2100 (12.6)	867 (12.1)	142 (12.1)	
Grade IV	306 (1.8)	116 (1.6)	16 (1.4)	
Tumor Deposits, n (%)				
No	11522 (69.0)	4916 (68.6)	776 (65.9)	*P<0.001*
Yes	1564 (9.4)	676 (9.4)	115 (9.8)	
Unknown	3620 (21.7)	1569 (21.9)	287 (24.4)	

(Continued)

TABLE 1 Continued

Variables	SEER database (N=23867)		External validation (N=1178)	*P* Value
	Training (N=16706)	Testing (N=7161)		
Perineural Invasion, n (%)				
No	11918 (71.3)	5170 (72.2)	818 (69.4)	*P<0.001*
Yes	1590 (9.5)	655 (9.1)	111 (9.4)	
Unknown	3198 (19.1)	1336 (18.7)	249 (21.1)	
CEA, n (%)				
Negative	5940 (35.6)	2521 (35.2)	370 (31.4)	*P<0.001*
Borderline	52 (0.3)	31 (0.4)	7 (0.6)	
Positive	4678 (28.0)	2007 (28.0)	376 (31.9)	
Unknown	6036 (36.1)	2602 (36.3)	425 (36.1)	
Tumor Size, n (%)				
≤5	12109 (72.5)	5169 (72.2)	821 (69.7)	*P<0.001*
>5	4597 (27.5)	1992 (27.8)	357 (30.3)	
Radiation, n (%)				
No	6961 (41.7)	2955 (41.3)	207 (17.6)	*P<0.001*
Yes	9745 (58.3)	4206 (58.7)	971 (82.4)	
Chemotherapy, n (%)				
No	5772 (34.6)	2460 (34.4)	394 (33.4)	*P<0.001*
Yes	10934 (65.4)	4701 (65.6)	784 (66.6)	
Distant Met, n (%)				
No	14729 (88.2)	6294 (87.9)	908 (77.1)	*P<0.001*
Yes	1977 (11.8)	863 (12.1)	270 (22.9)	

SEER, The Surveillance, Epidemiology, and End Results; CEA, Carcinoembryonic antigen.

We have analyzed the differences between patients in the SEER database by metastatic and non-metastatic groups, and we have some findings as follows. Thirteen clinicopathological factors were incorporated into our study: age, sex, marital status, race, tumor size, differentiation grade, T-stage, N-stage, preoperative CEA level, tumor deposits, PI, radiation, and chemotherapy. Patients in the SEER database were divided into DM (-) subgroups (21207 patients without distant metastasis,88.10%) and DM (+) (2840 patients with distant metastasis, 11.90%) subgroups. We found that DM (+) patients have a higher proportion of younger patients than DM (-) (*P<0.001*). Notably, the distant metastasis rate was significantly higher in men than women in the DM (+) subgroup (*P = 0.002*). Interestingly, the two subgroups had no statistical difference in race (*P = 0.138*). Consistent with our expectations, the incidence of distant metastasis was higher in singles (591/4103, 14.40%) than in married (1576/14059, 11.21%; *P<0.001*). In terms of the progression of rectal cancer, the proportion of patients with tumor size greater than 5 cm was higher in the DM (+) subgroup (45.9%) than in the DM (-) subgroup (25.1%; *P<0.001*). The subset with DM (+) had a significantly higher proportion of T-stage II-IV (*P < 0.001)* and a more advanced N-stage (*P < 0.001*). In addition, we observed higher levels of tumor deposits, PI, and preoperative CEA positivity in the subgroup of DM (+) than in the subgroup of DM (-) (*P < 0.001*). There was a significant difference between the DM (+) and DM (-) subgroups regarding patient access to treatment. (*P < 0.001*)

Univariate and multiple logistic regression analysis

Univariate and multiple logistic regression analyses were conducted for the training set data to identify the variables to be included in the machine learning model. Based on univariate logistic regression, age, sex, marriage, T-stage, N-stage, tumor size, tumor deposits, PI, CEA level, pathological grade, radiation, chemotherapy, and race were risk factors for distant metastasis in rectal cancer (*P<0.05*, Table 4). The results of including the above elements in the multiple logistic regression analysis showed that age, T-stage, N-stage, tumor size, tumor deposits, PI, preoperative CEA level, pathological grade, radiation, and chemotherapy were independent risk factors for distant metastasis of rectal cancer

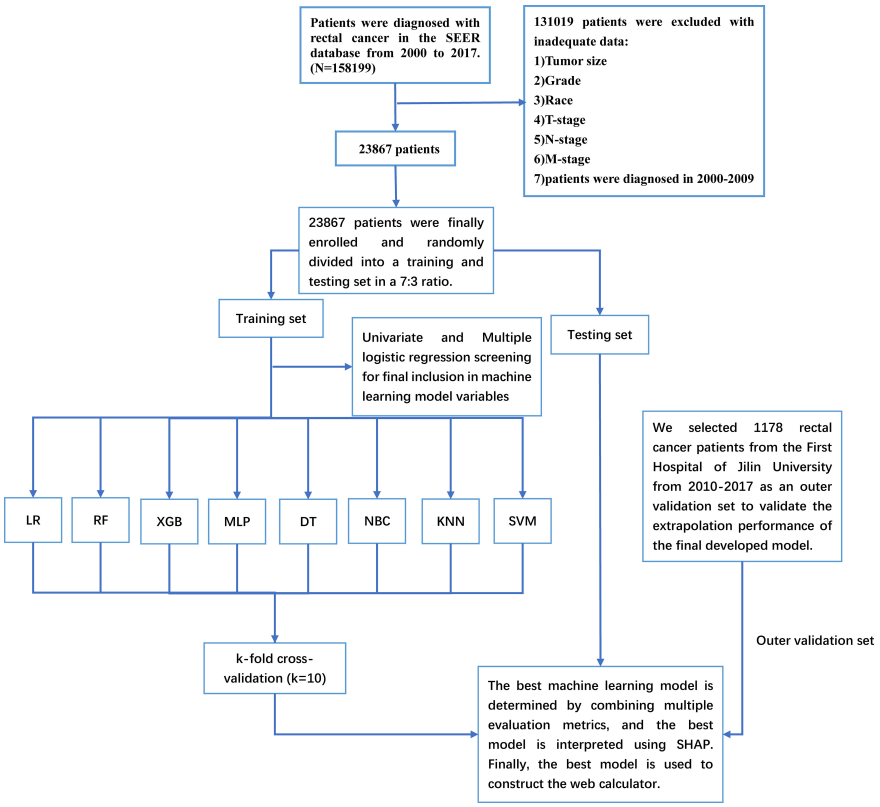

FIGURE 1
The Workflow diagram for study design and patient screening. SEER, The Surveillance, Epidemiology, and End Results; LR, logistic regression; DT, decision tree; RF, random forest; XGB, extreme gradient boosting; NBC, naive Bayesian classification; MLP, multilayer perceptron; SVM, support vector machine; KNN, k-nearest neighbor; SHAP, Shapley's Additive explanations.

($P<0.05$, Table 4). We included variables with $P<0.05$ in the multiple logistic regression analysis in the machine learning analysis.

Model performance

To compare the predictive performance of the eight models, we performed ten-fold cross-validation on the training set data (Figure 2A). The average AUC values of the eight machine learning models were between 0.793 and 0.859, demonstrating excellent predictive power. The XGB algorithm had the highest average AUC value (AUC=0.859, SD=0.013). Figure 2B shows the PR curves of the models in the training set, with the XGB model having a larger AUPRC than the other seven models (AUPRC=0.656). The XGB in the clinical decision curve analysis also demonstrated the ability to outperform the other models (Figure 2C). Figure 2D shows the calibration curve of the XGB model in the training set, showing that the XGB model has a more accurate predictive performance. In summary, the XGB model has a high degree of reliability. Figure 3 shows the ROC curves, PR curves, clinical decision curves, and calibration curves for the internal test set and external validation set of the eight models. The XGB model performed well in both datasets, demonstrating discriminative power beyond other models. The heat map analysis results, a comprehensive, clear, intuitive, and easy-to-judge analysis, are suitable for thorough evaluation as it allows for multiple dimensions (Figure 4) to more clearly reflect the performance of the models. After a comprehensive review of the performance of the models in the three datasets, we concluded that the XGB model performed best in predicting distant metastasis in patients with rectal cancer and designated the XGB model as the optimal model.

The relative importance of variables in machine learning algorithms

We use SHAP to interpret the XGB model. Generally, the higher the SHAP value of a feature, the higher the probability that the target event will occur. In the SHAP analysis, red indicates feature values that have a positive impact on the model, and blue indicates feature values that have a negative impact on the model (29). The study results showed that tumor deposits were the most crucial variable, followed by CEA, N-stage, radiation, chemotherapy, T-stage, PI, tumor size, age, and differentiation grade. (Figure 5)

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TABLE 2 Clinical and pathological characteristics of the study population for SEER database.

Variables	SEER Cohort			*P* Value
	All (N=23867)	DM (-) (N=21027)	DM (+) (N=2840)	
Age, n (%)				
≤50	4215 (17.7)	3556 (16.9)	659 (23.2)	P<0.001
>50	19652 (82.3)	17471 (83.1)	2181 (76.8)	
Sex, n (%)				
Male	14297(58.9)	12521(59.5)	1776(62.5)	P=0.002
Female	9570 (40.1)	8506 (40.5)	1064 (37.5)	
Race, n (%)				
White	19455 (81.5)	17170 (81.7)	2285 (80.5)	P=0.138
Black	2007 (8.4)	1736 (8.3)	271 (9.5)	
Asian or Pacific Islander	207 (0.9)	181 (0.9)	26 (0.9)	
American Indian/Alaska Native	2198 (9.2)	1940 (9.2)	258 (9.1)	
Marital status, n (%)				
Married (including common law)	14059 (58.9)	12483 (59.4)	1576 (55.5)	P<0.001
Single (never married)	4103 (17.2)	3512 (16.7)	591 (20.8)	
Widowed	2780 (11.6)	2504 (11.9)	276 (9.7)	
Divorced	2589 (10.8)	2245 (10.7)	344 (12.1)	
Separated	265 (1.1)	222 (1.1)	43 (1.5)	
Unmarried or Domestic Partner	71 (0.3)	61 (0.3)	10 (0.4)	
T stage, n (%)				
T1	4350 (18.2)	3984 (18.9)	366 (12.9)	P<0.001
T2	4149 (17.4)	3994 (19.0)	155 (5.5)	
T3	13038 (54.6)	11347 (54.0)	1691 (59.5)	
T4	2330 (9.8)	1702 (8.1)	628 (22.1)	
N stage, n (%)				
N0	13293 (55.7)	12456 (59.2)	837 (29.5)	P<0.001
N1	7798 (32.7)	6484 (30.8)	1314 (46.3)	
N2	2776 (11.6)	2087 (9.9)	689 (24.3)	
Grade, n (%)				
Grade I	2009 (8.4)	1861 (8.9)	148 (5.2)	p<0.001
Grade II	18469 (77.4)	16425 (78.1)	2044 (72.0)	
Grade III	2967 (12.4)	2394 (11.4)	573 (20.2)	
Grade IV	422 (1.8)	347 (1.7)	75 (2.6)	
Tumor Deposits, n (%)				
No	16438 (68.9)	15403 (73.3)	1035 (36.4)	P<0.001
Yes	2240 (9.4)	1752 (8.3)	488 (17.2)	
Unknown	5189 (21.7)	3872 (18.4)	1317 (46.4)	

(Continued)

TABLE 2 Continued

Variables	SEER Cohort			*P* Value
	All (N=23867)	DM (-) (N=21027)	DM (+) (N=2840)	
Perineural Invasion, n (%)				
No	17088 (71.6)	15707 (74.7)	1381 (48.6)	P<0.001
Yes	2245 (9.4)	1785 (8.5)	460 (16.2)	
Unknown	4534 (19.0)	3535 (16.8)	999 (35.2)	
CEA, n (%)				
Negative	8461 (35.5)	7980 (38.0)	481 (16.9)	P<0.001
Borderline	83 (0.3)	80 (0.4)	3 (0.1)	
Positive	6685 (28.0)	5044 (24.0)	1641 (57.8)	
Unknown	8638 (36.2)	7923 (37.7)	715 (25.2)	
Tumor Size, n (%)				
≤5	17278 (72.4)	15742 (74.9)	1536 (54.1)	P<0.001
>5	6589 (27.6)	5285 (25.1)	1304 (45.9)	
Radiation, n (%)				
No	9916 (41.5)	8446 (40.2)	1470 (51.2)	P<0.001
Yes	13951 (58.5)	12581 (59.8)	1370 (48.2)	
Chemotherapy, n (%)				
No	8232 (34.5)	7721 (36.7)	511 (18.0)	P<0.001
Yes	15635 (65.5)	13306 (63.3)	2329 (82.0)	

CEA, Carcinoembryonic antigen; SEER, The Surveillance, Epidemiology, and End Results; DM (+), patients with distant metastasis; DM (-), patients without distant metastasis.

TABLE 3 Clinical and pathological characteristics of the study population for Chinese Cohort.

Variables	Chinese Cohort			*P* Value
	All (N=1178)	DM (-) (N=908)	DM (+) (N=270)	
Age, n (%)				
≤50	242 (20.5)	177 (19.5)	65 (24.1)	*P=0.121*
>50	936 (79.5)	731 (80.5)	205 (75.9)	
Sex, n (%)				
Male	693 (58.8)	529 (58.3)	164 (60.7)	*P=0.511*
Female	485 (41.2)	379 (41.7)	106 (39.3)	
Race, n (%)				
White	0	0	0	*NA*
Black	0	0	0	
Asian or Pacific Islander	1178 (100.0)	908 (100.0)	270 (100.0)	
American Indian/Alaska Native	0	0	0	
Marital status, n (%)				
Married (including common law)	940 (79.8)	753 (90.6)	187 (69.3)	*P<0.001*
Single (never married)	0	0	0	

(Continued)

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TABLE 3 Continued

Variables	Chinese Cohort			*P* Value
	All (N=1178)	DM (-) (N=908)	DM (+) (N=270)	
Widowed	0	0	0	
Divorced	0	0	0	
Separated	0	0	0	
Unmarried or Domestic Partner	238 (20.2)	155 (9.4)	83 (30.7)	
T stage, n (%)				
T1	219 (18.6)	186 (20.5)	33 (12.2)	*P<0.001*
T2	192 (16.3)	171 (18.8)	21 (7.8)	
T3	657 (55.8)	490 (54.0)	167 (61.9)	
T4	110 (9.3)	61 (6.7)	49 (18.1)	
N stage, n (%)				
N0	644 (54.7)	542 (59.7)	102 (37.8)	*P<0.001*
N1	395 (33.5)	275 (30.3)	120 (44.4)	
N2	139 (11.8)	91 (10.0)	48 (17.8)	
Grade, n (%)				
Grade I	79 (6.7)	74 (8.1)	5 (1.9)	*p<0.001*
Grade II	941 (79.9)	728 (80.2)	213 (78.9)	
Grade III	142 (12.1)	97 (10.7)	45 (16.7)	
Grade IV	16 (1.4)	9 (1.0)	7 (2.6)	
Tumor Deposits, n (%)				
No	776 (65.9)	660 (72.7)	116 (43.0)	*P<0.001*
Yes	115 (9.8)	73 (8.0)	42 (15.6)	
Unknown	287 (24.4)	175 (19.3)	112 (41.5)	
Perineural Invasion, n (%)				
No	818 (69.4)	689 (75.9)	129 (47.8)	*P<0.001*
Yes	111 (9.4)	72 (7.9)	39 (14.4)	
Unknown	249 (21.1)	147 (16.2)	102 (37.8)	
CEA, n (%)				
Negative	370 (31.4)	321 (35.3)	49 (18.2)	*P<0.001*
Borderline	7 (0.6)	7 (0.8)	0 (0.0)	
Positive	376 (31.9)	232 (25.6)	144 (53.3)	
Unknown	425 (36.1)	348 (38.3)	77 (28.5)	
Tumor Size, n (%)				
≤5	821 (69.7)	681 (75.0)	140 (51.9)	*P<0.001*
>5	357 (30.3)	227 (25.0)	130 (48.1)	
Radiation, n (%)				
No	207 (17.6)	143 (15.7)	64 (23.7)	*P=0.003*
Yes	971 (82.4)	765 (84.3)	206 (76.3)	

(Continued)

TABLE 3 Continued

Variables	Chinese Cohort			*P* Value
	All (N=1178)	DM (-) (N=908)	DM (+) (N=270)	
Chemotherapy, n (%)				
No	394 (33.4)	339 (37.3)	55 (20.4)	*P<0.001*
Yes	784 (66.6)	569 (62.7)	215 (79.6)	

CEA, Carcinoembryonic antigen; SEER, The Surveillance, Epidemiology, and End Results; DM (+), patients with distant metastasis; DM (-), patients without distant metastasis.

TABLE 4 Univariate and multiple logistic regression analysis of variables in the training set.

Variables	Category	Univariate Analysis		Multiple Analysis	
		Odds Ratio (95% CI)	*P* value	Odds Ratio (95% CI)	*P* value
Age	**≤50**	Ref	*Ref*	Ref	*Ref*
	>50	0.65 (0.58-0.73)	*P<0.001*	0.72 (0.62-0.82)	*P<0.001*
Sex	**Male**	Ref	*Ref*	Ref	*Ref*
	Female	0.90 (0.82-0.99)	*P=0.031*	0.91 (0.81-1.03)	*P=0.128*
Race	**White**	Ref	*Ref*	Ref	
	Black	1.27 (1.08-1.49)	*P=0.003*	1.05 (0.87-1.27)	*P=0.603*
	Asian or Pacific Islander	1.16 (0.69-1.84)	*P=0.548*	1.12 (0.63-2.01)	*P=0.701*
	American Indian/Alaska Native	1.01 (0.85-1.19)	*P=0.913*	0.96 (0.79-1.16)	*P=0.692*
Marital status	**Married**	Ref	*Ref*	Ref	*Ref*
	Single (never married)	1.35 (1.20-1.53)	*P<0.001*	1.09 (0.94-1.26)	*P=0.247*
	Widowed	0.86 (0.73-1.01)	*P=0.069*	0.97 (0.80-1.18)	*P=0.782*
	Divorced	1.16 (1.00-1.35)	*P=0.052*	0.99 (0.83-1.18)	*P=0.901*
	Separated	1.67 (1.13-2.42)	*P=0.008*	1.68 (0.91-2.47)	*P=0.054*
	Unmarried or Domestic Partner	1.77 (0.84-3.38)	*P=0.104*	1.69 (0.73-3.89)	*P=0.220*
Grade	**Grade I**	Ref	*Ref*	Ref	*Ref*
	Grade II	1.56 (1.27-1.93)	*P<0.001*	1.22 (0.96-1.54)	*P=0.100*
	Grade III	2.98 (2.38-3.76)	*P<0.001*	1.71 (1.31-2.22)	*P<0.001*
	Grade IV	2.34 (1.61-3.36)	*P<0.001*	1.39 (0.91-2.12)	*P=0.124*
T stage	**T1**	Ref	*Ref*	Ref	*Ref*
	T2	0.47 (0.38-0.60)	*P<0.001*	0.59 (0.46-0.76)	*P<0.001*
	T3	1.68 (1.45-1.94)	*P<0.001*	1.11 (0.92-1.34)	*P=0.299*
	T4	4.20 (3.55-4.99)	*P<0.001*	1.74 (1.39-2.17)	*P<0.001*
N stage	**N0**	Ref	*Ref*	Ref	*Ref*
	N1	3.08 (2.76-3.44)	*P<0.001*	2.12 (1.85-2.42)	*P<0.001*
	N2	5.04 (4.42-5.76)	*P<0.001*	3.02 (2.55-3.58)	*P<0.001*
CEA	**Negative**	Ref	*Ref*	Ref	*Ref*
	Borderline	0.69 (0.11-2.23)	*P=0.606*	0.47 (0.10-2.14)	*P=0.328*
	Positive	5.58 (4.91-6.36)	*P<0.001*	3.93 (3.40-4.54)	*P<0.001*
	Unknown	1.57 (1.36-1.81)	*P<0.001*	1.47 (1.26-1.72)	*P<0.001*

(Continued)

TABLE 4 Continued

Variables	Category	Univariate Analysis		Multiple Analysis	
		Odds Ratio (95% CI)	*P* value	Odds Ratio (95% CI)	*P* value
Perineural Invasion	**No**	Ref	*Ref*	Ref	*Ref*
	Yes	2.85 (2.48-3.28)	*P<0.001*	1.37 (1.15-1.62)	*P<0.001*
	Unknown	3.19 (2.87-3.55)	*P<0.001*	1.53 (1.32-1.77)	*P<0.001*
Tumor Deposits	**No**	Ref	*Ref*	Ref	*Ref*
	Yes	4.13 (3.58-4.78)	*P<0.001*	1.78 (1.50-2.10)	*P<0.001*
	Unknown	5.09 (4.58-5.66)	*P<0.001*	4.01 (3.48-4.62)	*P<0.001*
Tumor size	**≤5**	Ref	*Ref*	Ref	*Ref*
	>5	2.58 (2.35-2.85)	*P<0.001*	1.46 (1.30-1.64)	*P<0.001*
Radiation	**No**	Ref	*Ref*	Ref	*Ref*
	Yes	0.60 (0.54-0.65)	*P<0.001*	0.16 (0.14-0.19)	*P<0.001*
Chemotherapy	**No**	Ref	*Ref*	Ref	*Ref*
	Yes	2.65 (2.35-2.99)	*P<0.001*	4.56 (3.87-5.38)	*P<0.001*

CEA, Carcinoembryonic antigen; CI, confidence interval; Ref, reference.

Web calculator

Although the XGB model is the best performing of the eight machine learning models, it is complex, challenging to understand, ad unsuitable for clinical generalization. We have therefore built a web calculator based on the XGB model, which allows the input of the patient's clinicopathological information on the left-hand side to obtain the probability of distant metastasis. An image of the web calculator is shown in Figure 6. The link to the web calculator is https://share.streamlit.io/woshiwz/rectal_cancer/main/distant.py.

Discussion

Rectal cancer is a common invasive tumor of the digestive system that is prone to distant metastasis. Metastasis is a significant driver of rectal cancer-related mortality, with the liver and lungs being the most commonly affected organs (30). Approximately 22% of patients with colorectal cancer have distant metastasis at the time of first presentation; also, the 5-year survival rate for these patients is less than 20% (31). The NCCN guidelines recommend routine CT of the chest and abdomen for patients with rectal cancer. Both tests can detect liver and lung metastasis, the two most common organs of metastasis in rectal cancer. However, patients often suffer unnecessary radiation damage because of the chest's high CT nodule detection and low diagnostic accuracy (32, 33). Positron emission tomography/computed tomography (PET/CT) is a standard diagnostic method for distant metastasis. However, it is not routinely used to screen for distant metastasis due to the high cost of treatment and the potential for radiation damage (34). It is, therefore, crucial to develop a clinical prediction model that can screen patients at high risk of distant metastasis from rectal cancer.

To date, many researchers have constructed different models to predict the distant metastasis of rectal cancer. However, all the data used for model development and validation comes from public databases, which has the disadvantage of needing more external data to validate the extrapolation of the model (35). Secondly, the method used to construct the models is logistic regression, which has specific requirements for data distribution and is sensitive to multivariate covariance and therefore has some limitations in its application (15). Chang et al. developed a model that incorporated a small sample size of data, making the developed model potentially biased (36). The paper uses big data from SEER to create the model, uses external data to validate the model, and finally develops a clickable web calculator to aid the clinical dissemination of the model.

As far as we know, this paper is the first to use machine learning algorithms to predict distant metastasis from rectal cancer and to construct a web calculator using the best model. This study found that the XGB algorithm best predicted distant metastasis from rectal cancer. The XGB model is an efficient, flexible, and scalable machine learning algorithm classifier widely used in medical fields such as COVID-19, chronic kidney disease diagnosis, and bone metastasis in prostate cancer (37–39). It has the advantage of using a large number of decision trees with low inverse correlation, and the number of included decision trees is optimized to achieve the lowest possible error rate, thus preventing over-fitting of the training model (40).

We used descriptive statistics and logistic regression to analyze the variables associated with distant metastasis in rectal cancer. We utilized SHAP values to assess the impact of each factor. Regarding SHAP visualization of variable importance, we found that each variable contributed to the model (Figure 5). In this study, tumor deposits were the most crucial variable in predicting distant metastasis in rectal cancer. Tumor deposits are isolated tumor

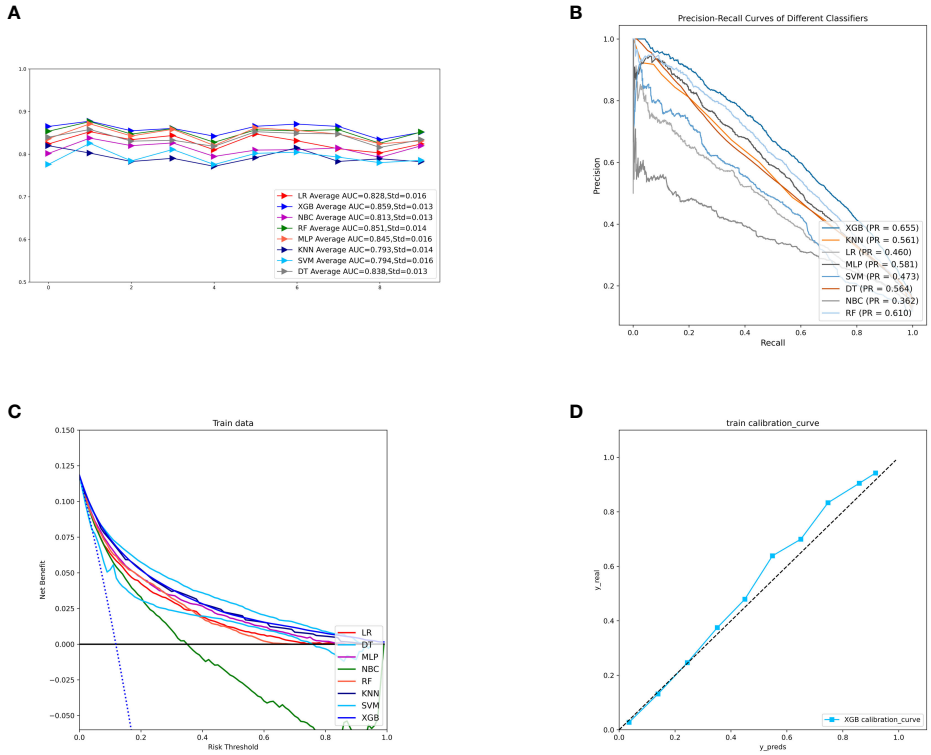

FIGURE 2
(A) Ten-fold cross-validation results of eight machine models in the training set. **(B)** PR curves of eight machine learning models in the training set. **(C)** DCA curves of eight machine learning models in the training set. **(D)** Calibration curves of the best models in the training set. LR, logistic regression; DT, decision tree; RF, random forest; XGB, extreme gradient boosting; NBC, naive Bayesian; MLP, multilayer perceptron; SVM, support vector machine; KNN, k-nearest neighbor; DCA, Decision curve analysis; PR, precision-recall; SD, Standard Deviation.

nodules present within the lymphatic drainage area of the primary tumor and without identifiable lymph nodes, blood vessels, or perineural structures within them (41). A meta-analysis of 17 retrospective studies found that tumor deposits were a stronger predictor of distant metastasis from rectal cancer than lymph node metastasis or vascular infiltration (42). In the importance ranking, the CEA was the second most crucial variable after tumor deposits. Several reports have pointed to preoperative CEA as an essential indicator of distant metastasis in rectal cancer, and our study confirms this (43–45). Although CEA is a broad-spectrum tumor marker and cannot be used as a specific indicator for diagnosing a particular malignancy, it still has significant clinical value in the differential diagnosis of malignancies, disease monitoring, and evaluation of the efficacy of treatment (46). Therefore, patients with rectal cancer with high preoperative CEA levels need enhanced postoperative screening. Logistic regression results showed that patients with regional lymph node involvement had a significantly higher risk of distant metastasis, 2-3 times higher than those with rectal cancer without lymph node metastasis (Figure 5). This may be because invaded regional lymph nodes can act as metastatic stations for tumor cell proliferation (47). Tumor size is another high-risk factor for developing distant metastasis from malignant tumors. Li et al. found that the risk of distant metastasis increased by 15% for each standard increase in rectal cancer tumor size, and our findings remain primarily consistent with them (48). Larger tumors may have invaded the surrounding soft tissues, which may explain the relationship between tumor size and distant metastasis. Tayyab et al. found that some lymphatic reflux was not present in some lymphatic tissues but could be found in larger tumor tissues (49). PI is a risk factor for distant metastasis in rectal cancer, but in-depth studies on how PI leads to distant metastasis remain elusive. Experts have emphasized the correlation between T-stage and distant metastasis. Our present study also found that T4 staging is an independent risk factor for distant metastasis of rectal cancer. We believe the reason for this is that the T4 stage implies that the tumor has grown through the plasma membrane layer, and the tumor cells can be implanted in the peritoneal tissue by direct metastasis, increasing the risk of distant metastasis of rectal cancer. Interestingly, the results of this study indicate that younger rectal cancer patients are more likely to develop distant metastasis, which is different from what we would expect (Figure 5). We believe that this may be because younger rectal cancer patients may have less differentiated tumors and are more likely to develop distant metastasis due to the tendency of younger patients to establish tumor mutations (50). According to George and Keklikoglou et al., chemotherapy may increase metastasis in malignant tumors, possibly because it promotes the expression of metastatic genes and increases the secretion of exosomes that promote metastasis (51, 52). This suggests that although chemotherapy may result in tumor shrinkage, it may also increase the chances of metastasis. Our study also shows that the administration of radiotherapy reduces

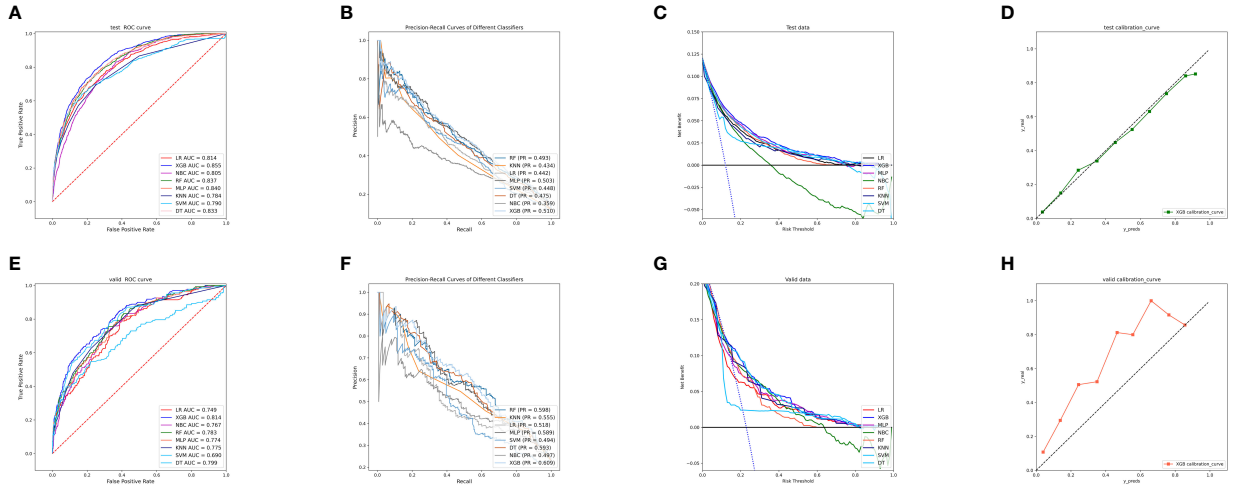

FIGURE 3
(A) ROC curves of eight machine learning models in the internal validation set. **(B)** PR curves of eight machine learning models in the internal test set. **(C)** DCA curves of eight machine learning models in the internal test set. **(D)** Calibration curves of eight machine learning models in the internal test set **(E)** ROC curves of eight machine learning models in the external validation set. **(F)** PR curves of eight machine learning models in the external validation set. **(G)** DCA curves of eight machine learning models in the external validation set. **(H)** Calibration curves of eight machine learning models in the external validation set. LR, logistic regression; DT, decision tree; RF, random forest; XGB, extreme gradient boosting; NBC, naive Bayesian classification; MLP, multilayer perceptron; SVM, support vector machine; KNN, k-nearest neighbor; DCA, Decision curve analysis; PR, precision-recall.

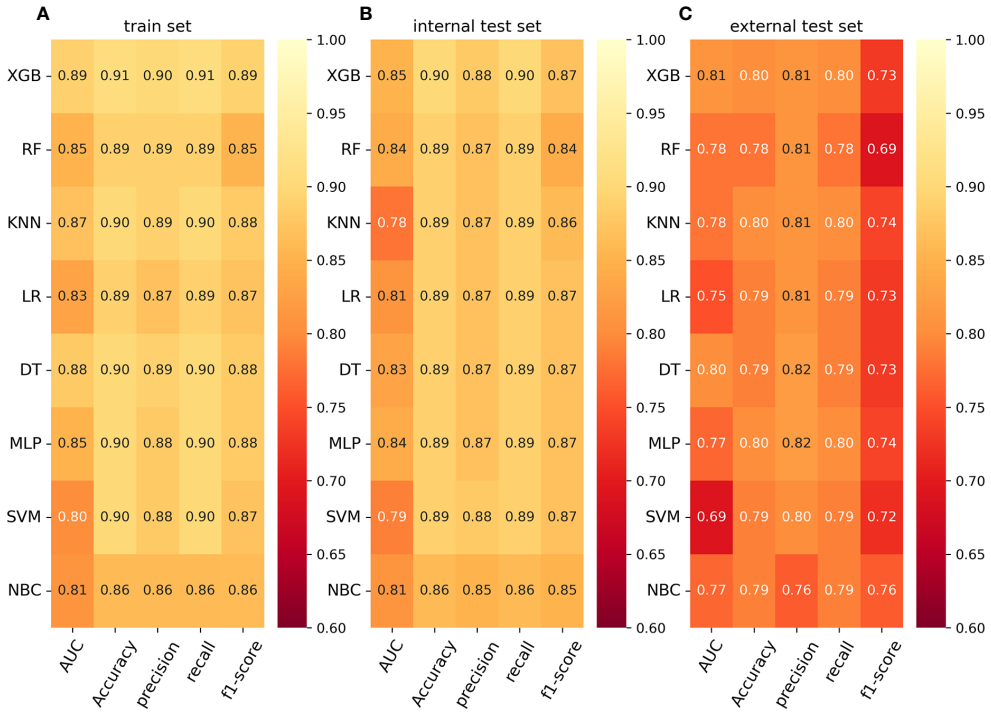

FIGURE 4
(A) Prediction performance of eight models in the training set. **(B)** Prediction performance of eight models in the internal test set. **(C)** Prediction performance of eight models in the external validation set. AUC, Area under the curve; LR, logistic regression; DT, decision tree; RF, random forest; XGB, extreme gradient boosting; NBC, naive Bayesian classification; MLP, multilayer perceptron; SVM, support vector machine; KNN, k-nearest neighbor.

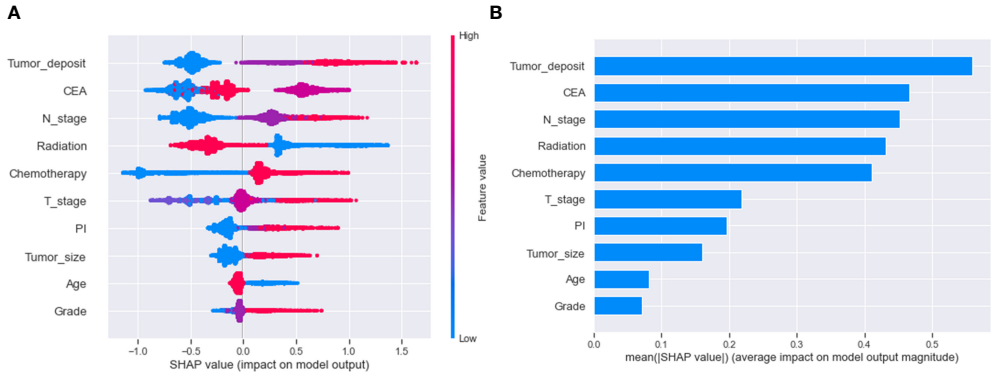

FIGURE 5
Relative importance of variables based on SHAP for XGB prediction model. SHAP, Shapley's Additive explanations; XGB, extreme gradient boosting; PI, perineural invasion; CEA, Carcinoembryonic antigen.

distant metastasis in patients with rectal cancer. There have been multiple potential theories to explain the protective effect of radiotherapy on distant metastasis from rectal cancer, including killing and reducing tumor cells at the primary site, eliminating micrometastasis from rectal cancer, and immunomodulatory effects. The impact of radiotherapy on controlling distant metastasis from rectal cancer depends on the mode of administration and dose (53, 54). Our model adequately incorporated various risk factors that may affect distant metastasis in patients with rectal cancer and achieved excellent predictive performance.

Despite the strengths of our study, there are some limitations to this study. Firstly, this is a retrospective study with data bias inherent to retrospective studies. Secondly, although the model demonstrated excellent performance in the external validation cohort, the data were only sourced from our one medical center, which may limit the model's generalization. Further independent validation sets are required to confirm our findings, and we will conduct a multi-center study in the future. Thirdly, because some variables in the SEER dataset were missing too much for multiple interpolations, we censored the missing data in the article, which may have caused a bias in the results. Finally, because of the limitations of the SEER database in terms of variables, we had some essential variables, such as blood biochemistry indicators, that were not available in time, thus limiting further optimization of our model, and we will investigate this issue further in the future. Of course, we hope to continue to improve the model in the future by incorporating a variety of other clinical factors to facilitate clinicians better.

Conclusion

In conclusion, we constructed eight prediction models for the risk of distant metastasis in patients with rectal cancer using machine learning algorithms. Among them, we found that the XGB model had the best predictive power, demonstrating strong discriminative power with high sensitivity, specificity, and accuracy in both the internal test set and the external validation set. We hope the XGB algorithm-based web calculator can help clinicians screen patients at high risk of distant metastasis from rectal cancer, intervene early and prevent distant metastasis from rectal cancer.

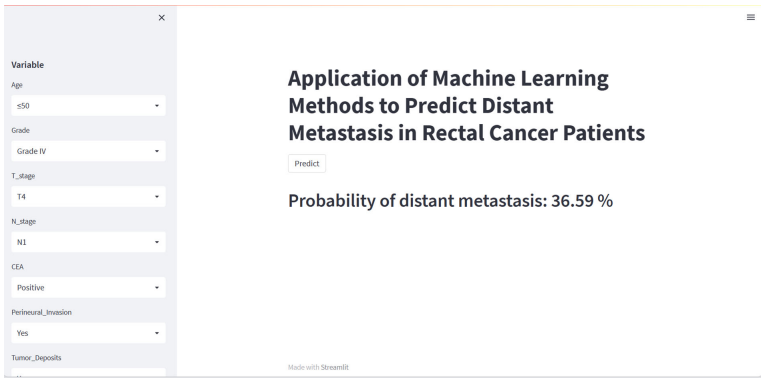

FIGURE 6
A web calculator for predicting distant metastasis from rectal cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

BQ and QW designed the study. BQ, ZS, SW, and DY conducted data analysis. BQ and XQ conceived the project and wrote the manuscript. QW and XQ revised and approved the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1235121/full#supplementary-material

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Refractory microsatellite stable metastatic colorectal cancer with ERBB2/ERBB3 mutation may be preferred population for regorafenib plus PD-1 inhibitor therapy: a real-world study

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Background: Microsatellite stable (MSS) colorectal cancer (CRC) has been referred to as the "cold tumor" because of almost no response to anti–programmed death-1 (PD-1) antibody. A recent REGONIVO trial showed that regorafenib plus nivolumab had an encouraging efficacy in MSS metastatic CRC (mCRC). However, only a small subset of patients may benefit from the combination therapy. We aim to evaluate the efficacy and safety data of immune checkpoint inhibitors combined with regorafenib in refractory MSS mCRC and to discover biomarkers that can effectively stratify the beneficial patient population.

Methods: We retrospectively analyzed patients with MSS mCRC who received regorafenib combined with anti–PD-1 antibody therapy. The objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and status of gene mutation were reviewed and evaluated.

Results: Twenty-one patients received combination treatment. At a median treatment duration of 4 months, one patient achieved complete response, three patients achieved partial response, and two patients achieved stable disease as the best response. The ORR and DCR were 19% and 28.5% in the overall population, respectively. The median PFS was 4 months, and the median OS was 25 months. Only erbb2 receptor tyrosine kinase 2/erbb3 receptor tyrosine kinase 3 (ERBB2/ERBB3) mutation status was confirmed to be a potential predictive factor for effective treatment. In patients with ERBB2/ERBB3 mutation, ORR, DCR, and PFS exhibited significant improvements in comparison with that in wild-type patients. Grade 3 or higher treatment-related adverse events occurred in three patients (14.3%).

Conclusions: Regorafenib in combination with PD-1 inhibitor provides a feasible treatment regimen for refractory MSS mCRC with tolerated toxicity. Patients with ERBB2/ERBB3 mutation may be the preferred population for this combination regimen.

KEYWORDS

colorectal cancer, immune checkpoint inhibitor, microsatellite stable, regorafenib, ErbB

Introduction

Immune checkpoint inhibitors (ICIs) including anti–programmed death-1 (PD-1) and anti–programmed death ligand-1 (PD-L1) have demonstrated a notable efficacy in metastatic colorectal cancer (mCRC) with mismatch repair deficiency (dMMR) or high microsatellite instability (MSI-H), which were characterized by a high mutational burden and a tumor-infiltrating lymphocyte enrichment (1, 2). However, MSI-H/dMMR cancer only accounts for 2%–4% of the total mCRC cases (3). The majority of patients with CRC exhibit a microsatellite stable (MSS) or mismatch repair proficient (pMMR) status, which is known as the "cold tumor" with less mutated oncogenes and less inflamed tumor immune microenvironment, resulting in a limited efficacy of ICIs (2). The inadequate recruitment and activization of immune cells to the tumor microenvironment were considered to be fundamental mechanisms underlying the inefficacy of ICIs in MSS mCRC (4). Combination strategies to enhance the immunogenicity of the tumor microenvironment and exploit the benefit of ICIs in patients with MSS are urgently needed.

Regorafenib, a small-molecule tyrosine kinase inhibitor, has been approved for treating chemotherapy refractory mCRC (5). Meanwhile, preclinical studies have shown that regorafenib could also (a) inhibit immune-suppressive cell infiltration, (b) inhibit the expression of immunosuppressive molecules, and (c) induce reprogramming of tumor-associated macrophages (TAMs) toward an M1 phenotype, which restored the immunosuppressive tumor microenvironment and synergistically enhanced the efficacy of ICIs (6–8). A phase Ib study (REGONIVO) reported the efficacy of regorafenib plus nivolumab with an objective response rate (ORR) of 33% and a prolonged median progression-free survival (PFS) of more than 6 months in 24 patients with refractory MSS mCRC (9). Another recent phase Ib/II study of regorafenib plus toripalimab showed a less ORR of 15.2% in 33 patients with mCRC (10). These studies have shown that a subset of patients may benefit from the combination therapy. It remains a compelling clinical challenge to further identify the beneficial subset in patients with mCRC.

In 2020, we treated one patient with refractory mCRC with extensive liver and pelvis metastases using regorafenib plus nivolumab regimen for a compassionate purpose. Unexpectedly, the multiple metastases completely regressed and achieved complete response (CR) after 8 months of treatment, although her tumor genotype is MSS with a low tumor mutation burden (TMB). It has been maintained for 28 months without any recurrence or metastasis. We performed second-generation sequencing of her tumor sample and identified a simultaneous G284R mutation and amplification in ERBB3. Preclinical study has shown that ERBB2/ERBB3 mutations could promote PD-L1–mediated immune escape in gallbladder cancer (11). Because dimerized human epidermal growth factor receptor 2/human epidermal growth factor receptor 3 (HER2/HER3), expressed by ERBB2 and ERBB3, respectively (12), are the tyrosine kinase targets of regorafenib (13), we hypothesize that regorafenib may reverse immunosuppressive tumor microenvironment and synergistically enhance the efficacy of ICIs in patients with ERBB2/ERBB3 mutation or amplification.

The combination of ICIs with regorafenib may be a promising treatment strategy for patients with MSS mCRC, especially for patients with ERBB2/ERBB3 mutation. To elucidate these issues, we conducted this retrospective study to evaluate the efficacy and safety of regorafenib combined with ICIs for patients with MSS mCRC with or without ERBB2/ERBB3 mutation for compassionate usage in the real world. The impact of ERBB2/ERBB3 mutation on the efficacy of combination treatment regimen was also investigated.

Materials and methods

Patients

We carried out a retrospective study of patients with mCRC with MSS status receiving regorafenib and anti–PD-1 antibody in Xinhua Hospital from November 2018 to April 2023. The usage of different types of anti–PD-1 antibody, including nivolumab, toripalimab, serplulimab, and sintilimab, was determined according to the doctor's decision. Eligibility for inclusion included the receipt of the combination of regorafenib and anti–PD-1 antibody in patients with MSS mCRC as the third- or later-line treatment for a compassionate purpose, following disease progression on standard of care therapy including Fluorouracil, Oxaliplatin, and Leucovorin (FOLFOX) and Fluorouracil, Irinotecan, and Leucovorin (FOLFIRI). Patients with prior exposure to regorafenib monotherapy were also included. The metastasis must be measurable with at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST; version1.1). The exclusion criteria included the following: (a) patients with less than two cycle of treatment, (b) patients with

little information on tumor response, and (c) patients with confirmed MSI-H/dMMR status. The study was approved by the hospital ethics committee (XHEC-C-2023-069) and was carried out in accordance with the 1964 Declaration of Helsinki. A written informed consent was obtained from the patients.

Treatment methods

Patients received oral regorafenib of 80–120 mg once a day for 21 days every 28-day cycle. The dose was reduced with a minimum dose of 80 mg for some patients to manage treatment-related toxicities. The PD-1 inhibitor (nivolumab, 3 mg/kg every 2 weeks; toripalimab, 3 mg/kg every 2 weeks; serplulimab, 3 mg/kg every 2 weeks; and sintilimab, 200 mg every 3 weeks) was intravenously administered on day 1 of oral regorafenib.

Outcome

ORR, disease control rate (DCR), PFS, overall survival (OS), status of gene mutation, and incidence of treatment-related adverse events (TRAEs) were reviewed and evaluated.

Tumor evaluation was based on RECIST (version1.1). The response evaluation included CR, partial response (PR), stable disease (SD), and progression disease (PD). The ORR was calculated as the sum of CR and PR, whereas the DCR was the sum of CR, PR, and SD. OS was defined as the time from treatment initiation to death from any cause. PFS was defined as the time from treatment initiation to the first documented disease progression or death. TRAEs were assessed according to the Common Terminology Criteria for Adverse Events (version 5.0).

The genetic status of the patients was evaluated through post-operative pathology tests performed by the pathology department. Immunohistochemistry (IHC) staining of four kinds of MMR protein (MLH1, MSH2, MSH6, and PMS2) or polymerase chain reaction (PCR) analysis of five microsatellite markers (BAT25, BAT26, D5S346, D2S123, and D17S250) were used to determine MSI/MMR status. IHC using anti–PD-L1, anti-HER2, and anti-HER3 antibodies was performed to assess the expression status of PD-L1 and HER2/HER3. Mutation status of kirsten rat sarcoma viral oncogene homolog (KRAS), neuroblastoma ras viral oncogene homolog (NRAS), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), ERBB2 and ERBB3 were determined by a PCR sequencing assay (Sanger or ARMS method). Fluorescence In Situ Hybridization (FISH) using the PathVysion HER-2 probe kit (Abbott Laboratories) was performed to assess the amplification status of HER2. All specimens in this study were reviewed by a pathologist. In addition, next-generation sequencing (NGS) analysis was conducted on the tumor samples of selected patients.

Statistical analysis

The study was done according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies (14). Difference between groups was determined by Pearson's chi-square test or Fisher's exact test. A multivariable logistic regression model was used to evaluate the risk of disease progression for ERBB2/ERBB3 mutation, adjusting for covariates determined *a priori* to be clinically relevant. PFS and OS were estimated by the Kaplan–Meier method and compared using the log-rank test. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 27 software. Statistical significance was defined at P-values <0.05.

Result

Baseline characteristics

A total of 21 patients with mCRC with MSS status met the study criteria and were enrolled in this study [15 men (71.4%); median age (range), 52 (32–73)] (Table 1). Eastern Cooperative Oncology Group Performance Status (ECOG PS) was 1 in 71.4% of patients, and ECOG PS was 0 in 28.6% of patients. Ten patients (47.6%) had synchronous metastases. Sixteen (76.2%) patients were diagnosed with left-sided primary CRC, and five (23.8%) patients were diagnosed with right-sided primary CRC. The most common metastatic sites included liver (66.7%), lung (33.3%), peritoneum (28.6%), and lymph node (19%). All patients had received ≥3 previous lines of chemotherapy, including anti-Vascular Endothelial Growth Factor (VEGF) treatment administered to 95.2% of patients. Nine (42.9%) patients had previously received regorafenib treatment, and all of them experienced disease progression before the combination treatment. The MSS/pMMR status was confirmed in 21 patients (100%). Eight patients (38.1%) had KRAS or NRAS mutant status, and three patients (14.3%) had BRAF V600E mutations. Five patients (23.8%) had ERBB2/ERBB3 mutation or amplification status (two patients with ERBB2 mutation, two patients with ERBB2 amplification, and one patient with synchronous ERBB3 mutation and amplification), whereas 16 patients (76.2%) were wild type. The types of anti–PD-1 antibody included nivolumab (61.9%), toripalimab (19%), serplulimab (9.5%), and sintilimab (9.5%). Among them, toripalimab, serplulimab, and sintilimab were Chinese domestic ICIs.

Efficacy

The median treatment duration was 4 months (range, 2–28 months). One patient (4.8%) achieved CR, three patients (14.3%) achieved PR, and two patients (9.5%) achieved SD as the best response (Table 2; Figures 1A, B). The ORR and DCR were 19% (four of the 21 patients) and 28.5% (six of the 21 patients) in the overall population, respectively. Three patients had ongoing responses at the time of analysis, including one patient with CR for 28 months. All 21 patients were evaluable for PFS and OS. The median PFS was 4 months (95% CI, 3.3–4.6) (Table 2; Figure 1C), and the median OS was 25 months (95% CI, 13.3–36.6) (Table 2; Figure 1D).

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TABLE 1 Baseline demographic and clinical characteristics of 21 patients with MSS mCRC.

Characteristics	Total no. (n = 21)
Median age, years (range)	52 (32–73)
Age	
≥ 60	9 (42.8%)
< 60	12 (57.2%)
Gender	
Male	15 (71.4%)
Female	6 (28.6%)
ECOG performance status	
= 1	15 (71.4%)
= 0	6 (28.6%)
Primary tumor location	
Cecum and ascending colon	4 (19%)
Transverse colon	1 (4.7%)
Descending colon	4 (19%)
Sigmoid colon	5 (23.8%)
Rectum	7 (33.3%)
Synchronous metastases	10 (47.6%)
Site of metastases	
Liver	14 (66.7%)
Lung	7 (33.3%)
Lymph node	4 (19%)
Peritoneum	6 (28.6%)
Bone	3 (14.2%)
Ovary	2 (9.5%)
Prior systemic treatment lines	
3	2 (9.5%)
4	15 (71.4%)
5	3 (14.2%)
Prior systemic treatment regimens	
Fluoropyrimidines	21 (100%)
Oxaliplatin	21 (100%)
Irinotecan	21 (100%)
Anti-EGFR treatment	9 (42.9%)
Anti-VEGF treatment	20 (95.2%)
Regorafenib	9 (42.9%)
Baseline NLR	
≥ 1.5	16 (76.2%)
< 1.5	5 (23.8%)
Anti–PD-1 antibodies	
Nivolumab	13 (61.9%)
Toripalimab	4 (19%)
Serplulimab	2 (9.5%)
Sintilimab	2 (9.5%)
KRAS/NRAS mutation	8 (38.1%)
BRAF mutation	3 (14.3%)
ERBB2/ERBB3 mutation or amplification	
Yes	5 (23.8%)
No	16 (76.2%)

ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil–lymphocyte ratio.

Subgroup analysis of predictive factors

We also performed univariate analysis to evaluate the predictive value of clinicopathologic factors for DCR, including age (≥ 60 *vs.* < 60), ECOG (1 *vs.* 0), primary tumor site (left colorectum *vs.* right colon), synchronous metastases (yes *vs.* no), liver metastasis (with *vs.* without), lung metastasis (with *vs.* without), previous regorafenib treatment (yes *vs.* no), baseline neutrophil–lymphocyte ratio (NLR) (≥ 1.5 *vs.* < 1.5), KRAS status (wide type *vs.* mutant), BRAF status (wide type *vs.* mutant), and ERBB2/ERBB3 status (wide type *vs.* mutant) (Table 3). Only ERBB2/ERBB3 mutation status was confirmed to be a potential predictive factor and associated with the increased risk of disease control [OR, 28 (95% CI, 1.9–394.4); p = 0.014] (Table 3). After adjusting for ECOG PS, Rat Sarcoma (RAS) mutation status, BRAF mutation status, and liver metastasis, the increased disease control risk for ERBB2/ERBB3 mutation remained significant [adjusted Odds Ratio (aOR), 54.8 (95% CI, 1.2–2497.3); p = 0.04).

Moreover, the ORR and DCR were significantly higher in patients with ERBB2/ERBB3 mutation (60% and 80%, respectively), compared with that in wild-type patients (6.2% and 12.5%, respectively) (Table 2). Median PFS was 15 months in patients with ERBB2/ERBB3 mutation and 4 months in wild-type patients (p = 0.01) (Table 2; Figure 1E). However, there were no significant differences observed in the OS between the groups (p = 0.238) (Table 2; Figure 1F).

Safety

Combination treatment was well tolerated, with no grade 4 or above toxicities being recorded while on treatment (Table 4). Of the 21 patients, 76% of patients had grade 1–2 TRAE and 14.3% of patients had grade 3 TRAE. The most common grade 1–2 TEAEs included hypertension (28.5%), decreased appetite (28.5%), fatigue (19%), diarrhea (19%), transaminase elevation (19%), and hand–foot skin reaction (14.2%). Two patients (9.5%) had grade 3 transaminase elevation, and one patient (4.7%) had grade 3 myocardial enzyme elevation.

TABLE 2 Anti-tumor efficacy of regorafenib plus PD-1 inhibitor in 21 patients with MSS mCRC.

Parameter	Patients with MSS mCRC, no. (%)			
	Total (n = 21)	ERBB2/ERBB3 mutation (n = 5)	ERBB2/ERBB3 wild type (n = 16)	*P* [a]
Best response				0.011*
CR	1 (4.8%)	1 (20%)	0 (0%)	
PR	3 (14.3%)	2 (40%)	1 (6.2%)	
SD	2 (9.5%)	1 (20%)	1 (6.2%)	
PD	15 (71.4%)	1 (20%)	14 (87.5%)	
ORR (CR + PR)	4/21(19%)	3/5 (60%)	1/16 (6.2%)	0.028*
DCR (CR + PR + SD)	6/21 (28.5%)	4/5 (80%)	2/16 (12.5%)	0.011*
PFS, median month (95% CI)	4 (3.3–4.6)	15 (5.8–24.1)	4 (3.3–4.6)	0.01*
OS, median month (95% CI)	25 (13.3–36.6)	25 (16.9–33)	12 (8.1–15.9)	0.238

ORR, objective response rate; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; PFS, progression-free survival; OS, overall survival.
[a]P-values indicate differences between the HER2/HER3 mutation group and the HER2/HER3 wild-type group. Fisher's exact test was used to in ORR and DCR comparison. Log-rank test was used in PFS and OS comparison. P < 0.05 was considered statistically significant. *P < 0.05.

A case report of complete response

Here, we present the case of a 32-year-old female patient diagnosed with a refractory metastatic colon cancer with a short expected survival time after progression of the third-line therapy. She was first diagnosed with sigmoid colon cancer in November 2019 with multiple liver and pelvic metastasis and hemorrhagic ascites. Because of the incomplete intestinal obstruction, she underwent colectomy with no metastasis excision on 11 November 2019. The post-operative pathology identified adenocarcinoma (pT4N2cM1) with KRAS/NRAS/BRAF wild-type MSS status in her tumor. Unfortunately, after five cycles of

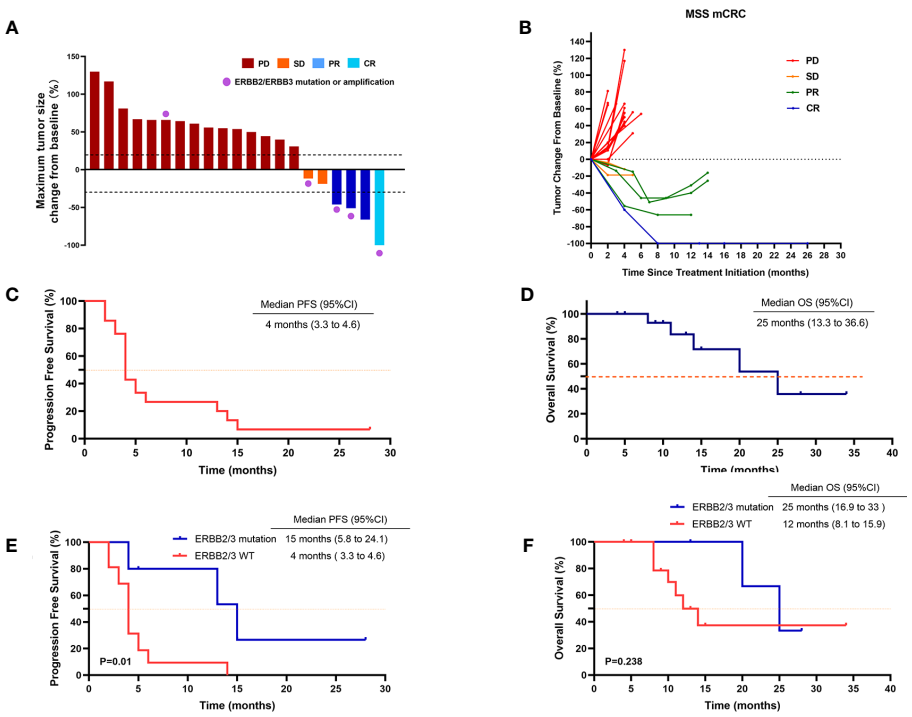

FIGURE 1
Tumor response in patients treated with regorafenib and anti–PD-1 antibody. **(A)** Waterfall plot of maximum percent change in tumor size from baseline as measured according to RECIST 1.1 in 21 evaluated patients. **(B)** Spider plot of longitudinal change in individual tumor burden over time in RECIST percentage from baseline. **(C)** Kaplan–Meier plot of progression-free survival (PFS) in 21 patients. **(D)** Kaplan–Meier plot of overall survival (OS) in 21 patients. **(E)** PFS in patients with or without HER2/HER3 mutation. **(F)** OS in patients with or without HER2/HER3 mutation. Data cutoff date for survival results was 1 May 2023. P < 0.05 was considered statistically significant.

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TABLE 3 Analysis of risk factors for disease progression and disease control.

	Disease control *vs.* disease progression		
Variable	DCR (CR + PR + SD) (n = 6)	PD (n = 15)	*P* [a]
Age			
≥ 60	3 (50%)	6 (66.7%)	1.00
< 60	3 (50%)	9 (75%)	
ECOG			
= 1	4 (66.7%)	11 (73.3%)	1.00
= 0	2 (33.3%)	4 (26.7%)	
Site of primary tumor			
Left	6 (100%)	10 (66.7%)	0.262
Right	0 (0%)	5 (33.3%)	
Synchronous metastases			
Yes	4 (66.7%)	6 (40%)	0.361
No	2 (33.3%)	9 (60%)	
Liver metastases			
Yes	5 (83.3%)	9 (60%)	0.613
No	1 (16.7%)	6 (40%)	
Lung metastases			
Yes	1 (16.7%)	7 (46.7%)	0.336
No	5 (83.3%)	8 (53.3%)	
Previous regorafenib			
Yes	3 (50%)	6 (40%)	1.00
No	3 (50%)	9 (60%)	
Baseline NLR			
≥ 1.5	4 (66.7%)	12 (80%)	0.598
< 1.5	2 (33.3%)	3 (20%)	
KRAS/NRAS mutation			
Yes	1 (16.7%)	7 (46.7%)	0.336
No	5 (83.3%)	8 (53.3%)	
BRAF mutation			
Yes	1 (16.7%)	2 (13.3%)	1.00
No	5 (83.3%)	13 (86.7%)	
ERBB2/ERBB3 mutation			
Yes	4 (66.7%)	1 (6.7%)	0.011*
No	2 (33.3%)	14 (93.3%)	

DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; ECOG, Eastern Cooperative Oncology Group Performance Status; NLR, neutrophil–lymphocyte ratio.

[a]P-values indicate differences between the disease control group and the disease progression group patients. Fisher's exact test was used for comparison between groups. $P < 0.05$ was considered statistically significant. *$P < 0.05$.

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TABLE 4 Adverse events of combination treatment of regorafenib and anti–PD-1 antibodies.

	Patients (n = 21)		
Adverse events	Any grade	Grades 1–2	Grade 3
All	16 (76%)	16 (76%)	3 (14.3%)
Fatigue	4 (19%)	4 (19%)	0
Hand–foot skin reaction	3 (14.2%)	3 (14.2%)	0
Hypertension	6 (28.5%)	6 (28.5%)	0
Decreased appetite	6 (28.5%)	6 (28.5%)	0
Diarrhea	4 (19%)	4 (19%)	0
Transaminase elevation	4 (19%)	2 (9.5%)	2 (9.5%)
Myocardial enzyme elevation	1 (4.7%)	0	1 (4.7%)
Rash	2 (9.5%)	2 (9.5%)	0
Pneumonia	1 (4.7%)	1 (4.7%)	0
Vomiting	2 (9.5%)	2 (9.5%)	0
Fever	1 (4.7%)	1 (4.7%)	0

mFOLFOX6 plus cetuximab and FOLFIRI plus bevacizumab, respectively, she was found to have ongoing disease progression with increased Carcinoembryonic Antigen (CEA) (from 230 ng/mL to 398 ng/mL), CA199 (from 570 U/mL to 9,620 U/mL), and liver metastatic lesion size (Figures 2, 3). After three cycles of regorafenib [120 mg daily (dq)] monotherapy used as the third-line treatment, minor decrease of CEA (from 398 ng/mL to 294 ng/mL) and CA199 (from 9,620 U/mL to 8,210 U/mL) were observed (Figure 2). However, CT scanning revealed a severe disease progression in liver in December 2020 (Figure 3).

The patient was subsequently treated with nivolumab [200 mg every two weeks (q2w)] plus regorafenib (120 mg qd) from December 2020 for a compassionate purpose (Figure 2). However, after 2 months of combination treatment, the patient developed immune-associated pneumonia (grade 2) with cough and fever. The patient successfully recovered from pneumonia after discontinuing the treatment for one month and received dexamethasone treatment for 1 week and, subsequently, opted to undergo re-challenge with the nivolumab (180 mg q2w) plus regorafenib (80 mg qd) regimen. Unexpectedly, the multiple liver and pelvic metastases exhibited a rapid regression and achieved a CR with abrupt decline of CEA (from 398 ng/mL to 3 ng/mL) and CA199 (from 8,210 U/mL to 35 U/mL) following an 8-month course of treatment. After combination treatment for 16 months, the patient received regorafenib (80 mg qd) for the maintenance therapy and resumed her regular occupational and daily activities. Until May 2023, this CR has been sustained for a duration of 28 months, without any evidence of recurrence or metastasis.

NGS using the patient's tumor tissue sample was tested and identified the ERBB3 G284R mutation and amplification as major oncogenic molecular alteration. Moreover, mutation of CDKN2A/B

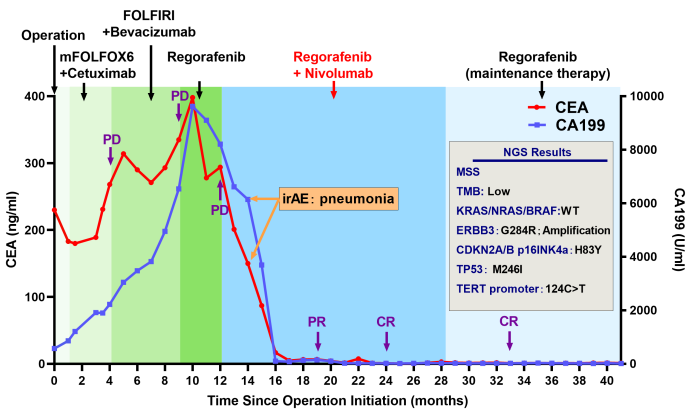

FIGURE 2
Clinical history and tumor marker levels of the patient with complete response during treatment. Treatment and molecular testing are indicated in the figure.

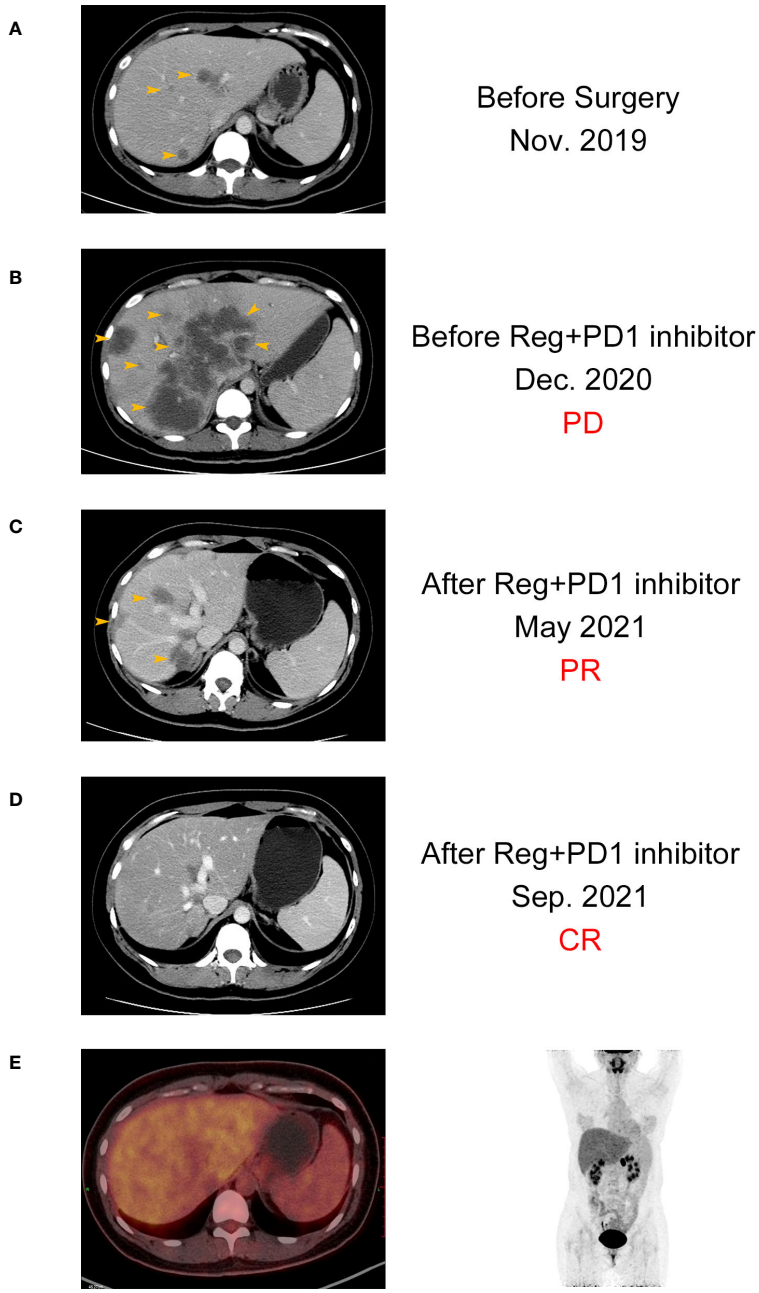

FIGURE 3
CT images of the patient with complete response before **(A, B)** and after **(C, D)** the combination treatment. **(E)** PET-CT images of the patient after the combination treatment.

p16INK4a (H83Y), TP53 (M246I), and TERT promter (124C>T) and low TMB were also detected (Figure 2).

Discussion

Although ICIs have demonstrated a remarkable efficacy in patients with MSI-H status, which only account for 2%–4% of the total mCRC cases (3). MSS-type CRC, characterized by a low tumor mutational burden and a negligible immune cell infiltration, has been referred to as the "cold tumor" because of almost no response to immunotherapy (4). Current research studies are actively investigating the viability of combination strategies as a means of converting MSS "cold tumor" into an immune-responsive "hot tumor." Although the combination of ICIs with chemotherapy, bevacizumab, cetuximab, and Mitogen-Activated Protein Kinase Kinase (MEK) inhibitor has been investigated in some clinical trials, these studies failed to show a significant improvement in ORR, PFS, or OS with this combination (15–18).

Immunosuppressive cells, including regulatory T cells (Tregs), and TAMs, are present within the tumor microenvironment of patients with MSS colorectal cancer. These cells can effectively

suppress the activity of T cells. Preclinical research studies had demonstrated that the multi-kinase inhibitor regorafenib can alleviate the immunosuppressive effects of Tregs and TAMs on T cells by inhibiting Colony Stimulating Factor 1 Receptor (CSF1R) and Vascular Endothelial Growth Factor Receptor (VEGFR) (6–8). This mechanism can be utilized to overcome ICI resistance in MSS CRC.

A recent Japanese trial, the REGONIVO study, reported an ORR of 36% and PFS of 7.9 months in 25 patients with MSS mCRC (9). In the North American REGONIVO trial, the ORR of regorafenib combined with nivolumab was 7% among patients with MSS mCRC. The PFS and OS were 1.8 and 11.9 months, respectively, both of which were inferior to those observed in Japanese REGONIVO study (19). In contrast to the findings of the REGONIVO study, a recent retrospective study of 18 patients with MSS mCRC revealed a poor clinical activity of regorafenib combined with nivolumab or pembrolizumab. Only 31% of the DCR was observed, and no patients demonstrated an objective response (20). The authors of the study suggest that the clinical use of this combination should be avoided in patients with MSS mCRC, particularly those with liver metastases.

Our present study evaluated the efficacy of regorafenib and anti–PD-1 antibodies as the third-line or above therapy in 21 patients with refractory MSS mCRC. In general, our treatment regimen demonstrated a certain degree of therapeutic efficacy in patients. The overall ORR and DCR reached 19% and 28.5%, respectively, with one patient with CR, three patients with PR, and two patients with SD being observed, although our response rates were lower than that in the Japanese REGONIVO study. Furthermore, the median PFS and OS were found to be 4 and 25 months, respectively. Notably, among patients with previously treated mCRC who had undergone regorafenib treatment and experienced disease progression, the median PFS and OS were 2 and 7 months, respectively (21, 22). Our study outcomes were found to be more favorable compared with that in the conventional treatment. Therefore, combination therapy could be a feasible treatment option for refractory MSS colorectal cancer.

Despite the promising results of the combination therapy, a considerable portion of MSS CRC (71.5%) have exhibited disease progression. Therefore, it is crucial to further investigate biomarkers that can effectively stratify the patient population and enhance the survival benefit. In the subgroup analysis of predictive factors for DCR, the clinical benefit of the treatment was correlated with ERBB2/ERBB3 status. Patients with ERBB2/ERBB3 mutation responded well to this combination regimen (ORR, 60% *vs.* 6.2%; $p = 0.028$). The median PFS in patients with ERBB2/ERBB3 mutation was significantly better than that in wild-type patients (15 months *vs.* 4 months; $p = 0.022$). In the only CR patient, we found that the patient had both ERBB3 G284R mutation and ERBB3 amplification.

HER2 and HER3, expressed by ERBB2 and ERBB3, respectively, are tyrosine kinase receptors that form heterodimers in cell membrane (12). Mutation of ERBB2/ERBB3 results in an abnormal activation of ERBB signaling pathway and promotes tumor proliferation and metastasis, which can be inhibited by regorafenib (13). Whole-exome sequencing identified ERBB2 and ERBB3 mutation (including short-variant mutation and copy number amplification) at a frequency of 6.5%–11.5% in CRC, and patients with ERBB2/ERBB3 mutations exhibited poorer prognoses (23, 24). HER2/HER3 may also serve as an attractive therapeutic target for the treatment of CRC with ERBB2/ERBB3 mutation (25, 26). Preclinical studies have revealed that genomic ERBB2/ERBB3 mutations promote PD-L1–mediated immune escape in gallbladder cancer through inhibiting the ability of tumor-reactive T cells and attenuating the release of Interferon (IFN-γ) and Interleukin-2 (IL-2) (11). Combination treatment with an ERBB signaling pathway inhibitor and anti–PD-1 antibody reversed these immunosuppressive effects and revealed promising therapeutic activities (11).

Previous reports have identified ERBB2 and ERBB3 alteration at a frequency of 6.5%–11.5% in CRC (23, 24), whereas 23.8% of patients with mCRC exhibited alterations in ERBB2/ERBB3 in our study. This study is a retrospective analysis in the real-world setting, where treatment regimens were based on the actual patients' conditions. For patients with refractory MSS mCRC with ERBB2/ERBB3 alterations, we are inclined to use either single-agent regorafenib or combination therapies, as mutated ERBB2/ERBB3 is one of the targets of regorafenib, which leads to a higher incidence of ERBB2/ERBB3 alterations in our study population. However, the elevated incidence of ERBB2/ERBB3 alterations does not impact the analysis of results. As reported by public databases (27, 28), alterations in ERBB2 and ERBB3 in patients with CRC are not correlated with DFS and OS. Higher alteration rates of ERBB2/ERBB3 do not predict a better prognosis or efficacy in our study.

The tolerability of the combination therapy's toxicity profile was comparable to that of the previous studies, and the incidence of TRAEs was similar to conventional treatments such as regorafenib monotherapy (9, 19, 20). Notably, the only patient in this study who achieved CR developed immune-related pneumonia (ir-pneumonia, grade 2) after 2 months of treatment, possibly due to overactive immune response. After the discontinuation of treatment for 1 month and the administration of dexamethasone, the patient recovered. During the onset of pneumonia, there was a significant decrease in CEA and CA199, and re-challenge treatment led to the achievement of CR. Hence, irTRAEs may predict a better antitumor effect of PD-1 therapy, and a careful consideration should be given to balancing the risk of irTRAEs with the potential efficacy benefits.

This study has several limitations. First, this is a retrospective study with a small sample size. Thus, any efficacy analysis was preliminary, and the role of ERBB mutation as a potential biomarker could not be fully evaluated. Prospective validations of this strategy in large cohorts are required. We are currently conducting a phase II prospective study to evaluate the potential efficacy of regorafenib plus toripalimab in patients with MSS mCRC with ERBB2/ERBB3 mutations. Second, this is a real-word study including four different anti–PD-1 antibodies used in this study. The doses of regorafenib and anti–PD-1 antibodies were not uniform between patients, which would further increase the heterogeneity of this study.

On the basis of our findings, we speculate that ERBB2/ERBB3 mutations lead to immune escape in patients with MSS, and regorafenib can reactivate the tumor microenvironment by targeting the ERBB pathway, transforming "cold tumor" into "hot tumor," thereby synergistically enhancing the therapeutic effects of anti–PD-1 antibodies. In conclusion, we found that regorafenib, in combination with PD-1 inhibitor, provides a feasible treatment

regimen for chemotherapy refractory MSS mCRC with tolerated toxicity. Patients with ERBB2/ERBB3 mutation may be sensitive to this combination regimen.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by xinhua hospital ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TW and YL: conceptualization, data curation, funding acquisition, supervision, and writing. XD and WD: data collection, data analysis, and methodology. SH and YH: data collection. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Can immunotherapy reinforce chemotherapy efficacy? a new perspective on colorectal cancer treatment

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As one of the main threats to human life (the fourth most dangerous and prevalent cancer), colorectal cancer affects many people yearly, decreases patients' quality of life, and causes irreparable financial and social damages. In addition, this type of cancer can metastasize and involve the liver in advanced stages. However, current treatments can't completely eradicate this disease. Chemotherapy and subsequent surgery can be mentioned among the current main treatments for this disease. Chemotherapy has many side effects, and regarding the treatment of this type of tumor, chemotherapy can lead to liver damage, such as steatohepatitis, steatosis, and sinus damage. These damages can eventually lead to liver failure and loss of its functions. Therefore, it seems that other treatments can be used in addition to chemotherapy to increase its efficiency and reduce its side effects. Biological therapies and immunotherapy are one of the leading suggestions for combined treatment. Antibodies (immune checkpoint blockers) and cell therapy (DC and CAR-T cells) are among the immune system-based treatments used to treat tumors. Immunotherapy targets various aspects of the tumor that may lead to 1) the recruitment of immune cells, 2) increasing the immunogenicity of tumor cells, and 3) leading to the elimination of inhibitory mechanisms established by the tumor. Therefore, immunotherapy can be used as a complementary treatment along with chemotherapy. This review will discuss different chemotherapy and immunotherapy methods for colorectal cancer. Then we will talk about the studies that have dealt with combined treatment.

KEYWORDS

colorectal cancer, tumor treatment, chemotherapy, immunotherapy, cell therapy

1 Introduction

According to GLOBOCAN data, colorectal cancer (CRC) is the third most common cancer in men and the second in women. It is also known that its mortality is higher in men than in women (1). This type of cancer has the highest incidence rate in Europe, Australia, North America, and New Zealand (2). Instead, the incidence of this disease in Africa and South-Central Asia is the lowest among others (3). It seems that diet, environment, and genetics are the most influential factors in the susceptibility to this disease. Colorectal cancer (CRC) is divided into two groups based on DNA stability, mutation, and repair (4). The first type is characterized by DNA mismatch repair (dMMR), a high level of microsatellite instability (MSI-H), and reduced expression of beta 2 microglobulin (B2MG) (presence in the MHC-1 structure and contributing to its stability at the cell surface) (5). The second type has stable microsatellites (MSI-L) and is mismatch-repair-proficient (pMMR) (6). In addition, these two types of CRC differ in immune checkpoint ligands expression level, MSI-H tumors have high expression, and MSI-L type has low expression of immune checkpoint ligands (7, 8). Due to this characteristic, the two types of CRC respond differently to different immunotherapy treatments and knowing the type of colorectal cancer is essential for immunotherapy. As expected, cancers with instability in DNA experience fewer repairs and stably undergo mutations (9, 10). These mutations lead to the production of protein antigens (presented and new peptides on the surface of tumor cells) called neoantigens which have better immunogenicity than tumors with a lower mutation and show a high level of DNA repair (11).

Also, in another type of classification, four consensus molecular subtypes (CMSs) with a distinguishing feature are described: CMS1 (microsatellite instability immune, 14%), hypermutated, microsatellite unstable and robust immune activation; CMS2 (canonical, 37%), epithelial, marked WNT and MYC signaling activation; CMS3 (metabolic, 13%), epithelial and evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), prominent TGF-β activation, stromal invasion, and angiogenesis. Samples with mixed features (13%) possibly represent a transition phenotype or intratumoral heterogeneity. So knowing the type of CRC is very important in choosing the proper treatment (12).

In addition, the results of studies have shown that chemotherapy as monotherapy cannot completely remove the tumor (13). Sometimes, even after surgery, the cancer recurs and can disrupt the patient's life (14). Therefore, the use of simultaneous and combined treatments is suggested. One of the most important treatments that have received much attention today is tumor immunotherapy, which has shown promising results (15). Immunotherapy includes various treatments based on antibodies and T cell transfer; these are among the most critical cells used in CRC immunotherapy, and they are used in three ways, expanded without any change, TCR genetic manipulation, and CAR-T cell application (16). Also, dendritic cells (DCs) can be used to treat various tumors (17). Antibodies (Ab) used in the treatment of tumors target multiple pathways of tumor progression, including angiogenesis, tumor growth, metastasis, and immune suppression mechanisms, which have been very promising (18, 19). One group of the most important antibodies used in treating CRC (even in MSS type) is immune checkpoint blockers, which suppress the pathways developed by tumor cells to suppress the immune system (20, 21). In this article, we will first talk about approved chemotherapy and their combined uses. Then we will discuss the various available immunotherapies. Finally, we will discuss the talk about studies that use the combination of chemotherapy and immunotherapy for CRC treatment.

2 Colorectal cancer chemotherapy

Chemotherapy is one of the first treatment strategies after tumor diagnosis (22). However, this type of treatment should be personalized according to the patient's tumor characteristics (23). Among the elements that should be checked include the general state of health, biology of the tumor (its aggressiveness), side effects of the chemotherapy regimen, left and right laterality and the primary location of the tumor, the drugs currently taken, other co-morbidities, and mutation status of important genes in colorectal cancer, including genes related to RAS and BRAF in tumor cells (24, 25). Mutations of RAS and BRAF genes can activate cell signaling pathways related to cell proliferation and differentiation (26). Investigating these types of mutations is very important because they can lead to resistance of cancer cells to treatment with EGFR inhibitors (27). Some chemotherapy drugs that the has approved include 5-fluorouracil, irinotecan, oxaliplatin, trifluoridine-tipiracil, and capecitabine (28, 29). These drugs exert their anti-tumor effects by different cells affecting growth pathways. However, using these drugs is not without harm to healthy cells, and they have different side effects, which we will discuss later.

2.1 Colorectal cancer treatment by 5-fluorouracil (5-FU)

5-FU (uracil analog) is an anti-metabolite drug (30) that replaces fluorine with hydrogen at the C5 position of uracil and ultimately leads to the formation of adenine-uracil/5-FU base pairs (31). 5-FU, after the entrance to a cell by the facilitated transport mechanism, converted intracellularly to several active metabolites, including 1) fluorouridine triphosphate (FUTP), 2) fluorodeoxyuridine triphosphate (FdUTP), and 3) fluorodeoxyuridine monophosphate (FdUMP) (31, 32). 5-FU normally exerts its antitumor effects (mediated by active metabolites) by three mechanisms. This drug can inhibit thymidylate synthase (TS) (33). This action disrupts intracellular deoxynucleotide pools required for DNA replication and cell proliferation (34). In addition, this drug can replace more than 40% of cellular RNA uracils, which can lead to the disruption of RNA synthesis (35). Also, this drug can be attached to cellular DNA after its anabolism inside the cell, leading to DNA fragmentation (36).

5-FU has been used orally or intravenously since 1990 (37, 38). However, due to the considerable variation in pharmacokinetics and unpredictable absorption, its oral use is not recommended and has been abandoned (39). The results of studies show that only 3% of 5-FU (prescribed dose) becomes toxic to cancer cells through anabolic actions (40). Although most of the administrated amounts

of 5-FU are catabolized in the liver through the activity of the dihydropyridine dehydrogenase enzyme and turn into a non-toxic and inactive metabolite (41). Also, studies have shown that 20% of 5-FU prescribed through infusion is excreted through urine directly and without any change (42).

One of the drugs that can be used alongside 5-FU and increase its therapeutic efficiency is leucovorin (LV) (43, 44). The simultaneous use of these two drugs leads to an increase in the survival of patients, a decrease in side effects (chemoprotection), and an increase in the therapeutic potential of 5-FU (45). Side effects associated with the therapeutic use of 5-FU are divided into three categories. The first category is related to the effects of this drug on general conditions, including fatigue, mucositis, vomiting, diarrhea, nausea, fever, and stomatitis (46). The second category is its effects on immune system cells, blood cells, and other healthy cells, and it includes neutropenia, anemia, leukopenia, thrombocytopenia, skin rashes, and neuropathy (47). The third category comprises neurological abnormalities, including changes in cognitive function and cerebellar ataxia, which occur less often than the previous two categories (48). Cardiotoxicity (although its pathogenesis has not been completely determined) is one of the side effects of chemotherapy with 5-FU, which rarely happens, but can seriously affect the patient's health (49).

Also, dihydropyrimidine dehydrogenase (DPYD), as a highly polymorphic gene, may be affected the treatment outcome in fluoropyrimidine-based treatments (50, 51). The product of this gene is the rate-limiting enzyme in fluoropyrimidine metabolism, or dihydropyrimidine dehydrogenase (DPD), whose function defects can lead to the accumulation of toxic metabolites from fluoropyrimidine (51). Investigations show that in patients with DPYD pathogenic variants receiving the standard dose of fluoropyrimidine chemotherapy, the risk of death due to treatment increases (52). Therefore, considering that the variants of this gene have been identified to a certain extent, it is recommended to investigate the variants of this gene before approving this type of drug. The results have shown that c.2194G>A is the most common polymorphism associated with DPYD, which is also associated with neutropenia (53). However, there is still no suitable single-nucleotide polymorphism (SNPs) panel to investigate DPYD variants, requiring more investigations.

The results of various studies show that this drug has multiple effects, including oxidative stress that causes myocardial damage, coronary artery spasm, ischemia caused by impaired oxygen transfer from red blood cells, and endothelial damage leading to thrombosis, and it causes cardiotoxicity and heart tissue damage (54). However, considering the therapeutic effects of 5-FU regardless of its side effects, this drug is being used in combination with other drugs in many clinical trials (Table 1).

2.2 Colorectal cancer treatment by capecitabine

Despite the success of using 5-FU in treating colorectal cancer, due to the short half-life of this drug (requiring multiple injections) and its rapid clearance from the body, researchers are looking for a way to redesign and use the therapeutic advantages of this drug (57). The results of researchers' efforts in 2009 led to the discovery of capecitabine, a prodrug of 5-FU, which has advantages over 5-FU (58). Unlike 5-FU, this drug is oral, and after being absorbed through the patient's digestive system, it is converted into 5-FU by successive enzymatic reactions, first in the liver and then in the tumor site (3 reactions) (58, 59). Thymidine phosphorylase (TP) is expressed in higher concentrations in neoplastic tissue (60, 61). This enzyme mediates capecitabine final conversion from 5'-deoxy-5-fluorouridine to 5-FU; therefore, the production of the active form of the drug is preferably done in tumor tissue (62, 63). Also, the results of studies have shown that the level of TP expression in tumor tissue is increased after exposure to radiotherapy and cytotoxic agents to help tumor eradication synergistically (64).

2.3 Colorectal cancer treatment by irinotecan (IRI)

IRI is a water-soluble semi-synthetic chemotherapy drug derived from camptothecin, which was approved for the treatment of lung cancer, cervical cancer, and ovarian cancer in Japan in 1994 (65, 66). This drug is also used to treat metastatic colorectal cancer (67). The results of clinical trials showed that the combination of IRI with 5-FU/LV can significantly increase the survival of patients compared to the 5-FU/LV receiving group (68). Also, this drug is used in combination with oxaliplatin and can help to improve patients' conditions by inhibiting metastasis (69–71). Irinotecan has an acceptable tolerability profile, increases the duration of treatment, is not associated with cumulative toxicity in patients with metastatic CRC, and leads to improved patient survival and quality of life (QOL) (66). Various studies have shown that exposure to ethyl-10-hydroxy-camptothecin (SN38), the active metabolite of irinotecan, shows different results in different people and can lead to severe toxicity in patients receiving the drug (72). Also, the drug dosage should be strictly controlled in some patients, including patients with severe renal failure and patients with UDP-glucuronosyltransferase 1A1 (UGT1A1) polymorphism (73).

IRI exerts its antitumor function usually by inhibiting topoisomerase I (74). However, the results of studies show that this is not the only functional mechanism of IRI. Cells exposed to IRI experience extensive gene expression changes. SN38 interacts with the various vital proteins for the cell, including BCL-xL (75), which has an anti-apoptotic role, up-regulation of FAS (76), mouse double minute 2 homolog (MDM2) involved in TP53-mediated cell death (77), and activation of MAPK signaling pathway can lead to increased cancer cells apoptosis (78). The excretion and pharmacokinetics of IRI depend on several factors, such as dosage, liver function status, age, administration time, and gender (79).

One of the side effects of IRI application is neutropenia (more common in women than in men) in receiving patients (80). Several side effects with the use of this chemotherapy drug, including delayed and severe diarrhea, abdominal pain, steatohepatitis, metabolic changes in patients' plasma (accumulation of acylcarnitines, nucleobases, and certain amino acids in plasma), similar cholinergic symptoms, oxidative stress in the liver and sweating they experience (81).

TABLE 1 Example of chemotherapy drugs application in combination with Fluorouracil in clinical trials.

Study name	Intervention Model	Estimated Enrollment	Drugs	Phase	Date	NTC number	Major findings
5FU/LV, Irinotecan, Temozolomide, and Bevacizumab for MGMT Silenced, Microsatellite Stable Metastatic Colorectal Cancer.	Sequential Assignment	18	1. Bevacizumab 2. Irinotecan 3. Leucovorin 4. 5-Fluorouracil 5. Temozolomide	Phase 1	2020	NCT04689347	Recruiting
Metformin and 5-fluorouracil for Refractory Colorectal Cancer	Single Group Assignment	50	Metformin and Fluorouracil	Phase 2	2013	NCT01941953	1. Metformin has anti-tumor activity (55). 2. Metformin decreases 5-FU side effects.
mFOLFOX6 Combined With Dalpiciclib in Patients With Metastatic Colorectal Cancer (FIND)	Single Group Assignment	18	1. Dalpiciclib 2. Oxaliplatin injection 3. Calcium folinate 4. 5-fluorouracil	Phase 2	2022	NCT05480280	Recruiting
Study of Magrolimab Given Together With FOLFIRI/BEV in Participants With Previously Treated Advanced Inoperable Metastatic Colorectal Cancer (mCRC)	Parallel Assignment	135	1. Magrolimab 2. Bevacizumab 3. Irinotecan 4. Fluorouracil 5. Leucovorin	Phase 2	2022	NCT05330429	Recruiting
Metastatic Colorectal Cancer (RAS-wildtype) After Response to First-line Treatment With FOLFIR Plus Cetuximab	Parallel Assignment	550	1. Irinotecan 2. Folinic Acid 3. 5-Fluorouracil 4. Cetuximab 5. Bevacizumab 6. Capecitabine 7. regorafenib 8. Irinotecan 125mg 9. Cetuximab wkly	Phase 3	2016	NCT02934529	Recruiting
Study in mCRC Patients RAS/BRAF wt Tissue and RAS Mutated LIquid BIopsy to Compare FOLFIRI Plus CetuxiMAb or BevacizumaB	Parallel Assignment	280	1. Bevacizumab 2. Cetuximab 3. 5-Fluorouracil 4. Irinotecan 5: Calcium levofolinate	Phase 3	2021	NCT04776655	Recruiting
Systemic Chemotherapy Plus HAI (FUDR) vs Systemic Chemotherapy Alone For CRCLM	Parallel Assignment	288	1. FUDR 2. Oxaliplatin 3. Leucovorin 4. 5-Fluorouracil	Phase 3	2018	NCT03500874	Recruiting
A Randomized Trial of Avastin + Gemcitabine + 5-Fluorouracil (5FU)/ Folinic Acid Versus Avastin + Oxaliplatin + 5FU/Folinic Acid in Metastatic Colorectal Cancer	Parallel Assignment	84	1. Gemcitabine 2. Avastin 3. 5-FU/folinic acid 4. Oxaliplatin	Phase 2	2009	NCT00192075	1. Folinic acid, 5-fluorouracil, gemcitabine (FFG), and FOLFOX4 were generally well tolerated. 2. FGG has no potential advantage over 5-FU/folinic acid (56).

2.4 Colorectal cancer treatment by oxaliplatin (Ox)

Oxaliplatin is a platinum-derived drug used to treat metastatic colorectal cancer distributed throughout the body by binding to plasma proteins (82). About half of the Ox injected into patients is eliminated through urine, but its excretion in feces is insignificant (clearance unrelated to liver function) (83). Most of the side effects of this drug are related to the release of platinum-active species (84) and its binding to DNA sequences (usually to GA or GG), which prevents DNA repair and synthesis in healthy cells (85). Currently, Ox is not usually used alone in treating colorectal cancer, but this drug is combined with other chemotherapy and biological drugs (83). For example, in three randomized clinical trials, adding

oxaliplatin to a regimen of leucovorin, capecitabine, and fluorouracil resulted in a 20% reduction in disease recurrence (86).

2.5 Colorectal cancer treatment by trifluridine-tipiracil

As it is clear from the name of the drug trifluridine/tipiracil, this drug consists of two parts. Trifluoridine is a thymidine-related nucleoid analog and replaces thymidine in DNA (87). This is while tipiracil strengthens the function of trifluridine by inhibiting the thymidine phosphorylase enzyme (88). Tipiracil leads to trifluridine replacement in DNA by preventing thymidine bases and ultimately prevents cell proliferation (87). The most common side effects associated with the use of this drug in metastatic colorectal cancer patients included neutropenia, anemia, thrombocytopenia, and leukopenia (89). Trifluoridine in this medicine, similar to what is seen in the therapeutic use of 5-FU, is converted into a monophosphorylated form by thymidine kinase (90). Still, unlike 5-FU, the monophosphorylated form of trifluorothymidine inhibits the activity of this enzyme by binding to the active site of the thymidylate synthase enzyme (90). It leads to cytotoxicity and non-production of thymidine by this enzyme. Subsequent phosphorylation by thymidine kinase produces trifluridine triphosphate, readily incorporated into the DNA of tumor cells (in place of thymidine bases), interferes with DNA function, and inhibits tumor growth (91). In clinical applications, this drug leads to the inhibition of tumor growth in a dose-dependent manner (92). The results of new studies show that this drug has high therapeutic efficiency, is very easy to use, and has fewer side effects than other chemotherapy drugs (93).

3 Colorectal cancer combinational chemotherapy

In many combination treatments related to colorectal cancer chemotherapy, 5-FU or capecitabine are usually the leading drugs. The results of the studies show that the therapeutic effects of capecitabine have more promising effects compared to the combined treatment of LV/5-FU (94, 95). However, chemotherapy regimens based on 5-FU have been designed over many years (96).

Among these treatments, we can mention FOLFOX, which includes folinic acid, 5-FU, and oxaliplatin (97, 98). The therapeutic efficacy of this combination is greater than the single use of oxaliplatin and 5-FU, but its primary mechanism is unclear (99). Today, this combination seems more effective in affecting the intestinal microbiota (100, 101). The 16S rRNA gene sequence analysis from new studies has shown that the abundance of *Akkermansia muciniphila* increases significantly in patients receiving the FOLFOX combination (102). Further studies on *Akkermansia muciniphila* showed that dipeptides containing branched-chain amino acids (BCAA) are one of the main factors in increasing the anticancer activity of the mentioned compound (102). The results of various studies have shown that CAPOX (folinic acid, capecitabine, oxaliplatin) and FOLFOX are the most effective regimens in treating advanced colon cancer (103), with the difference that in the CAPOX regimen, the infusion of 5 FU is replaced by an oral derivative of capecitabine (104).

FOLFIRI is one of the other combined chemotherapy options that consists of 400 mg/m^2 5-FU (day one iv bolus), 600 mg/m^2 5-FU (days 1 and 2 iv by ci), and 180 mg/m^2 irinotecan (day 1 iv) and are repeated every two weeks (105, 106). Due to the low therapeutic efficiency of 5-FU (about 10 to 15%), combination treatments of chemotherapy drugs, including FOLFIRI, are used, which leads to an increase in efficiency of up to 45% (105). The results of new studies show that the treatment outcome can be predicted based on the profile of the immune system cells of a person with colon cancer, especially the Treg/TH ratio (107). So patients with a higher Treg/TH ratio respond better to FOLFIRI treatment than patients with a lower ratio (108). Also, a specific decrease in the population of regulatory T cells was observed in patients receiving FOLFIRI (109). Therefore, it seems that some patients' high number of regulatory T cells does not have the immunological pressure to make the tumor more resistant. When these patients are faced with treatment, they respond to it in a better way (107).

The combination of capecitabine and irinotecan (CAPIRI) is another chemotherapy combination used less in studies than other combinations (110). However, the various results that have used chemotherapy compounds have acknowledged that these compounds cannot eradicate tumor cells. Additional treatments, including biological or immunological options, are needed in their cases. Table 2 summarizes some approved combined and single drug-based chemotherapy regimens.

4 Biological treatments for colorectal cancer

Many biological treatments are directly related to immunological treatments because the responsible for their function is an antibody or its derivatives; however, in this section, we will talk about drugs that can affect tumor biology. Currently, three biological agents, which are also considered immunological agents, include bevacizumab, an anti-VEGF-A antibody; cetuximab, an anti-EGF receptor antibody; and panitumumab, an anti-EGFR antibody, which has been approved for the first-line treatment of metastatic colorectal cancer (119). These antibodies prevent cancer cell proliferation by blocking growth receptors' function. In CRC patients who have mutated RAS in association with BRAF V600E mutation, along with chemotherapy, bevacizumab is the only agent that can lead to increased treatment efficiency (120). In addition, panitumumab and other anti-EGFR antibody applications are limited to tumors with mutations in RAS (121). Therefore, knowing the tumor type and properties can help choose the appropriate treatment with biological agents.

In addition to approved biologic therapies, researchers are currently searching for new biologic drugs to treat CRC. One of the most critical aspects of various tumors is mesenchymal-

TABLE 2 Summary of some approved combined and single drug-based chemotherapy regimens.

Chemotherapy regimen	Injection program	Type	Components	Ref.
Lokich	Daily IV injection	Single	5-FU (300 mg/m2)	(111)
TTD	IV infusion daily for 5 days, repeated every 28 days	Combined	1. Bolus leucovorin (200 mg/m2) 2. 5-FU (370 mg/m2)	(112)
FOLFOX-4	Programed infusion in 2 day	Combined	1. Leucovorin (200 mg/m2, day 1,2) 2. 5-FU bolus (400 mg/m2, day 1,2) 3. Continuous infusion 5-FU (600 mg/m2 for 22 h, day 1,2) 4. Oxaliplatin (85 mg/m2 day 1)	(113)
FOLFOX-6	Programed infusion in 2 day	Combined	1. Leucovorin (400 mg/m2 IV, day 1) 2. 5-FU (400 mg/m2 IV bolus on day 1; then a continuous infusion of 1,200–1,500 mg/m2/day × 2 days) 3. Oxaliplatin (100 mg/m2 IV, day 1)	(113)
FOLFIRI	Programed infusion in 2 day	Combined	1. Leucovorin (400 mg/m2 IV, day 1) 2. 5-FU (400 mg/m2 IV bolus, day 1; then a continuous infusion of 1,200–1,500 mg/m2/day × 2 days) 3. Irinotecan (180 mg/m2 IV, day 1)	(114)
FOLFOXIRI	Programed infusion in 2 day	Combined	1. Irinotecan (165 mg/m2 over 60 min) 2. Oxaliplatin (85 mg/m2) 3. Leucovorin (200 mg/m2 over 120 min) 4. 5-FU (3200 mg/m2 continuous infusion for 48 h)	(115)
Capecitabine	Twice daily, days 1–14	Single	Capecitabine (1000–1250 mg/m^2)	(116)
CAPOX/XELOX	Twice daily, days 1–14	Combined	1. Capecitabine (1,000 mg/m2 twice daily PO for 14 days) 2. Oxaliplatin (130 mg/m2 IV, day 1)	(117)
mXELIRI	Daily, days 1–14	Combined	2. Irinotecan 200 mg/m2 (Day1) 1. Capecitabine 1,600 mg/m2/day (Day 1–14),	(118)

epithelial transition (122). This phenomenon is facilitated by the binding of hepatocyte growth factor (HGF) to its tyrosine kinase receptor called c-MET (119, 123). Blocking this pathway is particularly important because of the significant role of mesenchymal-epithelial transition in metastasis (124). Therefore, the biological drugs under investigation are essential for this path. Onartuzumab, Tivantinib, Savolitinib, and Cabozantinib can be mentioned among these drugs (125). However, the use of biological treatments is not without side effects. For example, using bevacizumab may have serious side effects such as proteinuria, impaired wound healing, hypertension, arterial (but not venous) thromboembolic events, bowel perforation, bleeding, and leukoencephalopathy.

Due to the considerable overlap of biological treatments with immunological treatments, they are usually grouped together. However, because these treatments directly affect the biology of tumor cells and do not affect the immune system's responses, they are known by this name.

5 Colorectal cancer immunotherapy

The results of previous studies have shown that increasing the penetration of T cells into the colorectal tumor can control tumor growth (126). After identifying the MHC-peptide complex and in the presence of co-stimulatory signals, T cells identify tumor cells and destroy them using different methods, including releasing granules (cytotoxic T lymphocytes) (127). In a constant challenge, tumor cells are established in the body and destroyed by the immune system's cells (128). Tragedies begin when the immune cells cannot destroy malignant or neoplastic cells. An important point about the tumor is its microenvironment. As the cancer progresses, it evolves to suppress the immune system's responses (129, 130). Different types of tumors use different mechanisms, but for example, they can reduce the expression of MHC-1 molecules (131), reduce the expression of co-stimulatory molecules (132), increase the expression of growth factors (133), and increase the expression of anti-inflammatory cytokines and inhibitory surface molecules (CTLA-4, TIM-3, PD-1, PD-L1, LAG-3, and A2AR) (134–136). In addition, tumor cells secrete extracellular vesicles (EVs) into the tumor environment, reaching the immune system cells and disrupting their functions (137). Therefore, even if tumor cells are destroyed by chemotherapy, there must be a competent immune system that cleans the dead cells and also eliminates the remaining tumor cells with its abilities.

For this reason, tumor immunotherapy's importance has increased daily (Table 3). Tumor immunotherapy can be divided into several parts. Passive immunotherapy occurs through the transfer of antibodies (native or engineered) mRNA (151), and cytokines (152, 153), and active immunity, which is usually performed through transferring cells related to the immune system, including dendritic cells (DCs) (154) and T cells (155).

5.1 Colorectal cancer passive immunotherapy

MSI-H tumors usually experience infiltration of immune cells, including $TCD4^+$ (TH1) cells and $TCD8^+$ (156); however, the immune cells are functionally unresponsive. These cells (present in the tumor stroma) usually express immune checkpoints such as PD-1 and CTLA-4, which bind to ligands on the surface of tumor cells (such as PD-L1 and CD28) and suppress the function of immune cells, especially T cells (157). Also, as it was said, by reducing the expression of β2MG, these tumors express a low level of stable MHC-1 on the tumor cell surface and escape from being recognized by $TCD8^+$ cells (158). However, MSI-L colorectal cancers experience less infiltration of immune cells into the tumor stroma and have low levels of PD-1 expression (immune cells) and PD-L1 expression (tumor cells) (159, 160). Therefore, it seems that antibodies called immune checkpoint blockers (ICb) based on tumor type can be used to a greater extent in treating MSI-H tumors than MSI-L tumors (157).

In addition to the importance of the above classification, there is another molecular classification (Consensus Molecular Subtype (CMS)) for colorectal cancer, and knowing these characteristics is important for choosing the type of immunotherapy (161). From a molecular point of view, colorectal tumors are divided into four groups. This division simultaneously considers tumor and immune cells' main gene expression changes in the different environments mentioned. CMS1 is a group of CRC where many mutations are observed (161). They are synonymous with MSI-H type, and mutations associated with BRAF genes are frequently observed in these tumors, including about 1/7 of colorectal tumors (161). CMS2, also called the canonical type, comprises approximately 1/3 of CRC and is associated with mutations that activate the Wnt and Myc signaling pathways (12). CMS3 tumors are also called metabolic type and include 1/7 of colorectal tumors, which often have KRAS-related mutations and disrupt the metabolic pathways of cancer cells (12, 162). The last type and CMS4, also called mesenchymal type, constitute 1/4 of colorectal tumor cases and are associated with activation of the growth factor-β (TGF-β) pathway, increased stromal activation, angiogenesis, and inflammatory infiltration (12).

The importance of these classifications is that the tumor microenvironment of each of these classes is different and therefore requires different treatments (163). In terms of immunity, CMS2 and CMS3 tumors are called cold tumors, which are at a very low level in terms of immune system responses, and in fact, the immune response against these tumors is not well established (161, 164). However, CMS1 and CMS4 tumors are called hot tumors that have higher immune responses than the previous groups. But the noteworthy point is that CMS1 and CMS4 show different responses to treatments due to their features for the immunotherapy index (165). As mentioned before, the CMS1 group, which is similar to MSI-H, was diffuse immune tumor-infiltrating lymphocytes (TILs) rich in $CD8^+$ and $CD68^+$ macrophages (162), whereas CMS4 tumors differed with a different pattern of immune infiltration, including monocyte-derived cells, regulatory t cells (Treg), MDSCs, and TH17 cells (166). The main factor in immunosuppression in the CMS4 type is the production of TGF-β and its related mechanisms (167). A therapeutic strategy of combining selective TGFβ inhibitors with immune checkpoint blockers can reactivate a strong immune response in these models of colon cancer.

In addition to antibodies that play a role in inhibiting immune checkpoints, other antibodies, including antibodies against cytokines such as TGF-β, can reduce the suppression of tumor-related immune responses (168). Also, antibodies against specific tumor antigens can sometimes be used to increase the recognition

TABLE 3 Examples of FDA-approved novel therapeutics in colorectal cancer.

No	Drug name	Trade name	Properties	Disease	Date of approval	Ref.
Chemotherapy						
1	Irinotecan HCl	Camptosar	DNA topoisomerase I inhibitor	Metastatic colorectal cancer	06/14/1996	(138)
2	Oxaliplatin	Eloxatin	Organoplatinum alkylating agent	Colorectal cancer (in combination with leucovorin and 5-FU)	08/09/2002	(139)
Passive immunotherapy (Ab based)						
3	Cetuximab	Erbitux	EGFR-directed mAb	Colorectal cancer	02/12/2004	(140, 141)
4	Bevacizumab	Avastin	VEGF-A-directed mAb	Colorectal cancer	02/26/2004	(142)
5	Panitumumab	Vectibix	EGFR-directed mAb	Colorectal cancer	09/27/2006	(143, 144)
6	Pembrolizumab	Keytruda	PD-1 targeted Ab	Colorectal cancer (for (dMMR and MSI-H types)	23/05/2017	(145, 146)
7	Nivolumab	Opdivo	PD-1 targeted Ab	Colorectal cancer (for (dMMR and MSI-H types)	01/08/2017	(147)
8	Yervoy	Ipilimumab	CTLA-4 targeted Ab	Colorectal cancer (for (dMMR and MSI-H types)	10/07/2018	(148–150)

of tumor cells by the innate immune system (169). In this model, antibody attachment to tumor cells can lead to the tumor cells' apoptosis by mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), performed by macrophages and NK cells, and complement-mediated cytotoxicity (CDC).

5.2 Colorectal cancer active immunotherapy

Active immunotherapy, which usually occurs by cell transfer, uses T cells and DCs for treatment. The use of T cells occurs in 3 ways. In the first case, tumor-infiltrated lymphocytes (TILs) are collected from the tumor site, expanded, and injected into the patient as an autograft (170). This action leads to an increase in the number of tumor-specific T cells. In the second type of t cell therapy, T cell receptors (TCRs) are changed using genetic engineering methods, and their ability to identify antigens related to tumor cells increases (171). Another approach that has attracted much attention is using T cells with chimeric antigen receptors (CAR-T cells) (16, 172). In this technology, the Scfv part of an antibody that identifies tumor-specific or associated antigens (TSA or TAA) and its combination with different intracellular domains involved in T cell activation signal transmission is usually used (173).

In some cases, the extracellular part of CAR consists of different parts of a receptor whose ligand is abundantly produced in tumor cells (174). However, the applications of CAR-T have various limitations, and it is a dynamic field in cancer treatment, which is still being researched (175). CAR-T cell-based treatment is often used in clinical trial studies (Table 4).

In the case of CRC, there are limitations in the use of CAR-T cells, which include the low chemotactic ability of these cells to migrate to the tumor site, the acidic environment resulting from the metabolism of tumor cells, induced hypoxia, and lack of nutrients (173, 178). In addition to these cases, the severe immunosuppressive microenvironment in CRC is also one of the treatment obstacles. Among these substances that suppress transplanted CAR-T cells are anti-inflammatory cytokines, anti-inflammatory cells including Tregs, MDSCs, and tumor-associated macrophages, as well as metabolites derived from tumor cells (such as kynurenine produced from tryptophan by IDO) (178, 179).

In the study conducted by Jie Xu et al., they produced a human epidermal growth factor receptor 2 (HER2) based CAR-T cell. This receptor also is expressed on the surface of many healthy cells. However, its expression level is higher in tumor cells such as ovarian, stomach, colorectal, breast, and lung cancer (180). This study showed that HER2-specific CAR-T could increase anti-tumor responses in the CRC mouse model and has a high therapeutic capacity (181).

In addition to T cells, under the influence of TME, DCs acquire different functional and phenotypic characteristics, which leads to their non-functionality in anti-tumor responses and even suppression of immune responses (182, 183). According to the characteristics of tumor microenvironment-infiltrated DCs (TIDCs), such as their maturation level and their interaction with other cells in the tumor environment, including TILs, they can have positive or negative effects on CRC prognosis (184, 185). In addition to TIDCs, in CRC patients, the number and function of circulating DCs are generally reduced, and the immature or progenitor phenotype is also associated with an increase (186, 187). In general, it can be said that a large population of TIDCs (checked

TABLE 4 Examples of CAR-T cell clinical trials as novel therapeutics in colorectal cancer.

Study Name	Intervention Model	Estimated Enrollment	Antigen	Route	Phase	Dose	Date	NTC number	Major findings
NKG2D CAR-T Cells to Treat Patients With Previously Treated Liver Metastatic Colorectal Cancer	Sequential Assignment	9	NKG2DL	Hepatic artery transfusion	Early Phase 1	NA	2022	NCT05248048	Recruiting
Hepatic Transarterial Administrations of NKR-2 in Patients With Unresectable Liver Metastases From Colorectal Cancer (LINK)	Sequential Assignment	1	NKG2DL	Hepatic transarterial administrations	Phase 1	3 time administration: 3×10^8–3×10^9 cells/d(3ds)	2017	NCT03370198	No Results Posted
CAR-T Hepatic Artery Infusions or Pancreatic Venous Infusions for	Single Group Assignment	5	CEA	1. Hepatic artery infusions 2. Pancreatic	Phase 1	1×10^{10} cells/d	2016	NCT02850536	1. ↑ Overall survival time. 2. CAR-T safely and

(Continued)

TABLE 4 Continued

Study Name	Intervention Model	Estimated Enrollment	Antigen	Route	Phase	Dose	Date	NTC number	Major findings
CEA-Expressing Liver Metastases or Pancreas Cancer (HITM-SURE)				venous infusions					effectively target CEA-expressing LM and achieve anti-tumor activity (176).
Anti-CEA CAR-T Cells to Treat Colorectal Liver Metastases	Single Group Assignment	18	CEA	Intravenous infusion	Phase 1	1- 6×10^6/kg	2022	NCT05240950	Recruiting
EGFR-IL12-CART Cells for Patients With Metastatic Colorectal Cancer (EGFRCART)	Single Group Assignment	20	EGFR	NA	Phase 1	NA	2018	NCT03542799	No Results Posted
EGFR CART Cells for Patients With Metastatic Colorectal Cancer	Single Group Assignment	20	EGFR	NA	Phase 1 Phase 2	NA	2017	NCT03152435	No Results Posted
Binary Oncolytic Adenovirus in Combination With HER2-Specific Autologous CAR VST, Advanced HER2 Positive Solid Tumors (VISTA)	Single Group Assignment	45	HER2	Intra-tumor injection	Phase 1	1–100 × 10^6Cells (1d)	2018	NCT03740256	Recruiting
Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART133	Single Group Assignment	20	CD133	NA	Phase 1 Phase 2	0.5–2 × 10^6 cells/kg (2ds)	2015	NCT02541370	1. The 3-month disease control rate was 65.2%. 2. Repeated cell infusions provide a longer disease stability period 3. CD133+ cells elimination occurred after CART-133 infusions (177).
P-MUC1C-ALLO1 Allogeneic CAR-T Cells in the Treatment of Subjects With	Sequential Assignment	100	MUC1	NA	Phase 1	NA	2022	NCT05239143	Recruiting

(Continued)

TABLE 4 Continued

Study Name	Intervention Model	Estimated Enrollment	Antigen	Route	Phase	Dose	Date	NTC number	Major findings
Advanced or Metastatic Solid Tumors									
Autologous CAR-T/TCR-T Cell Immunotherapy for Malignancies	Single Group Assignment	73	c-Met	NA	Phase 1 Phase 2	NA	2018	NCT03638206	No Results Posted
αPD1-MSLN-CAR T Cells for the Treatment of MSLN-Positive Advanced Solid Tumors	Single Group Assignment	10	EpCAM	Intravenous injection	Early Phase 1	1×10^5-3×10^6aPD1 MSLN-CAR+ T cells/kg (1d)	2020	NCT04503980	No Results Posted

using the S100 marker) is associated with a good prognosis and less metastasis in CRC (188, 189). It has also been shown that the higher number of mature TIDCs in CRC is associated with better disease prognosis and TH and CTL responses (as seen in MSI-CRC) (190). It has been shown that the therapeutic use of DCs expressing PD-L1 leads to an increase in the lymph infiltration of $TCD8^+$ cells to the CRC site and is associated with increased patient survival (191). Both circulating DCs and TIDCs produce cytokines and anti-inflammatory factors, including VEGF, IL-10, and TGF-β, which ultimately suppress T-cell responses (184). Also, in these cells, the expression level of some genes, including genes related to COX-2 and HMGB1, increases and helps to suppress the immune system's responses (185). The important point is that tumor cells affect DCs by producing various factors such as CCL2, CXCL5, CXCL1, and VEGF. Therefore, if dendritic cells are isolated from CRC patients and autologously transplanted to patients after expansion and differentiation, they can exert strong anti-immune responses against the tumor by stimulating T cells (192). Another approach is to use the stimulating factors of DC responses and the processing and presenting of their antigens by *in situ* administration of their stimulating molecules, such as CpG, FLT3L, TLR, and STING agonists (124, 193).

6 Colorectal cancer combination therapy

Considering the TME and the complex behavior of tumor cells in the face of different treatments, it seems that monotherapy cannot achieve the desired results. As mentioned earlier, researchers had reached this conclusion many years ago and therefore used the combination of different chemotherapy drugs along with radiotherapy and surgery. With the emergence and increase of treatments based on immunology and biology to prevent the many side effects of chemotherapy, these treatments became more desirable options. In this way, researchers are interested in using multiple immunotherapies with fewer side effects to reduce the dose of chemotherapy drugs and help eradicate tumor cells.

6.1 Cytokine combination with chemotherapy

In the meantime, many achievements have been made that show that immunotherapy increases the effectiveness of chemotherapy. Studies have shown the existence of interactions between 5-FU and IFN-α in increasing cytotoxicity for different cancers (194). In a study by Laurent et al., 5-FU and IFN-α were used to treat colorectal cancer (194). The results of this study show that IFN-α, in combination with 5-FU, increases the amount of DNA single-stranded and double-stranded breaks in colorectal cancer cells *in vitro* by modulation of converting enzymes for anticancer prodrugs (194). Other studies also showed that IFN-γ could increase the activity of enzymes related to 5-FU anabolism (TP and UP) and thus help to remove more tumor cells by increasing the active form of this drug (195). Although the experimental results were promising, the combined use of 5-FU and IFN-α in clinical studies could not significantly affect patients' survival rate or tumor removal (196). For this reason, the combined use of this drug stopped.

6.2 Combination of immune checkpoint blockades and chemotherapy

According to the treatment experiences of immune checkpoint blockades with chemotherapy drugs, these drugs seem to increase immune system cells' capacity for anti-tumor responses (197). For example, it has been shown that using 5-FU can lead to apoptosis of MDSCs in the TME, which leads to the removal of immune inhibition induced by these cells (198, 199). In another way, the use of oxaliplatin, which leads to the apoptosis of tumor cells (199), causes the appearance of various antigens and their removal by DCs for the pancreas to T cells and leads to an increase in tumor-specific responses (200, 201). In one study, an *in vivo* assay using an immune checkpoint blockade mouse colon cancer model showed that an antitumor response was induced in the combined use of oxaliplatin with immune checkpoint inhibitors and resulted in

increased survival in this model (157). In addition, 5-FU and atezolizumab (202), a humanized antibody against PD-L1, were used in a clinical trial (202). To use this combination, patients are first treated with the FOLFOX chemotherapy combination; then, they are treated with 5-FU and atezolizumab (202). However, the results of this study showed that adding this therapeutic combination does not affect the progression-free survival (PFS) and overall survival (OS) of patients.

6.3 Combination of monoclonal antibodies with chemotherapy

Today, most patients with mCRC are treated with a first-line biologic agent, usually monoclonal antibodies against vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR), depending on their RAS mutation status (203). As shown in Table 5, bevacizumab is a humanized anti-angiogenic antibody used to treat various tumors as a front line in combination with chemotherapy (208). The binding of this antibody to its ligand (VEGFR) leads to the suppression of new angiogenesis and normalization of blood vessels, which allows T cells to penetrate the tumor and activate them effectively (209, 210). According to those mentioned earlier, FOLFOXIRI and bevacizumab (targeting VEGF-A) in combination with atezolizumab (targeting PD-L1) were used in a clinical trial in the front line with chemotherapy, and the results obtained from it with the therapeutic use of bevacizumab and chemotherapy have been compared (211). The number of patients participating in this study was 218 patients, the results of which show that the PFS for patients receiving combined treatment (about 13 months) was higher compared to those receiving bevacizumab and chemotherapy alone (11.5 months) ($p=0.07$) (211). This change was not significant. Further studies have used FOLFOX combined with bevacizumab and nivolumab versus FOLFOX and bevacizumab for the treatment of CRC to investigate the effect of chemotherapy combined with immunotherapy in a clinical trial study (212). The primary endpoint of PFS was not met in this study. However, patients receiving FOLFOX combined with bevacizumab and nivolumab showed a higher PFS rate than patients receiving FOLFOX and bevacizumab after 12 months (212). Also, higher and more durable immune response rates were observed in the combined treatment compared to FOLFOX and bevacizumab, which indicates that the combined treatment of chemotherapy and tumor adenoma targeting with two antibodies that target different pathways has a higher therapeutic efficiency (Figure 1).

Among other studies that have dealt with the combination of chemotherapy and immunotherapy, we can refer to the study of GOIRC-03-2018 in phase II, in which the combination of triple chemotherapy (FOLFOXIRI) with bevacizumab and nivolumab in patients with CRC containing mutations Noted in RAS/BRAF (213). The results of this study show that the combination of FOLFOXIRI with bevacizumab and nivolumab is safe and has shown promising results; that's why this group has continued its work in phase 3, the results of which have not been published yet. Also, in another clinical trial, although the POCHI trial (NCT04262687) is currently investigating the combination of CAPOX and bevacizumab with pembrolizumab as first-line treatment in eligible patients with MSS mCRC who have a high immune infiltrate, and the results are expected to show goodness in patients (214).

Cetuximab, a chimeric antibody (mouse V and human FC), is an IgG1 antibody that can increase the elimination of tumor cells by increasing ADCC and the expression of MHC-2 molecules on the surface of DCs (215, 216). It has been approved as part of the treatment regimen of CRC patients in combination therapy. In this way, to investigate the effectiveness of cetuximab in combination treatment with chemotherapy, treatment with cetuximab in combination with FOLFOX was used (phase 2 clinical trial), and it has been shown that this combination has potential therapeutic effects in selected patients (217, 218). The results of phase 3 clinical trial (TAILOR study) related to the therapeutic use of the combination of cetuximab and FOLFOX show that all the relevant clinical objectives and endpoints have been met cetuximab in combination with FOLFOX is an effective standard care first-line treatment regimen approved for patients with mCRC (219). Also, a study that investigates the therapeutic efficacy of adding avelumab to FOLFOX and cetuximab shows that this addition does not have a specific adverse effect (220). However, adding avelumab to the above combination did not reach its first endpoint, and no significant improvement was observed in the patient's condition.

6.4 Combination of CAR-T cells with chemotherapy

It seems that by modulating the phenotype and abundance of blood leukocytes, chemotherapy could facilitate the production of the most effective CAR-T cell products. Also, by increasing the activity of circulating CD8+ T lymphocytes and rebuilding the effective memory population, chemotherapy can strengthen immune system responses (221). However, chemotherapy's effect on other immune system cells, such as neutrophils, is harmful and can sometimes lead to neutropenia. Also, the results of various studies show inconclusive data about the effect of chemotherapy on $TCD4^+$ cells (Figure 2) (222). Interestingly, Bellone et al. found induced and spontaneous IFN-γ release by $TCD8^+$ cells after each course of combined chemotherapy with cisplatin, gemcitabine, and 5-fluorouracil (up to 4 cycles) are unchanged in comparison with pre-treatment values, indicating preservation of effect function throughout the treatment course (221, 223, 224). Therefore, it seems that the use of chemotherapy before transferring the CAR-T cells can provide suitable conditions in the body for the activity of these cells. However, during our search in the literature until March 2023, no study simultaneously used chemotherapy drugs and CAR-T cells to treat colorectal cancer. We recommend that researchers also use this method to treat CRC because chemotherapy can increase the therapeutic efficiency of CAR-T cells based treatments.

TABLE 5 Examples of Ab-based immunotherapy in combination with chemotherapy in clinical trials as novel therapeutics in colorectal cancer.

Study Name	Intervention Model	Estimated Enrollment	Drug	Phase	Date	NTC number	Major findings
LEAC-102 for Advanced Colorectal Cancer	Single Group Assignment	30	LEAC-102 500mg capsule and FOLFOX +Bevacizumab/ Cetuximab	Phase 1 Phase 2	2016	NCT02826837	No Results Posted
Reolysin in Combination With FOLFOX6 and Bevacizumab or FOLFOX6 and Bevacizumab Alone in Metastatic Colorectal Cancer	Parallel Assignment	109	1. FOLFOX + Bevacizumab + Reolysin 2. FOLFOX + Bevacizumab	Phase 2	2012	NCT01622543	The addition of Reolysin to FOLFOX6/bevacizumab increases the patient's overall response rate (204).
Efficacy of FOLFOX +Bevacizumab in Combination With Irinotecan in the Treatment of Metastatic Colorectal Cancer (CHARTA)	Parallel Assignment	250	1. Oxaliplatin, 5FU/LV, Bevacizumab 2. 5FU/LV, Oxaliplatin, Bevacizumab, Irinotecan	Phase 2	2011	NCT01321957	↑ Survival of patients (205).
Sequential Treatment Strategy for Metastatic Colorectal Cancer (ITACa)	Parallel Assignment	350	1. FOLFIRI or FOLFOX +Bevacizumab 2. FOLFIRI or FOLFOX 3. FOLFIRI or FOLFOX + CETUXIMAB 4. FOLFIRI or FOLFOX + BEVACIZUMAB and CETUXIMAB	Phase 3	2013	NCT01878422	Adding bevacizumab to standard first-line chemotherapy did not benefit progression-free survival, overall survival, and response rate (206).
2nd-line Treatment of Metastatic Colorectal Cancer (BEVATOMOX)	Parallel Assignment	83	1. Bevacizumab, oxaliplatin, and 5FU combination 2. Bevacizumab, oxaliplatin, and raltitrexed combination	Phase 2	2012	NCT01532804	Terminated No Results Posted
Neoadjuvant Treatment With mFOLFOXIRI Plus Cadonilimab (AK104) Versus mFOLFOX6 in Locally Advanced Colorectal Cancer (OPTICAL-2)	Parallel Assignment	82	1. mFOLFOXIRI + Cadonilimab 2. mFOLFOX6	Phase 2	2022	NCT05571644	Not yet recruiting
FOLFOXIRI + Bev + Atezo vs FOLFOXIRI + Bev as First-line Treatment of Unresectable Metastatic Colorectal Cancer Patients (AtezoTRIBE)	Parallel Assignment	201	Different combination of: Bevacizumab Irinotecan Oxaliplatin L-Leucovorin 5-fluorouracil Atezolizumab	Phase 2	2018	NCT03721653	↑ Progression-free survival in patients (207).
A Study of Biomarker-Driven Therapy in Metastatic Colorectal Cancer (mCRC) (MODUL)	Parallel Assignment	609	Different combination of: Cetuximab FOLFOX induction regimen Fluoropyrimidine (5-FU/LV or capecitabine) Atezolizumab	Phase 2	2014	NCT02291289	N/A

(Continued)

TABLE 5 Continued

Study Name	Intervention Model	Estimated Enrollment	Drug	Phase	Date	NTC number	Major findings
			Vemurafenib Bevacizumab Trastuzumab Pertuzumab Cobimetinib 5-FU/LV				
Study of Pembrolizumab Treatment After CYAD-101 With FOLFOX Preconditioning in Metastatic Colorectal Cancer	Sequential Assignment	24	Different combination of: CYAD-101 FOLFOX Pembrolizumab	Phase 1	2021	NCT04991948	Recruiting
Chemotherapy and Immunotherapy as Treatment for MSS Metastatic Colorectal Cancer With High Immune Infiltrate (POCHI)	Single Group Assignment	55	Different combination of: Capecitabine Oxaliplatin Bevacizumab Pembrolizumab	Phase 2	2020	NCT04262687	Recruiting
Tucatinib Plus Trastuzumab and Oxaliplatin-based Chemotherapy or Pembrolizumab-containing Combinations for HER2+ Gastrointestinal Cancers	Sequential Assignment	120	Different combination of: Tucatinib Trastuzumab Oxaliplatin Leucovorin Fluorouracil Capecitabine Pembrolizumab	Phase 1 Phase 2	2020	NCT04430738	Recruiting

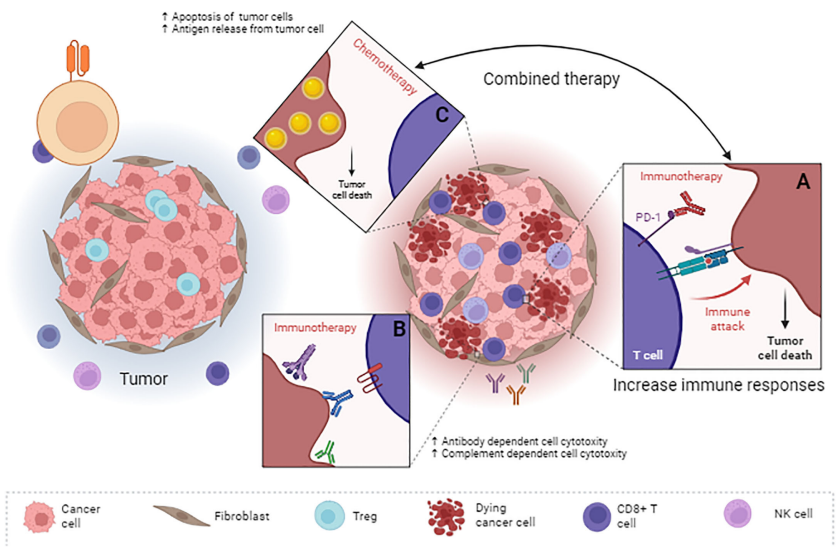

FIGURE 1
Combined use of chemotherapy and antibodies in the treatment of colorectal cancer. Antibodies can have different functions by targeting different tumor antigens. Some of them, such as immune checkpoint inhibitors **(A)** such as anti-PD-1 Ab and anti-CTLA-4 Ab, increase the response of immune cells, and some, by targeting a specific antigen **(B)**, leading to the activation of ADCC or CDC. Chemotherapy can lead to apoptosis of tumor cells and the release of various types of antigens derived from tumor cells **(C)**. On the one hand, the release of these antigens leads to an increase in the maturation of antigen-presenting cells. On the other hand, using immune checkpoint inhibitors leads to activating more immune system cells to deal with the tumor. In fact, these types of therapeutic compounds affect the tumor from different pathways and do not allow tumor recurrence after radiation or chemotherapy to the tumor cells. ADCC, Antibody-dependent cell cytotoxicity; CDC, Complement-dependent cell cytotoxicity.

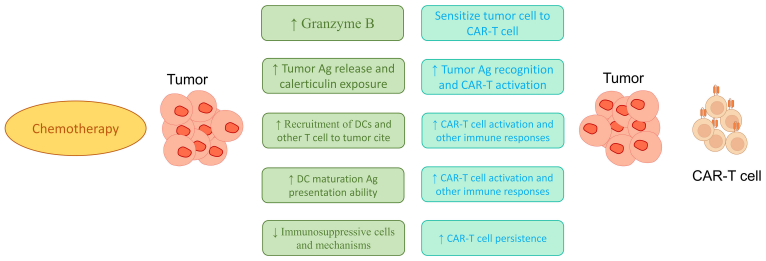

FIGURE 2
Possible mechanisms in increasing the effectiveness of treatments based on combinations of CAR-T cell and chemotherapy. As shown in the figure, the use of chemotherapy can increase the recruitment of immune cells, increase the maturation of dendritic cells, increase the activation of immune cells at the tumor site, and reduce the suppressive responses of immune cells to increase the potential therapeutic function of CAR-T cells.

7 Conclusion and future perspective

As mentioned in many articles and based on the opinion of scientists active in the field of tumor treatment, therapies based on a drug usually cannot overcome the complex TME created by tumor cells. Regarding chemotherapy, due to its side effects, the lower the dose of the drug, the better the patient's health and the fewer side effects. As discussed throughout this review, tumors use different methods and pathways to overcome various checkpoints that inhibit tumor growth. Also, many studies have shown that, in many cases, the use of a single treatment can lead to tumor resistance to that treatment. For example, cancer cells can become resistant to chemotherapy drugs by expressing some membrane pumps and creating new strains of tumor cells resistant to the given treatment. Also, using antibodies against immune checkpoints (For example, anti-PD-1 Ab) can lead to compensatory expression of other immune checkpoints (LAG-3 expression) on the surface of tumor cells (225). Therefore, it seems that combinational treatments that simultaneously target several different mechanisms related to the growth and immunosuppression induced by the tumor can be of great help in the treatment of tumors. As we know, the primary system that ultimately leads to tumor eradication is the immune system. Therefore methods based on activating this system more and more specifically against tumor cells can help eradicate the tumor faster. One of the ways to get the proper treatment and, at the same time, get the appropriate treatment is to use immunotherapy along with chemotherapy. As discussed in Section 6, in many studies, the use of this combination has helped improve patients' health. But it is worth noting that in some cases, no significant change in the improvement of the condition of patients was observed in the combined treatment group and single treatment with chemotherapy. The critical point about these treatments is that the combination of chemotherapy and the CAR-T cells in colorectal cancer was not observed in the literature. While theoretically and in clinical applications, this combination is highly effective in improving patients' conditions in other tumors. It seems that the use of new compounds, such as small molecules, chemotherapy, and immunotherapy, can affect this tumor treatment. However, more studies are needed to investigate the efficacy and safety of combination treatments.

Author contributions

All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Comparison of the efficacy and safety of third-line treatments for metastatic colorectal cancer: a systematic review and network meta-analysis

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Background: The objective of this study is to evaluate the efficacy and safety of different third-line treatment regimens for metastatic colorectal cancer (mCRC) through a comprehensive analysis and network meta-analysis (NMA). Additionally, the study aims to provide guidance on selecting appropriate third-line systemic treatment regimens for patients with mCRC.

Methods: We conducted a search of the PubMed, Embase, Web of Science, and Cochrane Central Register of Controlled Trials databases from January 1, 2005, to May 20, 2023, to include phase II/III randomized clinical trials (RCTs) of third-line treatments for mCRC. The primary outcome assessed in the NMA was median overall survival (mOS), and other outcomes included median progression-free survival (mPFS), disease control rate (DCR), and grade 3 or higher adverse events (≥3AEs).

Results: Ultimately, nine phase II/III RCTs involving five treatment regimens were included in this study. Trifluridine/tipiracil (TAS-102) plus bevacizumab (hazard ratio [HR] 0.41, 95% credible interval [CrI] 0.32-0.52) was found to be the most effective treatment for mOS compared to best supportive care (BSC). TAS-102 plus bevacizumab also significantly improved mPFS compared to BSC (HR 0.20, 95% CrI 0.16-0.25). In terms of adverse events (AEs), TAS-102 (RR 0.52, 95% CrI 0.35-0.74) had a lower incidence of ≥3AEs compared to fruquintinib, but fruquintinib (RR 1.79, 95% CrI 1.10-3.11) showed better improvement in DCR than TAS-102. Subgroup analysis using the Bayesian surface under the cumulative ranking curve (SUCRA) ranked the regimens based on the OS benefit. The results indicated that TAS-102 plus bevacizumab ranked first across age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS), and time from initial diagnosis of metastatic disease to randomization.

Conclusion: TAS-102, fruquintinib, TAS-102 plus bevacizumab, the regorafenib standard dose regimen (regorafenib), and the regorafenib dose-escalation regimen (regorafenib 80+) all demonstrated improved OS and PFS compared to BSC in mCRC patients. However, TAS-102 plus bevacizumab may be the optimal choice for third-line treatment in mCRC patients.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php, CRD42023434929.

KEYWORDS

colorectal cancer, third-line, neoplasm metastasis, network meta-analysis (NMA), treatment

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women (1). It accounts for approximately 10% of all cancer diagnoses and cancer-related deaths worldwide (2). Early-stage CRC patients often lack typical symptoms, and 20%–30% of them already have metastatic disease at the time of diagnosis (3). The prognosis for metastatic CRC (mCRC) is poor, with a 5-year survival rate of less than 20% (4).

The main treatments for early-stage CRC patients are surgery, radiotherapy, and chemotherapy. For patients with mCRC, first- and second-line treatments typically involve oxaliplatin or irinotecan combined with a fluoropyrimidine (5-fluorouracil or capecitabine), often in combination with targeted drug therapy such as vascular endothelial growth factor (VEGF) inhibitors or epidermal growth factor receptor (EGFR) inhibitors for patients with RAS wild-type (5, 6). However, most patients with mCRC eventually become insensitive or non-responsive to these treatments or intolerant to multiple cycles, leading to the need for third-line therapy. Therefore, the choice of appropriate treatment options plays a crucial role in prolonging survival.

Currently, several drugs have been approved for the standard third-line treatment of mCRC through validation in clinical trials. Regorafenib is the first small-molecule kinase inhibitor approved for the third-line treatment of mCRC. It improves patient survival by inhibiting multiple tumor growth-promoting protein kinases involved in tumor cell production, tumor angiogenesis, and maintenance of tumor microenvironment (TME) signaling (7). Trifluridine/tipiracil (TAS-102) is an oral cytotoxic antitumor drug composed of trifluridine (FTD) and tipiracil hydrochloride (TPI) in a specific ratio (8). It acts by incorporating into tumor cell DNA, thereby inhibiting tumor cell growth and proliferation (9). The presence of a thymidine phosphorylase inhibitor protects FTD from degradation and increases the concentration of the antitumor drug component (10). In the RECOURSE study, the TAS-102 group exhibited significantly higher disease control rates (DCR) (44% *vs*. 16%), longer survival (7.1 months *vs*. 5.3 months), and a 32% reduction in the risk of patient death compared to the best supportive care (BSC) group (11). The efficacy of TAS-102 was further confirmed in the 2013 TERRA study involving Asian populations (12). Fruquintinib, a highly selective oral tyrosine kinase inhibitor (TKI), gained global approval for the first time in China in 2018 for the treatment of mCRC patients who have failed at least second-line therapy, leading to benefits in both overall survival (OS) and progression-free survival (PFS) (13).

While most randomized controlled trials (RCTs) have assessed the efficacy and safety of these treatments compared to the BSC group, there is a lack of head-to-head comparisons between different treatment regimens. As a result, the selection of appropriate third-line treatment regimens for mCRC patients remains an unresolved issue. The objective of this study is to analyze the treatment effects, adverse events (AEs), and impact on relevant subgroups of various regimens through a systematic review and network meta-analysis (NMA) in the absence of direct comparisons. The aim is to evaluate the efficacy and tolerability of each regimen. The results of this study can help provide some clinical reference for the selection of third-line treatment options for mCRC patients.

Materials and methods

This study adheres to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement extension for network meta-analysis (NMA) (Supplementary Table 1) (14).

Literature search strategies and eligibility criteria

A comprehensive search was performed in the PubMed, Embase, Web of Science, and Cochrane Central Register of Controlled Trials databases from January 1, 2005, to May 20,

2023, using the search strategy outlined in Supplementary Table 2. We included phase II/III randomized controlled trials (RCTs) focusing on third-line treatment for metastatic colorectal cancer (mCRC) in the network meta-analysis (NMA). The inclusion criteria for this study were as follows: 1) phase II/III RCTs; 2) histologically confirmed mCRC in patients included in the trial; and 3) The hazard ratios (HRs) and 95% credible intervals (CrIs) for overall survival (OS) and progression-free survival (PFS), disease control rate (DCR), and adverse events (AEs) were available. Exclusion criteria: 1) non-RCTs, single-arm design studies, and dose-finding studies; 2) trial results limited to specific patient groups only, e.g., the patient group was elderly only, male only, or female only;3) studies with insufficient published data for analysis or unpublished final results.

Data extraction and risk of bias assessment

The following information was extracted from the articles: study title, study ID, publication year, first author, number of study subjects, baseline characteristics, OS, PFS, DCR, and grade 3 or higher adverse events (≥3AEs). The risk of bias in the included trials was assessed using the Cochrane risk of bias tool, which assessed seven aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Two reviewers (LLG and ZXH) independently conducted data extraction and assessed the risk of bias in the included studies. Any disagreements were resolved by a third reviewer (BL).

Statistical analysis

The primary outcome of this study was mOS. Secondary outcomes were median progression-free survival (mPFS), DCR, and ≥3AEs. The statistical heterogeneity between treatment effects across RCTs was assessed using the I^2 statistic. I^2 values below 25%, between 25% and 50%, or above 50% indicated low, moderate, and high heterogeneity, respectively (15). A network plot was generated using Stata 16.0 to visually display the comparative relationships among the various treatment regimens. Fixed and random effect models were considered and compared using deviance information criteria (DIC). If the difference in DIC between the random model and the fixed model was less than 5, the fixed model should be selected (16). The NMA was performed within a Bayesian framework using the Markov chain Monte Carlo simulation technique implemented with the GEMTC package in R-Statistics and the J.A.G.S. program (17). Each analysis involved 20,000 sample iterations with 5,000 burn-in cycles and a thinning interval of 1. Model convergence was assessed using Brooks-Gelman-Rubin diagnostic plots and trace plots (18). To estimate the probability of each treatment ranking, we calculated the surface under the cumulative ranking curve (SUCRA). A higher SUCRA value indicates a greater likelihood of a treatment regimen being the preferred option (19).

Results

Literature search and study characteristics

The flow chart depicting the study selection process is shown in Figure 1. Ultimately, we included nine phase II/III randomized controlled trials (RCTs) (11–13, 20–25), involving a total of 3456 patients and encompassing five treatment regimens. These treatments included chemotherapy (TAS-102), chemotherapy in combination with an anti-angiogenic agent (TAS-102 plus bevacizumab), best supportive care (BSC), and anti-angiogenic agents (regorafenib, regorafenib 80+, and fruquintinib). The included studies of regorafenib included two different dosage regimens: one of 160 mg/day, administered orally for 21 consecutive days over a 28-day treatment cycle (regorafenib); the other used a treatment regimen with a starting dose of 80 mg/day, which was increased by 40 mg per week up to 160 mg/day in the absence of any significant drug-related adverse effects (regorafenib 80+). The network diagram for direct and indirect comparison of all treatments is shown in Figure 2. The baseline characteristics of the study are shown in Table 1. Our NMA satisfied the transitivity assumption that the population baseline is relatively stable among the different interventions included in the study. (Supplementary Figure 7).

Overall outcomes

Regarding overall survival (OS), compared to BSC, regorafenib (HR 0.71, 95% CrI 0.60-0.84), TAS-102 (HR 0.67, 95% CrI 0.60-0.76), fruquintinib (HR 0.65, 95% CrI 0.51-0.83), regorafenib 80+ (HR 0.51, 95% CrI 0.32-0.81), and TAS-102 plus bevacizumab (HR

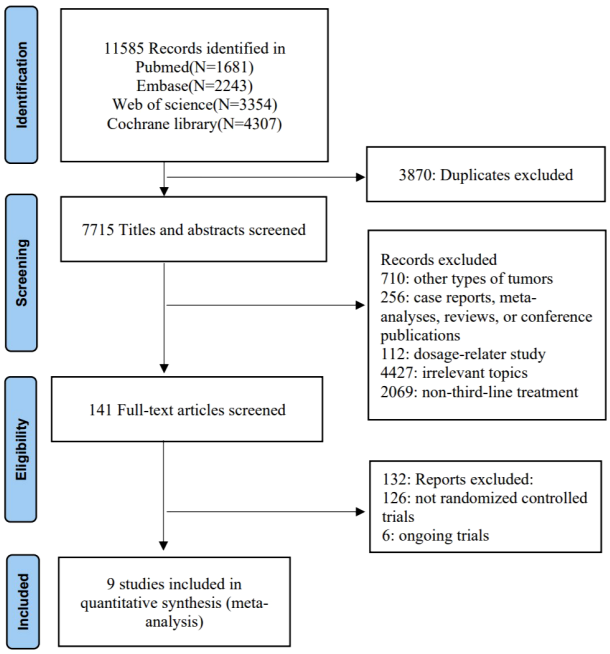

FIGURE 1
Screening and selection process.

0.41, 95% CrI 0.32-0.52) demonstrated superior efficacy (Figure 3A). According to the SUCRA results, TAS-102 plus bevacizumab (0.96) had the highest probabilities of ranking first, followed by regorafenib 80+ (0.76), fruquintinib (0.50), TAS-102 (0.44), and regorafenib (0.33) (Supplementary Figure 1A). In terms of progression-free survival (PFS), compared to BSC, regorafenib (HR 0.45, 95% CrI 0.39-0.53), TAS-102 (HR 0.46, 95% CrI 0.40-0.52), fruquintinib (HR 0.27, 95% CrI 0.21-0.34), regorafenib 80+ (HR 0.38, 95% CrI 0.25-0.58), and TAS-102 plus bevacizumab (HR 0.21, 95% CrI 0.16-0.25) were all more effective than BSC. TAS-102 plus bevacizumab also showed better PFS than regorafenib 80+ (HR 0.53, 95% CrI 0.33-0.85) (Figure 3A). The SUCRA value for TAS-102 plus bevacizumab (0.99) was higher than the other treatment regimens, followed by fruquintinib (0.80), regorafenib 80+ (0.53), and TAS-102 (0.34) (Supplementary Figure 1A). In terms of disease control rate (DCR) compared to BSC, regorafenib (RR: 3.28, 95% CrI 2.48-4.46), TAS-102 (RR: 2.88, 95% CrI 2.30-3.67), fruquintinib (RR: 5.15, 95% CrI 3.38-8.54), and TAS-102 plus bevacizumab (RR: 3.81, 95% CrI 2.52-5.92) demonstrated superiority. Fruquintinib (RR: 1.79, 95% CrI 1.10-3.11) was superior to TAS-102 (Figure 3B). The SUCRA values, in descending order, were as follows: fruquintinib (0.94), TAS-102 plus bevacizumab (0.71), regorafenib (0.52), and TAS-102 (0.33) (Supplementary Figure 1A). Regarding adverse events (AEs) with grade ≥3, the incidence rates of regorafenib (RR: 3.88, 95% CrI 2.98-5.23), TAS-102 (RR: 1.63, 95% CrI 1.43-1.88), fruquintinib (RR: 3.13, 95% CrI 2.26-4.59), and TAS-102 plus bevacizumab (RR: 1.72, 95% CrI 1.42-2.04) were all higher than BSC (Figure 3B). Gastrointestinal and hematologic toxicities were the major AEs associated with TAS-102 plus bevacizumab, although their incidence rates in the network meta-analysis were relatively low (Supplementary Figure 3).

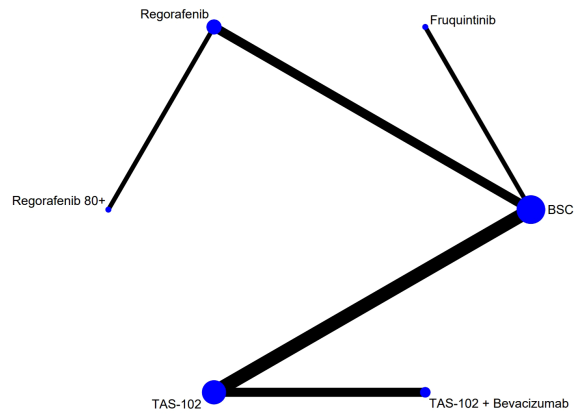

FIGURE 2
The network analysis diagram. Each circular node represented a treatment measure. The size of the nodes indicated the number of people involved in that treatment. The line between the two nodes represented the existence of a direct comparison between the two treatment options, and the thickness of the line indicated the number of direct comparisons. TAS-102, Trifluridine/Tipiracil; BSC, best supportive care.

NMA of age, gender, ECOG and region subgroup

In the age subgroup, for patients aged ≥65 years, TAS-102 (HR 0.57, 95% CrI 0.46-0.70) and TAS-102 plus bevacizumab (HR 0.33, 95% CrI 0.23-0.49) significantly prolonged survival compared to BSC. TAS-102 plus bevacizumab was also superior to regorafenib (HR 2.44, 95% CrI 1.48-4.02), TAS-102 (HR 1.71, 95% CrI 1.23-2.38), and fruquintinib (HR 2.85, 95% CrI 1.45-5.54). For patients aged <65 years, regorafenib (HR 0.67, 95% CrI 0.55-0.82), TAS-102 (HR 0.79, 95% CrI 0.67-0.93), fruquintinib (HR 0.56, 95% CrI 0.43-0.73), and TAS-102 plus bevacizumab (HR 0.51, 95% CrI 0.36-0.71) all significantly improved OS compared to BSC. Fruquintinib (HR 0.71, 95% CrI 0.52-0.97) and TAS-102 plus bevacizumab (HR 0.65, 95% CrI 0.48-0.87) were also superior to TAS-102 (Supplementary Figure 4A). In the gender subgroup, regorafenib (HR 0.74, 95% CrI 0.59-0.93), TAS-102 (HR 0.70, 95% CrI 0.59-0.82), fruquintinib (HR 0.52, 95% CrI 0.39-0.70), and TAS-102 plus bevacizumab (HR 0.42, 95% CrI 0.30-0.58) demonstrated an OS benefit in male patients compared to BSC. In female patients, regorafenib (HR 0.66, 95% CrI 0.51-0.86), TAS-102 (HR 0.72, 95% CrI 0.58-0.88), and TAS-102 plus bevacizumab (HR 0.42, 95% CrI 0.29-0.59) showed longer OS compared to BSC, except for fruquintinib (Supplementary Figure 4B). In patients with ECOG PS=0, regorafenib (HR 0.69, 95% CrI 0.53-0.90), TAS-102 (HR 0.71, 95% CrI 0.59-0.87), fruquintinib (HR 0.49, 95% CrI 0.31-0.79), and TAS-102 plus bevacizumab (HR 0.47, 95% CrI 0.33-0.68) prolonged survival significantly compared to BSC. In patients with ECOG PS=1, regorafenib (HR 0.69, 95% CrI 0.56-0.86), TAS-102 (HR 0.69, 95% CrI 0.58-0.83), fruquintinib (HR 0.68, 95% CrI 0.52-0.90), and TAS-102 plus bevacizumab (HR 0.39, 95% CrI 0.21-0.72) all demonstrated significantly better OS than BSC (Supplementary Figure 4C).

NMA of different KRAS status subgroup

In the KRAS wild-type subgroup, regorafenib (HR 0.64, 95% CrI 0.49-0.84), TAS-102 (HR 0.65, 95% CrI 0.55-0.78), and fruquintinib (HR 0.56, 95% CrI 0.40-0.78) demonstrated superior efficacy compared to BSC. In KRAS mutant patients, TAS-102 (HR 0.76, 95% CrI 0.63-0.92) achieved a significant OS benefit compared to BSC, while regorafenib and fruquintinib did not differ significantly from BSC (Supplementary Figures 1A, 4D).

NMA of primary sites subgroup

In patients with a primary tumor site in the colon, regorafenib (HR 0.71, 95% CrI 0.56-0.89) and TAS-102 (HR 0.70, 95% CrI 0.59-0.87) showed a benefit in OS compared to BSC. However, fruquintinib did not improve OS, and TAS-102 had a higher SUCRA value compared to regorafenib. In patients with rectal cancer, TAS-102 (HR 0.65, 95% CrI 0.53-0.81) and

TABLE 1 Baseline characteristics of studies included in the systematic review with Bayesian network meta-analysis of third-line treatments for metastatic colorectal cancer.

Study (phase)	Trial name	Register	Study design	Sample size	Median age	Previous chemotherapy	Intervention arm	Control arm	Primary endpoint
Grothey A et al. (III)	CORRECT	NCT01103323	Randomized double-blind trial	505/255	61/61	One or more	Regorafenib at 160 mg/day	Placebo +BSC	OS
Li J et al. (III)	CONCUR	NCT01584830	Randomized double-blind trial	136/68	57·5/55·5	Two or more	Regorafenib at 160 mg/day	Placebo +BSC	OS
Mayer RJ et al. (III)	RECOURSE	NCT01607957	Randomized double-blind trial	534/266	63/63	Two or more	TAS-102 at 35 mg/m^2 twice daily	Placebo +BSC	OS
Xu J et al. (III)	TERRA	NCT01955837	Randomized double-blind trial	271/135	58/56	Two or more	TAS-102 at 35 mg/m^2 twice daily	Placebo +BSC	OS
Li J et al. (III)	FRESCO	NCT02314819	Randomized double-blind trial	278/138	55.0/57.0	Two or more	Fruquintinib at 5mg/day	Placebo +BSC	OS
Yoshino T et al. (II)	NA	JapicCTI-090880	Randomized double-blind trial	112/57	63/62	Two or more	TAS-102 at 35 mg/m^2 twice daily	Placebo +BSC	OS
Bekaii-Saab TS et al. (II)	ReDOS	NCT02368886	Randomized open-label trial	54/62	62/61	Two or more	The starting dose of regorafenib was 80 mg/day in week 1, 120 mg/day in week 2, and 160 mg/day in week 3 for cycle 1. Weekly incremental dose escalation occurred up to the maximum of 160 mg/day if no significant drug-related toxicities were observed	Regorafenib at 160 mg/day	The proportion of patients in each group who completed two cycles of treatment and initiated the third cycle
Pfeiffer P et al. (II)	NA	EudraCT 2016–005241–23	Randomized open-label trial	46/47	64/67	One or more	TAS-102 at 35 mg/m^2 twice daily plus bevacizumab at 5 mg/kg intravenously on days 1 and 15 every 28 days	TAS-102 at 35 mg/m2 twice daily	PFS
Prager GW et al. (III)	SUNLIGHT	NCT04737187	Randomized open-label trial	246/246	62/64	One or more	TAS-102 at 35 mg/m^2 twice daily plus bevacizumab at 5 mg/kg intravenously on days 1 and 15 every 28 days	TAS-102 at 35 mg/m2 twice daily	OS

NA, not available; TAS-102, Trifluridine/Tipiracil; BSC, best supportive care; OS, overall survival; PFS, progression-free survival.

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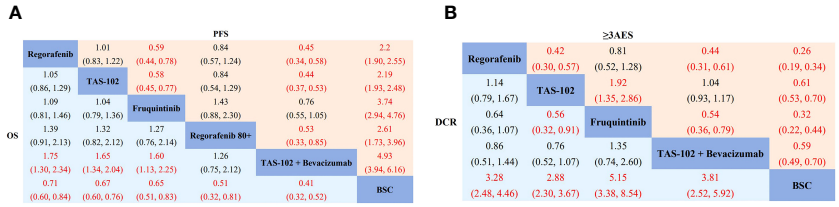

FIGURE 3
Network meta-analysis of the third-line treatments for mCRC. **(A)** Pooled hazard ratio (HR) [95% CrIs (credible intervals)] for overall survival (OS) and progression-free survival (PFS) in the overall population. **(B)** Pooled relative risk (RR) (95% CrIs) for disease control rate (DCR) and grade 3 or higher adverse events (≥3AEs) in the overall population. TAS-102, Trifluridine/Tipiracil; BSC, best supportive care.

fruquintinib (HR 0.59, 95% CrI 0.41-0.86) were superior to BSC. Regarding SUCRA values, fruquintinib (0.87) was higher than TAS-102 (0.76) and regorafenib (0.23) (Supplementary Figures 1A, 4E).

NMA of time since diagnosis of the first metastases

In the subgroup with a time of less than 18 months, regorafenib (HR 0.68, 95% CrI 0.49-0.93) and the combination of TAS-102 plus bevacizumab (HR 0.44, 95% CrI 0.29-0.67) demonstrated benefits in terms of OS compared to BSC. The combination of TAS-102 with bevacizumab was superior to TAS-102 alone (HR 1.94, 95% CrI 1.39-2.70). In the subgroup with a time greater than or equal to 18 months, regorafenib (HR 0.73, 95% CrI 0.60-0.88), TAS-102 (HR 0.65, 95% CrI 0.55-0.77), and TAS-102 plus bevacizumab (HR 0.46, 95% CrI 0.33-0.64) all improved OS compared to BSC. Furthermore, the combination of TAS-102 plus bevacizumab was superior to regorafenib (HR 1.58, 95% CrI 1.08-2.32) and TAS-102 alone (HR 1.42, 95% CrI 1.06-1.89) (Supplementary Figure 4F).

Rank probabilities

According to the SUCRA values, the ranking of different treatment options in different subgroups and the Bayesian ranking curve were estimated (Supplementary Figure 1B). The Bayesian ranking results were consistent with the NMA. TAS-102 plus bevacizumab had the highest SUCRA value for OS and PFS, indicating that it is a relatively effective treatment option for improving OS and PFS. Among regorafenib, TAS-102, fruquintinib, and TAS-102 plus bevacizumab, fruquintinib ranked first in DCR, and regorafenib ranked first in terms of ≥3AEs, indicating relatively higher toxicity. In the subgroups of age, gender, ECOG PS, and time since diagnosis of the first metastases, TAS-102 plus bevacizumab ranked first. In the subgroup of primary tumor site, compared with regorafenib and fruquintinib, TAS-102 ranked first in the colon group, while fruquintinib ranked first in the rectal group. Some treatment options were missing from subgroup analyses, resulting in relatively incomplete rankings.

Risk of bias assessment, model convergence, heterogeneity and inconsistency analysis

According to the results of the risk of bias assessment, the majority of RCTs had a low risk of bias. Please refer to Supplementary Figure 5 for the bias risk assessment chart. As seen from the trajectory plots and Brooks-Gelman-Rubin diagnostic plots, the chosen model demonstrated acceptable convergence (Supplementary Figure 6). The statistical heterogeneity of the studies, both in the primary and secondary outcomes, ranged from low to moderate ($I^2 < 50\%$, ranging from 1% to 50%) (Supplementary Table 3). In most comparisons, the fit of the consistency model was similar to or better than the inconsistency model (Supplementary Table 3).

Discussion

Regorafenib and TAS-102 have emerged as standard third-line treatments for refractory mCRC. The approval of fruquintinib in China in 2018, based on the FRESCO study, has provided an additional treatment option (13). Previous meta-analyses have reported comparable efficacy between regorafenib and TAS-102, with regorafenib showing relatively higher toxicity, which is consistent with the findings of this study (26, 27). A NMA presented at ESMO 2022 by H. Burnett et al. demonstrated that fruquintinib had the longest median progression-free survival (mPFS) and the highest reduction in the risk of disease progression or death among all currently approved third-line treatments for mCRC. Additionally, regorafenib 80+ showed superior overall survival (OS) compared to other treatment options, in line with our study results (28). However, due to the lack of comparison and analysis with the combination of TAS-102 and bevacizumab in these studies, we included this treatment option in our analysis for the first time. This allowed us to more accurately assess and optimize third-line treatment options through systematic review and NMA, offering guidance for selecting appropriate treatments for patients with mCRC.

Based on our research analysis, TAS-102 plus bevacizumab emerged as the most effective treatment in terms of both OS and PFS among all the included options, followed by regorafenib 80+ and fruquintinib. TAS-102 has demonstrated antitumor activity

against fluorouracil-resistant cell lines in preclinical xenograft models, which has important implications for CRC treatment (29, 30). Bevacizumab is a recombinant humanized immunoglobulin G1 (IgG1) monoclonal antibody that inhibits the binding of VEGF-A to VEGF receptor-2 (VEGFR-2). It can also modulate the immune system of CRC patients by inhibiting the maturation of tumor microenvironment (TME) dendritic cells (31). The combination of bevacizumab with TAS-102 may enhance the accumulation and phosphorylation levels of trifluorothymidine in tumor DNA without increasing systemic exposure or toxicity, thereby improving treatment efficacy (32).

In terms of adverse events (AEs), regorafenib exhibits a higher toxicity profile compared to other regimens. Common ≥3AEs include hand-foot syndrome (HFS), fatigue, and hypertension (20–22). Studies have shown that regorafenib-related AEs are dose-dependent, primarily occurring in the initial treatment cycles. In an effort to mitigate regorafenib toxicity, the REDOS study explored a dose escalation strategy to prolong the duration of treatment as tolerated by patients. The results demonstrated that treatment efficacy was not compromised in the dose-escalation group compared to the standard dose group, and the incidence of AEs was relatively low. Patients in the dose-escalation group also reported slightly higher overall quality of life (QOL) scores on the questionnaires, although the difference was not significant (22). However, due to the small sample size of this study, further research is needed to investigate the dosing aspects of regorafenib. The most common AEs associated with fruquintinib were hypertension, HFS, and proteinuria, similar to regorafenib but with much less fruquintinib toxicity across all classes of toxicity (13). The three regimens mentioned above generally have less hematological toxicity compared to TAS-102 (11, 12, 23). TAS-102 plus bevacizumab exhibits similar AEs to TAS-102 alone, with a higher incidence of severe neutropenia but no increased incidence of febrile neutropenia. These AEs are manageable (23, 25). Therefore, the choice of an appropriate treatment regimen can be based on the AEs associated with each option, taking into consideration the patient's individual condition.

Compared to other treatment protocols within the same subgroups, TAS-102 plus bevacizumab demonstrated the greatest improvement in survival among patients aged 65 years or older, female patients, and patients with a time of 18 months or more from the first diagnosis of metastatic disease to randomization. The SUNLIGHT study also demonstrated the efficacy of TAS-102 plus bevacizumab across different RAS mutation statuses (25). RAS mutations upregulate VEGF expression, promoting tumor angiogenesis in CRC, while bevacizumab effectively inhibits VEGF activity, delaying tumor growth and metastasis. The mechanism of action of TAS-102 involves the direct binding of FTD to DNA, indicating that RAS mutations do not directly affect the activity of TAS-102 plus bevacizumab (9, 33). The order of drug use can also impact treatment effectiveness, as demonstrated in the REVERCE study, a phase II clinical trial comparing two treatment sequences in patients with KRAS wild-type mCRC. The results showed that the regimen of regorafenib followed by cetuximab was superior to the regimen of cetuximab followed by regorafenib in terms of mOS (17.4 months *vs.* 11.6 months, P = 0.0293). This suggests that using regorafenib as the initial treatment may enhance the survival benefits for patients (34). Similar findings were observed in the RESOURCE trial, where patients previously treated with regorafenib maintained a longer survival benefit when retreated with TAS-102 (11). Notably, the CONCUR study reported a significantly greater OS benefit compared to the CORRECT study, which may be partly attributed to the inclusion of patients who had not received targeted therapy in the CONCUR trial, while the patients in the CORRECT study had received at least one targeted biological drug treatment (20, 21). These findings further support the consideration of early utilization of regorafenib.

In addition to the studies analyzed in this paper, there are other treatments worth considering. For instance, a meta-analysis conducted by Thomas Walter et al. on third-line treatment for mCRC included studies on selective internal radiation therapy (SIRT), which demonstrated that SIRT resulted in greater OS benefits for patients with liver metastases compared to systemic therapy while reducing the incidence of toxicity (26). Patients with mCRC with high microsatellite instability (MSI-H) or defective mismatch repair (dMMR) have shown better survival rates compared to those with microsatellite stability (MSS) or low microsatellite instability (MSI-L), and they have exhibited greater sensitivity to immune checkpoint inhibitor therapy (35, 36). In the REGONIVO study, the combination of regorafenib and nivolumab showed promising efficacy in MSS mCRC, with an objective remission rate (ORR) of 36% and a median progression-free survival (mPFS) of 7.9 months. This combination regimen demonstrated superior efficacy compared to regorafenib or nivolumab monotherapy, although the study had a small sample size and further validation is needed (37). Approximately 8–10% of mCRC patients have BRAF mutations, with over 90% of these mutations occurring at the V600E locus (38). In the randomized phase III BEACON study, encorafenib plus cetuximab, with or without binimetinib, showed longer OS and higher response rates compared to standard therapy (irinotecan or FOLFIRI and cetuximab) in patients with BRAF V600E-mutated mCRC who had received prior treatment. Based on the BEACON study, encorafenib in combination with cetuximab was approved by the FDA in 2020 for the treatment of patients with BRAF V600E-mutated mCRC (39).

There are several limitations to this study. Firstly, the number of clinical studies we included and the sample size of patients were limited. Furthermore, some of the included studies had inconsistent or incomplete content for subgroup analysis, which resulted in insufficient research results. Additionally, some of the definitions of AEs differed between the RCTs included in this study, which may have led to inconsistent findings. Moreover, our NMA was unable to create a closed loop, so no Bayesian method of nodal analysis or direct element analysis by the frequency method was performed. Therefore, we were unable to assess inconsistencies in the analysis due to heterogeneity (40). Although this NMA focused on third-line treatment studies, trials involving first-line, second-line, or more lines of treatment for patients were also included in the analysis. It is worth noting that different studies have different inclusion criteria, and ethnic differences in patients included in different studies may also lead to biased results. Therefore, we hope that more third-line

studies of patients with mCRC can be conducted in multiple centers worldwide, enabling direct comparison of the efficacy of different treatment regimens and detailed analysis for different subgroups, in order to provide guidance for the development of precise, individualized treatment plans for patients.

Conclusions

Based on the results of the analysis of treatment efficacy, safety, and subgroups in this study, it was found that regorafenib and TAS-102 had similar efficacy. However, regorafenib had the highest toxicity compared to other treatment options. TAS-102 combined with bevacizumab may be the optimal third-line therapy for patients with mCRC compared to the other treatment options included in this study. However, due to the limitations of the included studies in terms of number and quality, these results should be further confirmed by large-scale RCTs in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

LG: Data curation, Formal Analysis, Investigation, Software, Writing – original draft, Writing – review & editing. LT: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing. ZH: Data curation, Formal Analysis, Investigation, Writing – review & editing. JP: Formal Analysis, Investigation, Methodology, Writing – review & editing. XL: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – review & editing. BL: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1269203/full#supplementary-material

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Regorafenib alone or in combination with high/low-dose radiotherapy plus toripalimab as third-line treatment in patients with metastatic colorectal cancer: protocol for a prospective, randomized, controlled phase II clinical trial (SLOT)

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Combination strategies to improve immunotherapy response in microsatellite stable metastatic colorectal cancer (MSS mCRC) remain an unmet need. Several single-arm clinical trials have shown promising synergistic effects between regorafenib and ICIs; however, some contradictory results have also been reported. Randomized controlled trials are needed to further validate the combination of regorafenib with ICIs. In addition, low-dose radiotherapy has been demonstrated to induce local immune responses by reprogramming the tumor microenvironment when combined with high-dose radiotherapy and ICIs. In this study, we designed a prospective, randomized, controlled phase II trial to investigate the efficacy and safety of regorafenib in combination with high/low-dose radiotherapy plus toripalimab in MSS mCRC compared to regorafenib alone. Patients with MSS metastatic adenocarcinoma of the colon or rectum will be enrolled and randomly assigned into two arms: a control arm and an experimental arm. Patients in the control arm will receive regorafenib monotherapy (120 mg once daily on days 1-21 of each 28 days cycle). Patients in the experimental arm will first receive one cycle of regorafenib (80 mg once daily on days 1-21 of each 28 days cycle) and toripalimab (240mg, q3w), followed by high-dose (4-8 fractions of 8-12Gy) and low-dose (1-10Gy at 0.5-2Gy/fraction) radiotherapy, and then continue regorafenib and toripalimab

treatment. The primary endpoint is the objective response rate, and the secondary endpoints are disease control rate, duration of remission, median progress-free survival, median overall survival, and adverse events. Recruitment started in August 2023 and is ongoing.

Clinical Trial Registration: https://clinicaltrials.gov/study/NCT05963490?cond=NCT05963490&rank=1, identifier NCT05963490.

KEYWORDS

metastatic colorectal cancer, regorafenib, immunotherapy, low-dose radiotherapy, clinical protocol

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second most frequent cause of cancer deaths. Approximately 20% of patients with CRC have metastases at the time of diagnosis, and more than 50% of patients with CRC eventually develop metastases during their disease course (1). 95% of patients with metastatic CRC (mCRC) are microsatellite stable (MSS)/DNA mismatch repair proficient (pMMR) and unresponsive to immune checkpoint inhibitors (ICIs). More therapeutic options are needed to improve the outcomes of MSS mCRC patients.

Regorafenib is an oral multi-kinase inhibitor that targets signaling pathways involved in tumor angiogenesis (VEGFR1-3 and TIE2), oncogenesis (KIT, RET, RAF1, and BRAF), and the tumor microenvironment (PDGFR, FGFR, and CSF1R). It is currently approved as a salvage-line treatment for mCRC patients, but the objective response rate (ORR) is only 1%-4% (2, 3). Several mechanisms whereby regorafenib synergizes with ICIs have been evidenced by preclinical studies, including (1) reducing TAMs in tumors and modulating M1-like TAM polarization through inhibition of CSF1R (2); promoting trafficking and activity of effector T cells while decreasing recruitment of Tregs and MDSCs by targeting VEGFR (3); suppressing expression of PD-L1 and IDO1 by targeting the RET pathway (4). Multiple single-arm clinical trials have tested multi-kinase inhibitors such as regorafenib in combination with ICIs in mCRC and some of them have reported encouraging outcomes. The most impressive results were achieved in the REGONIVO study, with the ORR, median progress-free survival (mPFS), and median overall survival (mOS) of 33.3%, 7.9 months, and not reached, respectively (5). However, no objective responses were also observed when regorafenib was combined with pembrolizumab or avelumab (4). Therefore, the combination of regorafenib with ICIs warrants further validation in randomized, controlled trials.

Stereotactic ablative radiotherapy (SABR) is an effective local modality for the treatment of cancer metastases. Besides directly killing cancer cells, SABR simultaneously mobilizes innate and adaptive antitumor immune responses through (1) release of tumor antigens and damage-associated molecular molecules (DAMPs) (2); activation of the dsDNA-cGAS-STING pathway resulting in IFN production and DC maturation to prime tumor-specific T cells (3); secretion of cytokines and chemokines to promote T cell infiltration (6). A combination of SBRT and ICIs may synergistically unleash systemic T-cell responses and lead to abscopal effect. A phase II trial combining radiation of 8Gy×3Fx, ipilimumab, and nivolumab to treat patients with MSS mCRC reported a disease control rate (DCR) of 37% and an ORR of 15% outside of the irradiated field (7). This study provided proof of concept that SABR can increase the likelihood of responses to ICIs in MSS mCRC.

The clinical trials of low-dose radiotherapy (LDRT) started from the early 1930s in which patients with hematological or disseminated solid cancers were exposed to whole-and half-body LDRT totaling around 10Gy (8). The improved understanding of the interactions between radiation and the immune system has revived interest in LDRT's potential to enhance immunotherapy. Recent studies showed that LDRT could reprogram the TME through (1) inflaming tumors with an influx of T cells including those newly primed by SABR, monocytes, DCs, and NK cells (2); reversing the immunosuppressive microenvironment with M1-like macrophage polarization, TGF-β decrease, and Treg reduction (9–11). That is to say, LDRT might augment local responses to immunotherapy and further boost abscopal effect rates when added to the combination of SABR and ICIs. The *post-hoc* analysis of patients who received LDRT either unintentionally as scatter or intentionally from three prospective immune-radiation trials reported an ORR of 58% in low-dose lesions compared with 18% in no-dose lesions (12). A phase II trial examined immunotherapy plus high-dose radiotherapy (HDRT) with or without LDRT for metastatic NSCLC and melanoma. The ORR in the HDRT+LDRT cohort was 26%, which doubled that in the HDRT-only cohort. The lesion-specific response was significantly improved in low-dose lesions (53%) compared with no-dose lesions in the both HDRT+LDRT (23%) and HDRT cohort (11%) (13). These studies provide foundations for combining SABR and LDRT as a potentially paradigm-changing approach in patients with larger, diffuse, or previously radiated metastases.

Based on the above rationale, we are conducting a randomized, controlled phase II trial to compare the efficacy and safety of

regorafenib alone or in combination with SABR and LDRT plus toripalimab in MSS mCRC. Correlative studies will also be performed to explore potential predictive biomarkers and resistance mechanisms and inform better design of future clinical trials.

Methods and analysis

Study design

SLOT is a prospective, randomized, controlled, investigator-initiated phase II trial carried out at Fudan University Shanghai Cancer Center (FUSCC) in China. This is to our knowledge the first prospective trial investigating a novel radiotherapy regimen with SABR and LDRT in mCRC in addition to ICI and regorafenib. Patients with metastatic MSS CRC, who have failed or are intolerant of the standard first-and second-line therapies, will be enrolled and randomly assigned into two arms: a control arm and an experimental arm. Patients in the control arm will receive regorafenib monotherapy. Patients in the experimental arm will first receive one cycle of regorafenib and toripalimab, followed by SABR and LDRT radiotherapy, and then continue regorafenib and toripalimab treatment. The ORR, DCR, duration of remission (DoR), mPFS, mOS, and adverse effects will be analyzed. The study algorithm is presented in Figure 1.

Key eligibility criteria

Eligible patients should present histologically confirmed, MSS, metastatic (at least two evaluable lesions) CRC and have received two prior lines of chemotherapy. In addition, patients are required to have good performance status, normal organ function, no active autoimmune disease or infections, and no history of ICI or regorafenib treatment. Previous radiotherapy performed at least 4 weeks before enrollment is allowed. Inclusion and exclusion criteria are listed in detail in Table 1.

Randomization process

Eligible patients will be randomly assigned into the control arm with regorafenib monotherapy and the experimental arm with combination therapies in a 1:1 proportion. Randomization is performed via a secure software based on a stratified blocked randomization design. The block size is also randomized and the

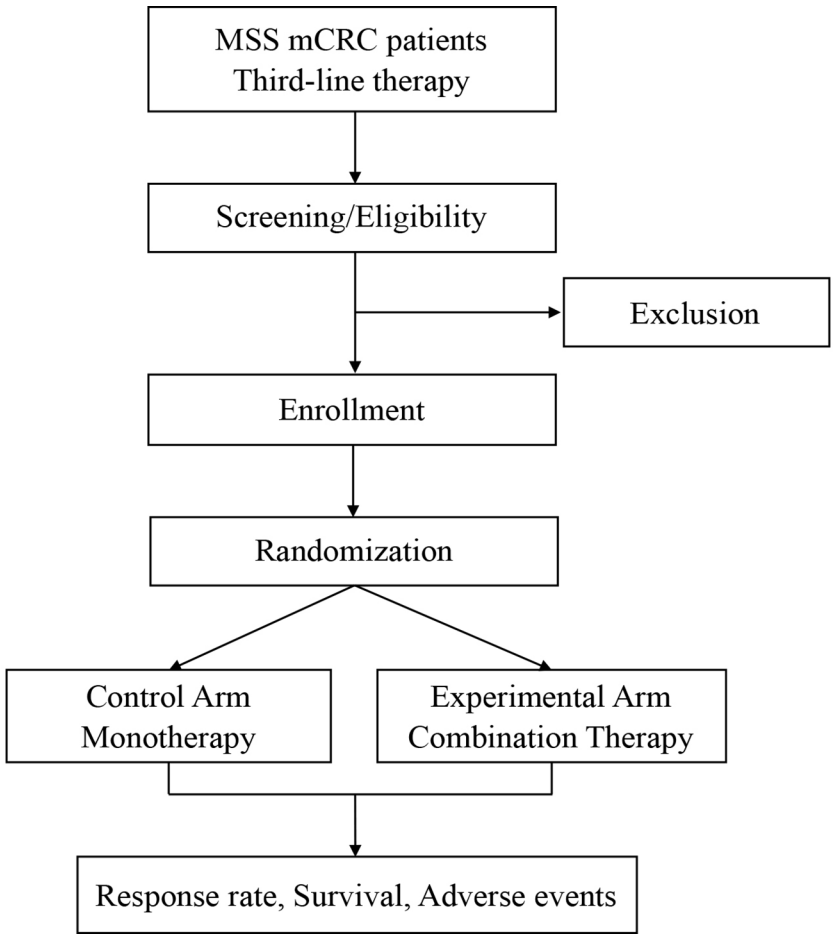

FIGURE 1
Study design of the SLOT trial.

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Age ≥18 years old 2. An Eastern Cooperative Oncology Group (ECOG) performance status ≤1 3. Life expectancy of at least 3 months 4. Histopathological confirmed MSS/pMMR adenocarcinoma of the colon or rectum 5. At least two evaluable metastatic lesions for SABR and LDRT according to RECIST 1.1 6. Progressed on or after the standard first-and second-line therapies or stopped standard therapy because of unacceptable toxic effects 7. Previous radiotherapy completed at least 4 weeks before randomization 8. Adequate bone-marrow, hepatic, and renal function: neutrophils ≥ 1.5 × 10^9/L, Hb ≥ 90 g/L, PLT ≥ 100 × 10^9/L, ALT/AST ≤ 2.5 ULN, Cr ≤ 1 ULN 9. Sign the informed consent and have good compliance	1. History of previous treatment with regorafenib and ICIs such as anti-PD-1 or anti-PD-L1 mAbs 2. Current severe cardiovascular diseases such as unstable angina, congestive heart failure, or serious cardiac arrhythmia requiring medication 3. Acute cardiac infarction or cerebral ischemic stroke occurred within 6 months before recruitment 4. Active autoimmune diseases and immunodeficiencies, known history of organ transplantation, or systematic use of immunosuppressive agents 5. Active Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection: HBsAg positive or HBV DNA positive, anti-HCV antibody testing positive and confirmatory HCV RNA positive 6. Positive human immunodeficiency virus (HIV) infection, active syphilis infection, or active pulmonary tuberculosis infection 7. Severe infections requiring systemic antibiotics, antifungal or antiviral therapy 8. Uncontrollable pleural effusion, pericardial effusion, or ascites 9. Other malignancies within 5 years before recruitment, except for non-melanoma skin cancer, superficial bladder cancer, cervical carcinoma *in situ*, or breast cancer *in situ* that had been effectively treated. 10. Known history of severe neurological or mental illness such as schizophrenia, dementia, or epilepsy 11. Known history of allergy to any component in this study. 12. Pregnancy or breast-feeding women

stratification factor is the presence of liver metastases (yes vs no). The result of randomization is immediately available in the software and will be forwarded to the investigator.

Interventions

Eligible patients will receive treatment as follows (1): control arm: regorafenib 120 mg orally once daily on days 1-21 of each 28 days cycle (2). experimental arm: patients will first receive one cycle of regorafenib and toripalimab followed by SABR/LDRT radiotherapy. Regorafenib and toripalimab will be continued after the completion of radiotherapy. In this arm, regorafenib is administered 80 mg once daily on days 1-21 of each 28 days cycle with intravenous toripalimab 240 mg every 3 weeks. Radiotherapy regimes include 4-8 fractions of 8-12Gy via SABR and up to 1-10Gy at 0.5-2Gy/fraction via LDRT (Figure 2). Patients will follow the treatment program until disease progression, unacceptable toxic effects, or withdrawal of consent.

Regorafenib: dose modifications are permitted to manage clinically significant treatment-related adverse events (AEs) of regorafenib. If AEs of grade 3 occur, regorafenib will be suspended and the AEs will be managed until the AEs have been resolved to grade 1 or baseline levels. The regorafenib dose may be reduced by 40 mg for the next treatment at the discretion of the investigator. Regorafenib will be discontinued permanently if the toxic effect did not recover after a 4-week interruption, after two consecutive dose reductions in the control arm and one dose reduction in the experimental arm (minimum permissible dose 40 mg per day), or if there are AEs of grade 4.

Radiotherapy: Radiotherapy will be initiated 3-7 days after the first administration of toripalimab, depending on actual time intervals from simulation localization to irradiation. Patients will first receive SABR and then LDRT after the completion of SABR. LDRT will be delivered using intensity-modulated radiotherapy (IMRT) or volumetric-modulated arc therapy (VMAT) and will target large tumors, previously radiated tumors, and those located in areas more sensitive to radiation's adverse effects. The choice of radiotherapy regimen is left to the discretion of the physician. For metastatic lesions in the liver, lungs, bones, and brain, the radiation field only includes gross tumor volumes (GTV) plus a 5-10 mm margin. Regional prophylactic irradiation of the lymph node drainage area may also be performed for metastatic lymph nodes. Four-dimensional CT (4DCT), passive breath gating (PBG), and abdominal spatula will be adopted to eliminate the influence of respiratory movement.

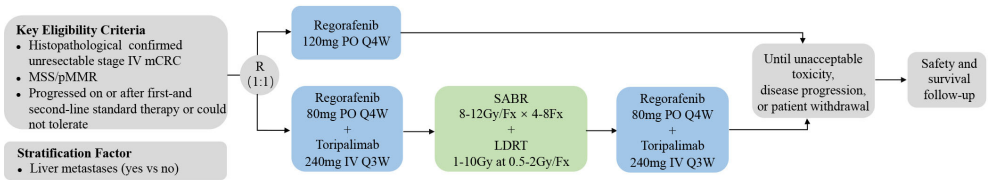

FIGURE 2
Flowchart of the SLOT trial.

Immunotherapy: the ICI used in this study will be toripalimab which is administrated intravenously at 240 mg on day 1 of every 3-week cycle. Toripalimab is provided free of charge for the first three cycles by Shanghai Junshi Biomedical Technology Co., Ltd., which has also purchased liability insurance for clinical trial subjects.

Endpoints

The primary endpoint of this study is ORR according to RECIST 1.1, and the secondary endpoints are DCR, DoR, mPFS, mOS, and adverse events. Exploratory objectives include potential predictive biomarkers and resistance mechanisms. Therefore, we will conduct genomic (DNA and RNA) sequencing and multicolor immunohistochemical staining using baseline tumor biopsies, FACS analyses of peripheral leukocytes and plasma cytokines using blood samples, and 16S rRNA sequencing of the gut microbiome using stool samples. Time points for sample collection are described in Figure 3.

Assessment and follow up

Regular radiological examinations consisting of pelvic MRI/CT, abdominal MRI/CT, chest CT, head MRI, or PET-CT (depending on the locations of metastases) will be conducted before treatment initiation and after every three cycles of toripalimab. Imaging efficacy is evaluated according to the Response Evaluation Criteria in solid tumors (RECIST v.1.1). The primary endpoint ORR and secondary DCR will be assessed based on LDRT-treated lesions in patients without non-irradiated lesions. For patients with non-irradiated lesions, ORR and DCR will be evaluated based on both LDRT-treated lesions and measurable non-irradiated lesions. Patients will also receive blood tests at baseline, after the completion of radiotherapy but before the second toripalimab, and after every three cycles of toripalimab. These tests include serum tumor markers (CEA, AFP, CA724, CA242, CA199, etc.), immune-related indicators (myocardial enzymes, thyroid hormones, and adrenal hormones), serum cytokines (IL-2, IL-6, IL-10, IFN-γ, etc.), and PBMC immunophenotyping of both lymphoid and myeloid cells (Figure 3). Adverse events will be evaluated throughout the treatment period and recorded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Quality of life during the treatment and follow-up period will be evaluated using the EORTC QLQ-C30 and EORTC QLQ-CR29 scales.

Patients who discontinue the protocol treatment will be scheduled for survival follow-up by telephone or clinic visits every two months for one year, every three months in the second year, every six months in the third to fifth year, and once a year thereafter. Subsequent therapy after progression, the cause of death, and the date of death or last follow-up visit will be recorded in detail.

Sample size

This is a prospective, randomized, controlled phase II trial and the primary endpoint is ORR. The CONCUR trial showed that regorafenib monotherapy yielded an ORR of 4%. A study of regorafenib plus toripalimab (NCT03946917) reported an ORR of 15.2% and a study combining high/low-dose radiotherapy and ICIs (NCT02710253) showed an ORR of 26% (13, 14). Based on these results, the ORR in the control arm is set as 4% (P1), and we assume that the ORR in the experimental arm can be increased to 25% (P2). The sample size is calculated using Z-test in PASS 2021. Patients will be randomly assigned into two arms at a ratio of 1:1. With a one-sided significance level (α) of 0.05 and statistical power (1-β) of 80%, 64 patients (32 patients per arm) need to be enrolled. Taking into account a maximum dropout rate of 10%, the final total sample size in this study will be 70 cases (35 cases per arm).

Statistical analysis

In this study, the SPSS 2021 software will be used for statistical analysis. Objective response and disease control rates between treatment groups will be compared using the Cochran-Mantel-Haenszel test adjusted for stratification factors. Fisher's exact test will be used to compare the patient characteristics between the two arms. Duration of Response, overall survival, and progression-free survival for each arm will be estimated using the Kaplan-Meier method. We will compare survival using a stratified log-rank test, and calculate HRs (with 95% CIs) using the Cox model, adjusting for baseline stratification factors. All statistical tests are two-sided and the level of significance is $P < 0.05$.

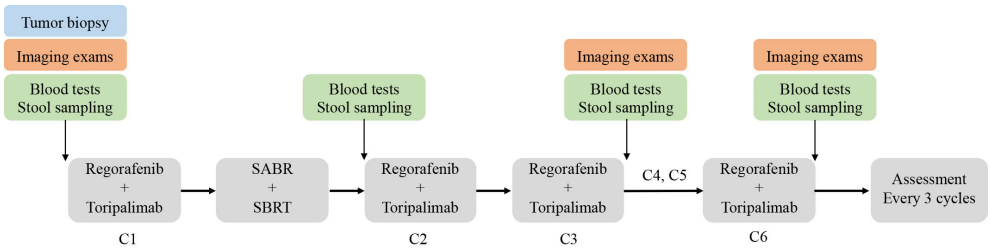

FIGURE 3
Timeline for assessment of the SLOT trial.

Discussion

The CORRECT and CONCUR study showed an ORR of 0%-4% and an mOS of 6.4-8.8 months with regorafenib monotherapy in MSS mCRC, which left much room for improvement. The efficacy data reported in multiple single-arm phase II clinical trials appear to favor regorafenib in combination with ICIs over either single agent, with ORR and mOS ranging between 7.1%-33.3% and 9.6-15.5 months (or not reached), respectively (4). However, two studies combining regorafenib with pembrolizumab or avelumab demonstrated an 0% of ORR (15, 16). Such inconsistency may have resulted from heterogeneity across studies in terms of geographic region, patient characteristics (especially treatment line and the proportion of patients with liver metastases), sample size, etc. What's more, there is a lack of randomized, controlled trials to provide the most robust evidence about the relative efficacy of regorafenib in combination with ICIs versus regorafenib alone. Therefore, we conduct the current study to further validate the efficacy and safety of combining regorafenib with toripalimab.

Besides synergizing with ICIs, regorafenib may also enhance the radiosensitivity of colorectal tumors by blocking radiation-induced activation of receptor tyrosine kinases, inhibiting VEGF-mediated angiogenesis, and suppressing DNA damage repair (17, 18). However, only a few studies have demonstrated these effects of regorafenib using tumor cell lines and mouse models, and no clinical trials investigating regorafenib in combination with radiotherapy have been reported. This is probably because the radiosensitization effect of regorafenib is not obvious enough; thus, combinations with other anticancer agents like ICIs are needed.

Different radiation doses vary in immunomodulatory effects. 8-12Gy high-dose radiation modulates systemic immune responses by facilitating tumor antigen release, presentation, and recognition to prime T cells which circulate throughout the body. Low-dose radiation mainly modulates the local TME by recruiting effector cells and reducing immunosuppressive stroma components (19). Recently, a pilot study (RACIN, NCT03728179) testing the efficacy of LDRT in combination with ICIs reported an ORR of 12.5% in eight patients with 'cold' solid tumors, and two additional patients achieved dramatic responses by PET (11). Despite this, we recommend the combined high-dose and low-dose radiotherapy plus ICIs as a better treatment regimen, based on the results of phase II studies (NCT0271025 and NCT02710253) and retrospective analysis (12, 13, 20, 21). The assumption is that high-dose radiation primes tumor-reactive T cells, while low-dose radiation facilitates the infiltration of these T cells into irradiated sites. Of note, we emphasize the importance of high-dose radiation to liver metastases which delete T cells and diminish immunotherapy efficacy (22). We also suggest low-dose radiation to all metastatic deposits, as tumor progressions were observed only outside the irradiated field in the RACIN study (11).

Overall, this prospective, randomized, controlled phase II trial investigates whether the addition of high/low-dose radiotherapy and immunotherapy can achieve better responses and prolonged survival with good tolerance compared to regorafenib monotherapy, aiming to provide an effective treatment strategy for patients with MSS mCRC.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Fudan University Shanghai Cancer Center. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SZ: Formal Analysis, Methodology, Software, Writing – original draft, Writing – review & editing. CW: Funding acquisition, Supervision, Writing – review & editing. LS: Investigation, Resources, Supervision, Writing – original draft. YanW: Project administration, Resources, Supervision, Writing – original draft. HZ: Data curation, Investigation, Project administration, Supervision, Writing – original draft. RW: Data curation, Investigation, Project administration, Writing – original draft. YaqW: Data curation, Software, Writing – original draft. YC: Investigation, Methodology, Project administration, Writing – original draft. YX: Data curation, Formal Analysis, Investigation, Writing – original draft. FX: Project administration, Supervision, Validation, Writing – review & editing. ZZ: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. JW: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Optimization of treatment strategies based on preoperative imaging features and local recurrence areas for locally advanced lower rectal cancer after lateral pelvic lymph node dissection

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Purpose: Local recurrence (LR) is the main cause of treatment failure in locally advanced lower rectal cancer (LALRC). This study evaluated the preoperative risk factors for LR in patients with LALRC to improve the therapeutic strategies.

Patients and Methods: LALRC patients who underwent total mesorectal excision (TME) with lateral pelvic lymph node (LPN) dissection (LPND) from January 2012 to December 2019 were reviewed. The log-rank test was used to assess local recurrence-free survival (LRFS), and multivariate Cox regression was used to identify the prognostic risk factors for LRFS. Follow-up imaging data were used to classify LR according to the location.

Results: Overall, 376 patients were enrolled, and 8.8% (n=33) of these patients developed LR after surgery. Multivariate analysis identified positive clinical circumferential resection margin (cCRM) as an independent prognostic factor for LRFS (HR: 4.94; 95% CI, 1.75-13.94; *P*=0.003). The most common sites for LR were the pelvic plexus and internal iliac area (PIA) (54.5%), followed by the central pelvic area (CPA) (39.4%) and obturator area (OA) (6.1%). Following a subgroup analysis, LR in the OA was not associated with positive cCRM. Patients treated with upfront surgery (n=35, 14.1%) had a lower cCRM positive rate when compared with patients treated with neoadjuvant chemoradiotherapy (nCRT) (n=12, 23.5%). However, the LR rate in the nCRT group was still lower (n=28, 36.4%) than that in the upfront surgery group (n=35, 14.%). Among patients with positive cCRM, the LR rate in patients with nCRT remained low (n=3, 10.7%).

Conclusion: Positive cCRM is an independent risk factor for LR after TME plus LPND in LALRC patients. LPND is effective and adequate for local control within the OA regardless of cCRM status. However, for LALRC patients with positive cCRM, nCRT should be considered before LPND to further reduce LR in the PIA and CPA.

KEYWORDS

lateral pelvic lymph node dissection, rectal cancer, local recurrence, neoadjuvant chemoradiotherapy, circumferential resection margin

Introduction

Since the introduction of total mesorectal excision (TME) in 1982, the local recurrence (LR) rate of rectal cancer was significant decreased (1, 2). However, the local control in patients treated with TME alone for locally advanced lower rectal cancer (LALRC) is still not satisfactory (3). The use of neoadjuvant chemoradiotherapy (nCRT) and lateral pelvic lymph node (LPN) dissection (LPND) to reduce LR in these patients is still controversial. The Japanese guidelines recommend the adoption of TME with prophylactic LPN dissection (LPND) to treat LALRC (T3/T4) (4). The Japanese clinical oncology group (JCOG) 0212 large-scale clinical trial demonstrated that TME with LPND reduced the LR in patients diagnosed with lateral tumors when compared with TME alone (7.4% versus 12.6%, P=0.024) (5). Conversely, for LALRC, the European guidelines recommend the use of nCRT instead of LPND to eradicate lateral disease and reduce the risk of LR (6, 7). However, studies have shown that the LR rate ranged between 7.2% and 13.7% after nCRT followed by TME, and the proportion of lateral pelvis recurrence is as high as 64.6%-82.7% (8). Furthermore, a multicenter collaborative study on LPNs showed that nCRT alone without LPND cannot completely eradicate metastatic LPNs. An additional LPND could significantly reduce recurrence within the lateral compartment (9). Therefore, LPND has a positive significance in improving LR in patients with LALRC.

However, some studies reported that LR rate remained between 5% to 10% after TME with LPND for LALRC patients with clear margins (5, 10). In recent years, the value and significance of comprehensive treatment strategies in LALRC have gradually emerged, and surgeons are now evaluating the use of nCRT before LPND to further reduce the risk of LR (11–13). However, immunosuppression and tissue edema caused by nCRT also increase the management difficulty and risk of complications following LPND. This highlights the need to identify factors leading to LR after LPND in patients with LALRC to optimize the treatment for these patients.

Therefore we conducted a multicenter retrospective study to identify the impact of preoperative clinical characteristics and radiologic features on the risk of developing LR after LPND in patients with LALRC. Meanwhile, the areas of LR and different preoperative treatment methods were analyzed in order to tailor appropriate comprehensive management approach according to different recurrence risk groups to improve the LR of patients with LALRC.

Abbreviations: TME,Total mesorectal excision; LR, Local recurrence; LALRC, Locally advanced lower rectal cancer; nCRT, Neoadjuvant chemoradiotherapy; LPN, Lateral pelvic lymph node; LPND, Lateral pelvic lymph node dissection; JCOG, Japanese clinical oncology group; JSCCR, Japanese Society for Cancer of the Colon and Rectum; CT, computed tomography; MRI, magnetic resonance imaging; cCRM, clinical circumferential resection margin; AJCC, American Joint Committee on Cancer; NAC, neoadjuvant chemotherapy; CPA, central pelvis area; PIA, pelvic plexus and internal iliac area; OA, obturator area; SPSS, statistical package for social science.

Methods

Study population

LALRC patients who underwent TME with LPND from January 2011 to December 2019 at three institutions of the Chinese Lateral Node Collaborative Group were identified. The clinical and radiographic characteristics were retrospectively extracted from the institutional databases and tumor registries.

The patients were included in the study if they underwent standard LPND according to the Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines for a clinical advanced rectal cancer (cT3-T4/cN+) and pathologically confirmed adenocarcinoma with the lower margin of the tumor located below the peritoneal reflection. All enrolled patients were followed for at least 36 months. Patients who underwent a palliative resection were excluded. In addition, patients with a history of other malignancies, incomplete follow-up data, and/or distant metastases were also excluded.

Ethical consideration

The study was conducted in accordance with the ethical standards of the World Medical Association Declaration of Helsinki and the STROBE Guidelines. The institutional ethics review boards of the three participating hospitals approved the study. The trial was registered (NCT04850027) in

ClinicalTrials.gov. Written informed consent was obtained from all patients enrolled in the study.

Preoperative diagnosis

All patients underwent preoperative examination, including colonoscopy, serum tumor marker analysis, computed tomography (CT), and pelvic magnetic resonance imaging (MRI). The images were evaluated by two radiologists who specialized in colorectal cancer, and the TNM stage, clinical circumferential resection margin (cCRM), extramural venous invasion (EMVI), and LPN status were recorded. Positive cCRM was defined as a distance below or equal to 1 mm between the tumor and mesenteric fascia or levator muscle (14). The EMVI status was assessed according to a 5-scale EMVI scoring system (15), whereby a score between 0 to 2 was defined as negative, and a score of 3 and 4 was defined as positive. The American Joint Committee on Cancer (AJCC) staging system (8th edition) was used to assess the TNM staging (16).

Treatment strategies

The indication of LPND was determined for the patients with cT4, cN2, or clinical suspected LPN metastasis. Clinically suspicious LPN metastasis was defined as a node with the shortest axis diameter above or equal to 5 mm with inhomogeneous or intense enhancement and an irregular shape with rough edges based on MRI. The treatment strategies for the patients were determined based on the patient's preferences and the recommendations of a multidisciplinary team that incorporated radiologists, medical oncologists, and surgical oncologists. nCRT or neoadjuvant chemotherapy (NAC) was recommended for patients with a high risk of distant metastasis or LR, such as T4b stage and multiple lymph node metastases. The indications for the use of nCRT and NAC were similar. Treatment strategies for LPN metastases were updated during the study period. Between 2011 and 2017, patients with clinically suspected LPN metastasis were mainly treated using upfront surgery without preoperative treatment. After 2018, preoperative chemotherapy or chemoradiation was performed before LPND for patients with a short LPN diameter above 8 mm.

The nCRT regimen consisted of a long radiotherapy treatment course using a prescription of 50 Gy in 25 fractions and capecitabine at a dose of 825 mg/m^2 administered twice daily on all days of radiotherapy. The NAC regimen consisted of 4 to 6 cycles of either FOLFOX or XEOLX. Surgical resections were performed 4 to 6 weeks after NAC and 6 to 8 weeks after nCRT.

LPND procedure

All chief surgeons involved in the study had completed at least 500 cases of laparoscopic colorectal surgery and mastered the mature LPND technique. Unilateral LPND was usually performed according to the location of enlarged LPN or the main invasion direction of the primary tumor, while bilateral LPND was only performed in bilateral LPN enlargement. The LPND was performed in accordance with the JSCCR guidelines for all patients (13, 17). The extent of the dissection included 4 areas: the internal iliac lymph node, obturator lymph node, external iliac lymph node, and common iliac lymph node (4).

Adjuvant therapy

Patients with stage III and high-risk stage II diseases (CRM ≤ 1 mm, pT4, tumor perforation, lymphatic invasion, perineural invasion) received adjuvant chemotherapy within 4 to 6 weeks after surgery. All patients treated with nCRT, irrespective of their pathological stage, received 6 months of perioperative chemotherapy.

Follow-up procedure

Patients were followed-up every 3 months for the first 3 years and every 6 months after 3 years. The examinations performed during each follow-up included a physical examination, assessment of tumor markers (CEA and CA19-9), and a CT of the chest, abdomen, and pelvis. In addition, a total endoscopy was performed annually. The endpoints of this study were 3-year LR-free survival (LRFS) and 3-year LR rate.

Classification of LR area

All patients were followed for more than 3 years, so only LR within 3 years were counted in this study. The LR sites were classified into central pelvis area (CPA), pelvic plexus and internal iliac area (PIA), or obturator area (OA) based on follow-up image data as described by Shiraishi et al. (18) (Figure 1). LR within the anastomosis region, presacral fascia, or perirectal soft tissue away from the pelvic plexus and neurovascular bundle was classified as CPA (Figure 2A), LR within the soft tissue area around the pelvic plexus or neurovascular bundle or the region along the internal iliac artery and veins was classified as PIA (Figure 2B), and LR between the internal iliac artery and pelvic sidewall was classified as OA (Figure 2C).

Statistical analysis

The continuous variables were expressed as mean ± standard deviation and compared using the paired T-test or Mann–Whitney's U-test. The categorical variables are presented as percentages, and the Chi-square test or Fisher's exact test was used to compare these variables. The 3-year cumulative LRFS was calculated by the Kaplan–Meier method, and univariate analysis was performed using the log-rank test. The statistically significant variables in the univariate analysis were subsequently tested by multivariate analysis using a Cox regression model. A *P*-value below 0.05 was considered statistically significant. The statistical package

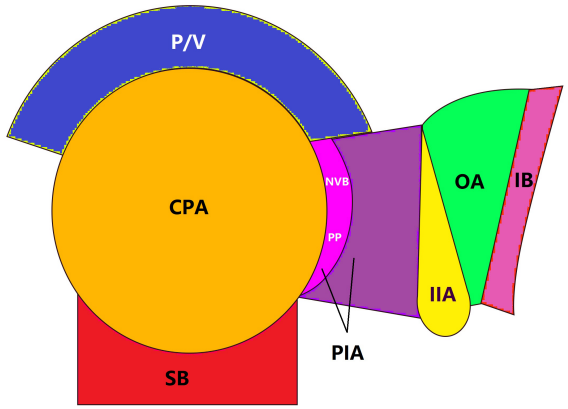

FIGURE 1
Local recurrence areas. Local recurrence was classified into 3 areas: CAP, central pelvic areal; P/V, prostate or vagina; SB, sacrum bone; PIA, pelvic plexus and internal iliac area; NVB, neurovascular bundle; PP, pelvic plexus; IIA, internal iliac artery; OA, obturator area; IB, ischial bone.

for social science (SPSS) software version 24.0 (IBM Inc., Armonk, NY, USA) was used for statistical analysis.

Results

Patient characteristics

A total of 376 patients were eligible for the study, of whom 33 had LR and 343 did not have LR. The patients' demographic data and clinical characteristics are summarized in Table 1. The cT stage, cN stage, enlarged LPN, cCRM, EMVI, and adjuvant therapy differed significantly between the LR and non-LR groups. (P<0.05).

Surgical outcomes and postoperative recovery

The surgical outcomes and postoperative recovery are shown in Table 2. The majority of the patients (n=267, 71.0%) had laparoscopic surgery as opposed to open surgery. The surgery involved either a low anterior resection (n=192, 51.1%), abdominoperineal resection (n=147, 39.1%), Hartmann procedure (n=12, 3.2%), or a total pelvic exenteration (n=25, 6.6%). There were no significant differences in the operation type, resection site, LPND procedure operation time, intraoperative blood loss, postoperative complications, and length of hospital stay between the LR and non-LR groups (P>0.05).

Pathological results

The pathological results between the LR and non-LR groups are compared in Table 3. The proportion of patients in the LR group with T3-T4 stage (90.9% versus 68.8%, P=0.008), N1-N2 stage (78.8% versus 45.2%, P<0.001), poor differentiation (42.4% versus 25.7%, P=0.039), and perineural invasion (60.6% versus 36.7%, P=0.007) was significantly higher than that in the non-LR group.

Preoperative risk factors for LR and LRFS

The median follow-up period for the entire group was 57 months. The incidence of 3-year LR was 8.8% (33/376), and the estimated 3-year LRFS rate was 90.1% (Figure 3A). The univariate and multivariate analyses of the preoperative risk factors influencing LRFS are presented in Table 4. The 3-year LRFS in positive cCRM patients was significantly worse than patients with negative cCRM (92.3% versus 81.3%, P=0.003) (Figure 3B). Univariate analysis demonstrated that enlarged LPN, histology, EMVI, cCRM, cT stage, and cN stage were the preoperative predictors for LRFS (P<0.05). Multivariate analysis revealed positive cCRM (HR: 4.94; 95% CI, 1.75-13.94; P=0.003) as an independent prognostic factor for LRFS.

LRFS and LR according to cCRM status, tumor site resection, and preoperative treatment

The most common LR site was PIA (n=18, 54.5%), followed by CPA (n=13, 39.4%) and OA (n=2, 6.1%). Table 5 summarizes the findings of the subgroup analysis evaluating the relationship between LR site and cCRM. The proportion of positive cCRM in patients with LR in the CPA (8.3% *vs* 2.3%, P=0.022) and PIA

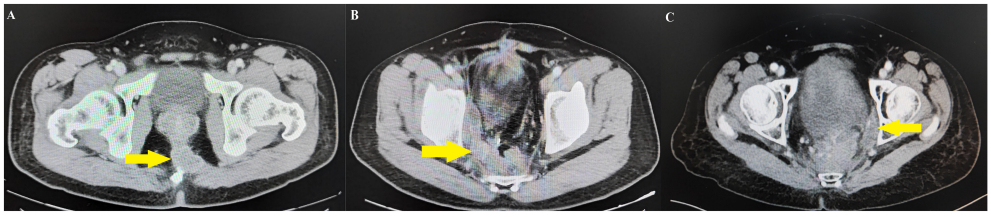

FIGURE 2
Follow-up image data of local recurrence areas. **(A)** Central pelvis area case identified the local recurrence on presacral fascia. **(B)** Pelvic plexus and internal iliac area case identified the local recurrence between the right pelvic plexus and the internal iliac artery. **(C)** Obturator area case identified the local recurrence between internal iliac artery and pelvic side wall.

TABLE 1 The demographic data and clinical characteristics between LR and non-LR groups.

Characteristics	LR (n=33)	Non-LR (n=343)	*P*
Age at operation (y, mean ± SD)	54.1 ± 12.2	57.2 ± 11.3	0.143
Gender (%)			0.340
Male	18 (54.5)	216 (63.0)	
Female	15 (45.5)	127 (27.0)	
BMI (Kg/m^2,mean ± SD)	23.3 ± 2.4	24.5 ± 5.6	0.228
ASA score (%)			1.000
I-II	32 (97.0)	333 (97.1)	
III	1 (3.0)	10 (2.9)	
Preoperative CEA level (ng/ml)			0.723
<5	22 (66.7)	218 (63.6)	
≥5	11 (33.3)	125 (36.4)	
Distance from AV (cm, mean ± SD)	4.3 ± 2.5	4.8 ± 2.4	0.333
Preoperative treatment			0.013
None	18 (54.5)	230 (67.1)	
Preoperative chemotherapy	10 (30.3)	41 (12.0)	
Preoperative Chemoradiotherapy	5 (15.2)	72 (20.9)	
cT stage (%)			0.022
T1-T2	0 (0)	44 (12.8)	
T3-T4	33 (100.0)	299 (87.2)	
cN stage (%)			0.012
N0	3 (9.1)	102 (29.7)	
N1-N2	30 (90.9)	241 (70.3)	
Enlarged LPN			0.042
Presence	19 (57.6)	135 (39.4)	
Absence	14 (42.4)	208 (60.6)	
EMVI			0.011
Positive	10 (30.3)	47 (13.7)	
Negative	23 (69.7)	296 (86.3)	
cCRM			<0.001
Positive	17 (51.5)	58 (16.9)	
Negative	16 (48.5)	285 (83.1)	
Adjuvant therapy			0.027
Presence	29 (87.9)	239 (69.7)	
Absence	4 (12.1)	104 (30.3)	

TABLE 2 The surgical outcomes and postoperative recovery between LR and non-LR groups.

Characteristics	LR (n=33)	Non-LR (n=343)	*P*
Operation type			0.303
Open	7 (21.2)	102 (29.7)	
Laparoscopic	26 (78.8)	241 (70.3)	
Surgical procedure			0.271
Low anterior resection	12 (36.4)	180 (52.5)	
Abdominoperineal resection	17 (51.4)	130 (37.9)	
Hartmann procedure	2 (6.1)	10 (2.9)	
Total pelvic exenteration	2 (6.1)	23 (6.7)	
LPND procedure			0.497
Unilateral dissection	19 (57.6)	218 (63.6)	
Bilateral dissection	14 (42.4)	125 (36.4)	
Operation time (min, mean ± SD)	303.3 ± 82.1	290.4 ± 112.1	0.519
Estimated blood loss (ml, mean ± SD)	214.6 ± 269.0	237.2 ± 379.8	0.739
Postoperative complications			0.695
Presence	9 (27.3)	83 (24.1)	
Absence	24 (72.7)	260 (75.9)	
Total hospital stay (day, mean ± SD)	12.3 ± 12.0	13.9 ± 12.6	0.695

(13.9% *vs* 2.6%, *P*<0.001) were significantly higher, suggesting that LR in these two areas were associated with positive cCRM. LR in the OA was not associated with positive cCRM. However, the number of LR events in the OA was too small.

Table 6 shows the LR and cCRM status according to the preoperative treatment. LR was observed in 18 (7.3%) of the 248 patients treated with upfront surgery, 10 (19.6%) of the 51 patients treated with NAC, and 5 (6.5%) of the 77 patients treated with nCRT. Patients who underwent nCRT had a lower LR rate. However, it is important to note that the cCRM positive rate in patients who underwent nCRT (n=28, 36.4%) was higher than that of patients treated with upfront surgery (n=35, 14.1%) and NAC (n=12, 23.5%). In addition, among patients with positive cCRM, the LR rate in patients who underwent nCRT remained low (n=3, 10.7%).

Discussion

The JCOG0212 trial demonstrated the benefits of LPND in LALRC patients in reducing LR in the lateral pelvic compartment following TME (5). However, even in patients with negative surgical

TABLE 3 Pathological results between LR and non-LR groups.

Characteristics	LR (n=33)	Non-LR (n=343)	*P*
(y)pT stage			0.008
T0-T2	3 (9.1)	107 (31.2)	
T3-T4	30 (90.9)	236 (68.8)	
(y)pN stage			<0.001
N0	7 (21.2)	188 (54.8)	
N1-N2	26 (78.8)	155 (45.2)	
Histologic grade			0.039
Moderate	19 (57.6)	255 (74.3)	
Poor/Mucinous/signet	14 (42.4)	88 (25.7)	
Pathological LPNM			0.233
Presence	10 (30.3)	73 (21.3)	
Absence	23 (69.7)	270 (78.7)	
CRM status			0.188
Positive	4 (12.1)	17 (5.0)	
Negative	29 (87.9)	326 (95.0)	
Perineural invasion			0.007
Presence	20 (60.6)	126 (36.7)	
Absence	13 (39.4)	217 (63.3)	
Lymphatic invasion			0.057
Presence	15 (45.5)	101 (29.4)	
Absence	18 (54.5)	242 (70.6)	
LPNs removed (n, mean ± SD)	7.3 ± 4.9	9.0 ± 6.2	0.120
Mesorectal LN removed (n, mean ± SD)	16.8 ± 6.7	17.4 ± 8.2	0.422

margins, the LR rate remains high at 5% to 10% after TME with LPND for LALRC patients (5, 10). Therefore, this study aimed to explore the preoperative risk factors associated with LR after TME with LPND. In addition, the LR sites were also classified and evaluated in detail to selected appropriate comprehensive management approach in specific patients.

Primarily, our study demonstrated that the 3-year LRFS in positive cCRM patients was significantly worse than that in patients with negative cCRM. (92.3% versus 81.3%, *P*=0.003). A positive cCRM was identified as an independent prognostic factor (HR: 4.94; 95% CI, 1.75-13.94; *P*=0.003) for LRFS. In addition, the highest LR rate occurred in the PIA (n=18, 54.5%), while LR in the OA (n=2, 6.1%) was the least common. Patients with positive cCRM had a high LR rate in the PIA and CPA but not in the OA. LPND followed by nCRT might effectively reduce the risk of LR in the PIA and CPA, especially in cases with positive cCRM.

Lateral lymphatic drainage is one of the most common metastatic pathways for tumors located below the peritoneal reflection. Our previous study demonstrated that patients with pathologically confirmed LPN metastasis could achieve satisfactory local control through LPND (19). This study identified LPN enlargement in 154 (40.9%) preoperative MRIs, which were subsequently confirmed by pathology in 83 (22.1%) patients. Univariate analysis showed that LPN enlargement was associated with worse LRFS (*P*=0.004). However, multiple lymph node metastases, poor differentiation, positive cCRM, and other adverse pathological factors can increase the risk of LR after surgery. Enlarged LPN was not an independent prognostic factor for LRFS after the above confounders were eliminated by multivariate analysis, suggesting that LPND can achieve satisfactory local control for patients with enlarged LPN.

Studies have shown that the accuracy of the preoperative cCMR assessment on MRI is comparable to that of the pathological gold standard (14, 18, 20). The present study revealed that positive cCRM was significantly associated with LR after LPND in patients with LALRC. Furthermore, the LR rate in the lateral pelvic area, except for LR in the OA, was high after LPND, especially in patients with positive cCRM. These findings suggest that the LPND procedure within the OA is easier to perform, minimizing the risk of residual microscopic disease after surgery. The lower local control rates in the PIA after TME with LPND in patients with positive cCRM could be attributed to cancer cells remaining in the pelvic plexus area when the tumor penetrates the proper fascia of the rectum via the lateral pathway. The LPND procedure that

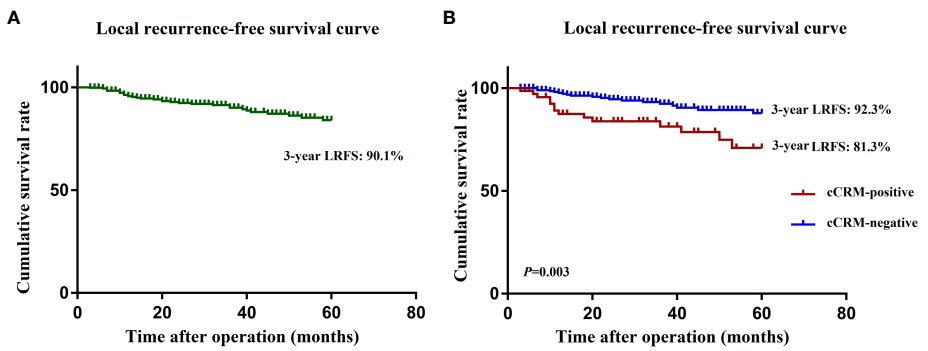

FIGURE 3
(A) Kaplan-Meier curves for local recurrence rate in all patients. **(B)** Kaplan-Meier curves for local recurrence rate in cCRM-positive and cCRM-negative patients. LRFS, local recurrence free survival; cCRM, clinical circumferential resection margin.

TABLE 4 The univariate and multivariate analyses of the preoperative risk factors influencing LRFS.

Variables	Local recurrence-free survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Gender: male/female	0.67 (0.34-1.34)	0.259		
Age at operation (≥65/<65years)	0.68 (0.28-1.64)	0.384		
CEA level (>5/≤5 ng/L)	1.13 (0.55-2.33)	0.748		
Distance from anal verge (>5/≤5 cm)	0.58 (0.26-1.29)	0.184		
Preoperative treatment (yes/no)	1.09 (0.69-1.72)	0.718		
None				
Preoperative chemotherapy	1.90 (0.88-4.11)	0.105		
Preoperative Chemoradiotherapy	1.46 (0.35-2.41)	0.464		
Enlarged LPN	2.80 (1.39-5.64)	0.004	1.83 (0.69-4.86)	0.226
Histology (Poor, Mucinous or signet/moderate)	2.59 (1.16-5.80)	0.021	1.88 (0.79-4.47)	0.152
EMVI (Positive/negative)	2.38 (1.15-4.92)	0.019	1.67 (0.49-5.65)	0.409
cCRM (Positive/negative)	2.81 (1.41-5.62)	0.003	4.94 (1.75-13.94)	0.003
cT stage	1.73 (1.05-2.99)	0.048	0.86 (0.46-1.60)	0.630
T1-T2	Reference		Reference	
T3	2.03 (0.77-5.36)	0.153	1.24 (0.49-3.35)	0.626
T4	2.85 (1.28-8.70)	0.010	1.86 (0.91-7.32)	0.102
cN stage				
N0	Reference		Reference	
N1	1.77 (0.77-4.11)	0.181	0.89 (0.27-2.29)	0.840
N2	2.71 (1.19-6.17)	0.017	1.22 (0.43-3.41)	0.712

preserves the pelvic plexus may increase the possibility of residual tumor tissue omission, potentially increasing the risk of recurrence in this area. Therefore, multidisciplinary strategies should be considered for patients with positive cCRM to improve local control in the lateral pelvic area (PIA), except for the OA.

In this study, all enrolled patients were followed for at least 36 months, and the median follow-up period for the entire group was 57 months. Because the median follow-up was less than 5 years, therefore, the endpoints of this study were 3-year LRFS and 3-year LR rate. The literature has reported that the 3-year LR rate of patients with LALRC after R0 resection is 5%-14% (21–23). This study also included patients with LALRC (cT4, cN2, or clinical suspected LPN metastasis), and 3-year LR rate after radical surgery was 8.8%, which was basically consistent with previous literature reports. Neoadjuvant therapy has positive prognostic value and significance in patients with LALRC (24, 25). From 2009 to 2010, Poulsen et al. treated 479 (29%) of 1633 patients with LALRC in Denmark, and only 68 patients (4.2%) developed local recurrence within 3 years, reflecting the satisfactory local control effect of neoadjuvant therapy (26). In present study, we also analyzed the

TABLE 5 Relationship between LR area and cCRM.

Local recurrence area	cCRM		*P*
	Positive (n=72)	Negative (n=304)	
Central pelvis area	6 (8.3)	7 (2.3)	0.022
Pelvic plexus and internal iliac area	10 (13.9)	8 (2.6)	<0.001
Obturator area	1 (1.4)	1 (0.3)	0.347

TABLE 6 LR and cCRM status according to preoperative treatment.

Local recurrence area	Preoperative treatment		
	None (n=248)	NAC (n=51)	NCRT (n=77)
Local recurrence	18 (7.3)	10 (19.6)	5 (6.5)
cCRM positive	35 (14.1)	12 (23.5)	28 (36.4)
Local recurrence in cCRM positive	10/35 (28.6)	4/12 (33.3)	3/28 (10.7)

relationship between different preoperative treatment models and LR and found that patients with nCRT (6.5%) had a significantly lower LR rate than patients who underwent NAC (19.6%) and upfront surgery (7.3%), even if the proportion of positive cCRM was higher in patients with nCRT (36.4%). In addition, nCRT still has an advantage over NAC in terms of local control in patients with positive cCRM (10.7% versus 33.3%). Similarly, a previous study reported that NAC could not control the LR in patients with a high risk of recurrence (27). In addition, Shiraishi et al. also revealed that LALRC patients with positive cCRM required nCRT instead of NAC to decrease LR (15). Therefore, nCRT should be considered as preoperative treatment in LALRC patients with positive cCRM.

Limitations

Our study has several limitations that have to be acknowledged. First of all, due to the limited number of participants, the study is prone to selection bias caused by variations in the population characteristics, surgical quality, and treatment strategies. The retrospective multicenter nature of the study may also limit the generalizability of the research findings. However, all three institutions involved in the study were tertiary hospitals from the Chinese Lateral Lymph Node Collaboration group. Therefore the treatment concept and technology can fully reflect the current diagnosis and treatment level of LPN metastasis in China. Moreover, the proportion of patients undergoing open surgery was higher in earlier years, and more recently the proportion undergoing laparoscopic surgery was higher. Changes in medical technology and treatment strategies can lead to different outcomes. In addition, while the indications for NAC and nCRT are similar, the surgeons' preference for treatment may have influenced the research findings. Furthermore, since the study was performed over 8 years, the continuous development and updating of laparoscopic equipment and technology may have influenced the results of this study. Finally, the median follow-up time of the whole study was only 37 months, so we only calculate 3-year LRFS. A longer follow-up period is required to identify the long term impact of these treatments on LR and survival.

Conclusions

The present study showed that positive cCRM is an independent risk factor for LR after TME with LPND in patients with LALRC. LPND is effective and adequate for local control in OA regardless cCRM status. However, for LALRC patients with positive cCRM, nCRT should be considered before LPND to further reduce LR in the PIA and CPA regions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by All enrolled patients sign written informed consent to participate in the study. The study was conducted per STARD reporting guidelines. All the procedures followed the ethical standards of the World Medical Association Declaration of Helsinki. The study was approved by the institutional review boards of the three hospitals, and the study design was registered (NCT04850027) at ClinicalTrials. gov. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ZX: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. MB: Writing – original draft, Writing – review & editing, Conceptualization. QCC: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. QC: Supervision, Validation, Writing – review & editing. QW: Formal analysis, Investigation, Project administration, Software, Writing – review & editing. WX: Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing. QL: Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JL declared a shared affiliation with the author(s) QL to the handling editor at the time of review.

Publisher's note

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