Original Article

The prognostic and predictive value of mismatch repair status in patients with locally advanced rectal cancer following neoadjuvant therapy

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Background: We examined the predictive value of mismatch repair (MMR) status in relation to responses to neoadjuvant therapy and the prognosis of locally advanced rectal cancer patients.

Methods: A total of 854 consecutive patients with locally advanced rectal cancer with MMR status who underwent neoadjuvant therapy followed by curative surgery between January 2013 and December 2018 were included this retrospective study. MMR status was determined by an analysis of MMR protein expression by immunohistochemistry (IHC). Propensity score matching was performed to reduce imbalances in baseline characteristics. The categorical variables were compared using the Chi-square test or Fisher's exact test. Local recurrence-free survival (LRFS) and disease-free survival (DFS) curves were estimated using the Kaplan-Meier method.

Results: Deficient MMR (dMMR) was detected in 63 of the 854 (7.4%) patients. Patients with dMMR had a lower proportion of tumor regression grade (TRG) of 0–1 compared with proficient MMR (pMMR) (28.6% *vs.* 43.7%; P=0.027). After propensity score matching at 1:4 for patients who received chemotherapy alone, proportion of TRG of 0–1 was observed to be significantly lower in dMMR than pMMR patients (9.1 *vs.* 30.3%%; P=0.013). For patients who received chemoradiation, after matching, no significant difference in the proportion of TRG 0–1 and the pathological complete response (pCR) rate was observed. The multivariable analysis revealed that patients whose tumors had dMMR had significantly longer DFS than those whose tumors had pMMR [hazards ratio (HR) =0.38, 95% confidence interval (CI): 0.18–0.81, P=0.013]. In the subgroup analysis, dMMR was only a statistically significant prognostic factor for DFS in patients with ypStage II/III (HR =0.38, 95% CI: 0.17–0.86; P=0.020).

Conclusions: We found that patients with dMMR responded worse to chemotherapy alone than patients with pMMR, in terms of TRG. Also, dMMR is a good prognostic marker for DFS in patients with ypStage II/III after neoadjuvant therapy.

Keywords: Rectal cancer; deficient mismatch repair (dMMR); neoadjuvant therapy

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Introduction

With 1.8 million new cases and 0.8 million deaths each year colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer, and the 2nd cause of cancer-related death worldwide (1). CRC is a disease that develops via 2 well-described heterogeneous pathways of colorectal carcinogenesis; that is, chromosomal instability and, less commonly, microsatellite instability (MSI) (2,3). MSI is a consequence of a deficient mismatch repair (dMMR) system that results in the accumulation of insertion and/or deletion mutations within microsatellite deoxyribonucleic acid (DNA) regions (4,5). The MMR system is an evolutionarily high conserved system that recognizes mismatches and repairs DNA errors (6). Deficient MMR can result from the inheritance of a germline mutation in an MMR gene (e.g., *MLH1*, *MSH2*, *MSH6*, or *PMS2*), a situation called Lynch Syndrome, or more commonly, from the epigenetic inactivation of MLH1 in sporadic cases, often associated with the CpG island methylator phenotype (7,8). Thus, dMMR refers to a loss of function of MMR system, while proficient MMR (pMMR) refers to a proficient function of MMR system (6). Several studies and systematic reviews have shown that dMMR is associated with a more favorable stage-adjusted prognosis in non-metastatic colon cancer, and that 5-fluorouracil (5-FU) adjuvant therapy provides no benefits in stage II patients (9-16). Emerging evidence also suggests that dMMR can be predictive of a durable response and survival gain from immune checkpoint inhibitors (e.g., programmed cell death protein 1) in advanced and metastatic CRCs (17-19).

To date, the implications of MMR status have not been fully evaluated in relation to locally advanced rectal cancer for which dMMR prevalence has been reported to be <10% with a gradual decrease in its distribution from the proximal colon to the rectum (20,21). It remains unknown whether dMMR could predict tumor responses to neoadjuvant therapy, including chemoradiation or chemotherapy alone, or whether dMMR could be used as a prognostic marker for oncological outcomes after neoadjuvant therapy. Li *et al.* reported the predictive value of MMR in gastric and gastroesophageal junction adenocarcinoma patients receiving neoadjuvant chemotherapy. No significant difference was found in the terms of tumor regression grade (TRG) between pMMR and dMMR tumors (22). However, the predictive value of MMR status in rectal cancer receiving neoadjuvant therapy was unknown. Thus, the present study sought to investigate the predictive and prognostic value of dMMR in locally advanced rectal cancer patients who had undergone neoadjuvant therapy including chemotherapy alone and chemoradiation therapy. We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-124/rc)

Methods

Study population

This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of the Sixth Affiliated Hospital, Sun Yat-sen University (No. 2022ZSLYEC-091) and individual consent for this retrospective analysis was waived.

This retrospective study included all consecutive patients with histologically confirmed locally advanced rectal cancer and MMR status who underwent neoadjuvant therapy followed by curative surgical resection from January 2013 to December 2018 at The Sixth Affiliated Hospital of Sun Yat-sen University. Patients in the last three years were not included due to immature survival data. Patients with synchronous multiple primary cancer, inflammatory bowel disease, or familial adenomatous polyposis were excluded from the study. The selection process for this study is outlined in *Figure 1*.

IHC analysis of MMR expression

MLH1, MSH2, MSH6, and PMS2 proteins were stained by immunohistochemistry (IHC), with formalin-fixed, paraffin-embedded tumors. Negative nuclear staining in neoplastic cells, with positive nuclear staining in lymphocytes and normal adjacent colonic epithelium, were defined as MMR loss (23). Primary monoclonal antibodies against MLH1 (clone ES05; Zhong Shan Jin Qiao, Beijing, China), MSH2 (clone RED2; Zhong Shan Jin Qiao, Beijing, China), MSH6 (clone UMAB258; Zhong Shan Jin Qiao, Beijing, China), and PMS2 (clone EP51; Zhong Shan Jin Qiao, Beijing, China) were applied. Representative images of IHC were provided in Figure S1.

MMR status determination

MMR status was determined by a detection of MMR protein expression by IHC, and MSI testing by polymerase

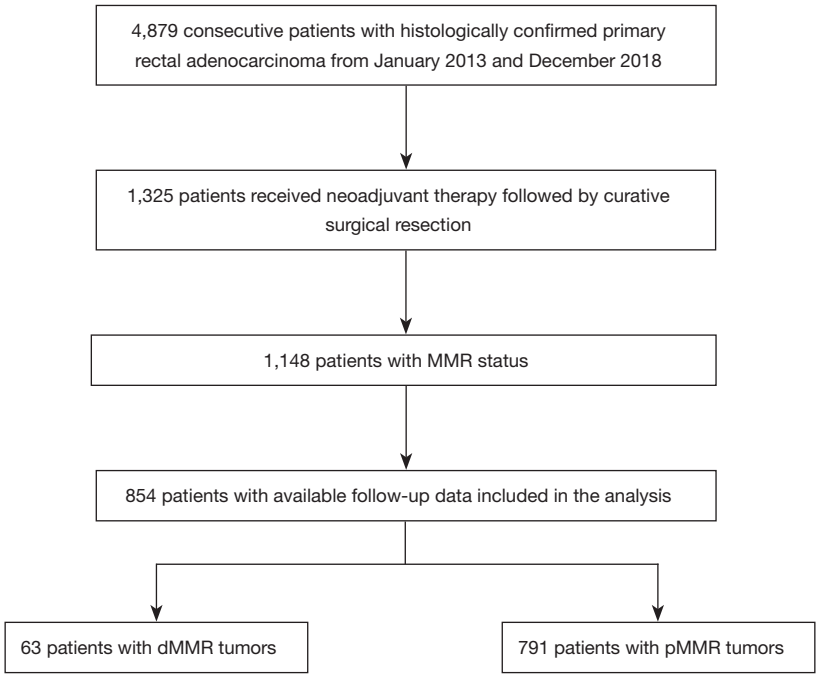

Figure 1 Flow diagrams of the study population. MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

chain reaction (PCR) was used if the result of IHC was uncertain. Deficient MMR phenotype tumors were defined as tumors with 1 or more loss expression of MMR proteins by IHC (23). Tumors with discordant results of MMR protein and DNA MSI testing were excluded from this study.

Treatment and follow-up

All patients received surgery with total mesorectal excision after neoadjuvant therapy, including infusional fluorouracil (the de Gramont regimen) or mFOLFOX6 plus radiotherapy, and neoadjuvant chemotherapy with mFOLFOX6 or the mFOLFOXIRI regimen alone. Radiotherapy was administered at 2.0 Gy for 25 fractions over 5 weeks, with a total dose of 50 Gy (24-26). A physical examination, serum carcinoembryonic antigen test, and computed tomography scan (chest/abdominal/pelvic) with a frequency of every 3–6 months for the first 3 years after surgery and then every 6 months for the following 2 years, were the routine follow-up strategy for all patients. The data were updated in August 2019.

Propensity score matching

Propensity score matching was performed to reduce bias of the baseline characteristics between patients with different MMR statuses in the chemotherapy and chemoradiation groups. A multivariable logistic regression model was constructed to generate propensity scores. Factors presumed to be associated with the patients' tumor responses after neoadjuvant therapy were selected in the propensity model. The following baseline data were included in the model: ≥65 years, sex, grade of differentiation, mucus, low location, clinical tumor (T) stage, and clinical node (N) stage. Patients with dMMR were matched to those with pMMR at a 1:4 ratio using a greedy nearest-neighbor matching algorithm with no replacement. Baseline characteristics were compared between the propensity score-matched group using standardized mean differences (SMDs). A SMD <0.1 indicated a negligible imbalance between groups (27).

Statistical analysis

The primary endpoint was the effect of dMMR on tumor response to neoadjuvant therapy in the overall cohort and

the different neoadjuvant patterns. TRG was evaluated semi-quantitatively on a scale of 0 to 3 (complete to poor response, respectively) according to the American Joint Committee on Cancer system. Pathologic complete response was defined as the absence of viable tumor cells in the surgical specimens, including in the primary tumor area, whole mesorectal fat, and the resected lymph nodes (ypT0N0).

The 2nd endpoint was the relationship between MMR status and Local recurrence-free survival (LRFS) and disease-free survival (DFS) in the overall cohort. LRFS was defined as the time from surgery to tumor regrowth within the pelvis or perineum. DFS was defined as the time from surgery to the first event of local or metastatic recurrence, second primary cancer, or death from any cause.

The categorical variables were compared using the Chi-square test or Fisher's exact test. LRFS and DFS curves were estimated using the Kaplan-Meier method, and were compared using a Cox proportional hazards regression model with HRs, 95% CI, and P values for the candidate prognostic factors. Variables with P values <0.05 in the univariate analysis or considered clinically significant were included in the multivariate analysis. Two-sided P values <0.05 were considered statistically significant. All the statistical analyses were performed with SPSS software (version 22, SPSS Inc., Chicago, IL, USA), except that the propensity score matching was implemented in R, version 3.3.2 (R Foundation), using the package MatchIt.

Results

Patient characteristics

A total of 854 patients with clinical stage II (23.4%) or III (76.6%) disease at the time of diagnosis were enrolled in this study. The patients had a median age of 55 years (range, 19–80 years) at diagnosis, and 71.5% were male. Among the 854 patients tested for MMR status by IHC, 63 patients (7.4%) had dMMR tumors. No significant differences were observed between the baseline characteristics and MMR status, except that dMMR patients were more likely to be younger at the time of diagnosis (<65 years, 88.9% *vs.* 77.7%; P=0.056) and have mucinous adenocarcinoma (12.7% *vs.* 4.4%; P=0.010; *Table 1*).

Tumor responses to neoadjuvant therapy according to MMR status

The associations between MMR status and the postoperative pathological characteristics that reflect tumor responses to neoadjuvant therapy are listed in *Table 2*. Among the 854 enrolled patients, 420 (49.2%) received neoadjuvant fluorouracil-based chemotherapy, consisting of mFOLFOX6 (n=264, 30.9%) or the De Gramont regimen (n=156, 18.3%), concurrently with long-course pelvic radiation, and 434 (50.8%) received neoadjuvant chemotherapy alone. The neoadjuvant therapy regimens were generally well balanced between patients with dMMR and pMMR status. After neoadjuvant therapy, patients with dMMR had a lower proportion of TRG of 0–1 compared with pMMR patients (28.6% *vs.* 43.7%; P=0.027). However, no significant association was observed between MMR status and neoadjuvant therapy efficacy with respect to ypT, ypN, and ypTNM stages, and pathological complete response (pCR). 15.9%, 28.6%, 38.1%, and 17.5% of dMMR patients, and 12.9%, 30.6%, 34.5%, and 22.0%, of pMMR patients had ypTNM stage T0N0, I, II, and III, respectively. pCR was achieved in 112 cases (13.1%), at a rate of 15.9% in dMMR patients and 12.9% in pMMR patients (*Table 2*).

Tumor responses to chemotherapy alone according to MMR status

For patients who received chemotherapy alone, after 1:4 propensity score matching, 33 patients with dMMR were matched to 132 patients with pMMR. After propensity score matching, the SMDs for most of the included covariates among patients with dMMR and pMMR were <0.1, indicating a well-balanced covariate distribution (Table S1). After matching, the proportion of TRG 0–1 was obviously lower in patients with dMMR (9.1% *vs.* 30.3%; P=0.013). However, the pCR rates were similar between the dMMR and pMMR groups (6.1% *vs.* 6.1%; *Table 3*).

Tumor responses to chemoradiation therapy according to MMR status

For patients who received chemoradiation, at 1:4 propensity score matching, 30 patients with dMMR were matched to 120 patients with pMMR. The SMDs of the included covariates are shown in Table S2. After matching, no significant difference in the proportion of TRG 0–1 and pCR rate was observed between patients with dMMR and pMMR (50.0% *vs.* 64.2%; P=0.224 and 26.7% *vs.* 22.5%; P=0.809; Table S2 and *Table 4*).

Table 1 Baseline characteristics of patients according to mismatch repair status

Variables	Total (n=854), n (%)	dMMR (n=63), n (%)	pMMR (n=791), n (%)	P
Age, years				0.056
<65	671 (78.6)	56 (88.9)	615 (77.7)	
≥65	183 (21.4)	7 (11.1)	176 (22.3)	
Gender				0.679
Male	611 (71.5)	47 (74.6)	564 (71.3)	
Female	243 (28.5)	16 (25.4)	227 (28.7)	
Grade of differentiation				0.223
Well/moderate	764 (89.5)	53 (84.1)	711 (89.9)	
Poor	90 (10.5)	10 (15.9)	80 (10.1)	
Mucinous adenocarcinoma				0.010
No	811 (95.0)	55 (87.3)	756 (95.6)	
Yes	43 (5.0)	8 (12.7)	35 (4.4)	
Location from anal verge, cm				0.153
<5	411 (48.1)	27 (42.9)	384 (48.5)	
5–10	376 (44.0)	27 (42.9)	349 (44.1)	
>10	67 (7.8)	9 (14.3)	58 (7.3)	
Clinical T stage				0.124
T2	15 (1.8)	2 (3.2)	13 (1.6)	
T3	659 (77.2)	43 (68.3)	616 (77.9)	
T4	180 (21.1)	18 (28.6)	162 (20.5)	
Clinical N stage				0.396
N0	200 (23.4)	18 (28.6)	182 (23.0)	
N1–2	654 (76.6)	45 (71.4)	609 (77.0)	

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Survival according to MMR status

With an overall median follow-up period of 37.6 months, the 3-year local recurrence rates were 5.7% and 7.8% in the dMMR and pMMR patients, respectively. In the univariate analysis, MMR status was not significantly associated with LRFS (HR =0.83, 95% CI: 0.26–2.63; P=0.746; *Figure 2A*). In the multivariate analysis, which was adjusted for age, gender, grade of differentiation, MMR status, clinical N stage, neoadjuvant therapy pattern, pCR, TRG, ypT, and adjuvant chemotherapy, patients with a higher clinical T stage and ypN stage had significantly shorter LRFS (Table S3).

The 3-year DFS rates were 93.2% in patients with dMMR, and 73.9% in patients with pMMR. In the multivariate analysis, which was adjusted for age, sex, grade of differentiation, clinical T stage, clinical N stage, neoadjuvant therapy pattern, postoperative chemotherapy, ypT stage, ypN stage, pCR, and TRG, dMMR status was independently and significantly associated with longer DFS than pMMR status (HR =0.38, 95% CI: 0.18–0.81; P=0.013; *Figure 2B*, Table S3). In the subgroup multivariate analysis, dMMR status was only significantly associated with a longer DFS than pMMR status in patients with ypStage II/III disease (HR =0.38, 95% CI: 0.17–0.86; P=0.020); no such association was found in patients with ypT0N0 and Stage I disease (HR =0.34, 95% CI: 0.05–2.55; P=0.294; Table S4, *Figure 2C,2D*).

Table 2 Tumor response to neoadjuvant therapy according to mismatch repair status

Variables	Total (n=854), n (%)	dMMR (n=63), n (%)	pMMR (n=791), n (%)	P
Neoadjuvant therapy				0.878
Infusional fluorouracil plus radiotherapy	156 (18.3)	10 (15.9)	146 (18.5)	
mFOLFOX6 plus radiotherapy	264 (30.9)	20 (31.7)	244 (30.8)	
Neoadjuvant chemotherapy alone	434 (50.8)	33 (52.4)	401 (50.7)	
ypT stage				0.686
T0	123 (14.4)	11 (17.5)	112 (14.2)	
T1	74 (8.7)	6 (9.5)	68 (8.6)	
T2	210 (24.6)	12 (19.0)	198 (25.0)	
T3	419 (49.1)	31 (49.2)	388 (49.1)	
T4	28 (3.3)	3 (4.8)	25 (3.2)	
ypN stage				0.088
N0	669 (78.3)	52 (82.5)	617 (78.0)	
N1	137 (16.0)	11 (17.5)	126 (15.9)	
N2	48 (5.6)	0 (0.0)	48 (6.1)	
ypTNM stage				0.743
T0N0	112 (13.1)	10 (15.9)	102 (12.9)	
Stage I	260 (30.4)	18 (28.6)	242 (30.6)	
Stage II	297 (34.8)	24 (38.1)	273 (34.5)	
Stage III	185 (21.7)	11 (17.5)	174 (22.0)	
pCR				0.631
No	742 (86.9)	53 (84.1)	689 (87.1)	
Yes	112 (13.1)	10 (15.9)	102 (12.9)	
TRG				0.027
0–1	364 (42.6)	18 (28.6)	346 (43.7)	
2–3	490 (57.4)	45 (71.4)	445 (56.3)	
Postoperative chemotherapy				0.480
Untreated	118 (13.8)	8 (12.7)	110 (13.9)	
Fluoropyrimidine-based	122 (14.3)	6 (9.5)	116 (14.7)	
Oxaliplatin-based	614 (71.9)	49 (77.8)	565 (71.4)	

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; pCR, pathologic complete response; TRG, tumor regression grade.

Survival according to neoadjuvant pattern and MMR status

Somewhat surprisingly, in the after-matched cohort of neoadjuvant chemotherapy and neoadjuvant chemoradiation, MMR status was not significantly associated with DFS in the univariate and multivariate analyses (Table S5). This may have been due to the limited number of patients.

Discussion

To our knowledge, our current study is the largest sample-

Table 3 Tumor response to chemotherapy alone according to mismatch repair status

Variables	Total (n=165), n (%)	dMMR (n=33), n (%)	pMMR (n=132), n (%)	P
ypT stage				0.760
T0	12 (7.3)	2 (6.1)	10 (7.6)	
T1	11 (6.7)	3 (9.1)	8 (6.1)	
T2	38 (23.0)	9 (27.3)	29 (22.0)	
T3	98 (59.4)	17 (51.5)	81 (61.4)	
T4	6 (3.6)	2 (6.1)	4 (3.0)	
ypN stage				0.187
N0	122 (73.9)	27 (81.8)	95 (72.0)	
N1	31 (18.8)	6 (18.2)	25 (18.9)	
N2	12 (7.3)	0 (0.0)	12 (9.1)	
ypTNM stage				0.562
T0N0	10 (6.1)	2 (6.1)	8 (6.1)	
Stage I	46 (27.9)	12 (36.4)	34 (25.8)	
Stage II	66 (40.0)	13 (39.4)	53 (40.2)	
Stage III	43 (26.1)	6 (18.2)	37 (28.0)	
pCR				1.000
No	155 (93.9)	31 (93.9)	124 (93.9)	
Yes	10 (6.1)	2 (6.1)	8 (6.1)	
TRG				0.013
0–1	43 (26.1)	3 (9.1)	40 (30.3)	
2–3	122 (73.9)	30 (90.9)	92 (69.7)	
Postoperative chemotherapy				1.000
Untreated	16 (9.7)	3 (9.1)	13 (9.8)	
Fluoropyrimidine-based	3 (1.8)	0 (0.0)	3 (2.3)	
Oxaliplatin-based	146 (88.5)	30 (90.9)	116 (87.9)	

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; pCR, pathologic complete response; TRG, tumor regression grade.

sized analysis to examine the predictive and prognostic value of MMR status in patients with locally advanced rectal cancer following neoadjuvant therapy. Neoadjuvant therapy is recommended for patients with clinical stage II/III rectal cancer (28). However, few studies have evaluated the effect of neoadjuvant therapy patterns in dMMR patients with rectal cancer. Our findings indicate that patients with pMMR respond better to neoadjuvant therapy in terms of TRG than patients with dMMR. However, after matching, no difference in relation to TRG between dMMR and pMMR tumors was observed in the subgroup of patients who underwent neoadjuvant chemoradiotherapy, but patients with dMMR had a worse response to chemotherapy alone in terms of TRG than patients with pMMR.

Five previous retrospective studies have evaluated the effect of MMR status in rectal cancer patients treated with neoadjuvant therapy, but conflicting results have been reported. Meillan *et al.* reported on a series of 296 locally advanced rectal cancer patients who received chemoradiation, 23 of whom had dMMR status. They found that dMMR was associated with a higher pathologic downstaging rate but worse TRG (29). One problem of

Table 4 Tumor response to chemoradiation therapy according to mismatch repair status

Variables	Total (n=150), n (%)	dMMR (n=30), n (%)	pMMR (n=120), n (%)	P
ypT stage				0.257
T0	39 (26.0)	9 (30.0)	30 (25.0)	
T1	14 (9.3)	3 (10.0)	11 (9.2)	
T2	36 (24.0)	3 (10.0)	33 (27.5)	
T3	58 (38.7)	14 (46.7)	44 (36.7)	
T4	3 (2.0)	1 (3.3)	2 (1.7)	
ypN stage				0.666
N0	124 (82.7)	25 (83.3)	99 (82.5)	
N1	21 (14.0)	5 (16.7)	16 (13.3)	
N2	5 (3.3)	0 (0.0)	5 (4.2)	
ypTNM stage				0.486
T0N0	35 (23.3)	8 (26.7)	27 (22.5)	
Stage I	45 (30.0)	6 (20.0)	39 (32.5)	
Stage II	44 (29.3)	11 (36.7)	33 (27.5)	
Stage III	26 (17.3)	5 (16.7)	21 (17.5)	
pCR				0.809
No	115 (76.7)	22 (73.3)	93 (77.5)	
Yes	35 (23.3)	8 (26.7)	27 (22.5)	
TRG				0.224
0–1	92 (61.3)	15 (50.0)	77 (64.2)	
2–3	58 (38.7)	15 (50.0)	43 (35.8)	
Postoperative chemotherapy				0.468
Untreated	33 (22.0)	5 (16.7)	28 (23.3)	
Fluoropyrimidine-based	37 (24.7)	6 (20.0)	31 (25.8)	
Oxaliplatin-based	80 (53.3)	19 (63.3)	61 (50.8)	

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; pCR, pathologic complete response; TRG, tumor regression grade.

downstaging is that clinical staging is highly variable, as the different imaging modalities are not always accurate due to the large differences between clinical and pathological stages (30). The pCR rate, which is a potential surrogate for longer-term outcomes, was not reported, as all patients with pCR were excluded from this study. In an analysis of 636 MSI (+) patients from the National Cancer Database, MSI (+) patients were reported to have a more reduced pCR rate than MSI (–) patients (5.9% *vs.* 8.9%; P=0.01) (31). Conversely, 3 previous studies reported results similar to those of our study, and found no significant difference in the pCR rates between patients with dMMR/MSI and pMMR/MSS (32-34).

Data on neoadjuvant chemotherapy in CRC are scarce. In a phase III FOXTROT trial, which evaluated the efficacy of neoadjuvant chemotherapy in treating locally advanced colon cancer, 95% of the 106 patients with dMMR tumors who received neoadjuvant chemotherapy showed little or no response (35). Another retrospective study at Memorial Sloan Kettering included 21 dMMR patients who received neoadjuvant chemotherapy (fluorouracil/oxaliplatin). Of these 21 patients, 6 (29%) had progression of disease,

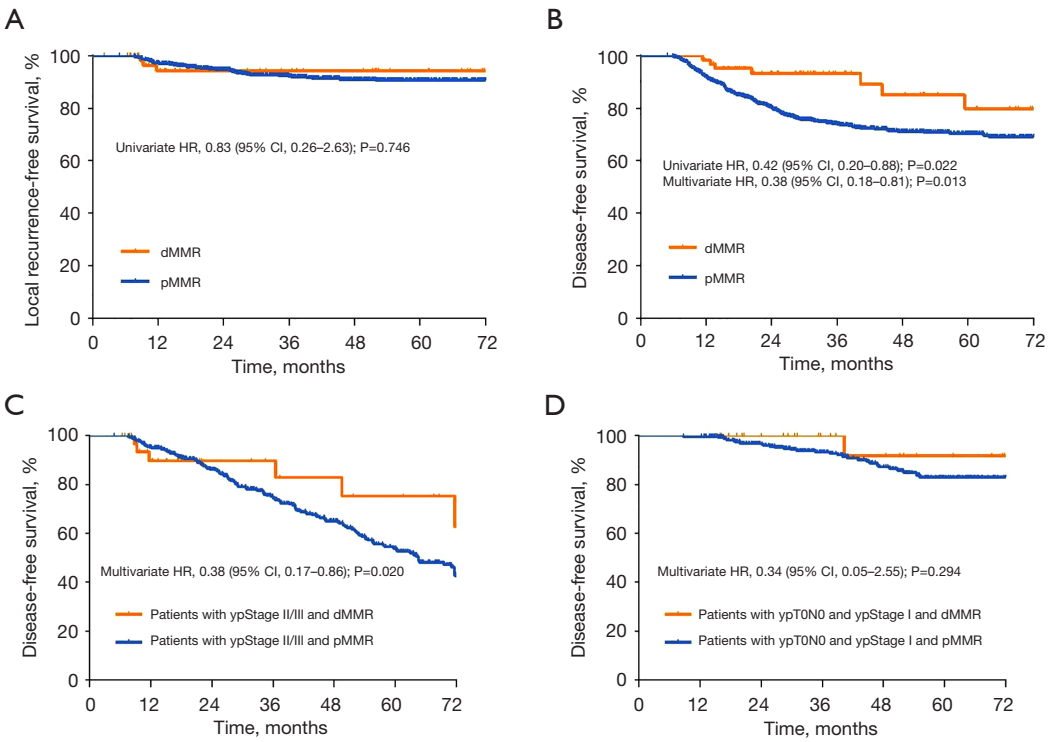

Figure 2 Survival by mismatch repair status. (A) LRFS and (B) DFS in patients by mismatch repair status; (C) DFS in patients with ypStage II/III by mismatch repair status; (D) DFS in patients with ypT0N0 and ypStage I by mismatch repair status. HR, hazards ratio; CI, confidence interval; LRFS, local recurrence-free survival; DFS, disease-free survival.

compared to no progression in the matched 63 pMMR rectal tumors (36). Our data showed that in rectal cancer patients who received neoadjuvant chemotherapy, pMMR patients had a better response. Thus, chemotherapy alone should be administered with caution to dMMR patients who have received neoadjuvant therapy, especially when the tumor volume is large and significant tumor regression is required. One possible explanation for dMMR resistance to fluorouracil-based chemotherapy is that, in the absence of a functional MMR system, repair may only occur through the "base excision repair" system, a process that is less affected by the disequilibrium disequilibrium induced by 5-FU (37).

Despite dMMR patients having a poorer response to neoadjuvant chemotherapy than pMMR patients, the DFS of dMMR patients was not inferior in our study after propensity score matching. This might be due to the small number of dMMR patients, and thus the poor statistical power of this study. Alternately, this might be due to the good prognosis of patients with dMMR, and a poor response to neoadjuvant therapy may not have a significant effect on survival. Du *et al.* found that patients with MSI-H tumors had significantly better DFS than those with MSI-L and MSS tumors in a ypN0 subgroup (34). Conversely, we found that dMMR was a significantly good prognostic marker for DFS in patients with ypStage II/III, and observed a non-significant trend toward better DFS in dMMR patients in the ypT0N0/stage I subgroup. These results might be explained by the good prognosis of all patients with ypT0N0/stage I; however, the small sample size may not have been powerful enough to reveal a statistical difference between the dMMR and pMMR tumors.

Several previous studies have investigated the prognostic effect of MMR status in patients with rectal cancer following upfront surgery. Colombino *et al.* demonstrated that patients with MSI-H rectal cancers had better DFS and overall survival than those with MSI-L/MSS (38), but others have found no significant survival advantages in patients with MSI/dMMR (39-41). These results need to be interpreted with caution because of the small number (range, 12 to 24) of MSI/dMMR patients.

The main limitation of our study is that it was a

retrospective study. Thus, selection bias cannot be excluded. The decision to administer neoadjuvant therapy was left to the investigators' discretion after discussion with a multidisciplinary team, which was mainly based on the estimated risk of recurrence, age, and each patient's physical condition and preferences; however, we used propensity score matching to reduce the imbalance. The other potential predictive or prognostic molecular markers, such as RAS and BRAF, were absent. A pooled analysis of resected stage III colon cancer patients suggested BRAF or KRAS mutations are independently associated with a shorter time to recurrence in patients with MSS but not MSI tumors (42). All the patients in this study were determined to have MMR status by IHC. IHC with antibodies directed against MLH1, MSH2, MSH6, and PMS2 is the preferred approach in daily clinical practice due to its availability and costs. A review of 16 studies of 3,494 cases demonstrated that the sensitivity and specificity of IHC was essentially concordant with PCR-based MSI testing (43). However, a few cases of MSI with rare missense mutations cannot be detected by IHC, which is likely due to the retained antigenicity in an otherwise non-functional protein (44,45). In these cases, MSI testing by PCR can help to determine whether there are true functional MMR proteins.

In conclusion, we demonstrated that in terms of TRG, the response to neoadjuvant chemotherapy of patients with dMMR locally advanced rectal cancer was worse than that of pMMR patients. Also, dMMR is a significantly good prognostic marker for DFS in patients with ypStage II/III after neoadjuvant therapy.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-124/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-124/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-124/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of the Sixth Affiliated Hospital, Sun Yat-sen University (No. 2022ZSLYEC-091) and individual consent for this retrospective analysis was waived.

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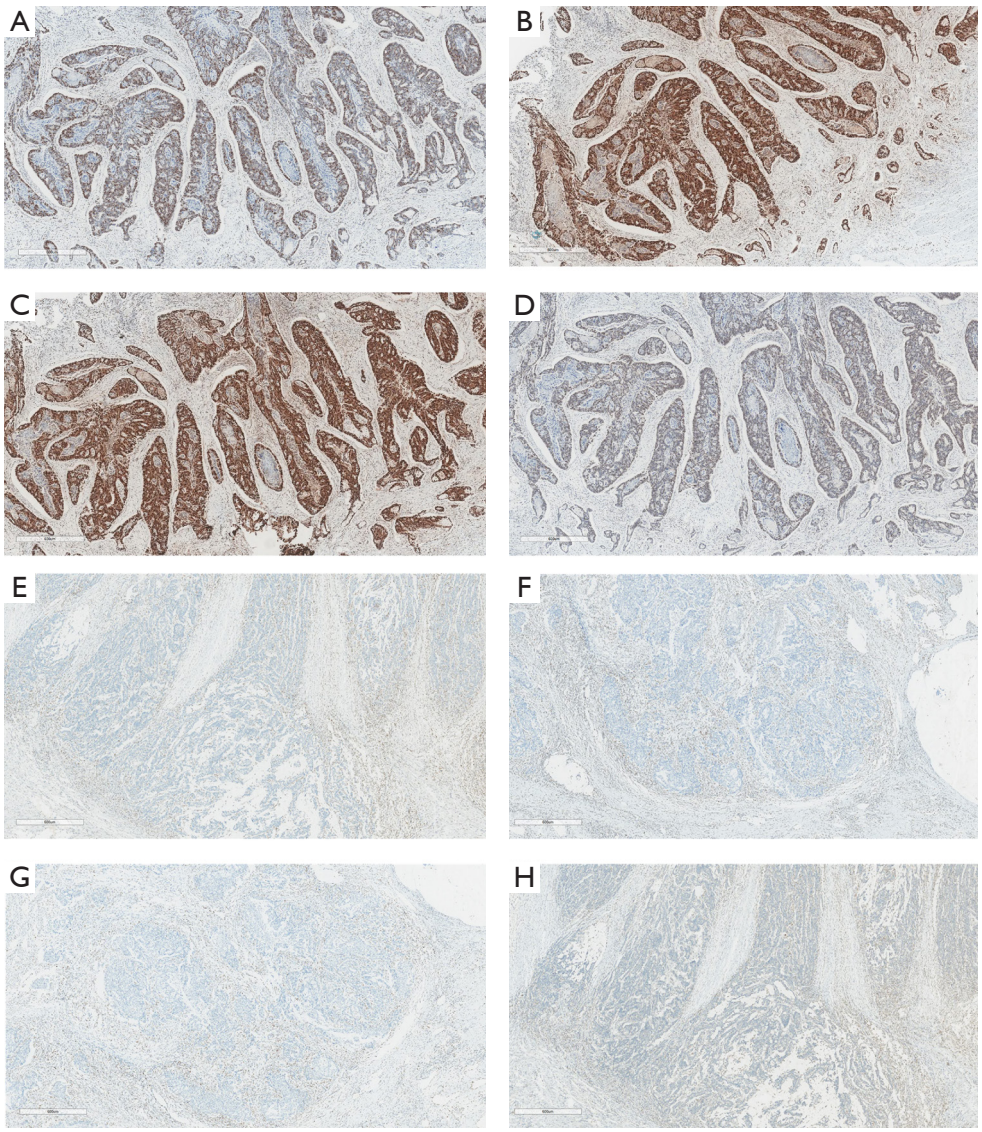

Figure S1 Representative images of MMR proteins. (A) Representative images of MLH1 being positive; (B) representative images of MSH2 being positive; (C) representative images of MSH6 being positive; (D) representative images of PMS2 being positive; (E) representative images of MLH1 being negative; (F) representative images of MSH2 being negative; (G) representative images of MSH6 being negative; (H) representative images of PMS2 being negative. Tumor tissues were stained with MLH1, MSH2, MSH6, PMS2 by IHC (600 μm). MMR, mismatch repair; IHC, immunohistochemistry.

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Table S1 Selected baseline characteristics before and after propensity score matching in chemotherapy group

Characteristics	Before matching				After matching (1:4)			
	pMMR, n=401, n (%)	dMMR, n=33, n (%)	P	Standardized difference	pMMR, n=132, n (%)	dMMR, n=33, n (%)	P	Standardized difference
Age, years			0.025	0.540			0.923	0.105
<65	301 (75.1)	31 (93.9)			127 (96.2)	31 (93.9)		
≥65	100 (24.9)	2 (6.1)			5 (3.8)	2 (6.1)		
Gender			0.974	0.042			0.930	0.017
Male	284 (70.8)	24 (72.7)			97 (73.5)	24 (72.7)		
Female	117 (29.2)	9 (27.3)			35 (26.5)	9 (27.3)		
Grade of differentiation			0.090	0.315			0.564	0.110
Well/moderate	352 (87.8)	25 (75.8)			106 (80.3)	25 (75.8)		
Poorly	49 (12.2)	8 (24.2)			26 (19.7)	8 (24.2)		
Mucinous adenocarcinoma			<0.001	0.495			0.278	0.201
No	381 (95.0)	26 (78.8)			114 (86.4)	26 (78.8)		
Yes	20 (5.0)	7 (21.2)			18 (13.6)	7 (21.2)		
Location from anal verge, cm			0.417	0.232			0.948	0.065
<5	172 (42.9)	11 (33.3)			44 (33.3)	11 (33.3)		
5–10	191 (47.6)	17 (51.5)			65 (49.2)	17 (51.5)		
>10	38 (9.5)	5 (15.2)			23 (17.4)	5 (15.2)		
Clinical T stage			0.232	0.222			0.928	0.080
T2	5 (1.2)	1 (3.0)			3 (2.3)	1 (3.0)		
T3	327 (81.5)	24 (72.7)			93 (70.5)	24 (72.7)		
T4	69 (17.2)	8 (24.2)			36 (27.3)	8 (24.2)		
Clinical N stage			1.000	0.025			0.797	0.050
N0	105 (26.2)	9 (27.3)			39 (29.5)	9 (27.3)		
N1-2	296 (73.8)	24 (72.7)			93 (70.5)	24 (72.7)		

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

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Table S2 Selected baseline characteristics before and after propensity score matching in chemoradiation group

Characteristics	Before matching				After matching (1:4)			
	pMMR, n=390, n (%)	dMMR, n=30, n (%)	P	Standardized difference	pMMR, n=120, n (%)	dMMR, n=30, n (%)	P	Standardized difference
Age, years			0.891	0.073			0.954	0.069
<65	314 (80.5)	25 (83.3)			103 (85.8)	25 (83.3)		
≥65	76 (19.5)	5 (16.7)			17 (14.2)	5 (16.7)		
Gender			0.717	0.112			1.000	0.040
Male	280 (71.8)	23 (76.7)			94 (78.3)	23 (76.7)		
Female	110 (28.2)	7 (23.3)			26 (21.7)	7 (23.3)		
Grade of differentiation			1.000	0.049			1.000	<0.001
Well/moderate	359 (92.1)	28 (93.3)			112 (93.3)	28 (93.3)		
Poorly	31 (7.9)	2 (6.7)			8 (6.7)	2 (6.7)		
Mucinous adenocarcinoma			1.000	0.028			1.000	0.050
No	375 (96.2)	29 (96.7)			117 (97.5)	29 (96.7)		
Yes	15 (3.8)	1 (3.3)			3 (2.5)	1 (3.3)		
Location from anal verge, cm			0.162	0.298			0.849	0.114
<5	212 (54.4)	16 (53.3)			69 (57.5)	16 (53.3)		
5–10	158 (40.5)	10 (33.3)			39 (32.5)	10 (33.3)		
>10	20 (5.1)	4 (13.3)			12 (10.0)	4 (13.3)		
Clinical T stage			0.321	0.235			0.312	0.228
T2	8 (2.1)	1 (3.3)			1 (0.8)	1 (3.3)		
T3	289 (74.1)	19 (63.3)			86 (71.7)	19 (63.3)		
T4	93 (23.8)	10 (33.3)			33 (27.5)	10 (33.3)		
Clinical N stage			0.268	0.239			1.000	0.036
N0	77 (19.7)	9 (30.0)			38 (31.7)	9 (30.0)		
N1-2	313 (80.3)	21 (70.0)			82 (68.3)	21 (70.0)		

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

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Table S3 Univariate and multivariate Cox proportional hazards regression models for LRFS and DFS

Variables	Local recurrence-free survival				Disease-free survival			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95%CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.189	1	0.230	1	0.192	1	0.186
≥65	0.62 (0.31–1.26)		0.64 (0.32–1.32)		0.79 (0.55–1.13)		0.78 (0.54–1.13)	
Gender								
Male	1	0.024	1	0.054	1	0.739	1	0.562
Female	0.46 (0.23–0.90)		0.51 (0.26–1.01)		0.95 (0.70–1.29)		1.10 (0.80–1.50)	
Grade of differentiation								
Well/moderate	1	0.001	1	0.081	1	<0.001	1	0.408
Poorly	2.73 (1.50–4.96)		1.74 (0.93–3.23)		1.95 (1.35–2.80)		1.17 (0.80–1.71)	
Mucinous adenocarcinoma								
No	1	0.478			1	0.191		
Yes	1.44 (0.52–3.98)				1.44 (0.83–2.47)			
Location from anal verge, cm								
≥5	1	0.108			1	0.628		
<5	1.52 (0.91–2.53)				1.06 (0.85–1.32)			
Clinical T stage								
T2–3	1	0.006	1	0.012	1	0.082	1	0.407
T4	1.20 (1.06–1.37)		1.19 (1.04–1.37)		1.07 (0.99–1.16)		1.04 (0.95–1.12)	
Clinical N stage								
N0	1	0.026	1	0.161	1	0.023	1	0.351
N1–2	2.45 (1.11–5.38)		1.79 (0.79–4.01)		1.52 (1.06–2.18)		1.19 (0.82–1.73)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone	1	0.074	1	0.143	1	0.09	1	0.753
Fluorouracil based-radiotherapy	0.63 (0.37–1.05)		0.65 (0.37–1.16)		0.79 (0.60–1.04)		0.95 (0.70–1.29)	
Postoperative chemotherapy								
No	1	0.747	1	0.305	1	0.008	1	0.114
Yes	1.14 (0.52–2.50)		0.65 (0.28–1.48)		2.09 (1.22–3.60)		1.56 (0.90–2.72)	
ypT stage								
T0-2	1	<0.001	1	0.059	1	<0.001	1	<0.001
T3-4	3.52 (1.91–6.49)		2.08 (0.97–4.46)		3.67 (2.63–5.11)		2.78 (1.84–4.21)	
ypN stage								
N0	1	<0.001	1	0.009	1	<0.001	1	<0.001
N1-2	3.29 (1.99–5.45)		2.09 (1.20–3.62)		3.38 (2.56–4.47)		2.27 (1.67–3.07)	
pCR								
No	1	0.066	1	0.811	1	0.002	1	0.653
Yes	0.34 (0.11–1.07)		0.85 (0.22–3.22)		0.39 (0.22–0.70)		1.17 (0.59–2.31)	
TRG				0.907		<0.001		
0-1	1	0.022	1		1		1	0.660
2-3	1.38 (1.05–1.81)		1.02 (0.73–1.43)		1.34 (1.15–1.55)		1.04 (0.87–1.24)	
MMR status								
pMMR	1	0.746	1	0.686	1	0.022	1	0.013
dMMR	0.83 (0.26–2.63)		0.79 (0.24–2.54)		0.42 (0.20–0.88)		0.38 (0.18–0.81)	

LRFS, local recurrence-free survival; DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

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Table S4 Univariate and multivariate Cox proportional hazards regression models for DFS in patients with ypStage II/III and patients with ypT0N0 and Stage I

Variables	ypStage II/III				ypT0N0 and Stage I			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95%CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.211	1	0.348	1	0.259	1	0.306
≥65	0.78 (0.53–1.15)		0.83 (0.56–1.23)		0.55 (0.19–1.56)		0.58 (0.2–1.65)	
Gender								
Male	1	0.246	1	0.371	1	0.550	1	0.530
Female	1.22 (0.87–1.71)		1.17 (0.83–1.64)		0.80 (0.38–1.68)		0.79 (0.37–1.67)	
Grade of differentiation								
Well/moderate	1	0.069	1	0.351	1	0.834	1	0.961
Poorly	1.42 (0.97–2.07)		1.20 (0.82–1.77)		0.81 (0.11–5.92)		1.05 (0.14–8.09)	
Mucinous adenocarcinoma								
No	1	0.437			1	0.996		
Yes	1.24 (0.72–2.15)				0 (0-Inf)			
Location from anal verge, cm								
≥5	1	0.398			1	0.271		
<5	1.14 (0.84–1.54)				1.49 (0.73–3.03)			
Clinical T stage								
T2–3	1	0.271	1	0.156	1	0.177	1	0.168
T4	1.05 (0.96–1.14)		1.06 (0.98–1.16)		0.78 (0.55–1.12)		0.78 (0.54–1.11)	
Clinical N stage								
N0	1	0.010	1	0.050	1	0.240	1	0.046
N1–2	1.77 (1.15–2.72)		1.56 (1.00–2.42)		0.65 (0.32–1.33)		0.47 (0.22–0.99)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone	1	0.156	1	0.429	1	0.373	1	0.431
Fluorouracil based-radiotherapy	0.8 (0.59–1.09)		0.87 (0.62–1.22)		1.38 (0.68–2.81)		1.34 (0.64–2.81)	
Postoperative chemotherapy								
No	1	0.251	1	0.527	1	0.068	1	0.039
Yes	1.39 (0.79–2.45)		1.21 (0.68–2.15)		6.39(0.87–46.81)		8.31 (1.12–61.84)	
ypT stage								
T0–2	1	0.911	1	0.350				
T3–4	1.03 (0.57–1.86)		1.37 (0.71–2.65)					
ypN stage								
N0	1	<0.001	1	<0.001				
N1–2	1.99 (1.47–2.69)		1.92 (1.38–2.66)					
pCR								
No					1	0.396	1	0.542
Yes					1.36 (0.67–2.76)		1.28 (0.58–2.80)	
TRG								
0–1	1	0.169	1	0.211	1	0.066	1	0.187
2–3	1.14 (0.95–1.36)		1.14 (0.93–1.40)		0.66 (0.42–1.03)		0.72 (0.44–1.17)	
MMR status								
pMMR	1	0.037		0.020	1	0.322	1	0.294
dMMR	0.42 (0.19–0.95)		0.38 (0.17–0.86)		0.37 (0.05–2.68)		0.34 (0.05–2.55)	

DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

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Table S5 Univariate and multivariate Cox proportional hazards regression models for DFS in chemotherapy and chemoradiation subgroup patients

Variables	Chemotherapy				Chemoradiation			
	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age, years								
<65	1	0.996	1	0.998	1	0.574	1	0.789
≥65	0 (0–Inf)		0 (0–Inf)		1.29 (0.53–3.14)		1.15 (0.41–3.18)	
Gender								
Male	1	0.702	1	0.662	1	0.665	1	0.853
Female	0.87 (0.42–1.79)		0.85 (0.40–1.80)		0.83 (0.36–1.92)		0.92 (0.38–2.23)	
Grade of differentiation								
Well/moderate	1	0.128	1	0.464	1	0.083	1	0.298
Poorly	1.31 (0.92–1.86)		1.15 (0.70–1.67)		1.52 (0.95–2.45)		1.34 (0.77–2.31)	
Mucinous adenocarcinoma								
No	1	0.385			1	0.327		
Yes	1.44 (0.63–3.27)				2.05 (0.49–8.58)			
Location from anal verge, cm								
≥5	1	0.490			1	0.805		
<5	0.78 (0.39–1.58)				0.92 (0.46–1.82)			
Clinical T stage								
T2–3	1	0.217	1	0.324	1	0.290	1	0.981
T4	1.11 (0.94–1.32)		1.09 (0.91–1.31)		1.10 (0.92–1.31)		1.00 (0.80–1.24)	
Clinical N stage								
N0	1	0.555	1	0.824	1	0.037	1	0.199
N1–2	1.24 (0.60–2.56)		0.92 (0.42–1.99)		2.76 (1.06–7.14)		2.00 (0.70–5.73)	
Neoadjuvant therapy pattern								
Neoadjuvant chemotherapy alone								
Fluorouracil based-radiotherapy								
Postoperative chemotherapy								
No	1	0.182	1	0.410	1	0.057	1	0.133
Yes	3.87 (0.53–28.22)		2.34 (0.31–17.73)		4.02 (0.96–16.84)		3.15 (0.70–14.04)	
ypT stage								
T0–2	1	0.002	1	0.082	1	0.006	1	0.180
T3–4	1.45 (1.14–1.83)		1.3 (0.97–1.74)		1.28 (1.07–1.52)		1.18 (0.93–1.52)	
ypN stage								
N0	1	0.008	1	0.174	1	0.002	1	0.065
N1–2	1.54 (1.12–2.13)		1.28 (0.90–1.84)		1.77 (1.24–2.52)		1.47 (0.98–2.21)	
pCR								
No	1	0.356	1	0.795	1	0.059	1	0.424
Yes	0.39 (0.05–2.86)		1.35 (0.14–13.05)		0.32 (0.10–1.05)		0.57 (0.15–2.24)	
TRG								
0–1	1	0.200	1	0.634	1	0.825	1	0.317
2–3	1.31 (0.87–1.97)		1.12 (0.69–1.82)		1.04 (0.73–1.47)		0.80 (0.51–1.25)	
MMR status								
pMMR	1	0.997	1	0.997	1	0.987	1	0.498
dMMR	0 (0–Inf)		0 (0–Inf)		0.99 (0.43–2.29)		0.73 (0.29–1.83)	

DFS, disease-free survival; HR, hazards ratio; CI, confidence interval; pCR, pathologic complete response; TRG, tumor regression grade; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

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