Original Article

Prognostic impact of high-risk factors and *KRAS* mutation in patients with stage II deficient mismatch repair colon cancer: a retrospective cohort study

Yuting Zhang[1,2#], Zehua Wu[1,2#], Bin Zhang[2,3#], Huabin Hu[1,2], Jianwei Zhang[1,2], Yi Chen[1,2], Miaomiao Ding[1,2], Yabing Cao[4], Yanhong Deng[1,2]

[1]Department of Medical Oncology, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; [2]Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; [3]Department of Colorectal Surgery, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; [4]Department of Oncology, Kiang Wu Hospital, Macau, China

Contributions: (I) Conception and design: Y Deng, Y Cao, Y Zhang, Z Wu, B Zhang; (II) Administrative support: Y Deng, Y Cao; (III) Provision of study materials or patients: H Hu, J Zhang; (IV) Collection and assembly of data: Y Zhang, Y Chen, M Ding; (V) Data analysis and interpretation: Y Zhang, Z Wu, B Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Prof. Yabing Cao. Department of Oncology, Kiang Wu Hospital, Macau 999078, China. Email: sumscaoyabing@hotmail.com; Prof. Yanhong Deng. Department of Medical Oncology, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou 510655, China. Email: dengyanh@mail.sysu.edu.cn.

Background: Deficient mismatch repair (dMMR) is associated with a good prognosis in patients with stage II colon cancer and observation is recommended after surgery in these patients. In contrast, patients with high-risk factors and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation is associated with a poor prognosis in colon cancer. However, the prognosis and treatment of patients with dMMR colon cancer combined with high-risk factors or *KRAS* mutation remains unclear. This study aimed to evaluate whether patients with dMMR colon cancer combined with high-risk factors or *KRAS* mutation require further treatment.

Methods: This single-center retrospective study included patients who received radical surgical resection and mismatch repair (MMR) immunohistochemical detection at The Sixth Affiliated Hospital of Sun Yat-sen University between May 2011 and March 2021. The high-risk factors and *KRAS* mutation were assessed by clinicopathological data and targeted sequencing. Associations with disease-free survival (DFS) were evaluated using multivariable Cox models.

Results: Among the 1,357 patients with stage II colorectal cancer included, 226 of these patients had dMMR. Patients in the dMMR group were more likely to be younger [<50 years: odds ratio (OR) =0.401, 95% CI: 0.288–0.558, P<0.001], with poor differentiation (OR =5.800, 95% CI: 3.437–9.787, P<0.001), no perineural invasion (OR =0.132, 95% CI: 0.047–0.368, P<0.001), and more than 12 excised lymph nodes (OR =0.427, 95% CI: 0.188–0.968, P=0.042). The disease-free survival (DFS) of patients with stage II dMMR colon cancer with high-risk factors was similar to that of patients without high-risk factors (hazard ratio (HR) =1.285, 95% CI: 0.273–6.051, P=0.607). A total of 836 patients had complete data regarding *KRAS* status. Compared with *KRAS* wild-type patients, patients with *KRAS* gene mutation had a trend of poor prognosis in patients with stage II colon cancer (HR=1.483, 95% CI: 0.983–2.239, P=0.061). In addition, dMMR appeared to be a protective factor in patients with *KRAS* mutation (HR =0.138, 95% CI: 0.019–1.002, P=0.0501).

Conclusions: The survival of patients with stage II dMMR colon cancer with high-risk factors was similar to that of patients without high-risk factors, regardless of the presence of *KRAS* mutation.

Keywords: Deficient mismatch repair status (dMMR status); high-risk factors; colorectal cancer; Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation

Submitted Apr 25, 2022. Accepted for publication Jun 16, 2022.
doi: 10.21037/atm-22-2803
View this article at: https://dx.doi.org/10.21037/atm-22-2803

Introduction

Malignant tumors are one of the main causes of human death. According to the 2020 global cancer database (1), colorectal cancer ranked third in incidence and second in mortality among all malignant tumors. In 2021, a study comparing epidemiological characteristics of gastrointestinal cancer in China and the United States found that the incidence rate of upper gastrointestinal tumors (gastric cancer and esophageal cancer) had decreased in China in recent decades, while the incidence rate of colorectal cancer was increasing each year (2).

In stage II colorectal cancer, deficient mismatch repair (dMMR) is associated with a good prognosis (3-6), and these patients can be followed up with observation after operation. In contrast, stage II colon cancer patients with high-risk factors (pathologic stage T4, poor differentiation [grade 3/4, excluding microsatellite instability-high (MSI-H)], vascular invasion, perineural invasion, initial bowel obstruction or perforation of tumor site, positive or unknown margins, insufficient surgical margin, and fewer than 12 excised lymph nodes) have a poorer prognosis and require combination chemotherapy with 2 drugs. In general, T4 stage is predicted and prognostic factors of stage II colon cancer, whereas other high-risk factors are prognostic factors of stage II colon cancer, including poor differentiation, vascular invasion, perineural invasion, initial bowel obstruction or perforation of tumor site, positive or unknown margins, insufficient surgical margin, and fewer than 12 excised lymph nodes (7). The prognosis of dMMR colon cancer patients with high-risk factors is still uncertain. The previous study has found that high-risk factors do not affect disease-free survival (DFS) or overall survival (OS) in patients with stage II dMMR colon cancer (8), and other study has proposed that MMR status is an independent prognostic factor for DFS in patients with stage II colon cancer (9). However, these studies did not compare the prognosis of dMMR patients with high-risk factors and those without high-risk factors. Therefore, the purpose of this study was to explore the prognosis of stage II dMMR colon cancer patients with high-risk factors and confirm whether patients with dMMR colon cancer combined with high-risk factors require further treatment.

The Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene is considered to be an oncogene (10), and its mutation can be an indicator of poor prognosis. Previous studies have found that cancer patients with *KRAS* mutation have a worse prognosis (11-17), and the recent retrospective study (18) have suggested that patients with *KRAS* gene mutation have worse DFS and OS in stage II/III colon cancer. Therefore, *KRAS* inhibitors treating stage II/III *KRAS* mutation colon cancer are an important treatment strategy. In 2020, Hallin *et al.* identified MRTX849 as a new *KRAS* mutation inhibitor (19). This *KRAS* mutation inhibitor showed obvious tumor inhibition in 26 (65%) *KRAS* positive cell lines and 17 human xenotransplantation models from various tumor types and demonstrated a good curative effect in patients with *KRAS*-positive colon adenocarcinoma. However, in the above study, the patients with stage II and III colon cancer were not distinguished for subgroup analysis. Therefore, the impact of *KRAS* gene mutation on the prognosis of stage II patients still needs to be clarified. In this study, we explored the prognostic impact of *KRAS* mutation on patients with stage II dMMR colon cancer and indicated that observation is recommended for patients with stage II dMMR colon cancer after surgery, regardless of the presence of *KRAS* mutation. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2803/rc).

Methods

Study design and patients

This retrospective cohort study included patients with histologically confirmed stage II colon cancer who received radical surgical resection and mismatch repair (MMR) immunohistochemical detection at The Sixth Affiliated Hospital of Sun Yat-sen University between May 2011 and March 2021. Patients with histologically confirmed stage I or III colon cancer, distant metastases, incomplete surgical

resection (R1 or R2 resection), and no MMR or MSI status were excluded. According to the 2021 Chinese Society of Clinical Oncology (CSCO) colorectal cancer diagnosis and treatment guidelines, the high-risk factors of stage II colon cancer are the following: pathologic stage T4, poor differentiation (grade 3/4, excluding MSI-H), vascular invasion, perineural invasion, initial bowel obstruction or perforation of tumor site, positive or unknown margins, insufficient surgical margin, and fewer than 12 excised lymph nodes. Among these factors, initial bowel obstruction or perforation of tumor site, positive or unknown margin, and insufficient surgical margin were not included in this analysis due to incomplete collection of clinical information. The recent study has found that the incidence and mortality rates of patients with early onset colorectal cancer (EOCRC; patients younger than 50 years old) are rising (20). Our study used 50 years of age as the age cutoff in our analysis. In addition, information concerning patient age, gender, human epidermal growth factor receptor 2 (HER2) status, adjuvant chemotherapy, and *KRAS* gene status were collected. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective cohort study was approved by the ethics committee of The Sixth Affiliated Hospital of Sun Yat-sen University (No. 2022ZSLYEC-125). Individual consent for this retrospective analysis was waived.

MMR protein immunohistochemistry

The formalin-fixed paraffin-embedded (FFPE) tumor samples were stained with MLH1, MSH2, MSH6, and PMS2 proteins. The loss of MMR proteins was defined as the absence of staining in the nuclei of tumor cells while the nuclei of lymphocytes and adjacent normal colonic epithelial cells were positive. MLH1 (clone M1, prediluted, Ventana, Roche, Basel, Switzerland), MSH2 (clone G219-1129, prediluted, Ventana), MSH6 (clone 44, prediluted, Ventana), and PMS2 (clone EPR3947, prediluted, Ventana) monoclonal primary antibodies were used.

MSI testing

DNA was extracted from the FFPE tumor tissues. Five mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, and NR-27) obtained by polymerase chain reaction (PCR) were used to compare and analyze the DNA of normal colon tissue and tumor tissue and to evaluate MSI. Specimens with at least 2 unstable markers were rated as highly unstable, while specimens with fewer than 2 unstable markers were rated as stable.

KRAS gene mutation detection

Mutation analysis was completed at the Molecular Diagnostic Laboratory of the Sixth Affiliated Hospital of Sun Yat-sen University under appropriate quality control procedures. Genomic DNA was extracted from surgical FFPE specimens with an EZgene Tissue gDNA Miniprep Kit (cat no. GD2211, Biomiga, Shanghai, China). *KRAS* (exon 2, 3, and 4) gene loci were sequenced by an ABI Prism 3 500 DX genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Follow-up

The patients were followed up through outpatient service once every 3 to 6 months in the first 3 years, once every 6 months in the next 3 to 5 years, and finally once a year after 5 years. The follow-up included a physical examination, serum carcinoembryonic antigen (CEA) detection, and a computed tomography (CT) scan (chest/abdomen/pelvis). At the same time, we will follow up the patient's condition by telephone every 6 months.

Statistical analysis

The data for this retrospective analysis were frozen on 30 May 2021. 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. All data were analyzed by univariate and multivariate logistic regression using SPSS 26.0 statistical software (IBM Corp., Armonk, NY, USA). Chi-square test was used for categorical variables. Continuous variables with normal distribution are expressed as mean ± standard deviation, and continuous variables with nonnormal distribution are expressed as median and interquartile spacing. To control confounding factors, we included variables with $P<0.05$ from the univariate analysis in the multivariate logistic regression analysis model and used the "enter" method for analysis. In the univariate analysis, we listed the odds ratio (OR) or hazard ratio (HR) and the 95% CIs of all variables. In the multivariate analysis, we listed the OR or HR and the 95% CIs of the variables included in the model. All analyses were performed using a two-tailed test. $P<0.05$ indicated that the difference was statistically significant.

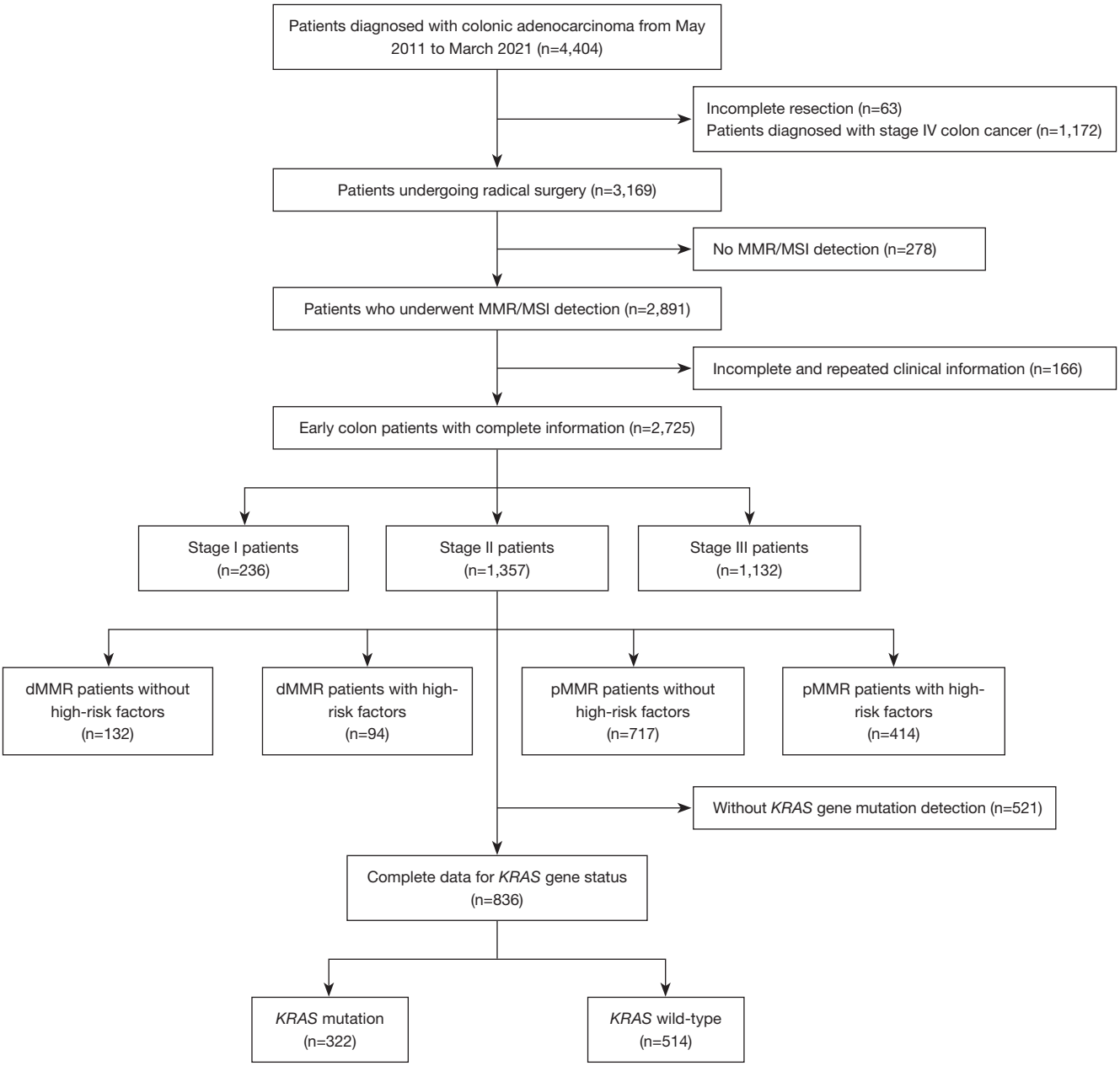


Figure 1 Flow chart of enrolled patients. MMR, mismatch repair; MSI, microsatellite instability; dMMR, deficient MMR; pMMR, proficient MMR; KRAS, Kirsten rat sarcoma viral oncogene homolog.

Results

Patient characteristics

A total of 1,357 patients with stage II colon cancer were included in the analysis. Of these patients, 1,131 had proficient MMR (pMMR) status and 226 had dMMR status. There were 94 dMMR patients with high-risk factors. The screening process is shown in *Figure 1*.

In the total population of patients with stage II colon cancer, patients aged 50 years or older were more common in the pMMR group than in the dMMR group (80.5% *vs.* 61.9%, P<0.001). In other words, patients in the dMMR group were more likely to be younger. Poor differentiation (9.6% *vs.* 31.9%, P<0.001) and mucinous components

in tumor tissues (7.3% *vs.* 18.6%, P<0.001) were more common in the dMMR group, while perineural invasion (11.9% *vs.* 1.8%, P<0.001) and fewer than 12 excised lymph nodes (8.0% *vs.* 3.1%, P=0.010) were more common in the pMMR group. Postoperative adjuvant chemotherapy was also statistically different between the pMMR and the dMMR groups (39.7% *vs.* 46.9%, respectively, P=0.044). The baseline characteristics of the 2 MMR statuses are presented in *Table 1*.

The results of the multivariate logistic regression analysis are shown in *Table 2*. Age ≥50 years (OR =0.401, 95% CI: 0.288–0.558, P<0.001), perineural invasion (OR =0.132, 95% CI: 0.047–0.368, P<0.001), and fewer than 12 excised lymph nodes (OR =0.427, 95% CI: 0.188–0.968, P=0.042) were independent risk factors for pMMR, while poor differentiation (OR =5.800, 95% CI: 3.437–9.787, P<0.001) was an independent risk factor for dMMR.

Prognostic analysis of stage II dMMR colon cancer patients with high-risk factors

The median overall follow-up was 18.9 months. We performed a Cox regression prognostic analysis on patients with stage II colon cancer, and the results are shown in *Table 3*. The multivariate analysis showed that patients with dMMR had a better prognosis than patients with pMMR (HR =0.328, 95% CI: 0.152–0.708, P=0.005), and the difference was statistically significant. This indicated that dMMR might be an independent prognostic factor in patients with stage II colon cancer, which was consistent with the conclusions of previous clinical studies. Pathologic stage T4 (HR =1.588, 95% CI: 1.058–2.384, P=0.026), perineural invasion (HR =3.101, 95% CI: 2.103–4.572, P<0.001), and fewer than 12 excised lymph nodes (HR =2.021, 95% CI: 1.250–3.267, P=0.004) were also independent prognostic factors in patients with stage II colon cancer. We also included some other factors which may influence patients' prognosis for cox regression model (Table S1), and the results were similar to that in *Table 3*.

We divided the stage II colon cancer population into 4 groups: dMMR patients without high-risk factors (n=132), dMMR patients with high-risk factors (n=94), pMMR patients without high-risk factors (n=717), and pMMR patients with high-risk factors (n=414). The DFS of each group is shown in *Figure 2*. The prognosis of the pMMR with high-risk factors group was worse than that of the other 3 groups, and the difference was statistically significant. There was no significant difference in DFS among dMMR patients without high-risk factors, dMMR patients with high-risk factors, and pMMR patients without high-risk factors. The survival curve of the dMMR with high-risk factors group was similar to that of the dMMR without high-risk factors group (HR =1.285, 95% CI: 0.273–6.051, P=0.607) and separated from that of the pMMR without high-risk factors group (HR =0.573, 95% CI: 0.245–1.337, P=0.542). This indicated that dMMR patients with high-risk factors still had a relatively good prognosis.

Prognostic impact of KRAS mutation on patients with stage II colon cancer

We further investigated the prognostic impact of *KRAS* mutation on patients with stage II colon cancer. A total of 836 patients had complete data regarding *KRAS* status, of whom 514 (61.5%) had *KRAS* wild-type and 322 (38.5%) had *KRAS* mutation. The survival curves are shown in *Figure 3*. There was no statistical difference between the survival of patients with *KRAS* wild-type and *KRAS* mutation (HR =1.483, 95% CI: 0.983–2.239, P=0.061), but patients with *KRAS* mutation tended to have a worse prognosis than patients with *KRAS* wild-type. The baseline characteristics and Cox analysis of the 836 patients are presented in Tables S2-S4.

Prognostic impact of KRAS mutation on patients with different MMR statuses

The prognostic impact of *KRAS* mutation and *KRAS* wild-type on patients with different MMR statuses is shown in *Figure 4*. The patients were divided into 4 groups: dMMR patients with *KRAS* mutation, dMMR patients with *KRAS* wild-type, pMMR patients with *KRAS* mutation, and pMMR patients with *KRAS* wild-type. Among these 4 groups, pMMR patients with *KRAS* mutation had the worst prognosis. The survival curve of dMMR patients with *KRAS* mutation was similar to that of dMMR patients with *KRAS* wild-type, and both were better than that of pMMR patients with *KRAS* wild-type. These results indicated that the prognosis of dMMR patients was better than that of pMMR patients, regardless of whether they had *KRAS* mutation or wild-type.

To explore whether dMMR status was a protective factor for patients with *KRAS* mutation, we analyzed the prognosis of different MMR statuses in patients with *KRAS* mutation. The results are shown in *Figure 5*. Among the patients

Table 1 Basic characteristics of patients with stage II colon cancer

Characteristic	Total population, N=1,357, No. (%)	pMMR group, N=1,131, No. (%)	dMMR group, N=226, No. (%)	P value
Age (year)				<0.001
<50	307 (22.6)	221 (19.5)	86 (38.1)	
≥50	1,050 (77.4)	910 (80.5)	140 (61.9)	
Gender				0.646
Female	522 (38.5)	432 (38.2)	90 (39.8)	
Male	835 (61.5)	699 (61.8)	136 (60.2)	
Grade of differentiation				<0.001
Well or moderately	1,176 (86.7)	1,022 (90.4)	154 (68.1)	
Poorly	181 (13.3)	109 (9.6)	72 (31.9)	
Mucus component				<0.001
Negative	1,233 (90.9)	1,049 (92.7)	184 (81.4)	
Positive	124 (9.1)	82 (7.3)	42 (18.6)	
T4				0.102
Negative	1,193 (87.9)	987 (87.3)	206 (91.2)	
Positive	164 (12.1)	144 (12.7)	20 (8.8)	
Vascular invasion				0.100
Negative	1,292 (95.2)	1,072 (94.8)	220 (97.3)	
Positive	65 (4.8)	59 (5.2)	6 (2.7)	
Perineural invasion				<0.001
Negative	1,218 (89.8)	996 (88.1)	222 (98.2)	
Positive	139 (10.2)	135 (11.9)	4 (1.8)	
No. of lymph nodes excised				0.010
≥12	1,260 (92.9)	1,041 (92.0)	219 (96.9)	
<12	97 (7.1)	90 (8.0)	7 (3.1)	
HER2				0.301
Negative	1,334 (98.3)	1,110 (98.1)	224 (99.1)	
Positive	23 (1.7)	21 (1.9)	2 (0.9)	
Adjuvant chemotherapy				0.044
Negative	802 (59.1)	682 (60.3)	120 (53.1)	
Positive	555 (40.9)	449 (39.7)	106 (46.9)	
KRAS mutation				0.087
Negative	514 (61.5)	417 (60.2)	97 (67.8)	
Positive	322 (38.5)	276 (39.8)	46 (32.2)	
Missing value	521			

pMMR, proficient mismatch repair; dMMR, deficient mismatch repair; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma viral oncogene homolog.

Table 2 Multivariate logistic regression analysis predicting patients with dMMR status in stage II colon cancer

Characteristic	OR	95% CI	P value
Age (year)			
<50			
≥50	0.401	0.288–0.558	<0.001
Grade of differentiation			
Well or moderately			
Poorly	5.800	3.437–9.787	<0.001
Mucus component			
Negative			
Positive	0.582	0.313–1.080	0.086
Perineural invasion			
Negative			
Positive	0.132	0.047–0.368	<0.001
No. of lymph nodes excised			
≥12			
<12	0.427	0.188–0.968	0.042
Adjuvant chemotherapy			
Negative			
Positive	1.235	0.903–1.687	0.186

dMMR, deficient mismatch repair; OR, odds ratio; CI, confidence interval.

with *KRAS* mutation, the dMMR group appeared to have a better prognosis (HR =0.138, 95% CI: 0.019–1.002, P=0.0501). Although there was no significant difference, the risk ratio was 0.138 and the 95% CI was 0.019–1.002, suggesting that the prognosis of dMMR patients was better.

Discussion

dMMR status is an indicator of good prognosis in patients with stage II dMMR colon cancer; therefore, CSCO guidelines suggest follow-up and observation after operation in these patients. However, patients with high-risk factors are recommended to receive adjuvant chemotherapy with doublet regimens. *KRAS* mutation is a poor prognostic factor in patients with stage II–III colon cancer. dMMR status and high-risk factors have opposite effects on prognosis and affect the treatment strategy, yet little is known about the effect of dMMR status combined with high-risk factors and *KRAS* mutation on the prognosis of patients with colon cancer. This study found that patients with stage II dMMR colon cancer were more likely to have a good prognosis regardless of the presence of high-risk factors. This suggests that dMMR status is a significant protective factor. Therefore, observation without postoperative adjuvant treatment is appropriate for these patients, and this recommendation fills the gap in the CSCO guidelines. In addition, the prognostic impact of *KRAS* mutation in patients with stage II colon cancer did not show a statistical difference, although it showed a tendency for worse prognosis. This may be related to the short follow-up time.

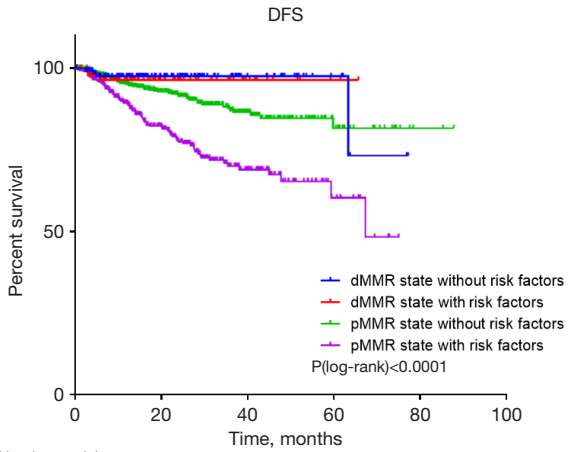
In our study, patients in the dMMR group were more likely to be younger and to have poor differentiation but less perineural invasion, which was consistent with the results of previous studies (21,22). Patients with dMMR status are younger, and this may be related to Lynch syndrome, a familial genetic disease that is associated with the incidence of colorectal cancer, endometrial cancer, small bowel cancer, ureteral cancer, renal pelvis cancer, gastric cancer, hepatobiliary tract cancer, and ovarian cancer (23). The onset age of colorectal cancer in patients with Lynch syndrome is young. Therefore, the current clinical practice guidelines in Europe, the United States, Canada, Australia, and New Zealand unanimously recommend that patients with Lynch syndrome should receive a colonoscopy every 1, 2, or 3 years from the age of 25 to 35 (24). The early onset of colorectal cancer in patients with Lynch syndrome may also be related to Knudson's two hit hypothesis (25,26).

The prognostic analysis of this study revealed that postoperative pathological stage T4 is an independent prognostic factor in the stage II colon cancer population. Previous studies have shown that MSI status did not affect the prognosis of patients in the T4 and N2 groups (27,28). Taken together, these results indicate that postoperative pathological stage has a great impact on the prognosis of patients, especially T4 and N2. In rectal cancer, if the preoperative imaging stage is T3, T4, or N+, preoperative neoadjuvant therapy should be considered first in the treatment plan. Should the treatment plan of colon cancer also use such a treatment model? In our univariate analysis of the prognosis of patients with stage II colon cancer, there were statistic significant differences in the prognosis between patients who had received adjuvant chemotherapy and those who had not (HR =1.524, 95% CI: 1.095–2.121, P=0.013). This suggested that patients who had received adjuvant chemotherapy had a worse prognosis. However, adjuvant chemotherapy was not

Table 3 Univariate and multivariate analysis of DFS in patients with stage II colon cancer

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
MMR status						
pMMR						
dMMR	0.250	0.117–0.535	<0.001	0.328	0.152–0.708	0.005
Gender						
Female						
Male	0.833	0.598–1.160	0.279			
Age (year)						
<50						
≥50	1.283	0.857–1.922	0.226			
Grade of differentiation						
Well or moderately						
Poorly	0.844	0.502–1.421	0.524			
Mucus component						
Negative						
Positive	0.670	0.341–1.316	0.245			
T4						
Negative						
Positive	2.223	1.504–3.286	<0.001	1.588	1.058–2.384	0.026
Vascular invasion						
Negative						
Positive	1.663	0.874–3.163	0.121			
Perineural invasion						
Negative						
Positive	4.011	2.779–5.791	<0.001	3.101	2.103–4.572	<0.001
No. of lymph nodes excised						
≥12						
<12	2.410	1.501–3.869	<0.001	2.021	1.250–3.267	0.004
HER2						
Negative						
Positive	2.081	0.918–4.715	0.079			
Adjuvant chemotherapy						
Negative						
Positive	1.524	1.095–2.121	0.013	1.212	0.857–1.716	0.277
KRAS mutation						
Negative						
Positive	1.479	0.980–2.232	0.063			
Unknown	1.016	0.685–1.508	0.936			

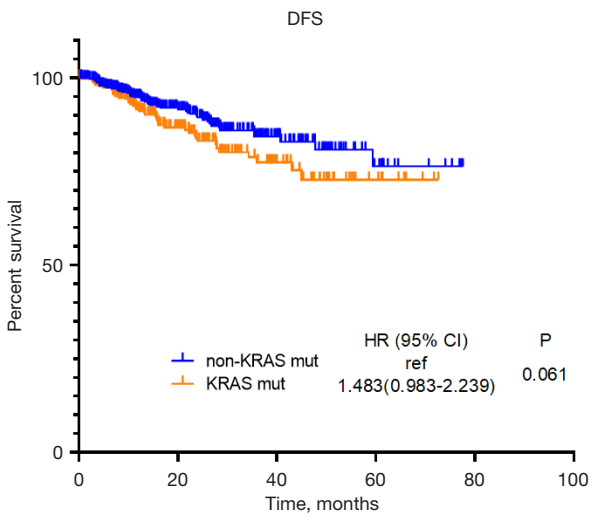
DFS, disease-free survival; MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; CI, confidence interval; HR, hazard ratio.



Number at risk

Months	0	12	24	36	48	60	72
dMMR state without risk factors	132	98	63	33	17	7	3
dMMR state with risk factors	94	53	28	15	6	3	1
pMMR state without risk factors	717	439	259	144	64	29	12
pMMR state with risk factors	414	268	150	75	34	12	4

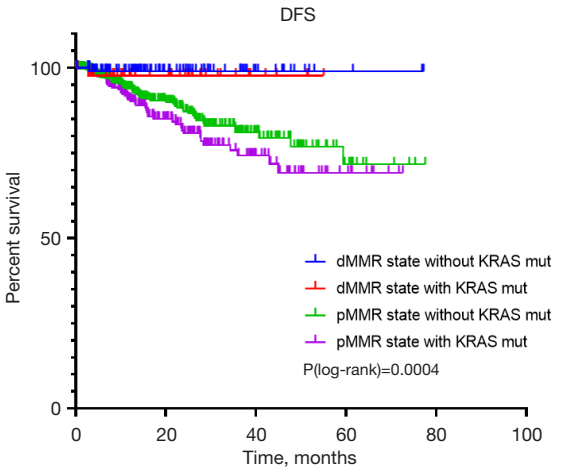
Figure 2 Prognostic analysis of patients with stage II colon cancer grouped according to high-risk factors and MMR status. DFS, disease-free survival; MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR.



Number at risk

Months	0	12	24	36	48	60	72
Non-KRAS mut	514	329	192	98	38	16	6
KRAS mut	322	199	113	57	25	11	3

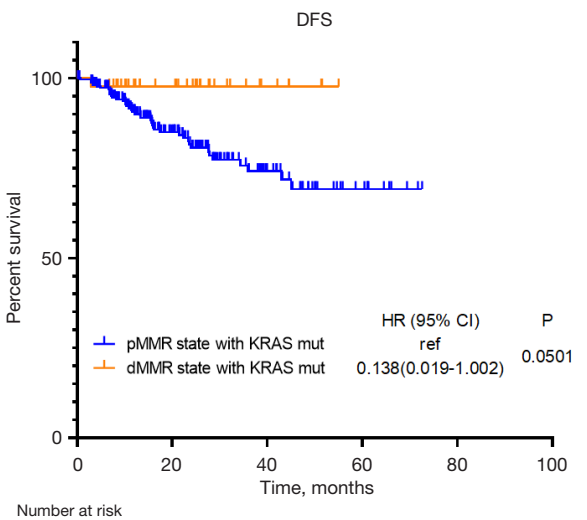
Figure 3 Survival curves of DFS comparing *KRAS* mutations in patients with stage II colon cancer. DFS, disease-free survival; KRAS, Kirsten rat sarcoma viral oncogene homolog; HR, hazard ratio; CI, confidence interval.



Number at risk

Months	0	12	24	36	48	60	72
dMMR state without KRAS mut	97	66	41	22	8	4	3
dMMR state with KRAS mut	46	29	21	9	4	1	0
pMMR state without KRAS mut	417	264	152	77	31	13	4
pMMR state with KRAS mut	276	171	93	49	22	11	2

Figure 4 Survival curves of DFS comparing *KRAS* mutation and *KRAS* wild-type in stage II colon cancer patients with dMMR or pMMR status. DFS, disease-free survival; KRAS, Kirsten rat sarcoma viral oncogene homolog; dMMR, deficient mismatch repair; pMMR, proficient MMR.



Number at risk

Months	0	12	24	36	48	60	72
pMMR state with KRAS mut	276	171	93	49	22	11	2
dMMR state with KRAS mut	46	29	21	9	4	1	0

Figure 5 Survival curves of DFS comparing MMR status in stage II colon cancer patients with *KRAS* mutation. DFS, disease-free survival; MMR, mismatch repair; KRAS, Kirsten rat sarcoma viral oncogene homolog; pMMR, proficient MMR; dMMR, deficient MMR; HR, hazard ratio; CI, confidence interval.

an independent prognostic factor after multivariate analysis, and adjuvant chemotherapy was not a prognostic factor after adjusting for pathologic stage T4 or perineural invasion. This indicated that the influence of postoperative adjuvant chemotherapy on prognosis was confounded by pathologic stage T4 and perineural invasion, which might be attributable to doctors preferring to recommend postoperative chemotherapy for patients with high-risk factors. The previous study has suggested that postoperative adjuvant chemotherapy may have little effect on the prognosis of patients with pathologic stage T4 and perineural invasion. Baxter *et al.* found that the prognosis of patients with stage II colon cancer in the T4 group was worse, and it is still unknown whether postoperative adjuvant chemotherapy can benefit patients with perineural invasion (7). In our study, perineural invasion was also an independent prognostic factor in the stage II colon cancer population, with the highest HR among all independent prognostic factors (HR =3.101). This suggests that perineural invasion has a great impact on patient prognosis.

This study had some limitations. First, this study was a single-center retrospective study, and thus selection bias and recall bias cannot be excluded. Second, the median follow-up was short, which limited the analysis of patient prognosis. Prospective research can be considered to improve the evidence levels. Third, this study found that dMMR status had an obvious protective effect on patients, but we did not explore its mechanism. Fourth, only common mutation sites of the *KRAS* gene, exons 2, 3, and 4, were detected. There may have been some patients with rare mutation sites that were not detected, which might account for why the prognostic impact of *KRAS* mutation on patients had no statistical difference.

In conclusion, stage II dMMR colon cancer patients with high-risk factors had similar survival to those without high-risk factors. The prognosis of dMMR patients was better than that of pMMR patients regardless of whether they had *KRAS* mutation or *KRAS* wild-type.

Acknowledgments

Funding: The study was supported by grants from the Natural Science Foundation of Guangdong Province of China (Nos. 2019A1515010071, 2021A1515010568), the National Natural Science Foundation of China (No. 81974369), the program of Guangdong Provincial Clinical Research Center for Digestive Diseases (No. 2020B1111170004).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2803/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2803/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2803/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of The Sixth Affiliated Hospital of Sun Yat-sen University (No. 2022ZSLYEC-125). Individual consent for this retrospective analysis was waived.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Xie Y, Shi L, He X, et al. Gastrointestinal cancers in China, the USA, and Europe. Gastroenterol Rep (Oxf) 2021;9:91-104.
3. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23:609-18.
4. Lanza G, Gafà R, Santini A, et al. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J

Clin Oncol 2006;24:2359-67.
5. Alwers E, Jansen L, Bläker H, et al. Microsatellite instability and survival after adjuvant chemotherapy among stage II and III colon cancer patients: results from a population-based study. Mol Oncol 2020;14:363-72.
6. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010;28:3219-26.
7. Baxter NN, Kennedy EB, Bergsland E, et al. Adjuvant Therapy for Stage II Colon Cancer: ASCO Guideline Update. J Clin Oncol 2022;40:892-910.
8. Qin Q, Zhou AP, Yang L, et al. Prognostic and predictive roles of DNA mismatch repair status in colon cancer patients treated with oxaliplatin-based chemotherapy: a retrospective study. J Physiol Pharmacol 2020. doi:10.26402/jpp.2020.4.12.
9. Kim JE, Hong YS, Kim HJ, et al. Defective Mismatch Repair Status was not Associated with DFS and OS in Stage II Colon Cancer Treated with Adjuvant Chemotherapy. Ann Surg Oncol 2015;22 Suppl 3:S630-7.
10. Ryan MB, Corcoran RB. Therapeutic strategies to target RAS-mutant cancers. Nat Rev Clin Oncol 2018;15:709-20.
11. Buscail L, Bournet B, Cordelier P. Role of oncogenic KRAS in the diagnosis, prognosis and treatment of pancreatic cancer. Nat Rev Gastroenterol Hepatol 2020;17:153-68.
12. Slebos RJ, Kibbelaar RE, Dalesio O, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 1990;323:561-5.
13. Pan W, Yang Y, Zhu H, et al. KRAS mutation is a weak, but valid predictor for poor prognosis and treatment outcomes in NSCLC: A meta-analysis of 41 studies. Oncotarget 2016;7:8373-88.
14. Dahabreh IJ, Terasawa T, Castaldi PJ, et al. Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. Ann Intern Med 2011;154:37-49.
15. Lin Z, Liu Y, Cai S, et al. Not All Kirsten Rat Sarcoma Viral Oncogene Homolog Mutations Predict Poor Survival in Patients With Unresectable Colorectal Liver Metastasis. Technol Cancer Res Treat 2021;20:15330338211039131.
16. Amini N, Andreatos N, Margonis GA, et al. Mutant KRAS as a prognostic biomarker after hepatectomy for rectal cancer metastases: Does the primary disease site matter? J Hepatobiliary Pancreat Sci 2022;29:417-27.
17. Zhu G, Pei L, Xia H, et al. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. Mol Cancer 2021;20:143.
18. Formica V, Sera F, Cremolini C, et al. KRAS and BRAF Mutations in Stage II and III Colon Cancer: A Systematic Review and Meta-Analysis. J Natl Cancer Inst 2022;114:517-27.
19. Hallin J, Engstrom LD, Hargis L, et al. The KRASG12C Inhibitor MRTX849 Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. Cancer Discov 2020;10:54-71.
20. Siegel RL, Fedewa SA, Anderson WF, et al. Colorectal Cancer Incidence Patterns in the United States, 1974-2013. J Natl Cancer Inst 2017;109:djw322.
21. Baek DW, Kang BW, Lee SJ, et al. Clinical Implications of Mismatch Repair Status in Patients With High-risk Stage II Colon Cancer. In Vivo 2019;33:649-57.
22. Zhao N, Cao Y, Yang J, et al. Serum Tumor Markers Combined With Clinicopathological Characteristics for Predicting MMR and KRAS Status in 2279 Chinese Colorectal Cancer Patients: A Retrospective Analysis. Front Oncol 2021;11:582244.
23. Sinicrope FA. Lynch Syndrome-Associated Colorectal Cancer. N Engl J Med 2018;379:764-73.
24. International Mismatch Repair Consortium. Variation in the risk of colorectal cancer in families with Lynch syndrome: a retrospective cohort study. Lancet Oncol 2021;22:1014-22.
25. Sudhir PR, Lin ST, Chia-Wen C, et al. Loss of PTPRM associates with the pathogenic development of colorectal adenoma-carcinoma sequence. Sci Rep 2015;5:9633.
26. Díaz-Gay M, Franch-Expósito S, Arnau-Collell C, et al. Integrated Analysis of Germline and Tumor DNA Identifies New Candidate Genes Involved in Familial Colorectal Cancer. Cancers (Basel) 2019;11:362.
27. Cohen R, Taieb J, Fiskum J, et al. Microsatellite Instability in Patients With Stage III Colon Cancer Receiving Fluoropyrimidine With or Without Oxaliplatin: An ACCENT Pooled Analysis of 12 Adjuvant Trials. J Clin Oncol 2021;39:642-51.
28. Kim JE, Hong YS, Kim HJ, et al. Microsatellite Instability was not Associated with Survival in Stage III Colon Cancer Treated with Adjuvant Chemotherapy of Oxaliplatin and Infusional 5-Fluorouracil and Leucovorin (FOLFOX). Ann Surg Oncol 2017;24:1289-94.

(English Language Editor: C. Gourlay)

Cite this article as: Zhang Y, Wu Z, Zhang B, Hu H, Zhang J, Chen Y, Ding M, Cao Y, Deng Y. Prognostic impact of high-risk factors and KRAS mutation in patients with stage II deficient mismatch repair colon cancer: a retrospective cohort study. Ann Transl Med 2022;10(12):702. doi: 10.21037/atm-22-2803

Supplementary

Table S1 Univariate and multivariate analysis of stage II colon cancer patients

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
MMR status						
pMMR						
dMMR	0.250	0.117–0.535	<0.001	0.333	0.153–0.725	0.006
Gender						
Female						
Male	0.833	0.598–1.160	0.279			
Age (year)						
<50						
≥50	1.283	0.857–1.922	0.226			
Grade of differentiation						
Well or moderately						
Poorly	0.844	0.502–1.421	0.524	1.141	0.669-1.945	0.628
Mucus component						
Negative						
Positive	0.670	0.341–1.316	0.245			
T4						
Negative						
Positive	2.223	1.504–3.286	<0.001	1.609	1.045-2.477	0.031
Vascular invasion						
Negative						
Positive	1.663	0.874–3.163	0.121	1.166	0.602-2.257	0.649
Perineural invasion						
Negative						
Positive	4.011	2.779–5.791	<0.001	3.154	2.122-4.688	<0.001
No. of lymph nodes excised						
≥12						
<12	2.410	1.501–3.869	<0.001	1.997	1.235-3.230	0.005
HER2						
Negative						
Positive	2.081	0.918–4.715	0.079	2.100	0.918-4.807	0.079
Adjuvant chemotherapy						
Negative						
Positive	1.524	1.095–2.121	0.013	1.207	0.850-1.713	0.292
KRAS mutation						
Negative						
Positive	1.479	0.980–2.232	0.063	1.504	0.986-2.294	0.058
Unknown	1.016	0.685–1.508	0.936	0.951	0.627-1.443	0.815

KRAS, Kirsten rat sarcoma viral oncogene homolog; HR, hazard ratio; MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR; HER2, human epidermal growth factor receptor 2; CI, confidence interval.

https://dx.doi.org/10.21037/atm-22-2803

Table S2 Basic characteristics of stage II colon cancer patients with *KRAS* gene testing

Characteristic	Total population, n=836, No. (%)	pMMR group, n=693, No. (%)	dMMR group, n=143, No. (%)	P value
Age (year)				<0.001
<50	191 (22.8)	131 (18.9)	60 (42.0)	
≥50	645 (77.2)	562 (81.1)	83 (58.0)	
Gender				0.795
Female	331 (39.6)	273 (39.4)	58 (40.6)	
Male	505 (60.4)	420 (60.6)	85 (59.4)	
Grade of differentiation				<0.001
Well or moderately	721 (86.2)	624 (90.0)	97 (67.8)	
Poorly	115 (13.8)	69 (10.0)	46 (32.2)	
Mucus component				<0.001
Negative	765 (91.5)	648 (93.5)	117 (81.8)	
Positive	71 (8.5)	45 (6.5)	26 (18.2)	
T4				0.109
Negative	767 (91.7)	631 (91.1)	136 (95.1)	
Positive	69 (8.3)	62 (8.9)	7 (4.9)	
Vascular invasion				0.428
Negative	796 (95.2)	658 (94.9)	138 (96.5)	
Positive	40 (4.8)	35 (5.1)	5 (3.5)	
Perineural invasion				<0.001
Negative	751 (89.8)	609 (87.9)	142 (99.3)	
Positive	85 (10.2)	84 (12.1)	1 (0.7)	
No. of lymph nodes excised				0.050
≥12	782 (93.5)	643 (92.8)	139 (97.2)	
<12	54 (6.5)	50 (7.2)	4 (2.8)	
HER2				0.318
Negative	822 (98.3)	680 (98.1)	142 (99.3)	
Positive	14 (1.7)	13 (1.9)	1 (0.7)	
Adjuvant chemotherapy				0.035
Negative	504 (60.3)	429 (61.9)	75 (52.4)	
Positive	332 (39.7)	264 (38.1)	68 (47.6)	
KRAS mutation				0.087
Negative	514 (61.5)	417 (60.2)	97 (67.8)	
Positive	322 (38.5)	276 (39.8)	46 (32.2)	

KRAS, Kirsten rat sarcoma viral oncogene homolog; pMMR, proficient mismatch repair; dMMR, deficient MMR; HER2, human epidermal growth factor receptor 2.

https://dx.doi.org/10.21037/atm-22-2803

Table S3 Multivariate logistic regression analysis predicting patients with dMMR status in stage II colon cancer patients with *KRAS* gene testing

Characteristic	HR	95% CI	P
Age (year)			
<50			
≥50	0.308	0.204-0.463	<0.001
Grade of differentiation			
Well or moderately			
Poorly	5.530	2.812-10.875	<0.001
Mucus component			
Negative			
Positive	0.587	0.313-1.080	0.199
Perineural invasion			
Negative			
Positive	0.041	0.005-0.302	0.002
Adjuvant chemotherapy			
Negative			
Positive	1.443	0.903-1.687	0.068

dMMR, deficient mismatch repair; KRAS, Kirsten rat sarcoma viral oncogene homolog; HR, hazard ratio; CI, confidence interval.

https://dx.doi.org/10.21037/atm-22-2803

Table S4 Univariate and multivariate analysis of stage II colon cancer patients with *KRAS* gene testing

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
MMR status						
pMMR						
dMMR	0.104	0.026-0.423	0.002	0.152	0.037-0.626	0.009
Gender						
Female						
Male	0.966	0.637-1.465	0.871			
Age (year)						
<50						
≥50	1.380	0.840-2.269	0.204			
Grade of differentiation						
Well or moderately						
Poorly	0.625	0.302-1.291	0.204			
Mucus component						
Negative						
Positive	1.662	0.935-2.956	0.084			
T4						
Negative						
Positive	5.263	3.288-8.424	<0.001	3.561	2.146-5.910	<0.001
Vascular invasion						
Negative						
Positive	1.813	0.877-3.748	0.108			
Perineural invasion						
Negative						
Positive	4.098	2.621-6.406	<0.001	2.671	1.653-4.316	<0.001
No. of lymph nodes excised						
≥12						
<12	2.290	1.247-4.205	0.008	2.274	1.236-4.183	0.008
HER2						
Negative						
Positive	1.003	0.247-4.077	0.996			
Adjuvant chemotherapy						
Negative						
Positive	1.828	1.203-2.778	0.005	1.210	0.762-1.923	0.419
KRAS mutation						
Negative						
Positive	1.483	0.983-2.239	0.061			

KRAS, Kirsten rat sarcoma viral oncogene homolog; HR, hazard ratio; MMR, mismatch repair; pMMR, proficient MMR; dMMR, deficient MMR; HER2, human epidermal growth factor receptor 2; CI, confidence interval.

https://dx.doi.org/10.21037/atm-22-2803