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**Clinical implication of adjuvant chemotherapy according to mismatch repair status in patients with intermediate-risk stage II colon cancer: a retrospective study**

**Abstract**

**Background:** The present study evaluated the clinical implications of adjuvant chemotherapy according to the mismatch repair (MMR) status and clinicopathologic features of patients with intermediate- and high-risk stage II colon cancer (CC).

**Methods:** This study retrospectively reviewed 5,774 patients who were diagnosed with CC and underwent curative surgical resection at OOO Hospital. The patients were enrolled according to the following criteria: (1) pathologically diagnosed with primary CC; (2) stage II CC classified based on the 7th edition of the American Joint Committee on Cancer staging system; (3) intermediate- and high-risk features; and (4) available test results for MMR status. A total of 286 patients met these criteria and were included in the study.

**Results:** Among the 286 patients, 54 (18.9%) were identified as microsatellite instability-high (MSI-H) or deficient MMR (dMMR). Although all the patients identified as MSI-H/dMMR showed better survival outcomes, T4 tumors and adjuvant chemotherapy were identified as independent prognostic factors for survival. For the intermediate-risk patients identified as MSI-low (MSI-L)/microsatellite stable (MSS) or proficient MMR (pMMR), adjuvant chemotherapy exhibited a significantly better disease-free survival (DFS) but had no impact on overall survival (OS). Oxaliplatin-containing regimens showed no association with DFS or OS. Adjuvant chemotherapy was not associated with DFS in intermediate-risk patients identified as MSI-H/dMMR.

**Conclusion:** The current study found that the use of adjuvant chemotherapy was correlated with better DFS in MSI-L/MSS or pMMR intermediate-risk stage II CC patients.

**Keywords:** Adjuvant chemotherapy; Colon cancer; Intermediate risk; Mismatch repair; Stage II

**Introduction**

Complete surgical resection followed by adjuvant chemotherapy according to pathologic stage is the current standard of care for patients with locoregional colon cancer (CC). For patients with stage III disease, the standard adjuvant chemotherapy is usually FOLFOX (infusional 5-fluorouracil [5-FU], leucovorin, and oxaliplatin) or CAPOX (capecitabine and oxaliplatin) [1,2]. However, for patients with stage II disease, the additional survival benefit from adjuvant chemotherapy varies according to clinicopathological parameters, including microsatellite instability (MSI). Thus, standard guidelines do not recommend adjuvant therapy for patients with low-risk stage II disease, while recommending adjuvant chemotherapy for patients with high-risk stage II disease (T3N0 with high-risk factor for recurrence or T4N0). High-risk factors include poorly differentiated histology, lymphovascular invasion, perineural invasion, bowel obstruction, perforation, positive margin, and inadequately sampled lymph nodes, according to National Comprehensive Cancer Network (NCCN) guidelines [3-6].

MSI, the abnormal shortening or lengthening of DNA by 1–6 repeating base pair units, results from the inactivation of the DNA mismatch repair (MMR) system and is found in approximately 15% of CCs [7]. Thus, MMR status is an important factor to consider when deciding whether to use adjuvant chemotherapy in patients with stage II CC [8]. According to previous studies, CC patients with MSI-high (MSI-H) tumors have a more favorable prognosis than those with microsatellite stable (MSS) tumors [9-11]. In addition, patients with MSI-low (MSI-L) or MSS tumors exhibited improved outcomes with 5-FU-based adjuvant chemotherapy, while adjuvant treatment was seemingly detrimental for patients with MSI-H stage II CC [10].

Recently, the European Society for Medical Oncology (ESMO) subdivided high-risk stage II CC into high-risk (T4, <12 lymph nodes or multiple risk factors) and intermediate-risk (lymphatic invasion, perineural invasion, vascular invasion, histologic grade 3, obstruction, or carcinoembryonic antigen [CEA] >5 ng/mL) groups. In addition, they recommended adjuvant FOLFOX or CAPOX for high-risk stage II CC regardless of MMR status and 5-FU or capecitabine chemotherapy alone for intermediate-risk stage II CC with MSS [12]. However, there are discrepancies in the chemotherapy recommendations for high- and intermediate-risk stage II CC between the ESMO and NCCN guidelines [6].~~~~~~~~~~

**Methods**

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| **Ethical statements:** This study was approved by the Institutional Review Board (IRB) of OOO Hospital (IRB No: 2017-11-009), and the requirement for informed consent was waived. |

**1. Patients and treatment**

This study retrospectively reviewed 5,774 patients who were diagnosed with CC and underwent curative surgical resection at OOO Hospital between January 2011 and December 2019. The patients were enrolled according to the following criteria: (1) pathologically diagnosed with primary CC; (2) stage II CC based on the 7th edition of the American Joint Committee on Cancer staging system [13]; (3) intermediate- and high-risk features [12]; and (4) available test results for MMR status. A total of 286 patients met all of these criteria and were included in the study (Fig. 1). Patient records were also reviewed for data on their medical history, age, sex, adjuvant chemotherapy regimen, surgical metAhods, ~~~~~~~~~~

**2. Definition of high-risk stage II disease by National Comprehensive Cancer Network guidelines**

For patients with MSS, stage II disease was classified as high risk if they exhibited at least one of the poor prognosis features, while all patients with MSI-H were excluded from the high-risk group [6].

**3. Statistical analysis**

Descriptive statistics are reported as proportions and medians. Categorical variables were evaluated using chi-square and Fisher exact tests, as appropriate. Disease-free survival (DFS) was measured from the date of surgery to the date of tumor recurrence or all-cause mortality. Overall survival (OS) was calculated from the date of surgery to that of all-cause mortality. Data were censored if patients were free of recurrence or were alive at the last follow-up. The Kaplan-Meier method was used to estimate DFS and OS. The survival curves were compared using a log-rank test according to MMR status or adjuvant chemotherapy. Multivariate survival analyses were performed using the Cox proportional hazard regression model. The hazard ratio and 95% confidence interval were estimated for each factor. Statistical significance was set at *p*<0.05. Statistical analyses were performed using IBM SPSS ver. 21.0 for Windows (IBM Corp., Armonk, NY, USA).

**Results**

**1. Patient and tumor characteristics**

The patient and tumor characteristics are summarized in Table 1. The median age was 70 years (range, 25–88 years) at the time of diagnosis, and 153 patients (53.5%) were male. According to the MMR status results, 54 patients (18.9%) were identified as MSI-H/dMMR. The primary tumors were located in the ascending colon in 100 patients (35.0%), transverse colon in 56 patients (19.6%), and descending colon in 130 patients (45.5%). Right-sided CC was observed in 147 patients (51.4%), and left-sided CC was observed in 139 patients (48.6%). The frequencies of intermediate- and high-risk features were as follows: T4 tumor (n=51, 17.8%), fewer than 12 lymph nodes examined (n=28, 9.8%), obstruction (n=22, 7.7%), perforation (n=3, 1.0%), high-grade tumor (n=25, 8.7%), perineural invasion (n=162, 56.6%), and lymphovascular invasion (n=184, 64.3%). Among the 286 eligible patients, 201 (70.3%) received adjuvant therapy. Among these 201 patients, 99 (49.3%) received capecitabine alone, four (2.0%) received 5-FU/leucovorin, 95 (47.3%) received FOLFOX, and three (1.5%) received CAPOX as adjuvant chemotherapy. The incidence of MSI-H/dMMR was higher with right-sided CC (n=41, 27.9%) and high-grade tumors (n=11, 44.0%).

**2. Survival outcomes**

With a median follow-up duration of 36.0 months (range, 0.5–105.2 months), the estimated 3-year DFS and OS rates were 88.9% and 93.8%, respectively. During the analyses, 32 patients (11.2%) experienced disease relapse, and 19 patients (6.6%) died. Among the patients with MSI-H, only two experienced relapse, and only one died. According to ESMO guidelines, 115 patients (40.2%) were classified as intermediate risk and 171 (59.8%) as high risk (Table 1). The incidence of MSI-H/dMMR was higher among intermediate-risk patients (n=29, 25.2%) than among high-risk patients (n=25, 14.6%). For the intermediate-risk patients identified as MSI-L/MSS or pMMR, adjuvant chemotherapy produced a significantly better DFS (*p*=0.002), yet had no impact on OS (*p*=0.176) (Fig. 2). The oxaliplatin-containing regimens were not associated with DFS or OS (Fig. 3). ~~~~~~~~~~

**Discussion**

Accumulating data suggest that MMR status and clinicopathologic features are both important determinants in deciding whether to pursue adjuvant chemotherapy for patients with stage II CC. However, the use of adjuvant chemotherapy in intermediate-risk stage II patients remains debatable. Therefore, the present study investigated the clinical impact of adjuvant chemotherapy in a relatively large cohort of intermediate-risk stage II CC patients. As a result, the intermediate-risk patients identified as MSI-L/MSS or pMMR exhibited improved outcomes with adjuvant chemotherapy, but the addition of oxaliplatin showed no survival benefit. Thus, a further prospective randomized study is needed to explore the benefit of oxaliplatin in adjuvant therapy for MSI-L/MSS or pMMR intermediate-risk stage II patients. Meanwhile, the intermediate-risk patients with tumors identified as MSI-H/dMMR in the present study showed no statistically significant benefit from adjuvant chemotherapy.

Several guidelines suggest that certain clinicopathologic high-risk features may be predictive of benefit from adjuvant chemotherapy for patients with stage II CC [20]. According to NCCN guidelines, high-risk features include T4 tumors; poorly differentiated/undifferentiated histology; lymphovascular invasion; perineural invasion; tumor budding; bowel obstruction; lesions with localized perforations or close, indeterminate, or positive margins; and inadequately sampled lymph nodes (<12 nodes) [6]. Thus, for high-risk patients, adjuvant therapy can be considered in conjunction with patient/physician discussions personalized for each patient [3,21]. Meanwhile, ESMO guidelines propose both major prognostic parameters (pathological [p] T4 stage including perforations and lymph node sampling <12) and minor prognostic parameters (high-grade tumor, vascular invasion, lymphatic invasion, perineural invasion, tumor presentation with obstruction, and high preoperative CEA levels) [12]. For intermediate-risk patients (non-MMR/MSI and any risk factor except pT4 or <12 lymph nodes assessed), 6 months of 5-FU treatment is recommended [12]. However, most studies addressing the role of adjuvant treatment in high-risk stage II settings have been retrospective or unplanned analyses [22]. Moreover, the limitations of these studies are the biologic heterogeneity of the various factors and the lack of an unequivocal definition of clinicopathologic conditions [23]. Nevertheless, the current findings confirm a significant survival benefit for MSI-L/MSS or pMMR intermediate-risk stage II CC patients treated with adjuvant therapy when compared to patients not receiving adjuvant therapy. Furthermore, the current analyses excluded high-risk patients with pT4 and/or <12 lymph nodes and several intermediate-risk factors known as robust risks of relapse after CC resection [4]. The current findings also narrow the indications for adjuvant chemotherapy and may help in establishing appropriate treatment strategies and disease prognosis for patients with stage II CC. ~~~~~~~~~~~~~~~~~~~~

The use of adjuvant chemotherapy was found to correlate with better DFS in MSI-L/MSS or pMMR intermediate-risk stage II CC patients, thereby warranting further clarification of the role of adjuvant chemotherapy and benefit of oxaliplatin-containing regimens for MSI-L/MSS or pMMR intermediate-risk stage II CC patients after curative resection.

**Notes**

**Conflicts of interest**

No potential conflict of interest relevant to this article was reported.

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**References**

1. Des Guetz G, Uzzan B, Morere JF, Perret G, Nicolas P. Duration of adjuvant chemotherapy for patients with non-metastatic colorectal cancer. Cochrane Database Syst Rev 2010;(1):CD007046.

2. André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343–51.

3. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 2004;22:3408–19.

4. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000;124:979–94.

5. André T, de Gramont A, Vernerey D, Chibaudel B, Bonnetain F, Tijeras-Raballand A, et al. Adjuvant fluorouracil, leucovorin, and oxaliplatin in stage II to III colon cancer: updated 10-year survival and outcomes according to BRAF mutation and mismatch repair status of the MOSAIC Study. J Clin Oncol 2015;33:4176–87.

6. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. Colon cancer, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2021;19:329–59.

7. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science. 1993;260:816–9.

8. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. N Engl J Med 2009;361:2449–60.

9. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, 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.

10. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247–57.

11. Klingbiel D, Saridaki Z, Roth AD, Bosman FT, Delorenzi M, Tejpar S. Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. Ann Oncol 2015;26:126–32.

12. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020;31:1291–305.

13. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471–4.

14. Twelves C, Wong A, Nowacki MP, Abt M, Burris H 3rd, Carrato A, et al. Capecitabine as adjuvant treatment for stage III colon cancer. N Engl J Med 2005;352:2696–704.

15. Haller DG, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, et al. Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. J Clin Oncol 2011;29:1465–71.

16. Haller DG, Catalano PJ, Macdonald JS, O’Rourke MA, Frontiera MS, Jackson DV, et al. Phase III study of fluorouracil, leucovorin, and levamisole in high-risk stage II and III colon cancer: final report of Intergroup 0089. J Clin Oncol 2005;23:8671–8.

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**Figure legends**

**Fig. 1.** Flow diagram of patient selection. CC, colon cancer; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, MSI-high; dMMR, deficient MMR; MSI-L, MSI-low; MSS, microsatellite stable; pMMR, proficient MMR.

**Fig. 2.** Kaplan-Meier survival curves for (A) disease-free and (B) overall survival of patients with intermediate-risk stage II colon cancer and microsatellite instability-low/microsatellite stable according to adjuvant chemotherapy.

**Fig. 3.** Kaplan-Meier survival curves for (A) disease-free and (B) overall survival of patients with intermediate-risk stage II colon cancer and microsatellite instability-low/microsatellite stable or proficient mismatch repair according to type of adjuvant chemotherapy.

**Fig. 4.** Kaplan-Meier survival curves for disease-free survival of patients with intermediate-risk stage II colon cancer and microsatellite instability-high or deficient mismatch repair according to adjuvant chemotherapy.

**Table 1.** Patient characteristics

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristic | Total | MMR status | | *p*-value |
| MSI-H/dMMR | MSI-L/MSS or pMMR |
| No. of patients | 286 (100) | 54 (18.9) | 232 (81.1) |  |
| Age | 70 (25–88) | 71 (40–86) | 70 (25–88) | 0.747 |
| Sex |  |  |  | 0.139 |
| Male | 153 (53.5) | 24 (15.7) | 129 (84.3) |  |
| Female | 133 (46.5) | 30 (22.6) | 103 (77.4) |  |
| Primary tumor location |  |  |  | <0.001 |
| Ascending colon | 100 (35.0) | 30 (30.0) | 70 (70.0) |  |
| Transverse colon | 56 (19.6) | 13 (23.2) | 43 (76.8) |  |
| Descending colon | 130 (45.5) | 11 (8.5) | 119 (91.5) |  |
| Primary tumor sidedness |  |  |  | <0.001 |
| Right | 147 (51.4) | 41 (27.9) | 106 (72.1) |  |
| Left | 139 (48.6) | 13 (9.4) | 126 (90.6) |  |
| T stage |  |  |  | 0.299 |
| T4 | 51 (17.8) | 7 (13.7) | 44 (86.3) |  |
| T3 | 235 (82.2) | 47 (20.0) | 188 (80.0) |  |
| No. of sampled LNs |  |  |  | 0.245 |
| <12 | 28 (9.8) | 3 (10.7) | 25 (89.3) |  |
| ≥12 | 258 (90.2) | 51 (19.8) | 207 (80.2) |  |
| Obstruction |  |  |  | 0.579 |
| Yes | 22 (7.7) | 5 (22.7) | 17 (77.3) |  |
| No | 264 (92.3) | 49 (18.6) | 215 (81.4) |  |
| Perforation |  |  |  | 1.000 |
| Yes | 3 (1.0) | 0 (0) | 3 (100) |  |
| No | 283 (99.0) | 54 (19.1) | 229 (80.9) |  |
| Positive margins |  |  |  |  |
| Yes | 0 (0) | 0 (0) | 0 (0) |  |
| No | 286 (100) | 54 (18.9) | 232 (81.1) |  |
| High-grade tumor |  |  |  | 0.002 |
| Yes | 25 (8.7) | 11 (44.0) | 14 (56.0) |  |
| No | 261 (91.3) | 43 (16.5) | 218 (83.5) |  |
| Perineural invasion |  |  |  | 0.900 |
| Yes | 162 (56.6) | 31 (19.1) | 131 (80.9) |  |
| No | 124 (43.4) | 23 (18.5) | 101 (81.5) |  |
| Lymphovascular invasion |  |  |  | 0.935 |
| Yes | 184 (64.3) | 35 (19.0) | 149 (81.0) |  |
| No | 102 (35.7) | 19 (18.6) | 83 (81.4) |  |
| ESMO guidelines |  |  |  | 0.025 |
| Intermediate risk | 115 (40.2) | 29 (25.2) | 86 (74.8) |  |
| High risk | 171 (59.8) | 25 (14.6) | 146 (85.4) |  |
| Adjuvant chemotherapy |  |  |  | 0.753 |
| Yes | 201 (70.3) | 37 (18.4) | 164 (81.6) |  |
| No | 85 (29.7) | 17 (20.0) | 68 (80.0) |  |
| Oxaliplatin-contained |  |  |  | 0.281 |
| Yes | 98 (48.8) | 21 (21.4) | 77 (78.6) |  |
| No | 103 (51.2) | 16 (15.5) | 87 (84.5) |  |
| Relapse |  |  |  | 0.053 |
| Yes | 32 (11.2) | 2 (6.3) | 30 (93.8) |  |
| No | 254 (88.8) | 52 (20.5) | 202 (79.5) |  |
| Death |  |  |  | 0.140 |
| Yes | 19 (6.6) | 1 (5.3) | 18 (94.7) |  |
| No | 267 (93.4) | 53 (19.9) | 214 (80.1) |  |

Values are presented as number (%) or median (range).

MMR, mismatch repair; MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; LN, lymph node; ESMO, European Society for Medical Oncology.

**Table 2.** Univariate and multivariate analyses for disease-free survival

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Disease-free survival | | | |
| Univariate analysis | | Multivariate analysis | |
| HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| Age, 65 yr  Male sex  Primary tumor sidedness, right  Tumor stage, T4  Sampled LNs, <12  Obstruction, yes  Perforation, yes  High-grade tumor, yes  Perineural invasion, yes  Lymphovascular invasion, yes  Adjuvant chemotherapy, no  Oxaliplatin-contained, no  MMR status, low/MSS | 3.782 (1.325–10.794)  1.120 (0.559–2.262)  1.425 (0.708–2.865)  4.027 (2.002–8.098)  1.715 (0.697–4.219)  2.268 (0.309–16.667)  20.366 (0.000–6.956×109)  22.896 (0.087–6.050×103)  1.040 (0.516–2.098)  2.644 (1.284–5.448)  3.592 (1.783–7.240)  2.720 (0.866–8.544)  3.804 (0.907–15.915) | 0.013  0.741  0.321  <0.001  0.240  0.420  0.764  0.271  0.913  0.008  <0.001  0.087  0.068 | 2.335 (0.795–6.857)  1.016 (0.481–2.146)  1.558 (0.727–3.339)  4.679 (2.020–10.838)  2.053 (0.745–5.658)  1.393 (0.625–3.106)  3.967 (1.910–8.239)  2.434 (0.550–10.768) | 0.123  0.967  0.255  <0.001  0.165  0.418  <0.001  0.241 |

HR, hazard ratio; CI, confidence interval; LN, lymph node; MMR, mismatch repair; MSS, microsatellite stable.

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