Prognostic implication of mucinous histology in colorectal cancer patients treated with adjuvant FOLFOX chemotherapy

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Background: There have been controversies in prognostic impact of mucinous histology on colorectal cancer, and its implication in patients treated with adjuvant 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) is unclear.

Methods: Stage II and III colorectal cancer patients who underwent curative resection followed by adjuvant FOLFOX were included. Patients were grouped according to the mucinous content: >50%, mucinous adenocarcinoma (MAC); <50%, adenocarcinoma with intermediated mucinous component (AIM); and without any mucinous component, non-MAC (NMA). Clinicopathological features and disease-free survival (DFS) were compared.

Results: Among a total of 521 patients, 27 patients (5.2%) had MAC, 41 patients (7.9%) had AIM, and 453 patients (86.9%) had NMA. Mucinous adenocarcinoma and AIM had higher frequency of proximal location and microsatellite instability, but lower frequency of angiolymphatic invasion. Disease-free survival was significantly worse in the MAC compared with NMA (3-year DFS 57% and 86%, respectively; \( P < 0.001 \)) and AIM (3-year DFS 87%, \( P = 0.01 \) vs MAC). Multivariate analysis revealed MAC as an independent negative prognostic factor of DFS (adjusted hazard ratio 7.96, 95% confidence interval 3.76–16.8).

Conclusion: Adenocarcinoma with intermediated mucinous component and MAC have distinct clinicopathological features compared with NMA. Mucinous adenocarcinoma has an adverse prognostic impact on stage II or III colorectal cancer treated with adjuvant FOLFOX.

Colorectal mucinous adenocarcinoma (MAC) is a subtype of colorectal adenocarcinoma with prominent mucin (MUC) production. In the World Health Organisation (WHO) classification, MAC is defined as an adenocarcinoma in which >50% of the lesion is composed of pools of extracellular mucin (Bosman, 2010). Tumour with <50% of the lesion composed of mucin is categorised as having mucinous component. Mucinous adenocarcinoma is associated with proximal location of tumour, advanced stage at diagnosis, microsatellite instability (MSI), and BRAF mutation compared with non-MAC (Green et al, 1993; Younes et al, 1993; Kakar et al, 2004; Song et al, 2005; Leopoldo et al, 2008). The frequency of MAC among colorectal cancer varies according to geographic origin, Western studies exhibiting higher frequencies compared with Asian studies, suggesting possible ethnic difference in pathogenesis (Wu et al, 1996; Kanemitsu et al, 2003; Du et al, 2004; Papadopoulos et al, 2004; Kang et al, 2005; Lee et al, 2007; Leopoldo et al, 2008; Xie et al, 2009). Previous studies have reported poor response and survival following palliative chemotherapy in advanced colorectal cancer with mucinous histology (Negri et al, 2005; Catalano et al, 2009). Moreover, a recent meta-analysis showed 2–8% increased hazard of death associated with mucinous histology (Verhulst et al, 2012).

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However, other studies have failed to demonstrate the adverse prognostic impact of mucinous histology on treatment outcome (Farhat et al., 2008; Catalano et al., 2012; Langner et al., 2012). Different outcomes of MAC according to MSI status have been reported exhibiting better outcome with MSI compared with microsatellite stable (MSS) among MAC (Kakar et al., 2004; Leopoldo et al., 2008).

Adjuvant chemotherapy using 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) is commonly used in stage III colon cancer, as it can reduce recurrence and mortality in these patients (Andre et al., 2009). However, the exact prognostic impact of mucinous histology on colorectal cancer patients treated with adjuvant FOLFOX is unclear. This study aimed at elucidating the prognostic implication of MAC among colorectal cancer in the current era of adjuvant FOLFOX. Furthermore, we investigated clinicopathological features and prognostic effect of mucinous component of <50%, of which only limited data have been reported.

**MATERIALS AND METHODS**

**Patients and treatment.** We retrospectively analysed patients with stage II or III colorectal cancer treated with adjuvant FOLFOX chemotherapy between April 2005 and December 2011. Patients were eligible if they met the following criteria: age over 18, adenocarcinoma histology, stage III or high-risk stage II, complete resection of the tumour with negative margin, and completion of at least six cycle of adjuvant FOLFOX chemotherapy. High-risk stage II was defined if they had any of the following: T4 lesion, obstruction or perforation, lymphovascular invasion, perineural invasion, or poorly differentiated histology. Patients with upper rectal cancer were included if the patient did not receive pre- or post-operative radiation. Exclusion criteria were the following: previous chemotherapy for CRC, previous radiotherapy for CRC, signet ring cell histology, distant metastasis, and history of other malignancy within 5 years. All patients received surgery and chemotherapy at Seoul National University Hospital, and eligible patients were identified from the electronic medical record of Seoul National University Hospital. The study protocol was reviewed and approved by the institutional review board of Seoul National University Hospital.

Patient received FOLFOX chemotherapy as either FOLFOX-4 or modified FOLFOX-6 regimen. Patients who started chemotherapy before July 2009 received FOLFOX-4 and after July 2009 received modified FOLFOX-6. Each cycle of FOLFOX-4 consisted of oxaliplatin (85 mg m⁻²) on day 1, folic acid (200 mg m⁻²), and a bolus of 5-fluorouracil (FU) (400 mg m⁻²) followed by a 22-h infusion of 5-FU (600 mg m⁻²) on days 1 and 2, which was repeated every 2 weeks. Modified FOLFOX-6 consisted of oxaliplatin (85 mg m⁻²), folic acid (400 mg m⁻²), and a bolus of 5-FU (400 mg m⁻²) followed by a 46-h infusion of 5-FU (2400 mg m⁻²) repeated every 2 weeks. Adjuvant chemotherapy was planned for a total of 12 cycles. Patients were assessed every 2 weeks during chemotherapy treatment, and then at least every 6 months for 5 years. The post-chemotherapy period assessment included a medical history taking, physical examination, measurement of the carcinoembryonic antigen level, chest computed tomography, and abdominal computed tomography. The diagnosis of recurrence was made on the basis of imaging and, if necessary, biopsy.

**Pathological examination.** Mucinous adenocarcinoma was defined according to the WHO classification, which is >50% of the tumour lesion composed of pools of extracellular MUC (Bosman, 2010). In the present study, we defined tumours with mucinous area of <50% as adenocarcinoma with intermediate mucinous component (AIM) in order to avoid confusion with MAC. Non-MAC (NMA) was defined as cancer without any mucinous component.

The microsatellite status of each tumour was determined by evaluating five microsatellite markers (D2S123, D5S346, D17S250, BAT25, and BAT26). Either forward or reverse primer for each marker was labelled with fluorescence, and PCR products were electrophoresed and analysed. We classified MSI status as follows: high MSI (MSI-H; instability at ≥2 microsatellite markers), low MSI (MSI-L; instability at 1 marker), or MSS (no instability). Only MSI-H was regarded as having MSI, and MSI-L was grouped with MSS.

**Statistical analysis.** The objective of this study was to investigate the effect of mucinous histology (MAC and AIM) on the treatment outcome (disease-free survival (DFS)) of colorectal cancer patients treated with adjuvant FOLFOX chemotherapy. Disease-free survival was calculated from the date of operation to the first date of documented progressive disease or the date of death from any cause. Data from patients who were free of progression were censored at the date of the last follow-up visit for DFS. Categorical variables were compared using χ²-test or Fisher’s exact test as appropriate. Disease-free survival was calculated using the Kaplan–Meier method, and comparisons were made using the log-rank tests. Hazard ratios (HRs) were calculated using the Cox proportional hazard model. To adjust for the baseline characteristics, Cox proportional hazard analysis of DFS included age, sex, T stage, N stage, tumour location, angiolymphatic invasion, venous invasion, perineural invasion, and MSI status. Two-sided P-values of <0.05 were considered statistically significant. Statistical analysis was performed with SPSS software for Windows, version 17.0 (SPSS, Chicago, IL, USA).

**RESULTS**

**Patient characteristics.** A total of 521 patients with stage II or III colorectal cancer, treated with adjuvant FOLFOX chemotherapy, were included. Baseline characteristics are summarised in Table 1. Tumour location was caecum in 24, ascending colon in 118, transverse in 39, descending in 33, sigmoid in 275, and rectum in 32 patients. Collectively, 181 patients had tumour in proximal (from caecum to transverse colon) location and 340 patients had tumour in distal location. Tumour stage was stage II in 78 patients (IIA in 53, IIB in 21, and IIC in 4) and stage III in 443 patients (IIIA in 39, IIB in 289, and IIC in 115). All stage II patients had high-risk features. Microsatellite instability (MSI-H) was shown in 6.8% of tumours. MSI was more frequently observed in proximal location (13.9% vs 3.0% in distal location, P<0.001). There was no significant difference in MSI incidence according to tumour stage, sex, or age. According to the inclusion criteria, all patients received at least 6 cycles of chemotherapy, and 88.8% of patients completed planned 12 cycles of chemotherapy.

**Clinicopathological characteristics of mucinous histology.** Twenty-seven patients (5.2%) had MAC and 41 patients (7.9%) had AIM. The remaining 453 patients (86.9%) had NMA. Tumours with mucinous component (AIM and MAC) were more frequently found in proximal location (61.0% in AIM and 59.3% in MAC vs 30.9% in NMA), less likely to show angiolymphatic invasion (29.3% in AIM and 22.2% in MAC vs 45.3% in NMA), and more likely to have microsatellite instability (14.6% in AIM and 14.8% in MAC vs 5.6% in NMA) compared with NMA (Table 1). Venous invasion and perineural invasion tended to be also less frequently observed in AIM and MAC.

Although AIM and MAC showed similar clinicopathological features described above, differences were also found. Adenocarcinoma with intermediated mucinous component was more frequently observed in patients over 65 years old (46.3% in AIM vs 5.6% in MAC).
vs 29.6% in MAC and 28.3% in NMA) and in female (68.3% in AIM vs 40.7% in MAC and 37.5% in NMA) patients compared with MAC and NMA. In contrast, higher proportion of MAC had T4 tumour (40.7% in MAC vs 14.6% in AIM and 13.0% in NMA) compared with AIM and NMA.

### Prognostic implication of mucinous histology

After a median follow-up duration of 38 months, 72 recurrent events (62 distant metastases and 10 local recurrences) have occurred. The 3-year DFS of the entire cohort was 84.9%.

Disease-free survival was significantly worse in the MAC compared with NMA (3-year DFS 57% and 86%, respectively; \( P < 0.001 \)) (Figure 1). Interestingly, DFS of AIM (3-year DFS 87%) was similar to NMA (\( P = 0.40 \)) and significantly better than MAC (\( P = 0.01 \)).

At the time of analysis, 10 patients in the MAC group, 4 patients in AIM group, and 58 patients in the NMA group had recurrence. There was no difference in pattern of recurrence (local recurrence vs distant metastasis) between each group (Table 2). Regarding initial sites of distant metastasis, AIM and MAC had higher percentage of peritoneal seeding compared with NMA, whereas lymph node was not involved in MAC. However, we could not make meaningful comparisons as only limited number of recurrent cases, especially for MAC and AIM, are involved in the present analysis. Among the patients with recurrence, we had tissue specimen from metastatic site in seven patients with MAC and two patients with AIM. Review of the pathological specimen from the

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**Table 1. Patient characteristics and mucinous histology**

|                      | Total (\( N = 521 \)) | NMA (\( N = 453 \)) | AIM (\( N = 41 \)) | MAC (\( N = 27 \)) | AIM vs NMA | MAC vs NMA | AIM vs MAC |
|----------------------|------------------------|----------------------|---------------------|---------------------|------------|------------|------------|
| Age                  |                        |                      |                     |                     |            |            |            |
| Median (range)       | 60 (25–81)             | 62 (25–81)           | 56 (30–81)          | 0.015               | 0.88       | 0.17       |
| >65 years            | 155 (29.8%)            | 128 (28.3%)          | 19 (46.3%)          | 8 (29.6%)           |            |            |            |
| Sex                  |                        |                      |                     |                     |            |            |            |
| Male                 | 312 (59.9%)            | 283 (62.5%)          | 13 (31.7%)          | 16 (59.3%)          | < 0.001    | 0.74       | 0.025      |
| Female               | 209 (40.1%)            | 170 (37.5%)          | 28 (68.3%)          | 11 (40.7%)          |            |            |            |
| Location             |                        |                      |                     |                     |            |            |            |
| Proximal             | 181 (34.7%)            | 140 (30.9%)          | 25 (61.0%)          | 16 (59.3%)          | < 0.001    | 0.002      | 0.89       |
| Distal               | 340 (65.3%)            | 313 (69.1%)          | 16 (39.0%)          | 11 (40.7%)          |            |            |            |
| Stage                |                        |                      |                     |                     |            |            |            |
| II                   | 78 (15.0%)             | 67 (14.8%)           | 7 (17.1%)           | 4 (14.8%)           | 0.70       | 1.0        | 1.0        |
| III                  | 443 (85.0%)            | 386 (85.2%)          | 34 (82.9%)          | 23 (85.2%)          |            |            |            |
| T stage              |                        |                      |                     |                     |            |            |            |
| T1–3                 | 445 (85.4%)            | 394 (87.0%)          | 35 (85.4%)          | 16 (59.3%)          | 0.77       | < 0.001    | 0.015      |
| T4                   | 76 (14.6%)             | 59 (13.0%)           | 6 (14.6%)           | 11 (40.7%)          |            |            |            |
| N stage              |                        |                      |                     |                     |            |            |            |
| NO–1                 | 378 (72.6%)            | 332 (73.3%)          | 29 (70.7%)          | 17 (63.0%)          | 0.72       | 0.24       | 0.50       |
| N2                   | 143 (27.4%)            | 121 (26.7%)          | 12 (29.3%)          | 10 (37.0%)          |            |            |            |
| Angiolymphatic invasion |                      |                      |                     |                     |            |            |            |
| Present              | 223 (42.8%)            | 205 (45.3%)          | 12 (29.3%)          | 6 (22.2%)           | 0.048      | 0.019      | 0.52       |
| Absent               | 298 (57.2%)            | 248 (54.7%)          | 29 (70.7%)          | 21 (77.8%)          |            |            |            |
| Venous invasion      |                        |                      |                     |                     |            |            |            |
| Present              | 57 (10.9%)             | 54 (11.9%)           | 1 (2.4%)            | 2 (7.4%)            | 0.070      | 0.76       | 0.56       |
| Absent               | 464 (89.1%)            | 399 (88.1%)          | 40 (97.6%)          | 25 (92.6%)          |            |            |            |
| Perineural invasion  |                        |                      |                     |                     |            |            |            |
| Present              | 120 (23.0%)            | 112 (24.7%)          | 5 (12.2%)           | 3 (11.1%)           | 0.084      | 0.16       | 1.00       |
| Absent               | 401 (77.0%)            | 341 (75.3%)          | 36 (87.8%)          | 24 (88.9%)          |            |            |            |
| Microsatellite status |                        |                      |                     |                     |            |            |            |
| MSS = MSI-L          | 483 (93.2%)            | 425 (94.4%)          | 35 (85.4%)          | 23 (85.2%)          | 0.022      | 0.073      | 1.00       |
| MSI-H                | 35 (6.8%)              | 25 (5.6%)            | 6 (14.6%)           | 4 (14.8%)           |            |            |            |

Abbreviations: AIM = adenocarcinoma with intermediate mucinous component; MAC = mucinous adenocarcinoma; MSI-H = high microsatellite instability; MSI-L = low microsatellite instability; MSS = microsatellite stable; NMA = non-mucinous adenocarcinoma.
metastatic site in patients with initial MAC showed that four patients had MAC, two patients had AIM, and one patient had AIM, whereas the other patient had NMA in the metastasis. Collectively, 78% (7 out of 9) of patients with initial tumour with mucinous component (MAC and AIM) retained MUC production in the recurrent metastatic tumour.

In addition to MAC histology, following clinicopathological features were associated with poor treatment outcome: T4 stage, N2 stage, angiolymphatic invasion, venous invasion, and perineural invasion (Table 3). Other factors including MSI were not significantly associated with DFS. Although MSI-H showed trend towards favourable 3-year DFS compared with MSS/MSI-L (90.0% vs 84.1%), there was no statistically significant difference ($P = 0.46$). There was no significant DFS difference according to MSI in any subgroup of patients (tumour location, stage, or sex). We could not compare the DFS according to MSI because only limited number of patients showed MSI among MAC (four patients) and AIM (six patients).

To examine whether MAC was independently associated with poor DFS, we used the Cox proportional hazard model in a forward stepwise manner with variables in Table 3 as covariates. Multivariate analysis revealed that MAC was an independent negative prognostic factor (Table 4). Mucinous adenocarcinoma had significantly higher risk of recurrence (adjusted HR 7.96) compared with non-MAC (NMA and AIM). We also conducted multivariate analysis using combined stage (i.e., Ila, Iib, Iic, IIa, IIb, and IIIc) instead of individual T stage and N stage as covariates. Similar result was obtained showing MAC as an independent negative prognostic factor (adjusted HR 7.42; $P < 0.001$).

The aim of the present study was to examine whether mucinous histology (MAC and AIM) has prognostic value in stage II and III colorectal cancer patients treated with adjuvant FOLFOX. There have been controversies in prognostic impact of MAC, and some studies showed poorer prognostic role of MAC compared with NMA (Green et al, 1993; Kanemitsu et al, 2003; Negri et al, 2005; Catalano et al, 2009), whereas others did not (Farhat et al, 2008; Catalano et al, 2012; Langner et al, 2012). Most of the previous studies focused only on MAC but not on AIM. Moreover, only limited proportion of patients had been treated with adjuvant FOLFOX, which is the current standard of care in stage III disease. Using a homogeneous cohort of patients treated with adjuvant FOLFOX, we found that MAC was independently associated with a higher risk of recurrence. In contrast, AIM showed similar treatment outcome compared with NMA.

Mucinous adenocarcinoma having poor treatment outcome is in line with previous studies performed with stage IV patients receiving palliative chemotherapy (Negri et al, 2005; Catalano et al, 2009). A recent study including stage II and III colon cancer showed no prognostic role of mucinous histology (Catalano et al, 2012). However, only 16% of patients received oxaliplatin-based adjuvant chemotherapy (Catalano et al, 2012). In contrast, all patients received at least six cycles of adjuvant FOLFOX chemotherapy in the present study. As a result, 88.8% of patients had completed 12 cycles of chemotherapy, and this maybe one explanation for the relatively high 3-year DFS in this study.

Mucins are high-molecular-weight heavily glycosylated proteins that may function as chemical barriers. Over 20 MUC genes are known, which are classified into secreted or transmembrane MUCs

Figure 1. Kaplan–Meier curves of DFS according to mucinous histology.

![Figure 1](image)

### Table 2. Pattern of recurrence

| Pattern of recurrence | Total (N = 72) | NMA (N = 58) | AIM (N = 4) | MAC (N = 10) | AIM vs NMA | MAC vs NMA | AIM vs MAC |
|-----------------------|---------------|-------------|-------------|--------------|------------|------------|------------|
| **Local recurrence**  |               |             |             |              |            |            |            |
| N (%)                 | 10 (13.9%)    | 8 (13.8%)   | 1 (25.0%)   | 1 (10.0%)    | 0.48       | 1.00       | 0.505      |
| **Distant metastasis**| 62 (86.1%)    | 50 (86.2%)  | 3 (75.0%)   | 9 (90.0%)    |            |            |            |

**Initial site involved in case of distant metastasis**

| Site                | Total (N = 72) | NMA (N = 58) | AIM (N = 4) | MAC (N = 10) | AIM vs NMA | MAC vs NMA | AIM vs MAC |
|---------------------|---------------|-------------|-------------|--------------|------------|------------|------------|
| Lung                | 25 (40.3%)    | 22 (44.0%)  | 1 (33.3%)   | 2 (22.2%)    | 1.00       | 0.29       | 1.00       |
| Liver               | 19 (30.6%)    | 17 (34.0%)  | 0 (0%)      | 2 (22.2%)    | 0.54       | 0.70       | 1.00       |
| Lymph node          | 19 (30.6%)    | 17 (34.0%)  | 2 (66.7%)   | 0 (0%)       | 0.29       | 0.048      | 0.045      |
| Peritoneum          | 20 (32.3%)    | 13 (26.0%)  | 2 (66.7%)   | 5 (55.6%)    | 0.19       | 0.12       | 1.00       |
| Bone                | 2 (3.2%)      | 2 (4.0%)    | 0 (0%)      | 0 (0%)       | 1.00       | 1.00       | —          |

**Abbreviations**: AIM = adenocarcinoma with intermediate mucinous component; MAC = mucinous adenocarcinoma; NMA = non-mucinous adenocarcinoma.

*a*Analysis among patients with recurrence.

*b*Analysis among patients having distal metastasis.
(Senapati et al, 2010). It has been shown that loss of MUC expression is an early event in colorectal carcinogenesis in a recent study (Grivennikov et al, 2012). In contrast, a number of secreted MUCs, such as MUC2, have been shown to be preserved in MAC compared with NMA (Byrd and Bresalier, 2004). Taken together, these data suggest that MAC and NMA have different mechanisms of carcinogenesis. Distinct expression pattern of MUCs and higher evidence that MUC-producing cells are resistant to adjuvant chemotherapy and responsible for the recurrence. However, it is based on a limited number of patients and should be investigated in a larger series of paired tissues from primary and metastatic tumour. The exact mechanism of poor prognosis of MAC should be elucidated in future studies to improve treatment outcome of these patients with MAC. In the meantime, patients with MAC may benefit from a more stringent follow-up. In addition, future studies analysing the role of mucinous histology as a prognostic marker in non-high-risk stage II CRC may help identify patients that may potentially benefit from adjuvant chemotherapy.

Mucinous adenocarcinoma and MSI show geographical variation in terms of its frequencies. The reported frequencies of MAC are 3–12% in Asian studies compared with 10–39% in Western studies (Wu et al, 1996; Kanemitsu et al, 2003; Du et al, 2004; Papadopoulos et al, 2004; Kang et al, 2005; Lee et al, 2007; Leopoldo et al, 2008; Xie et al, 2009). Microsatellite instability is also more frequently found in Western patients. In stage III colorectal cancer, 11–21% show MSI in Western studies, whereas 5–8% have MSI in Asian studies (Watanabe et al, 2001; Ribic et al, 2003; Kim et al, 2010; Yoon et al, 2011; Zaanan et al, 2011; Hong et al, 2012). Mucinous adenocarcinoma is frequently found in MAC compared with NMA. Moreover, AIM MSI-L is also observed among patients with MAC (Kakar et al, 2008). In the present study, MSI was more frequently found in MAC compared with NMA (6.8% in this study compared with 10–20% in Western studies) and limited number of events in the entire cohort (3-year DFS of 84.9%). Better outcome of MSI compared with MSS is also observed among patients with MAC (Lee et al, 2007). In terms of prognosis, MSI is generally associated with favourable outcome in colorectal cancer (Popat et al, 2005; Sargent et al, 2010; Zaanan et al, 2011). However, we could not find significant role for MSI. This study was underpowered to detect significant difference of DFS according to MSI status, considering the low frequency of MSI-H in Korean patients (6.8% in this study compared with 10–20% in Western studies) and limited number of events in the entire cohort (3-year DFS of 84.9%). Better outcome of MSI compared with MSS is also observed among patients with MAC (Kakar et al, 2004; Leopoldo et al, 2008). In the present study, MSI was more frequently found in MAC compared with NMA. Moreover, AIM showed similar frequency of MSI with MAC. However, we could not examine the prognostic value of MSI among MAC or AIM, because of the small number of such patients included.
In addition to MSI, MAC is frequently associated with CpG island methylation phenotype (CIMP) and BRAF mutation (Ogino et al., 2006; Kakar et al., 2012). Among the present patient cohort, we had CIMP and BRAF mutation data available in 322 and 269 patients, respectively, which had been reported previously (Han et al., 2013). The frequency of high CIMP in AIM (25%) was similar to MAC (35.3%), which was significantly higher than that in NMA (4.6%). BRAF mutation was found in 5.3% (1/19 patients) of AIM, 6.7% (1/15) of MAC, and 2.1% (5/235) of NMA. The number of BRAF mutations was too small to make statistical comparisons. Even though BRAF mutation has been associated with poor prognosis in colorectal cancer (Pai et al., 2012; Phipps et al., 2012; Safaei Ardekani et al., 2012), it cannot explain the poor prognosis of MAC in the present study, considering its low frequency.

In the present patient cohort, we found that AIM has similar features with MAC in terms of tumour location, angio-lymphatic invasion, MSI status, CIMP status, and BRAF mutation. In contrast, AIM showed differences compared with MAC in regard to patient age, sex, and T stage. Most importantly, the prognosis of AIM was significantly better than MAC but comparable to NMA. Although MAC had higher percentage of T4 stage, MAC was independently associated with poor prognosis in the multivariate analysis in which T4 stage was also included as a covariate. Although we could not find differences in the molecular markers tested between AIM and MAC, it is highly likely that AIM and MAC are distinct disease entities, considering the differences in prognosis and clinicopathological characteristics. Only few prior studies have focused on comparing AIM and MAC (Ogino et al., 2006; Langner et al., 2012). Differences were observed in frequencies of MGMT loss, p16 loss, and KRAS mutation according to the degree of mucinous component (Ogino et al., 2006). However, we could not explain the poor DFS of MAC compared with AIM and NMA in the present study with these differences. Genetic or epigenetic mechanism leading to the two mucinous phenotypes, AIM and MAC, and poor prognosis of MAC should be investigated in future studies.

The major limitation of this study is that only patients treated with adjuvant FOLFOX are included. Therefore, we cannot answer whether the higher recurrence of MAC is due to its innate aggressive biology or its resistance to adjuvant chemotherapy. Nevertheless, this is the first study to evaluate the prognostic implication of mucinous histology in colorectal cancer patients treated with adjuvant FOLFOX. The study cohort was relatively homogeneous in terms of stage (only including high-risk stage II and stage III) and treatment (surgery and single chemotherapy regimen at a high-volume single institution). Our findings need further validation in an independent cohort. Another limitation is the relatively short duration of follow-up (median 38 months). We could not evaluate the impact of mucinous histology on overall survival (OS) because only limited number of events (15 deaths) was recorded. However, the median follow-up duration had passed 3 years, DFS at which point has been shown to have good correlation with 5-year OS in colon cancer (Sargent et al., 2005). Further analysis including OS will be performed after longer follow-up in the future.

In conclusion, AIM and MAC have distinct clinicopathological features compared with NMA. Only MAC, but not AIM, has an adverse prognostic impact on stage II or III colorectal cancer treated with adjuvant FOLFOX compared with NMA.

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CONFLICT OF INTEREST
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