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Gastrointestinal Cancer

Immunohistochemistry-Based Consensus Molecular Subtypes as a Prognostic and Predictive Biomarker for Adjuvant Chemotherapy in Patients with Stage II Colorectal Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Colorectal cancer • Consensus molecular subtype • Tumor location • Adjuvant chemotherapy • Stage II

Abstract

Background. For stage II colorectal cancer (CRC), the efficacy of adjuvant chemotherapy remains controversial. Consensus molecular subtype (CMS) has been validated to be a prognostic tool for CRCs. In this study, CMS status was investigated as a prognostic biomarker for the efficacy of adjuvant chemotherapy for stage II colorectal cancer.

Materials and Methods. The tissue microarray was retrospectively constructed of 165 nonconsecutive, primary, and sporadic stage II CRCs. CMS status was determined by immunohistochemistry staining of CDX2, HTR2B, FRMD6, and ZEB1, combining with microsatellite instability testing. The prognostic for adjuvant chemotherapy efficacy of CMS status was calculated by Kaplan-Meier curves and Cox regression analysis. Subgroup analyses were conducted according to tumor location.

Results. Kaplan-Meier curves indicated that CMS was associated with overall survival (OS) and disease-free survival for stage II CRCs. Cox regression analysis showed that CMS was an independent risk factor for OS. Among high-risk clinicopathological factors, patients with CMS2/3 (hazard ratio [HR]: 0.445, 95% confidence interval [CI]: 0.227–0.875), left-sided tumors (HR: 0.488, 95% CI: 0.247–0.968), or fewer than 12 lymph nodes examined (HR: 0.307, 95% CI: 0.097–0.974) had survival benefit from adjuvant chemotherapy. Subgroup analysis showed that adjuvant chemotherapy only improved OS for patients with left-sided tumors of CMS2/3 subtype. Regardless of CMS, right-sided tumors had no benefit from adjuvant chemotherapy.

Conclusion. CMS is a better prognostic factor for adjuvant chemotherapy for stage II CRCs. Together with tumor location, CMS classification will aid in personalized treatment for stage II CRCs. ***The Oncologist*** 2020;25:e1968–e1979

Implications for Practice: For stage II colorectal cancer (CRC), the efficacy of adjuvant chemotherapy remains controversial, in that its minimal benefit (no more than 5% on average) is considered not worth the toxic effects of the drugs. There are still no effective prognostic and predictive biomarkers. This study showed that consensus molecular subtype (CMS) status is a predictive marker for adjuvant chemotherapy efficacy. Patients with left-sided tumors of CMS2/3 subtype have survival benefit by receiving adjuvant chemotherapy, which will aid in personalized treatment for stage II CRCs. Moreover, this test of CMS based on immunohistochemistry is cheap, not time consuming, and easily conducted in the laboratories of most hospitals.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second most common cause of cancer death worldwide [1]. Approximately 20%–25% of patients with CRC present with stage II disease [2]. Patients with stage II CRC are often cured with surgery alone, but 15%–20% of patients have a recurrence and eventually succumb to their disease [3]. Owing to the wide use of 5-fluorouracil (5-FU)-based adjuvant chemotherapy, reduced relapses have been observed in patients with stage III CRC [4–6]. However, the application of adjuvant chemotherapy to patients with stage II CRC remains controversial because of its minimal benefit that is usually considered to be not worth the toxic effects of the drugs [7–9].

To date, a variety of high-risk clinicopathological features correlated with prognosis in stage II disease have been proposed to assist the decision for adjuvant chemotherapy, including microsatellite stability (MSS)/proficient mismatch repair (pMMR), poorly differentiated histology (exclusive of those cancers that are microsatellite instability-high [MSI-H]), lymphatic/vascular invasion, bowel obstruction, <12 lymph nodes examined, perineural invasion, localized perforation, or close, indeterminate, or positive margins [10–12]. According to recurrence risk by the National Comprehensive Cancer Network (NCCN) guidelines [13], patients with stage II CRC are divided into three groups: low-risk group as T3 (MSI-H) with no high-risk factors; mid-risk group as T3 (MSS/MSI-L) with no high-risk factors; and high-risk group as T3 with high-risk factors or T4. Patients in the low-risk group have been validated to have a good prognosis and do not benefit from adjuvant chemotherapy [14, 15]. However, for up to 90% of patients in the mid-risk and high-risk groups, clinicopathological high-risk factors combined with MSI status are not enough [16, 17]. Thus, identifying novel biomarkers that could reliably screen out patients with stage II CRC who could benefit from treatment with adjuvant chemotherapy is a research priority.

Several molecular subtypes of CRC have been identified by unsupervised clustering analyses of gene expression profiles. The Colorectal Cancer Subtyping Consortium integrated these subtypes and established four robust transcriptome-based subtypes known as consensus molecular subtypes (CMSs), dividing CRCs into one of four CMS groups: CMS1, MSI immune; CMS2, canonical; CMS3, metabolic; and CMS4, mesenchymal [18]. CMS status have been validated to be a prognostic tool [19]. However, its translation into clinical practice has been hampered by its complex testing procedure, lack of qualified laboratories, and high cost. Recently, an immunohistochemistry (IHC) assay and an online classification tool have been successfully established, in combination with MSI testing, delivering objective and accurate scoring to classify patients with CRC into the main CMSs [20, 21]. This rapid classifier, based on semiquantitative pathology scoring, improves clinical utility of CMS status to promote its prognostic and predictive value.

To date, no clinical evidence for CMS status predicting the efficacy of adjuvant chemotherapy for stage II CRC has been reported. In this study, by adopting the IHC-baed classifier, we classified 165 cases with stage II CRC from a single center into the main CMSs to validate its feasibility, assess the prognostic and predictive accuracy of CMSs as biomarkers for adjuvant chemotherapy, and compare with traditional clinicopathological high-risk factors.

Materials and Methods

Patients

This study retrospectively enrolled 165 nonconsecutive, primary, and sporadic CRCs treated between May 2008 and December 2010 in Fudan University Shanghai Cancer Center (FUSCC). The inclusion criteria of eligible patients were as follows: aged between 18 and 80 years; located at colon or upper rectum of more than 10 cm distal from the anus; pathologically confirmed colorectal adenocarcinoma, mucinous adenocarcinoma, or signet-ring cell carcinoma with stage II disease (T3–4, N0, M0) according to the American Joint Committee on Cancer/Union for International Cancer Control TNM staging system 8th edition; without neoadjuvant chemotherapy or radiotherapy; had radical resection of the primary tumor. The exclusion criteria were as follows: had emergency surgery because of an acute intestinal obstruction, bleeding, or perforation; had evidence of distant metastases; received neoadjuvant therapy; diagnosed as hereditary colorectal cancer, such as familial adenomatous polyposis and Lynch syndrome; had a history of other malignancies; tissue specimen or follow-up data unavailable. This study was approved by the institutional review board of FUSCC and was carried out in accordance with the Declaration of Helsinki. All patients provided written and oral informed consent. Patients' demographic and clinicopathological variables, including age, gender, primary site, histological type, T stage, tumor differentiation, vascular/perineural invasion, lymph node examined, MSI status, pretreatment carcinoembryonic antigen (CEA) level, molecular characteristics and treatment type, were retrieved from the FUSCC database. Tumors proximal to the splenic flexure were defined as right-sided and tumors at or distal to the splenic flexure as left-sided.

Patients were followed up regularly according to Chinese guidelines for CRC. Physical examination and serum tumor biomarkers, including CEA, were performed every 3–6 months for the first 2 years, every 6 months within the third to fifth year, and then annually. Chest/abdominal/pelvis computed tomography was performed annually for up to 5 years, and colonoscopy was performed the first year after treatment and repeated in the third year if no advanced adenoma was found and then every 5 years. As this study described the prognosis of patients with CRC, analysis of overall survival (OS) and disease-free survival (DFS) were ascertained. The OS was defined as the time from surgical resection to death from any cause, and the DFS was defined as the time from surgical resection to the first recurrence or death caused by disease progression. The survival data were provided by the Clinical Statistics Center of FUSCC, relying on the hospital medical records follow-up platform or contact with patients by phone or e-mail. Patients who were alive at last follow-up were censored for analysis.

Table 1. Baseline characteristics of patients with stage II CRC in FUSCC (*n* = 165)

Characteristics	Cases, *n* (%)	CMS1	CMS2/3	CMS4	*p* value
Age, years					.083
<60	81 (49.1)	19 (63.3)	52 (43.7)	10 (62.5)	
≥60	84 (50.9)	11 (36.7)	67 (56.3)	6 (37.5)	
Gender					.614
Male	92 (55.8)	19 (63.3)	65 (54.6)	8 (50)	
Female	73 (44.2)	11 (36.7)	54 (45.4)	8 (50)	
Primary site					**<.001**
Right sided	45 (27.3)	16 (53.3)	28 (23.5)	1 (6.3)	
Left sided	120 (72.7)	14 (46.7)	91 (76.5)	15 (93.7)	
Histological type					0.137
Adenocarcinoma	136 (82.4)	21 (70.0)	101 (84.9)	14 (87.5)	
Mucinous	29 (17.6)	9 (30.0)	18 (15.1)	2 (12.5)	
T stage					.817
T3	81 (49.1)	14 (46.7)	58 (48.7)	9 (56.3)	
T4	84 (50.9)	16 (53.3)	61 (51.3)	7 (43.8)	
Differentiation					.529
Well/moderate	122 (73.9)	19 (63.3)	90 (75.6)	13 (81.3)	
Poor	33 (20.0)	8 (26.7)	22 (18.5)	3 (18.8)	
Unknown	10 (6.1)	3 (10.0)	7 (5.9)	0 (0.0)	
Vascular invasion					.187
Negative	147 (89.1)	28 (93.3)	105 (88.2)	14 (87.5)	
Positive	14 (8.5)	0 (0.0)	12 (10.1)	2 (12.5)	
Unknown	4 (2.4)	2 (6.7)	2 (1.7)	0 (0.0)	
Perineural invasion					.177
Negative	130 (78.8)	26 (86.7)	94 (79.7)	10 (66.7)	
Positive	33 (20.0)	4 (13.3)	24 (20.3)	5 (33.3)	
Unknown	2 (1.2)	0 (0.0)	1 (0.8)	1 (6.3)	
Lymph nodes examined					**.025**
<12	36 (21.8)	1 (3.3)	31 (26.1)	4 (25.0)	
≥12	129 (78.2)	29 (96.7)	88 (73.9)	12 (75.0)	
CEA, μL/mL					.658
<5	110 (66.7)	22 (73.3)	77 (64.7)	11 (68.8)	
≥5	55 (33.3)	8 (26.7)	42 (35.3)	5 (31.3)	
KRAS					.369
Wild type	71 (53.4)	5 (35.7)	58 (55.8)	8 (53.3)	
Mutant	62 (46.6)	9 (64.3)	46 (44.2)	7 (46.7)	
NRAS					.357
Wild type	126 (94.7)	14 (100)	97 (93.3)	15 (100)	
Mutant	7 (5.3)	0 (0.0)	7 (6.7)	0 (0.0)	
BRAF					.954
Wild type	122 (91.7)	13 (92.9)	95 (91.3)	14 (93.3)	
Mutant	11 (8.3)	1 (7.1)	9 (8.7)	1 (6.7)	
Risk group for recurrence					**<.001**
Low-risk	15 (9.1)	14 (46.7)	1 (0.8)	0 (0.0)	
Mid-risk	26 (15.8)	0 (0.0)	22 (18.5)	4 (25.0)	
High-risk	124 (75.1)	16 (53.3)	96 (80.7)	12 (75.0)	
Adjuvant chemotherapy					.974
No	63 (38.2)	12 (40.0)	45 (37.8)	6 (37.5)	
Yes	102 (61.8)	18 (60.0)	74 (62.2)	10 (62.5)	

Bold values are statistically significant.
Abbreviations: CEA, carcinoembryonic antigen; CMS, consensus molecular subtype; FUSCC, Fudan University Shanghai Cancer Center.

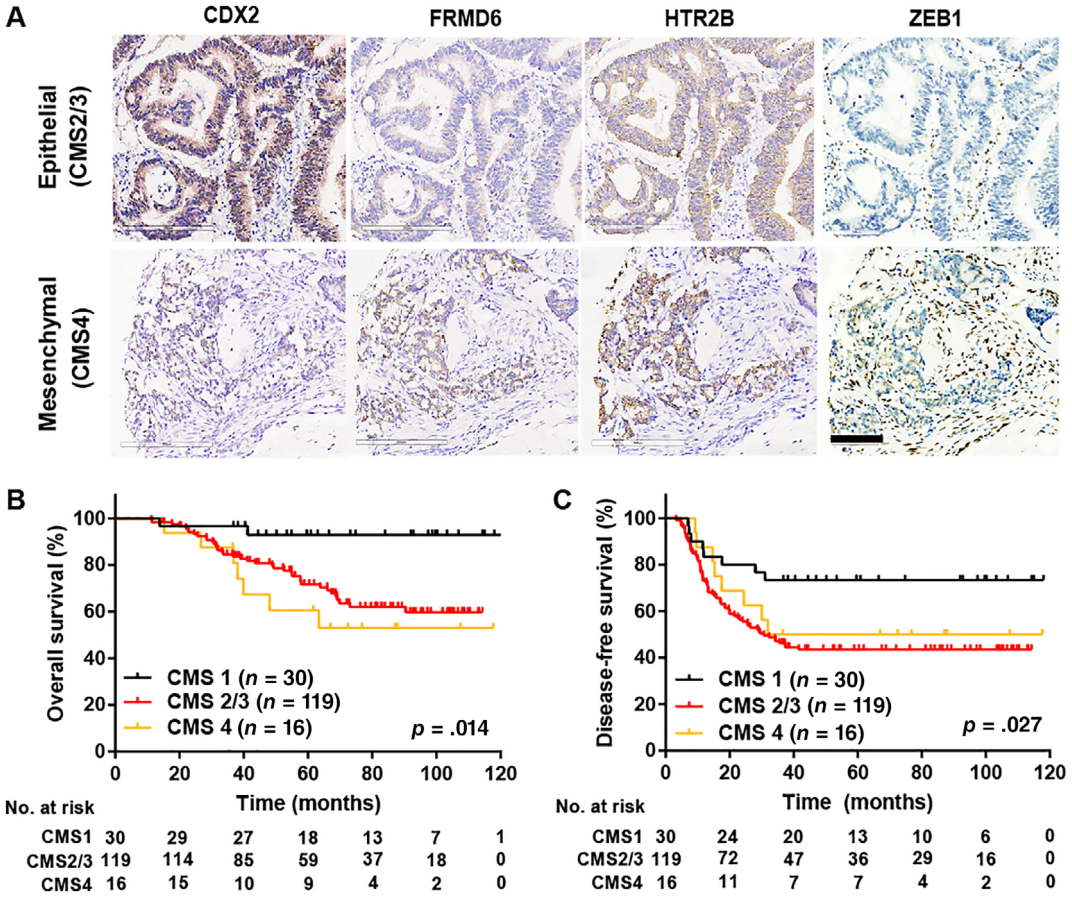

Figure 1. CMS subtype and its prognostic value in stage II colorectal cancer. **(A):** Staining of representative epithelial-like or mesenchymal-like patients. **(B):** Comparing the overall survival curves of patients with different CMS status. **(C):** Comparing the disease-free survival curves of patients with different CMS status.
Abbreviation: CMS, consensus molecular subtype.

TMA Construction, IHC Staining, and MMR Status

A tissue microarray (TMA) of tumor tissue was constructed as described previously [22, 23]. Briefly, formalin-fixed, paraffin-embedded tissue blocks from resected CRC were obtained. Tissue cylinders with a 0.6-mm diameter were punched from representative tissue areas of each donor tissue block and brought into one recipient paraffin block (30 × 25 mm). Each TMA spot included at least 50% tumor cells. The histological types were confirmed by experienced pathologists (D.H. and W.S.).

Four markers were selected from previous transcriptomic analysis for the IHC-based CMSs classifier, including CDX2, HTR2B, FRMD6, and ZEB1 [21]. In addition, pan-cytokeratin was selected to normalize the other markers for tumor content. IHC staining was performed according to standard protocol. TMA slides were baked overnight at 58°C, deparaffinized in xylene, rehydrated through graded ethanol, quenched for endogenous peroxidase activity in 0.3% hydrogen peroxide at 37°C for 15 minutes, and processed for antigen retrieval. Sections were then incubated at 4°C overnight with anti-HTR2B (1:100; HPA012867; Sigma-Aldrich, St. Louis, MO), anti-FRMD6 (1:500; HPA001297; Sigma-Aldrich), anti-CDX2 (1:200; NB100-2136; Novus Biologicals, Centennial, CO), anti-ZEB1 (1:500; HPA027524; Sigma-Aldrich), or anticytokeratin (AE1/AE3; 1:1000; Thermo Fisher Scientific, Waltham, MA). Immunostaining was performed using the EnVision^{+}System-HRP (AEC) (K4005; Dako, Glostrup, Denmark), which resulted in a brown-colored precipitate at the antigen site. Subsequently, sections were counterstained with hematoxylin (Sigma-Aldrich) and mounted in a nonaqueous mounting medium. All runs included a no primary antibody control. MMR status was identified by IHC with antibodies against hMLH1, hMSH2, hMSH6, and hPMS2 and reported by the Department of Pathology in FUSCC.

CMS Status by the IHC-Based Classifier

To classify patients into their colorectal cancer subtype, MSI status was first used to define patients who belong to the CMS1 subtype. The remaining patients were classified into "epithelial" (CMS2/3) or "mesenchymal" (CMS4) subtypes using the online classification tool according to its detailed instructions (crcclassifier.shinyapps.io/appTesting). Briefly, by entering the staining intensity and percentage of CDX2, HTR2B, FRMD6, and pan-keratin, and the presence of ZEB1, defined by two independent pathologist (D.H. and W.S.), the prediction probability for "mesenchymal" or "epithelial" will be calculated. Cores with a random forest probability of 60% were scored as "mesenchymal" (CMS4); otherwise, they were scored as "epithelial" (CMS2/3).

Table 2. Univariate and multivariate analyses of OS and DFS for stage II CRC patients in FUSCC (n = 165).

	OS						DFS					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
Age, years			.103			.177			.242			.933
<60	1.000			1.000			1.000			1.000		
≥60	1.632	0.906–2.940		1.120	0.583–2.154		1.296	0.839–2.001		0.980	0.613–1.568	
Gender			.429			.897			.511			.464
Male	1.000			1.000			1.000			1.000		
Female	1.260	0.711–2.233		1.041	0.564–1.921		0.864	0.559–1.336		0.841	0.530–1.336	
Tumor location			.958			**.042**			.503			.599
Right-sided	1.000			1.000			1.000			1.000		
Left-sided	0.982	0.499–1.931		0.457	0.214–0.973		1.188	0.718–1.964		0.864	0.501–1.490	
Histological type			.281			.264			.161			**.017**
Adenocarcinoma	1.000			1.000			1.000			1.000		
Mucinous	0.624	0.265–1.471		0.536	0.179–1.601		0.635	0.337–1.198		0.376	0.168–0.842	
T stage			.374			.216			.785			.629
T3	1.000			1.000			1.000			1.000		
T4	0.77	0.433–1.369		0.684	0.375–1.248		0.942	0.612–1.448		0.895	0.571–1.403	
Differentiation			.407			.135			**.007**			**<.001**
Well/moderate	1.000			1.000			1.000			1.000		
Poor	1.189	0.604–2.342		2.237	0.978–5.114		1.904	1.173–3.089		3.688	2.068–6.577	
Vascular invasion			.983			.898			.844			.390
Negative	1.000			1.000			1.000			1.000		
Positive	1.091	0.431–2.761		0.792	0.295–2.128		0.795	0.366–1.725		0.569	0.254–1.274	
Perineural invasion			.580			.622			.905			.653
Negative	1.000			1.000			1.000			1.000		
Positive	0.719	0.335–1.541		0.743	0.331–1.664		0.893	0.517–1.542		0.866	0.492–1.521	
Lymph nodes examined			**.036**			0.150			.149			.977
<12	1.000			1.000			1.000			1.000		
≥12	0.518	0.280–0.957		0.606	0.306–1.198		0.698	0.428–1.137		0.992	0.583–1.688	
CEA, μL/mL			**<.001**			**<.001**			**<.001**			**<.001**
≤5	1.000			1.000			1.000			1.000		
>5	2.787	1.571–4.947		3.404	1.765–6.564		2.316	1.500–3.578		2.990	1.847–4.841	
MMR status			**.015**						**.012**			
pMMR	1.000			undefined			1.000			undefined		
dMMR	1.171	0.041–0.705					0.394	0.190–0.817				

(continued)

Table 2. (continued)

	OS						DFS					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
CMS subtype			**.035**			**.029**			**.035**			.112
CMS1	1.000			1.000			1.000			1.000		
CMS2/3	5.586	1.347–23.163		6.287	1.395–28.332		2.613	1.254–5.443		2.137	0.983–4.645	
CMS4	7.862	1.632–37.878		9.89310.147	1.819–53.793		2.053	0.770–5.474		1.429	0.495–4.130	
Risk group for recurrence			.357						.266			
Low-risk	1			undefined			1			undefined		
Mid-risk	3.070	0.662–14.231					2.347	0.772–7.134				
High-risk	2.562	0.616–10.660					2.290	0.834–6.288				
Adjuvant chemotherapy			.134			.132			.725			.968
No	1.000			1.000			1.000			1.000		
Yes	0.645	0.364–1.144		0.602	0.312–1.165		1.083	0.694–1.690		0.990	0.603–1.625	

Bold values are statistically significant.
All factors that do not interact with others in univariate analysis were included into multivariate analysis.
Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; CMS, consensus molecular subtype; DFS, disease-free survival; dMMR, dificent mismatch repair; HR, hazard ratio; MMR, mismatch repair; MSI-L/H, microsatellite instability low/high; MSS, microsatellite stable; OS, overall survival; pMMR, proficient mismatch repair.

Statistical Analysis

Patient baseline characteristics were summarized using descriptive statistics. Categorical variables were compared using the two-sided Pearson χ^2 test or Fisher's exact test as appropriate. Continuous variables were compared using a *t* test or the Wilcoxon rank test as appropriate. Summary statistics on time-to-event variables, such as DFS and OS, were calculated according to the Kaplan-Meier method and compared by the log-rank test. Cox regression was used for univariate and multivariate analyses with hazard ratios (HRs) and 95% confidence intervals (CI). All factors that did not interact with others in the univariate analysis were included in the multivariate analysis. To compare the prognostic efficacy of different biomarkers, Harrell concordance index (C-index, calculated using the Hmisc package, Soft R version 2.11.1) was used [24]. The higher the C-index, the more effective the biomarker is. All *p* values were two-sided and considered significant when <.05.

Results

Baseline Characteristics of Patients According to CMS Status

In this study, 165 eligible patients with stage II CRC were enrolled. The median age was 60, ranging from 24 to 80 years. Of the patients, 55.8% (92/165) were male and 44.2% (73/165) were female. The median follow-up time was 63.2 months, and 47 patients died during follow-up. The overall 5-year OS was 74.5%, and the overall 5-year DFS was 64.8%. According to MSI status and IHC staining of selected makers, 30 (18.2%) patients were classified as CMS1 subgroup, 119 (72.1%) patients as CMS2/3, and 16 (9.7%) patients as CMS4 (Table 1; Fig. 1A). CMS subtype was compared with clinicopathological features (Table 1) and CMS status was significantly associated with tumor primary site ($p < .001$), lymph nodes examined ($p = .025$), and risk group for recurrence ($p < .001$). Right-sided tumors had quite different CMS distribution from left-sided tumors (Fig. 1B). Of right-sided tumors, 35.6% were classified as CMS1, 62.2% as CMS2/3, and only 2.2% as CMS4. Of left-sided tumors, only 11.7% were classified as CMS1, 75.8% as CMS2/3, and 12.5% as CMS4.

CMS Status as a Better Prognostic Biomarker for Stage II CRC

Kaplan-Meier analysis showed that CMS status was associated with OS and DFS of patients with stage II CRC ($p < .05$; Fig. 1B, 1C). The 5-year OSs for CMS1, CMS2/3, and CMS4 were 92.9%, 71.6%, and 60.6%, respectively, and the 5-year DFSs were 73.3%, 43.5%, and 50.0%, respectively. The univariate Cox regression analysis indicated that lymph nodes examined, CEA level, MMR status, and CMS subtype were associated with OS for patients with stage II CRC ($p < .05$), whereas differentiation, CEA level, MMR status, and CMS subtype were associated with DFS ($p < .05$; Table 2). Multivariate analysis after adjustment demonstrated that tumor location, CEA, and CMS subtypes were independent prognostic factors for OS ($p < .05$) and that histological type, differentiation, and CEA were independent prognostic factors

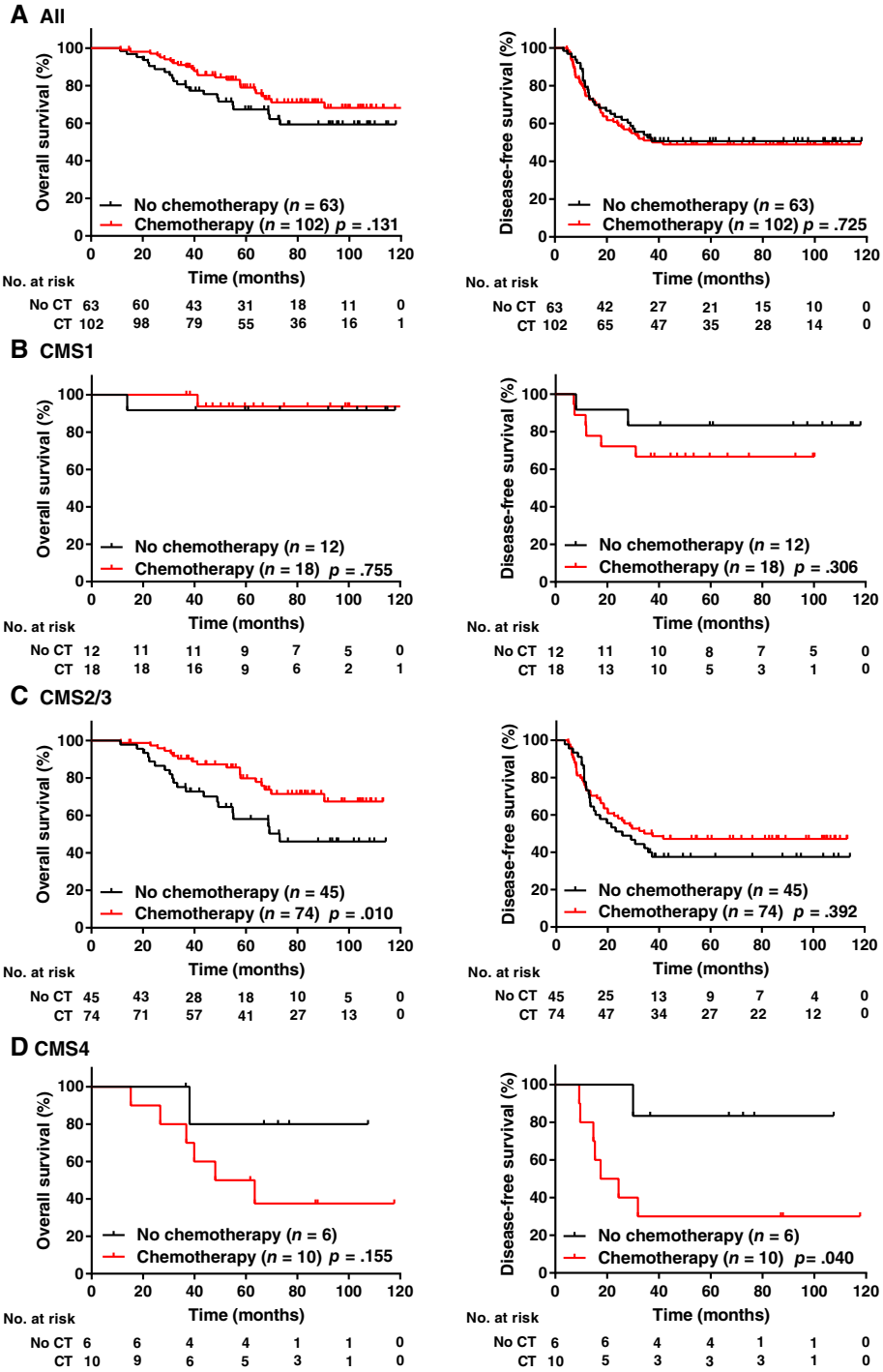

Figure 2. CMS status as a prognostic factor for the efficacy of adjuvant chemotherapy for stage II colorectal cancer. Comparing the overall survival (left) and disease-free survival (right) curves of patients receiving chemotherapy or not in all patients **(A)** and patients with CMS1 **(B)**, CMS2/3 **(C)**, and CMS4 **(D)** subtypes.
Abbreviations: CMS, consensus molecular subtype; CT, chemotherapy.

for DFS ($p < .05$) and CMS subtype lost its significance for DFS ($p > .05$; Table 2).

The efficacy of prognostic biomarkers was compared by C-index (supplemental online Table 1). Although second to CEA (OS, C-index = 0.624; DFS, C-index = 0.598), the efficacy of CMS subtype as a prognostic biomarker for OS (C-index = 0.594) and DFS (C-index = 0.545) was better than risk group and other high-risk factors alone.

CMS Status as a Prognostic Biomarker for the Efficacy of Adjuvant Chemotherapy

Adjuvant chemotherapy had no significant benefit on OS ($p = .131$) or DFS ($p = .725$; Figs. 2, 3A). For patients with CMS1 subtype, adjuvant chemotherapy had no benefit on OS ($p = .755$) or DFS ($p = .306$). For patients with CMS2/3 subtype, adjuvant chemotherapy significantly improved the OS rate from 58.0% to 79.8% at 5 years ($p = .010$), whereas DFS rate

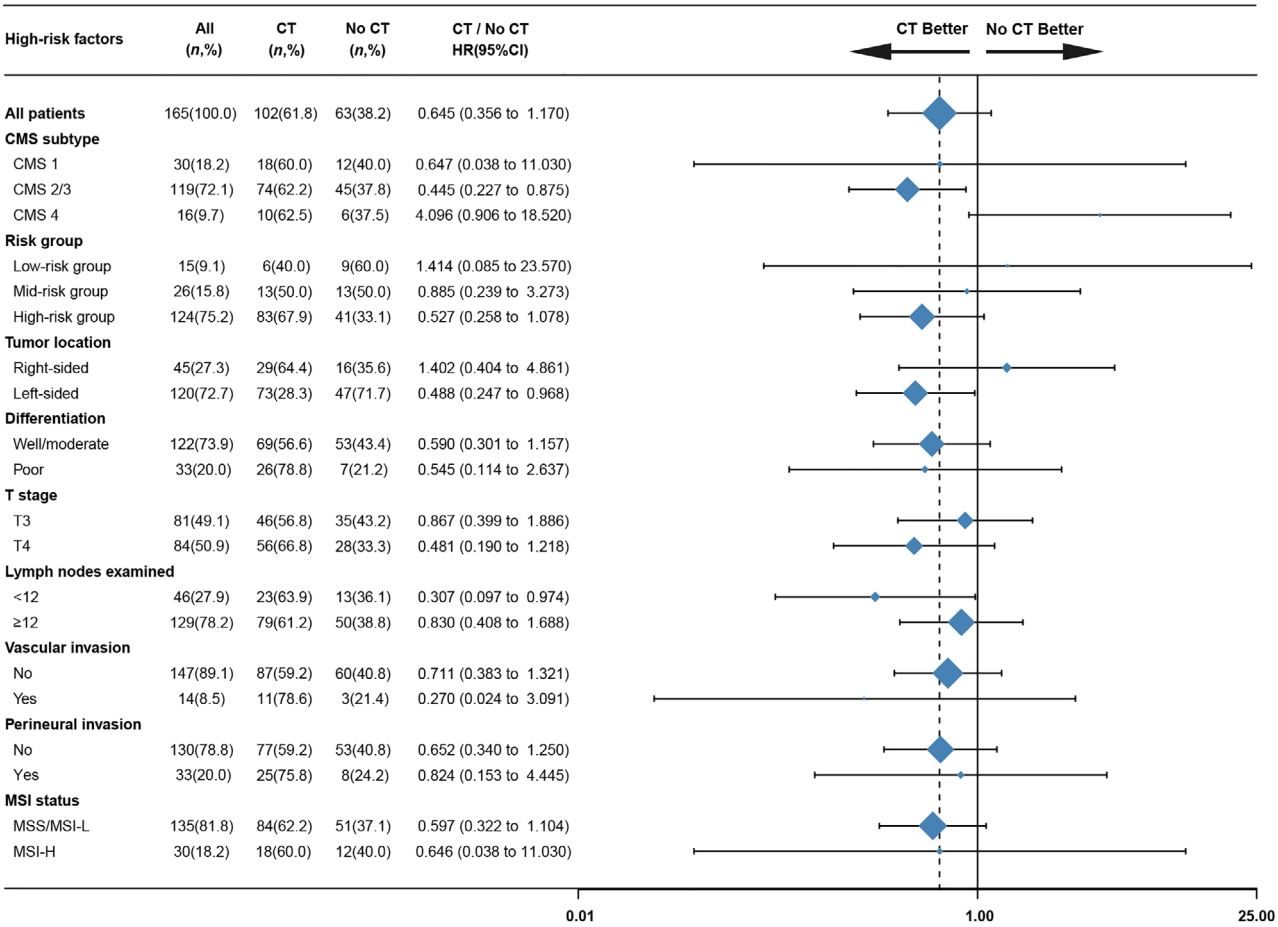

Figure 3. Efficacy of adjuvant chemotherapy on each high-risk clinicopathological factor for overall survival. The subgroups were divided according to the traditional clinicopathologic high-risk factors and CMSs. The efficacy of adjuvant chemotherapy was compared in each subgroup.
Abbreviations: CI, confidence interval; CMS, consensus molecular subtype; CT, chemotherapy; HR, hazard ratio; MSI-L/H, microsatellite instability low/high; MSS, microsatellite stable.

was improved from 37.5% to 47.3%, although without statistical significance (p = .392). However, for patients with CMS4 subtype, adjuvant chemotherapy significantly decreased the 5-year DFS rate from 83.3% to 30.0% (p = .040), and 5-year OS rate from 80.0% to 50.0%, although without statistical significance (p = .155).

Clinicopathological high-risk factors were then evaluated to predict the efficacy of adjuvant chemotherapy. For OS, patients with CMS2/3 (p = .010, HR: 0.445, 95% CI: 0.227–0.875), left-sided tumors (p = .028, HR: 0.488, 95% CI: 0.247–0.968), or < 12 lymph nodes examined (p = .015, HR: 0.307, 95% CI: 0.097–0.974) had survival benefit from adjuvant chemotherapy (Fig. 3). For DFS, no survival benefit was achieved by CMS subtype or traditional clinicopathologic high-risk factors from adjuvant chemotherapy, whereas patients with CMS4 subtype who had adjuvant chemotherapy even had worse DFS (p = .040, HR: 6.547, 95% CI: 1.636–26.200; supplemental online Fig. 1).

Subgroup Analysis of the CMS Subtype as a Prognostic Biomarker for the Efficacy of Adjuvant Chemotherapy According to Tumor Location

CMS subtypes were distinctly distributed in right-sided and left-sided CRCs (Fig. 4A). Of right-sided tumors, 35.6% were classified as CMS1; 75.6% of left-sided tumors were defined as CMS2/3. Almost all CMS4 (93.7%) tumors were left-sided. For left-sided tumors, adjuvant chemotherapy significantly improved the OS (p = .028), whereas DFS was not improved (p = .713; Fig. 4B). For right-sided tumors, adjuvant chemotherapy had no benefit on OS (p = .616) or DFS (p = .200; Fig. 4C).

Subgroup analysis according to tumor location was further conducted to investigate the prognostic value of CMS subtype for the efficacy of adjuvant chemotherapy. In the left-sided subgroup, no death occurred for tumors with CMS1. A significant benefit of adjuvant chemotherapy for OS was observed in patients with CMS2/3 (p = .001; Fig. 5A), whereas no significant benefit for OS or DFS was observed in patients with CMS4 (Fig. 5B). In the right-sided subgroup, only one patient was classified as CMS4. No significant benefit of adjuvant chemotherapy for OS or DFS was observed, regardless of CMS subtypes.

Discussion

5-FU based adjuvant chemotherapy has been widely accepted for the treatment of stage III CRCs. However, for stage II CRCs, this therapeutic benefit has not been replicated [25, 26]. The NCCN guidelines recommend that

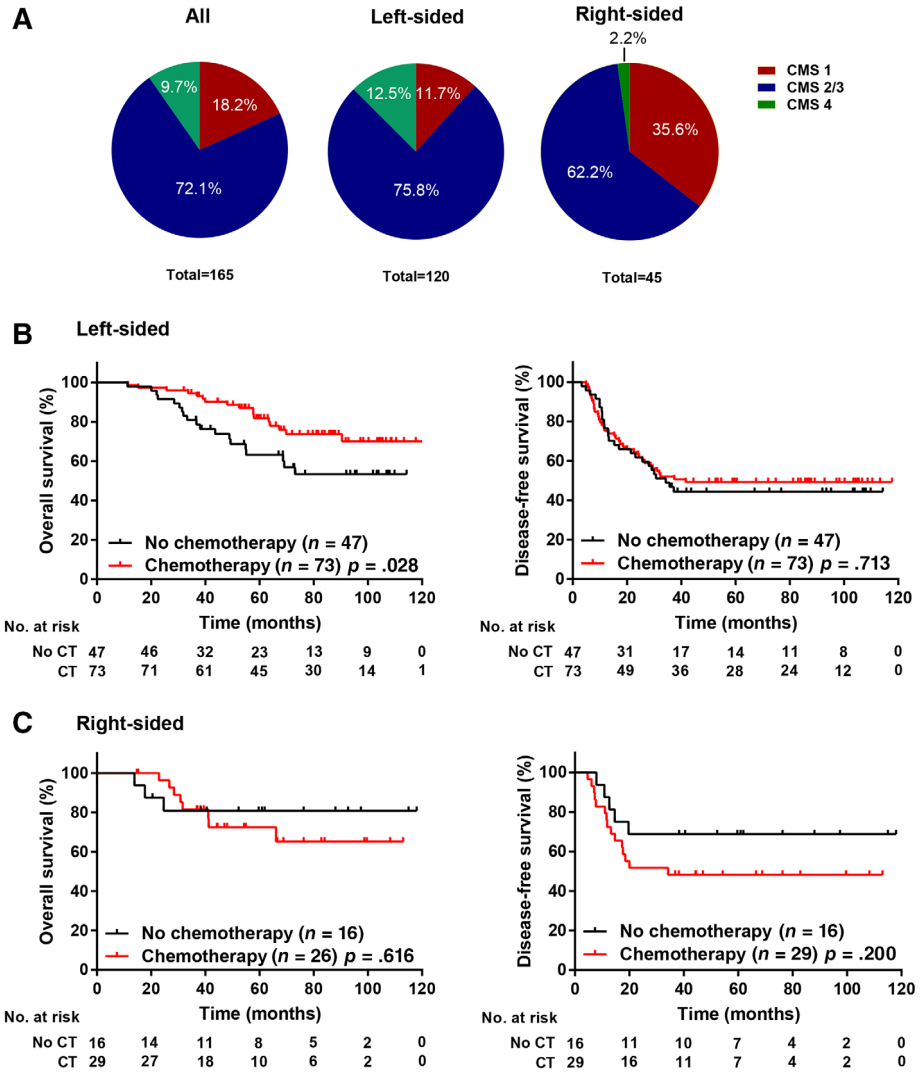

Figure 4. Tumor location as a prognostic factor for the efficacy of adjuvant chemotherapy for stage II colorectal cancer. **(A):** CMS distribution in all tumors, left-sided tumors, and right-sided tumors. **(B):** Comparing the overall survival (left) and disease-free survival (right) curves of patients receiving chemotherapy for left-sided stage II tumors. **(C):** Comparing the overall survival (left) and disease-free survival (right) curves of patients receiving chemotherapy for right-sided stage II tumors.
Abbreviations: CMS, consensus molecular subtype; CT, chemotherapy.

patients with high-risk stage II CRCs could be considered for adjuvant chemotherapy [13], but most of these high-risk factors are not well validated. In recent years, there has been some progress in molecular biomarkers. Dalerba et al. [27] revealed that lack of CDX-2 expression could identify a subgroup of patients with high-risk stage II colon cancer who appeared to benefit from adjuvant chemotherapy. In another study, Rohr et al. [28] found that pMMR/MACC1-low tumors have a similar favorable prognosis to those with deficient mismatch repair (dMMR) with potential implications for the role of adjuvant therapy. However, the clinical transition of these studies is impeded as few high-risk patients were identified (CDX-2 negative, 7.2%; pMMR/MACC1-low, 5-7%). Gao et al. [29] developed a hallmark gene signature that identifies a subset of patients with stage II CRC who could have survival benefit from adjuvant chemotherapy, but it is difficult to put this signature into clinical practice because of its high cost and complex test methods. In addition, studies have shown that patients with stage II CRC with human epidermal growth receptor 2–positive expression or high CD206/CD68 ratio benefited from adjuvant chemotherapy [30, 31], but the cut-off values cannot be widely applied to clinical practice. Therefore, there is still a need for better predictive biomarkers for adjuvant chemotherapy.

CMS subtyping emerged in 2015, by an international effort dedicated to sharing large-scale data and integrating six independent transcriptomic-based subtyping systems [18]. The four CMS groups represent the present best description of CRC heterogeneity. However, the original method of CMS subtyping is based on gene-expression profiling, which requires sufficient tumor tissue, cost, and time. An IHC-based CMS classifier was then established and validated as a rapid, cost-effective, and reliable surrogate [21]. Currently, the IHC-based classifier does not distinguish between different epithelial-like subtypes (canonical Wnt signaling CMS2 and metabolic CMS3 subtypes), which have similar prognosis. Although not

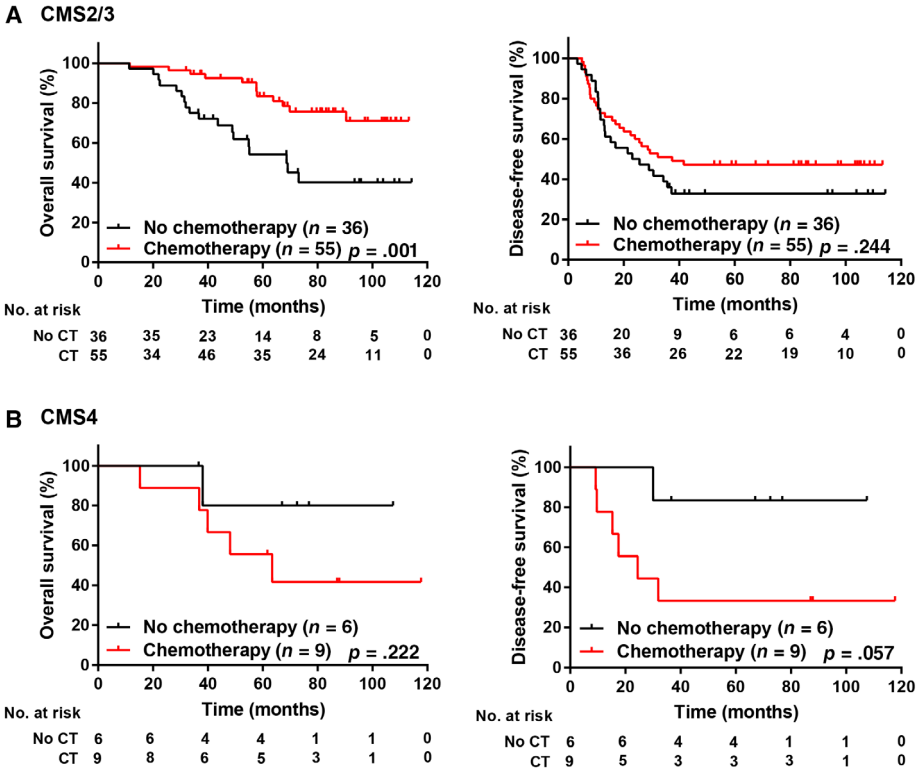

Figure 5. The prognostic value of CMS subtype for the efficacy of adjuvant chemotherapy for left-sided stage II colorectal cancer. Comparing the overall survival (left) and disease-free survival (right) curves of patients receiving chemotherapy or not in patients with right-sided tumors of CMS2/3 **(A)** and CMS4 **(B)** subtypes.
Abbreviations: CMS, consensus molecular subtype; CT, chemotherapy.

completely accurate, we believe it has little influence on the prognostic value research of CMS status. CMS classification has been reported as an independent prognostic factor in patients with metastatic CRC who undergo first-line therapy and a potential biomarker to guide selection of anti–vascular endothelial growth factor and anti–epidermal growth factor receptor (EGFR) therapy [19]. In the present study, we sought to investigate whether CMS status determined by IHC-based CMS classifier could be used as a biomarker for the efficacy of adjuvant chemotherapy for stage II CRCs.

Our study identified 18.2% of patients as CMS1, 72.1% as CMS2/3, and 9.7% as CMS4 in stage II CRCs, according to MSI status and IHC staining of selected makers. CMS1 subtype represents patients with MSI-H tumors, who do not benefit from 5-FU–based adjuvant chemotherapy, similar to previous studies [14, 15]. For the reminder of patients, we found that patients with CMS2/3 tumors have OS benefit from adjuvant chemotherapy, whereas patients with CMS4 tumors even have DFS decrease from adjuvant chemotherapy. CMS4 tumors represents an aggressive subtype, characterized by clear upregulation of genes implicated in epithelial-to-mesenchymal transition and of signatures associated with the activation of transforming growth factor-β signaling, angiogenesis, matrix remodeling pathways, and stromal infiltration. Several previous studies have found the drug-resistant feature of CMS4 subtype of CRC. Roepman et al. [32] reported that C-type CRCs at stage III, which were referred to as mesenchymal phenotype, showed no benefit from adjuvant chemotherapy treatment (HR: 1.4, $p = .542$), partly linked to its low proliferative but invasive activity [33, 34]. De Sousa et al. [23] found that for metastatic disease, CMS4 CRCs are resistant to anti-EGFR therapy, independent of *KRAS* mutation status.

Previous studies have validated that advanced right-sided CRCs (stage III–IV) have inferior OS and treatment response for adjuvant chemotherapy and anti-EGFR therapy. But no conclusion has been reached on a potential different chemosensitivity between right-sided and left-sided CRCs. Our study found that right-sided stage II CRCs have no benefit from chemotherapy independent of CMS subtype, whereas significant benefit of OS was observed for left-sided CRCs. Subgroup analysis further revealed that only left-sided CRCs with CMS2/3 subtype have OS benefit from chemotherapy, which represents 55.2% of all stage II CRCs. Thus, together with tumor location, CMS classification will aid in personalized treatment for stage II CRCs.

There were several limitations to this study. First, owing to the nature of retrospective study, the lack of some clinical information (such as chemotherapy regimen, etc.) may affect the richness of research results. Second, a relatively small sample of the cohort may result in lack of power for the Cox regression analysis in each subgroup of CMS status and some of potential correlation between CMS status and chemotherapy efficacy may fail to manifest. Third, the cases included in the present cohort were nonconsecutively collected and more patients who had relapse or metastases

were included, which may lead to selection bias. Fourth, the predictive value of CMS status was only conducted in one cohort from a single medical center, lacking internal and external validation, which limited the extrapolation of the results. Fifth, our evaluation method of IHC results was not automated as it still needed the involvement of pathologists. Although the evaluation method has been validated to have good stability and repeatability, bias in manual detection could affect the results. Last, additional biomarkers should be added to further classify CMS2 and CMS3, which may also have different response to adjuvant chemotherapy.

Conclusion

Our study shows that CMS status can effectively predict the OS of stage II CRC and that only patients with stage II CRC in the left-sided CMS2/3 subgroup have survival benefit from adjuvant chemotherapy. Moreover, CMS classification using IHC methods is affordable, is not time consuming, and can be easily applied in most hospitals. With further large-scale clinical validation, the CMS status will aid in precision treatment for patients with stage II CRC.

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Disclosures

The authors indicated no financial relationships.

References

1. Bray F, Ferlay J, Soerjomataram I et al. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

2. Zhang JX, Song W, Chen ZH et al. Prognostic and predictive value of a microRNA signature in stage II colon cancer: A microRNA expression analysis. Lancet Oncol 2013;14:1295–1306.

3. Oliphant R, Nicholson GA, Horgan PG et al. Contribution of surgical specialization to improved colorectal cancer survival. Br J Surg 2013;100:1388–1395.

4. Andre T, Boni C, Mounedji-Boudiaf L et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343–2351.

5. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005;352:476–487.

6. Saltz LB, Cox JV, Blanke C et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan study group. N Engl J Med 2000;343:905–914.

7. Marshall JL. Risk assessment in stage II colorectal cancer. Oncology (Williston Park) 2010;24(1 suppl 1):9–13.

8. Benson AB 3rd, Schrag D, Somerfield MR et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 2004;22:3408–3419.

9. Gray R, Barnwell J, McConkey C et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: A randomised study. Lancet 2007;370:2020–2029.

10. Quah HM, Chou JF, Gonen M et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. Dis Colon Rectum 2008;51:503–507.

11. Kucukzeybek Y, Dirican A, Demir L et al. Adjuvant chemotherapy and prognostic factors in stage II colon cancer--Izmir Oncology Group Study. Asian Pac j Cancer Prev 2015;16:2413–2418.

12. Okada K, Sadahiro S, Suzuki T et al. The size of retrieved lymph nodes correlates with the number of retrieved lymph nodes and is an independent prognostic factor in patients with stage II colon cancer. Int J Colorectal Dis 2015;30:1685–1693.

13. Kang H, O'Connell JB, Maggard MA et al. A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum. Dis Colon Rectum 2005;48:1161–1168.

14. 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;28:3219-3226.

15. Ribic CM, Sargent DJ, Moore MJ et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247–257.

16. Andre T, de Gramont A, Vernerey D 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;33:4176–4187.

17. O'Connor ES, Greenblatt DY, LoConte NK et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. J Clin Oncol 2011;29:3381–3388.

18. Guinney J, Dienstmann R, Wang X et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–1356.

19. Lenz HJ, Ou FS, Venook AP et al. Impact of consensus molecular subtype on survival in patients with metastatic colorectal cancer: Results from CALGB/SWOG 80405 (Alliance). J Clin Oncol 2019;37:1876–1885.

20. Ten Hoorn S, Trinh A, de Jong J et al. Classification of colorectal cancer in molecular subtypes by immunohistochemistry. Methods Mol Biol 2018;1765:179–191.

21. Trinh A, Trumpi K, De Sousa EMF et al. Practical and robust identification of molecular subtypes in colorectal cancer by immunohistochemistry. Clin Cancer Res 2017;23:387–398.

22. Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Discov 2003;2:962–972.

23. De Sousa EMF, Wang X, Jansen M, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 2013;19:614–618.

24. Harrell FE Jr, Califf RM, Pryor DB et al. Evaluating the yield of medical tests. JAMA 1982;247:2543–2546.

25. Kohne CH. Should adjuvant chemotherapy become standard treatment for patients with stage II colon cancer? Against the proposal. Lancet Oncol 2006;7:516–517.

26. Sobrero A. Should adjuvant chemotherapy become standard treatment for patients with stage II colon cancer? For the proposal. Lancet Oncol 2006;7:515–516.

27. Dalerba P, Sahoo D, Paik S et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 2016;374:211–222.

28. Rohr UP, Herrmann P, Ilm K et al. Prognostic value of MACC1 and proficient mismatch repair status for recurrence risk prediction in stage II colon cancer patients: The BIOGRID studies. Ann Oncol 2017;28:1869–1875.

29. Gao S, Tibiche C, Zou J et al. Identification and construction of combinatory cancer hallmark-based gene signature sets to predict recurrence

and chemotherapy benefit in stage II colorectal cancer. JAMA Oncol 2016;2:37–45.

30. Feng Y, Li Y, Huang D et al. HER2 as a potential biomarker guiding adjuvant chemotherapy in stage II colorectal cancer. Eur J Surg Oncol 2019; 45:167–173.

31. Feng Q, Chang W, Mao Y et al. Tumor-associated macrophages as prognostic and predictive biomarkers for postoperative adjuvant chemotherapy in patients with stage II colon cancer. Clin Cancer Res 2019;25:3896–3907.

32. Roepman P, Schlicker A, Tabernero J et al. Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. Int J Cancer 2014;134:552–562.

33. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: An emerging axis of evil in the war on cancer. Oncogene 2010;29: 4741–4751.

34. Yang AD, Fan F, Camp ER et al. Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. Clin Cancer Res 2006;12:4147–4153.

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