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Optimal Criteria for G3 (Poorly Differentiated) Stage II Colon Cancer

Prospective Validation in a Randomized Controlled Study (SACURA Trial)

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Abstract: Grade 3 (G3, poorly differentiated) is an important treatment-decision factor in stage II colon cancer, but no unified diagnostic criteria are established. According to previous studies, an intratumoural poorly differentiated area with no glandular formation (POR) that fills the microscopic field of a ×40 objective lens was an essential factor that defined G3. We aimed to prospectively validate this in a randomized controlled study of adjuvant chemotherapy (SACURA trial). We enrolled 991 patients with stage II colon cancer. POR was graded according to the ×40 objective lens rule and the intensity of poorly differentiated clusters ($Grade^{POR}$), and its prognostic power was compared with that of the conventional tumor grade on the basis of predominant histology rule ($Grade^{conv}$). According to $Grade^{POR}$, 313, 526, and 152 tumors were classified as $G1^{POR}$, $G2^{POR}$, and $G3^{POR}$, respectively, and the 5-year relapse-free survival (RFS) rates were 91.1%, 82.9%, and 74.7%, respectively ($P < 0.0001$). When $G3^{POR}$ and $G3^{conv}$ were alternatively added to the prognostic model consisting of 8 conventional factors, only $G3^{POR}$ was a significant factor for RFS ($P = 0.040$, Wald test). The adverse impact of $G3^{POR}$ on RFS was greater in the microsatellite stable/microsatellite instability–low subset than that in the full analysis set. In the microsatellite stable/microsatellite instability–low subset, the 5-year RFS rate of patients with $G3^{POR}$ tumors in the chemotherapy group achieved greater improvement (9.1%) than the surgery-alone group. The least differentiation policy with the ×40 objective lens rule may be highlighted as the diagnostic criterion for G3 because of its validated prognostic value.

Key Words: tumor grading, poorly differentiated component, tumor budding, microsatellite instability status, adjuvant chemotherapy

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Tumor grade is the numerical description of a malignant tumor based on how the tumor cells are microscopically different from normal cells from which the tumor originated and is regarded as an indicator of potential tumor growth and spread. Generally, a higher grade cancer may have a

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higher malignant potential and be associated with worse clinical outcomes. Starting in the 1920s, the degree of tumor differentiation was primarily assessed on the basis of architecture, specifically gland or tubule formation, in colorectal cancer (CRC),[1,2] and this method has long been used as the most essential characteristic of the tumor to be recorded in pathology reports. In the Union for International Cancer Control (UICC) classification, grade (G) 3 is applied to poorly differentiated tumors.[3]

Internationally, an appropriate pathologic diagnosis of G3 is especially important for stage II CRC because G3 is regarded as an essential risk factor for recurrence according to the guidelines of the National Comprehensive Cancer Network (NCCN)[4] and the European Society for Medical Oncology[5] and is used to identify a subgroup of patients with stage II CRC that could benefit from postoperative adjuvant therapy. However, a single widely accepted and uniform standard for grading is lacking for CRCs, most of which have intratumoural heterogeneity in their differentiation patterns.[6,7] The issues to be resolved with this system include the uncertainty of how we define the least differentiated area, which could convey important prognostic information and should be reflected in the tumor grade. More specifically, the extent of an intratumoral poorly differentiated lesion that should be diagnosed as G3 is currently unclear.

In 2008, we reported the results of a retrospective exploratory study using 1075 patients with stage I to III CRC to clarify how a large intratumoral poorly differentiated area impacts the postoperative survival of these patients.[8] As a result, we found that an intratumoural poorly differentiated histologic area that fills a ×40 objective lens field (a field of 0.196 mm^2) was an essential index for the diagnosis of G3 in terms of the postoperative prognosis of CRC. The ×40 microscopic objective lens field may also represent the specific extent of how an intratumoral poorly differentiated phenotype of the tumor can determine oncologic outcomes, such as lymph node metastasis and recurrence of T1 CRC.[9] This was reasonably validated in a recent multicenter study of 2057 patients with T1 CRC that was conducted by the Japanese Society for Cancer of the Colon and Rectum (JSCCR).[10] We had expected these findings to help clarify the long-pending issue of uncertainty of tumor grade criteria.

The SACURA (Surgical Adjuvant Chemotherapy with UFT for Curatively Resected Stage II Colon Cancer) trial is a multicenter, randomized controlled study that evaluated the superiority of 1 year of adjuvant treatment with oral tegafur-uracil (UFT) compared with surgery alone for stage II colon cancer (ClinicalTrials.gov NCT00392899).[11] The 5-year disease-free survival (DFS) rate was 78.4% in the surgery-alone group and 80.2% in the UFT group (hazard ratio [HR], 0.91; 95% confidence interval [CI]: 0.75-1.10; $P = 0.31$), and, although the superiority of adjuvant treatment with UFT over surgery alone was not shown, the recurrence rate was lower in the UFT group than in the surgery-alone group (10.4% vs. 13.4%).[12]

As one of the predetermined translational studies in the SACURA trial, the grading system based on the extent of intratumoural poorly differentiated histology, which was reported in 2008,[8] was prospectively evaluated to validate its value for the grading of stage II colon cancer.[11] Hence, the primary aims of the present study were to determine the accuracy of the prognostic stratification power of the grading system and to verify its superiority to the conventional tumor grading system for stage II colon cancer. Because it has long been acknowledged that a part of poorly differentiated colonic tumors constitutes an entity of microsatellite instability (MSI) in tumors, which markedly differs from those without MSI in their prognosis,[13,14] we analyzed a microsatellite stable (MSS)/MSI-low subset in addition to a full analysis set (FAS).

PATIENTS AND METHODS

Patients

The study protocol was approved by the Institutional Review Boards of each participating institution and was conducted in accordance with the tenets of the Declaration of Helsinki and comparable Japanese ethical standards. Furthermore, written informed consent was obtained from all study participants.

Overall, 2024 patients with stage II colon cancer and no preoperative treatment from 270 hospitals in Japan were enrolled in the SACURA trial between October 2006 and July 2010 and were randomly assigned to the surgery-alone group or the UFT group.[11] After the exclusion of 42 ineligible patients, data from 1982 patients were analyzed for DFS as the primary endpoint, and overall survival, relapse-free survival (RFS), and the incidence and severity of adverse events, as secondary endpoints, and were compared (Fig. 1). Consequently, 5 years after the last patient was enrolled, a primary analysis concluded that UFT was statistically nonsuperior in regard to any of the endpoints.[12]

It was predetermined in the study protocol that pathologic specimens for the translational study of new histopathologic prognostic factors would be collected from 1000 patients out of those enrolled in the SACURA trial. Consequently, 123 hospitals participated in the translational study for new histopathologic factors, and pathologic specimens were collected from 1003 eligible patients in these hospitals. After 12 patients were excluded because of noncompliance with the allocated protocol, 991 patients with curatively resected stage II colon cancer from 123 institutions (surgery-alone group, 501 patients; UFT group, 490 patients) were enrolled as the FAS in this study. Of these, 807 patients had colon cancer (cecum, 73; ascending colon, 209; transverse colon, 123; descending colon, 60; and sigmoid colon, 342) and 184 had rectosigmoid cancer. As regards the extent of lymph node dissection per the *Japanese Classification of Colorectal Carcinoma* (second English edition) edited by the JSCCR,[15] most patients underwent D3 (complete dissection of all regional lymph nodes) or D2 (complete dissection of pericolic/perirectal and intermediate lymph nodes) procedures (800 and 188 patients, respectively) with only 3 patients classified as D1 (complete dissection

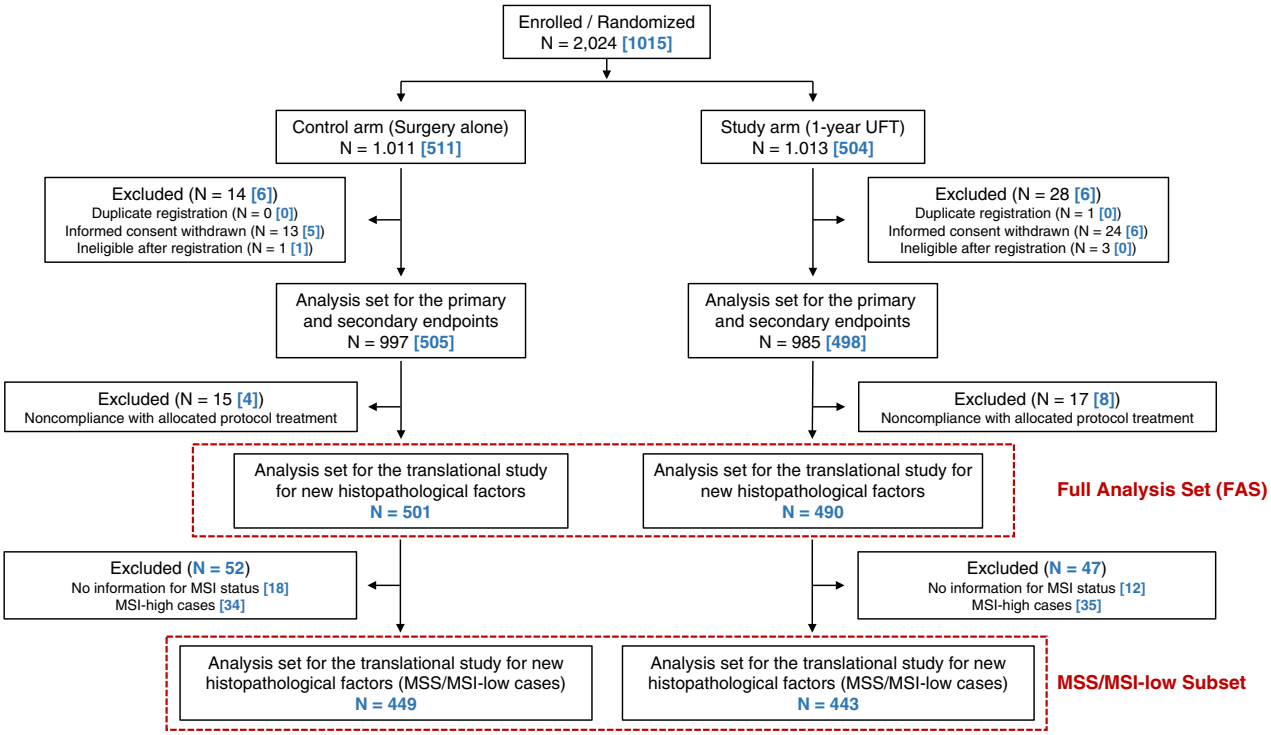

FIGURE 1. CONSORT diagram. Number of patients in the translational study for new histopathologic factors are indicated in blue.

of pericolic/perirectal lymph nodes). The median follow-up period was 69.7 (range: 2.1 to 105.6) months, and the 5-year RFS rate for all patients was 84.2% (85.3% in the UFT group and 83.2% in the surgery-alone group).

Pathologic Examination

Postoperatively, glass slides containing tissue stained with hematoxylin and eosin were prepared according to routine pathology practice at each participating institution and were collected in the study office at Tokyo Medical and Dental University and then submitted to National Defense Medical College, the institution responsible for the central review of new histopathologic factors; those who performed the assessments were blinded to both patient and tumor information. The glass slides were prepared from a whole tumor section that included the deepest part of the tumor and were prospectively examined by one of the authors (H.U.) to evaluate the extent of poorly differentiated components (PORs) and tumor budding according to the criteria detailed below. Evaluated data on new histopathologic factors were sent to the study office at Tokyo Medical and Dental University, where these data were prospectively registered in an internet-based electronic data capture system managed by the Translational Research Center for Medical Innovation.

The conventional pathologic factors, including conventional tumor grade (Grade^{conv}), T-stage, lymphatic invasion, venous invasion, and the number of lymph nodes examined, were evaluated by pathologists at each participating institution and were prospectively registered in the electronic data capture system together with the clinical data.

Conventional Tumor Grade Based on the Predominant Histologic Type

According to the definition of *the Japanese Classification of Colorectal Carcinoma*,[15] tumors were diagnosed as papillary adenocarcinoma, well-differentiated tubular adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, mucinous adenocarcinoma, or signet-ring cell carcinoma (Fig. 2A). Tumors that contain >1 histologic type of carcinoma are classified on the basis of the predominant histologic type by definition.[15] G1^{conv} was assigned for papillary adenocarcinoma (N = 19) and well-differentiated tubular adenocarcinoma (N = 401), G2^{conv} was reserved for moderately differentiated adenocarcinoma (N = 526), and G3^{conv} was reserved for poorly differentiated adenocarcinoma (N = 14) and mucinous adenocarcinoma (N = 31). No patient in this study population was diagnosed with signet-ring cell carcinoma.

Tumor Grade According to the Extent of Poorly Differentiated Component

Tumor grade according to the extent of poorly differentiated component (Grade^{POR}) was evaluated as previously reported.[8] POR was simply defined as an area composed of adenocarcinoma with no gland formation irrespective of the growth pattern or mucin production. Consequently, POR included mucinous carcinoma comprising tumor nests with no gland formation, and signet-ring cell carcinoma as well.

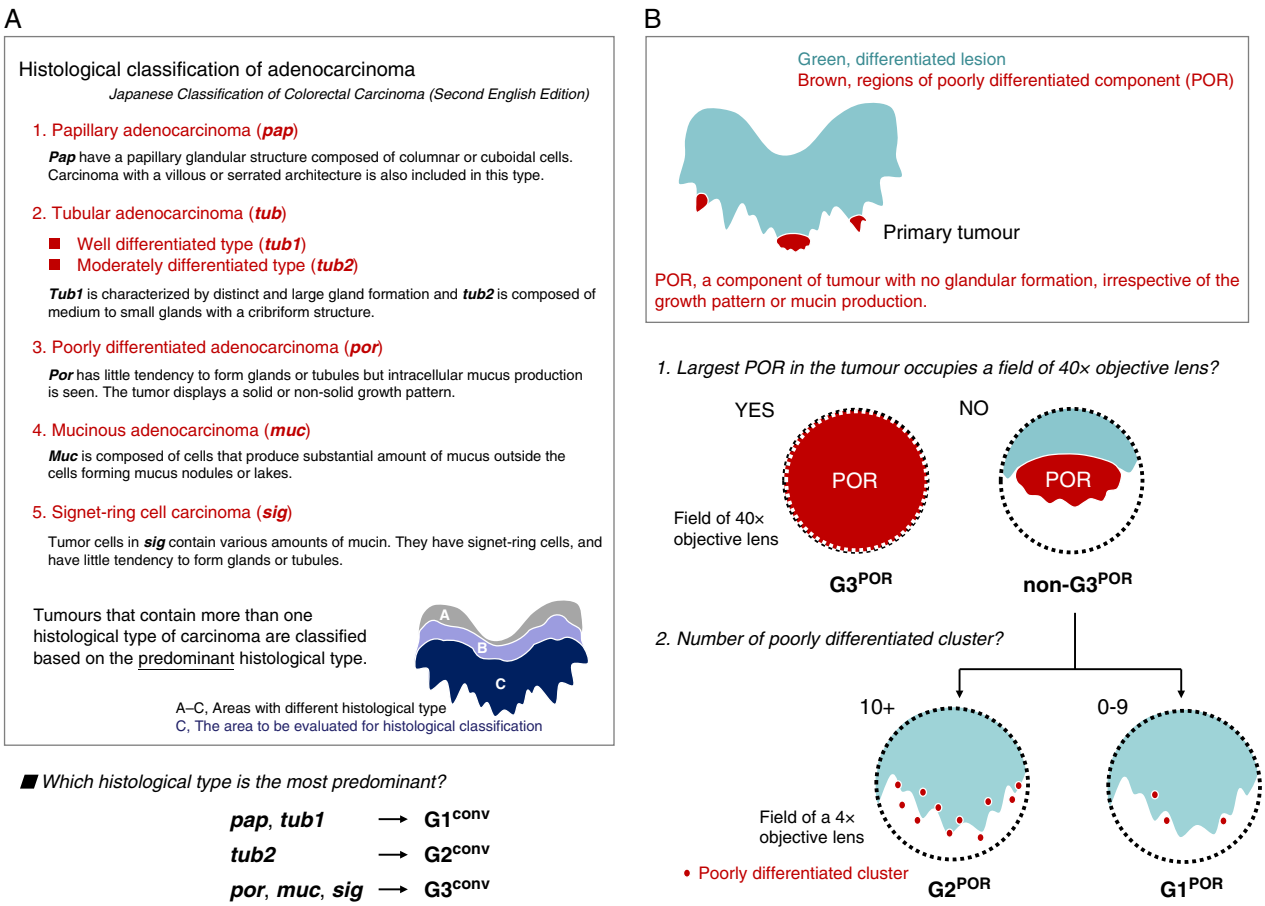

FIGURE 2. Schema for evaluation of Grade^{conv} and Grade^{POR}.[8] A, Adenocarcinomas in the colon are classified as papillary adenocarcinoma (*pap*), well-differentiated tubular adenocarcinoma (*tub1*), moderately differentiated tubular adenocarcinoma (*tub2*), poorly differentiated adenocarcinoma, mucinous adenocarcinoma (*muc*), or signet-ring cell carcinoma (*sig*) according to the definitions in the Japanese Classification of Colorectal Carcinoma.[15] For tumors that contain >1 histologic type of carcinoma, the diagnosis is based on the predominant finding.[15] Colon cancers have conventionally been categorized into 3 groups: G1^{conv} (*pap* and *tub1*), G2^{conv} (*tub2*), and G3^{conv} (*por*, *muc*, and *sig*). B, The POR was defined as an area composed of adenocarcinoma with no gland formation irrespective of the growth pattern or mucin production. To determine Grade^{POR}, the largest POR in the tumor was selected first with a low-power microscopic field, and whether this field filled the microscopic field of a ×40 objective lens (a field of 0.196 mm^2) was evaluated. Tumors that satisfied this condition were classified as G3^{POR}. When the largest POR did not fill the field of a ×40 objective lens, the tumors were classified as G1^{POR} (<10) or G2^{POR} (10 or more) according to the number of poorly differentiated cancer clusters (without a glandular structure composed of 5 or more cancer cells) in the microscopic field of a ×4 objective lens (a field of 196.250 mm^2) at its hotspot.

The largest POR of the tumor was first determined at low magnification (Fig. 2B). It was then determined whether this area had filled the microscopic field of a ×40 objective lens (a field of 0.196 mm^2). Tumors that satisfied this condition were regarded as G3^{POR} (Fig. 3). In order to classify patients for whom the largest POR did not fill the field of a ×40 objective lens, we focused on cancer clusters without glandular structure that were composed of 5 or more cancer cells (poorly differentiated clusters). After a field with poorly differentiated clusters with the highest frequency was identified, the clusters in the microscopic field of a ×4 objective lens (a field of 196.250 mm^2) were counted. Tumors with <10 clusters were regarded as G1^{POR}, whereas those with ≥10 clusters were regarded as G2^{POR}.

Tumor Budding

Tumor budding was defined as an isolated cancer cell or cluster of <5 cells at the invasive front and was graded according to the number of cells or clusters in the microscopic field of a ×20 objective lens (0.785 mm^2) in the hotspot.[16] Tumors with <5, 5 to 9, and ≥10 budding foci were classified as grades BD1, BD2, and BD3, respectively. These assessment criteria were subsequently adopted in the Japanese guidelines (2009)[17] and as international criteria by the International Tumour Budding Consensus Conference (ITBCC) in 2016.[18]

MSI Status

Macrodissected specimens prepared from formalin-fixed, paraffin-embedded blocks of the primary tumor that

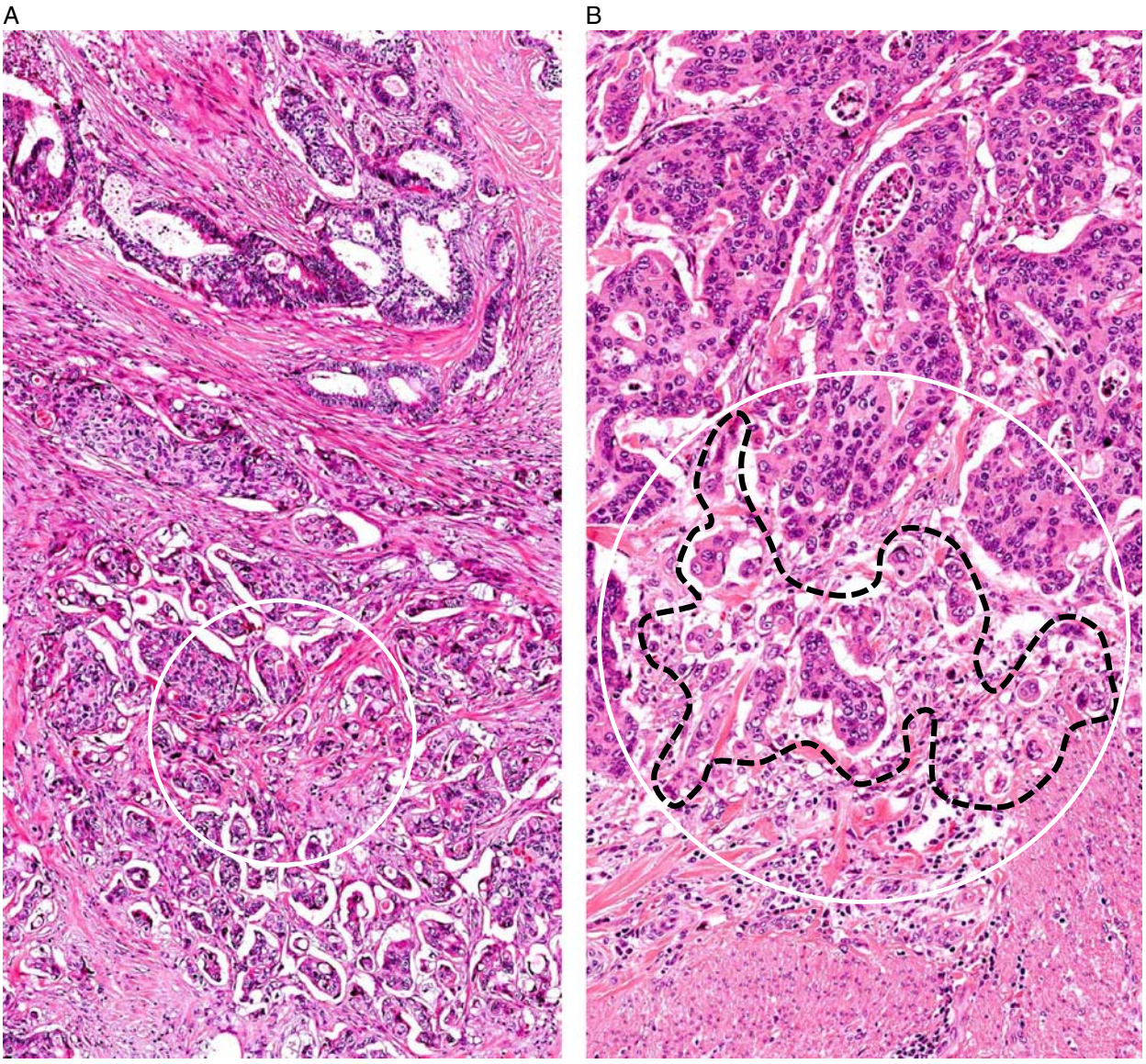

FIGURE 3. Definition of G3 based on the least differentiation policy with the ×40 objective lens rule.[8] A, The area of the POR observed at the tumor front is extensive enough to fully fill the microscopic field of a ×40 objective lens; thereby, this tumor is classified as $G3^{POR}$. B, Non-$G3^{POR}$. Note that the POR (area circled with black dotted line) does not fill the field of a ×40 objective lens. A and B, Hematoxylin and eosin staining. White circles indicate the microscopic field of a ×40 objective lens (a field of 0.196 mm^2).

were collected from each participating institution were used to extract the genomic DNA of individual tumors. MSI status was evaluated using 5 markers (BAT25, BAT26, D2S123, D5S346, and D17S250) in accordance with the international guidelines adopted by the National Cancer Institute collaborative meeting.[19] Analyses were performed using an ABI 3130 Genetic Analyser (Applied Biosystems, Carlsbad, CA) with GenoMapper Software, version 3.0 (www-archbac.u-psud.fr/Genomap/GenomapBrowser.html). MSI status was defined according to the number of positive markers as follows: that is, MSI-high, 2 or more; MSI-low, 1; and MSS, 0.

Statistical Analyses

Prognostic analyses were performed 5 years after the completion of patient registration. The endpoint definition in the SACURA trial is reported elsewhere.[11,12] DFS was defined as the time from randomization to recurrence, or the development of a secondary cancer or death, whichever occurred first. Secondary cancers included cancers that developed metachronously in the colorectum or other organs. In the SACURA trial, ~9% of the patients had secondary cancers, which comprised 40.7% of the DFS events.[12] RFS was defined as the time from randomization to first recurrence or death. In this validation analysis, we

selected RFS as an endpoint because RFS could be a more suitable outcome by which the clinical value of the prognostic factors is appraised.

Given that a part of poorly differentiated adenocarcinomas of the colon is MSI-high, of which the prognosis is generally favorable,[13,14] we provided the MSS/MSI-low subset for prognostic analyses to avoid underestimation of the prognostic value of the tumor grading systems. More specifically, after 69 patients with MSI-high tumors and 30 patients with tumors for which the MSI status could not be evaluated were excluded from the FAS, 892 patients with MSS/MSI-low tumors were assigned to the MSS/MSI-low subset (Fig. 1). In the MSS/MSI-low subset, the median follow-up period was 69.5 months, and the 5-year RFS rate for all patients was 83.5% (84.6% in the UFT group and 82.5% in the surgery-alone group).

To assess the correlation among the Grade^{POR}, clinicopathologic characteristics, and postoperative oncologic events, the Kruskal-Wallis test was used for continuous variables, and the χ^2 test was used for categorical variables. The RFS was estimated using the Kaplan-Meier method. The 95% CI at a specific time was estimated using Greenwood's formula, and comparisons of the RFS among groups were performed using the log-rank test. Univariate analyses using the Cox proportional hazards regression model were performed to calculate the HR and 95% CI values for the RFS of 14 prognostic factors, which included conventional factors (sex, age, tumor location, tumor size, number of lymph nodes examined, Grade^{conv}, T-stage, lymphatic and venous invasion, preoperative carcinoembryonic antigen level, treatment arm, and MSI status), tumor budding, and Grade^{POR}.

We performed multivariate analyses for the following 3 combinations of prognostic factors: (1) standard prognostic model including 8 prespecified, elemental prognostic factors (number of lymph nodes examined, tumor size, T-stage, lymphatic invasion, venous invasion, MSI status, treatment arm, and tumor budding), (2) standard model factors and Grade^{conv}, and (3) standard model factors and Grade^{POR}. The value of the addition of Grade^{conv} or Grade^{POR} to the standard model was estimated using the Wald test. Additional multivariate analyses using the Cox proportional hazards regression model were also performed as sensitivity analyses for other sets of prognostic factors.

We also evaluated G3^{POR} as a predictive factor to determine the treatment effect of adjuvant chemotherapy with UFT (rather than as a prognostic factor) using a test of treatment-by-grade interaction. All statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Grade^{POR} and Clinicopathologic Characteristics

On the basis of Grade^{POR}, 313 (31.6%), 526 (53.1%), and 152 (15.3%) tumors were classified as G1^{POR}, G2^{POR},

TABLE 1. Grade^{POR} and Other Clinicopathologic Characteristics (FAS)

Parameters	Category	Grade^{POR}, N (%)			*P*
		G1^{POR}	G2^{POR}	G3^{POR}	
Sex	Male	200 (63.9)	308 (58.6)	93 (61.2)	0.3060
	Female	113 (36.1)	218 (41.4)	59 (38.8)	
Age (y)		66.0	65.5	64.4	0.4833*
Tumor location	Right-sided colon	107 (34.2)	225 (42.8)	73 (48.0)	0.0195
	Left-sided colon	145 (46.3)	199 (37.8)	58 (38.2)	
	Rectosigmoid	61 (19.5)	102 (19.4)	21 (13.8)	
Maximum diameter (mm)		47.7	48.8	51.6	0.1075*
No. LNs examined	< 12	81 (25.9)	124 (23.6)	37 (24.3)	0.7538
	≥ 12	232 (74.1)	402 (76.4)	115 (75.7)	
Grade^{conv}	G1^{conv}	136 (43.5)	246 (46.8)	38 (25.0)	< 0.0001
	G2^{conv}	174 (55.6)	269 (51.1)	83 (54.6)	
	G3^{conv}	3 (1.0)	11 (2.1)	31 (20.4)	
T-stage	T3	288 (92.0)	423 (80.4)	112 (73.7)	< 0.0001
	T4	25 (8.0)	103 (19.6)	40 (26.3)	
Lymphatic invasion	Negative	158 (50.5)	213 (40·5)	45 (29.6)	0.0001
	Positive	155 (49.5)	313 (59.5)	107 (70.4)	
Venous invasion	Negative	124 (39.6)	199 (37.8)	63 (41.4)	0.6931
	Positive	189 (60.4)	327 (62·2)	89 (58·6)	
Preoperative CEA (ng/mL)	≤ 5·0	233 (74.4)	338 (64.3)	99 (65.1)	0.0262
	> 5·0	66 (21.1)	165 (31.4)	45 (29.6)	
	Not available	14 (4.5)	23 (4.4)	8 (5.3)	
Microsatellite status	MSI-high	9 (2.9)	27 (5.1)	33 (21·7)	< 0.0001
	MSS/MSI-low	295 (94.2)	483 (91.8)	114 (75.0)	
	Not available	9 (2.9)	16 (3.0)	5 (3·3)	
Tumor budding	BD1	273 (87.2)	82 (15.6)	21 (13.8)	< 0.0001
	BD2	35 (11.2)	273 (51.9)	23 (15.1)	
	BD3	5 (1.6)	171 (32.5)	108 (71.1)	

*Kruskal-Wallis test.
CEA indicates carcinoembryonic antigen; LN, lymph node.

and $G3^{POR}$, respectively. The proportion of right-sided tumors, higher $Grade^{conv}$ tumors, T4 tumors, those with positive lymphatic invasion, those with preoperative serum carcinoembryonic antigen values >5.0 ng/mL, and tumors with a higher tumor budding grade were increased with $Grade^{POR}$ ($P<0.0001$ to 0.03; Table 1). Although a significant positive correlation between $Grade^{conv}$ and $Grade^{POR}$ was observed, the number of tumors classified

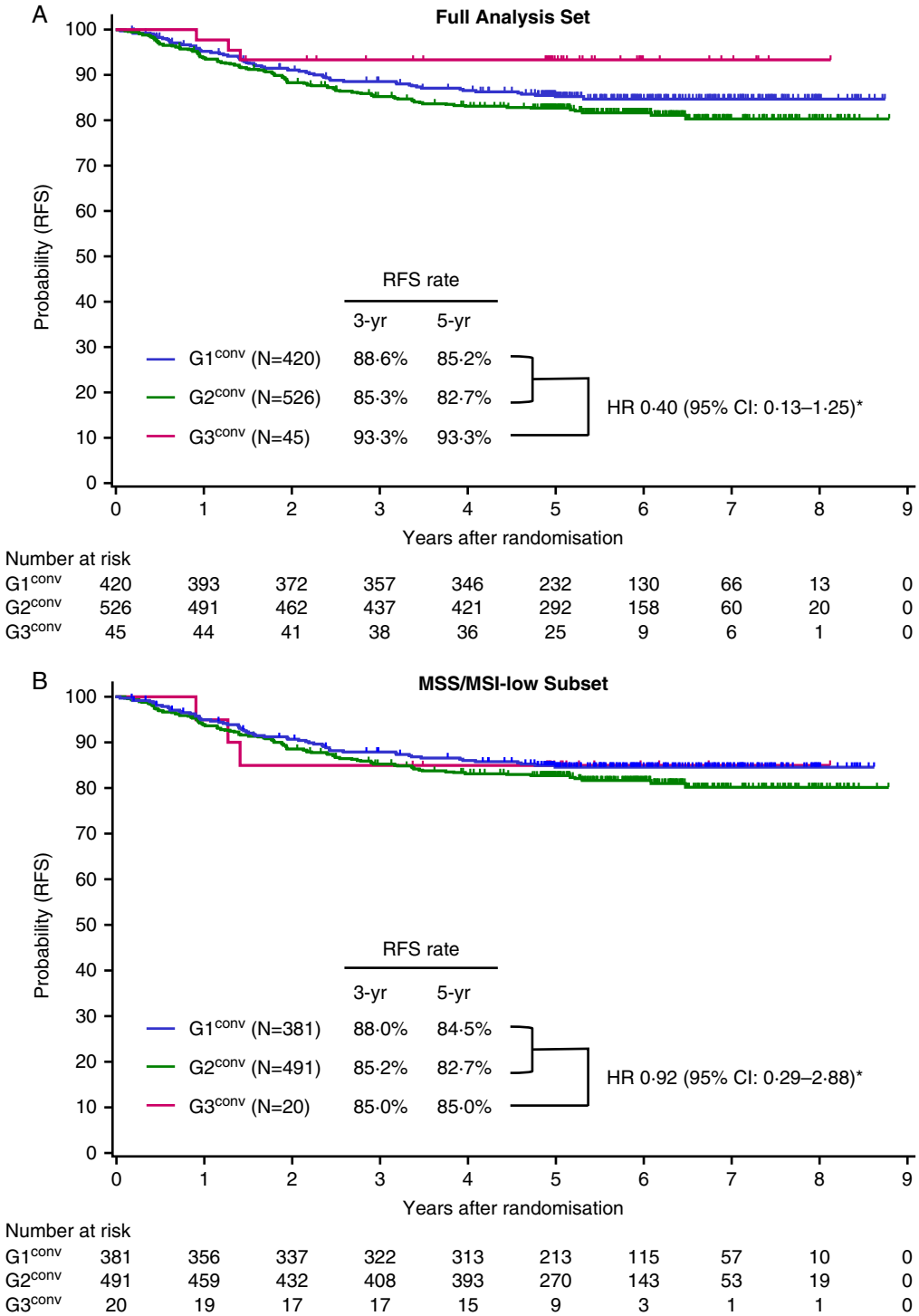

FIGURE 4. Kaplan-Meier estimates of the RFS rate in patients with stage II colon cancer based on the conventional tumor grade, which was based $Grade^{conv}$. Log-rank test: A, $P=0.1003$. B, $P=0.5089$. *The HR for $G3^{conv}$ (reference, $G1^{conv}$+$G2^{conv}$), the corresponding 95% CI, and the *P*-value were calculated.

as $G3^{POR}$ was >3 times higher than that classified as $G3^{conv}$, and ~30% of $G3^{conv}$ tumors were classified as other $Grade^{POR}$ tumors (non-$G3^{POR}$ tumors). The proportion of MSI-high in 961 cases with values of MSI status was 22.5% for $G3^{POR}$ tumors, 5.3% for $G2^{POR}$, and 3.0% for $G1^{POR}$ tumors ($P<0.0001$). No significant correlation was

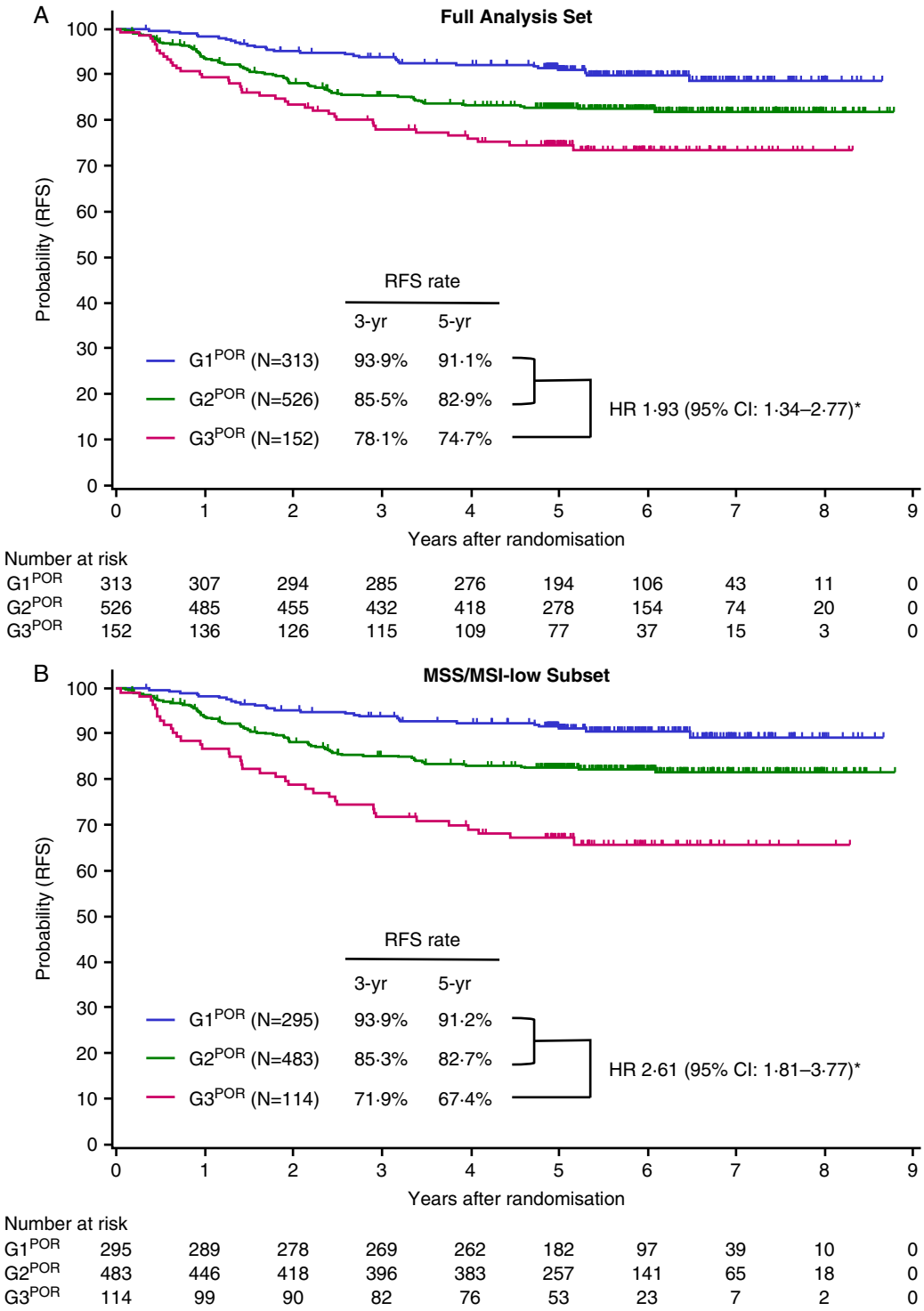

FIGURE 5. Kaplan-Meier estimates of the RFS rate in patients with stage II colon cancer based on the tumor grade, which was based on $Grade^{POR}$. Log-rank test: A, $P<0.0001$. B, $P<0.0001$. *The HR for $G3^{POR}$ (reference, $G1^{POR}$+$G2^{POR}$), the corresponding 95% CI, and the *P*-value were calculated.

observed between Grade^{POR} and the conventional factors of tumor diameter, number of lymph nodes examined, and venous invasion.

Grade^{conv} and Relapse-free Survival

In the FAS, the RFS rate was better in patients with G3^{conv} tumors, as compared with that in patients with non-G3^{conv} tumors (Fig. 4A), although the difference was not statistically significant (HR, 0.40; 95% CI: 1.81-3.77). The MSS/MSI-low subset included only 20 patients with G3^{conv} tumors, and their 5-year RFS rate (85.0%) was comparable to that of patients with G1^{conv} tumors (85.2%) and those with G2^{conv} tumors (82.7%; Fig. 4B).

Grade^{POR} and Relapse-free Survival

In the FAS, based on Grade^{POR}, the 5-year RFS rate was 91.1% (95% CI, 87.2%-93.8%), 82.9% (79.4%-85.9%), and 74.7% (66.9%-80.9%) for G1^{POR}, G2^{POR}, and G3^{POR} tumors, respectively (Fig. 5A). The significant impact of Grade^{POR} on the RFS was similarly observed in the MSS/MSI-low subset (Fig. 5B), in which 295 (33.1%), 483 (54.1%), and 114 (12.8%) tumors were classified as G1^{POR}, G2^{POR}, and G3^{POR}, respectively. The gap in the 5-year RFS rates between the G3^{POR} group and the non-G3^{POR} group tended to be greater in the MSS/MSI-low subset. The HR of the RFS of the G3^{POR} vs. non-G3^{POR} group was 1.93 (95% CI: 1.34-2.77) in the FAS, whereas it was 2.61 (95% CI: 1.81-3.77) in the MSS/MSI-low subset.

Adjustment for the Effect of Standard Prognostic Factors on RFS

In the FAS, among the 10 prespecified prognostic factors, T-stage, MSI status, tumor budding, and Grade^{POR} were correlated with the RFS according to the univariate analysis, which was performed using the Cox proportional hazards regression model (Table 2). Table 3 shows the results of the multivariate analysis of prognostic factors for RFS in the FAS. When G3^{conv} and G3^{POR} were alternatively added to the standard model consisting of 8 conventional prognostic factors, only G3^{POR} was a statistically significant factor that could determine the RFS (HR, 1.54; 95% CI: 1.02-2.33, $P = 0.040$). A similar observation was made in the MSS/MSI-low subset in which G3^{POR}, rather than G3^{conv}, significantly affected the RFS (HR, 1.63; 95% CI: 1.08-2.47, $P = 0.022$; Table 4).

Supplementary Tables 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/B5) and 2 (Supplemental Digital Content 2, http://links.lww.com/PAS/B6) show the results of the sensitivity analysis with another set of combined prognostic factors for the FAS and the MSS/MSI-low subset. The results were similar in terms of Grade^{POR}, which was selected as an independent factor for RFS.

Grade^{POR}, Recurrence Rate, and Recurrence Pattern (First Relapse Organs)

The recurrence rates during the study period were 6.7%, 14.3%, and 23.0% for the G1^{POR}, G2^{POR}, and G3^{POR} groups, respectively ($P < 0.0001$; Table 5). The rates ranged

TABLE 2. Univariate Analyses of Prespecified Variables for RFS Using the Cox Proportional Hazards Regression Model

		FAS				MSS/MSI-low Subset			
Parameters	**Category**	**N**	**5-Year RFS (%)**	**HR (95% CI)**	***P***	**N**	**5-Year RFS (%)**	**HR (95% CI)**	***P***
No. lymph nodes examined	≥ 12	749	84.8	1		672	84.0	1	
	< 12	242	82.4	1.26 (0.90-1.78)	0.180	220	82.0	1.20 (0.84-1.72)	0.313
Size of tumor	< 50 mm	529	85.4	1		486	85.2	1	
	≥ 50 mm	462	82.9	1.24 (0·91-1.72)	0.171	406	81.6	1.34 (0.97-1.85)	0.072
T-stage	T3	823	87.4	1		737	87.0	1	
	T4	168	68.4	2.76 (1.98-3.84)	< 0.001	155	67.0	2.83 (2.01-3.97)	< 0.001
Lymphatic invasion	Negative	416	85.3	1		378	84.6	1	
	Positive	575	83.5	1.10 (0.80-1.51)	0.568	514	82.8	1.11 (0.80-1.54)	0.531
Venous invasion	Negative	386	86.3	1		333	85.0	1	
	Positive	605	82.9	1.29 (0.93-1.80)	0.129	559	82.7	1.18 (0.84-1.66)	0.346
MSI status*	MSS/MSI-low	892	83.5	1		—	—	—	—
	MSI-high	69	94.0	0.33 (0.12-0.90)	0.030	—	—	—	—
Treatment arm	Surgery-alone	501	83.2	1		449	82.5	1	
	UFT	490	85.3	0.85 (0.62-1.16)	0.309	443	84.6	0.86 (0.62-1.18)	0.344
Tumor budding	BD1	376	90.9	1		340	90.6	1	
	BD2	331	85.1	1.58 (1.03-2.42)	0.035	300	84.6	1.61 (1.04-2.52)	0.034
	BD3	284	74.4	2.93 (1.97-4.36)	< 0.001	252	72.8	3.12 (2.07-4.70)	< 0.001
Grade^{conv}	G1^{conv}	420	85.2	1		381	84.5	1	
	G2^{conv}	526	82.7	1.25 (0.91-1.73)	0.173	491	82.7	1.22 (0.87-1.70)	0.250
	G3^{conv}	45	93.3	0.45 (0.14-1.44)	0.181	20	85.0	1.03 (0.32-3.28)	0.963
	Non-G3^{conv}	946	83.9	1		872	83.5	1	
	G3^{conv}	45	93.3	0.40 (0.13-1.25)	0.117	20	85.0	0.92 (0.29-2.88)	0.882
Grade^{POR}	G1^{POR}	313	91.1	1		295	91.2	1	
	G2^{POR}	526	82.9	1.91 (1.27-2.89)	0.002	483	82.7	2.04 (1.32-3.14)	0.001
	G3^{POR}	152	74.7	3.00 (1.87-4.83)	< 0.001	114	67.4	4.25 (2.59-6.96)	< 0.001
	Non-G3^{POR}	839	86.0	1		778	85.9	1	
	G3^{POR}	152	74.7	1.93 (1.34-2.77)	< 0.001	114	67.4	2.61 (1.81-3.77)	< 0.001

*961 cases with values of MSI status were analyzed.

TABLE 3. Multivariate Analyses of 8 Prespecified Variables and Tumor Grades ($Grade^{conv}$ and $Grade^{POR}$) for RFS Using the Cox Proportional Hazards Regression Model in the FAS

		Standard Model (Prespecified 8 Factors)		Tested Model 1 (8 Factors and $Grade^{conv}$)		Tested Model 2 (8 Factors and $Grade^{POR}$)	
Parameters	**Category**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
No. lymph nodes examined	≥12	1		1		1	
	<12	1.30 (0.91-1.87)	0.155	1.30 (0.9-1.87)	0.164	1.30 (0.90-1.87)	0.165
Size of tumor	<50 mm	1		1		1	
	≥50 mm	1.39 (1.00-1.93)	0.052	1.40 (1.01-1.95)	0.046	1.35 (0.97-1.88)	0.075
T-stage	T3	1		1		1	
	T4	2.40 (1.70-3.39)	<0.001	2.43 (1.71-3.44)	<0.001	2.33 (1.65-3.30)	<0.001
Lymphatic invasion	Negative	1		1		1	
	Positive	0.91 (0.65-1.28)	0.595	0.92 (0.66-1.29)	0.633	0.90 (0.65-1.26)	0.551
Venous invasion	Negative	1		1		1	
	Positive	1.13 (0.80-1.59)	0.502	1.11 (0.78-1.56)	0.573	1.16 (0.82-1.64)	0.410
MSI status	MSS/MSI-low	1		1		1	
	MSI-high	0.31 (0.11-0.83)	0.020	0.38 (0.14-1.09)	0.071	0.27 (0.10-0.73)	0.010
Treatment arm	Surgery-alone	1		1		1	
	UFT	0.86 (0.63-1.19)	0.358	0.85 (0.62-1.17)	0.321	0.85 (0.62-1.17)	0.308
Tumor budding	BD1	1		1		1	
	BD2	1.53 (0.98-2.38)	0.059	1.51 (0.97-2.35)	0.067	1.34 (0.77-2.35)	0.302
	BD3	2.77 (1.82-4.21)	<0.001	2.71 (1.78-4.13)	<0.001	2.09 (1.18-3.70)	<0.001
$Grade^{conv}$	G1	—	—	1		—	—
	G2	—	—	1.16 (0.84-1.62)	0.370	—	—
	G3	—	—	0.57 (0.17-1.91)	0.363	—	—
$Grade^{POR}$	G1	—	—	—	—	1	
	G2	—	—	—	—	1.23 (0.69-2.20)	0.481
	G3	—	—	—	—	1.86 (0.96-3.62)	0.068
Test to investigate the addition of $Grade^{conv}$ or $Grade^{POR}$ to standard model*							
Wald test for ordered grade ($df=1$)		—		1.05 (0.78-1.40)	0.763	1.40 (1.02-1.93)	0.036
Wald test for grade with non-G3/G3 ($df=1$)		—		0.53 (0.16-1.75)	0.296	1.54 (1.02-2.33)	0.040

*Estimated model was constructed with all variables included in the standard model and a tumor grading system ($Grade^{conv}$ or $Grade^{POR}$).

more widely in the MSS/MSI-low subset (from 6.4% to 29.8%), in which the 3-tiered $Grade^{POR}$ was significantly associated with the incidence of recurrence in the liver, lung, lymph node, and peritoneum.

Impact of Adjuvant Chemotherapy on RFS According to the $Grade^{POR}$

In the interaction analysis, the treatment effect of UFT was not significantly different between the $G3^{POR}$ and non-$G3^{POR}$ groups in the FAS ($P=0.247$) and in the MSS/MSI-low subset ($P=0.349$), but we observed a greater difference in the 5-year RFS rate between the UFT group and the surgery-alone group in patients with $G3^{POR}$ tumors in the MSS/MSI-low subset. More specifically, the RFS was quite similar between the UFT group and surgery-alone group in patients with non-$G3^{POR}$ tumors (HR, 0.92; 95% CI: 0.63-1.24; Fig. 6A), whereas the 5-year RFS rate was better by ~9% in the UFT group than in the surgery-alone group in patients with $G3^{POR}$ tumors (HR, 0.65; 95% CI: 0.34-1.24; Fig. 6B).

DISCUSSION

Even now, the method of tumor grading is not unified worldwide. Grading differs depending on the pathologic reporting protocols, and there is some debate whether grading should be based on the predominant pattern of differentiation[20] or the area of least differentiation.[21] In contrast from these reporting protocols, the guidelines of the College of American Pathologists (CAP)[7] have adopted the World Health Organization (WHO) classification, which defines the grade based on the percentage of gland formation (>95% as G1, 5% to 95% as G2, and <50% as G3).[22] None of these grading methods have been fully validated in terms of their effectiveness in the prognostic stratification in stage II colon cancer.

Although it may be reasonable to use the representative histologic findings to determine the diagnostic designation of the tumor, the predominant differentiation policy lacks the scientific evidence to support its prognostic value.[23] On the contrary, the least differentiation policy would be logical in terms of what determines the patients' oncologic outcome, which would be the absolute quantity of the cancer component with highly malignant potential rather than the percentage of the component in the whole tumor. However, the extent of the poorly differentiated area to be judged as G3 has not been specified, and the distinction between G3 and non-G3 has been left to the discretion of the pathologist; therefore, grading is largely subjective and uncertain.[6] Given these backgrounds, we expect the results of the present study to address the stalemate associated with such issues with the G3 criteria.

According to a previous study that investigated the prognostic impact of the extent of POR, the area of POR

TABLE 4. Multivariate Analyses of 7 Prespecified Variables and Tumor Grades ($Grade^{conv}$ and $Grade^{POR}$) for RFS Using the Cox Proportional Hazards Regression Model in the MSS/MSI-low Subset

		Standard Model (Prespecified 7 Factors)		Tested Model 1 (7 Factors and $Grade^{conv}$)		Tested Model 2 (7 Factors and $Grade^{POR}$)	
Parameters	**Category**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
No. lymph nodes examined	≥ 12	1		1		1	
	< 12	1.35 (0.94-1.95)	0.110	1.34 (0.93-1.93)	0.118	1.35 (0.93-1.94)	0.114
Size of tumor	< 50 mm	1		1		1	
	≥ 50 mm	1.45 (1.04-2·02)	0.031	1.45 (1.04-2.02)	0.029	1.40 (1.00-1.96)	0.048
T-stage	T3	1		1		1	
	T4	2.34 (1.65-3.32)	< 0.001	2.36 (1.66-3.36)	< 0.001	2.25 (1.58-3.20)	< 0.001
Lymphatic invasion	Negative	1		1		1	
	Positive	0.91 (0.65-1.27)	0.562	0.91 (0.65-1.28)	0.598	0.90 (0.64-1.26)	0.531
Venous invasion	Negative	1		1		1	
	Positive	1.11 (0.78-1.57)	0.576	1.09 (0·77-1.55)	0.628	1.14 (0.80-1.62)	0.465
Treatment arm	Surgery-alone	1		1		1	
	UFT	0.87 (0.63-1.20)	0.381	0.86 (0.62-1.19)	0.360	0.85 (0.61-1.17)	0.323
Tumor budding	BD1	1		1		1	
	BD2	1.55 (0.99-2.42)	0.057	1.54 (0.98-2.41)	0.059	1.31 (0.74-2.31)	0.352
	BD3	2.83 (1.85-4.34)	< 0.001	2.78 (1.81-4.27)	< 0.001	2.01 (1.12-3.61)	0.019
$Grade^{conv}$	G1	—	—	1		—	—
	G2	—	—	1.19 (0.85-1.66)	0.320	—	—
	G3	—	—	0.90 (0.28-2.89)	0.858	—	—
$Grade^{POR}$	G1	—	—	—	—	1	
	G2	—	—	—	—	1.30 (0.72-2.35)	0.385
	G3	—	—	—	—	2.07 (1.05-4.07)	0.036
Test to investigate the addition of $Grade^{conv}$ or $Grade^{POR}$ to the standard model*							
Wald test for ordered grade ($df = 1$)		—		1.12 (0.83-1.52)	0.447	1.48 (1.07-2.04)	0.017
Wald test for grade with non-G3/G3 ($df = 1$)		—		0.82 (0.26-2.57)	0.727	1.63 (1.08-2.47)	0.022

*Estimated model was constructed with all variables included in the standard model and a tumor grading system ($Grade^{conv}$ or $Grade^{POR}$).

corresponding to the full microscopic field of a ×40 objective lens, rather than that of a ×20 or ×10 objective lens and 50% of the tumor area, was the significant factor that influenced the survival outcome of patients with stage I to III CRC.[8] In the population studied in the SACURA trial, the prognostic value of POR based on the ×40 objective lens rule was prospectively validated, and its impact on RFS was compared with that of the predominant differentiation policy. Consequently, a Cox proportional hazard model with other prespecified factors showed that $G3^{POR}$, but not $G3^{conv}$, was an independent adverse prognostic factor for stage II colon cancer; this result was further verified by other sensitivity analyses. Specifically, only 5% of stage II tumors met the diagnostic criteria for $G3^{conv}$ in the FAS, and, crucially, G3 determined by the predominant differentiation policy was not so much an adverse factor but was more likely to be a favorable factor in terms of its effect on the RFS (HR, 0.40; 95% CI: 0.13-1.25). In contrast, ∼15% of stage II tumors were classified as $G3^{POR}$, which was able to effectively select the patient population with unfavorable outcomes, that is, the population with an HR of 1.93 (95% CI: 1.34-2.77).

Apart from the robustness of the prognostic value of the ×40 objective lens field rule as the criterion of tumor grade, some important findings were presented in the current study. First, tumor grading based on the extent of a poorly differentiated lesion was improved by including

TABLE 5. $Grade^{POR}$ and the Incidence of Postoperative Oncologic Events

		$Grade^{POR}$, N (%)			
Data Set	**Events**	**$G1^{POR}$**	**$G2^{POR}$**	**$G3^{POR}$**	***P***
FAS	Recurrence	21 (6.7)	75 (14.3)	35 (23.0)	< 0.0001
	Liver	14 (4.5)	31 (5.9)	15 (9.9)	
	Lungs	5 (1.6)	22 (4.2)	10 (6.6)	
	Lymph node	1 (0.3)	10 (1.9)	6 (3.9)	
	Peritoneum	1 (0.3)	12 (2.3)	6 (3.9)	
	Local	3 (1.0)	14 (2.7)	4 (2.6)	
	Secondary malignancy	36 (11.5)	39 (7.4)	12 (7.9)	0.1183
MSS/MSI-low subset	Recurrence	19 (6.4)	71 (14.7)	34 (29.8)	< 0.0001
	Liver	12 (4.1)	30 (6.2)	15 (13.2)	
	Lungs	5 (1.7)	21 (4.3)	10 (8.8)	
	Lymph node	1 (0.3)	10 (2.1)	6 (5.3)	
	Peritoneum	1 (0.3)	12 (2.5)	5 (4.4)	
	Local	3 (1.0)	12 (2.5)	4 (3.5)	
	Secondary malignancy	33 (11.2)	35 (7.2)	8 (7.0)	0.1336

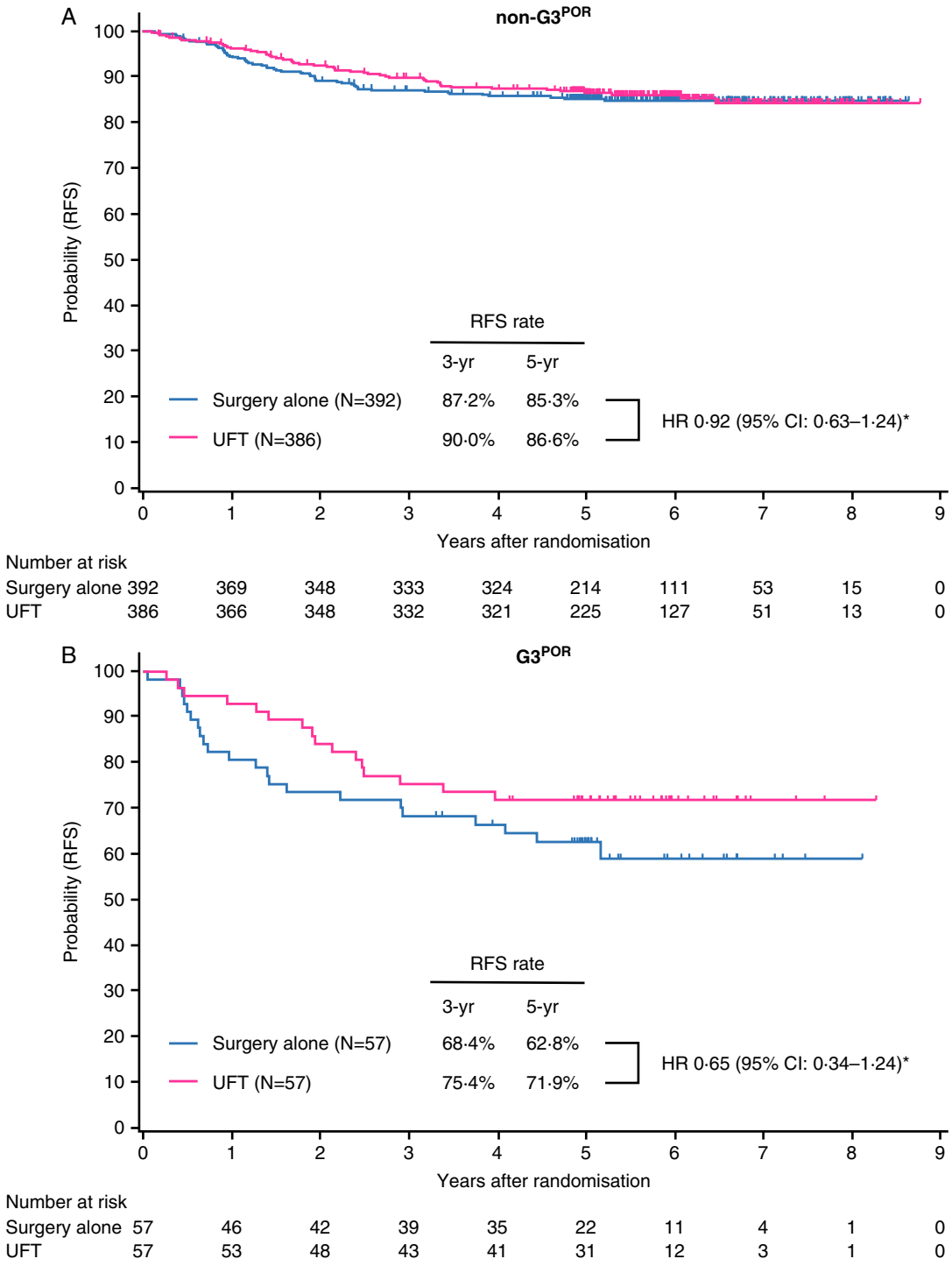

FIGURE 6. Comparison of Kaplan-Meier estimates of the RFS rate between the surgery-alone group and the chemotherapy group in the MSS/MSI-low subset. Log-rank test: A, $P=0.6595$. B, $P=0.1889$. *The HR for UFT (reference, surgery alone), the corresponding 95% CI, and the *P*-value were calculated.

the MSI status. More specifically, by excluding MSI-high tumors from tumors classified as $G3^{POR}$, the HR of the RFS rate of $G3^{POR}$ compared with that of non-$G3^{POR}$ tumors increased from 1.93 to 2.61, which indicates the improved value of the tumor differentiation grade. This result suggests that poorly differentiated colonic carcinoma with no glandular structures constitutes 2 different entities, which markedly differ in their genotype and

prognosis,[13] and that MSI-high tumors should be treated separately even if they are poorly differentiated, as stated in the NCCN guidelines.[4] Unfortunately, in Japan, MSI testing for patients with early-stage CRC is not currently covered by the national health insurance program, but this situation is expected to change so that clinicians can more accurately determine the indication of adjuvant chemotherapy for patients with stage II colon cancer based on the MSI status of the tumor.

Second, the study results showed that $G3^{POR}$ affected survival results independently of tumor budding, an important prognostic determinant for patients included in the SACURA trial.[16] The ITBCC2016 concluded that tumor budding is not the same as tumor grade,[18] which was originally derived from the differential pathologic characteristics, but the results of our prognostic analyses strongly support the conclusion of the ITBCC2016 in terms of its independent impact on survival. We believe both tumor grade and tumor budding are important factors to be recorded in pathology reports as factors for clinicians to consider when selecting an appropriate treatment regimen for colon cancer.

Third, the present study may be the first to prospectively evaluate the predictive value of tumor differentiation grade exclusively for stage II colon cancer. Consequently, the RFS results were quite similar regardless of the administration of UFT adjuvant chemotherapy for 1 year in patients with non-$G3^{POR}$ tumors, whereas adjuvant chemotherapy improved the 5-year RFS rate by ~9% in patients with $G3^{POR}$ tumors (63% in the surgery-alone group and 72% in the UFT group). The *P*-values for interaction were statistically insignificant, which is likely due to the lack of statistical power caused by the sample size, as this was not predetermined to clarify the effectiveness of adjuvant chemotherapy in patients with a high risk of recurrence. However, the results of our randomized controlled study suggest that the benefit derived from adjuvant therapy would differ between patients with $G3^{POR}$ tumors and those with non-$G3^{POR}$ tumors and that the reduction in recurrence with adjuvant chemotherapy is highly expected in patients with $G3^{POR}$ tumors. Future studies are needed to clarify the definite value of the current global-standard adjuvant chemotherapy, such as 5-fluorouracil/leucovorin, capecitabine, and oxaliplatin-based regimens, against $G3^{POR}$ tumors.

Multivariate analyses in the present study demonstrated that the distinction between $G1^{POR}$ and $G2^{POR}$ tumors, as determined using the cutoff of 10 poorly differentiated clusters in a ×4 objective lens field, had no independent prognostic value, although a significant difference in survival was observed by univariate analysis. A poorly differentiated cluster is a histologic feature that predominantly appears at the leading front of the tumor, thereby indicating its possible relevance to epithelial-mesenchymal transition, likely as tumor budding.[24] A 3-tiered grading system with 2 cutoff values of clusters at the hotspot in a microscopic field of a ×20 objective lens (5 and 10) is an alternative method by which poorly differentiated clusters are evaluated.[25] More specifically, this is the method where the framework of the tumor budding grading method would be adopted, which has been used in the Japanese guidelines since 2009[17] and was thereafter accepted as the standard of budding evaluation criteria at the ITBCC2016.[18] Since the first report in 2012,[25] an increasing number of studies has shown the prognostic value of this grading scheme,[26–28] thereby indicating that this alternative method is likely more appropriate for the evaluation of poorly differentiated clusters, rather than the method adopted in the present study. Future studies should aim to optimize the selection of patients at a high risk of recurrence who would benefit from chemotherapy on the basis of comprehensive assessment of new risk factors, including $G3^{POR}$, a high grade of budding, and the presence of poorly differentiated clusters.

A poorly differentiated phenotype is conventionally defined as a tumor with "highly irregular glands or loss of glandular differentiation and loss of nuclear polarity" (Association of Directors of Anatomic and Surgical Pathology)[29] or a tumor that is "either irregularly folded, distorted and often small tubules or the absence of any tubular formation" (The Royal College of Pathologists).[20] Because these subjective and multifactorial criteria may cause increased interobserver disagreement in the grading process,[30] we provided a simple definition for POR in 2008 as "an area composed of adenocarcinoma with no gland formation,"[8] which is expected to decrease the ambiguity of judgment. Consequently, $G3^{POR}$, which is characterized by a simple definition for POR and the least differentiation policy with the ×40 objective lens rule, is expected to have an advantage in the objectiveness of the criteria compared with G3 defined by the UICC/TNM or the WHO classification. The robust prognostic value of $G3^{POR}$ validated in the present study is promising for future global initiatives to solve the long-pending issue of the lack of unified tumor grading criteria.

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