Received: 27 September 2017 | Revised: 11 January 2018 | Accepted: 13 January 2018

DOI: 10.1111/cas.13521

ORIGINAL ARTICLE

WILEY Cancer Science

Area of residual tumor is a robust prognostic marker for patients with rectal cancer undergoing preoperative therapy

Naoki Sakuyama[1] | Motohiro Kojima[2] | Shingo Kawano[1] | Yoko Matsuda[3] | Mari Mino-Kenudson[4] | Atsushi Ochiai[2] | Masaaki Ito[1]

[1]Department of Colorectal and Pelvic Surgery, National Cancer Center Hospital East, Chiba, Japan

[2]Division of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, Japan

[3]Department of Pathology, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan

[4]Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

Correspondence
Motohiro Kojima, Division of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, Japan.
Email: mokojima@east.ncc.go.jp

Funding information
Ministry of Health, Labor and Welfare of Japan.

The aim of this study was to elucidate differences in the histological features of rectal cancer between patients treated with preoperative chemoradiotherapy and those treated with preoperative chemotherapy. Area of residual tumor (ART) was also evaluated for its utility as a potential prognostic marker between them. Sixty-eight patients with rectal cancer who underwent sphincter-saving surgery were enrolled in this study. Of these, 39 patients received preoperative chemoradiotherapy (CRT group) and 29 patients received preoperative (neoadjuvant) chemotherapy (NAC group). Area of residual tumor was determined by using morphometric software. Tumors in the two groups were compared for differences in their histological features and clinical outcomes. Tumors in the CRT and NAC groups varied greatly with regard to their histological features after preoperative therapy. Tumors in the CRT group showed more marked fibrosis than those in the NAC group. The total ART were significantly smaller in tumors in the CRT group than those in the NAC group. However, in circumferential resection margin-negative pathologic stage 0-III cases, clinical outcomes were not statistically different between the CRT and NAC groups. Both ART and pathologic TNM classification were associated with clinical outcome in preoperative CRT and NAC groups, but Dworak regression grade and fibrotic change were not. Tumors in those undergoing preoperative CRT and NAC were shown to differ significantly in their histological features. Area of residual tumor-based assessment may provide useful prognostic information, regardless of preoperative therapy.

KEYWORDS
area of residual tumor, neoadjuvant chemotherapy, pathological assessment, preoperative chemoradiotherapy, rectal cancer

1 | INTRODUCTION

Surgical resection after preoperative CRT represents the global standard in advanced rectal cancer.[1-3] It is reported that CRT is associated with anal dysfunction, while it improves post-resection local control.[4-6]

Abbreviations: ART, area of residual tumor; BM-ART, ART beyond the muscular layer with perirectal adipose tissue; CRM, circumferential resection margin; CRT, chemoradiotherapy; DFS, disease-free survival; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; NAC, neoadjuvant chemotherapy; ROC, receiver operating characteristic; T-ART, total ART; TRG, tumor regression grade; WM-ART, ART within the muscular layer; ypN, pathologic N stage; ypT, pathologic T stage.

Given that preoperative NAC could represent a potential alternative to CRT and provide better local control without anal dysfunction,[7,8] NAC with FOLFOX was attempted and a detailed pathological assessment was compared patients undergoing NAC with those undergoing other preoperative therapy, to provide information on how preoperative therapies differ in their histological effects. Next, the effect of preoperative therapy was pathologically evaluated in terms of TRG according to the Dworak or CAP criteria, although TRG remains yet to be standardized.[9,10] We have reported the utility of measuring ART in gastric, lung, and rectal cancer.[11-13] However, no study has evaluated the availability of these assessment methods for various therapeutic regimens.

In this study, therefore, tumors from patients undergoing preoperative CRT vs those undergoing NAC[14] were compared for their histologic and clinicopathologic features. Histologic assessments reported after preoperative CRT and NAC were evaluated to investigate whether or not one single assessment may be applicable across a variety of preoperative therapies.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical data collection

This study was approved by the institutional review board of the National Cancer Center Hospital East, Chiba, Japan (2012-067, 2015-013). From January 2001 to April 2014, a total of 2184 patients underwent surgery for rectal cancer at the National Cancer Center Hospital East. Of these, this study included a total of 39 pStage I-III, and CRM-negative patients who had undergone preoperative CRT (5-fluorauracil and radiation with a total dose of 45 Gy in 25 fractions) and surgical resection. All of these patients underwent surgery 4-6 weeks after the completion of preoperative CRT. Another 29 pStage I-III and CRM-negative patients who underwent preoperative NAC (six courses of FOLFOX) and surgical resection were also entered. All of these patients had undergone surgery during the 4-8 weeks after the completion of preoperative NAC. Patients undergoing preoperative CRT and those undergoing NAC were included from 2001 to 2006 and from 2010 to 2014, respectively. Preoperative clinical staging was carried out before and after preoperative therapy using the 7th UICC classification and staging system.

2.2 | Histologic assessment

All resected surgical specimens were fixed in 10% formalin. Tumor tissue was serially sliced in 5-mm longitudinal sections. Hematoxylin-eosin-stained sections were evaluated by two independent reviewers (M.K. and N.S.) who were blinded to their associated clinical findings. Discrepancies between their findings were resolved by discussion. All residual tumor was pathologically staged according to the UICC system. In the present study, any ypT reduced from clinical T stage and any ypN reduced from clinical N stage was regarded as down-staging. Histological TRG was semiquantitatively evaluated according to the method described by Dworak et al., in which grades are defined as follows: grade 1, dominant tumor mass with obvious fibrosis and/or vasculopathy; grade 2, dominant fibrotic changes with few tumor cells or groups (easy to find); grade 3, very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance; and grade 4, no tumor cells, only fibrotic mass (total regression or response).[9]

All tumors were examined for vascular, lymphovascular, and perineural invasion. To assess any histological alteration after therapy, all tumors were evaluated for the presence or absence of mucus lakes. Tumors in which the mucus lakes constituted <10% of the entire tumor area were classified as grade A. Grades B and C reflected mucus lakes of 10%-30% and >30% of the tumor area, respectively.[15,16] Tumor budding was defined as an isolated single cancer cell or a cluster composed of <5 cancer cells. After choosing one field where budding was the most intensive, a budding count was made in the field measuring 0.785 mm^2 using a ×20 objective lens. The presence of ≥5 buds per field was considered positive.[17]

Tumor differentiation in an initial biopsy specimen before preoperative treatment was reviewed and classified as low-grade (poorly differentiated) or high-grade (well- to moderately differentiated) adenocarcinomas, or no grade if prominent tumor regression (ie, prominent colloid formation) made accurate histological evaluation difficult.[15]

All primary tumors were evaluated for degree of fibrosis on a 4-point scale. Grades 0, 1, 2, and 3 reflected <10%, 10%-24%, 25%-50% and >50% replacement of tumor tissue by fibrosis, respectively. They were also evaluated for other histologic features of acidophilic degeneration of cytoplasm and calcification.[15,16,18]

Pathologic finding of CRM positivity was defined as the distance between the tumor and the CRM of <1 mm in H&E staining.[19,20]

2.3 | Measurement of ART

HE-stained slides from the maximum slice of each tumor were photographed using a NanoZoomer Digital Pathology Virtual Slide Viewer (Hamamatsu Photonics, Hamamatsu, Japan) and were used for morphometric analysis.

The depth of tumor invasion beyond the muscular layer was measured as the distance between the inferior margin of the muscular layer and the outermost portion of the tumor. In tumors in which the muscular layer had been destroyed or replaced by fibrosis, the shortest line between the residual muscular layers was drawn on the picture and the distance between the line and outermost portion of the tumor was measured.

The WM-ART and BM-ART were morphometrically measured, and T-ART was calculated using tumor slices of the largest residual tumor. The ART was measured using viewer software, and mucus lakes were excluded. All tumor nests >0.1 mm^2 were measured for ART. The ART inside the inferior margin of the muscular layer was defined as WM-ART, and ART outside the inferior margin was defined as BM-ART. If the muscular layer was disrupted by inflammation, necrotic tissue, or fibrosis, a connecting line between the residual tumor muscular layers was drawn on the picture to

discriminate between WM-ART and BM-ART (Figure 1).[13] Excluded from ART analysis was mucosa showing ulceration, inflammation, necrosis, or adenoma components.

2.4 | Statistical analysis

The associations between ART and histopathologic tumor features were evaluated using the *t*-test. All calculated *P*-values were two-sided, and $P < .05$ was considered statistically significant.

The overall accuracy of the potential variables in predicting the prognosis was summarized using the area under the ROC curves and compared by a non-parametric test for comparing areas under correlated ROC curves.[21,22]

Receiver operating characteristic curves to predict patient recurrence were drawn in the NAC and CRT groups. Cut-off values were obtained and used for the association between ART and clinicopathological features. All statistical analyses were carried out using JMP 13 software (SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Clinicopathologic characteristics

Table 1 summarizes the clinicopathological characteristics of the 29 patients receiving NAC and 39 patients receiving CRT. There were no significant differences in age or sex between the NAC and CRT groups. Clinical and pathologic stage III tumors tended to be more frequently present in the NAC group than in the CRT group, although the intergroup difference was not statistically significant. All patients in the CRT group underwent intersphincteric resection. The NAC groups included six patients who had undergone other operative procedures, including abdominoperineal resection and low anterior resection.

3.2 | Downstaging

Among the 29 patients who had undergone NAC with six cycles of FOLFOX, the rate of downstaging was shown to be 68.9%, with the ypT and ypN downstaging rates being 55.1% and 62.0%, respectively. Four lesions (13.8%) in the NAC group were diagnosed as having a complete response or Dworak grade 4. Among the 39 patients who had received preoperative CRT, the rate of downstaging was 43.6% (NAC vs CRT, $P = .29$) with ypT and ypN downstaging rates of 46.1% and 23.1%, respectively. Nine lesions (23.1%) in the CRT group were diagnosed as having a complete response or Dworak grade 4. The ypN downstaging was more frequently seen in the NAC group. Although ypT downstaging was similar between the groups, Dworak grade 3 and 4 regression was seen more frequently in the CRT group than that in the NAC group (NAC, 7/29 [18.2%]; CRT, 22/39 [56.4%]; $P = .0095$).[9] Therefore, NAC and CRT differed with regard to their respective therapeutic effect on primary tumors and lymph nodes.

Complete response rate was not significantly different between the NAC and CRT groups. ($P = .33$). Again, although ypT downstaging was not different between the groups, ypN downstaging was more frequently seen in the NAC group than in the CRT group ($P = .001$).

3.3 | Histopathologic features

The histopathologic features of tumors in the preoperative CRT and NAC groups are shown in Table 2. Positive lymphatic vessel invasion was higher, and fibrosis grade was less in the NAC group than in the CRT group ($P < .05$). Grade 3 fibrosis was seen in >50% of patients who had undergone preoperative CRT. Histologic features reported post-therapy did not differ between the NAC and CRT groups, including acidophilic degeneration of cytoplasm, calcification, mucus lake, and budding grade.

3.4 | Area of residual tumor

Areas of residual tumors in the NAC and CRT groups are shown in Table 3. Although there was no statistical difference in WM-ART and BM-ART between the groups, the CRT group was associated

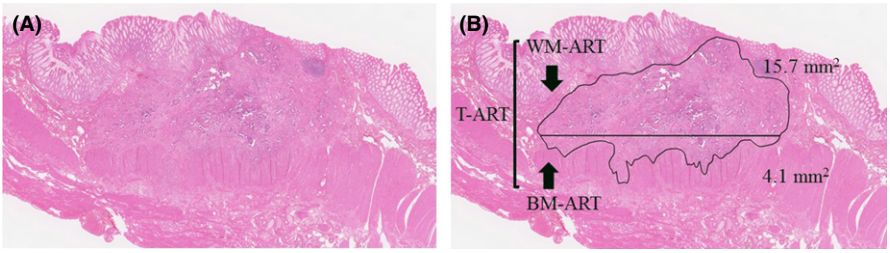

FIGURE 1 A, Low magnification view of H&E-stained section of rectal tumor tissue. B, Morphometric analysis used NanoZoomer Digital Pathology. Area of residual tumor (ART) was measured by tracing the outline of the tumor nests (black line). When the tumor was larger than 32 mm in size, we separated the slide and measured size. The border between the ART within the muscular layer (WM-ART) and the ART beyond the muscular layer (BM-ART) was measured by machine. The WM-ART was determined as the ART inside the inferior margin of the muscular layer, and BM-ART was measured as the ART outside the inferior margin of the muscular layer. If the muscular layer was not identified or had been displaced by inflammation, necrosis, and fibrosis, a connecting line between the muscular layers was drawn on the picture. In such cases, the area inside the line was measured as WM-ART and the area outside the line was measured as BM-ART. The total ART consisted of both areas

TABLE 1 Characteristics of patients with rectal cancer who received preoperative chemoradiotherapy (CRT group) or neoadjuvant chemotherapy (NAC group)

	NAC group (n = 29)	CRT group (n = 39)	*P*-value
Male/female	20/9	30/9	.46
Median age, years (range)	59 (34-76)	56 (27-77)	.42
Median AV, cm (range)	3.5 (0.0-6.0)	4.0 (0.0-5.0)	.92
Operative procedure, n (%)			
ISR	23 (79.3)	39 (100)	NS
Other	6 (20.7)	0 (0)	
cT, 0/1/2/3/4	0/0/1/20/8	0/0/10/29/0	.01*
cN, 0/1/2/3/4	2/9/4/14/0	27/5/6/1/0	.01*
pT, 0/1/2/3/4	4/2/8/13/2	9/3/10/17/0	.50
pN, 0/1/2/3/4	15/9/2/3/0	26/6/7/0/0	.21
Clinical stage, 0/I/II/IIIA/IIIB/IV	0/0/2/9/16/2	0/6/20/7/6/0	.10
Pathologic stage, 0/I/II/IIIA/IIIB/IV	4/6/5/9/5/0	9/12/10/4/4/0	.86
Tumor downstaging (UICC)			
Present	21	22	.29
Absent	8	17	
Dworak grade of regression, 0/1/2/3/4	0/7/15/3/4	0/3/14/13/9	.33

*$P < .05$.
Dworak grade of regression: grade compared in 4.
AV, anal verge; cN, clinical lymph node metastasis; cT, clinical T stage; ISR, intersphincteric resection; NS, not significant.

TABLE 2 Histologic features of rectal tumors in patients who received preoperative chemoradiotherapy (CRT group) or neoadjuvant chemotherapy (NAC group)

	NAC (n = 29)	CRT (n = 39)	*P*-value, NAC vs CRT
Ly, n (%)	13 (44.9)	4 (10.3)	.0020*
V, n (%)	12 (41.4)	17 (43.6)	.8600
PN, n (%)	7 (24.1)	11 (23.2)	.7100
Acidophilic degeneration of cytoplasm, n (%)	1 (3.4)	2 (5.1)	.6800
Calcification n (%)	0 (0)	1 (2.6)	.4700
Mucus lake, n (%)			
Grade A	4 (13.8)	5 (12.8)	
Grade B	1 (3.4)	3 (7.7)	
Grade C	1 (3.4)		
Present	6	8	.7300
Absent	23	31	
Budding grade			
−	24	35	.6200
+	5	4	
Fibrosis grade			
0-2 (0%-50%)	28	15	.0004*
3 (>50%)	1	24	

*$P < .05$.
Ly, lymphovascular invasion; PN, perineural invasion; V, venous invasion.

TABLE 3 Area of residual tumor (ART) in patients with rectal cancer who received preoperative chemoradiotherapy (CRT) or neoadjuvant chemotherapy (NAC)

ART	NAC (n = 29)	CRT (n = 39)	*P*-value, NAC vs CRT
T-ART, mm^2	75.4 ± 95.4	38.1 ± 36.5	.048*
WM-ART, mm^2	33.7 ± 46.6	17.6 ± 20.1	.078
BM-ART, mm^2	41.7 ± 79.5	20.5 ± 24.7	.168

*$P < .05$.
BM-ART, ART beyond muscular layer; T-ART, total ART; WM-ART, ART within muscular layer area.

with smaller T-ARTs than that of the NAC group ($P < .05$), suggesting that preoperative CRT may have a greater role in reducing tumors than NAC.

3.5 | Prognostic factors of rectal cancer treated by CRT and NAC

Total ART cut-off values 140 mm^2 and 49.5 mm^2 and BM-ART cut-off values 92.8 mm^2 and 35.3 mm^2 were obtained for NAC and CRT, respectively. The NAC and CRT groups were assessed for clinicopathologic features associated with ART.

Table 4 shows the association between the clinicopathologic features and T-ART in NAC and CRT. Dworak and fibrosis grade were not associated with T-ART in either the NAC or CRT groups. There was a significant association between downstaging and T-ART in the NAC group. There was also a significant association between tumor budding/vascular invasion and T-ART in both the NAC and CRT groups. However, significant association between lymphatic/neural invasion and T-ART was shown to be associated only in the NAC group.

Table 5 shows the results of the univariate analyses on the DFS of patients treated with NAC and CRT. In the CRT group, lower T, BM-ART ≤35.3 mm^2, and T-ART ≤49.5 mm^2, as well as negativity for lymphovascular invasion, were significantly associated with better DFS ($P = .016$, $P = .04$, $P = .02$, and $P = .03$, respectively). However, fibrosis and Dworak grades (0-3/4) were not associated with DFS. In NAC, downstaging, lower T and N grades, BM-ART (≤92.8 mm^2), T-ART (≤140.2 mm^2), and negativity for vascular and neural invasion were significant prognostic factors for better DFS ($P = .0001$, $P = .016$, $P = .008$, $P = .0001$, $P = .015$, $P = .0012$, and $P = .0008$, respectively). Fibrosis and Dworak grades (0-3/4) were not associated with DFS.

Multivariate analysis identified downstaging as an independent prognostic factor for NAC (Table 5), but no independent prognostic factor for CRT.

TABLE 4 Relationship between clinicopathologic features and total area of residual tumor (T-ART) in in patients with rectal cancer who received preoperative chemoradiotherapy (CRT group) or neoadjuvant chemotherapy (NAC group)

(a) NAC	T-ART <140 mm^2 (n = 23)	T-ART ≥140 mm^2 (n = 6)	*P*-value	(b) CRT	T-ART <49.5 mm^2 (n = 25)	T-ART ≥49.5 mm^2 (n = 14)	*P*-value
Dworak, 1-2/3-4	16 (69.6%)/7	6 (100%)/0	.5300	Dworak, 1-2/3-4	9 (36%)/16	8 (57.1%)/6	.1900
Downstaging, present/absent	20 (87.0%)/3	1 (16.7%)/5	.0006*	Downstaging, present/absent	15 (60%)/10	7 (50%)/7	.5400
V, n (%)	6 (26.1)	6 (100)	.0011*	V, n (%)	7 (28)	10 (71.4)	.0087*
LY, n (%)	8 (34.8)	5 (83.3)	.0330*				
PN, n (%)	3 (13.0)	4 (66.7)	.0060*				
Budding grade, n (%)				Budding grade, n (%)			
-	21 (91.3)	3 (50.0)	.0100*	-	24 (96.0)	10 (71.4)	.0280*
+	2 (8.7)	3 (50.0)		+	1 (4.0)	4 (28.6)	
Fibrosis grade, n (%)				Fibrosis grade, n (%)			
0-2 (0%-50%)	22 (95.7)	6 (100.0)	.6000	0-2 (0%-50%)	9 (36.0)	6 (42.9)	.6700
3 (>50%)	1 (4.3)	0 (0.0)		3 (>50%)	16 (64.0)	8 (57.1)	

*$P < .05$.

Ly, lymphovascular invasion; PN, perineural invasion; V, venous invasion.

T-ART was determined based on cut-off values 140 mm^2 and 49.5 mm2 for NAC and CRT groups, respectively. Cut-off values were obtained by using receiver operating characteristic curves to estimate recurrence.

TABLE 5 Univariate and multivariate analyses on disease-free survival (DFS) of patients treated with CRT and NAC

	Univariate analysis NAC (n = 29) (3-year DFS) *P*	Multivariate analysis			Univariate analysis CRT (n = 39) (3-year DFS) *P*	Multivariate analysis		
		NAC Hazard ratio	NAC 95% CI	*P*		CRT Hazard ratio	CRT 95% CI	*P*
Down stage (present/absent)	.0001*	6.974	0.003-0.509	.0083*	NS	-	-	-
Down T (present/absent)	.016*	-	-	-	.016*	0.242	0.070-3.910	.622
Down N (present/absent)	.008*	-	-	-	NS	-	-	-
WM-ART (NAC 17.5: CRT 11.9)	.90	-	-	-	.58	-	-	-
BM-ART (NAC 92.8: CRT 35.3)	.0001*	-	-	-	.04*	-	-	-
T-ART (NAC 140.2: CRT 49.5)	.015*	0.638	0.059-3.335	.424	.02*	2.906	0.809-37.258	.257
Ly (present/absent)	NS	-	-	-	.03*	3.395	0.866-44.862	.065
V (present/absent)	.0012*	1.160	0.320-94.916	.299	NS	-	-	-
NE (present/absent)	.0008*	0.065	0.180-11.701	.799	NS	-	-	-
Fibrosis (0-2/3)	.59	-	-	-	.68	-	-	-
Dworak grade (0-3/4)	.81	-	-	-	.87	-	-	-

*$P < .05$.

T-ART, total area of residual tumor; WM-ART, within muscular layer area of residual tumor; BM-ART, beyond muscular layer area of residual tumor. T-ART, BM-ART and WM-ART were determined based on cut-off values for NAC and CRT obtained using ROC curves to estimate recurrence. Ly, lymphovascular invasion; V, vein invasion; PN, perineural invasion.

3.6 | Area of residual tumor as a factor predicting recurrence of rectal cancer treated with NAC and CRT

Figure 2 shows DFS curves according to T-ART in the NAC and CRT groups. In 29 NAC group patients, T-ART value <140 mm^2 was shown to be associated with a better outcome (Figure 2A) with the 3-year DFS: 86.9% in 24 patients vs 50% in the remaining 5 patients with T-ART value ≥140 mm^2 ($P = .015$). Similarly, 39 patients in the CRT group with T-ART value <49.5 mm^2 were shown to be associated with a better outcome (Figure 2B) with 3-year DFS of 86.7% in 24 patients vs 58.2% in the remaining 15 patients with T-ART value ≥49.5 mm^2 ($P = .028$).

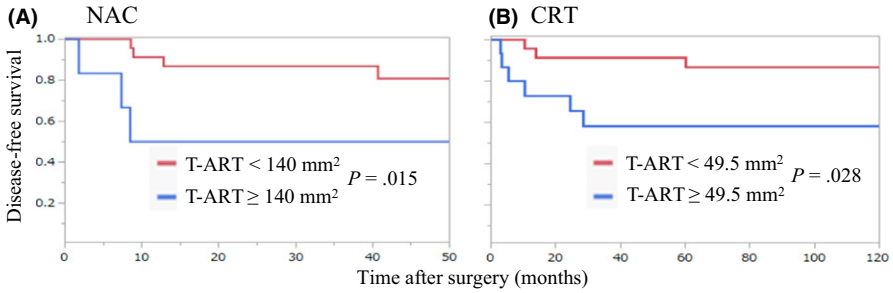

FIGURE 2 Kaplan-Meier curves for disease-free survival curves for the impact of area of residual tumor (ART) on outcomes in circumferential resection margin-negative and pStage I-III patients with rectal cancer treated with neoadjuvant chemotherapy (NAC; n = 29) (A) and chemoradiotherapy (CRT; n = 39) (B). *$P < .05$. T-ART, total ART

4 | DISCUSSION

Recently, the pathological assessment of ART was reported to be clinically useful in patients with gastric, lung, and rectal cancer undergoing preoperative therapy.[11-13] Although many pathological assessment methods have been reported for various cancers, their utility after different preoperative therapeutic regimens have not been well described.[9,23] This study revealed that not only ART-based assessment but ypTNM might have a role in predicting patient outcome after various preoperative therapies. Although morphometric analysis may not be practical in routine practice, semiquantitative ART-based assessment in terms of microscopic field numbers may be more readily available for routine clinical use. Indeed, our results appear to provide support for ART-based assessment (Figure 1). Given that one field of ×10 ocular lens measures 31.4 mm^2, being smaller than the cut-off in NAC and CRT, while two fields of ×4 ocular lens measures 157.0 mm^2, being larger than the cut-off in NAC and CRT, we propose an ART-based pathological assessment, coupled with marking around the residual tumor area before evaluation, as follows: grade 1, those with ART extending over two fields of ×4 ocular lens (high-risk group); grade 2, those with ART extending over one field of ×10 to two fields of ×4 ocular lens (intermediate-risk group); grade 3, those with ART confined within one field of ×10 ocular lens (low-risk group); and grade 4, those with complete response (Figure 3). Marking around the residual tumor area is recommended before the evaluation. This assessment method should be validated in another cohort in the future. Previous assessment methods are using fibrosis, and residual tumor since fibrosis was assumed to be an original tumor area. In contrast, our study revealed that the fibrosis grade is different between NAC and CRT groups, and may not reflect the original tumor area, but depend on the applied therapeutic regimens. Furthermore, fibrosis was not associated with clinical survival. Thus, tumor regression should be assessed independently of the grade of fibrosis present. This is the first study to provide evidence for an ART-based assessment method for residual tumor after various therapy in rectal cancer. In our previous study, we compared the clinicopathologic characteristics of tumors in relation to preoperative CRT and NAC in rectal cancer, revealing marked differences in clinicopathologic features, which reflected different systemic effects between preoperative CRT and NAC.[14] In this study, we showed frequent ypN downstaging with large T-ART in those treated with NAC, suggesting that NAC could have a limited effect on primary tumors, while affecting both primary tumors and distant lymph node metastases.

In accord with such a variation in systemic effect, we reported earlier that the tumor microenvironment differed with preoperative

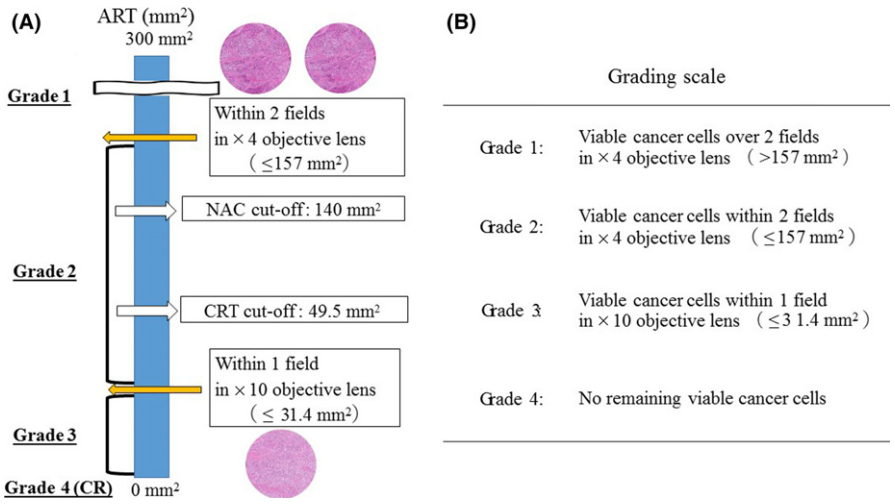

FIGURE 3 A, Based on our area of residual tumor (ART) results, we propose an ART-based assessment method for patients with rectal cancer undergoing preoperative therapy. CR, complete response; CRT, chemoradiotherapy; NAC, neoadjuvant chemotherapy. B, The proposed new grading after preoperative therapy consists of four grades based on the microscopic magnifications and number of fields involved

CRT vs NAC, where immune cells have been reported to be associated with postoperative convalescence and clinical outcome.[24-30] Therefore, other physiological features reflecting the state of the tumor microenvironment, such as hypoxia, might also be different between NAC and CRT. It has been thought that tumor hypoxia reduces sensitivity to chemotherapy and radiation, and tumor hypoxia also associated with prognosis factor to CRT.[31,32] Further biological investigation is also required in the future.

The main limitation of this study is that the number of cases used to determine the cut-off level for ART with CRT vs NAC was small. Furthermore, a validation study is required to determine the utility of the proposed ART-based pathologic assessment.

In conclusion, histologic features of the preoperative CRT and NAC were elucidated. Pathological assessment based on ART could provide useful prognostic information for rectal cancer irrespective of therapeutic regimens. Further study is required to validate the proposed assessment method in the future.

ACKNOWLEDGMENTS

This work was funded by a Grant-in-Aid for Cancer Research A26-19 from the Ministry of Health, Labor and Welfare of Japan.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ORCID

Naoki Sakuyama http://orcid.org/0000-0003-2711-1168
Motohiro Kojima http://orcid.org/0000-0002-6150-6545

REFERENCES

1. Bosset JF, Calais G, Daban A, et al. Preoperative chemoradiotherapy versus preoperative radiotherapy in rectal cancer patients: assessment of acute toxicity and treatment compliance. *Eur J Cancer*. 2004;40:219-224.
2. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351:1731-1740.
3. Sebag-Montefiore D, Stephens RJ, Steele R, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet*. 2009;373:811-820.
4. Rullier E, Zerbib F, Laurent C, et al. Intersphincteric resection with excision of internal anal sphincter for conservative treatment of very low rectal cancer. *Dis Colon Rectum*. 1999;42:1168-1175.
5. Kohler A, Athanasiadis S, Ommer A, Psarakis E. Long-term results of low anterior resection with intersphincteric anastomosis in carcinoma of the lower one-third of the rectum: analysis of 31 patients. *Dis Colon Rectum*. 2000;43:843-850.
6. Nishizawa Y, Saito N, Fujii S, et al. Association between anal function and therapeutic effect after preoperative chemoradiotherapy followed by intersphincteric resection. *Dig Surg*. 2012;29:439-445.
7. Nishizawa Y, Fujii S, Saito N, et al. Differences in tissue degeneration between preoperative chemotherapy and preoperative chemoradiotherapy for colorectal cancer. *Int J Colorectal Dis*. 2012;27:1047-1053.
8. Chau I, Brown G, Cunningham D, et al. Neoadjuvant capecitabine and oxaliplatin followed by synchronous chemoradiation and total mesorectal excision in magnetic resonance imaging-defined poor-risk rectal cancer. *J Clin Oncol*. 2006;24:668-674.
9. Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis*. 1997;12:19-23.
10. Trakarnsanga A, Gonen M, Shia J, et al. Comparison of tumor regression grade systems for locally advanced rectal cancer after multimodality treatment. *J Natl Cancer Inst*. 2014;106 pii: dju248. https://doi.org/10.1093/jnci/dju248.
11. Nakamura K, Kuwata T, Shimoda T, et al. Determination of the optimal cutoff percentage of residual tumors to define the pathological response rate for gastric cancer treated with preoperative therapy (JCOG1004-A). *Gastric Cancer*. 2015;18:597-604.
12. Yamane Y, Ishii G, Goto K, et al. A novel histopathological evaluation method predicting the outcome of non-small cell lung cancer treated by neoadjuvant therapy: the prognostic importance of the area of residual tumor. *J Thorac Oncol*. 2010;5:49-55.
13. Kojima M, Ishii G, Yamane Y, Nishizawa Y, Saito N, Ochiai A. Area of residual tumor beyond the muscular layer is a useful predictor of outcome in rectal cancer patients who receive preoperative chemoradiotherapy. *Pathol Int*. 2009;59:857-862.
14. Sakuyama N, Kojima M, Kawano S, et al. Histological differences between preoperative chemoradiotherapy and chemotherapy for rectal cancer: a clinicopathological study. *Pathol Int*. 2016;66:273-280.
15. Rullier A, Laurent C, Vendrely V, Le Bail B, Bioulac-Sage P, Rullier E. Impact of colloid response on survival after preoperative radiotherapy in locally advanced rectal carcinoma. *Am J Surg Pathol*. 2005;29:602-606.
16. Shia J, McManus M, Guillem JG, et al. Significance of acellular mucin pools in rectal carcinoma after neoadjuvant chemoradiotherapy. *Am J Surg Pathol*. 2011;35:127-134.
17. Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC. Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. *Histopathology*. 2002;40:127-132.
18. Shia J, Guillem JG, Moore HG, et al. Patterns of morphologic alteration in residual rectal carcinoma following preoperative chemoradiation and their association with long-term outcome. *Am J Surg Pathol*. 2004;28:215-223.
19. Nagtegaal ID, Quirke P. What is the role for the circumferential margin in the modern treatment of rectal cancer? *J Clin Oncol*. 2008;26:303-312.
20. Taylor FG, Quirke P, Heald RJ, et al. One millimetre is the safe cut-off for magnetic resonance imaging prediction of surgical margin status in rectal cancer. *Br J Surg*. 2011;98:872-879.
21. DeLong ER. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837-845.
22. Yamamoto R, Nagasawa Y, Marubashi S, et al. Early plasma exchange for progressive liver failure in recipients of adult-to-adult living-related liver transplants. *Blood Purif*. 2009;28:40-46.
23. Ryan R, Gibbons D, Hyland JM, et al. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology*. 2005;47:141-146.
24. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313:1960-1964.
25. Forssell J, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with

improved survival in colon cancer. *Clin Cancer Res*. 2007;13:1472-1479.
26. Gao J, Knutsen A, Arbman G, Carstensen J, Franlund B, Sun XF. Clinical and biological significance of angiogenesis and lymphangiogenesis in colorectal cancer. *Dig Liver Dis*. 2009;41:116-122.
27. Kojima M, Ishii G, Atsumi N, Nishizawa Y, Saito N, Ochiai A. CD133 expression in rectal cancer after preoperative chemoradiotherapy. *Cancer Sci*. 2010;101:906-912.
28. Mantovani A. B cells and macrophages in cancer: yin and yang. *Nat Med*. 2011;17:285-286.
29. Shinto E, Hashiguchi Y, Ueno H, et al. Pretreatment CD133 and cyclooxygenase-2 expression as the predictive markers of the pathological effect of chemoradiotherapy in rectal cancer patients. *Dis Colon Rectum*. 2011;54:1098-1106.
30. Shinto E, Hase K, Hashiguchi Y, et al. CD8 + and FOXP3 + tumor-infiltrating T cells before and after chemoradiotherapy for rectal cancer. *Ann Surg Oncol*. 2014;21(Suppl 3):S414-S421.
31. Harrison LB, Chadha M, Hill RJ, Hu K, Shasha D. Impact of tumor hypoxia and anemia on radiation therapy outcomes. *Oncologist*. 2002;7:492-508.
32. Korkeila EA, Sundstrom J, Pyrhonen S, Syrjanen K. Carbonic anhydrase IX, hypoxia-inducible factor-1alpha, ezrin and glucose transporter-1 as predictors of disease outcome in rectal cancer: multivariate Cox survival models following data reduction by principal component analysis of the clinicopathological predictors. *Anticancer Res*. 2011;31:4529-4535.

How to cite this article: Sakuyama N, Kojima M, Kawano S, et al. Area of residual tumor is a robust prognostic marker for patients with rectal cancer undergoing preoperative therapy. *Cancer Sci*. 2018;109:871–878. https://doi.org/10.1111/cas.13521