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Distribution of Residual Cancer Cells in the Bowel Wall after Neoadjuvant Chemoradiation in Patients with Rectal Cancer

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Abstract

Background—The standard treatment for locally advanced rectal cancer is pre-operative chemoradiation and total mesorectal excision. After surgery, tumors are classified according to the depth of tumor invasion, nodal involvement, and tumor regression grade. However, these staging systems do not provide information about the distribution of residual cancer cells within the bowel wall.

Objective—To determine the distribution of residual cancer cells in each layer of the bowel wall in rectal cancer specimens.

Design—Prospective Phase II study.

Setting—Multi-institutional.

Patients—153 patients with stage II or stage III rectal cancer.

Interventions—Patients were treated with chemoradiation and surgery. Surgical specimen tumor tissue was analyzed and the distribution of residual cancer cells in each layer of the bowel wall was determined.

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Main Outcome Measures—Statistical analysis was used to examine the correlation of residual cancer cells in each layer of the bowel wall with the clinical/pathological stage and tumor regression grade.

Results—Forty-two of 153 (27%) patients had complete response in the bowel wall (ypT0). Of the remaining 111 patients who had residual cancer cells, 5 (3%) were ypTis, 12 (8%) were ypT1, 41 (27%) were ypT2, 50 (33%) were ypT3, and 3 (2%) were ypT4. Of the 94 patients with ypT2-4 tumors, 12 (13%) had cancer cells in the mucosa and 53 (56%) had cancer cells in the submucosa; 92 (98%) had cancer cells in the muscularis propria. Pretreatment cT stage correlated with distribution of residual cancer cells. Tumor regression grade was not associated with distribution of residual cancer cells after chemoradiation.

Limitations—Patients received different chemotherapy regimens.

Conclusions—Residual cancer cells in rectal cancer specimens after chemoradiation are preferentially located close to the invasive front. This should be considered when designing strategies to diagnose complete pathologic response and when investigating the mechanisms of tumor resistance to chemoradiation.

Keywords

Rectal cancer; Residual cancer cells; Bowel wall; Neoadjuvant chemoradiation

Introduction

The goal of neoadjuvant chemoradiation therapy (CRT) in patients with locally advanced rectal cancer is to increase local tumor control and improve the potential for sphincter preservation.^{1, 2} Although most rectal cancers regress to some extent after CRT, the degree of tumor response is variable. Whereas some tumors change minimally in size, others are completely eradicated by CRT and have a pathologic complete response (pCR). Numerous studies have demonstrated that rectal cancer patients with a pCR after CRT have lower recurrence and improved survival compared to patients who do not have a pCR.^{3, 4}

The improved prognosis associated with a pCR questions the need for a total mesorectal excision (TME) or any surgery at all in these patients. A number of retrospective case series have reported promising results with a “wait and watch” approach in selected patients who refused surgery or were considered unfit for laparotomy and bowel resection and in select patients who developed a clinical complete response (cCR).^{5–7} The idea of sparing the rectum in patients who respond to CRT is also being prospectively explored by a number of institutions worldwide.^{5–10} However, not every patient with a cCR has a pCR, and imaging studies cannot accurately identify patients with minimal residual disease.^{11–14} Presently, histopathological assessment of the surgical specimen after TME is the only reliable way to identify patients with a pCR after CRT.^{8, 10, 15}

The seventh edition of the American Joint Committee on Cancer (AJCC) staging manual recognizes a modified pathologic staging for tumors that have received neoadjuvant therapy.¹⁶ This postneoadjuvant therapy tumor staging system (ypTNM) is based on the maximal depth of tumor penetration in the bowel wall (ypT) and the involvement of the

regional lymph nodes (ypN). The AJCC also ranks the tumor regression grade (TRG) on a 4-point scale, with TRG0 representing a complete response and TRG3 indicating a poor response.¹⁶ However, these histological classifications do not specify the distribution of the residual cancer cells (RCCs) within the different layers of the bowel wall (mucosa, submucosa, muscularis propria, and subserosa). Understanding the distribution of RCCs within the layers of the bowel wall after CRT may help clinicians develop strategies to diagnose a pCR before removing the rectum. We examined the distribution of RCCs in the different layers of the bowel wall in 153 patients with rectal cancer treated with neoadjuvant CRT and TME.

Materials and Methods

Patients and treatment

This study included patients with clinical stage II (cT3-4, cN0) or stage III (any cT, cN1-2) invasive adenocarcinoma of the rectum with a distal tumor border within 12cm of the anal verge, as measured on rigid proctoscopic exam, who were enrolled in the Timing of Rectal Cancer Response to Chemoradiation study, a multi-institutional clinical trial investigating the effect of increasing the CRT-to-surgery interval, and adding chemotherapy, modified FOLFOX-6 (mFOLFOX-6) during the waiting period ([ClinicalTrials.org](https://clinicaltrials.org) Identifier: NCT00335816). This trial was designed in a series of sequential Phase II trials or study groups (SGs), each with a progressively longer CRT-to-surgery interval and increasing cycles of preoperative mFOLFOX-6. This study was approved by an Institutional Review Board (IRB) at each participating institution as well as a central IRB, and informed written consent was obtained from each patient prior to enrollment in the trial. Patients included in the present study were pooled from SG1 (n=49), SG2 (n=54), and SG3 (n=50); total n=153. Only patients with available pretreatment tumor biopsy and surgical specimen tumor blocks were included in this study. Pretreatment tumor biopsies were used to confirm the diagnosis of adenocarcinoma. Further details of patient eligibility for this trial are presented elsewhere.¹⁷

Treatment protocol

Patients in all SGs were treated with CRT; 5-Fluorouracil (FU) and a total of 50.4Gy of radiation as described previously.¹⁷ Patients in SG1 underwent TME an average of 6 weeks after completing CRT (standard of care). Following CRT, patients in SG2 and SG3 with no evidence of stable disease received 2 and 4 cycles of additional chemotherapy (mFOLFOX-6), respectively, as described previously.^{17, 18} Patients in SG2 underwent TME an average of 11 weeks after completing CRT, and patients in SG3 underwent TME an average of 16 weeks after completing CRT. The clinical outcomes for these patients are presented elsewhere.^{17, 18}

Pathologic staging

Pathologic staging (ypTNM) and TRG were determined according to AJCC guidelines.¹⁶ Surgical specimens were processed according to the College of American Pathologists guidelines¹⁹ as described previously ([ClinicalTrials.org](https://clinicaltrials.org) Identifier: NCT00335816). For all patients, histological sections from pretreatment tumor biopsy and surgical specimen blocks

were prepped, stained with hematoxylin and eosin (H&E), and examined by two independent surgical pathologists. A pCR was defined as the absence of viable tumor in the entire resected surgical specimen and in the regional lymph nodes (ypTON0). In patients with residual tumor after CRT, the presence of RCCs in the mucosa, submucosa, muscularis propria, and subserosa layers of the bowel wall was recorded.

Statistical analysis

Patient demographics, clinical tumor characteristics, and pathological staging overall were compared with RCC distribution in the different layers of the bowel wall. The association of individual SGs with tumor staging was determined using the Fisher exact test. The association of individual SGs with depth of tumor invasion was analyzed using a repeated measures logistic regression model with invasion as outcome and an auto-regressive covariance matrix. The association of maximum invasion (the deepest level of tumor invasion) with continuous measures - age and anal verge distance - was determined using Kendall's Tau rank correlation test; and for categorical measures - pretreatment T- and N stage, pathologic T and N stage, and TRG (not including TRG0) - the 2-sided Jonckheere-Terpstra test was used. Two-sided p -values of <0.05 were considered statistically significant.

Results

Patient demographics and tumor response

Patient demographics overall and stratified by SG are shown in Table 1. Overall, the average patient age was 57 years (range 25–87 years) and more than half (57%) of the patients were male. Most patients (77%) had clinical AJCC Stage III rectal carcinoma. On clinical examination, the average distance from the anal verge was 7cm (range 1–14cm). Patient characteristics including age, gender, clinical T stage (cT), clinical N stage (cN), and distance from anal verge were not significantly different between SGs.

Overall, 40 of 153 patients (26%) had a pCR after CRT. Of the 113 patients (74%) with residual disease (non-pCR), 79 (53%) had a moderate response (TRG1), 28 (18%) had minimal response (TRG2), and 4 (3%) had no response to CRT (TRG3). There was a slight increase in the pCR rates in SG2 and SG3 compared to SG1, but the differences were not statistically significant. The distribution of TRGs were similar among the SGs. ypT stage ypN stage were also similar between SGs.

Distribution of cancer cells in layers of the bowel wall

The distribution of RCCs in each layer of the bowel wall overall and stratified by SG is presented in Table 2. Overall, 42 patients (27%) had no RCCs in any layer of the bowel wall (ypT0 or TRG0), 21 (14%) had RCCs in the mucosa, 65 (42%) in the submucosa, 92 (60%) in the muscularis propria, and 53 (35%) in the subserosa/perirectal fat. The distribution of RCCs in the different layers of the bowel wall was similar among SGs ($p=0.52$). Of note, 2 patients without RCCs in the bowel wall (ypT0 or TRG0), had RCCs in the mesorectal lymph nodes and were staged as ypTON1.

Distribution of cancer cells in the bowel wall by pathologic stage (ypT)

The distribution of RCCs in the different layers of the bowel wall according to ypT stage is presented in Figure 1. All 5 patients staged as ypTis had RCCs present only in the mucosa. In contrast, only 4 of 12 (33%) patients with ypT1 tumors had RCCs present in the mucosa. In patients with ypT2 tumors, 6 (15%) had RCCs in the mucosa, and 23 (56%) in the submucosa. For the 50 patients with ypT3/4 tumors (n=53), 6 (12%) had RCCs in the mucosa and 30 (60%) in the submucosa.

A total of 88 tumors were described as ulcerated by the surgical pathologist who assessed the surgical specimens. As tumor ulceration could potentially affect the distribution of RCCs in the different layers of the bowel wall, we repeated the analysis stratifying by tumor ulceration. The distribution of the RCCs in the different layers of the bowel wall was similar in ulcerated and nonulcerated tumors (data not shown).

Correlation between clinical stage (cT and cN) and pathologic stage (ypT)

To determine whether it is possible to predict the distribution of RCCs in the different layers of the bowel wall preoperatively, we examined the correlation between the clinical (cT and cN) stage and the pathological stage (ypT). We found a strong correlation between cT stage and ypT stage ($p<0.0001$); cT2 tumors correlated with ypT0 and ypT1-2 stages, and cT3-4 tumors correlated with ypT3 stage. There was no correlation between cN stage and ypT stage ($p=0.33$) (Table 3).

Distribution of cancer cells in the bowel wall by clinical stage (cT and cN)

We next examined the distribution of RCCs in the different layers of the bowel wall after CRT based on the clinical T and N stages (Figure 2). Of the 12 patients with cT2 tumors, 7 (58%) had no RCCs in the bowel wall, 2 (17%) had RCCs in the mucosa, 2 (17%) in the submucosa, and 3 (25%) in the muscularis propria. None of the cT2 patients had RCCs within the subserosa or perirectal fat. Of the 106 cT3/4 patients with RCCs in the bowel wall after CRT, 19 (18%) had cancer RCCs in the mucosa, 63 (59%) in the submucosa, 89 (84%) in the muscularis propria, and 53 (50%) in the subserosa or perirectal fat. The association between the cT stage and the distribution of RCCs in the different layers of the bowel wall after CRT was statistically significant ($p=0.0001$). The distribution of RCCs in the different layers of the bowel wall was not associated with cN stage ($p=0.67$).

Distribution of cancer cells in the bowel wall by TRG

We found a strong correlation between TRG and ypT stage for patients with RCCs (Table 4). Most patients with TRG1 were ypTis, ypT1 or ypT2, and most patients with TRG2 and TRG3 tumors were ypT3 (79% and 75%, respectively). These differences were statistically significant ($p<0.0001$). The distribution of RCCs in the different layers of the bowel wall according to TRG is presented in Figure 3. With the exception of the mucosa, RCCs were distributed relatively evenly in the submucosa, muscularis propria, and subserosa/perirectal fat.

Discussion

Our study shows that RCCs in rectal cancer specimens of patients treated with neoadjuvant CRT are distributed unequally in the different layers of the bowel wall. Whereas RCCs are found preferentially in the layers of the bowel wall corresponding to the invasive front of the tumor based on the clinical and pathologic stage of the tumor, the distribution of RCCs in the bowel wall is independent of the clinical or pathological lymph node status. The findings of this study confirm previous observations that many patients with tumor eradicated from the mucosa and submucosa in response to CRT still have RCCs in different layers of the bowel wall;²⁰ but to our knowledge, ours is the first study to systematically analyze the distribution of RCCs in the different layers of the bowel wall in rectal cancer patients treated with CRT.

We found a correlation between cT stage and ypT stage, and cT2 tumors were more likely to have a pCR after CRT compared to cT3 or T4 tumors. A similar pCR rate for clinically staged T2 tumors has already been reported in the literature.^{21, 22} Therefore, it was not surprising to find that the distribution of RCCs in the bowel wall according to cT stage was similar to the distribution by ypT stage. However, the distribution of RCCs in the bowel wall was independent of cN stage and ypN stage, suggesting that tumor response in the bowel wall is probably more dependent on local factors related to the depth of tumor penetration (T stage) than on the overall AJCC disease stage (II or III). The high rate of the primary tumor response to chemotherapy and radiation in patients with stage IV disease seems to confirm this observation. The distribution of RCCs in the bowel wall was not significantly different by TRG.

Our findings are important because they highlight the limitations of clinical exam and imaging techniques to assess tumor response, and offer insight into developing new strategies to identify patients with pCR. Clinical exam is particularly unreliable to identify patients with a pCR after CRT. Although many patients with a cCR have a pCR, most patients with a pCR do not meet the most recent criteria for cCR.^{10, 14} Consequently, a “wait and watch” protocol based on clinical criteria - blanching of the mucosa, telangiectasia, and nonulceration - will not identify many patients with a true pCR. The identification of all patients with a pCR in the bowel wall will most likely require a biopsy of the tumor site in patients with significant tumor regression in response to CRT.¹⁴ Our data suggest that a superficial sampling of the mucosa and submucosa obtained with an endoscopic biopsy will fail to detect cancer cells in at least 25% of patients with cT2 tumors and more than 40% of patients with cT3/T4 tumors. Instead, a full thickness biopsy of the muscularis propria will provide adequate sampling for all rectal cancers treated with CRT.

Similar to previous studies^{23–25} we found that 2 of the 42 (5%) patients with no residual tumor in the bowel wall had residual tumor detected in the lymph nodes. Although the number of patients is relatively small, the inability to accurately identify these patients before surgery remains one of the main hurdles to the implementation of an organ-sparing approach - with or without full-thickness biopsy of the primary tumor - in rectal cancer patients treated with CRT.

The ultimate reasons for the differences in the distribution of RCCs in the rectal wall after CRT are unknown, but they are most likely related to heterogeneous tumor response to the genotoxic insult within the tumor. Colorectal cancer is characterized by genetic instability that results in the accumulation of activating mutations or amplifications of oncogenes and inactivating mutations and deletions of tumor suppressor genes.^{26, 27} The accumulation of different genetic alterations results in phenotypic tumor heterogeneity through clonal evolution and selection.^{26–28} This intratumor heterogeneity is best exemplified at the invasive front that is considered more biologically active than the center of the tumor.^{29–31} A number of studies have shown different protein expression profiles at the invasive front compared to other regions of the tumor.^{28–34} It is, therefore, possible that differences in the genetic and molecular profile at the invasive front of the tumor may be responsible for the differences in response and the distribution of the RCCs observed in our study.

A different dimension of the phenotypic heterogeneity is the existence of cancer stem cells (CSCs) that have been considered responsible for tumor resistance to therapeutic intervention.^{35, 36} The preferential location of CSCs at the invasive front could also explain the distribution of the RCCs in the bowel wall after CRT.^{36, 37}

Alternatively, the differences in response to therapy may not depend on the cancer cells themselves, but on the tumor microenvironment. The effect of radiation depends on adequate tissue oxygenation, and the delivery of chemotherapy agents requires adequate blood supply.³⁸ It is possible that the portion of the tumor located close to the submucosa - the most highly vascularized layer of the gastrointestinal tract - may respond better than parts of the tumor located near to less vascularized tissues.³⁸

Finally, it is possible that heterotypic signaling between tumor and various stromal cells present in the different layers of the bowel wall may contribute to variation in response and ultimately to the differences in the distribution of RCCs in the rectum and perirectal tissues.^{39, 40} However, it is currently not known which stromal factors derived from the microenvironment may contribute to response to chemotherapy. Further investigation addressing the mechanism contributing to the difference in tumor response across the bowel wall may provide additional insight as to how to improve rectal cancer response to CRT.

The limitations to our study deserve mention. Patients included in this study were treated according to three different neoadjuvant protocols.^{17, 18} Patients receiving consolidation chemotherapy (2 or 4 cycles of mFOLFOX-6 after CRT) after CRT had a slightly higher, although not significantly different, pCR rate compared to patients receiving CRT alone. It is, therefore, possible that the study may be underpowered to detect small differences between SGs, both in the pCR rates and in the distribution of RCCs in the different layers of the bowel wall. Therefore, while the purpose of the study was not to compare pCR rates between SGs, the possibility that the study may be underpowered cannot be excluded.

In conclusion, rectal cancer response to CRT is heterogeneous within the tumor and RCCs are preferentially located close to the invasive front of the tumor. A significant number of locally advanced tumors do not have RCCs in the mucosa or submucosa after CRT. For a biopsy to provide useful information about the tumor response in the bowel wall, it must

include the muscularis propria. These findings should be taken into consideration when designing strategies to identify patients who may benefit from a “wait and watch” approach after CRT. They may also help direct the efforts to understand the mechanisms of rectal cancer resistance to neoadjuvant CRT.

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Figure 1.
Distribution of residual cancer cells (RCCs) in the different layers of the bowel wall according to the pathologic (ypT) stage; $p < 0.001$.

Figure 2.
Distribution of residual cancer cells (RCCs) in the different layers of the bowel wall according to the clinical T (cT) stage; $p < 0.001$.

Figure 3.
Distribution of residual cancer cells (RCCs) in the different layers of the bowel wall according to tumor regression grade (TRG); $p=0.1$.

Table 1

Patient Demographics and Characteristics overall and stratified by SG

Demographics	Total n=153	SG1 n=49	SG2 n=54	SG3 n=50	p
Age, years *	57 (25–87)	60 (37–87)	56 (32–84)	56 (25–76)	0.17
Male	87 (57%)	30 (61%)	29 (54%)	28 (56%)	0.75
Clinical T Stage (cT)					
cT2	12 (8%)	2 (4%)	6 (11%)	4 (8%)	0.54
cT3	136 (89%)	46 (94%)	47 (87%)	43 (86%)	
cT4	5 (3%)	1 (2%)	1 (2%)	3 (6%)	
Clinical N Stage (cN)					
cN0	35 (23%)	15 (31%)	10 (18%)	10 (20%)	0.30
cN1-2	118 (77%)	34 (69%)	44 (82%)	40 (80%)	
Distance anal verge, cm **†	7 (1–14)	7 (1–12)	7 (2–12)	7 (2–14)	0.89
Pathologic Response					
Non-pCR	113 (74%)	39 (80%)	38 (70%)	36 (72%)	0.57
pCR	40 (26%)	10 (20%)	16 (30%)	14 (28%)	
TRG					
0	42 (27%)	12 (20%)	16 (30%)	14 (28%)	0.15
1	79 (53%)	29 (63%)	30 (56%)	20 (40%)	
2	28 (18%)	7 (14%)	8 (15%)	13 (26%)	
3	4 (3%)	1 (2%)	0 (0%)	3 (6%)	
Pathologic T Stage (ypT)					0.52
ypT0	42 (27%)	12 (24%)	16 (30%)	14 (28%)	
ypTis	5 (3%)	2 (4%)	3 (6%)	0 (0%)	
ypT1	12 (8%)	3 (6%)	3 (6%)	6 (12%)	
ypT2	41 (27%)	11 (22%)	15 (28%)	15 (30%)	
ypT3	50 (33%)	21 (43%)	15 (28%)	14 (28%)	
ypT4	3 (2%)	0 (0%)	2 (4%)	1 (2%)	
Pathologic N Stage (ypN)					

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Demographics	Total n=153	SG1 n=49	SG2 n=54	SG3 n=50	p
ypN0	120 (78%)	38 (78%)	40 (74%)	42 (84%)	0.77
ypN1-2	33 (22%)	11 (22%)	14 (26%)	8 (16%)	

Abbreviations: SG: study group; pCR: pathologic complete response; TRG: tumor regression grade.

* Mean (range) shown.

[†]Missing data: SG: n=6; SG2: n=10; SG3: n=4.

Distribution of RCCs in the Different Layers of the Bowel Wall overall and stratified by SG

Table 2

Distribution of RCCs	Total n=153	SG1 n=49	SG2 n=54	SG3 n=50	p
No cancer cells (ypT0)	42 (27%)	12 (24%)	16 (30%)	14 (28%)	0.52
Mucosa	21 (14%)	5 (10%)	11 (20%)	5 (10%)	
Submucosa	65 (42%)	21 (43%)	25 (46%)	19 (38%)	
Muscularis propria	92 (60%)	31 (63%)	31 (57%)	30 (60%)	
Subserosa/perirectal fat	53 (35%)	21 (43%)	17 (32%)	15 (30%)	

Abbreviations - Residual Cancer Cells; RCCs; SG: study group; pCR: pathologic complete response, TRG: tumor regression grade.

Table 3

Correlation between pathologic (ypT) and clinical (cT and cN) stage

Clinical Stage	n=153	Pathologic T Stage (ypT)						p
		ypT0 n=42	ypT1s n=5	ypT1 n=12	ypT2 n=41	ypT3 n=50	ypT4 n=3	
T Stage (cT)								<0.0001
cT2	12	7 (58%)	1 (8%)	1 (8%)	3 (25%)	0 (0%)	0 (0%)	
cT3	136	35 (26%)	4 (3%)	11 (8%)	38 (28%)	46 (34%)	2 (1%)	
cT4	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (80%)	1 (20%)	
N Stage (cN)								0.33
cN0	35	6 (17%)	2 (6%)	4 (11%)	9 (26%)	14 (40%)	0 (0%)	
cN+	118	36 (31%)	3 (3%)	8 (7%)	32 (27%)	36 (31%)	3 (3%)	

Table 4

Maximal Depth of Tumor Invasion (ypT) by TRG

TRG	n=153	Pathologic T Stage (ypT)					p
		ypT0	ypTis	ypT1	ypT2	ypT3	
0 ^{†**}	42	42 (100%)	–	–	–	–	
1	79	2 (2%)	5 (6%)	12 (15%)	34 (42%)	28 (35%)	<0.0001
2	28	–	–	–	6 (21%)	22 (79%)	
3	4	–	–	–	1 (25%)	3 (75%)	

Abbreviations – TRG: tumor regression grade.

[†] Patients with TRG0 excluded from analysis.^{**} Two patients with TRG0 had residual cancer cells (RCCs) in the mesotectal lymph nodes.