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Clinicopathologic Determinants of Pathologic Treatment Response in Neoadjuvant Treated Rectal Adenocarcinoma

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Abstract

Neoadjuvant treatment (NAT) followed by total mesorectal excision is currently considered the standard of treatment for rectal adenocarcinoma. The degree of pathologic treatment response (pTR) correlates significantly with the recurrence free survival and overall survival (OS). However, it remains unclear which clinical and pathologic factors are associated with a more robust response to NAT, including showing pathologic complete response (pCR). Chemokine receptor 4 (CXCR4) overexpression has been associated with unfavorable OS in some studies. In this study, we sought to evaluate the clinicopathologic determinants of pTR in neoadjuvant treated rectal adenocarcinoma (NAT-RA). We retrospectively identified 91 patients who underwent pre-treatment diagnostic biopsy, NAT, and surgical resection at our institution. The archival slides were reviewed for pathologic features in the pre-treatment biopsies and for assessment of pTR in the resection specimens according to the current College of American Pathologist (CAP)'s guidelines. pCR was obtained in 16.5% of the cases, whereas 20.9% had near pCR, 30.8% had partial response, and 31.9% had a poor/no response. CXCR4 immunohistochemical analysis was also performed on the pre-treatment biopsies. Lower pre-treatment cT-stage (p=0.019) and pre-

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treatment AJCC cTNM stage groups (p=0.004), longer time interval between completion of NAT and resection (p=0.022), and presence of tumor-infiltrating lymphocytes in the pre-treatment biopsies (p=0.019) were significantly associated with a better pTR. CXCR4 nuclear expression was associated with a lower percentage of residual tumor (p=0.036). Pre-treatment CEA levels, tumor differentiation, CAP treatment response groups and lower percentage of residual tumor were associated with a better OS.

Keywords

Rectal adenocarcinoma; neoadjuvant treatment; NAT; treatment response; complete response; CXCR4; tumor infiltrating lymphocytes; TILs

1. INTRODUCTION

Colorectal cancer (CRC) remains the third most common cancer in the United States, with approximately 135,430 new cases and 50,260 deaths in 2017[1]. Rectal adenocarcinoma (RA) differs from other CRCs, given its unique anatomy and treatment modalities available. The anatomic location of the rectum in the pelvis and lack of a serosal lining increases the risk of local invasion and distant metastasis compared to the rest of the colon[2,3]. RA most commonly presents with locally advanced (stage II-III) disease, for which neoadjuvant treatment (NAT) followed by total mesorectal excision (TME) and adjuvant chemotherapy is currently considered the standard of care[2–5].

Pathologic complete response (pCR) following NAT is achieved in approximately 20% of RA cases[2,3,5], and another 60% of patients show some degree of tumor regression[6]. The degree of pathologic treatment response (pTR) strongly correlates with recurrence-free survival (RFS), with a 5-year RFS rate of 95% for pCR compared to 61% for minor response[5]. It remains unclear, however, which patients respond best to NAT and, moreover, achieve a pCR. With the increasing interest in non-operative management in RA, prediction of response to NAT has become a critically important clinical question[7,8].

We sought to evaluate which clinicopathologic factors in the pre-treatment diagnostic biopsies for RA could predict treatment response. Pre-treatment clinical stage, pre-treatment carcinoembryonic antigen (CEA), histologic differentiation, tumor budding (TB), tumor infiltrating lymphocytes (TILs), and desmoplastic reaction are among other factors that are currently being considered in multiple other neoplasms to show a prognostic association[9–20]. Similarly, the novel marker C-X-C chemokine receptor 4 (CXCR4) is a membrane bound heptahelical receptor which is low or absent in healthy tissues but highly expressed in multiple tumor types[21,22]. CXCR4 binds to its corresponding ligand chemokine stromal-derived factor 1α (SDF-1α) (CXCL12)[23], and its overexpression is associated with chemotaxis, invasion, angiogenesis and proliferation, independent of the specific tumor histologic findings[22,24]. The goal of the study was to identify factors that are predictive of a pCR or a near-complete response.

2. MATERIALS AND METHODS

2.1 Data Collection

A retrospective review was performed for patients with a confirmed pathologic diagnosis of RA followed by NAT and total mesorectal excision at our institution. A total of 91 patients were identified from 2008 to 2016, for which the archival hematoxylin and eosin (H&E) stained slides from both the diagnostic biopsy and the surgical resection were available. Clinical data were obtained from a prospectively maintained quality improvement rectal cancer database. Approval from the Institutional Review Board of Washington University School of Medicine was obtained prior initiating the study.

2.2 Clinicopathologic Parameters

Demographic and clinical parameters included gender, age at diagnosis, pre-treatment clinical T-, N-, and M-stages, pretreatment CEA levels, NAT modality, duration of NAT, time interval from end of treatment to surgical resection, RFS and overall survival (OS). NAT modalities for this cohort of patients included four different regimens: chemoradiation, short-course radiation alone, short-course total neoadjuvant therapy, and other modalities. Chemoradiation consisted of 28 fractions of 180cGy of pelvic radiation delivered 5 days per week for 6 weeks with concurrent single agent chemotherapy. Short-course radiation consisted of 5 consecutive treatments of 500cGy of pelvic radiation followed by surgery within 2 weeks. Short-course total neoadjuvant therapy included a combination of a short-course radiation followed by 2–6 months of multi-agent FOLFOX-equivalent chemotherapy. The other modalities included chemotherapy alone and long-course radiotherapy alone.

All the archival H&E slides of the diagnostic RA biopsies were examined, and the following pathologic findings were noted: identifiable precursor lesion if any (tubular adenoma, tubulovillous adenoma, villous adenoma, traditional serrated adenoma, or other), tumor histologic differentiation (well-, moderately-, or poorly-differentiated), intratumoral budding (TB), tumor infiltrating lymphocytes (TILs), type of desmoplastic reaction if any, lymphovascular invasion (LVI), perineural invasion (PNI), and mitotic count per 10/high-power field (HPF). TB was defined according to the International Tumor Budding Consensus Conference 2016 (ITBCC) as a single tumor cell or cell clusters of up to 4 tumor cells[17]. TILs was defined as the presence of >4 intratumoral lymphocytes per HPF[12,25]. The type of desmoplastic reaction was classified as myxoid/immature desmoplastic stroma, collagenous/mature desmoplastic stroma or absent for significant stromal response[12].

All the slides of the surgical resection specimens were examined for each case. The presence or absence of pCR was assessed and a percentage of residual tumor was assigned to each case by eyeballing after reviewing all tumor bed slides submitted. The pTR was also recorded as indicated in the current CAP guideline for primary carcinoma of the colon and rectum (version 4.0.1.0) as: group 0 (no viable cancer cells), group 1 (single cells or rare small groups of cancer cells), group 2 (residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells), and group 3 (extensive residual tumor with no evident tumor regression)[26]. The pathologic stage was established according to the American Joint Committee on Cancer (AJCC) staging system (8th edition)[27]. The

resection specimens were grossed according to our institutional protocol: when an evident tumor was identified at least 1 block per cm was included. If the tumor measured less than 3 cm, the entire lesion was submitted, and if no tumor was grossly identified the entire recognizable tumor bed/area of fibrosis was submitted.

All the biopsy and resection slides were reviewed by two pathologist independently involved in recording the findings. The method followed was objective and reproducible, since only minor differences arose in a small subset of cases which were easily resolved by re-review.

2.3 Immunohistochemistry

The archival formalin-fixed paraffin embedded (FFPE) blocks of the diagnostic endoscopic biopsy were available in 85 patients and were used for immunohistochemical (IHC) analysis. In cases where more than one FFPE block or biopsy parts were available, one representative block per case was selected. IHC was performed using mouse monoclonal IgG2 antibodies against human CXCR4 (R&D systems, clone #44716) on 5 μm unstained whole slides. Invasive breast caricnoma, renal cell carcinoma, and non-small cell lung carcinoma were used as positive controls[23,28]. Inflammatory cells present in the biopsy served as internal positive control[23]. Appropriate negative controls were used. The location of reactivity was recorded as cytoplasmic only, nuclear only, or cytoplasmic and nuclear, as previously reported[29]. The intensity of the reactivity was interpreted as weak, intermediate, or strong, and an estimated percentage of reactivity in the tumor cells was also noted.

2.4 Statistical Analysis

The clinical characteristics were summarized using descriptive statistics. Continuous and categorical variables were compared by Kruskal-Wallis test and Chi-square test, respectively. OS was defined as the years from the date of surgery to death. Alive patients were censored at the last follow-up. RFS was defined as the years from the date of surgery to recurrence. Patients without recurrence were censored at the last follow-up. Recurrence free probabilities were calculated using Kaplan-Meier plot. Differences between groups were determined by logrank test. Cox proportional-hazards models were used to evaluate the relationship of the interested variables for OS and RFS analysis. The proportionality assumption was tested by adding a time-dependent covariate for each variable. The variables with $p<0.25$ from the univariate models were considered in the multivariable model. The stages included pre-treatment clinical N-stage, T-stage, and cTNM prognostic stage groups, and post-treatment pathologic N-stage, T-stage, and ypTNM prognostic stage groups. Given the possible correlation among these, ypTNM prognostic stage groups had the highest priority. The final multivariable model was built using the backward stepwise selection approach to identify all significant risk factors. Factors significant at a 10% level were kept in the final model. All statistical tests were two-sided using an $\alpha = 0.05$ level of significance. SAS Version 9.4 (Cary, NC) was used to perform all statistical analyses.

3. RESULTS

3.1 Patient Characteristics

The demographic and clinical characteristics are summarized in Table-1. The male-to-female ratio was 1.4:1 with a mean age of diagnosis of 58.6 ±12.3 years. The pretreatment CEA level was available in 77 patients with a mean value of 11.0 ±32.3 ng/mL (reference range: 0.0 – 5.0 ng/mL). The majority of the patients were pre-treatment clinical stage T3 (73.6%). Clinical node-positive disease was more prevalent than node-negative disease (62.7%). The majority of patients received chemoradiation (51.7%) followed by short course total neoadjuvant therapy (29.7%), short course radiation alone (13.2%), and other treatment modalities (5.5%) including chemotherapy alone (2 cases, 2.2%) and radiotherapy alone (3 cases, 3.3%). The time interval from end of treatment to surgical resection was available in 84 patients with a mean duration of 94.3 ±91.8 days. The mean clinical follow-up time was 4.1 ±2.4 years.

3.2 Tumor Characteristics

The majority of the RA were moderately-differentiated (76.9%), followed by well-, and poorly-differentiated, in 15.4% and 7.7% of the cases, respectively (Table-1). In 29.7% of the cases, a precursor lesion was not identified. The most common pre-neoplastic lesion identified was tubular adenoma (42.9%), followed by tubulovillous adenoma (20.9%) and villous adenoma (6.6%). TB was present in 29 cases (31.9%; Figure-1a), and TILs were present in 17 cases (18.7%; Figure-1b). LVI and PNI were identified in 5 (5.5%) and 2 (2.2%) of the cases, respectively. The mean number of mitoses in 10HPF was 28.2 ±19.4. Collagenous/mature desmoplastic stroma was present in 28 cases (30.8%; Figure-1c), myxoid/immature desmoplastic stroma in 32 cases (35.2%; Figure-1d), and in 31 cases (34.1%), prominent desmoplastic reaction was not identified.

On review of the resection specimens, a pathologic complete response was achieved in 15 cases (16.5%; Figure-2a), and the mean percentage of residual tumor was 38.3 ±38.4% (Table-1). Nineteen cases (20.9%) were considered as pTR group 1 (Figure-2b), 28 cases (30.8%) group 2 (Figure-2c), and 29 cases (31.9%) group 3 (Figure-2d). The majority of the cases were post-treatment pathologic T-stage 3 (44 cases, 48.4%), ypN0 (64 cases, 70.3%), and post-treatment pathologic TNM prognostic stage group II (30 cases, 33.0%).

3.3 Correlation of Clinicopathologic Characteristics with CAP Treatment Response Groups

Patients in treatment response groups 0, 1 and 2 were associated with a longer time interval from the end of NAT to surgical resection (Table-2) than those with poor or no pathologic response (group 3, $p = 0.022$). Patients in treatment response groups 2 and 3 were associated with a higher pre-treatment clinical T-stage ($p = 0.019$) and pre-treatment clinical stage ($p = 0.004$) than those in treatment response groups 0 and 1. The presence of TILs in the pre-treatment biopsies was associated with a greater treatment response ($p = 0.019$). No other clinicopathologic variables were associated with treatment response (Table-2). There was no statistical significance difference between the treatment modalities and a complete response ($p = 0.273$).

3.4 Recurrence-Free Survival and Overall Survival

On Cox-univariate analysis there were no clinical or pathologic characteristics associated with RFS (Table-3). On Cox-multivariate analysis, pre-treatment TILs was significantly associated with RFS (p = 0.040). CAP treatment response groups was not associates with RFS on cox-multivariate analysis (p = 0.098). On Cox-univariate analysis, OS was associated with gender (p = 0.043), age (p = 0.023), pre-treatment CEA levels (p = 0.001), pre-treatment tumor differentiation (p = 0.033), percentage of residual tumor (p = 0.003), CAP treatment response groups (p = 0.029), and mitotic count in 10HPF (p = 0.052) (Table-4). On Cox-multivariate analysis, gender (p = 0.034), age (p = 0.002), mitotic count in 10HPF (p = 0.007), and CAP treatment response groups (p = 0.001) were associated with OS.

3.5 Correlation of Immunohistochemical Characteristics with Treatment Response

All tumors showed some degree of reactivity for CXCR4 in the pre-treatment biopsies. CXCR4 expression was divided into 3 groups: cytoplasmic (45 cases, 52.9%), cytoplasmic and nuclear (31 cases, 36.5%), and nuclear (9 cases, 10.6%) (Figure-3). There was no significant correlation between CXCR4 expression and pCR (p = 0.219), CAP treatment response group (p = 0.355), ypT-stage (p = 0.206), ypN-stage (p = 0.562), or ypTNM prognostic stage groups (p = 0.761) (Table-5). There was no significant association between CXCR4 expression and the percentage of residual tumor (p = 0.111).

CXCR4 was further analyzed and divided in 2 groups: cytoplasmic (45 cases, 52.9%) and nuclear staining (40 cases, 47.1%). Nuclear CXCR4 expression was associated with a lower percentage of residual tumor compared to cytoplasmic expression (20.3 ±29.9% vs 34 ±36.3%, p = 0.036). Similarly, cases with cytoplasmic expression were associated with higher ypT-stage compared to nuclear expression (p = 0.039). No significant association was seen with pCR, CAP treatment response group, ypN-stage, and ypTNM prognostic stage groups.

4. DISCUSSION

In the treatment of rectal cancer with neoadjuvant therapy, pretreatment clinical T-stage and clinical TNM prognostic stage groups, therapy-to-surgery time interval, and TILs were associated with improved pathologic treatment response. TILs on pretreatment biopsy was also associated with a longer recurrence-free survival, and age, gender, treatment response, and mitotic rate were associated with improved overall-survival. CXCR4 nuclear expression was associated with a lower percentage of residual tumor.

In this study, we sought to evaluate the relationship between clinical and pathologic parameters and pathologic treatment response, recurrence-free survival, and overall survival. In our cohort, 16.5% of the cases achieved a pCR, which is similar to previously reported rates of 15 to 20%[3,5,30–33]. It's well stablished that patients with pCR have significantly better 5-year RFS compare to those without a pCR[32]. Additionally, patients who achieve a cCR to NAT may be able to be treated without surgery[7]. While a cCR is determined by a combination of imaging and endoscopic evaluation, there are borderlines cases where it is

difficult to differentiate treatment response from residual tumor on imaging. Perhaps in these borderline cases tumor characteristics associated with treatment response may guide clinicians.

A shorter time interval from end of NAT to surgical resection was significantly associated with a worse pTR ($p = 0.022$). The appropriate time interval between NAT and surgical resection has previously investigated. One of the first clinical trials to target this question was done by Francois Y. et al.[34], where patients were divided into two groups based on the interval between the end of radiation therapy and resection (short: < 2 weeks, and long: 6 to 9 weeks). The long interval group had a significantly better pTR compared to the short interval group[34]. Another prospective study included 233 stage II and III rectal cancer patients and divided them into two groups: short (≤7 weeks) and long interval (>7 weeks). The long interval group was significantly associated with a better pTR and 3-year local recurrence rate[35]. It has been proposed that radiotherapy produces DNA damage with subsequent cellular death over subsequent weeks to months; therefore, a longer interval allows for continued therapy[33,36].

Alternatively, some studies have shown that a longer neoadjuvant therapy-to-surgery interval is not always beneficial. Calvo FA et al. demonstrated that a surgical delay of ≥6 weeks was not associated with pCR or improved pTR[37]. Likewise, a multicenter clinical trial (GRECCAR-6) randomized patients into a short interval group (7 weeks) and long interval group (11 weeks) and found that the long interval group was associated with a higher morbidity and did not have a significant difference in achievement of pCR[33]. In our cohort, the mean time interval between NAT and resection in the pCR group was 12.6 weeks compared to 8.5 weeks in cases with poor or no response. Our findings suggest that a longer time interval is associated with a better pTR.

In our cohort, pre-treatment CEA level was not associated with pTR, however, a trend of a higher level of CEA was noted in the pTR group 3. In a recent study, a decreased CEA clearance pattern with NAT was associated with pTR and pCR in RA[9]. A similar study found no correlation between pre-treatment CEA and response; however, the post-treatment CEA was significantly lower in the pCR group[10]. A separate study with clinical stage II and III RA found that an elevated pre-treatment CEA level was associated with a worse pTR and OS[13]. Similar to these studies, in our cohort, a lower pre-treatment CEA was significantly associated with a better OS ($p = 0.001$). A limitation of our study is the unavailability of a post-treatment CEA level in all the cases.

Lower pre-treatment clinical T-stage and cTNM prognostic stage were associated with pCR and pTR. As expected, the majority of the clinical stage IV cases (70%) had a poor pTR (group 3) and none of them achieved a pCR. On the other hand, 77.8% of the cases with a pre-treatment clinical stage I were pTR groups 0 and 1. Similar results were noted in prior studies where cases with pCR were significantly associated with a lower pre-treatment clinical stage[14,15]. Patients with non-obstructive, well- or moderately-differentiated tumors and no clinically apparent nodal or distant metastatic disease have been reported to achieve the best pTR[15].

TB has a well-established association with infiltrative tumor growth, perineural and lymphovascular invasion, lymph node metastasis, and advanced pathologic stage[16–19]. Recent studies have showed TB to be more prevalent in cecal adenocarcinomas, and associated with *KRAS* mutations and microsatellite stability[18]. TB has also been identified as an independent prognostic factor for lymph node involvement and as a poor prognostic factor[38–44]. One prior study evaluated TB in RA biopsies before NAT and found TB to be predictive of a poor pathologic response[45]. In our cohort, TB in the pre-treatment biopsies was not associated with pTR grade or pCR. Although not significant, the presence of TB was more predominant in cases with poor or no pTR (12 cases, 41.4%). TB is usually seen at the deep infiltrative edge of the tumor, and biopsy tissue from the luminal aspect is not an ideal site to assess for TB. Although we noted this finding as present or absent due to its prognostic associations, it might not reflect the TB status of the entire tumor and may explain our lack of association between TB and pTR.

TILs has been associated with a better RFS in stage II mismatch repair proficient (pMMR) colon cancer[46] and with better RFS in node-negative colon cancer[47]. It is considered as an independent favorable prognostic marker in right colon and rectal cancers[20]. A decreased number of CD3+ or CD8+ TILs is associated with a worse RFS and OS, regardless of MMR status[48]. In the setting of NAT-RA one prior study evaluated the significance of CD3+ and CD8+ lymphocytes, defined as a percentage of tumor stromal positivity, in pre-treatment biopsy and the post-treatment resected specimens[11]. This study found a significantly higher lymphocyte density in the post-treatment tumors. In addition, a higher pre-treatment CD3+ and CD8+ was associated with better pTR, RFS, and OS[11]. In our study, TILs was significantly associated with a better pTR on univariate analysis and better RFS on multivariate analysis. A limitation in our study is the lack of MMR protein status for many of the included patients. Since our cohort included patients from 2008 to 2016, many of the patients from early on the study period did not have MMR testing performed, as it was not routinely performed on all colorectal tumors at that time. More studies with larger cohorts, the development of novel biomarkers, and advancement in imaging techniques are needed to better predict or detect a pCR, or near pCR.

In our study, we analyzed CXCR4 expression as a possible biomarker of prognosis in rectal cancer. A recent meta-analysis reported nuclear CXCR4 expression to correlate with a poor prognosis in older patients, advanced stage of disease, and poorly differentiated tumor grade[21]. CXCR4 expression is reported to be present in half of the CRCs in both a nuclear and/or cytoplasmic pattern, but only nuclear staining has been associated with worse survival[29]. A recent study analyzed CXCR4 and CXCL12 expression in metastatic rectal adenocarcinoma before and after local radiotherapy and systemic neoadjuvant treatment, and found no correlation between the expression of CXCR4 and CXCL12 and pTR[23]. Similarly, we found no association between CXCR4 expression and pCR, although there was a decrease in the percentage of residual tumor in cases with nuclear, or combined cytoplasmic and nuclear CXCR4 staining, compared to cases with only cytoplasmic staining (29.4% vs 44.7%; $p = 0.036$).

Pre-treatment assessment of tumor differentiation was significantly associated with OS. This is consistent with the established prognostic significance of tumor differentiation in CRC[49].

A lower percentage of residual tumor was also associated with better OS, which is concordant with previous investigations[5]. When controlling for multiple clinical and pathologic variables, CAP pTR group was associated with better OS, as was the mitotic count in 10HPF. A limitation of our study in terms of RFS and OS analysis is the small cohort of cases and a short follow-up period available for analysis.

In conclusion, pathologic factors such as TILs in combination with clinical staging can help predict response to neoadjuvant treatment and may also provide additional prognostic data for survival. These factors may help inform treatment decisions in patients with a borderline complete clinical response who wish to pursue non-operative management of their rectal cancer.

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HIGHLIGHTS

- Lower pre-treatment cT-stage was associated with better treatment response
- Lower AJCC cTNM stage groups was associated with better treatment response
- Tumor-infiltrating lymphocytes was associated with better treatment response
- CXCR4 nuclear expression was associated with a lower percentage of residual tumor

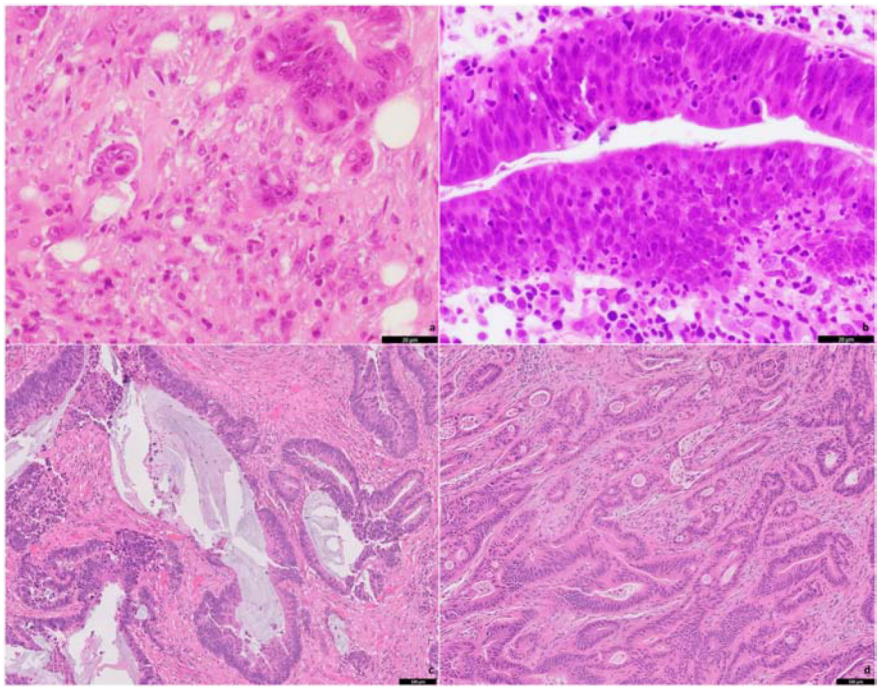

Figure 1.

Separate cases of pre-treatment rectal adenocarcinoma biopsies showing intratumoral budding (a), tumor-infiltrating lymphocytes (b), collagenous stroma/mature fibrosis (c) and myxoid stroma/immature fibrosis (d).

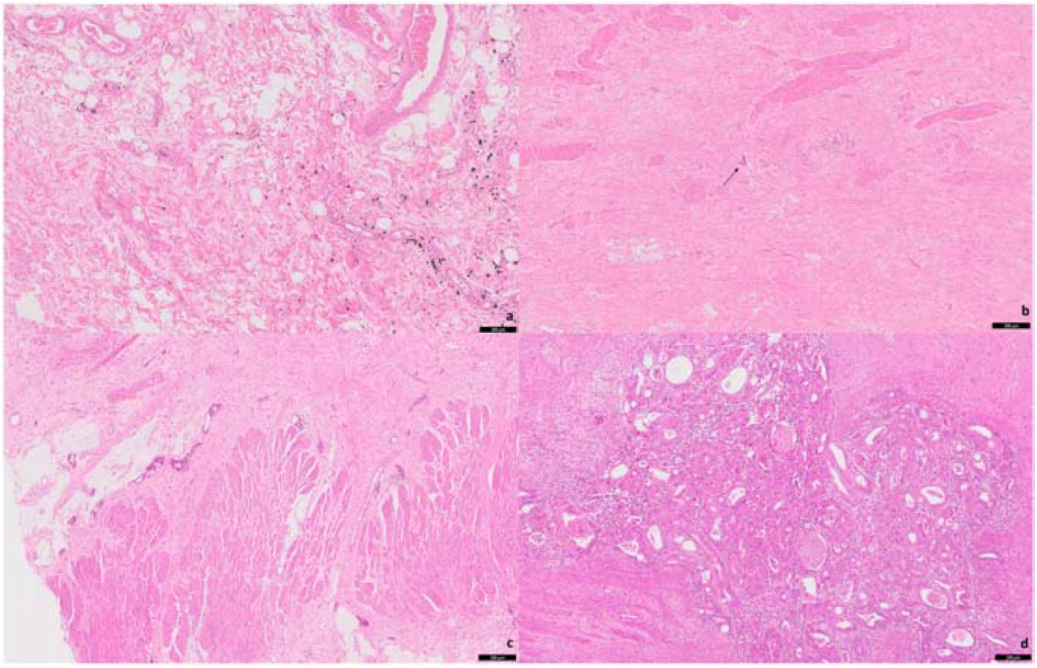

Figure 2.

Post-treatment rectal adenocarcinoma resections showing: complete pathologic response (a); a single group of small residual tumor (b, arrow) consistent with CAP treatment response group 1; evident tumor regression consistent with CAP treatment response group 2 (c); extensive residual tumor with no evident tumor regression consistent with CAP treatment response group 3 (d).

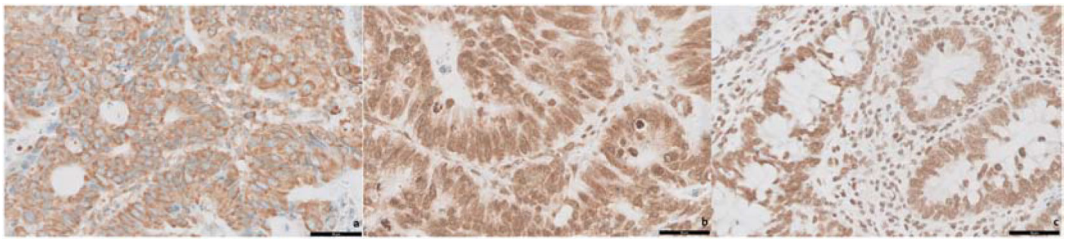

Figure 3.
CXCR4 immunohistochemical reactivity in pre-treatment biopsies showing cytoplasmic (a), cytoplasmic and nuclear (b), and nuclear (c) reactivity.

Table-1.

Patient and Tumor Characteristics (N = 91)

	n, %
Gender	
Male	53, 58.2%
Female	38, 41.8%
Age at diagnosis (mean, SD)	58.6 years, 12.3
Pre-treatment cT-stage	
T1	2, 2.2%
T2	11, 12.1%
T3	67, 73.6%
T4	11, 12.1%
Pre-treatment cN-stage	
N0	34, 37.4%
N1	44, 48.4%
N2	13, 14.3%
Pre-treatment cM-stage	
M0	81, 89.0%
M1	10, 11.0%
Pre-treatment cTNM prognostic stage groups	
I	9, 9.9%
II	23, 25.3%
III	49, 53.9%
IV	10, 11.0%
Pre-treatment CEA level, ng/mL (n, mean, SD)	77, 11.0, 32.3
NAT modality	
Chemoradiation	47, 51.7%
Total neoadjuvant therapy	27, 29.7%
Short course radiotherapy alone	12, 13.2%
Other	5, 5.5%
Time interval from end of NAT to resection, days (n, mean, SD)	84, 94.3, 91.8
Precursor lesion	
Tubular adenoma	39, 42.9%
Tubulovillous adenoma	19, 20.9%
Villous adenoma	6, 6.6%
Not identified	27, 29.7%
Histologic differentiation	

	n, %
Well	14, 15.4%
Moderate	70, 76.9%
Poor	7, 7.7%
Intratumoral budding	
No	62, 68.1%
Yes	29, 31.9%
Tumor infiltrating lymphocytes	
No	74, 81.3%
Yes	17, 18.7%
Desmoplastic reaction	
Myxoid/immature	28, 30.8%
Collagenous/mature	32, 35.2%
Absent	31, 34.1%
Lymphovascular invasion	5, 5.5%
Perineural invasion	2, 2.2%
Mitotic count/10HPF (mean, SD)	28.2, 19.4
Pathologic complete response	
No	76, 83.5%
Yes	15, 16.5%
CAP treatment response groups	
Group 0	15, 16.5%
Group 1	19, 20.9%
Group 2	28, 30.8%
Group 3	29, 31.9%
Percentage of residual tumor (mean, SD)	38.3, 38.4
ypT-stage	
T0	15, 16.5%
T1	5, 5.5%
T2	19, 20.9%
T3	44, 48.4%
T4	8, 8.8%
ypN-stage	
N0	64, 70.3%
N1	18, 19.8%
N2	9, 9.9%
ypTNM prognostic stage groups	

	n, %
0	14, 15.4%
I	18, 19.8%
II	30, 33.0%
III	19, 20.9%
IV	10, 11.0%
Mean follow-up, SD (years)	4.1, 2.4

Abbreviations: T- Tumor; N- Node; M- Metastasis; SD - Standard Deviation; CEA - Carcinoembryonic Antigen; NAT - Neoadjuvant Treatment; Percentages may not add up to 100% due to rounding.

Table-2.

Correlation of Clinicopathologic Features with CAP Pathologic Treatment Response Groups (N = 91)

	Group 0 (n = 15)	Group 1 (n = 19)	Group 2 (n = 28)	Group 3 (n = 29)	*P*
Pre-treatment cT-stage					
T1	0	2, 10.5%	0	0	**0.019**
T2	3, 20%	5, 26.3%	1, 3.6%	2, 6.9%	
T3	11, 73.3%	8, 42.1%	23, 82.1%	25, 86.2%	
T4	1, 6.7%	4, 21.1%	4, 14.3%	2, 6.9%	
Pre-treatment cN-stage					
N0	5, 33.3%	6, 31.6%	7, 25%	16, 55.2%	0.295
N1	9, 60%	10, 52.6%	15, 53.4%	10, 34.5%	
N2	1, 6.7%	3, 15.8%	6, 21.4%	3, 10.3%	
Pre-treatment cTNM prognostic stage groups					
I	1, 6.7%	6, 31.6%	0	2, 6.9%	**0.004**
II	5, 33.3%	1, 5.3%	8, 28.6%	9, 31%	
III	9, 60%	11, 57.9%	18, 64.3%	11, 37.9%	
IV	0	1, 5.26%	2, 7.1%	7, 24.1%	
Pre-treatment CEA levels (mean, SD)	8.4, 23.1	3.3, 3.6	6.3, 10.2	23.4, 55.5	0.120
Time interval from end of NAT to resection, days (median, SD)	88.5, 34.3	101.3, 86.9	123.6, 123.0	59.8, 68.5	**0.022**
Histologic differentiation					
Well	3, 20%	4, 21.1%	5, 17.9%	2, 6.9%	0.357
Moderate	12, 80%	14, 73.7%	22, 78.6%	22, 75.9%	
Poor	0	1, 5.3%	1, 3.6%	5, 17.2%	
Intratumoral budding					
No	12, 80%	12, 63.2%	21, 75%	17, 58.6%	0.421
Yes	3, 20%	7, 36.8%	7, 25%	12, 41.4%	
Tumor infiltrating lymphocytes					
No	11, 73.3%	12, 63.2%	27, 96.4%	24, 82.8%	**0.019**
Yes	4, 26.7%	7, 36.8%	1, 3.6%	5, 17.2%	
Desmoplastic reaction					
Myxoid/immature	3, 20%	5, 26.3%	12, 42.9%	12, 41.4%	0.641
Collagenous/mature	6, 40%	6, 31.6%	9, 32.1%	7, 24.1%	
Absent	6, 40%	8, 42.1%	7, 25%	10, 34.5%	
Lymphovascular invasion					
Yes	0	0	4, 14.3%	1, 3.5%	0.172
No	15, 100%	19, 100%	24, 85.7%	28, 96.5%	
Perineural invasion					

	Group 0 (n = 15)	Group 1 (n = 19)	Group 2 (n = 28)	Group 3 (n = 29)	*P*
Yes	0	1, 5.3%	1, 3.6%	0	0.667
No	15, 100%	18, 94.7%	27, 96.4%	29, 100%	
Mitotic count/10HPF (mean, SD)	32.3, 23.7	31.0, 17.4	24.1, 12.9	28.1, 23.3	0.566

Abbreviations: CEA - Carcinoembryonic Antigen; SD - Standard Deviation; NAT - Neoadjuvant Treatment. Percentages may not add up to 100% due to rounding.

Table-3.

Cox Proportional Hazard Models of Recurrence-free Survival

	Univariate Analysis	Multivariate Analysis	
	P	**HR (95% CI)**	***P***
Gender	0.298		
Age	0.601		
Pre-Tx CEA	0.378		
Pre-Tx cT-stage	0.474		
Pre-Tx cN-stage	0.256		
Pre-Tx cTNM prognostic stage groups	0.052		
Time from Tx to Sx	0.372		
Intratumoral budding	0.464		
Tumor differentiation	0.065		
Tumor infiltrating lymphocytes	0.159	3.085 (1.054 – 9.024)	**0.040**
Type of desmoplastic reaction	0.422		
Pathologic complete response	0.144		
Percentage of residual tumor	0.054		
CAP treatment response groups	0.197		0.098
0		1	
1		1.174 (0.195 – 7.082)	
2		4.219 (0.837 – 21.267)	
3		4.324 (0.911 – 20.517)	
Mitotic count/10HPF	0.553		
ypT-stage	0.146		
ypN-stage	0.243		
ypTNM prognostic stage groups	0.240		

Abbreviations: HR - Hazard ratio; CI - Confidence interval; CEA - Carcinoembryonic antigen; Tx - Treatment; Sx - Surgery; TB- Tumor budding; TILs - Tumor intraepithelial lymphocytes

Table-4.

Cox Proportional Hazard Models of Overall Survival

	Univariate Analysis	Multivariate Analysis	
	P	**HR (95% CI)**	***P***
Gender	**0.043**		**0.034**
Male		1	
Female		0.380 (0.155 – 0.929)	
Age	**0.023**	1.054 (1.019 – 1.091)	**0.002**
Pre-Tx CEA	**0.001**		
Pre-Tx cT-stage	0.975		
Pre-Tx cN-stage	0.061		
Pre-Tx cTNM prognostic stage groups	0.140		
Time from Tx to Sx	0.774		
Intratumoral budding	0.181		
Tumor differentiation	**0.033**		
Tumor infiltrating lymphocytes	0.868		
Type of desmoplastic reaction	0.918		
Pathologic complete response	0.095		
Percentage of residual tumor	**0.003**		
CAP treatment response groups	**0.029**		**0.001**
0		1	
1		3.806 (0.755 – 19.196)	
2		3.983 (1.027 – 15.446)	
3		14.604 (3.477 – 61.333)	
Mitotic count/10HPF	0.052	0.969 (0.948 – 0.991)	**0.007**
ypT-stage	0.096		
ypN-stage	0.609		
ypTNM prognostic stage groups	0.245		

Abbreviations: HR - Hazard ratio; CI - Confidence interval; CEA - Carcinoembryonic antigen; Tx - Treatment; Sx - Surgery

Table-5.

Clinicopathologic features and CXCR4 Expression (N = 85)

	Cytoplasmic (n = 45)	Cytoplasmic and Nuclear (n = 31)	Nuclear (n = 9)	*P*
Pathologic complete response				
No	40, 88.9%	23, 74.2%	8, 88.9%	0.220
Yes	5, 11.1%	8, 25.8%	1, 11.1%	
CAP treatment response groups				
Group 0	5, 11.1%	7, 22.6%	1, 11.1%	
Group 1	7, 15.6%	8, 25.8%	4, 44.4%	0.355
Group 2	16, 35.6%	9, 29%	2, 22.2%	
Group 3	17, 37.8%	7, 22.6%	2, 22.2%	
Percentage residual tumor (mean, SD)	44.7, 37.6	30.0, 37.7	27.3%, 36.8	0.111
ypT-stage				
T0	5, 11.1%	7, 22.6%	1, 11.1%	
T1	2, 4.4%	2, 6.5%	1, 11.1%	
T2	11, 24.4%	6, 19.4%	2, 22.2%	0.206
T3	19, 42.2%	16, 51.6%	5, 55.6%	
T4	8, 17.8%	0	0	
ypN-stage				
N0	29, 64.4%	22, 70.9%	8, 88.9%	
N1	11, 24.4%	6, 19.4%	0	0.562
N2	5, 11.1%	3, 9.7%	1, 11.1%	
ypTNM prognostic stage groups				
0	4, 8.9%	7, 22.6%	1, 11.1%	
I	9, 20%	6, 19.4%	3, 33.3%	
II	15, 33.3%	9, 29%	4, 44.4%	0.761
III	11, 24.4%	6, 19.4%	1, 11.1%	
IV	6, 13.3%	3, 9.7%	0	

Abbreviations: SD - standard deviation; yp – post-treated pathologic. Percentages may not add up to 100% due to rounding.