REVISION LETTER

REVİEWER 03551974: This is a good retrospective review of a series of 113 consecutive patients with locally advanced mid or distal rectum cancer who underwent preoperative neoadjuvant CRT followed by radical resection with total mesorectal excision (TME) for curative intent od locally advanced mid or distal rectal cancer between January 2009 and January 2014 at Sisli Hamidiye Etfal Training & Research Hospital, General Surgery and Oncology departments. The authors have concluded that Studies investigating the optimal time between neoadjuvant CRT and surgery and its effect on pre and postoperative outcomes should be encouraged for better oncological outcomes and lowest morbidity. The manuscript can be accepted after some minor revisions. 1. fufilled the figure legends. 2. Make sure the expression,"Overall survival showed statistical significance in when both groups was compared. (p=0.02). (Figure 1). ...." is right in section"Factors Predicting Local Recurrence, Disease-Free Survival, and Overall Survival."

REVİEWER 03551974:

1. fufilled the figure legends.

REPLY:

We have revised and fulfilled the all figure legends, and highlighted changes as follows:

Figure 1. Distributions of groups with regard to interval time between neoadjuvant therapy and surgery. Median interval periods ± std were 5 ± 1,28 (2-7,8) weeks in group I and 10,1 ± 2,2 (8,2-20,2) weeks in group II (p<0,001).

Figure 2. Comparison of overall survival and disease-free survival between the groups by Kaplan-Meier curves. The median DFS duration in group II was better than group I (p=0.01).

Figure 3. Effect of presence of tumor in lymph nodes and its correlation with OS (A) and DFS (B). Survival rates were better in patients who achieved nodal down-staging (p=0,001).

Figure 4. Correlation between level of the pathologic tumor responses and disease-free survival. Only a poor pathologic response (TRG IV) was associated with worse DFS (p=0,009).

Figure 5. Local recurrences were similar in both interval groups. The interval time did not show any association with local recurrence (p=0,79).

REVİEWER 03551974:

2. Make sure the expression,"Overall survival showed statistical significance in when both groups was compared. (p=0.02). (Figure 1). ...." is right in section"Factors Predicting Local Recurrence, Disease-Free Survival, and Overall Survival.

REPLY:

The expression "(Figure 1)" was removed from end of the sentence ,"Overall survival showed statistical significance in when both groups was compared. (p=0.02)."

REVİEWER 03552168:

I. Major Comments:

1. The Lyon R90-01 trial is the research about the interval between preoperative radiotherapy and surgery in rectal cancer. However, radiotherapy was described as chemoradiotherapy (CRT) in the sentences about the Lyon trial in introduction and discussion. Please check these.

REPLY:

1. We have corrected the expression "chemoradiotherapy (CRT)" in the sentences about the Lyon trial in introduction and discussion, and highlighted changes as follows:

   In the introduction section, page 7, line 8: " …….. between radiotherapy and…"

   In the introduction section, page 7, line 11: "…… interval has been accepted as the appropriate treatment interval between neoadjuvant therapy and surgery (6)."

   In the discussion section, page 15, line 9: "..week treatment interval between radiotherapy and surgery to improve tumor.."

   In the discussion section, page 15, line 11: "… neoadjuvant therapy and surgery (6)."

REVİEWER 03552168:

2. In pathological examination of materials and methods, the criteria of tumor regression grade (TRG) seem to be different from those in other papers. For example, Rödel C et al. described TRG 4 as tumor regression (when no viable tumor cells were detected). However, the authors of this study presented TRG IV as poor response. Please present a reference for TRG in this study and check if the grading criteria are correct.

Reference

Rödel C, Martus P, Papadoupolos T, et al. Prognostic significance of tumor reg ression after preoperative chemoradiotherapy for rectal cancer. J Clin Oncol 20 05 (23);34:8688-96 [PMid:16246976 DOI: 10.1200/JCO.2005.02.1329].

REPLY:

2. Several scoring systems for tumor regression have been described and advocated. Our Department of Pathology have been prefering Ryan scheme for grading of pathologic tumor response, which is suggested by College of American Pathologists. We have presented the reference "Ryan R, Gibbons D, Hyland JMP, Treanor D, White A, Mulcahy HE, O'Donoghue DP, Moriarty M, Fennelly D, Sheahan K. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. Histopathology 2005, 47, 141–146 [PMID: 16045774 DOI: 10.1111/j.1365-2559.2005.02176.x]" for tumor regression grade in pathological examination of materials and methods, page 9, line 21, and reference no 9: "….. according to the Ryan scheme for tumor regression score [9], which is suggested by…."

REVİEWER 03552168:

3. The poor response TRG was described as TRG IV or TRG-4 in the manuscript. Please unify the notation for TRG.

REPLY:

3. After checking the numeral system used for TRG in the manuscript, we have unified all the notation for TRG in the arabic numerals.

   In materials and methods section, page 9, line 24: "..complete response (TRG 1)."

   In materials and methods section, page 9, line 25: "..were assessed as near complete response (TRG 2) while residual cancer outgrown…"

   In materials and methods section, page 9, line 26: "..fibrosis were considered as the minimal response (TRG 3)."

   In materials and methods section, page 9, line 27: "..extensive residual cancer in specimens were found as a poor response (TRG 4)."

   In results section, page 14, line 17: "..moderate pathological regression grades (TRG 1-2-3) provided similar survival.."

   In results section, page 14, line 18: "..but only a poor pathologic response (TRG 4) was associated with worse DFS.."

REVİEWER 03552168:

4. The authors described that continuous variables were represented as mean ± SD (standard deviation) and categorical variables as numbers and percentages in statistical analysis. However, in table 1 and 2, the names of variables were represented with mean or median instead of 'mean ± SD'. In addition, the variables in table 2 and 3 were displayed as only percentage without numbers. Please correct these.

REPLY:

4. In table 1, names of variables have been corrected as 'mean ± SD', as follows:

Table 1. Demographic and clinical charactheristics of patients.

	GROUP I (n=45)	**GROUP II (n=42)**	**p value**
Age (mean± SD)	53,7 ±13,4	58 ±13,2	0,82
Sex (Male/Female)	32/13	31/11	0,62
Localization of tumor from the anal verge (cm) (mean± SD)	5,6±3	6,1±2,8	0,39
T stage (T2/T3/T4)	8/28/9	6/32/4	0,56
Stage (II/III)	4/41	5/37	0,53
Preoperative radiation dose (Gy) (mean)	49,5±1,99	49,5±2	0,78
Follow-up time (month) (mean± SD)	37,2±19,6	31,1±20,7	0,51

In table 2, the names of continuous variables have been corrected and represented with mean ± SD, and categorical variables have been showed as percentages with numbers.

Table 2. The effect of interval time on the perioperative variables.

	Group I (n=45)	**Group II (n=42)**	**p value**
Procedure Type LAR	% 62,2 (n=28)	% 73,8 (n=31)	0,06
ULAR	% 24,4 (n=11)	% 16,7 (n=7)	0,09
APR	% 13,3 (n=6)	% 9,5 (n=4)	0,50
Diverting ileostomy	% 93,3 (n=42)	% 92,9 (n=39)	0,90
Operative time (min) (mean± SD)	134,2±19,9	133,4±23,5	0,62
Intraoperative complications	% 8,9 (n=8)	% 7,1 (n=3)	0,48
Postoperative complications	% 28,9 (n=13)	% 26,1 (n=11)	0,42
Early postoperative mortality	% 2,3 (n=1)	% 4,7 (n=2)	0,37
Hospital stay (day) (mean± SD)	11±10,5	10±9,3	0,32

Variables in the table 3 have been checked and showed as percentages with numbers.

Table 3. Comparison of pre and post treatment stages in both groups.

	Group I (n=45)		**Group II (n=42)**		**Comparison of Group I and Group II**
	Pre-treatment	Post-Treatment	Pre-Treatment	Post-Treatment	P value
T stage T0	-	% 18,9 (n=9)	-	% 19 (n=8)	0,17
T1	-	% 4,4 (n=2)	-	% 4,8 (n=2)	
T2	% 17,8 (n=8)	% 31,7 (n=14)	% 14,3 (n=6)	% 33,4 (n=14)	
T3	% 62,2 (n=28)	% 42,8 (n=19)	% 76,2 (n=32)	% 38,1 (n=16)	
T4	% 20 (n=9)	% 2,2 (n=1)	% 9,5 (n=4)	% 4,8 (n=2)	
Stage					**0,002**
Stage 0	-	% 8,9 (n=4)	-	% 19 (n=8)	
Stage 1	-	% 24,4 (n=11)	-	% 35,7 (n=15)	
Stage 2	% 8,9 (n=4)	% 17,8 (n=8)	% 11,9 (n=5)	% 26,2 (n=11)	
Stage 3	% 91,1 (n=41)	% 48,9 (n=22)	% 88,1 (n=37)	% 19 (n=8)	
Postop LN without metastasis	% 46,7 (n=21)		% 81 (n=34)		**0,001**

REVİEWER 03552168:

5. In results, it would be better that the grading (A, B, and C) of anastomotic leakage would be explained without the concrete presentation of the cases such as diagnosis and treatment of post-operative complications.

REPLY:

5. In line with suggestions, in results section, anastomotic complications were recomposed and explained without the concrete presentation of the cases.

   In results section, page 12, lines 2-6: "In group I, three cases with diverting stoma developed peri-anastomotic abscess in the pelvis. These patients were classified as Grade B anastomotic complications, and managed with percutaneous abscess drainage and antibiotics successfully. Two cases who were not diverted at the time of TME were required re-operations for Grade C anastomotic leakages."

   In results section, page 12, lines 11-18: "Grade A anastomotic complications appeared in four patients with diverting ileostomy who were treated with antibiotics without the need for invasive interventions or surgical procedures. Grade B anastomotic complications was occured in three cases, one of them had not diverting stoma. These patients were underwent percutaneous abscess drainage and treated with antibiotics. One patient who was not diverted with a stoma at the time of TME developed anastomotic leakages classified as Grade C. Surgical procedure was performed for this case."

REVİEWER 03552168:

6. Please describe whether there were the patients who received adjuvant chemotherapy after surgery in this study in the manuscript.

REPLY:

6. We have added the patients who received adjuvant therapy after surgery in this study in the manuscript.

   In results section, page 13, lines 19-23: "A total of 60 patients who diagnosed stage 2 or 3 disease after histopathological examination of TME specimens, including 30 patients in each group, were recommended postoperative adjuvant therapy. However, a total of 57 patients eventually received adjuvant therapy after surgery due to early postoperative mortality in three patients."

REVİEWER 03552168:

7. Table 4 in results is too complex. It would be better that the information on the relationship between group I and II and TRG would be presented by creating another table or reorganizing table 4.

REPLY:

7. We have reorganized the Table 4 for better understanding, as follows:

Table 4. Analysis of the effect of factors on pathologic tumor regression grades.

	Relationship between patient's demographics and TRG (p*)					**Distribution and comparison of TRG rates in both groups**		
TRG**	**Age**	**Sex**	**Tumor Localization**	**Preop T Stage**	**Preop Stage**	**Group I (n=45) %**	**Group II (n=42) %**	**p value**
Complete response	0,46	0,84	0,17	0,24	0,48	(n=4) 8,9	(n=8) 19	0,36
Near complete response	0,91	0,79	0,38	0,75	0,80	(n=9) 20	(n=14) 33,3	0,35
Minimal Response	0,79	0,59	0,12	0,66	0,38	(n=12) 26,7	(n=16) 38,1	0,15
Poor response	0,48	0,95	0,11	0,19	0,70	**(n=20) 44,4**	**(n=4) 9,5**	**0,002**

REVİEWER 03552168:

8. In results, there was no presentation about disease-free survival rates at 60 months of group II on page 13. Please check this.

REPLY:

8. Disease-free survival rates at 60 months of group II remained same as at 24 months due to no recurrence of the disease in the period between 24 and 60 months.

We have restated the case in results section, page 14, lines 9-10:
"Disease-free survival rates were 85.1% at 24-months, and remained unchanged at 85.1% until the sixtieth month in group II."

REVİEWER 03552168:

9. It would be better that hazard or odds ratio and 95% confidence interval for each variable would be presented in table 4 and 5.

REPLY:

9. We have reorganized table 4 and table 5, in line with suggestions. Odds ratio in table 4 and hazard ratio in table 5 were presented with 95% confidence intervals for each variable.

Table 4. Analysis of the effect of factors on pathologic tumor regression grades.

	Relationship between patient's demographics and TRG (p) and OR with 95% CI					**Distribution and comparison of TRG rates in both groups**		
TRG	**Age**	**Sex**	**Tumor Localization**	**Preop T Stage**	**Preop Stage**	**Group I (n=45) %**	**Group II (n=42) %**	**p value**
Complete response	(0.46) 2.19, 95% CI 0.55-8.72	(0.84) 0.54, 95% CI 0.12-2.29	(0.17) 0.66, 95% CI 0.15-2.92	(0.24) 1.00, 95% CI 0.90-1.50	(0.48) 1.21, 95% CI 0.12-11.8	(n=4) 8,9	(n=8) 19	0,36
Near complete response	(0.91) 1.02, 95% CI 0.37-2.79	(0.79) 1.11, 95% CI 0.36-3.38	(0.38) 1.93, 95% CI 0.64-5.8	(0.75) 0.50, 95% CI 0.12-2.57	(0.80) 0.36, 95% CI 0.10-1.51	(n=9) 20	(n=14) 33,3	0,35
Minimal Response	(0.79) 0.67, 95% CI 0.25-1.76	(0.59) 1.25, 95% CI 0.43-3.63	(0.12) 0.45, 95% CI 0.16-1.20	(0.66) 2.25, 95% CI 0.36-13.8	(0.38) 2.02, 95% CI 0.37-10.9	(n=12) 26,7	(n=16) 38,1	0,15
Poor response	(0.48) 0.98, 95% CI 0.35-2.76	(0.95) 0.94, 95% CI 0.31-2.82	(0.11) 1.65, 95% CI 0.56-4.84	(0.19) 3.22, 95% CI 0.92-11.2	(0.70) 1.34, 95% CI 0.23-7.62	**(n=20) 44,4**	**(n=4) 9,5**	**0,002**

p<0,05 is statistical significance

OR: Odss Ratio

TRG: Tumor Regression Grade

Table 5. Effect of factors on the overall and disease-free survivals

	Overall Survival		Disease-free Survival	
	p value	**HR with 95% CI**	**p value**	**HR with 95% CI**
Sex	0.61	0.97, 95% CI 0.19-4.99	0.69	0.50, 95% CI 0.46-4.46
Age	0.57	1.01, 95% CI 0.95-1.08	0.60	1.00, 95% CI 0.94-1.06
Tumor localization	0.53	0.97, 95% CI 0.72-1.30	0.88	1.17, 95% CI 0.80-1.70
Pre-Treatment Stage	0.94	0.77, 95% CI 0.80-7.50	0.45	0.90, 95% CI 0.80-1.50
Pre Treatment T stage	0.59	1.08, 95% CI 0.21-5.48	0.39	0.39, 95% CI 0.15-3.02
Post Treatment Stage	**0.01**	18.07, 95% CI 0.60-53.9	**0.007**	0.82, 95% CI 0.10-6.23
Post Treatment T stage	0.13	0.62, 95% CI 0.34-11.3	0.07	0.25, 95% CI 0.19-8.54
Postoperative metastatic lymph node (+)	**0.001**	0.91, 95% CI 0.69-1.20	**0.001**	1.25, 95% CI 0.93-1.67
Pathologic TRG	0.11	0.90, 95% CI 1.28-6.35	**0.04**	1.19, 95% CI 0.17-8.41

HR: Hazard Ratio

CI: Confidence Interval

TRG: Tumor Regression Grade

REVİEWER 03552168:

10. The authors commented the previous studies about the interval time between neoadjuvant CRT and surgery in rectal cancer in discussion. It would be better to organize these studies into a new table.

REPLY:

10. We have organized the previous studies about the interval time between neoadjuvant CRT and surgery in rectal cancer into a new table, and indicated in the discussion section, page 15, line 19: "Several studies that have examined the effect of different interval times after neoadjuvant CRT on tumor response, pCR, local tumor control and survival have presented with conflicting findings (Table 6)."

Table 6. Studies comparing the effects of the interval periods between neoadjuvant therapy and surgery on oncological outcome in locally advanced rectal cancer.

Reference	Total number of patients	Design	Interval time (week)	pCR	Local recurrence	Overall survival
Francois et al. [6], 1999	201	Prospective, randomized	2 / 6-8	7% / 14%[a]	13% / 10%[b]	69% / 66%[b]
Wolthuis et al.[20], 2012	356	Retrospective	≤7 / >7	16% / 28%[a]	6% / 3%[a]	NA
Kalady et al. [21], 2009	306	Prospective	<8 / ≥8	16.3% / 28%[a]	NA	NA
Garcia-Aguilar et al.[22], 2011	136	Prospective, nonrandomized	6 / 11	18% / 25%[a]	NA	NA
De Campos-Lobato et al.[23], 2011	177	Retrospective	<8 / ≥8	16.5% /30.8%[a]	1.2% / 10.5%[a]	NA
Tulchinsky et al. [24], 2008	132	Retrospective	≤7 / >7	17% / 35%[a]	6% / 4%[a]	81% / 93%[b]
Sloothaak et al.[25], 2013	1593	Prospective	<13/ 13-14 /15-16	10% / 13% /18%[a]	NA	NA
Sağlam et al.[31], 2014	153	Prospective, randomized	4 / 8	19.7% / 14.3%[b]	11.8% / 10.3%[b]	76.5% / 74.2%[b]
Rödel et al.[36], 2005	385	Prospective	>6	10.4%	3%	85%
Kerr et al.[42], 2008	189	Retrospective	Median 76 day (6-215 day)	15.9%	21%	NA

[a] significant difference statistically
[b] not significant difference statistically
w: week
NA: not available

REVİEWER 03552168:

11. In discussion, the authors described that this study established a negative correlation between TRG 4 (poor response) and disease-free survival (DFS), which has not been mentioned in previous studies. Then, please describe its clinical meaning and significance more clearly in discussion.

REPLY:

11. We have described the clinical meaning and significance of TRG 4 (poor response) in discussion section, page 16, lines 1-2 and lines 4-7.

"It is questionable whether the poor tumor response could also reduce DFS, while pCR is assumed as an indicator of improved DFS as noted in many studies. Our findings further established a negative correlation between TRG 4 (poor response) and DFS, an outcome that has not been mentioned in previously published studies. Its clinical significance represent the poor prognosis in terms of recurrence of the disease. Thus, the possibility of early recurrence of the disease should be considered in follow up of patients who had TRG 4."

REVİEWER 03552168:

II. Minor Comments:

1. It would be better to use a term, ‘standard deviation’ rather than the abbreviation, ‘std’ in the phrase, 'median interval periods ± std' of abstract and results.

REPLY:

1. We have corrected the abbreviation of ‘std’ and used the term ‘standard deviation’ in the phrase, 'median interval periods ± std' of abstract and results.

In abstract, page 5, line 12: “…comparison of median interval periods ± standard deviation of groups..”

In results, page 11, line 7: “..comparison of median interval periods ± standard deviation of groups…”

REVİEWER 03552168:

2. Page 6, line 14: The phrase ‘disease-free (DFS)’ should be changed into ‘disease-free survival (DFS)’.

REPLY:

2. We have changed the phrase ‘disease-free (DFS) into ‘disease-free survival (DFS)’, coinciding in page 7, line 14: “….disease-free survival (DFS) and ….”

REVİEWER 03552168:

3. The explanation of the abbreviation for total mesorectal excision (TME) was described repeatedly in introduction, materials and methods, and discussion. Please correct these.

REPLY:

3. We have removed repeated explanation of the abbreviation for total mesorectal excision (TME) in introduction page 7, line 3; materials and methods, page 8, line 4 and 13; and in discussion page 15, line 2.

REVİEWER 03552168:

4. The explanation of the abbreviation for tumor regression grade (TRG) was described repeatedly in materials and methods, discussion, and conclusion. Please correct these.

REPLY:

4. We have removed repeated explanation of the abbreviation for tumor regression grade (TRG) in materials and methods, page 9, line 19; in discussion, page 18, line 9; and in conclusion, page 20, line 4.