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Microscopic intramural extension of rectal cancer after neoadjuvant chemoradiation: a meta-analysis based on individual patient data

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Gabriel Paiva Fonseca, Murillo Bellezzo, Frank Verhaegen, Evert Jan Van Limbergen and Maaïke Berbee have a patent EP16204735, rectal brachytherapy applicator with royalties paid.

The other authors have no potential conflicts of interest to disclose.

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Microscopic intramural extension of rectal cancer after neoadjuvant chemoradiation: a meta-analysis based on individual patient data

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Keywords: rectal cancer; response; chemoradiation; microscopic spread; margin concept; brachytherapy; contact therapy; boost

Abstract

Objective: In selected rectal cancer patients with residual local disease following neoadjuvant chemoradiation (CRT) and the preference of an organ preservation pathway, additional treatment with dose escalation by endoluminal radiotherapy (RT) may ultimately result in a clinical complete response. To date, the widespread introduction of selective endoluminal radiation techniques is hampered by a lack of evidence-based guidelines that describe the radiation treatment volume in relation to the residual tumor mass. In order to convert an incomplete response into a complete one with additional treatment such as dose-escalation with endoluminal RT from a theoretical perspective, it seems important to treat all remaining microscopic tumor cells after CRT. In this setting, residual tumor extension beneath normal appearing mucosa (microscopic intramural spread – MIS) becomes relevant for accurate tumor volume and margin estimation. With the goal of providing evidence-based guidelines that define an appropriate treatment volume and patient selection, we present results from a meta-analysis based on individual patient data of studies that have assessed the extent or range of MIS of rectal cancers after neoadjuvant CRT. This meta-analysis should provide an estimate of the residual tumor volume/extension that needs to be targeted by any additional radiation therapy boost in order to achieve complete tumor eradication after initial incomplete or near-complete response following standard CRT.

Methods and Materials: A PubMed search was performed. Additional articles were selected based on identification from reference lists. Papers were eligible when reporting MIS in patients who were treated by total mesorectal excision or local excision/transanal endoscopic microsurgery (TEM) after neoadjuvant long-course CRT. The mean MIS was calculated for the entire group along with the 70th until 95th percentiles. Additional exploratory subgroup analyses were performed.

Results: Individual patient data from 349 patients with residual disease from five studies were analyzed. 80% of tumors showed no MIS. In order to appropriately treat MIS in 95% of rectal cancer patients after CRT, a margin of 5.5 mm around the macroscopic tumor would suffice. An exploratory subgroup analysis showed that T-stage after CRT (ypT) and time interval between neoadjuvant CRT and surgery are

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9 significant factors predicting the extent of MIS ($p < 0.001$.) The group of ypT1 had the smallest MIS,
10 followed by the ypT3-4 group, while the ypT2 group had the largest MIS ($p < 0.001$). Regarding time
11 interval between CRT and surgery, a statistically significant difference was seen when comparing the
12 three time-interval groups (less than 8 weeks, 8-12 weeks, and more than 12 weeks), where waiting more
13 than 12 weeks after CRT resulted in the largest MIS ($p < 0.0001$).
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16 *Conclusion:* Based on this meta-analysis, in order to treat the MIS for 95% of rectal cancer patients after
17 CRT, a Clinical Target Volume (CTV) margin of 5.5 mm from the lateral most edge of the macroscopic
18 tumor would suffice. 80% of tumors showed no MIS and would not require an extra CTV margin for
19 treatment. These findings support the feasibility of localized radiotherapy boosts for dose-escalation to
20 improve response among patients with incomplete response after standard CRT and can also be applied
21 in the surgical setting.
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25 26 **Introduction**

27 The treatment and outcomes for rectal cancer patients have dramatically improved in the last decades.
28 The implementation of total mesorectal excision (TME), which enables an R0 resection of the primary
29 tumor and potentially involved mesorectal lymph nodes, has resulted in a decrease of locoregional
30 recurrences¹. The introduction of neoadjuvant therapy (radiotherapy or chemoradiation (CRT)) based on
31 high-risk factors has led to a further decline in locoregional failure^{2,3}. Despite these improvements, the
32 combination of neoadjuvant CRT and a TME-based rectal cancer resection is associated with an increased
33 risk of fecal incontinence, low anterior resection syndrome, as well as sexual and urinary dysfunction⁴⁻⁷.
34 For elderly patients, significant peri-operative morbidity and mortality risk also exist^{8,9}. Additionally,
35 patients with distally located rectal tumors often face a permanent colostomy, which may have a
36 significant impact on quality of life^{10,11}.
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42 Following long-course neoadjuvant CRT using standardized doses (usually 50 Gy or 50.4 Gy in 25
43 or 28 fractions, respectively), a pathologic complete response is seen in 8-20% of patients after
44 surgery^{3,12}. Phase I-II trials have shown that in highly selected patients with a complete clinical response
45 after neoadjuvant treatment, a watch and wait protocol might be considered instead of surgery^{13,14}. This
46 could spare selected patients an extensive operation and, for patients with distal tumors, a permanent
47 colostomy. The number of complete responses is likely to increase if higher radiation doses to the tumor
48 could be used, as shown in a phase II trial using a boost dose given by brachytherapy^{15,16}. The radiation
49 boost can be given to the tumor using either external beam radiotherapy (EBRT) or an endoluminal
50 technique such as brachytherapy or contact X-ray radiotherapy (CXT)^{15,17,18}. This boost can be
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administered before or after CRT. Giving the boost dose following CRT has the advantage that (a) it could potentially be delivered to a smaller tumor volume resulting in less toxicity (as tumor volume generally shrinks during CRT) and (b) that it may even be completely avoided in case of complete clinical response.

Important advantages of endoluminal techniques include the possibility to apply a more selective/localized boost compared to EBRT. Selective irradiation allows tumor dose escalation to higher levels and limits the chance of radiation-induced toxicity¹⁹. Hence, CXT according to the Papillon method has been re-introduced in a limited number of clinics. Due to the sharply falling depth-dose characteristics of CXT, fractional doses up to 30 Gy and total doses up to 90 Gy can be applied without causing significant normal tissue toxicity^{17,20}. As described above, a brachytherapy boost has also been used, showing an increase in the rate of pathological complete response¹⁵.

To date, the widespread introduction of selective endoluminal radiation techniques is hampered by the lack of evidence-based guidelines that describe the radiation treatment volume. In order to obtain a durable complete response, it would seem important from a theoretical perspective to treat all tumor cells remaining after CRT. This entails treating not only any visible mucosal lesion, in radiotherapy terms called the Gross Tumor Volume (GTV), but also potential microscopic intramural spread (MIS) or fragments of the tumor in the wall, called the Clinical Target Volume (CTV). Hence, the CTV should include the GTV as well as a margin for potential MIS. To provide evidence-based guidelines that define an appropriate treatment volume, we performed a meta-analysis based on individual patient data of studies that have assessed the extent or range of MIS of rectal tumors after neoadjuvant CRT.

The data generated by this meta-analysis can also be applied in the surgical setting. Local excision via transanal approaches including Transanal Endoscopic Microsurgery (TEM) or Transanal Minimally Invasive Surgery (TAMIS) of a residual (small) tumor after CRT are surgical organ-preserving alternatives to the selective radiation boost^{21,22}. Here too, there is no clear consensus on the margin of “healthy” tissue surrounding the residual tumor containing potential microscopic disease that should be excised²³. The results of this meta-analysis could therefore also be used to determine the surgical margin for local surgical techniques or the distal margin when a sphincter-sparing Low Anterior Resection with coloanal anastomosis is being considered in patients with an ultra-distal rectal cancer.

As certain tumor characteristics, such as tumor size, lymphatic, vascular or perineural invasion, may be predictive for the presence of MIS²⁴, the secondary aim of this meta-analysis was to identify potential factors that may be predictive for the absence or presence and the extent of MIS. Such factors may be useful in the future to select patients who are suitable candidates for selective endoluminal

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9 boosting and omission of surgery or very localized surgery, or who are likely better off with non-organ
10 preserving surgery.
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12 **Methods**

13 *Protocol and registration*

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15 This paper was written using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses
16 (PRISMA) *Checklist of items to include when reporting a systematic review and meta-analysis 2009*²⁵.
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19 *Search strategy*

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21 A search was performed in November 2016 by the first and second-to-last authors and updated on May
22 9th, 2018 by the first author. The PubMed search strategy used is listed below:
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- 24 • "Rectal Neoplasms"[Mesh] AND (("neoadjuvant therapy"[MeSH Terms] OR ("neoadjuvant"[All
25 Fields] AND "therapy"[All Fields]) OR "neoadjuvant therapy"[All Fields] OR "neoadjuvant"[All
26 Fields]) AND spread[All Fields])
- 27 • "Rectal Neoplasms"[Mesh] AND (lateral[All Fields] AND spread[All Fields] AND ("CRT"[MeSH
28 Terms]
- 29 • "Rectal Neoplasms"[Mesh] AND (intramural[All Fields] AND spread[All Fields] AND ("CRT"[MeSH
30 Terms] OR "CRT"[All Fields] OR "CRT"[All Fields]))
- 31 • "Rectal Neoplasms"[Mesh] AND (intramural[All Fields] AND spread[All Fields])
- 32 • "Rectal Neoplasms"[Mesh] AND spread[All Fields] AND ("CRT"[MeSH Terms]
- 33 • "Rectal Neoplasms"[Mesh] AND microscopic[All Fields] AND ("CRT"[MeSH Terms]

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41 Additional articles were selected based on identification from reference lists.
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43 *Study selection*

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45 Published articles were selected and evaluated by the first and second-to-last authors. First, eligibility was
46 determined based on title and abstract screening. Remaining articles were selected based on full-text
47 screening. Studies were eligible when reporting in English, experimental or observational studies, and
48 reporting submucosal or otherwise MIS in patients who received a total mesorectal excision or local
49 excision/TEM after neo-adjuvant long-course CRT. Studies only including patients who received surgery
50 immediately after neo-adjuvant treatment were excluded, as little to no pathological response was
51 expected. Publication dates between 1970 and 2018 were included. We determined 1970 as cut-off value
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due to differences in standard treatment for rectal cancer and advances in the technical aspects of radiation oncology in the recent decades. Conference abstracts were excluded. Authors of selected papers were approached by e-mail and asked whether they were willing to collaborate on this meta-analysis project. Authors who agreed were asked to fill in a data transfer agreement to ensure confidentiality from both parties, after which the anonymized individual patient data were transferred. Selected papers of which the authors eventually did not send their individual patient data or from which no response was received were excluded from analysis after several attempts of communication via mail and phone.

Data extraction and analysis

Data was extracted by full-text screening of the study as well as from the individual patient data using a self-made format reporting on (1) basic study demographics (country, study design, years of patient inclusion, number of patients and stages of disease); (2) treatment demographics (radiation dose, type of chemotherapy, median length of follow up and primary endpoints); (3) reporting of intramural spread; (4) risk of bias assessment. Patients with a pathological complete response were excluded. Descriptive and statistical analyses of the combined individual patient data were performed.

Statistics

The mean MIS was calculated for the entire group along with the percentiles between the 70th and the 95th by increments of 5. 95% Confidence intervals for these different percentiles were calculated using a bootstrap procedure with 10.000 samples.

An explorative analysis (percentiles with confidence intervals) was also performed on subgroups to test whether certain factors were predictive for MIS. Subgroups were made on the basis of ypT stage (ypT1 vs. 2 vs. 3-4), tumor size (median split), tumor diameter (median split), tumor grade of differentiation in the surgical specimen (1 vs 2 vs 3), vascular invasion in the surgical specimen (yes/no), lymphatic invasion in the surgical specimen (yes/no), perineural invasion in the surgical specimen (yes/no), and time between CRT and surgery (less than 8 weeks vs. more than 8 weeks, less than 12 weeks vs. more than 12 weeks, and less than 8 weeks vs. 8-12 weeks vs. more than 12 weeks). All subgroups were compared using a non-parametric test, the Mann-Whitney U test in case of two groups and the Kruskal-Wallis test (with post-hoc pairwise Mann-Whitney U tests if applicable) in case of 3 or more groups.

Comment [AV1]: It was specified that the subgroup analysis was performed on the surgical specimens.

Results

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Study Selection

For the study selection flow chart, we refer to Figure 1. The PubMed search resulted in 168 records. Two additional records were included on identification of reference lists. Based on title and abstract screening, 143 publications were excluded due to various reasons, including the absence of pathology assessment and absence of neo-adjuvant treatment. After full text screening, eleven studies met the inclusion criteria and were included in this systematic review. The search was last updated in May 2018. Nine out of eleven authors responded that they were willing to send us their individual patient data. Two authors were unable to retrieve their databases due to changes of workplace and their papers were thus excluded. Of the seven studies that were then included, we received the individual patient data of five papers^{23,24,26-28}. Two of the papers reported on the same study and therefore we received one dataset for these two papers^{26,27}.

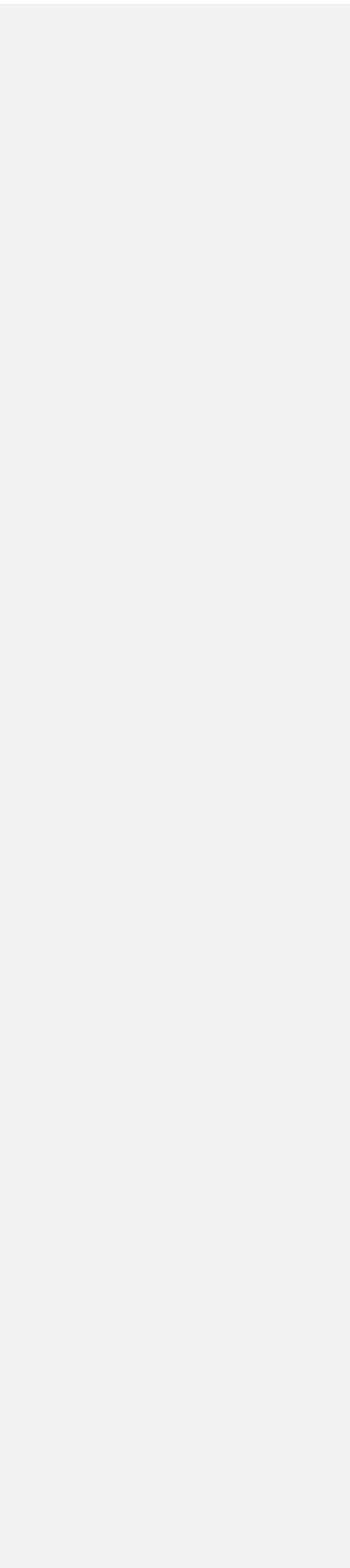
Study characteristics

For a summary of the study demographics, we refer to Table 1. Five studies with individual patient data from 349 patients were included in this meta-analysis^{23,24,26-28}. Two papers reported on the same prospective randomized trial comparing short-course radiotherapy (5x5 Gy) followed by immediate resection and CRT followed by delayed surgery. We excluded the patients in the short-course radiotherapy arm as for the purpose of this meta-analysis the response after CRT was of interest^{26,27}. The remaining three studies included two prospective observational studies and one retrospective observational study^{23,24,28}.

Treatment characteristics

For a summary of treatment characteristics, we refer to Table 2. All included patients received long course CRT followed by delayed surgery. The most commonly used radiation scheme was 50.4 Gy delivered in fractions of 1.8 Gy to the primary tumor, pathological regional lymph nodes and elective lymph node areas. The most commonly used chemotherapy was 5-fluorouracil-based. The time from CRT to surgery varied from 4-6 weeks in the prospective randomized trial to a median of 16.5 weeks in the observational retrospective study^{23,26,27}. All studies included patients who received TME surgery after neo-adjuvant CRT except for one study, in which all patients received TEM²³.

Pathological analysis



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In the prospective randomized trial, workshops for the participating pathologists were held before and during the trial to align the protocol and measurement methods of margins^{26,27}. In the two observational prospective studies, pathological examination was done by one or two dedicated pathologists^{24,28}. In all studies, pathological analysis was performed according to each institution's standards.

We define MIS as the greatest distance between the microscopic tumor cells in the bowel wall and the nearest edge of the macroscopic ulcer/tumor residue parallel to and perpendicular to the bowel wall between the microscopic tumor cells in the bowel wall and the nearest superficial edge of the macroscopic ulcer/tumor residue. In the prospective randomized trial, as well as in the papers by Guillem et al. and Guedj et al., MIS parallel to the bowel wall in the distal direction of the tumor (closer to the anus) were analyzed and measured^{26,27}. The study by Guedj et al. also examined the mesorectal spread of tumor²⁸. Perez et al. inspected MIS in all directions, parallel to the bowel wall²³.

Results of individual studies

For the measured MIS as well as other results in each study, we refer to Supplementary table S1. Remarkably, there was quite a range of percentage of patients with MIS. Two studies showed MIS in 1.8% and 2.4% of patients with residual tumor, while the three other studies with smaller patient populations reported >50% of patients having MIS^{23,24,26-28}. All cases of MIS were restricted to the bowel wall. However, one exception was made for a case in the study by Guedj et al., which included a tumor deposit in the mesorectal fat. As this pertained to a cT3 tumor, the possibility exists that this tumor deposit remained there due to tumor fragmentation. For this reason, we did not exclude this case from our analysis.

Syntheses of results

80% of patients showed no evidence of MIS. MIS ranged from 0 to 20mm, with a mean of 4.3 mm when only including patients with MIS. Figure 2a illustrates the total patient population included in this meta-analysis. Figure 2b shows a more detailed graph of only the patients with MIS. The CTV or local excision margin around the macroscopically visible tumor needed to treat all microscopic intramural disease in increasing percentages of patients are shown in Table 3. For example, the MIS for the 90th percentile was calculated to be 3 mm with a 95% confidence interval between 2 and 5 mm based on a bootstrap procedure of 10,000 samples. The analysis was performed including patients with a ypT0, as residual disease cannot be completely excluded when facing a ycT0 with a scar or other residual mucosal abnormality. However, results were also shown with exclusion of 48 patients who had a ypT0 in an

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attempt to assess robustness of our study data. This analysis showed very similar results (the 95th percentile became 6 mm instead of 5.5 mm), revealing that the impact of the ypT0 subgroup on the total cohort is negligible. When only including the patients with MIS in the analysis (n = 69), the 95th percentile becomes 10mm with a confidence interval of 9 – 19mm.

Comment [AV2]: The sentence was also added to reveal our extra analysis to test robustness of our study data.

Additionally, the entire group was split in two based on the median tumor diameter after surgery (excluding one study for which the tumor diameters based on pathology were not provided²⁴), being 24mm. No significant differences were seen in MIS percentiles when comparing tumors with diameters <24mm with those having diameters ≥24mm.

Comment [AV3]: This analysis was added in response to the interesting question by the reviewer concerning the 95th percentile of MIS for just the group of patients with MIS.

Additional exploratory analyses

Exploratory analyses were done to identify subgroups of patients who might have a higher risk of MIS, considering factors such as grade 3 tumors, lymphatic invasion, vascular invasion, perineural invasion, T-stage and time interval between CRT and surgery. The correlating mean MIS for these factors is shown in Table 4. Using post-hoc Mann-Whitney U test, significant differences were seen for all comparisons: ypT1 vs ypT2 (p < 0.001), ypT2 vs ypT3-4 (p=0.010) and ypT1 vs ypT3-4 (p=0.008). This means that the group of ypT1 has the smallest MIS, followed by the ypT3-4 group, while the ypT2 group had the largest MIS. Regarding time interval between CRT and surgery, a statistically significant difference was seen when comparing the three time-interval groups (less than 8 weeks, 8-12 weeks, and more than 12 weeks), where patients waiting for longer than 12 weeks after CRT had the largest MIS (p<0.0001). Due to the large group of tumors showing no MIS (80% in this meta-analysis) as well as missing information and skewed data, no other significant observations were made.

Discussion

This meta-analysis suggests that, to treat all microscopic intramural disease in 95% of patients with rectal cancer who achieve incomplete pathological response after standard CRT, a margin of 5.5 mm would be required around the macroscopically visible tumor. This is clinically relevant information when giving a radiation boost to these patients to improve primary tumor regression and achieve cCR. Additionally, this information can potentially be used when performing a local excision or a sphincter-sparing LAR after CRT in order to optimize chances of an R0 resection.

Because a true complete response is often difficult to discern after chemoradiation, we included the ypT0 patients in our meta-analysis. However, we performed the statistical analysis again on the group after excluding the ypT0 patients, and saw only minor changes in our results. For example, the 95th percentile increased by 0.5 mm and became 6mm, showing that our analysis was robust. The median

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tumor diameter after CRT was 24mm. Comparing the group <24mm and ≥24mm showed no significant differences in the MIS percentiles, suggesting that the required margins would be the same regardless of size of tumor.

Overall, 80% of the tumors showed no MIS. This suggests that there are two major groups of tumor response: those that retain MIS during tumor response and those that do not, the latter being by far the largest group. More research is needed to improve our ability to predict which tumors will display MIS, as these patients may need a larger margin for local radiation boost or surgical approaches.

Previous literature has shown that some rectal tumors show tumor fragmentation rather than concentric tumor shrinkage after CRT. This tumor fragmentation, or discontinuous spread of tumor, has been described in the studies by Perez et al., Chmielik et al., Rutkowski et al., and Guedj et al.^{23,26-28} A possible explanation for different patterns of tumor regression may be the presence of distinct degrees of intratumoral heterogeneity²⁹. The coexistence of multiple subpopulations of radiosensitive and radioresistant cancer cells may have resulted in isolated foci of cancer cells, reflected by significant fragmentation of the cancer. This concept does pose some unexplained dilemmas, as it may mean that there may be residual disease in the entire area of original tumor, which would require that a radiation boost also be given on this original tumor volume²³. However, given the reported small distances of MIS in the studies included in this meta-analysis, it seems that most tumors show a predominantly concentric shrinkage after CRT. This would mean that giving a radiation boost on a smaller volume should be feasible and safe for most patients. The same conclusion can be made for local excisions. In this meta-analysis, tumor fragmentation was reported for 36/349 (10.3%) patients and continuous intramural extension was reported for 39/349 (11.2%) patients. Patients without MIS, constituting the clear majority (80%) of patients in this meta-analysis, showed concentric shrinkage of tumor, as tumor fragments surrounding the central residual lesion would otherwise have been reported as MIS. The study by Guillem et al. only mentioned continuous intramural extension, thus it was assumed that there was no tumor fragmentation²⁴. Conclusions could not be drawn about the distance of intramural spread related to the pattern of tumor response due to the small number of tumors showing MIS. Biomarkers that help predict type of tumor response are not yet known for this group of patients. MRI could potentially aid in differentiating between these two different types of regression. A few papers have described patterns of response using diffusion-weighted MRI's during chemoradiation; however, data on accurate radiological detection of tumor fragmentation is still lacking^{30,31}.

The potential effect of the time interval between neoadjuvant CRT and surgery also warrants additional research. Rectal tumor regression has been noted beyond 12 weeks following completion of

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CRT^{32,33}. In a parallel to anal cancer where optimal assessment of response is considered to be 26 weeks, a significant proportion of patients with rectal cancer will only develop a complete clinical response after 16 weeks from CRT completion^{33,34}. In our meta-analysis, tumor response was usually measured at an earlier time and in the prospective randomized trial, tumor response was already measured between four to six weeks after neo-adjuvant treatment^{26,27}. The largest extension of MIS was seen at >12 weeks' interval. For patients with a near complete response who prefer an organ-sparing pathway, often a longer waiting period is chosen in the hopes of gaining a complete response. The increased MIS after longer intervals could potentially be explained by the fact that delayed tumor evaluation may result in more MIS due to uneven shrinkage of the macroscopic tumor versus the microscopic tumor at this stage. The relative importance of the two processes may vary depending on the time interval after treatment. However, as there were variable time intervals between neo-adjuvant CRT and surgery across different institutions, this is clearly a potential source of bias.

As the use of contact therapy in the adjuvant setting is becoming more frequent³⁵, parameters of the surgical specimen were considered potentially relevant and, therefore, reported in this study. In a recent study, MIS was seen as far as 4cm away from the visible tumor/ulcer and in up to half of patients³⁶. It must be taken into account, however, that most of these patients would not be considered appropriate candidates for a local excision or a radiation boost as the residual cancers were large and advanced³⁶. The maximum distance of MIS found in this meta-analysis was 20mm²⁸. The possible correlation between ypT stage and extent of MIS is another factor requiring more research. In our analysis, ypT2 had the highest MIS as opposed to ypT1 or ypT3-4. Reasons for this outcome remain unclear, but perhaps the relatively low amount of MIS cases could contribute to this statistical outcome. This analysis was exploratory and must be confirmed by more studies.

Perez et al. describe in their paper that according to their measurements, a 1cm margin around the visible tumor (which is now generally used during local excision) would be inadequate and a 1.5cm margin would be safer³⁶. In this series, a high number of tumors (70%) showed MIS. The time interval between neo-adjuvant chemoradiation and surgery in this series was also by far the highest, with a median of 16.5 weeks²³. Curiously, this series also had a majority of patients with residual ypT2 tumors, consistent with the present analysis showing the largest extension for MIS. Altogether, restaging of patients with incomplete response showing residual yct2 after more than 12 weeks from CRT may warrant additional CTV margin requirements. This analysis was exploratory and must be confirmed by further studies.

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Limitations of our meta-analysis include the fact that individual patient data was only received from five out of eleven eligible studies, leaving out potentially useful data for 240 patients with residual disease after preoperative RT. The studies that had to be excluded due to unavailable data included a retrospective study that assessed distal MIS parallel to the bowel wall and found that 49.1% of 55 patients with residual cancer had MIS, with three patients showing a MIS of more than 2cm³⁶. An Indian study including 41 patients with residual tumor of which 2 patients (5%) showed distal MIS³⁷. A Japanese study compared two groups of patients with T2-T4 lower rectal cancer; one in which patients received preoperative radiation followed by surgery and one in which patients received immediate surgery. The goal was to analyze the effect of preoperative radiation on distal MIS among other endpoints. 47 patients were irradiated of which it is unclear how many of these patients had MIS. Interestingly, the mean extent of distal MIS was significantly lower in the irradiated group³⁸. The same author published a second study comparing distal MIS in flattened- type and raised-type residual tumors after neoadjuvant chemoradiation in 34 patients. It is unclear how many patients showed distal MIS, yet the authors conclude that flattened-type tumors showed more diffusely distributed MIS compared to raised-type tumors³⁹. Mezhir et al. published a study in which out of 18 patients with residual rectal cancer after neoadjuvant chemoradiation, 11 patients (61%) showed distal MIS, 91% of which was < 1cm (ref Mezhir). Another study analyzing 45 patients showed that 71% of patients had distal MIS⁴⁰. Although the inclusion of these studies could have revealed some valuable insights, all possible attempts had been made to retrieve this data, leaving the authors convinced that no more actions could be undertaken. Another limitation of this analysis is that three out of the four patient populations only analyzed distal MIS parallel to the bowel wall (for surgical purposes), whereas we are interested in the spread parallel as well as perpendicular to the bowel wall, as this is relevant in the case of a radiation boost. Currently, a prospectively collected database of TME resections after neo-adjuvant chemoradiation is being analyzed for MIS in all directions parallel as well as perpendicular to the bowel wall, with the hopes of having more detailed information about the possible extent of MIS.

Additionally, little is known about the extent of shrinkage after formalin fixation of TME resections. Most papers report an enlargement/shrinkage of tumor diameter after fixation of around 5%⁴¹⁻⁴³. However, Goldstein et al. have reported longitudinal shrinkages of up to 30%, after which he would suggest a correction factor of approximately 2x when interpreting margin lengths⁴⁴. Eid et al. also observed histological processing variability of the lateral resection margins in rectal cancer, including increases and decreases in margins depending on the day the margin was measured⁴⁵. Possibly the extent of tumor enlargement/shrinkage will be quite limited due to the status after CRT, after which at least

Comment [AV4]: A summary of excluded studies due to data non-availability was added.

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9 partial fibrosis is expected which is less subject to deformation. Clearly, more research and
10 standardization of pathological analysis needs to be carried out to clearly define the effect of pathological
11 processing on tissue volume and MIS.
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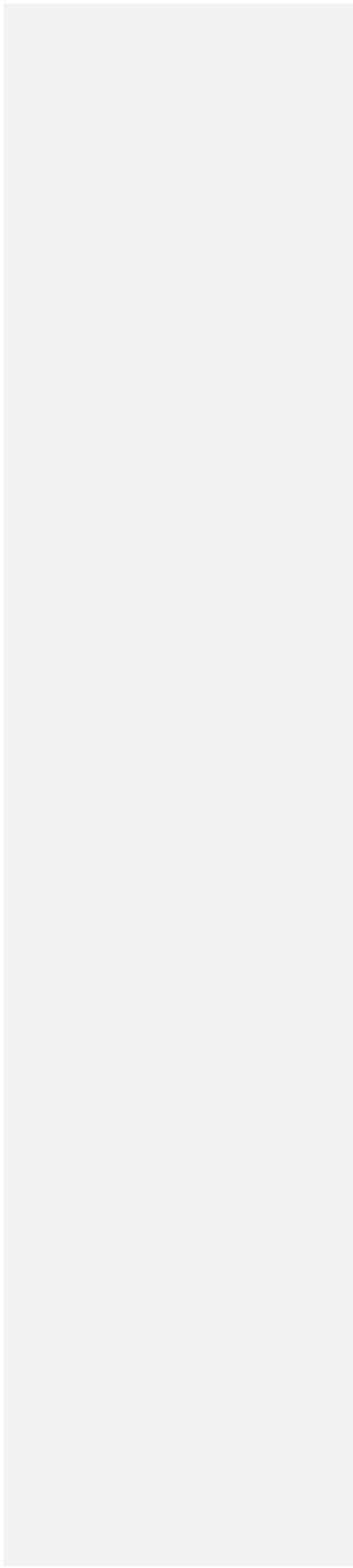
13 In conclusion, based on the meta-analysis of 5 studies including 349 patients, 80% of patients
14 with rectal cancer will not have microscopic intramural spread (MIS) following CRT. In cases where MIS is
15 detected, it is usually limited. Based on our calculations, it appears that in order to treat residual mural
16 tumor and MIS successfully in 95% of rectal cancer patients with significant tumor response after CRT, a
17 margin of 5.5 mm around the visible tumor would suffice. These data are of clinical importance,
18 specifically when planning additional radiotherapy treatments or for surgical approaches, being local
19 excisional approaches as well as sphincter sparing LAR.
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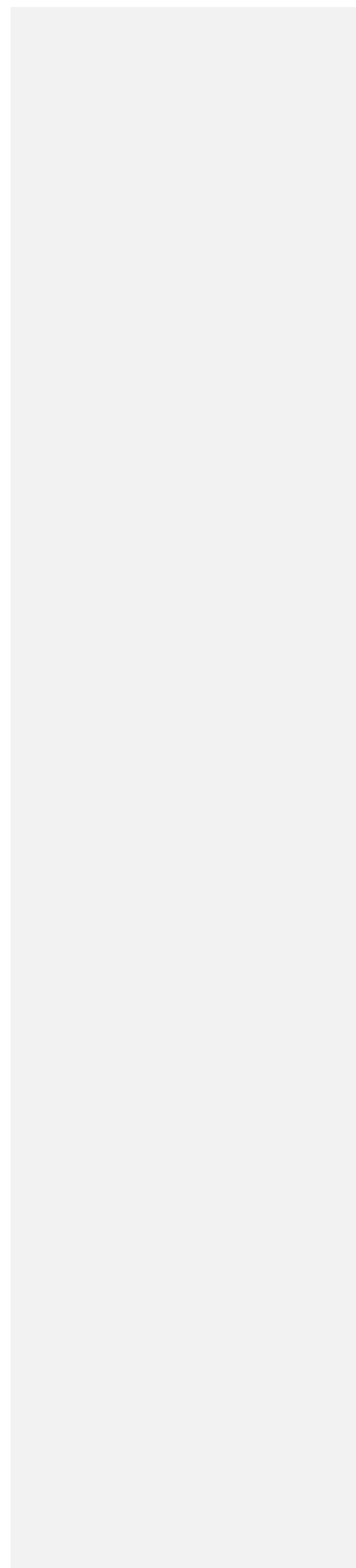
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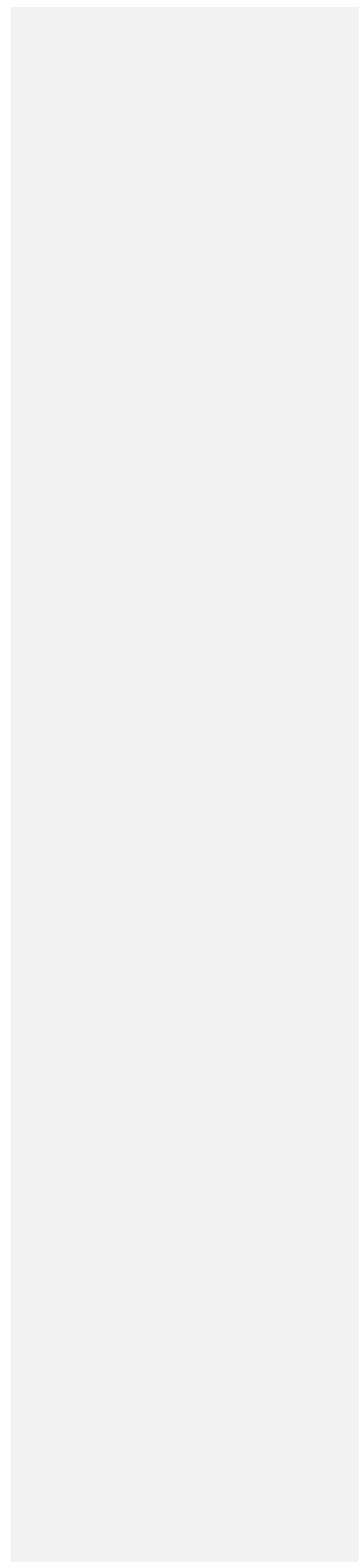
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4 Microscopic intramural extension of rectal cancer after neoadjuvant chemoradiation: a meta-
5 analysis based on individual patient data
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60 Disclosure of potential conflicts of Interest:
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The other authors have no potential conflicts of interest to disclose.

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4 Microscopic intramural extension of rectal cancer after neoadjuvant chemoradiation: a meta-analysis
5 based on individual patient data
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13 **Keywords:** rectal cancer; response; chemoradiation; microscopic spread; margin concept; brachytherapy;
14 contact therapy; boost
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17 **Abstract**
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19 *Objective:* In selected rectal cancer patients with residual local disease following neoadjuvant
20 chemoradiation (CRT) and the preference of an organ preservation pathway, additional treatment with
21 dose escalation by endoluminal radiotherapy (RT) may ultimately result in a clinical complete response.
22 To date, the widespread introduction of selective endoluminal radiation techniques is hampered by a lack
23 of evidence-based guidelines that describe the radiation treatment volume in relation to the residual
24 tumor mass. In order to convert an incomplete response into a complete one with additional treatment
25 such as dose-escalation with endoluminal RT from a theoretical perspective, it seems important to treat
26 all remaining microscopic tumor cells after CRT. In this setting, residual tumor extension beneath normal
27 appearing mucosa (microscopic intramural spread – MIS) becomes relevant for accurate tumor volume
28 and margin estimation. With the goal of providing evidence-based guidelines that define an appropriate
29 treatment volume and patient selection, we present results from a meta-analysis based on individual
30 patient data of studies that have assessed the extent or range of MIS of rectal cancers after neoadjuvant
31 CRT. This meta-analysis should provide an estimate of the residual tumor volume/extension that needs to
32 be targeted by any additional radiation therapy boost in order to achieve complete tumor eradication
33 after initial incomplete or near-complete response following standard CRT.
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46 *Methods and Materials:* A PubMed search was performed. Additional articles were selected based on
47 identification from reference lists. Papers were eligible when reporting MIS in patients who were treated
48 by total mesorectal excision or local excision/transanal endoscopic microsurgery (TEM) after neo-
49 adjuvant long-course CRT. The mean MIS was calculated for the entire group along with the 70th until 95th
50 percentiles. Additional exploratory subgroup analyses were performed.
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53 *Results:* Individual patient data from 349 patients from five studies were analyzed. 80% of tumors
54 showed no MIS. In order to appropriately treat MIS in 95% of rectal cancer patients after CRT, a margin of
55 5.5mm around the macroscopic tumor would suffice. An exploratory subgroup analysis showed that T-
56 stage after CRT (ypT) and time interval between neoadjuvant CRT and surgery are significant factors
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4 predicting the extent of MIS ($p < 0.001$). The group of ypT1 had the smallest MIS, followed by the ypT3-4
5 group, while the ypT2 group had the largest MIS ($p < 0.001$). Regarding time interval between CRT and
6 surgery, a statistically significant difference was seen when comparing the three time-interval groups (less
7 than 8 weeks, 8-12 weeks, and more than 12 weeks), where waiting more than 12 weeks after CRT
8 resulted in the largest MIS ($p < 0.0001$).

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11 *Conclusion:* Based on this meta-analysis, in order to treat the MIS for 95% of rectal cancer patients after
12 CRT, a Clinical Target Volume (CTV) margin of 5.5 mm from the lateral most edge of the macroscopic
13 tumor would suffice. 80% of tumors showed no MIS and would not require an extra CTV margin for
14 treatment. These findings support the feasibility of localized radiotherapy boosts for dose-escalation to
15 improve response among patients with incomplete response after standard CRT and can also be applied
16 in the surgical setting.
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25 **Introduction**

26 The treatment and outcomes for rectal cancer patients have dramatically improved in the last decades.
27 The implementation of total mesorectal excision (TME), which enables an R0 resection of the primary
28 tumour and potentially involved mesorectal lymph nodes, has resulted in a decrease of locoregional
29 recurrences¹. The introduction of neoadjuvant therapy (radiotherapy or chemoradiation (CRT)) based on
30 high-risk factors has led to a further decline in locoregional failure^{2,3}. Despite these improvements, the
31 combination of neoadjuvant CRT and a TME-based rectal cancer resection is associated with an increased
32 risk of fecal incontinence, low anterior resection syndrome, as well as sexual and urinary dysfunction⁴⁻⁷.
33 For elderly patients, significant peri-operative morbidity and mortality risk also exist^{8,9}. Additionally,
34 patients with distally located rectal tumors often face a permanent colostomy, which may have a
35 significant impact on quality of life^{10,11}.
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44 Following long-course neoadjuvant CRT using standardized doses (usually 50 Gy or 50.4 Gy in 25
45 or 28 fractions, respectively), a pathological complete response is seen in 8-20% of patients after
46 surgery^{3,12}. Phase I-II trials have shown that in highly selected patients with a complete clinical response
47 after neoadjuvant treatment, a watch and wait protocol might be considered instead of surgery^{13,14}. This
48 could spare selected patients an extensive operation and, for patients with distal tumors, a permanent
49 colostomy. The number of complete responses is likely to increase if higher radiation doses to the tumor
50 could be used, as shown in a phase II trial using a boost dose given by brachytherapy^{15,16}. The radiation
51 boost can be given to the tumor using either external beam radiotherapy (EBRT) or an endoluminal
52 technique such as brachytherapy or contact X-ray radiotherapy (CXT)^{15,17,18}. This boost can be
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4 administered before or after CRT. Giving the boost dose following CRT has the advantage that (a) it could
5 potentially be delivered to a smaller tumor volume resulting in less toxicity (as tumor volume generally
6 shrinks during CRT) and (b) that it may even be completely avoided in case of complete clinical response.
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10 Important advantages of endoluminal techniques include the possibility to apply a more
11 selective/localized boost compared to EBRT. Selective irradiation allows tumor dose escalation to higher
12 levels and limits the chance of radiation-induced toxicity¹⁹. Hence, CXT according to the Papillon method
13 has been re-introduced in a limited number of clinics. Due to the sharply falling depth-dose
14 characteristics of CXT, fractional doses up to 30 Gy and total doses up to 90 Gy can be applied without
15 causing significant normal tissue toxicity^{17,20}. As described above, a brachytherapy boost has also been
16 used, showing an increase in the rate of pathological complete response¹⁵.
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20 To date, the widespread introduction of selective endoluminal radiation techniques is hampered
21 by the lack of evidence-based guidelines that describe the radiation treatment volume. In order to obtain
22 a durable complete response, it would seem important from a theoretical perspective to treat all tumor
23 cells remaining after CRT. This entails treating not only any visible mucosal lesion, in radiotherapy terms
24 called the Gross Tumor Volume (GTV), but also potential microscopic intramural spread (MIS) or
25 fragments of the tumor in the wall, called the Clinical Target Volume (CTV). Hence, the CTV should include
26 the GTV as well as a margin for potential MIS. To provide evidence-based guidelines that define an
27 appropriate treatment volume, we performed a meta-analysis based on individual patient data of studies
28 that have assessed the extent or range of MIS of rectal tumors after neoadjuvant CRT.
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32 The data generated by this meta-analysis can also be applied in the surgical setting. Local excision
33 via transanal approaches including Transanal Endoscopic Microsurgery (TEM) or Transanal Minimally
34 Invasive Surgery (TAMIS) of a residual (small) tumor after CRT are surgical organ-preserving alternatives
35 to the selective radiation boost^{21,22}. Here too, there is no clear consensus on the margin of “healthy”
36 tissue surrounding the residual tumor containing potential microscopic disease that should be excised²³.
37 The results of this meta-analysis could therefore also be used to determine the surgical margin for local
38 surgical techniques or the distal margin when a sphincter-sparing Low Anterior Resection with colo-anal
39 anastomosis is being considered in patients with ultra-distal rectal cancer.
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43 As certain tumor characteristics, such as tumor size, lymphatic, vascular or perineural invasion,
44 may be predictive for the presence of MIS²⁴, the secondary aim of this meta-analysis was to identify
45 potential factors that may be predictive for the absence or presence and the extent of MIS. Such factors
46 may be useful in the future to select patients who are suitable candidates for selective endoluminal
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4 boosting and omission of surgery or very localized surgery, or who are likely better off with non-organ
5 preserving surgery.
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8 9 **Methods**

10 *Protocol and registration*

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12 This paper was written using the Preferred Reporting Items for Systematic Reviews and Meta-
13 Analyses (PRISMA) *Checklist of items to include when reporting a systematic review and meta-*
14 *analysis 2009*²⁵.
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19 *Search strategy*

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21 A search was performed in November 2016 by two of the authors and updated on May 9th 2018
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23 by the first author. The PubMed search strategy used is shown in Fig. 1. Additional articles were
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25 selected based on identification from reference lists.
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29 *Study selection*

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31 Published articles were selected and evaluated by the first and second-to-last authors. First,
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33 eligibility was determined based on title and abstract screening. Remaining articles were
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35 selected based on full-text screening. Studies were eligible when reporting in English,
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37 experimental or observational studies, and reporting submucosal or otherwise MIS in patients
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39 who received a total mesorectal excision or local excision/TEM after neo-adjuvant long-course
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41 CRT. Studies only including patients who received surgery immediately after neo-adjuvant
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43 treatment were excluded, as little to no pathological response was expected. Publication dates
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45 between 1970 and 2018 were included. We determined 1970 as cut-off value due to differences
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47 in standard treatment for rectal cancer and advances in the technical aspects of radiation
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49 oncology in the recent decades. Conference abstracts were excluded. Authors of selected papers
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51 were approached by e-mail and asked whether they were willing to collaborate on this meta-
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53 analysis project. Authors who agreed were asked to fill in a data transfer agreement to ensure
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55 confidentiality from both parties, after which the anonymized individual patient data were
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57 transferred. Selected papers of which the authors eventually did not send their individual patient
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4 data or from which no response was received were excluded from analysis after several
5 attempts of communication via mail and phone.
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10 *Data extraction and analysis*

11 Data was extracted by full-text screening of the study as well as from the individual patient data
12 using a self-made format reporting on (1) basic study demographics (country, study design, years
13 of patient inclusion, number of patients and stages of disease); (2) treatment demographics
14 (radiation dose, type of chemotherapy, median length of follow up and primary endpoints); (3)
15 reporting of intramural spread; (4) risk of bias assessment. Descriptive and statistical analyses of
16 the combined individual patient data were performed.
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25 *Statistics*

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27 The mean MIS was calculated for the entire group along with the percentiles between the 70th
28 and the 95th by increments of 5. 95% Confidence intervals for these different percentiles were
29 calculated using a bootstrap procedure with 10.000 samples.
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33 An explorative analysis (percentiles with confidence intervals) was also performed on subgroups
34 to test whether certain factors were predictive for MIS. Subgroups were made on the basis of
35 ypT stage (ypTis, 0, 1 vs. 2 vs. 3-4), tumor size (median split), tumor diameter (median split),
36 tumor grade of differentiation (1 vs 2 vs 3), vascular invasion (yes/no), lymphatic invasion
37 (yes/no), perineural invasion (yes/no), and time between CRT and surgery (less than 8 weeks vs.
38 more than 8 weeks, less than 12 weeks vs. more than 12 weeks, and less than 8 weeks vs. 8-12
39 weeks vs. more than 12 weeks). All subgroups were compared using a non-parametric test, the
40 Mann-Whitney U test in case of two groups and the Kruskal-Wallis test (with post-hoc pairwise
41 Mann-Whitney U tests if applicable) in case of 3 or more groups.
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52 *Risk of bias in individual studies*

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54 Items reported are random sequence generation; allocation concealment; blinding; incomplete
55 outcome data; and selective reporting. Bias reporting in randomized controlled trials was
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4 performed using the Cochrane risk of bias tool²⁶. Bias reporting in observational studies was
5 performed using *The Newcastle-Ottawa Scale (NOS) for cohort studies*²⁷.
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10 **Results**

11 *Study Selection*

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13 For the study selection flow chart, we refer to Figure 1. The PubMed search resulted in 168 records. Two
14 additional records were included on identification of reference lists. Based on title and abstract screening,
15 143 publications were excluded due to various reasons, including the absence of pathology assessment
16 and absence of neo-adjuvant treatment. After full text screening, eleven studies met the inclusion criteria
17 and were included in this systematic review. The search was last updated in May 2018. Nine out of eleven
18 authors responded that they were willing to send us their individual patient data. Two authors were
19 unable to retrieve their databases due to changes of workplace and their papers were thus excluded. Of
20 the seven studies that were then included, we received the individual patient data of five papers^{23,24,28-30}.
21 Two of the papers reported on the same study and therefore we received one dataset for these two
22 papers^{28,29}.
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32 *Study characteristics*

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34 For a summary of the study demographics, we refer to Table 1. Five studies with individual patient data
35 from 349 patients were included in this meta-analysis^{23,24,28-30}. Two papers reported on the same
36 prospective randomized trial comparing short-course radiotherapy (5x5 Gy) followed by immediate
37 resection and CRT followed by delayed surgery. The patients in the short-course radiotherapy arm were
38 excluded as, for the purpose of this meta-analysis, the response after CRT was of interest^{28,29}. The
39 remaining three studies included two prospective observational studies and one retrospective
40 observational study^{23,24,30}.
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48 *Risk of bias within studies*

49 Risk of bias in randomized trials

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51 We refer to Table S1, Supplementary info for a summary of the risk of bias assessment. There was one
52 prospective randomized trial, reported by two authors: Chmielik et al. and Rutkowski et al.^{28,29} Overall, a
53 low risk of bias can be concluded for this trial. Blinding of patients and personnel, as well as outcome
54 reporters, was impossible due to the fact that short-course radiotherapy was compared with long-course
55 neo-adjuvant chemoradiation in the study.
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6 Risk of bias in observational studies

7 Bias in observational studies was assessed using the NOS for cohort studies²⁷ (Table S2, Supplementary
8 material).
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13 *Treatment characteristics*

14 For a summary of treatment characteristics, we refer to Table 2. All included patients received long
15 course CRT followed by delayed surgery. Most commonly used radiation scheme was 50.4 Gy delivered in
16 fractions of 1.8 Gy to the primary tumor, pathological regional lymph nodes and elective lymph node
17 areas. The most commonly used chemotherapy was 5-fluorouracil-based. The time from CRT to surgery
18 varied from 4-6 weeks in the prospective randomized trial to a median of 16.5 weeks in the observational
19 retrospective study^{23,28,29}. All studies included patients who received TME surgery after neo-adjuvant CRT
20 except for one study, in which all patients received TEM²³.
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29 *Pathological analysis*

30 In the prospective randomized trial, workshops for the participating pathologists were held before and
31 during the trial to align the protocol and measurement methods of margins^{28,29}. In the two observational
32 prospective studies, pathological examination was done by one or two dedicated pathologists^{24,30}. In all
33 studies, pathological analysis was performed according to each institution's standards.
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38 We define MIS as the greatest distance between the microscopic tumor cells in the bowel wall and the
39 nearest edge of the macroscopic ulcer/tumor residue parallel to and perpendicular to the bowel wall
40 between the microscopic tumor cells in the bowel wall and the nearest superficial edge of the
41 macroscopic ulcer/tumor residue. In the prospective randomized trial, as well as in the papers by Guillem
42 et al. and Guedj et al., MIS parallel to the bowel wall in the distal direction of the tumor (closer to the
43 anus) were analyzed and measured^{28,29}. The study by Guedj et al. also examined the mesorectal spread of
44 tumor³⁰. Perez et al. inspected MIS in all directions, parallel to the bowel wall²³.
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52 *Results of individual studies*

53 For the measured MIS as well as other results in each study, we refer to Table 3. Remarkably, there was
54 quite a range of percentage of patients with MIS. Two studies reported MIS in 2.4% and 1.8% patients,
55 respectively, while the three other studies with smaller patient populations reported >50% of patients
56 having MIS^{23,24,28-30}. All cases of MIS were restricted to the bowel wall. However, one exception was made
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4 for a case in the study by Guedj et al., which included a tumor deposit in the mesorectal fat. As this
5 pertained to a cT3 tumor, the possibility exists that this tumor deposit remained there due to tumor
6 fragmentation. For this reason, we did not exclude this case from our analysis.
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10 11 *Syntheses of results*

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13 80% of patients showed no evidence of MIS. MIS ranged from 0 to 20mm, with a mean of 0.83mm. Figure
14 3a illustrates the total patient population included in this meta-analysis. Figure 3b shows a more detailed
15 graph of the patients with MIS. The CTV or local excision margin around the macroscopically visible tumor
16 needed to treat all microscopic intramural disease in increasing percentages of patients are shown in
17 Table 4. For example, the MIS for the 90th percentile was calculated to be 3mm with a 95% confidence
18 interval between 2 and 5 mm based on a bootstrap procedure of 10,000 samples. Additionally, the entire
19 group was split in two based on the median tumor diameter after surgery (excluding one study for which
20 the tumor diameters based on pathology were not provided²⁴), being 24mm. No significant differences
21 were seen in MIS percentiles when comparing tumors with diameters <24mm with those having
22 diameters ≥24mm.
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32 33 *Additional exploratory analyses*

34 Exploratory analyses were done to identify subgroups of patients who might have a higher risk of MIS,
35 considering factors such as grade 3 tumors, lymphatic invasion, vascular invasion, perineural invasion, T-
36 stage and time interval between CTR and surgery. The correlating mean MIS for these factors is shown in
37 Table 5. Using post-hoc Mann-Whitney U test, significant differences were seen for all comparisons: ypT1
38 vs ypT2 ($p < 0.001$), ypT2 vs ypT3-4 ($p=0.005$) and ypT1 vs ypT3-4 ($p=0.011$). This means that the group of
39 ypT1 has the smallest MIS, followed by the ypT3-4 group, while the ypT2 group had the largest MIS.
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45 Regarding time interval between CRT and surgery, a statistically significant difference was seen when
46 comparing the three time-interval groups (less than 8 weeks, 8-12 weeks, and more than 12 weeks),
47 where patients waiting for longer than 12 weeks after CRT had the largest MIS ($p<0.0001$). Due to the
48 large group of tumors showing no MIS (80% in this meta-analysis) as well as missing information and
49 skewed data, no other significant observations were made. We did not find evidence that the diameter (\geq
50 24mm vs < 24mm) of the residual tumor is associated with smaller or larger of extent MIS.
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57 **Discussion**

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4 This meta-analysis suggests that, to treat all microscopic intramural disease in 95% of patients with rectal
5 cancer who achieve incomplete pathological response after standard CRT, a margin of 5.5 mm would be
6 required around the macroscopically visible tumor. This is clinically relevant information when giving a
7 radiation boost to these patients to improve primary tumor regression and achieve cCR. Additionally, this
8 information can potentially be used when performing a local excision or a sphincter-sparing LAR after CRT
9 in order to optimize chances of an R0 resection. The median tumor diameter after CRT, in cases where
10 there was measurable residual disease, was 24mm. Comparing the group <24mm and ≥24mm showed no
11 significant differences in the MIS percentiles, suggesting that the required margins would be the same
12 regardless of size of tumor.
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20 Overall, 80% of the tumors showed no MIS. This suggests that there are two major groups of
21 tumor response: those that retain MIS during tumor response and those that do not, the latter being by
22 far the largest group. More research is needed to improve our ability to predict which tumors will display
23 MIS, as these patients may need a larger margin for local radiation boost or surgical approaches.
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27 Previous literature has shown that some rectal tumors show tumor fragmentation rather than
28 concentric tumor shrinkage after CRT. This tumor fragmentation, or discontinuous spread of tumor, has
29 been described in the studies by Perez et al., Chmielik et al., Rutkowski et al., and Guedj et al.^{23,28-30} A
30 possible explanation for different patterns of tumor regression may be the presence of distinct degrees of
31 intratumoral heterogeneity³¹. The coexistence of multiple subpopulations of radiosensitive and
32 radioresistant cancer cells may have resulted in isolated foci of cancer cells, reflected by significant
33 fragmentation of the cancer. This concept does pose some unexplained dilemmas, as it may mean that
34 there may be residual disease in the entire area of original tumor, which would require that a radiation
35 boost also be given on this original tumor volume²³. However, given the reported small distances of MIS
36 in the studies included in this meta-analysis, it seems that most tumors show a predominantly concentric
37 shrinkage after CRT. This would mean that giving a radiation boost on a smaller volume should be feasible
38 and safe for most patients. The same conclusion can be made for local excisions. In this meta-analysis,
39 tumor fragmentation was reported for 37/349 (10.6%) patients and continuous intramural extension was
40 reported for 39/349 (11.2%) patients. Patients without MIS, constituting the clear majority (80%) of
41 patients in this meta-analysis, showed concentric shrinkage of tumor, as tumor fragments surrounding
42 the central residual lesion would otherwise have been reported as MIS. The study by Guillem et al. only
43 mentioned continuous intramural extension, thus it was assumed that there was no tumor
44 fragmentation²⁴. Conclusions could not be drawn about the distance of intramural spread related to the
45 pattern of tumor response due to the small amount of tumors showing MIS. Biomarkers that help predict
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4 type of tumor response are not yet known for this group of patients. MRI could potentially aid in
5 differentiating between these two different types of regression. A few papers have described patterns of
6 response using diffusion-weighted MRI's during chemoradiation; however, data on accurate radiological
7 detection of tumor fragmentation is still lacking^{32,33}.
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11 The potential effect of the time interval between neoadjuvant CRT and surgery also warrants
12 additional research. Rectal tumor regression has been noted beyond 12 weeks following completion of
13 CRT^{34,35}. In a parallel to anal cancer where optimal assessment of response is considered to be 26 weeks,
14 a significant proportion of patients with rectal cancer will only develop a complete clinical response after
15 16 weeks from CRT completion^{35,36}. In our meta-analysis, tumor response was usually measured at an
16 earlier time and in the prospective randomized trial, tumor response was already measured between four
17 to six weeks after neo-adjuvant treatment^{28,29}. The largest extension of MIS was seen at >12 weeks'
18 interval. For patients with a near complete response who prefer an organ-sparing pathway, often a longer
19 waiting period is chosen in the hopes of gaining a complete response. The increased MIS after longer
20 intervals could potentially be explained by the fact that delayed tumor evaluation may result in more MIS
21 due to uneven shrinkage of the macroscopic tumor versus the microscopic tumor at this stage. The
22 relative importance of the two processes may vary depending on the time interval after treatment.
23 However, as there were variable time intervals between neo-adjuvant CRT and surgery across different
24 institutions, this is clearly a potential source of bias.
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36 In a recent study, MIS was seen as far as 4cm away from the visible tumor/ulcer in up to half of
37 patients³⁷. It must be taken into account, however, that most of these patients would not be considered
38 appropriate candidates for a local excision or a radiation boost as the residual cancers were large and
39 advanced³⁷. The maximum distance of MIS found in this meta-analysis was 20mm³⁰. The possible
40 correlation between ypT stage and extent of MIS is another factor requiring more research. In our
41 analysis, ypT2 had the highest MIS as opposed to ypT1 or ypT3-4. Reasons for this outcome remain
42 unclear, but perhaps the relatively low amount of MIS cases could contribute to this statistical outcome.
43 This analysis was exploratory and must be confirmed by more studies.
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50 Perez et al. describe in their paper that according to their measurements, a 1cm margin around
51 the visible tumor (which is now generally used during local excision) would be inadequate and a 1.5cm
52 margin would be safer²³. In this series, a high number of tumors (70%) showed MIS. The time interval
53 between neo-adjuvant chemoradiation and surgery in this series was also by far the highest, with a
54 median of 16.5 weeks²³. Curiously, this series also had a majority of patients with residual ypT2 tumors,
55 consistent with the present analysis showing the largest extension for MIS. Altogether, restaging of
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4 patients with incomplete response showing residual yct2 after more than 12 weeks from CRT may
5 warrant additional CTV margin requirements. This analysis was exploratory and must be confirmed by
6 further studies.
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10 Limitations of our meta-analysis include the fact that individual patient data was only received
11 from five out of eleven eligible studies, leaving out potentially useful data for 255 patients with residual
12 disease after preoperative RT. However, all possible attempts had been made to retrieve this data,
13 leaving the authors convinced that no more actions could be undertaken. Another limitation of this
14 analysis is that three out of the four patient populations only analyzed distal MIS parallel to the bowel
15 wall (for surgical purposes), whereas we are interested in the spread parallel as well as perpendicular to
16 the bowel wall, as this is relevant in the case of a radiation boost. Currently, a prospectively collected
17 database of TME resections after neo-adjuvant chemoradiation is being analyzed for MIS in all directions
18 parallel as well as perpendicular to the bowel wall, with the hopes of having more detailed information
19 about the possible extent of MIS.
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27 Additionally, little is known about the extent of shrinkage after formalin fixation of TME
28 resections. Most papers report an enlargement/shrinkage of tumor diameter after fixation of around
29 5%³⁸⁻⁴⁰. However, Goldstein et al. have reported longitudinal shrinkages of up to 30%, after which he
30 would suggest a correction factor of approximately 2x when interpreting margin lengths⁴¹. Eid et al. also
31 observed histological processing variability of the lateral resection margins in rectal cancer, including
32 increases and decreases in margins depending on the day the margin was measured⁴². Possibly the extent
33 of tumor enlargement/shrinkage will be quite limited due to the status after CRT, after which at least
34 partial fibrosis is expected which is less subject to deformation. Clearly, more research and
35 standardization of pathological analysis needs to be carried out to clearly define the effect of pathological
36 processing on tissue volume and MIS.
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45 In conclusion, based on the meta-analysis of 5 studies including 349 patients with rectal cancer,
46 80% of patients with rectal cancers that have a persistent tumor lesion following CRT will not have
47 microscopic intramural spread (MIS). In cases where MIS is detected, it is usually limited. Based on our
48 calculations, it appears that in order to treat residual mural tumor and MIS successfully in 95% of rectal
49 cancer patients with significant tumor response after CRT, a margin of 5.5 mm around the visible tumor
50 would suffice. These data are of clinical importance, specifically when planning additional radiotherapy
51 treatments or for surgical approaches, being local excisional approaches as well as sphincter sparing LAR.
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Table 1. Study Demographics

Reference	Country	Study design	Years of patient inclusion	Total patients / patients with residual disease	Stage of disease with no. of patients (%)	End points
Chmielik et al. ²⁷ , Rutkowski et al. ²⁶	Poland	Randomized trial	1999-2002	85* / 79 (CRT)	cT3-cT4: 85 (100)	Comparison of long course CRT with short course RT in regard to sphincter preservation rate; compare distal intramural spread in 2 different RT groups
Guedj et al. ²⁸	France	Observational prospective	2012-2014	124 / 102	cT2: 9 (7.3) cT3: 94 (75.8) cT4: 9 (7.3) N+: 83 (66.9) N0: 25 (20.2) T- and/or N-stage missing: 28 (22.6)	Intramural and mesorectal cancer spread
Guillem et al. ²⁴	USA	Observational prospective	2000-2004	110** / 89	cT2: 7 (6.4) cT3: 95 (86.4) cT4: 1 (0.9) N1: 79 (71.8) N0: 23 (20.9) T- and/or N-stage missing: 15 (13.6)	Microscopic patterns of residual disease and circumferential and distal resection margins; identify clinicopathologic factors associated with residual disease
Perez et al. ²³	Brazil	Observational retrospective	2009-2011	30 / 30	cT2: 12 (40) cT3: 18 (60) N1: 7 (23.3) N0: 23 (76.7)	Patterns of tumor response
Total				349 / 300		

Comment [AV2]: As suggested by the reviewer, numbers are now given of numbers of patients in each stage.

Comment [AV1]: Showing patients with residual disease as well

*In the paper 86 patients are described as having received chemoradiation; however, one of these patients had only received 6 fractions of 1.8 Gy (total 7.2 Gy) and was thus excluded from our analysis.

** In the paper 109 patients are included; however, the individual patient data that was delivered comprised of 110 patients.

Table 2. Treatment Characteristics

Reference	Treatment given	RT Dose	Chemotherapy	Median time between neoadjuvant therapy and surgery in weeks	Type of surgery
Chmielik et al. ²⁷ , Rutkowski et al. ²⁶	Chemoradiation *	50.4 Gy / 1.8 Gy fx/day; 5 fx per week	5-Fluorouracil and leucovorin	5.6 (range 3.1-18.6)	Abdominoperineal (n=35), Low anterior (n=48), Hartmann (n=3)
Guedj et al. ²⁸	Chemoradiation	45-50 Gy; 5 fx per week over 5- 6 weeks	5-Fluorouracil - based	9 (range 1.3-18)	Proctectomy with TME (n=118), Abdominoperineal (n=6)
Guillem et al. ²⁴	Chemoradiation	48.6 Gy-54 Gy / 1.8 Gy fx/day, with 3.6 Gy boost to tumor; 5 fx per week	5-Fluorouracil - based	6.9 (range 2.7-22.1)	Abdominoperineal (n=22), Low anterior (n=87)
Perez et al. ²³	Chemoradiation	50.4-54 Gy / 1.8Gy fx/day	5-Fluorouracil - based	16.5 (range 6-160)	TEM

Comment [AV1]: As suggested by the reviewer, where possible standardized RT treatment regimen were provided.

Comment [AV2]: As correctly suggested by the reviewer, time between neoadjuvant therapy and surgery was standardized as the median.

*Only the chemoradiation arm in Chmielik et al.²⁷ was analyzed for the purpose of this meta-analysis.

Table 3. Margins needed to treat percentiles of patients and their confidence intervals. In this table, margins for the total group are shown, as well as after exclusion of 48 patients with a ypT0 after chemoradiation for comparison.

Percentiles	Margin (mm)	95% Confidence intervals	Margin after exclusion ypT0 (mm)	95% Confidence intervals	Margin only patients with MIS (mm)	95% Confidence intervals
70	0	0 - 0	0	0 - 0	5	4 - 6.3
75	0	0 - 0	0	0 - 1	6	5 - 8
80	0	0 - 2	1.8	0 - 2	6.4	5 - 9
85	2	1 - 3	2	2 - 3	8	6 - 9.3
90	3	2 - 5	3	3 - 5	9	7 - 10
95	5.5	4.5 - 8	6	5 - 8.95	10	9 - 19

Comment [AV1]: Columns were added to include patient group after excluding ypT0 and after excluding patients without MIS

Comment [AV2]: A robust analysis was done by excluding patients who appeared to have a complete response after chemoradiation and so would not need a radiation boost or local excision.

Table 4. Predictive Factors

Factor		Percentile	Microscopic intramural spread (mm)	95% Confidence intervals	Comparison	Statistical significance ***
Tumor grade*	1	90	5	0 - 8	1 vs. 2	no
		95	7.8	2 - 10	1 vs. 3	no
	2	90	4.2	1 - 9	2 vs. 3	no
		95	9	3.2 - 11		
	3	90	4.5	0 - 6		
		95	NA	NA		
Vascular invasion**	no	90	2	0 - 4	no vs. yes	no
		95	5.3	2 - 8		
	yes	90	0	0 - 2.4		
		95	1.2	0 - 20		
Perineural invasion**	no	90	0.5	0 - 3	no vs. yes	no
		95	5	2 - 6.75		
	yes	90	1.8	0 - 10		
		95	9.65	0 - 20		
ypT stage	1	90	0	0 - 2.1	ypT1 vs. ypT2	$p < 0.001$
		95	1.05	0 - 7.05		
	2	90	5	3 - 6		
		95	6.6	5 - 8	ypT2 vs. ypT3-4	$p = 0.010$
	3-4	90	3	1 - 3		
		95	4.5	3 - 9.5	ypT1 vs. ypT3-4	$p = 0.008$
Time interval CRT - surgery (weeks) ****	≤ 8	90	3	2 - 5	≤ 8 vs. > 8	no
		95	5	3 - 8.7		
	8 - 12	90	0.6	0 - 5.3	≤ 8 vs. > 12	$p = 0.002$

		95	5.15	0 - 11.5		
	> 12	90	7	4 - 9	≤ 12 vs. > 12	$p < 0.001$
		95	8.5	6 - 9	≤ 8 vs. 8-12 vs. > 12	$p < 0.001$

* Only data by Perez et al.²³ and Guedj et al.²⁸

** Data not available for Chmielik et al.²⁷ and Rutkowski et al.²⁶

*** Statistical significance level is $p = 0.05$

**** Comparison using Kruskal-Wallis test

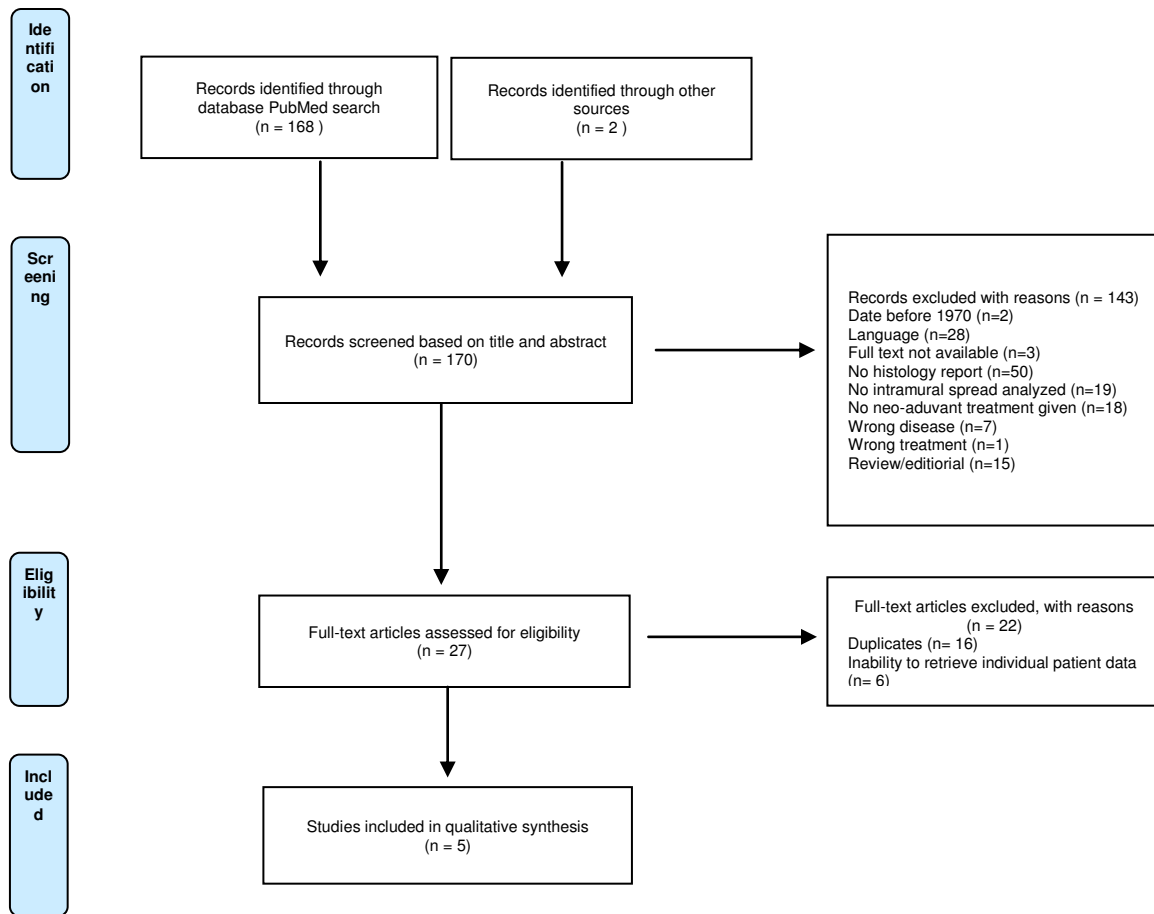


Fig. 1 Study Selection Flowchart. This figure shows the selection process, as well as reasons for exclusion of papers.

Figure

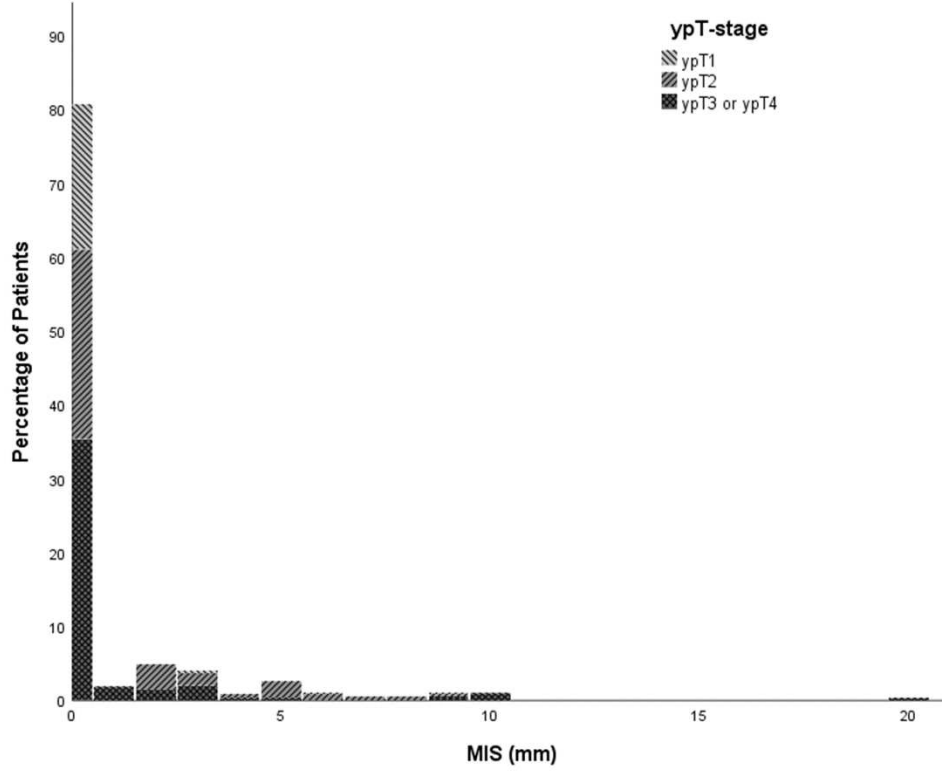


Figure 2a: Percentage of MIS in total meta-analysis population. This figure shows the percentage of patients with respective MIS according to ypT stage.

Figure

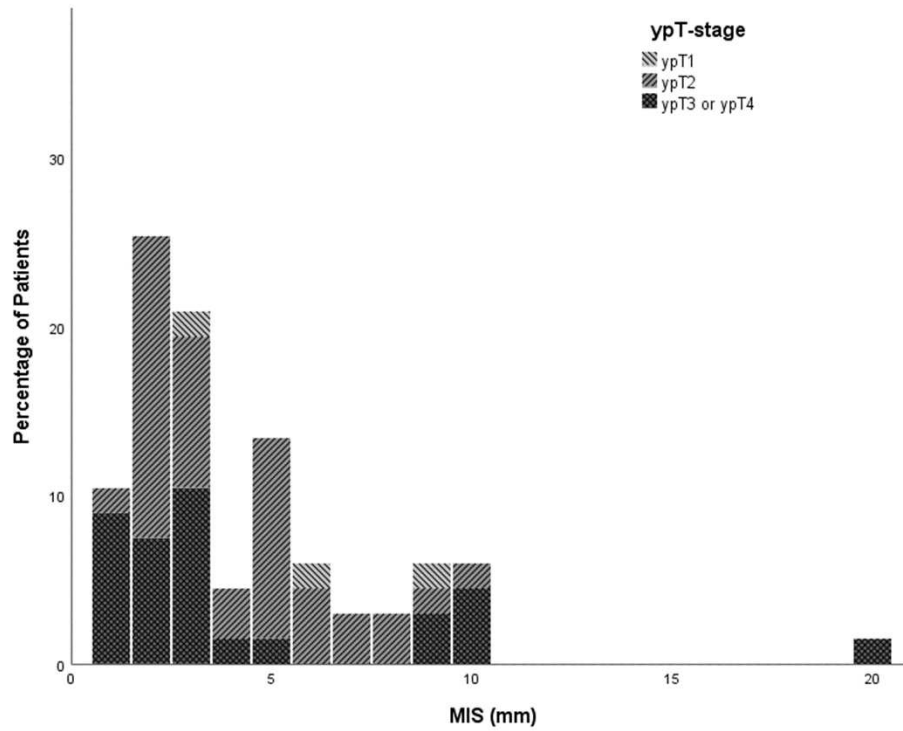


Figure 2b: Percentage of MIS in group excluding tumors without MIS. This figure shows the percentage of patients with respective MIS according to ypT stage after exclusion of patients without MIS.