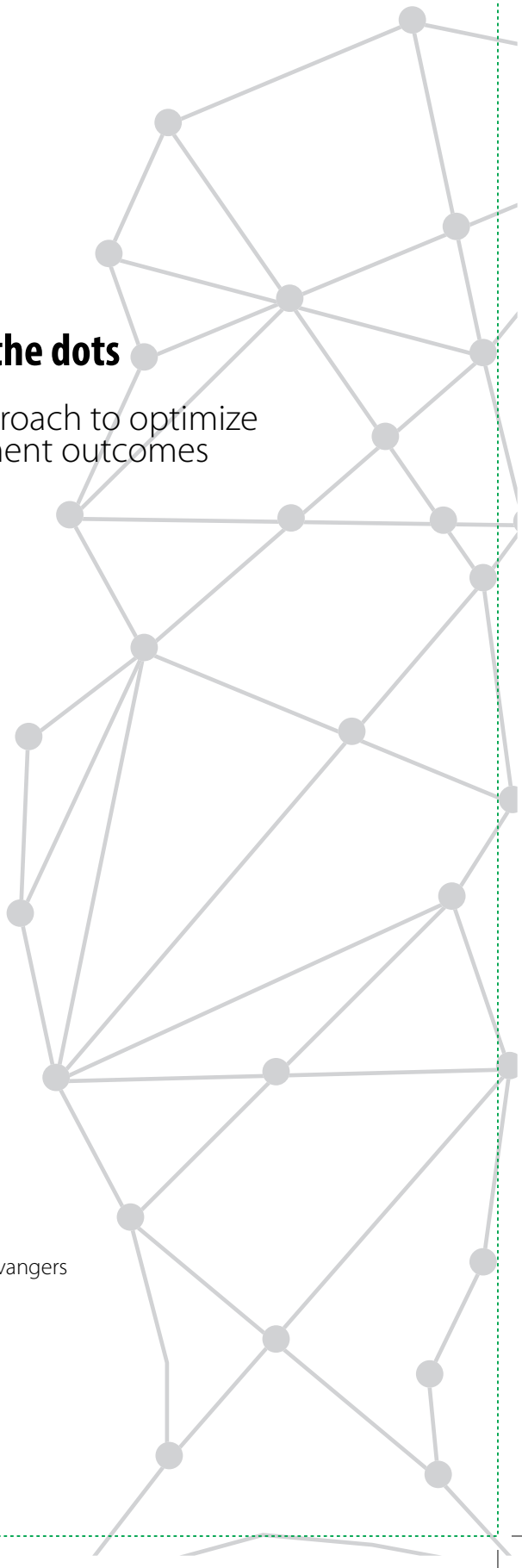
Connecting the dots

A multidisciplinary approach to optimize rectal cancer treatment outcomes



Sieske Hoendervangers

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Connecting the dots

A multidisciplinary approach to optimize rectal cancer treatment outcomes

Een multidisciplinaire aanpak ter optimalisatie
van de behandeling van rectumcarcinoom
(met een samenvatting in het Nederlands)

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aan de Universiteit Utrecht
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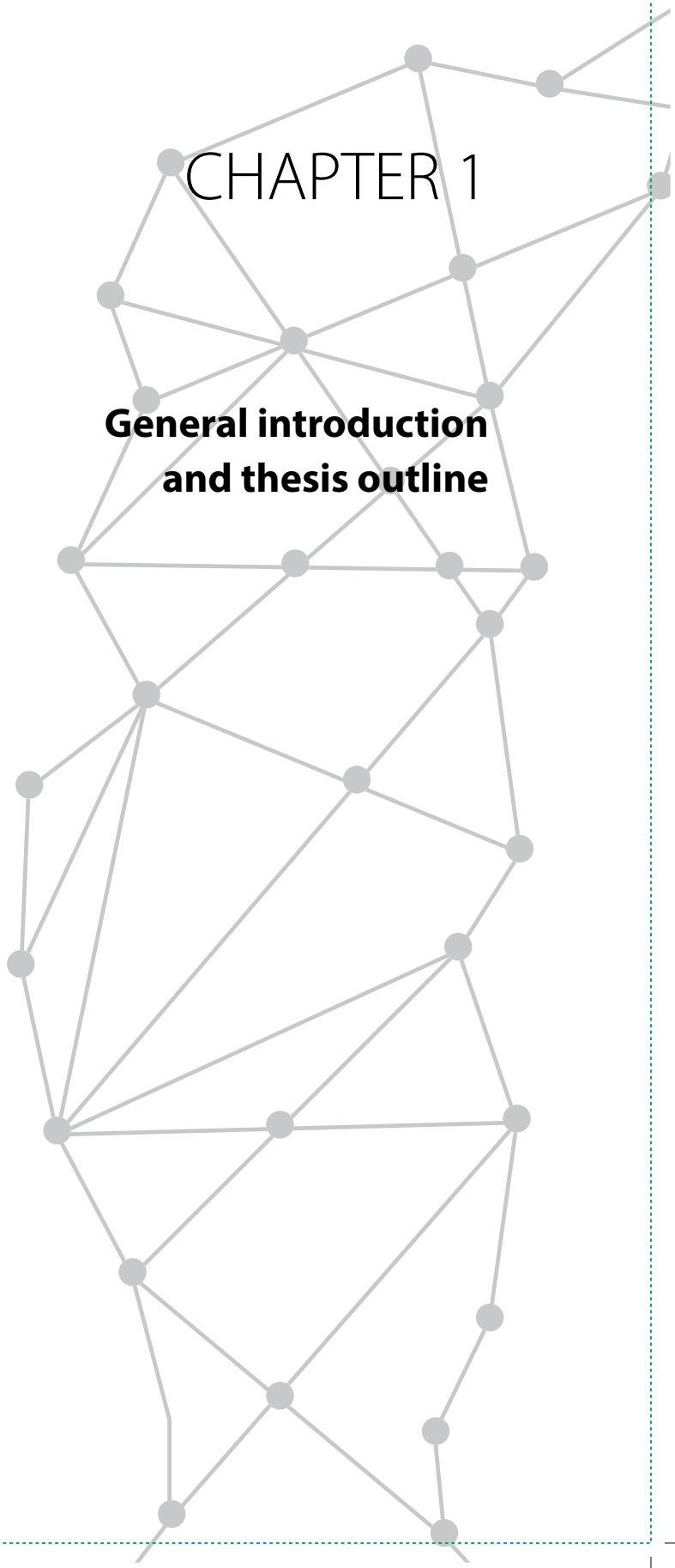
CHAPTER 1

General introduction and thesis outline



Incidence and staging of rectal cancer

Worldwide, 1,8 million people per year are diagnosed with colorectal cancer [1]. One third of these cancers is located in the rectum. In the Netherlands, this results in a yearly incidence of approximately 4,000 patients [2]. Rectal cancer treatment is based on several tumor dimensions, including tumor invasion depth (clinical tumor stage) and lymph node involvement (clinical nodal stage) assessed by Magnetic Resonance Imaging (MRI), the extent of locoregional disease, expressed as the relation with the mesorectal fascia (MRF), and the presence of distant metastases.

Table 1. Tumor, nodes and metastases (TNM) staging for rectal cancer [3]

Clinical tumor stage (cT)	T	Tumor is confined to the submucosal layer
	T2	Tumor extends into the muscularis propria
	T3	Tumor extends through the muscularis propria into the perirectal fat (mesorectum) without reaching the mesorectal fascia or adjacent organs
		- T3A: tumor extends <1 mm beyond muscularis propria
		- T3B: tumor extends 1-5 mm beyond muscularis propria
		- T3C: tumor extends 5-15 mm beyond muscularis propria
		- T3D: tumor extends 15 mm beyond muscularis propria
	T4	Tumor extends to surface of visceral peritoneum or into an adjacent organ
Clinical nodal stage (cN)	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Metastasis in 1-3 regional lymph nodes
	N2	Metastasis in > 3 regional lymph nodes
Relation with mesorectal fascia (MRF)	MRF+	Distance between tumor and MRF ≤1 mm
	MRF-	Distance between tumor and MRF >1 mm

In the TNM staging system, the cT-stage describes how deep the tumor has grown into the bowel lining (**Table 1** and **Figure 1**). Strictly speaking, TNM staging does not subclassify cT3, but this subclassification does have prognostic significance [4]. Disease involving only the regional (mesorectal and internal iliac) nodes accounts for the cN-stage. Involvement of other nodes is regarded as metastasis [5,6]. Lymph node metastases usually occur within the proximal 5 cm of the tumor. The mesorectal nodes are usually the first to get involved [7]. Extramesorectal nodes (i.e.

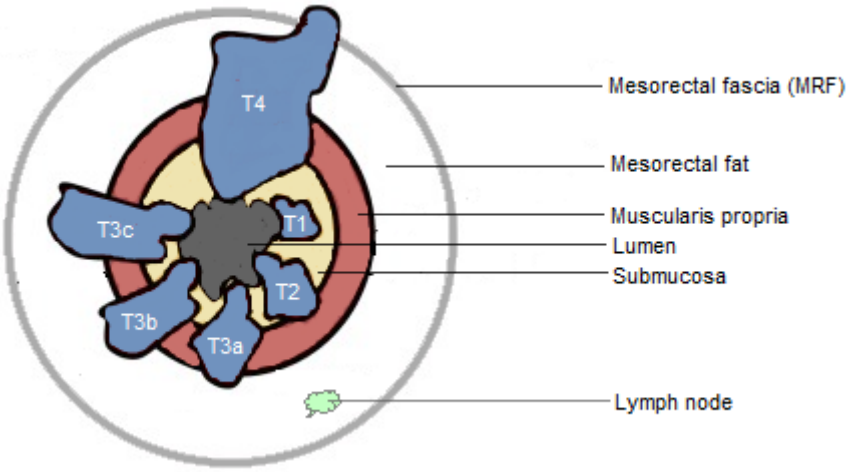


Figure 1. Transversal view on rectum with clinical rectal cancer stages (adapted from Engin et al.[5]).

iliac, superior rectal or inferior mesenteric nodes) are generally involved in locally advanced cancers [8]. Morphologic features, such as a size, an irregular contour and an inhomogeneous signal, are used to determine if the node is pathological [9].

The mesorectal fascia (MRF) is a connective tissue sheath that encloses the rectum and the perirectal fatty tissue, including lymph nodes and lymphatic vessels and acts as a natural barrier for tumor spread [10]. It is an important anatomic landmark for the assessment of local tumor extent. A distance from tumor to MRF is ≤1 mm (involved MRF) may impede radical resection and should therefore be treated with neoadjuvant therapy.

Neoadjuvant treatment

The indication for neoadjuvant treatment is determined by the risk on local recurrence according to the cTNM stage (**Table 2**). Patients with early stage rectal cancer have a low risk of local recurrence (2% in 5 years) and are therefore treated with surgery alone [11]. Patients with a cT1 tumor with low risk characteristics can be treated by local excision [12]. Patients with intermediate risk rectal cancer receive short-course radiotherapy (SCRT), which consists of 25 Gray (Gy) in 5 fractions of 5 Gy, followed by surgery. Traditionally, rectum resection was performed within 10 days following the start of SCRT, but recent studies show improved postoperative outcomes when surgery is delayed with 4 to 8 weeks after SCRT (SCRT-delay) [13].

Patients with locally advanced rectal cancer (LARC, **Table 2**) and a high risk of recurrence are treated with neoadjuvant chemoradiation (CRT) [14], which entails a combination of radiotherapy (25x2 Gy or 28x1.8 Gy) and fluoropyrimidine-based chemotherapy (e.g. capecitabine or 5FU). In addition to tumor downstaging and enabling radical resection, this treatment aims to improve survival and prevent local recurrence, while limiting treatment-related morbidity and preserving bowel, sexual and genitourinary function [14-16]. SCRT-delay is recommended as an alternative to CRT in older patients with comorbidities or frail patients with a poor performance status, because of their higher risk of treatment related complications [14]. This alternative regimen may also induce tumor downstaging [17-19]. Lastly, according to latest insights, an interval between SCRT and surgery may be used to deliver chemotherapy and herewith treat (or reduce the risk of) distant metastases and improve survival while maintaining locoregional control [20-22].

Table 2. Overview of risk classification according to the cTNM stage and recommended neoadjuvant treatment.

cTNM stage	Recurrence risk	Treatment
cT1-2N0 cT3a-bN0, distance to MRF >1 mm	Low	Surgery or local excision for low risk cT1 tumors
cT1-3c-dN1 cT3c-dN0, distance to MRF >1 mm	Intermediate	Short-course radiotherapy + surgery
cT4 cT3, distance to MRF ≤1 mm cN2 involved extramesorectal lymph nodes	High	Chemoradiation + surgery

MRF = mesorectal fascia, TME = total mesorectal excision

Surgery

Till day, surgery is the curative cornerstone of rectal cancer management. Patients with small (<3 cm) superficial (cT1) rectal cancer without lymph node involvement can be treated with local excision, in which the surrounding lymphatic tissue is not resected [23]. For larger or more extended tumors, surgery is performed according to the Total Mesorectal Excision (TME) technique, introduced by dr. R.J. Heald in 1979 (**Figure 2**) [24]. He showed that by sharply dissection the rectum along the mesorectum (where tumor deposits can be located), local recurrence rates could be reduced [25].

TME can be performed as a sphincter-sparing low anterior resection (LAR) with colorectal anastomosis, or as an abdominoperineal resection (APR), which includes resection of the anal sphincter and a permanent colostomy. The choice for LAR or APR depends on tumor location, since sufficient bowel length distal from the tumor is needed to allow the construction of a colorectal anastomosis. In addition, disease stage, age, performance status, culture and patients' or surgeons' preference play a role in this decision.

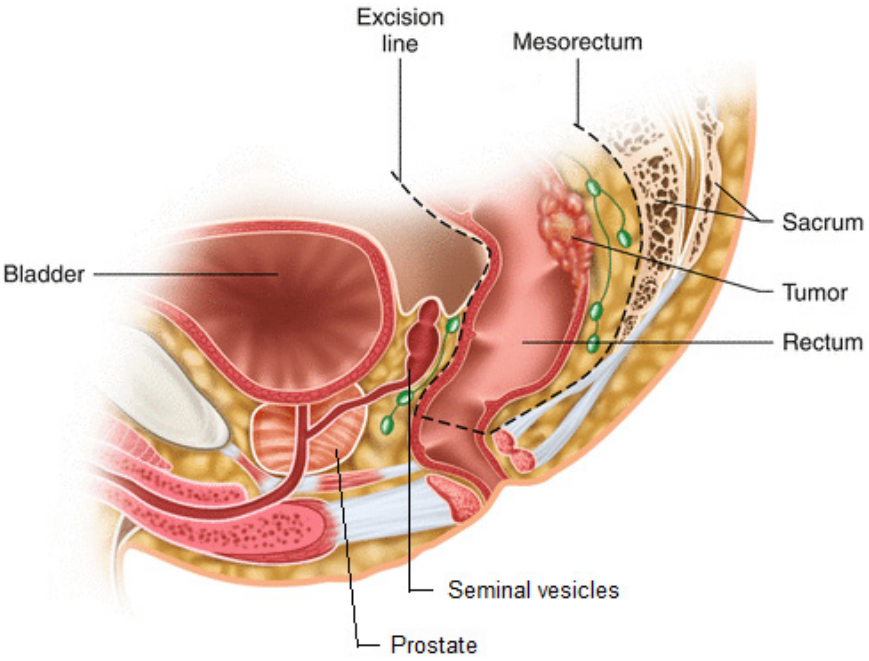


Figure 2. Total mesorectal excision dissection plane (adapted from Hakiman et al.[30]).

Rectum preservation

Following surgical resection, the rectum is analyzed by the pathologist on, among other things, tumor response to neoadjuvant therapy. The ultimate form of tumor downstaging is a complete pathological response (pCR), defined as no viable tumor in the surgical resection specimen. 15-27% of LARC patients achieve pCR after neoadjuvant chemoradiation [26-28]. Patients with a pCR after neoadjuvant therapy are reported to have better survival, lower local recurrence and less distant failure rates [27,29].

In the past decade, there has been a paradigm shift in the management of rectal cancer. The observation of pCR resulted in the introduction of a watch-and-wait strategy by Angelita Habr-Gama [31]. When a complete response is detected clinically, radiographically and endoscopically before surgery (i.e. clinical complete response, cCR), organ-preserving treatment strategies can be considered. This strategy may withhold patients from surgery-associated morbidity and the associated impairment in quality of life [32,33]. As such, patients with a cCR following neoadjuvant treatment are increasingly being offered rectum-sparing strategies, such as active surveillance, indicated as watch-and-wait, or local excision [31,34].

There are several reasons to pursue rectum-preserving strategies. First, some patients may feel a strong aversion towards having a permanent colostomy. Having a permanent ostomy may result in long-term impairment in quality of life [35]. Second, rectum-preserving treatment will avert surgical complications. Although quality of care has improved over the last years [36], postoperative complications are common following rectal surgery. Approximately 30% of patients experience one or more postoperative complications, which may have a long-term negative effect on quality of life [37]. Long-term morbidity, including urogenital- and sexual dysfunction and the low anterior resection syndrome (LARS), which entails increased stool frequency, urgency, flatulence and incontinence, are common [37-39]. The risk of postoperative complications may be higher following neoadjuvant therapy [40]. Furthermore, anastomotic leakage results in significantly more anorectal and urinary symptoms and higher Low Anterior Resection Syndrome (LARS) scores [41,42]. In the elderly population, postoperative morbidity and mortality are often increased as a result of comorbidities [43]. Postoperative complications result in a large decline in physical- and role functioning and increased mortality [44-46]. This patient group might therefore benefit from altered treatment, especially when they are more susceptible to treatment-related complications [45].

Current issues in management of patients with rectal cancer

To support individualized treatment, including rectum-preserving strategies, some issues need clarification. Many different neoadjuvant treatment strategies have been investigated in the past, but it is still unknown how they perform in achieving a complete response compared to the current standard of care. In addition, it is unclear who might benefit most from altered treatment and how new neoadjuvant regimens affect (pathological) response or surgical complications.

An example of a new neoadjuvant treatment strategy is the addition of a stereotactic radiation boost to the tumor prior to neoadjuvant CRT. Dose-escalated radiotherapy might increase tumor regression and herewith enhance the opportunity for rectum preservation [26,47]. However, no reliable methods are available to predict who might benefit from this dose-escalated therapy and who will have residual disease despite this higher dose. There is increasing evidence that tumor-infiltrating lymphocytes (TILs) may help to predict tumor regression after neoadjuvant CRT in LARC [48,49]. Higher levels of TIL subsets, such as CD3+ (a general T-lymphocyte that plays an essential role in the adaptive immune response [50]) and CD8+ (a cytotoxic T-lymphocyte that promotes apoptosis of cancer cells [50]), have been associated with increased immune response. Both radiotherapy and chemotherapy potentiate systemic antitumor immune effects by causing tumor cell death and inducing T-cell response [51,52]. Pretreatment biopsies with high CD3+ and CD8+ levels are associated with tumor regression after CRT [51,53-55]. This suggests that tumors that attract T-cells are more sensitive to CRT and are thus more likely to respond better to treatment.

To justify the omission of surgery, accurate detection of cCR is essential. However, pre-operative identification of complete responders remains challenging. The negative and positive predictive values of T2 weighted (T2w) MRI at these magnetic field strengths vary from 35-92% and 23-94%, respectively [56]. Radiation induced fibrosis is often misinterpreted as viable tumor, while undetected tumor residuals lead to incorrectly classifying patients as complete responders [57,58]. More accurate diagnostic tools may be helpful in this context.

Lastly, the effect of alternative neoadjuvant strategies on short- and long-term quality of life (QoL) remains unclear. As survival for rectal cancer treatment improves, QoL following treatment is becoming increasingly important [3,59]. As more and more treatment options become available, information on patient-reported outcome measurements is needed to enable a deliberate choice for a specific treatment.

This thesis aims to clarify the abovementioned issues regarding oncological outcomes, (pre-)treatment response prediction and long-term quality of life after different treatment modalities. Since rectal cancer treatment is a team effort, this is done with a multidisciplinary view.

A multidisciplinary approach to optimize treatment outcomes: overview per chapter

Each member of the Multidisciplinary Team (MDT) contributes to optimal and individualized rectal cancer management. The medical and radiation oncologist can alter/intensify neoadjuvant treatment, while the surgeon determines whether or not the rectum can be preserved and has to consider surgical outcomes. **Part I** of this thesis focusses on the oncological perspective, and investigates how the medical/radiation oncologist and the surgeon can contribute to treatment optimization. **Chapter 2** gives an overview of available neoadjuvant strategies and how they perform in achieving pCR, as compared to standard neoadjuvant CRT in randomized trials. **Chapter 3** and **4** compare the effect of a less intensive treatment (short course radiotherapy) and standard chemoradiation on pCR and surgical outcomes.

The response to treatment might be improved if reliable methods become available to predict who might benefit from (altered) neoadjuvant therapy and who will have residual disease despite this treatment. Hereto, the pathologist can help to identify individual tumor characteristics prior to treatment and the radiologist can assist in improving outcome evaluation following neoadjuvant treatment. **Part II** concentrates on the diagnostic perspective of the pathologist and the radiologist. **Chapter 5** analyzes whether pre-treatment tumor infiltrating lymphocytes (TILs) in rectal biopsies can contribute to better patient selection for intensified neoadjuvant treatment. **Chapter 6** looks into the role of 7 Tesla MRI in more accurate response assessment following (chemo)radiotherapy.

And last, but certainly not least, **part III** emphasizes the patients perspective, i.e. the quality of life following altered rectal cancer treatment. **Chapter 7** investigates the long-term effect on quality of life following dose-escalated radiotherapy, while **Chapter 8** looks at the short-term patient reported toxicity following short-course radiotherapy.

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PART I

Oncological perspective

CHAPTER 2

Pathological complete response following different neoadjuvant treatment strategies for locally advanced rectal cancer: a systematic review and meta-analysis

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ABSTRACT

Background

A pathological complete response (pCR) following neoadjuvant treatment for locally advanced rectal cancer (LARC) is associated with better survival, less local recurrence and less distant failure. Furthermore, pCR indicates that the rectum may have been preserved. This meta-analysis gives an overview of available neoadjuvant treatment strategies for LARC and analyzed how they perform in achieving pCR as compared to the standard of care.

Methods

Pubmed, EMBASE and Cochrane Central were searched. Randomized controlled trials (RCTs) in which patients received neoadjuvant treatment for MRI-staged, non-metastatic, resectable LARC were included. The primary outcome was pathological complete response (pCR), defined as ypT0N0. A meta-analysis of studies that compared an intervention to standard fluoropyrimidine-based chemoradiation (CRT) was performed.

Results

17 articles were included in the systematic review, of which 11 were used for the meta-analysis. Addition of oxaliplatin to fluoropyrimidine-based CRT resulted in significantly more pCR compared to fluoropyrimidine-based CRT only (OR 1.46), but at the expense of more ≥grade 3 toxicity. Other treatment strategies, including consolidation/induction chemotherapy and short-course radiotherapy did not improve pCR rates. None of the included trials reported benefit in local control or OS. 5-year DFS was significantly worse after SCRT-delay compared to CRT (59% vs. 75.1%, HR 1.93)

Conclusion

All included trials fail to deliver high level evidence to show an improvement in pCR compared to standard fluoropyrimidine-based CRT. The addition of oxaliplatin might result in more pCR, but at the expense of more toxicity. Furthermore, this benefit does not translate into less local recurrence or improved survival.

Introduction

The aim of rectal cancer treatment is to improve survival and prevent local recurrence, while limiting treatment-related morbidity and preserving bowel, sexual and genitourinary function [1,2]. Consequently, patients with locally advanced rectal cancer (LARC) generally undergo neoadjuvant chemoradiation (CRT) followed by surgery [3,4]. This combined modality approach decreases recurrence rates and improves survival compared to surgery only [4,5]. The most frequent used neoadjuvant treatment strategy for LARC is a combination of radiotherapy (25x2 Gy or 28x1.8 Gy) and fluoropyrimidine-based chemotherapy (e.g. capecitabine or 5FU). Hereby 15-20% of LARC patients achieve a pathological complete response (pCR) in which no tumor is found in the surgical resection specimen [6-8].

Unfortunately, still 30% of patients that received this treatment will die within 5 years due to a local or distant recurrence [9]. However, patients with a pCR after neoadjuvant therapy are reported to have better survival, lower local recurrence and less distant failure rates [10]. The observation of pCR after surgery has led to a paradigm shift in rectal cancer management, in which organ preservation has become an increasingly important endpoint after neoadjuvant treatment in combination with reduction of local recurrence and survival rates [9]. Organ-preserving treatment strategies can be considered when a complete response is detected clinically, radiographically and/or endoscopically before surgery (i.e. clinical complete response, cCR). , this strategy may withhold patients from surgery-associated morbidity and the associated impairment in quality of life [11,12]. As such, patients with a cCR following neoadjuvant treatment are increasingly being offered watch-and-wait regimens or organ-sparing strategies, such as local excision [13,14]. In order to further increase the number of eligible patients for such organ preservation strategies, physicians are searching for (new) neoadjuvant treatments with a higher organ-sparing potential than the current standard of care .

Previous studies suggested that treatment intensification, i.e. adding chemotherapy or dose-escalated radiotherapy to standard chemoradiation, might enhance rectum preservation and/or improve oncological outcomes [15]. Theoretically, intensified treatment would further downstage the tumor and any nodal disease prior to surgery, and/or target potential micrometastatic disease [4]. On the contrary, others prefer a short-course (radiation) schedule over long-course chemoradiation, based on its lower rates of toxicity, better compliance and lower cost [16-19].

This systematic review and meta-analysis gives an overview of available neoadjuvant treatment strategies for LARC and analyzes how they perform in achieving pCR (as a surrogate endpoint for cCR) compared to the current standard of care in patients with locally advanced rectal cancer, based on available evidence from randomized trials.

Methods

This systematic review and meta-analysis was registered in the PROSPERO database under number CRD 42017058674.

Pubmed, EMBASE and Cochrane Central were searched (last update June 20, 2019) for randomized controlled trials on neoadjuvant treatment for locally advanced rectal cancer, restricted to full text and English language. The search strategy, search syntax and characteristics of excluded studies can be accessed through Supplementary data (**Supplementary Table 1 and 2**, available online). Cross referencing was performed.

Phase II-III randomized controlled trials (RCTs), conducted after the introduction of TME surgery in the 1980s [20], in which patients received neoadjuvant treatment for MRI-staged, non-metastatic, LARC were included. LARC was defined as stage II-III (cT3-4N0 or T1-4N1-2) rectal cancer. All neoadjuvant treatment modalities that entailed systemic therapy and/or radiotherapy were eligible. Radiotherapy, delivered in either a short-course or a long-course, and optionally accompanied by radiation dose-escalation, was considered suitable. Inclusion was restricted to studies using an interval of at least 4 weeks between end of neoadjuvant therapy and surgery. The primary outcome was pathological complete response (pCR), defined as ypT0N0. Studies that did not report ypTN-stage were excluded. Secondary outcomes were ≥ grade 3 toxicity (according to Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 or 4.0), surgical outcomes (complication rate and R0 resection rate), local recurrence (LR), disease free survival (DFS), and overall survival (OS). Administration of postoperative systemic therapy was not an exclusion criterion, since this could not influence our primary outcome. Study selection was solely based on the primary outcome.

Identified studies were listed in Endnote (© 1988-2012 Thomson Reuters). Two authors (SH and MB) independently screened on title and abstract. Full text reports were retrieved and examined for eligibility criteria. Studies that only partially fulfilled the eligibility criteria were excluded. Disagreements were resolved by discussion between the two raters. Duplicates were removed and multiple reports

of the same study were linked together. Lastly, the corresponding author of each included study was contacted to obtain additional information or information on individual patient level.

Risk of bias was assessed by the first author using the Cochrane risk of bias tool [21], including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. All studies were included in the analyses, irrespective of their risk of bias.

From each included trial, information about trial characteristics (study year/ duration, year of publication and country), methodology (phase II or III RCT, number of arms and sample size), characteristics of study participants (clinical tumor and nodal stage, involvement of the mesorectal fascia (MRF) and distance from the anus in cm), characteristics of intervention (agent(s), (radiotherapy) dose, duration and interval to surgery in weeks), and outcomes (pCR (ypT0N0) rate, ≥ grade 3 toxicity (CTCAE), percentage of patients who received complete dose chemotherapy, percentage of patients that proceeded to surgery, surgical complications, R0 resection rate and oncological outcomes (local recurrence (LR), disease free survival (DFS) and overall survival (OS)) was collected. Survival data are reported as 3-year cumulative incidence rates. If available from the report, hazard ratios (HR) are also presented.

Four subgroups were created based on neoadjuvant treatment: multi-agent chemoradiation (n=9), induction chemotherapy (n=5), consolidation chemotherapy (n=2), and short-course radiotherapy and delayed surgery (SCRT-delay, n=1). A systematic review was performed of all included studies. A quantitative meta-analysis was conducted on the studies that compared an intervention to standard fluoropyrimidine-based chemoradiation (25-28 x 1.8-2Gy + capecitabine/5FU) in order to investigate their effect size. The Mantel-Haenszel random-effects model (REM) was applied, assuming that heterogeneity between studies was not a result of chance alone. Heterogeneity was expressed with I^2 [22]. The pooled effect size was calculated from per protocol data and is expressed as the odds ratio (OR) and its 95% confidence interval.

All analyses were performed using Review Manager (RevMan), version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Results were reported according the PRISMA guidelines [23].

Results

The literature search obtained 586 records after removal of duplicates, of which 526 records were excluded at title/abstract screening (**Figure 1**). After full-text review, 17 articles met the in- and exclusion criteria and were included in the systematic review. Of those, 11 papers were included in the quantitative (meta-)analysis. Four studies were excluded from meta-analysis, because they did not include a fluoropyrimidine-based (standard) CRT control arm. Two trials were excluded from quantitative analysis, because they were the only one in their subgroup [24,25].

Risk of bias

In general, random sequence generation and allocation concealment were well performed and described (**Figure 2**). Participants and personnel were not blinded in most studies. However, this was considered as low risk of bias, since the primary outcome pCR was unlikely to be influenced by this. On the contrary, most studies lacked a blinded assessment of pCR, which could have increased the risk of detection (observer) bias.

Characteristics of included studies

Ten phase II and 9 phase III trials were conducted between 2001 and 2018 (**Table 1**). Interval to surgery varied from 4 to 12 weeks after end of neoadjuvant therapy. Detailed patient and tumor characteristics as well as an overview of administered therapy doses are available as Supplementary data (Supplementary Tables 3 and 4, online accessible). The majority of patients had cT3N+ tumors (Supplementary Table 3). MRF involvement was reported in 8 studies and varied from 0-94.7%. Tumors located <5cm from the anus were present in 4-69.6% of included patients. The outcomes of included randomized controlled trials, stratified by neoadjuvant treatment regimen, are presented in **Table 2**.

Fluoropyrimidine-based CRT vs. multi-agent CRT

Nine trials compared fluoropyrimidine-based CRT with multi-agent CRT. Six trials (two phase II trials and four phase III trials), including 2,502 participants, entered the quantitative analysis. Overall, the pooled OR for pCR after multi-agent CRT (n=1,248) versus standard CRT (n=1,254) was statistically significant at 1.46 (95% CI 1.18 - 1.79, I^2 0%). Subgroup analysis revealed that the pooled OR resulting from phase II trials was not significant (OR 1.19, 95% CI 0.56 – 2.52, I^2 34%), and the pooled OR from phase III trials remained statistically significant in favor of multi-agent CRT (OR 1.50, 95% CI 1.20 – 1.87, I^2 0%, **Figure 3A**).

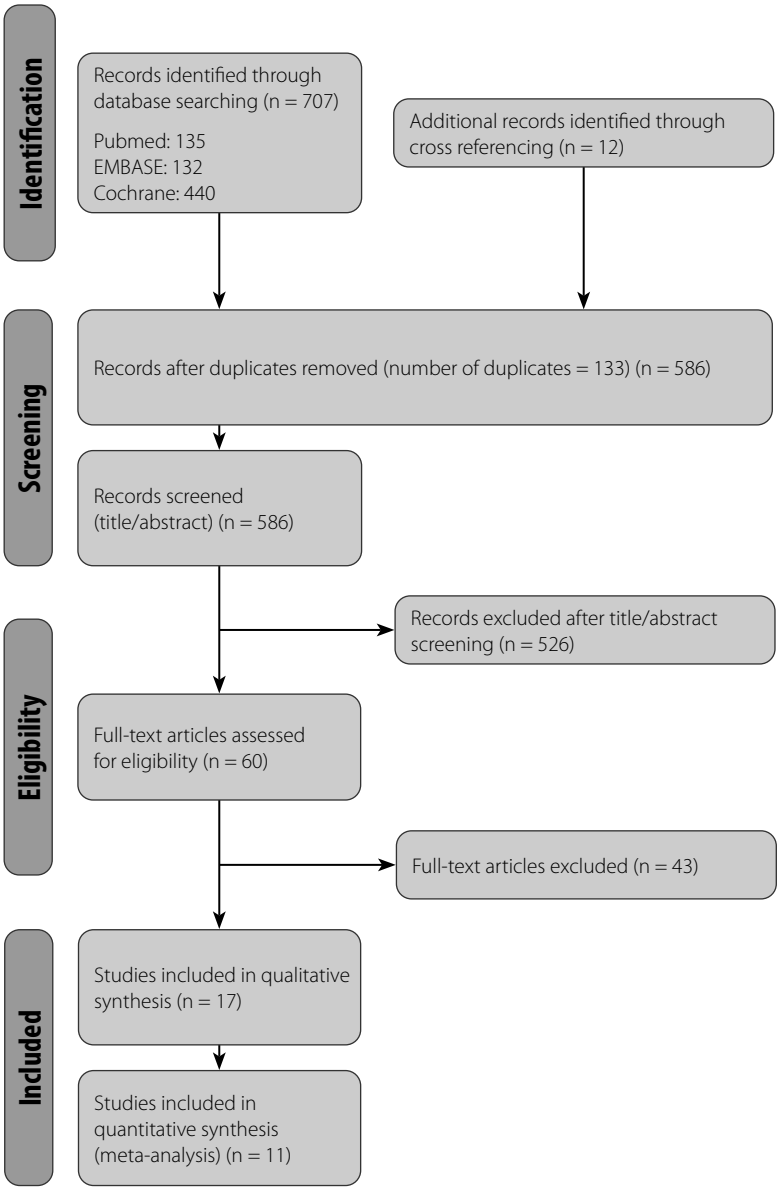
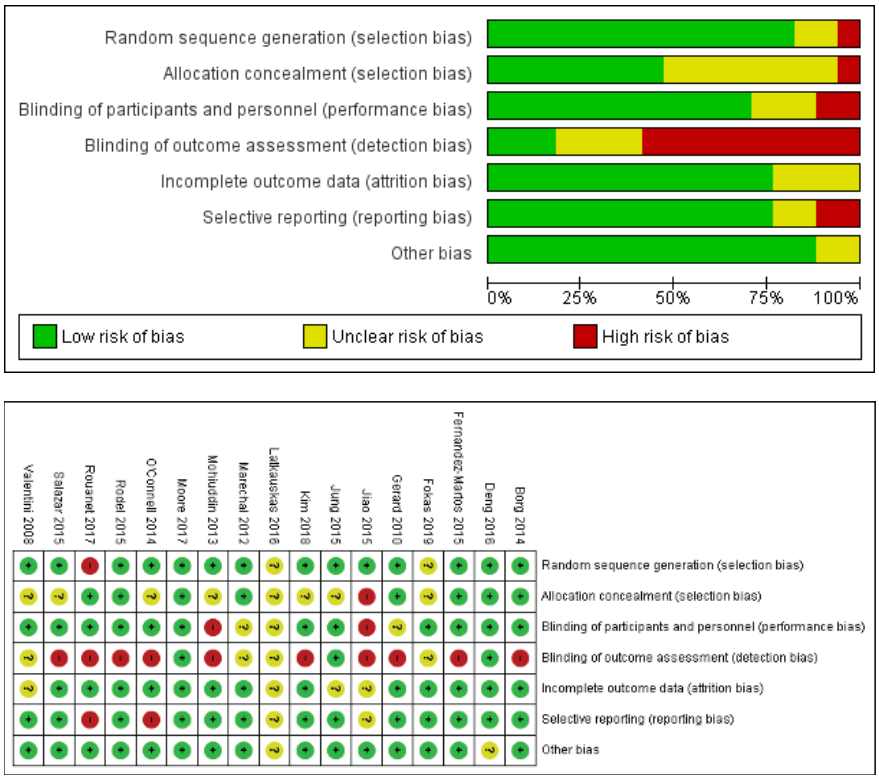


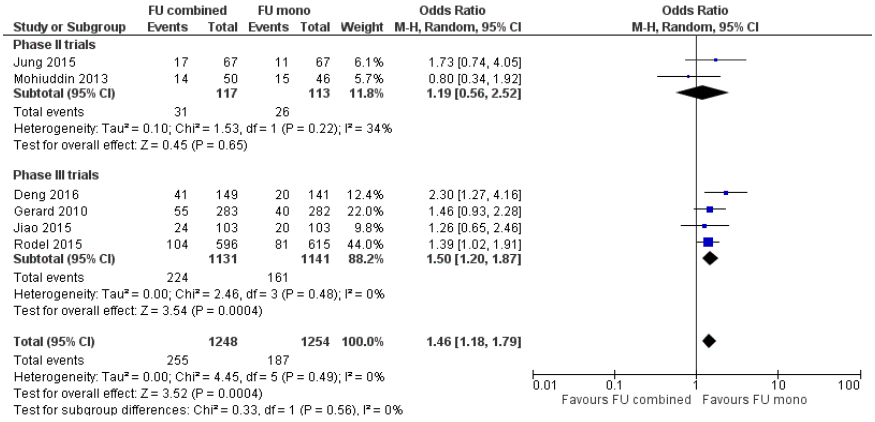
Figure 1. PRISMA flowchart of included studies. Reasons for exclusion are provided as Supplementary data (available online).

Figure 2. Review authors' judgements about each risk of bias item presented as percentages across all included studies; a) risk of bias graph, b) risk of bias summary

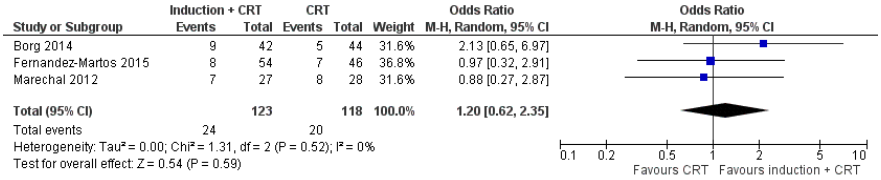


In five trials, the experimental group received a combination of fluoropyrimidine-based chemotherapy and oxaliplatin [26-30]. In patients that received fluoropyrimidine-based CRT, ≥ grade 3 toxicity occurred in 10.7-40%. In the oxaliplatin CRT group ≥ grade 3 toxicity rates were significantly higher (21.4-49.1%), but this did not affect the number of patients that completed neoadjuvant therapy or the percentage of participants that proceeded to surgery. Neoadjuvant fluoropyrimidine-based CRT resulted in pCR in 13.2-28.3% of patients. When oxaliplatin was added to this regimen, pCR rates were 17.4-28.4%. This was statistically significant in 2 trials [26,30]. No differences were seen in R0 resections or surgical complications. Two trials compared 5FU-based CRT with multi-agent CRT containing irinotecan [31-33]. One trial described significantly less complete dose administration in the experimental group [31]. No differences in pCR, nor in surgical and survival outcomes were seen. One trial evaluated the effect of targeted therapy (bevacizumab) added to

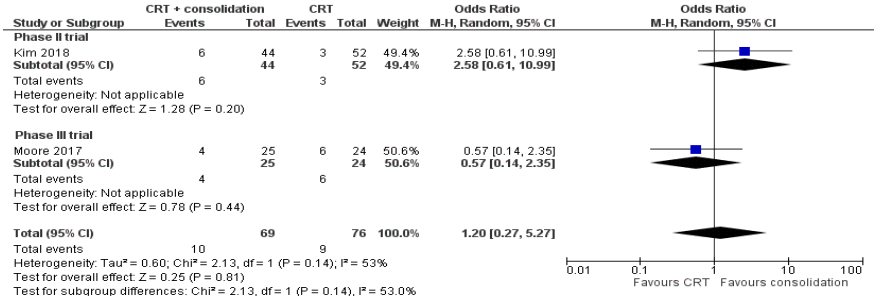
Figure 3. Pooled OR of pCR rates following multi-agent chemoradiation, consolidation chemotherapy and induction chemotherapy compared to standard fluoropyimidine-based CRT



A. Multi-agent chemoradiation vs. standard fluoropyimidine-based chemoradiation, analyzed per trial type



B. Induction chemotherapy and chemoradiotherapy vs. standard fluoropyimidine-based chemoradiation



C. Chemoradiotherapy and consolidation chemotherapy vs. standard fluoropyimidine-based chemoradiation, analyzed per trial type

capecitabine-based CRT in 44 patients [24]. Compared with patients that received capecitabine-based CRT (n= 46), no differences were seen in toxicity or treatment compliance. All but one patient (investigational group) underwent surgery after a median interval of 7.3 weeks. pCR was achieved in 10.9% of patients in the capecitabine-group and 16.3% of patients in the bevacizumab group. This difference was not statistically significant. Survival data were not available.

For all multi-agent comparisons, survival and recurrence data were available from five studies [27,28,30-32]. No significant differences were reported in LR or OS. 3-year cumulative incidence rates for LR and OS in the monotherapy group varied from 4.6% to 6.1% and 86.4% to 88.0%, respectively. For the multi-agent group these rates were 2.9-4.4% and 88.3-90.3%, respectively. One study reported a significant better 3-year DFS after fluoropyrimidine plus oxaliplatin-based CRT (71.2% vs. 75.9%, HR 0.79 (95% CI 0.64 - 0.98), **Table 2**) [30].

Induction chemotherapy

Five trials investigated the effect of CRT on pCR when it was preceded by induction chemotherapy. Induction chemotherapy plus CRT was compared to standard CRT in three phase II trials [34-36]. In these trials, induction therapy consisted of multi-agent chemotherapy (i.e. CAPOX or FOLFOX). Toxicity was higher after induction chemotherapy, and resulted in significantly lower compliance to CRT in one trial [35,37]. There were no differences in surgical outcomes or survival. There was no significant difference for pCR after induction chemotherapy (n=123) versus standard CRT (n=118) with a pooled OR of 1.20 (95% CI 0.62 - 2.35, I^2 0%, **Figure 3B**).

Two trials (GRECCAR-4 and CAO/ARO/AIO-12) in this subgroup were not used for quantitative analysis. The GRECCAR-4 trial randomized patients based on their response to induction FOLFIRINOX [38]. Good responders either received additional capecitabine-based CRT or underwent surgery. Poor responders were randomized to either capecitabine-based CRT or capecitabine-based CRT with dose-escalated radiotherapy (60Gy). The trial was stopped prematurely due to low accrual rates in the good-responders arm. In the good responders arm (n=20), pCR was achieved in 1 of 11 (9.1%) patients after FOLFIRINOX alone and 11 of 19 (57.9%) patients after induction chemotherapy with FOLFIRINOX and capecitabine-based CRT. In the poor responder group (n=103), CRT with dose-escalated radiotherapy resulted in pCR in 9 of 51 (17.6%) patients, compared to 7 of 52 (13.5%) patients in the standard CRT group. This was not a significant difference. The higher radiation dose in the poor responders arm increased R0 resection from 83% to 88%. The CAO/ARO/AIO-12 trial

compared CRT and consolidation chemotherapy with CRT and induction therapy [39]. Acute ≥ grade 3 toxicity occurred in 21.8% and 35.9% after induction chemotherapy alone and CRT after induction chemotherapy, respectively, compared to 27.3% in participants undergoing CRT before consolidation chemotherapy and 20% during consolidation therapy. There were no differences in number of R0 resections. pCR was significantly higher in the consolidation group. Long-term survival outcomes were not available.

Consolidation chemotherapy

Two RCTs (one phase II and one phase III trial) compared standard CRT with CRT followed by consolidation chemotherapy with either CAPOX or 5FU [40,41]. Acute ≥ grade 3 toxicity was reported in one trial and did not differ between groups [40]. R0 resections were achieved in 91.7-100% of patients after standard CRT and 88.6-92% of patients after CRT with consolidation CAPOX. This was a non-significant difference. The quantitative analysis for pCR in standard CRT (n=76) versus CRT with consolidation CAPOX (n=69) resulted in a non-significant difference with pooled OR of 1.17 (95% CI 0.33 - 4.23, I^2 54%). In subgroup analysis, the phase II trial was in favor of CRT with consolidation therapy (OR 2.58, 95% CI 0.61 – 10.99) [42], and the phase III trial was in favor of standard CRT (OR 0.57, 95% CI 0.14 – 2.35) [43]. None of the ORs were statistically significant (**Figure 3C**). Survival data were not reported.

Short-course radiotherapy and delayed surgery

One trial compared SCRT-delay to capecitabine-based CRT [25,44], resulting in a non-significant different pCR rate (4.4% vs. 11.1%, respectively). There were no differences in radicality or surgical complications. 5-year DFS was significantly worse after SCRT-delay compared to CRT (59% vs. 75.1%, HR 1.93, **Table 2**).

Table 1. Study characteristics of randomized controlled trials, stratified by neoadjuvant treatment regimen. Underlined trials were included in the meta-analysis.

Source			Study protocol		
Author Year Country	**Study ID**	**Period**	**Study design**	**Tumor stage**	**Number of arms**
Fluoropyrimidine-based chemoradiotherapy vs. multi-agent chemoradiotherapy					
Deng [26] 2016 China	FOWARC	2010-2015	Phase III	Stage II (cT3-4 N0) and stage III (cT1-4 N1-2)	3
Gerard [27] 2010 France	ACCORD 12/0405-Prodige 2	2005-2008	Phase III	cT2 in the anterior and lower rectum, cT3 or resectable cT4	2
Jiao [28] 2015 China	-	2007-2010	Phase III	clinical stage II/III (cT2 in the distal anterior or lower rectum, any cT3, resectable cT4 or cN1-2)	2
Jung [31] 2015 South Korea		2009-2011	Phase II	cT3-4 or any cN	2
Mohiuddin [32] 2013 USA	RTOG-0012	2001-2003	Phase II	cT3-4	2
O'Connell [29] 2014 USA	NSABP R-04	2004 - 2010	Phase III	stage II-III (cT3-4N0 or T1-4N1-2)	4

Study protocol				
Number of patients	**Neoadjuvant chemotherapy**	**Neoadjuvant radiotherapy total dose (Gy) (number of fractions*fraction dose)**	**Adjuvant treatment**	**Interval to surgery (weeks)**
165	5FU	46-50.4 Gy (23-28 * 1.8-2)	7 cycles 5FU	4-6
165	mFOLFOX6	46-50.4 Gy (23-28 * 1.8-2)	7 cycles mFOLFOX6	
165	mFOLFOX6	Before or after surgery at physician discretion	6–8 cycles mFOLFOX6	
293	Capecitabine	45 Gy (25 * 1.8)	decision left to institution	6
291	Capecitabine + Oxaliplatin	50 Gy (25 * 2)		
103	Capecitabine	50 Gy (25 * 2)	6–8 cycles FOLFOX	6–10
103	Capecitabine + Oxaliplatine	50 Gy (25 * 2)		
71	5FU	45-50.4 Gy + 4.5-9.0 Gy (25-28 * 1.8)	4 cycles 5FU	4-8
70	Irinotecan + S-1	45-50.4 Gy + 4.5-9.0 Gy (25-28 * 1.8)		
50	5FU	45.6 Gy + 9.6 Gy for cT3 / 14.4 Gy for cT4 (19 * 1.2 b.i.d.)	recommended for patients with residual disease	4-10
53	5FU + Irinotecan	45 Gy + 5.4 Gy for cT3 / 9 Gy for cT4 (25 * 1.8)		
477	5FU	45 Gy + 5.4 Gy for cT3 / 10.8 Gy for cT4 (25 * 1.8)	decision left to institution	6-8
329	5FU + Oxaliplatin	45 Gy + 5.4 Gy for cT3 / 10.8 Gy for cT4 (25 * 1.8)		
472	Capecitabine	45 Gy + 5.4 Gy for cT3 / 10.8 Gy for cT4 (25 * 1.8)		
330	Capecitabine + Oxaliplatin	45 Gy + 5.4 Gy for cT3 / 10.8 Gy for cT4 (25 * 1.8)		

Table 1 continued I

Source			Study protocol		
Author Year Country	**Study ID**	**Period**	**Study design**	**Tumor stage**	**Number of arms**
Rodel [30] 2015 Germany	CAO/ARO/AIO-04	2006 - 2010	Phase III	any cT3-4 or cN1–2	2
Valentini [45] 2008 Italy		2002-2005	Phase II	cT3N0-N2	2
Salazar [24] 2015 Spain		2009 - 2011	Phase II	Stage II-III	2
Induction chemotherapy and chemoradiotherapy vs. standard fluoropyimidine-based chemoradiation					
Borg [34] 2014 France	INOVA	2007 - 2010	Phase II	cT3N0–2 in the lower rectum, cT3N0 in the mid-rectum or cT3N1–2	2
Fernandez-Martos [35] 2015 Spain	GCR-3	2006-2007	Phase II	< 2mm from MRF, ≤ 6cm from anal verge, cT3, resectable cT4 or any cT3N+	2
Marechal [36] 2012 Belgium			Phase II	cT2-4 N+	2

Study protocol				
Number of patients	**Neoadjuvant chemotherapy**	**Neoadjuvant radiotherapy total dose (Gy) (number of fractions*fraction dose)**	**Adjuvant treatment**	**Interval to surgery (weeks)**
623	5FU	50.4 Gy (28 * 1.8)	4 cycles 5FU	5-6
613	5FU + Oxaliplatin	50.4 Gy (28 * 1.8)	8 cycles 5FU-OX	
83	5FU + Cisplatin	50.4 Gy (25 * 1.8 + 5.4)	recommended for ypN+, regimen depended on physician preference	6-8
81	Raltitrexed + Oxaliplatin	50.4 Gy (25 * 1.8 + 5.4)		
46	Capecitabine	45 Gy (25 * 1.8)	administered at the investigators discretion	6-8
44	Capecitabine + Bevacizumab	45 Gy (25 * 1.8)		
45	5FU + Bevacizumab	45 Gy (25 * 1.8)	left to the investigators discretion	6-8
46	Induction: Bevacizumab + FOLFOX4 CRT: 5FU + Bevacizumab	45 Gy (25 * 1.8)		
52	Capecitabine + Oxaliplatin	50.4 Gy (28 * 1.8)	4 cycles CAPOX	5-6
56	Induction Capecitabine + Oxaliplatin CRT: Capecitabine + Oxaliplatin	50.4 Gy (28 * 1.8)	-	
29	5FU	45 Gy (25 * 1.8)		6-8
28	Induction: mFOLFOX6 CRT: 5FU	45 Gy (25 * 1.8)		

Table 1 Continued II

Source			Study protocol		
Author Year Country	**Study ID**	**Period**	**Study design**	**Tumor stage**	**Number of arms**
Rouanet [38] 2017 France	GRECCAR-4	2011-2014	Phase II	cT3-4; CRM ≤1 mm, inferior tumor margin ≥1cm from anal verge	4
Fokas [39] 2019 Germany	CAO/ARO/AIO-12	2015-2018	Phase II	cT3 < 6cm from anal verge, cT3b in mid-rectum (≥6 to 12 cm), cT4, or any N+	2
Chemoradiotherapy and consolidation chemotherapy vs. standard fluoropyimidine-based chemoradiation					
Kim [40] 2018 South Korea	KCSG CO 14-03	2014-2016	Phase II	cT3-4	2
Moore [41] 2017 Australia	WAIT	2012-2014	Phase III	NS	2
SCRT-delay vs. CRT					
Latkauskas [25] 2016 Lithuania		2007-2013	Phase III	Stage II-III (T3-4N0 or N+)	2

Abbreviations: 5FU = 5-Fluorouracil, AJCC = American Joint Committee on Cancer, BEV = Bevacizumab, C = Cetuximab, CAP = Capecitabine, CAPOX = Capecitabine + Oxaliplatin, cN = clinical nodal stage, Cons. = consolidation chemotherapy, CRT= chemoradiotherapy, cT = clinical tumor stage,

Study protocol				
Number of patients	**Neoadjuvant chemotherapy**	**Neoadjuvant radiotherapy total dose (Gy) (number of fractions*fraction dose)**	**Adjuvant treatment**	**Interval to surgery (weeks)**
11	FOLFIRINOX	None	left to the investigators discretion. Advise: ypT0-1N0 no adjuvant treatment. ypT ≥ 2 or ypN ≥ 1: 6 cycles cycles FOLFOX	NR
19	Induction FOLFIRINOX CRT: Capecitabine	50 Gy (25 * 2)		
52	Induction FOLFIRINOX CRT: Capecitabine	50 Gy (25 * 2)		
51	Induction FOLFIRINOX CRT: Capecitabine	60 Gy (30 * 2)		
156	Induction: 5FU + Oxaliplatin CRT: 5FU + Oxaliplatin	50.4 Gy (28 * 1.8)	Not recommended	6-12
150	CRT: 5FU + Oxaliplatin Consolidation: 5FU + Oxaliplatin	50.4 Gy (28 * 1.8)		
55	Capecitabine	50.4 Gy (28 * 1.8)	ypStage 0-1: 6 cycles CAP	6-10
53	CRT: Capecitabine Consolidation: Capecitabine + Oxaliplatin	50.4 Gy (28 * 1.8)	ypStage II-III: 6 cycles CAPOX	8-10
24	5FU	45 Gy + 5.4Gy (25 * 1.8)		10
25	CRT: 5FU Consolidation: 5FU	45 Gy + 5.4Gy (25 * 1.8)		
68	None	25 Gy (5 * 5)	4 cycles 5FU	6
72	5FU	50 Gy (25 * 2)		

EMVI = Extramural venous invasion, Gy = Gray, Ind.= Induction chemotherapy, MRF = mesorectal fascia, NR= not reported, OX = Oxaliplatin, S1 = tegafur/gimeracil/oteracil,

Table 2. Overview of outcomes of included randomized controlled trials, stratified by neoadjuvant treatment regimen. Underlined trials were included in the meta-analysis.

Author Year	Treatment summary (CT, RT, adjuvant treatment)	Included cT4 (%)	Included cN+ (%)	N_{CRT}	Any ≥ grade 3 CT/CRT toxicity [a]	Full CT dose
Fluoropyrimidine-based chemoradiotherapy vs. multi-agent chemoradiotherapy						
Deng [26] 2016	5FU 46-50.4 Gy Adj. 7 x 5FU	34.5	77.6	155	CTC 3.0 49 (31.6)	88.4%
	FOLFOX6 46-50.4 Gy Adj. 7 x FOLFOX6	33.9	81.8	158	**87 (55.1)**	94.9%
	FOLFOX6 No RT Adj. 6–8 x FOLFOX6	30.3	72.1	163	40 (24.5)	94.5%
Gerard [27,46] 2010	CAP 45 Gy Adj.: decision left to institute	5.1	70.7	293	CTC 3.0 32 (10.9)	97.2%
	CAPOX 50 Gy Adj.: decision left to institute	6.5	73	291	**74 (25.4)**	91.2%
Jiao [28] 2015	CAP 50 Gy Adj. 6–8 x FOLFOX	37.9	77.7	103	CTC 3.0 11 (10.7)	85.4%
	CAPOX 50 Gy Adj. 6–8 x FOLFOX	34.0	78.6	103	**22 (21.4)**	81.5%
Jung [31] 2015	5FU 45-50.4 Gy Adj. 4 x 5FU	19.7	88.7	71	CTC 4.0 0	71 (100%)
	Irinotecan-S1 45-50.4 Gy Adj. 4 x 5FU	21.4	90	70	8 (11.4)	**S-1 90% Irinotecan 87.4%**
Mohiuddin [32,33] 2013	5FU 45.6 Gy + 9.6 / 14.4 Adj.: advised for residual disease	32	38	50	CTC NS 20 (40.0)	*NR*
	5FU-Irinotecan 45 Gy + 5.4 / 9 Adj.: advised for residual disease	26.4	38	53	26 (49.1)	

Numbers are presented as n(%), unless stated otherwise. Outcomes expressed in bold numbers are statistically significant.

$N_{surgery}$ [b]	**Weeks to surgery** [c]	**Surgical complications** [d]	**pCR**	**R0 resection**	**3-year LR** [e]	**3-year DFS** [e]	**3-year OS** [e]
141	7.6	*NR*	20 (14.2)	128 (90.8)	*NR*	*NR*	*NR*
149	7.4		**41 (27.5)**	134 (89.9)			
152	7.4		10 (6.6)	136 (89.5)			
282	6	37 (13.1)	40 (14.2)	131 [f]	6.1%	67.9%	87.6%
283	6	36 (12.7)	55 (19.4)	131 [f]	4.4%	72.7% HR 0.88 [0.65;1.18]	88.3% HR 0.94 [0.59;1.48]
103	7.4	*NR*	20 (19.4)	98 (95.1)	*NR*	69.9%	86.4%
103	8		24 (23.3)	100 (97.1)		80.6%	90.3%
67	*NR*	11 (16.4)	11 (16.4)	65 (98.5)	4.5%	79.7%	*NR*
67		12 (17.9)	17 (25.4)	65 (97)	4.2%	76.6%	
46	8.1	*NR*	15 (32.6)	*NR*	5-year 16%	5-year DSS 78% [66-90][g]	5-y OS 61% [47-74]
50	6.9		14 (28.0)		5-year 17%	5-year DSS 85% [75-95][g]	5-year OS 75% [61-85]

Table 2 Continued I

Author Year	Treatment summary (CT, RT, adjuvant treatment)	Included cT4 (%)	Included cN+ (%)	N_{CRT}	Any ≥ grade 3 CT/CRT toxicity [a]	Full CT dose
O'Connell [29] 2014	5FU 45 Gy+5.4/10.8 Adj,: decision left to institution	NR	42.1	477	CTC 4.0 129 (27.0)	*NR*
	5FU-OX 45 Gy+5.4/10.8 Adj,: decision left to institute	NR	38.3	329	**129 (39.2)**	
	CAP 45 Gy+5.4/10.8 Adj,: decision left to institute	NR	42.6	472	153 (32.4)	
	CAPOX 45 Gy+5.4/10.8 Adj,: decision left to institute	NR	38.5	330	**135 (40.9)**	
Rodel [30] 2015	5FU 50.4 Gy Adj. 4 x 5FU	8	72.4	623	CTC 3.0 128 (20.5)	79%
	5FU-OX 50.4 Gy Adj. 8 x 5FU-OX	6.7	73.7	613	144 (23.5)	(85%
Valentini [45] 2008	Cisplatin-5FU 50.4 Gy Adj.: physician dependent	0	67.5	83	RTOG 6 (7.1)	*NR*
	Raltitrexed-OX 50.4 Gy Adj.: physician dependent	0	63	81	13 (16.4)	
Chemoradiotherapy vs. chemoradiotherapy + targeted therapy						
Salazar [24] 2015	CAP 45 Gy Adj.: physician dependent	15.2	89.1	46	CTC 3.0 6 (13.0)	93.5%
	CAP-BEV 45 Gy Adj.: physician dependent	22.7	84.1	44	7 (16.0)	CAP 95.5% BEV 97.7%

$N_{surgery}$ [b]	Weeks to surgery [c]	Surgical complications [d]	pCR	R0 resection	3-year LR [e]	3-year DFS [e]	3-year OS [e]
636	*NR*	158 (33.1)	113 (17.8) (FU/CAP)[h]	*NR*	*NR*	*NR*	*NR*
640		116 (35.3)	125 (19.5) (FU/CAP)[h]				
		159 (33.7)					
		125 (37.9)					
615	6	272 (44.2)	81 (13.2)	584 (95.0)	4.6%	71.2% [67.6-74.9]	88.0% [85.3-90.7]
596	6	291 (48.8)	**104 (17.4)**	567 (95.1)	2.9%	75.9% [72.4-79.5]	88.7% [86.0-91.3]
						HR 0.79 [0.64;0.98]	HR 0.96 [0.72;1.26]
83	*NR*	15 (18.1)	18 (21.7)	*NR*	*NR*	*NR*	*NR*
81		8 (9.9)	23 (28.4)				
46	7.3	*NR*	5 (10.9)	-	*NR*	*NR*	*NR*
43	7.3		7 (16.3)	-			

Table 2 Continued II

Author Year	Treatment summary (CT, RT, adjuvant treatment)	Included cT4 (%)	Included cN+ (%)	N_{CRT}	Any ≥ grade 3 CT/CRT toxicity [a]	Full CT dose
Induction chemotherapy and chemoradiotherapy vs. standard fluoropyimidine-based chemoradiation						
Borg [34] 2014	BEV-5FU 45 Gy Adj.: physician dependent	0	82.2	45	CTC 3.0 9 (20.0)	100%
	Ind.: BEV-FOLFOX4 CRT: BEV-5FU 45 Gy Adj.: physician dependent	0	78.3	46	Overall 23 (50.0)	93.5%
Fernandez-Martos [35] 2015	CAPOX 50.4 Gy Adj. 4 x CAPOX	5.8	NR	52	CTC 3,0 15 (30.6)*	93.9%
	Ind.: CAPOX CRT: CAPOX 50.4 Gy Adj.: -	13.5	NR	56	Induction 10 (18.5) CRT 12 (22.6)	Induction 94.4% **CRT** **77.8%**
Marechal [36] 2012	5FU 45 Gy Adj.: -	10.3	86.2	29	CTC 3.0 2 (6.9)	97%
	Ind.: FOLFOX6 CRT 5FU 45 Gy Adj.: -	7.1NR	92.9	28	**Induction** **8 (28.6)** **CRT** **2 (7.1)**	Induction 96% CRT 86%
Rouanet [38] 2017	FOLFIRINOX No RT Adj.: ypT ≥ 2 / ypN ≥ 1: 6 x FOLFOX	0	81.8	11	CTC 4.0 7 (63.6)	Induction 73%
	Ind.: FOLFIRINOX CRT: CAP 50 Gy Adj.: ypT ≥ 2 / ypN ≥ 1: 6 x FOLFOX	0	73.7	19	Induction 8 (42.1) CRT 5 (26.3)	Induction 68%
	Ind. FOLFIRINOX CRT: CAP 50 Gy Adj.: ypT ≥ 2 / ypN ≥ 1: 6 x FOLFOX	23.1	96.2	52	Induction 19 (36.5) CRT 11 (21.2)	Induction 73%
	Ind. FOLFIRINOX CRT: CAP 60 Gy Adj.: ypT ≥ 2 / ypN ≥ 1: 6 x FOLFOX	25.5	98	51	Induction 8 (15.7) CRT 12 (23.5)	Induction 86%

$N_{surgery}$ [b]	Weeks to surgery [c]	Surgical complications [d]	pCR	R0 resection	3-year LR [e]	3-year DFS [e]	3-year OS [e]
44	*NR*	15 (34.1) (≥ gr. 3)	5 (11.4)	43 (97.8)	*NR*	*NR*	*NR*
42		14 (33.3)	9 (21.4)	41 (97.6)			
46	*NR*	21 (45.7)	7 (15.2)	45 (97.8)	5-year 2% [0-10.2%]	5-year DFS 64% [49.5-75.8]	5-year OS 78% [63.6-87.1]
54		27 (50.0)	8 (14.8)	48 (88.9)	5-year 5% [1.1-14.8]	5-year DFS 62% [48-73.4]	5-year OS 75% [61-84.1]
28	*NR*	9 (32.1)	8 (28.6)	*NR*	*NR*	*NR*	*NR*
27		7 (25.9)	7 (25.9)				
11	4.4	5 (50.0)	1 (9.1)	10 (90.9)	*NR*	*NR*	*NR*
19	7.6	8 (42.1)	11 (57.9)	19 (100)			
52	7	16 (31.4)	7 (13.5)	43 (82.7)			
51	7	23 (53.5)	9 (17.6)	43 (84.3)			

Table 2 Continued III

Author Year	Treatment summary (CT, RT, adjuvant treatment)	Included cT4 (%)	Included cN+ (%)	N_{CRT}	Any ≥ grade 3 CT/CRT toxicity [a]	Full CT dose
Fokas [39] 2019	Ind.: 5FU-OX CRT: 5FU-OX 50.4 Gy Adj.: -	11.5	85.9	156	CTC 4.0 Induction: 34 (21.8) **CRT: 56 (35.9)**	78%
	CRT: 5FU-OX Cons.: 5FU-OX 50.4 Gy Adj.: -	18	90	150	CRT: 41 (27.3) Cons.: 30 (20.0)	76%
Chemoradiotherapy and consolidation chemotherapy vs. standard fluoropyimidine-based chemoradiation						
Kim [40] 2018	CAP 50.4 Gy Adj.: ypStage 0-1: 6 x CAP, ypStage II-III: 6 x CAPOX	18.2	92.7	52	CTC 4.0 Overall 2 (3.8)	*NR*
	CRT: CAP Cons.: CAPOX 50.4 Gy Adj.: ypStage 0-1: 6 x CAP, ypStage II-III: 6 x CAPOX	17	92.5	44	Overall 5 (11.4)	
Moore [41] 2017	5FU 45 Gy + 5.4 Adj.: -	20.8	91.7	24	*NR*	*NR*
	CRT: 5FU Cons.: 5FU 45 Gy + 5.4 Adj.: -	4	100	25		
SCRT-delay vs. CRT						
Latkauskas [25] 2016	No CT 25 Gy Adj.: 4 x 5FU	NR	76.5	68	*NR*	*NR*
	5FU 50 Gy Adj.: 4 x 5FU	NR	79.2	72		

a. CRT toxicity is reported according to CTCAE 3.0, unless stated otherwise; **b.** Number of participants that proceeded to surgery after neoadjuvant treatment; **c.** Median interval in weeks between last radiation dose and surgery; **d.** Any grade surgical complication; **e.** expressed as cumulative incidence; **f.** 40-45% missing data; **g.** DSS = Disease specific survival, defined as death from study cancer or complications of protocol treatment; **h.** CAP/5FU reported as one group with or without OX

$N_{surgery}$ [b]	Weeks to surgery [c]	Surgical complications [d]	pCR	R0 resection	3-year LR [e]	3-year DFS [e]	3-year OS [e]
142	6.4	59 (41.6)	27 (19.0)	130 (91.5)	*NR*	*NR*	*NR*
142	12.9	47 (33.1)	**38 (26.8)**	128 (90.1)			
52	7.6	*NS*	3 (5.8)	52 (100)	*NR*	*NR*	*NR*
44	8.8		6 (13.6)	39 (88.6)			
24	10.6	10 (41.7)	6 (25.0)	22 (91.7)	*NR*	*NR*	*NR*
25	10.9	13 (52.0)	4 (16.0)	23 (92.0)			
68	6.9	24 (35.3)	3 (4.4)	57 (83.8)	3.1%	**59%** **HR 1.93** **[1.08-3.43]**	78% HR 1.64 [0.8-3.43]
72	6.7	19 (26.8)	8 (11.1)	64 (88.9)	5.6%	**75.1%**	82.4%

Abbreviations: 5FU = 5-Fluorouracil, adj. = adjuvant therapy, APR = abdominoperineal resection, BEV = Bevacizumab, C = Cetuximab, CAP = Capecitabine, CAPOX = Capecitabine + Oxaliplatin, cN = clinical nodal stage, Cons. = consolidation chemotherapy, CRT= chemoradiotherapy, cT = clinical tumor stage, CT = chemotherapy, CTC(AE) = Common Terminology Criteria for Adverse Events, DFS = disease free survival, Ind.= Induction chemotherapy, LR = local recurrence, MRF = mesorectal fascia, NR = not reported, OS = overall survival, OX = Oxaliplatin, pCR = pathological complete response, RT = radiotherapy, S1 = tegafur/gimeracil/oteracil, SCRT = short-course radiotherapy,

Discussion

This systematic review evaluated whether pCR rates were higher following alternative neoadjuvant treatment strategies as compared to standard neoadjuvant fluoropyrimidine-based chemoradiation. All included trials fail to deliver high level evidence to show an improvement in pathological outcomes and survival compared to standard fluoropyrimidine-based CRT. The addition of oxaliplatin to fluoropyrimidine-based CRT might result in significantly more pCR, but at the expense of more ≥ grade 3 toxicity. Furthermore, this benefit does not translate into lower rates of local recurrence or improved overall survival. Other neoadjuvant treatment strategies, including consolidation/induction chemotherapy and short-course radiotherapy with delayed surgery, were not associated with improved pCR rates. None of the included trials reported benefit in local recurrence or overall survival.

pCR following neoadjuvant therapy has been associated with improved survival [7], and may reflect the organ-sparing potential of a treatment protocol. To increase clinical response rates after neoadjuvant treatment and herewith enable rectum preservation, different intensification strategies have been investigated in phase I-II trials, e.g. multi-agent CRT, targeted therapy, radiotherapy dose-escalation, or additional chemotherapy before or after CRT (total neoadjuvant treatment, TNT). In multivariable meta-regression the addition of a second concurrent chemotherapy agent was not associated with improved pCR rates [47]. In accordance with our findings, previous meta-analyses showed that the addition of oxaliplatin to preoperative chemoradiotherapy improves pCR rate, decreases LR rate and improves DFS, but significantly worsens toxicity [48,49]. Also, no significant difference was found in R0 resection rate, sphincter preservation rate, permanent stoma rate, postoperative complication, mortality, and overall survival [49]. Dose-escalated radiotherapy could be associated with higher pCR rates [47,50]. However, this is not yet confirmed by a randomized controlled trial and could therefore not be further investigated in the present study [6]. TNT might manage micro-metastases, increase tumor regression that enhances R0 resection rates and increases probabilities for organ preservation [39]. A recent meta-analysis showed that patients who received TNT followed by surgery more often had pCR (OR 1.39 (1.08–1.81), better DFS (HR 0.75 (0.52–1.07) and OS (HR 0.73 (0.59–0.9) than those who received CRT only. However, this analysis was largely based on nonrandomized comparative studies, and in subgroup analyses (prospective and retrospective series) there were no statistically significant differences between TNT and CRT arms [15]. Several trials are still ongoing [51,52], but till day the superiority of TNT over standard CRT remains inconclusive.

Targeted therapy is latest development in rectal cancer management. Translational research has led to better understanding of molecular pathways and increased interest in targeted therapy. For example, cancer cells can express epidermal growth factor receptor (EGFR), which stimulates cell proliferation, as well as vascular endothelial growth factor receptor (VEGFR), enabling vessels formation for growth [53,54]. EGFR signaling might promote resistance to radiotherapy. Retrospective analyses demonstrated worse DFS and lower pCR rates in patients with rectal tumors expressing EGFR, and elevated VEGF expression in tumors has been associated with inferior survival [53]. The addition of cetuximab, a monoclonal antibody that can sensitize cells with overexpression of EGFR to radiotherapy [53], has been shown not to affect pCR rate, but to significantly improve OS [55]. Bevacizumab, an anti-VEGF antibody reducing tumor vascular density [53,54], did not improve pCR rates [24]. However, these translational results are still preliminary and clinical trials are needed.

In specific patient populations (elderly or frail) or in some countries SCRT-delay is preferred over CRT because of its lower costs, better compliance and less demanding nature [56]. However, the use of SCRT remains elusive outside of Europe [9]. Unsurprisingly, pCR rates are lower with this regimen based on its lower biological effective radiation dose compared to long course chemoradiation. The largest randomized trial that investigated the effect of SCRT-delay was the Swedish Stockholm III trial [57]. pCR was found in 10.4% of patients after SCRT-delay and the risk of postoperative complications was significantly lower after SCRT-delay compared to SCRT and immediate surgery [18,58]. However, this trial could not be included in this study due to the lack of baseline tumor characteristics. Additionally, a combination of (induction/consolidation) chemotherapy and SCRT-delay could increase pCR rates and improve survival [44,59,60]. The results of a large RCT on this topic are still awaited [60]. Therefore, at this moment, SCRT-delay only seems appropriate for frail LARC patients that are unfit to undergo CRT.

This is the first systematic review that gives an overview of the most widely used and available neoadjuvant treatment modalities that are investigated in a randomized trial. The evaluation of pathological outcomes in relation to toxicity, surgical and survival data provides more insight in the overall effect of these regimens. Nonetheless, this meta-analysis also encountered several limitations. First, only RCTs were included, whereas a lot of new interventions are trialed in prospective single-arm phase II trials. However, these trials are prone to selection bias as well

as optimism in the intervention effect and often fail to demonstrate superiority in subsequent phase III trials [47,61,62]. Nonetheless, randomized phase II trials may also overestimate the treatment effect [63]. We showed these differences between phase II and phase III trials in the analyses for multi-agent CRT and for CRT plus consolidation chemotherapy. In addition, the RCT-limited analysis might represent a relatively well-conditioned study population [64], resulting in an underestimation of compliance and toxicity rates. Second, the generalizability might be limited due to strict MRI criteria and pCR definitions. Although MRI is considered to be the most optimal staging method [2,65], it may not be as widely available and easy accessible in all countries. In addition, the primary outcome was restricted to ypT0N0, because the inter-observer agreement of other methods for tumor regression grading is low [66]. The TRG definition of pCR varies between approaches and the application of a TRG is not recommended in the present TNM classification [66,67]. Moreover, subgroups were small and secondary outcomes could not be extracted from all included trials, which might reduce power. Third, despite strict inclusion criteria and the use of a random effects model, uncorrected heterogeneity in study protocols might still influence the pooled effect estimates [68]. This is for instance reflected in the different intervals between the end of neoadjuvant treatment and surgery. A prolonged interval may increase pCR rates and recurrence-free survival without compromising surgical morbidity [69,70]. As such, higher pCR rates after consolidation therapy compared to induction therapy may be the result of an increased interval between surgery and CRT, rather than the therapy itself. And lastly, only those treatments compared to a similar baseline, namely standard fluoropyrimidine-based CRT, could be used in a formal meta-analysis. The opportunity to perform an extended network meta-analysis was explored, but was not reliable due to the large heterogeneity in study design and small amount of available RCTs.

The currently available data shows that there is a wide variety of neoadjuvant treatment strategies available, but that there is no high level evidence to show an improvement in pathological outcomes and survival compared to standard of care in terms of pCR achievement and organ-sparing potential. This is probably caused by the large number of confounding factors, resulting from differences in diagnosis and treatment, but, more importantly, also from differences in patient and tumor characteristics. In the era of personalized treatment, more high-level evidence on tumor characteristics, (pre-)treatment response prediction, long-term quality of life and oncological outcomes after different treatment modalities is needed to support optimal and individualized rectal cancer management. This requires new, efficient and innovative research infrastructures, such as large prospective cohorts

in which trials can be conducted according to the 'Trials within Cohorts' (TwiCs) design [71,72]. This enables investigation of novel prognostic and predictive factors in large populations as well as in small subgroups of patients, and simultaneously provides the platform to conduct (partly) overlapping randomized trials with robust and validated analysis methods that provide clinically relevant answers that can directly translated into changes for routine care [73].

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CHAPTER 3

Comparison of pathological complete response rates after neoadjuvant short-course radiotherapy or chemoradiation followed by delayed surgery in locally advanced rectal cancer

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ABSTRACT

Introduction

Patients with locally advanced rectal cancer (LARC), who are unfit for chemoradiation (CRT) are often offered short-course radiotherapy followed by delayed surgery (SCRT-delay). This entails a lower radiation dose, no chemotherapy and a shorter treatment period. This may lower their chances for complete tumor response and, as such, organ-sparing approaches. The purpose of this study was to compare the pathological complete response (pCR) rates between neoadjuvant CRT and SCRT-delay in patients with LARC in a nationwide database from the Netherlands.

Methods

In the population based Netherlands Cancer Registry, clinical stage III rectal cancer patients, diagnosed between 2008 and 2014, who underwent CRT or SCTR-delay were selected. pCR (ypT0N0), near pCR (ypT0-1N0), and tumor and nodal downstaging were compared between the treatment groups using multivariable logistic regression analysis.

Results

386 patients underwent SCRT-delay and 3,659 patients underwent CRT. The pCR-rate in the SCRT-delay group was significantly lower compared to the CRT-group (6.4% vs. 16.2%, $p<0.001$). After adjustment for clinical tumor stage, clinical nodal stage and time interval to surgery, SCRT-delay patients were significantly less likely to reach pCR (adjusted odds ratio 0.3, 95%CI 0.2-0.5). Also, near-pCR (ypT0–1N0) as well as tumor and nodal downstaging was observed less often in the SCRT-delay group.

Conclusion

Compared to patients treated with neoadjuvant CRT, those receiving SCRT and delayed surgery are less likely to develop pCR. Novel neoadjuvant treatment strategies for patients not fit enough for CRT are needed to increase their eligibility for organ-sparing treatments.

Introduction

Neoadjuvant chemoradiation (CRT) followed by surgery after 8 to 12 weeks interval is the standard of care for patients with locally advanced rectal cancer (LARC). CRT lowers the risk of local recurrence and leads to downsizing of the tumor [1]. The ultimate form of downsizing is a pathological complete response (pCR), which is achieved in 15-20% of LARC patients after neoadjuvant CRT [2-4]. Patients with pCR have lower local recurrence rates and better disease-free survival rates compared to patients without pCR [3,5-10]. If there is no tumor found on MRI and endoscopy after neoadjuvant treatment (clinical complete response, cCR), rectum-sparing treatment, such as a watch-and-wait approach, can be considered [11]. Until now, non-randomized studies have shown oncological safety and good survival rates in patients managed by a watch-and-wait strategy following CRT [12-14].

In the Netherlands, guideline deviation occurs in approximately 20% of rectal cancer patients older than 75 years [15]. When patients can't tolerate chemoradiation, it is often replaced by neoadjuvant short-course radiotherapy followed by surgery after 8 to 12 weeks (SCRT-delay) [1]. This regimen is less demanding for the patient, involves a lower radiation dose (25 Gy instead of 45-50 Gy), no chemotherapy and a shorter treatment period (5 days instead of 25 days) compared with CRT.

In small tumors, it has been shown that SCRT-delay may induce downsizing [16-18]. Nevertheless, the downsizing effect of this regimen in LARC is unknown. With the increasing use of the watch-and-wait strategy, it is important to know the probability of pCR following SCRT-delay, to enable a well-informed choice between intensive neoadjuvant treatment and a higher probability of reaching a complete response versus less intensive neoadjuvant treatment with a reduced chance of becoming eligible for organ-sparing treatment. The purpose of this study was to compare the pathological complete response (pCR) rates between neoadjuvant CRT and SCRT-delay in patients with LARC in a nationwide database from the Netherlands.

Methods

Data from the nationwide population-based Netherlands Cancer Registry (NCR) were used. In this registry, information on patient and tumor characteristics, diagnosis and treatment is routinely extracted from medical records. The quality of the data is high due to thorough training of the registration team and computerized consistency checks at regional and national level. We selected patients diagnosed with locally advanced rectal cancer between 2008 and 2014 with an indication

for neoadjuvant CRT according to the Dutch guidelines (cT2N2M0, cT3N1-2M0 or $cT4N_{any}M0$), and who underwent CRT followed by surgery, or SCRT with delayed surgery. Delayed surgery was defined as a minimum of four weeks interval between completion of neoadjuvant radiotherapy and surgery. Patients with a neuroendocrine tumor, patients with recurrent rectal cancer and or synchronous metastases and patients who received neoadjuvant therapy otherwise than standard CRT or SCRT were excluded.

Standard neoadjuvant therapy in patients with locally advanced disease consisted of CRT (45-50 Gy in 25 fractions of 1.8-2 Gy in 5 weeks with concurrent Capecitabine 2 times daily 825 mg/m^2) [1]. SCRT-delay consisted of 25 Gy in 5 fractions of 5 Gy in 1 week without chemotherapy. Rectal surgery was performed by total mesorectal excision (TME) (low anterior resection (LAR), abdominoperineal resection (APR), intersphincteric resection, Hartmann resection), extended TME including proctocolectomy, local excision including transanal endoscopic microsurgery (TEM) or transanal excision (TAE).

The primary outcome was pCR, defined as no viable tumor cells and no positive lymph node invasion after chemoradiation (ypT0N0). Secondary outcomes were near pCR (ypT0-1N0), tumor and nodal downstaging (ypT-N less than cT-N) and number of examined lymph nodes and the ratio of positive and examined lymph nodes.

Determinants of interest were systematically recorded in the NKR and included patient, tumor and treatment characteristics i.e. sex, age, year of incidence, follow-up time (in days), vital status, comorbidity (number and type), differentiation grade of the tumor, clinical TNM-stage, disease stage, tumor location, tumor distance for the anal verge, type of neoadjuvant therapy, chemotherapy received (yes or no), type of surgical resection, time between start of radiotherapy and date of surgery (in days), pathological TNM-stage and number of retrieved and positive lymph nodes. Since the stop date of radiotherapy was unknown, time until surgery was calculated by the date of surgery minus the start date of radiotherapy, corrected for the duration of the neoadjuvant therapy.

Statistics

Baseline characteristics were presented in descriptive statistics and stratified by neoadjuvant treatment group, i.e. SCRT-delay and CRT. Differences between SCRT-delay and CRT in terms of pCR rate, tumor and nodal downstaging and lymph node (yield and ratio) were compared with Chi-square tests or Fischer exact test for categorical variables and with Independent T or Mann-Whitney U tests for

continuous variables depending on the distribution. To adjust for clinical tumor and nodal stage, and time interval to surgery on the association between pCR and treatment group, multivariable logistic regression was used. The association between pCR and neoadjuvant therapy group was stratified for tumor stage (cT2-3 and cT4), nodal status (cN0 and cN+) and categories of time interval ($\leq$7 weeks, 8 to 9 weeks, 10 to 11 weeks and $\geq$12 weeks). The level of significance was defined as $P<0.05$. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) software version 23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.).

Results

Of the 20,950 rectal cancer patients registered in the NCR database between 2008 and 2014 4,050 (19.3%) patients met the inclusion criteria. Three hundred and ninety-one (9.7%) patients underwent SCRT-delay and 3,659 (90.3%) patients underwent CRT.

The SCRT-delay group had a higher mean age compared to the CRT group (76 years vs. 63 years) and consisted of relatively more female patients (49.4% vs. 36.1%) (**Table 1**). In both groups most tumors were located in the distal rectum and clinically stage T3. Clinical nodal stage was higher in the CRT group than in the SCRT-delay group (cN0 in 5.2% vs. 9.5%, cN1 in 55.2% vs. 42.9% and cN2 in 50.3% vs. 34.0% resp.). Median time interval to surgery was similar between the groups (9.1 vs. 9.4 weeks in the SCRT-delay resp. the CRT group). When analyzing time interval as a categorical variable, the highest proportion of patients in the SCRT-delay group underwent surgery within 7 weeks (27.1%) or after 12 weeks (32.2%) while in the CRT group most patients had surgery between 8 and 9 weeks (29.8%) or 10 and 11 weeks (28.3%). In the SCRT-delay group more patients underwent a Hartmann resection (14.3% vs. 7.4% in the CRT group) instead of sphincter-sparing surgery (37.8% vs. 48.3% in the CRT group). The median follow-up time was shorter in the SCRT-delay group compared to the CRT group (2.4 vs. 3.2 years resp.).

In univariable analysis, pCR-rate in the SCRT-delay group was significantly lower than in the CRT-group (6.4% vs. 16.2%, $p<0.001$) (**Table 2**). Also, near-pCR rates (11.0% vs. 20.6%, $p<0.001$), tumor downstaging rates (46.8 vs. 58.1%, $p<0.001$) and nodal downstaging rates (58.1% vs. 72.4%, $p<0.001$) were significantly lower in the SCRT-delay than in the CRT-group. Pathological tumor and nodal stage, number of examined lymph nodes and the ratio of positive and examined lymph nodes were significantly higher in the SCRT-delay group compared to the CRT-group (**Table 2**).

Table 1. Baseline characteristics of selected rectal cancer patients from the Netherlands Comprehensive Cancer Organisation (IKNL)

	SCRT-delay N= 391 (%)	CRT N= 3,659 (%)
Mean age in years ±SD	76±9	63±10
Sex		
Male	198 (50.6)	2,338 (63.9)
Female	193 (49.4)	1,321 (36.1)
Tumour distance		
≤5cm	176 (45.0)	1,680 (45.9)
6-10cm	146 (37.3)	1,324 (36.2)
≥11cm	43 (11.0)	437 (11.9)
Missing	26 (6.6)	218 (6.0)
cT-stage		
2	13 (3.3)	143 (3.9)
3	298 (76.2)	2,769 (75.7)
4	80 (20.5)	747 (20.4)
cN-stage		
0	37 (9.5)	192 (5.2)
1	216 (55.2)	1,569 (42.9)
2	133 (34.0)	1,840 (50.3)
Missing	5 (1.3)	58 (1.6)
Time interval to surgery (Median weeks, IQR)	9.1 (6.9-12.0)	9.4 (8.0-11.3)
Time interval categorical		
≤7 weeks	106 (27.1)	533 (14.6)
8-9 weeks	87 (22.3)	1,090 (29.8)
10-11 weeks	72 (18.4)	1,034 (28.3)
≥12 weeks	126 (32.2)	1002 (27.4)
Surgical procedure		
TEM/TAE	5 (1.3)	18 (0.5)
LAR	146 (37.3)	1,768 (48.3)
APR	169 (43.2)	1,498 (40.9)
Intersphincteric resection	10 (2.6)	34 (0.9)
Hartmann resection	56 (14.3)	270 (7.4)
Sigmoid resection	1 (0.3)	3 (0.1)
Extended TME	4 (1.0)	68 (1.9)
Vital status		
Alive	268 (68.5)	3,021 (82.6)
Dead	123 (31.5)	638 (17.4)
Median years follow-up (IQR)	2.4 (1.5-3.5)	3.2 (2.0-4.7)

APR: abdominoperineal resection; CRT: chemoradiation; LAR: low anterior resection; SCRT: short-course radiotherapy; TAE: transanal excision; TEM: Transanal endoscopic microsurgery; TME: total mesorectal excision.

Table 2. Differences in pathology outcomes between short-course radiotherapy and delayed surgery (SCRT-delay) and chemoradiation (CRT). Numbers are presented as n (%), unless stated otherwise.

	SCRT-delay N = 391	CRT N = 3,659	p-value
pCR (ypT0-N0)	25 (6.4)	592 (16.2)	< 0.001
Near-pCR (ypT0–1 N0)	43 (11.0)	755 (20.6)	<0.001
Tumour downstaging (ypT<cT)	182 (46.8)	2,079 (58.1)	<0.001
Node downstaging (ypN<cN)	225 (58.1)	2,618 (72.4)	<0.001
ypT-stage			< 0.001
0	31 (7.9)	673 (18.4)	
1	21 (5.4)	210 (5.7)	
2	99 (25.3)	934 (25.5)	
3	206 (52.7)	1,581 (43.2)	
4	32 (8.2)	183 (5.0)	
Missing	2 (0.5)	78 (2.1)	
ypN-stage			< 0.001
0	215 (55.0)	2,413 (65.9)	
1	109 (27.9)	805 (22.0)	
2	63 (16.1)	400 (10.9)	
Missing	4 (1.0)	41 (1.1)	
Median no. of examined lymph nodes (IQR)	14 (11-19)	12 (9-16)	< 0.001*
Median lymph node ratio (IQR)#	0 (0-0.09)	0 (0-0.14)	< 0.001*

CRT: chemoradiation; pCR: pathological complete response; SCRT: short-course radiotherapy. * Based on a Mann-Whitney U-test # The number of positive lymph nodes divided by number of examined lymph nodes

In multivariable analysis, the probability of pCR was significantly lower in the SCRT-delay group compared to the CRT group (OR=0.3, 95%CI 0.2-0.5, p<0.001 adjusted for cT-stage, cN-stage and categories of time interval) (**Table 3**).

In stratified analysis, SCRT-delay was associated with a lower pCR-rate in patients with a cT2-3, cN1-2 and all categories of time interval (**Table 4**). In patients diagnosed with cT4 and patients with cN0 the association between neoadjuvant treatment group and pCR-rate was not significant.

Table 3. Multivariable analysis of the association between neoadjuvant short-course with delayed surgery (SCRT-delay) or chemoradiation (CRT) and pathological complete response (pCR).

	crude OR (95%CI)	adjusted OR (95%CI)	p-value
Neoadjuvant therapy			
SCRT-delay	0.4 (0.2-0.5)	0.3 (0.2-0.5)	<0.001
CRT	Ref .	Ref.	
Clinical T-stage			
cT2	2.0 (1.3-3.1)	2.3 (1.4-3.8)	0.001
cT3	1.6 (1.2-2.0)	1.8 (1.3-2.4)	<0.001
cT4	Ref.	Ref.	
Clinical N-stage			
cN0	0.7 (0.4-1.0)	1.2 (0.7-2.0)	0.445
cN1	1.0 (0.8-1.2)	1.1 (0.9-1.3)	0.447
cN2	Ref.	Ref.	
Time interval			
<7weeks	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.477
8-9 weeks	1.0 (0.8-1.3)	1.0 (0.7-1.2)	0.831
10-11 weeks	1.1 (0.9-1.4)	1.1 (0.9-1.4)	0.440
>12 weeks	Ref.	Ref.	

CI: confidence interval; CRT: chemoradiation; OR: odds ratio. pCR: pathological complete response; SCRT: short-course radiotherapy.

Table 4. Stratified analysis of the association between neoadjuvant short-course with delayed surgery (SCRT-delay) or chemoradiation (CRT) and pathological complete response (pCR) by clinical tumor stage, clinical nodal stage and categories of time interval.

Strata	pCR in SCRT-delay N(%)	pCR in CRT N(%)	p-value*
Clinical tumour stage			
cT2-3	21/311 (6.8)	505/2,912 (17.3)	<0.001
cT4	4/80 (5.0)	87/747 (11.6)	0.071
Clinical nodal stage			
cN0	1/37 (2.7)	24/192 (12.5)	0.080
cN1-2	23/349 (6.6)	557/3,409 (16.3)	<0.001
Time interval to surgery			
<7 weeks	6/106 (5.7)	82/533 (15.4)	0.008
8-9 weeks	6/87 (6.9)	171/1,090 (15.7)	0.027
10-11 weeks	4/72 (5.6)	180/1,034 (17.4)	0.009
>12 weeks	9/126 (7.1)	159/1,002 (15.9)	0.010

*p-values are based on Chi-square tests

Discussion

Pathological compete response rates are lower in patients treated with neo-adjuvant short-course radiotherapy with delayed surgery than in patients treated with neoadjuvant chemoradiation for locally advanced rectal cancer. This is far from unexpected, since the administered biological effective radiation dose is lower in SCRT. SCRT followed by surgery within 10 days does not induce downstaging, let alone pCR [19]. Moreover, since chemotherapy is eliminated, the lack of a radiosensitizing agent may reduce radiotherapy efficacy [20]. A prolonged interval to surgery increases the pCR rate [16,21]. In previous studies the pCR rates following SCRT-delay range from 4.4% to 25%, with intervals to surgery from 4 up to 19 weeks [16,22-29]. A systematic review found an average increase in pCR rate of 10% in the delayed-surgery group compared to immediate surgery (3-12% and 0-0.4%, respectively) [21]. The Stockholm III trial showed that prolonging the interval to surgery from 1 week to 4-8 weeks increases pCR rates from 2.1% to 11.8% [16]. No differences were found in local recurrence, recurrence-free survival and 5-year overall survival [30]. A comparison between SCRT-delay and CRT for cT1-4 N0-3 tumors showed clinical complete response (cCR) rates of 20% and 34% after a median interval to response evaluation of 10.3 and 8.9 weeks, respectively. cCR was only achieved in cT2N0 and cT3N0 tumors [31]. Similar effects on cT3 tumors have been published [17,18]. In our study, pCR rate was 6.5% after SCRT-delay, with a mean interval to surgery of 9.1 weeks. This relatively low pCR rate may be attributed to higher tumors stages (Stage III or cT4N0) in our study population.

In contrast to literature, we did not observe a higher pCR rates when interval to surgery was increased within the CRT group. This corresponds to the results of the phase III randomized GRECCAR-6 trial, where no benefit of a prolonged interval beyond a minimal interval of 11 weeks was found [32].

We observed a lower proportion of near-pCR (ypT0-1) in the SCRT-delay group than in the CRT group. Near-pCR is relevant when local excision as an alternative treatment option is considered, for example for frail patients, to reduce acute and long-term morbidity of surgery, such as anastomotic leakage, bleeding, urinary, and fecal incontinence [33-35].

A higher pathological nodal stage, a higher mean number of examined lymph nodes and a higher mean metastatic/examined lymph nodes ratio (LNR) were observed in the SCRT-delay group compared to the CRT-group. An increase in lymph node yield after neoadjuvant therapy for locally advanced rectal cancer is associated with

improved cancer-specific and 5-year survival and lower local recurrence rates [36,37]. However, in tumors with (near) pCR a lower number of lymph nodes is found [38]. A high LNR is prognostic for decreased overall and disease-free survival, even in patients with fewer than 12 lymph nodes examined [39]. These findings suggest that prognosis after SCRT-delay for LARC might be worse than after CRT, but the clinical relevance of LNR is still under debate.

To the best of our knowledge, this is the first large, nationwide study that describes the difference in pathological response rates between chemoradiation and short-course radiotherapy in LARC. A limitation of our study is the missing information about involvement of the mesorectal fascia (MRF). According to Dutch Guidelines, SCRT might have been justified in cT3N1 patients with a wide margin (>1mm) to the MRF. An increased interval to surgery could have increased pCR rates in this group. However, since cT3N1 MRF- patients require immediate surgery after SCRT and we selected an interval to surgery of more than 4 weeks, we assumed most of the cT3N1 in our dataset had an involved MRF and thus needed CRT.

The group of patients that receive SCRT-delay instead of CRT is not clearly defined. Studies have shown that treatment deviation is more common in elderly patients [15,40-45], with age and comorbidities being predictive factors for altered treatment [42-44]. Since rectal cancer incidence increases with age and the general population is getting older, clinicians increasingly consider alternative, less aggressive treatment options for the frail LARC patient. More importantly, since per- and postoperative morbidity and mortality rates are increased in the older patient due to complications [44,46-48] and postoperative complications have a larger negative impact on physical- and role functioning in older patients [49], organ preservation would be favorable in this particular population. However, if SCRT-delay is mostly offered to elderly patients, and pCR rates are lower after SCRT-delay, elderly treated with SCRT-delay have a lower probability to become eligible for organ-sparing approaches. Novel neoadjuvant treatment strategies for frail patients are needed in order to increase their eligibility for organ-sparing treatments, while focusing on a balance between morbidity on the one hand and optimal function and cure rates on the other. Current studies investigate a combination of short-course radiotherapy and chemotherapy, resulting in increased pCR rates [22,23,27,29,50]. However, the risk of toxicity associated with this strategy is likely to be unacceptable in the frail rectal cancer patient. Future studies should look into new treatment modalities where therapy doses can be increased to a level where response rates are higher and toxicity is still tolerable.

Conclusion

Compared to patients treated with neoadjuvant CRT, those receiving SCRT and delayed surgery are less likely to develop pCR. Novel neoadjuvant treatment strategies for patients not fit enough for CRT are needed to increase their eligibility for organ-sparing treatments.

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CHAPTER 4

The effect of neoadjuvant short-course radiotherapy and delayed surgery versus chemoradiation on postoperative outcomes in locally advanced rectal cancer patients – a propensity score matched nationwide audit-based study

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ABSTRACT

Background

Previous trials suggest that SCRT-delay could serve as an adequate neoadjuvant treatment for LARC. Therefore, in frail LARC patients SCRT-delay is recommended as an alternative to CRT. However, data on postoperative outcomes after SCRT-delay in comparison to CRT is scarce. This study investigates differences in postoperative outcomes between short-course radiotherapy and delayed surgery (SCRT-delay) and chemoradiation (CRT) in patients with locally advanced rectal cancer (LARC).

Methods

This was an observational study with data from the Dutch ColoRectal Audit (DCRA). LARC patients who underwent surgery (2014-2017) after an interval of ≥6 weeks were included. Missing values were replaced by multiple imputation. Propensity score matching (PSM), using age, Charlson Comorbidity Index, cT-stage and surgical procedure, was applied to create comparable groups. Differences in postoperative outcomes were compared between SCRT-delay and CRT.

Results

2,926 patients were included. In total, 288 patients received SCRT-delay and 2,638 patients underwent CRT. Patients in the SCRT-delay group were older, had more comorbidities. Also, ICU-admissions and permanent colostomies were more common, as well as pulmonic, cardiologic, infectious and neurologic complications. After PSM, both groups comprised 246 patients with equivalent age, comorbidities and tumor stage. There were no differences in postoperative complications.

Conclusion

Postoperative complications were not increased in LARC patients undergoing SCRT-delay as neoadjuvant treatment. Regarding treatment-related complications, SCRT-delay is a safe alternative neoadjuvant treatment option for frail LARC patients.

Introduction

In compliance with European guidelines, neoadjuvant treatment for locally advanced rectal cancer (LARC) in the Netherlands comprises neoadjuvant chemoradiation (CRT), followed by surgery according to total mesorectal excision (TME) principles. Short-course radiotherapy followed by surgery after a prolonged interval (SCRT-delay) is recommended as an alternative to chemoradiation in older patients with comorbidities or frail patients with a poor performance status, because of their higher risk of treatment related complications [1].

Postoperative morbidity and mortality are often increased in frail or elderly patients as a result of concomitant comorbidities [2]. Data on surgical management of rectal cancer in these patients is scarce and reported postoperative morbidity and mortality figures vary widely in the population >65 years old [2-4]. This patient group might benefit from altered treatment, especially when they are more susceptible to treatment-related complications [5]. Moreover, inadequate treatment is associated with poor survival [2]. Unfortunately, the heterogeneity of this group and the lack of data impede an evidence-based choice of neoadjuvant treatment in this patient group [6]. With the aging population, there is need for evidence to justify the choice of the most optimal neoadjuvant treatment in frail patients with LARC.

In addition, previous trials suggest that SCRT-delay could also serve as an adequate neoadjuvant treatment for intermediate to high risk rectal cancer [7-9]. Although previous trials showed that an interval <10 days between SCRT and surgery is associated with anastomotic leakage and postoperative mortality [10,11], the rate of postoperative complications in the Stockholm III trial was lower when surgery was delayed for 4–12 weeks after SCRT [12], suggesting that it is better to prolong the interval between SCRT and surgery. Before adding this regimen to current guidelines, more data is needed on postoperative outcomes of SCRT-delay in comparison to CRT. The aim of this study was to investigate the effect of SCRT-delay on postoperative outcomes in comparison with CRT, in both the general and the frail population.

Methods

This was an observational study with data from the Dutch ColoRectal Audit (DCRA), a nationwide audit which registers clinical outcomes of all patients undergoing primary colorectal surgery in the Netherlands. The DCRA is based on evidence-based guidelines and is validated on a yearly basis with data from the Netherlands

Cancer Registry (NCR) [13]. Because data could not be traced back to individual patients, neither informed consent nor ethical approval was required for this study.

All patients with ≥cT2 rectal cancer who underwent surgery between May 2014 (after implementation of a new Dutch colorectal cancer guideline) and December 2017, were selected from the DCRA database. Based on Dutch guidelines, LARC was defined as cT4, cT_{any} with mesorectal fascia (MRF) involvement, or $cT_{any}N2$. All patients with LARC were included in the study. Clinical tumor stage was based on imaging. Patients were excluded in case of metastatic disease, tumors located outside the rectum, emergency or urgent surgery. Also, patients who did not receive neoadjuvant treatment, with a missing start date of neoadjuvant therapy, or patients who underwent surgery after an interval of less than 6 weeks after the end of radiotherapy were excluded. Furthermore, patients who underwent surgery after an initial watch and wait strategy were excluded from the dataset, since the prolonged interval in this group could be associated with higher morbidity and a more difficult surgical resection [14]. Finally, patients who received intraoperative Hyperthermic Intraperitoneal Chemotherapy (HIPEC) or Intraoperative Radiation Therapy (IORT) were excluded.

Neoadjuvant treatment entailed either SCRT (25 Gy in fractions of 5 Gy in 5 days) or CRT (45-50 Gy in fractions of 1.8-2 Gy in 5 weeks and concurrent oral Capecetabine 825-1000 mg/m2 twice daily 5-7 days a week). Interval to surgery was calculated from the end of neoadjuvant treatment. The interval between the end of SCRT and surgery was calculated by subtracting 4 days from the interval when treatment started on Monday or by subtracting 6 days from the interval when treatment started on another day, accounting for discontinuation of therapy in the weekend. The same syntax was used for CRT patients, subtracting 32 or 34 days, respectively.

Patient characteristics included gender, age at surgery, BMI (kg/m^2), number and type of comorbidities, and ASA score. Charlson Comorbidity Index was calculated according to the weighted index of comorbidity [15]. Tumor characteristics included clinical TNM-stage, MRF involvement and tumor distance from the anus (measured at colonoscopy). Treatment characteristics included type of neoadjuvant treatment (SCRT or CRT), date of surgery, surgical procedure and approach, intraoperative complications, conversion, and ostomy creation. The subgroup 'Minimally invasive approaches' included transanal endoscopic microsurgery (TEM), local excision and transanal minimally invasive local excision (TAMIS). Hartmann procedure was incorporated in the subgroup '(Low) Anterior Resection'. Subtotal colectomy, proctocolectomy and sigmoid resection were combined in the subgroup 'other

surgical procedures' because of low prevalence. Intraoperative complications comprised injury of intra-abdominal structures, complications requiring blood transfusion or other non-specified complications.

Follow-up time was 30 days after surgery. The primary outcome measure was the occurrence of postoperative complications. Complications were defined according to standards of the DCRA [13]. Postoperative complications comprised both surgical and non-surgical complications and were defined as hospital stay of ≥ 14 days and/or a complication, re-intervention due to a complication, and/or death during hospital stay or within 30 days after surgery. Postoperative surgical complications included anastomotic failure, abscess, bleeding, ileus, dehiscent fascia, iatrogenic bowel injury, ureter/urethra injury, or other non-specified complications. Postoperative non-surgical complications included pulmonary, cardiac, thrombotic, infectious, neurologic or other non-specified complications.

Postoperative outcome measures included re-intervention, prolonged hospital stay, intensive care unit (ICU) stay and re-admission. Re-intervention involved any laparotomy-, laparoscopic- or radiology-assisted treatment for a complication. Admission to the ICU and length of hospital stay were dichotomized based on the median length of admission. ICU stay was defined as admission to the ICU for at least 1 day. Prolonged hospital stay was defined as admission to the surgical ward for more than 7 days. Pathological outcomes included pathological tumor and nodal stage, pathological complete response rate (ypT0N0) and resection margin.

Statistical analyses

On average, there was 2.3% missing data. Missing values were classified as random and replaced by multiple imputation. All observed data, including the outcome, that were applied to the dataset after imputation were used as predictors [16,17]. The number of imputations depended on the average percentage rate of missingness [18,19]. The imputed data were checked with convergence plots. Imputation was successful if the streams intermingled and were free of any trend [17]. Finally, 5 imputed datasets were produced with 5 iterations.

Since frail patients are more likely to receive SCRT-delay or to experience postoperative complications, and the type of surgery is a determinant of postoperative complications, the likelihood of confounding by indication needed to be accounted for. To enable a comparison in equivalent groups, propensity score matching was performed [20]. The propensity score was calculated for each patient using logistic regression with the variables age, Charlson Comorbidity

Index, cT-stage, and surgical procedure. These covariates were chosen based on their clinical relevance. Propensity score matching was performed using 'nearest-neighbor matching' without replacement and a 1:1 ratio. The average within-pair difference in propensity scores was minimized by setting a caliper of 0.25 multiplied by the standard deviation of the logit of the propensity score [21]. The balance in the matched dataset was expressed in 'standardized mean difference' (SMD), with an SMD < 0.10 indicating a well-balanced set [22-24].

Differences in baseline characteristics and treatment outcomes were analyzed using Chi-square test for categorical variables and independent sample t-test for continuous variables. Mann-Whitney U test was used for non-parametric data. The Bonferroni correction was applied to account for multiple testing. The level of significance was set at $p<0.05$. All analyses were performed in IBM SPSS Statistics (version 23 - © 2015 IBM Corporation) and RStudio (Version 1.0.143 – © 2009-2016 RStudio, Inc.,'mice', 'tableone', 'MatchIt' and 'optmatch' packages).

Results

Between May 2014 and December 2017, 8,318 patients with ≥cT2 rectal cancer were registered in the DCRA database. Patients without locally advanced tumors (n = 3,541), with metastatic disease (n = 986), with tumors located outside the rectum (n = 257), who underwent urgent or emergency surgery (n = 15), who did not receive neoadjuvant treatment or if the start date of neoadjuvant therapy was missing (n = 31), who received chemotherapy only or chemotherapy combined with short-course radiotherapy (n = 102), who underwent surgery after an interval of less than 6 weeks after the end of radiotherapy (n = 298) or after an initial watch and wait strategy (n = 34) and who underwent HIPEC or IORT (n = 128) (**Supplementary Figure 1**) were excluded. Finally, 2,926 patients were included in the analysis. Non-imputed data are provided in **Supplementary Table 1 and 2**.

Pre-matching results

Patients in the SCRT-delay group had a higher mean age, had more comorbidities and a higher ASA score (**Table 1**). Mean BMI was higher in the CRT group. The MRF was less often involved in the SCRT-delay group and clinical N-stage was higher. There was no difference in the distance from the anus at colonoscopy. With an equal interval between neoadjuvant treatment and surgery, patients in the SCRT-delay group more often underwent abdominoperineal resection (APR) and more often received a permanent colostomy. In the SCRT-delay group, 63 patients

Table 1. Patient, tumor and pathological characteristics in the pre-matching cohort. Data are presented as number (percentage) unless stated otherwise.

	SCRT-delay n = 288	CRT n = 2,638	p-value
Patient characteristics			
Gender			0.021 *
Male	161 (55.9)	1,663 (63.0)	
Female	127 (44.1)	975 (37.0)	
Age (mean years (sd))	77.89 (8.76)	64.59 (10.25)	<0.001 *
BMI (mean kg/m²(sd))	25.54 (3.94)	26.27 (4.35)	0.006 *
Comorbidities	249 (86.5)	1,765 (66.9)	<0.001 *
Charlson Comorbidity Index			<0.001 *
0	103 (35.8)	1,645 (62.4)	
1	85 (29.5)	554 (21.0)	
2	48 (16.7)	277 (10.5)	
3	31 (10.8)	111 (4.2)	
≥4	21 (7.3)	51 (1.9)	
ASA score			<0.001 *
1	20 (6.9)	575 (21.8)	
2	157 (54.5)	1,686 (63.9)	
3	103 (35.8)	363 (13.8)	
4	8 (2.8)	14 (0.5)	
Tumor characteristics			
Distance from anus at colonoscopy (mean cm (sd))	6.69 (4.69)	6.36 (4.42)	0.224
cT			0.425
2	8 (2.8)	95 (3.6)	
3	225 (78.1)	1,971 (74.7)	
4	55 (19.1)	572 (21.7)	
Distance to MRF > 1mm	58 (20.1)	721 (27.3)	0.003 *
cN			<0.001 *
0	77 (26.7)	346 (13.1)	
1	80 (27.8)	648 (24.6)	
2	129 (44.8)	1,637 (62.1)	
x	2 (0.7)	7 (0.3)	

Table 1. Continued I

	SCRT-delay n = 288	CRT n = 2,638	p-value
Treatment characteristics			
Interval between end of neoadjuvant treatment and surgery (median weeks [IQR])	11.00 [9.00, 15.00]	11.00 [10.00, 13.00]	0.472
Surgical approach			<0.001 *
Transabdominal open	58 (20.1)	561 (21.3)	
Transabdominal scopic	204 (70.8)	1,933 (73.3)	
TaTME or TAMIS TME	18 (6.2)	134 (5.1)	
Minimally invasive	8 (2.8)	10 (0.4)	
Surgical procedure			<0.001 *
Local excision	8 (2.8)	6 (0.2)	
(Low) Anterior Resection	155 (53.8)	1,607 (60.9)	
Abdominoperineal Resection	124 (43.1)	996 (37.8)	
Other	1 (0.3)	29 (1.1)	
Conversion	39 (13.5)	341 (12.9)	0.839
Reason conversion			0.658
Extensive tumor growth	8 (2.8)	6 (0.2)	
Accessibility	28 (9.7)	216 (8.2)	
Intraoperative complication	3 (1.0)	47 (1.8)	
Intraoperative complication			0.954
No	275 (95.5)	2,506 (95.0)	
Bleeding	1 (0.3)	17 (0.6)	
Spleen injury	0 (0.0)	1 (0.0)	
Bowel injury	3 (1.0)	19 (0.7)	
Ureter / urethra injury	4 (1.4)	26 (1.0)	
Bladder injury	1 (0.3)	17 (0.6)	
Vagina injury	1 (0.3)	12 (0.5)	
Other	3 (1.0)	40 (1.5)	
Primary anastomosis	64 (22.2)	1,257 (47.6)	<0.001 *
Ostomy			<0.001 *
No	57 (19.8)	521 (19.7)	
Diverting ileostomy	27 (9.4)	773 (29.3)	
Permanent ileostomy	1 (0.3)	29 (1.1)	
Diverting colostomy	7 (2.4)	125 (4.7)	
Permanent colostomy	195 (67.7)	1,186 (45.0)	
Stoma, unknown type	1 (0.3)	4 (0.2)	

Table 1. Continued II

	SCRT-delay n = 288	CRT n = 2,638	p-value
Pathological characteristics			
pT			0.001 *
0	27 (9.4)	512 (19.4)	
1	15 (5.2)	157 (6.0)	
2	75 (26.0)	623 (23.6)	
3	152 (52.8)	1,202 (45.6)	
4	19 (6.6)	125 (4.7)	
x	0 (0.0)	13 (0.5)	
Unknown	0 (0.0)	6 (0.2)	
pN			0.831
0	185 (64.2)	1,776 (67.3)	
1	67 (23.3)	571 (21.6)	
2	35 (12.2)	282 (10.7)	
x	1 (0.3)	7 (0.3)	
Unknown	0 (0.0)	2 (0.1)	
Pathological complete response, ypT0N0	23 (8.0)	425 (16.1)	<0.001 *
Radicality (R0 resection)	267 (92.7)	2,512 (95.2)	0.087

Abbreviations: sd = standard deviation, IQR = interquartile range, BMI = Body Mass Index (kg/m^2), ASA = American Society of Anesthesiologists, TaTME = Transanal Total Mesorectal Excision, TAMIS TME = TransAnal Minimal Invasive Surgery - Total Mesorectal Excision, pT = pathological tumor stage, pN = pathological nodal stage

(21.9%) received a primary anastomosis, compared to 1,253 patients (47.5%) in the CRT group. Pulmonic, cardiologic, infectious and neurologic complications were significantly more common in the SCRT-delay group (**Table 2**). When stratified for procedure, there were significant more complications after APR in the SCRT-delay group; however,pulmonic, cardiologic, infectious and neurologic complications in the SCRT-delay group occurred independent of type of surgical procedure (**Supplementary Table 3**). There was no difference in the number of re-interventions. In patients that received a primary anastomosis, the frequency of re-interventions for anastomotic leakage was not different after SCRT-delay or CRT (6.3% vs 7.6%, respectively). Patients in the SCRT-delay group were more often admitted to the ICU and hospital stay was more often prolonged. Furthermore, SCRT-delay less often resulted in a pathological complete response compared to CRT (8.0% vs. 16.1%, **Table 1**). There were no differences in surgical radicality after SCRT-delay or CRT (92.7% vs. 95.2% R0 resections, respectively).

Table 2. Postoperative outcomes in the pre-matching cohort. Data are presented as number percentage) unless stated otherwise

	SCRT-delay n = 288	CRT n = 2,638	p-value
Postoperative complications < 30 days after surgery	124 (43.1)	987 (37.4)	0.070
Non-surgical complications < 30 days after surgery			
Pulmonic	25 (8.7)	102 (3.9)	<0.001 *
Cardiologic	18 (6.2)	71 (2.7)	0.002 *
Infectious	32 (11.1)	136 (5.2)	<0.001 *
Thrombotic	1 (0.3)	14 (0.5)	1.000
Neurological	12 (4.2)	38 (1.4)	0.002 *
Other	48 (16.7)	360 (13.6)	0.188
Surgical complications < 30 days after surgery	66 (22.9)	653 (24.8)	0.538
Re-intervention	31 (10.8)	317 (12.0)	0.598
Type of re-intervention			0.334
Radiologic	2 (0.7)	35 (1.3)	
Surgery, laparoscopic	3 (1.0)	69 (2.6)	
Surgery, open	19 (6.6)	138 (5.2)	
Other	7 (2.4)	76 (2.9)	
Reason re-intervention			0.572
Anastomotic failure [$]	4 of 64 (6.3)	96 of 1,257 (7.6)	
Abscess	9 (3.1)	74 (2.8)	
Bleeding	1 (0.3)	11 (0.4)	
Ileus	2 (0.7)	40 (1.5)	
Fascial dehiscence	2 (0.7)	17 (0.6)	
Iatrogenic bowel injury	0 (0.0)	5 (0.2)	
Ureter / urethra injury	2 (0.7)	5 (0.2)	
Other	9 (3.1)	62 (2.4)	
≥1 day on ICU	98 (34.0)	712 (27.0)	0.014 *
Hospital stay ≥ 7 days	145 (50.3)	1,076 (40.8)	0.002 *
Readmission < 30 days after surgery	36 (12.5)	384 (14.6)	0.539
Death during or ≤ 30 days after surgery	4 (1.4)	23 (0.9)	0.268

Abbreviations: ICU = intensive care unit. [$] Data shown for patients that received a primary anastomosis

Post-matching results

Baseline characteristics that entered the propensity score model are presented in **Supplementary Table 4**. After matching, both groups comprised 246 patients and characteristics were well-balanced. Differences in patient, treatment and pathological characteristics between SCRT-delay and CRT in the post-matching

cohort are presented in **Table 3**. After matching, BMI was higher in the CRT group. 57 patients in the SCRT-delay group (23.2%) received a primary anastomosis, compared to 73 patients (29.7%) in the CRT group. Permanent colostomies were more frequent in the SCRT-delay group, but this difference was not significant in the post-matching cohort. There were no differences in pathological outcomes. Overall, there were no differences in postoperative (surgical) complications (**Table 4**). The number of re-interventions for anastomotic leakage in patients that received a primary anastomosis was not significantly different between groups (5.3% vs. 2.7% after SCRT-delay vs. CRT, respectively). There were no differences in ICU admission and hospital stay.

Table 3. Differences in patient, treatment and pathological characteristics between SCRT-delay and CRT in the matched cohort. Data are presented as numbers (percentage), unless stated otherwise.

	SCRT-delay n = 246	CRT n = 246	p-value
Patient characteristics			
Gender			0.078
Male	142 (57.7)	162 (65.9)	
Female	104 (42.3)	84 (34.1)	
Age (mean years (sd))	76.72 (8.86)	75.90 (8.39)	0.294
BMI (mean kg/m² (sd))	25.51 (3.96)	26.30 (3.91)	0.027 *
Comorbidities	208 (84.6)	223 (90.7)	0.091
ASA score			0.148
1	20 (8.1)	18 (7.3)	
2	132 (53.7)	152 (61.8)	
3	87 (35.4)	74 (30.1)	
4	7 (2.8)	2 (0.8)	
Tumor characteristics			
Distance from anus at colonoscopy (mean cm (sd))	5.91 (4.17)	6.62 (4.54)	0.071
cT			0.681
2	7 (2.8)	9 (3.7)	
3	190 (77.2)	182 (74)	
4	49 (19.9)	55 (22.4)	

Table 3. Continnued I

	SCRT-delay n = 246	CRT n = 246	p-value
Distance to MRF > 1mm	52 (21.1)	61 (24.8)	0.123
cN			0.047 *
0	61 (24.8)	37 (15)	
1	68 (27.6)	69 (28)	
2	115 (46.7)	138 (56.1)	
x	2 (0.8)	2 (0.8)	
Treatment characteristics			
Interval between end of neoadjuvant treatment and surgery (median weeks [IQR])	11.00 [9.00, 15.00]	11.00 [10.00, 13.00]	0.361
Surgical approach			0.660
Transabdominal open	48 (19.5)	54 (22)	
Transabdominal scopic	179 (72.8)	175 (71.1)	
TaTME or TAMIS TME	17 (6.9)	13 (5.3)	
Minimally invasive	2 (0.8)	4 (1.6)	
Surgical procedure			0.849
Local excision	2 (0.8)	4 (1.6)	
(Low) Anterior Resection	133 (54.1)	128 (52)	
Abdominoperineal Resection	110 (44.7)	113 (45.9)	
Other	1 (0.4)	1 (0.4)	
Conversion	33 (13.4)	33 (13.4)	1.000
Reason conversion			0.469
Extensive tumor growth	5 (2.0)	10 (4.1)	
Accessibility	24 (9.8)	21 (8.5)	
Intraoperative complication	4 (1.6)	2 (0.8)	
Intraoperative complication			0.967
Bleeding	1 (0.4)	1 (0.4)	
Spleen injury	0	1 (0.4)	
Bowel injury	1 (0.4)	2 (0.8)	
Ureter / urethra injury	3 (1.2)	2 (0.8)	
Bladder injury	1 (0.4)	1 (0.4)	
Vagina injury	1 (0.4)	1 (0.4)	
Other	2 (0.8)	1 (0.4)	
Primary anastomosis	57 (23.2)	73 (29.7)	0.125

Table 3. Continnued II

	SCRT-delay n = 246	CRT n = 246	p-value
Ostomy			0.054
No	50 (20.3)	34 (13.8)	
Diverting ileostomy	25 (10.2)	42 (17.1)	
Permanent ileostomy	1 (0.4)	4 (1.6)	
Diverting colostomy	7 (2.8)	8 (3.3)	
Permanent colostomy	163 (66.3)	158 (64.2)	
Pathological characteristics		**38 (15.4)**	
pT		14 (5.7)	0.293
0	23 (9.3)	62 (25.2)	
1	12 (4.9)	115 (46.7)	
2	64 (26)	17 (6.9)	
3	131 (53.3)		
4	16 (6.5)	168 (68.3)	
pN		48 (19.5)	0.478
0	154 (62.6)	27 (11)	
1	59 (24)	1 (0.4)	
2	32 (13)	31 (12.6)	
x	0	219 (89)	
Pathological complete response, ypT0N0	19 (7.7)		0.101
Radicality (R0 resection)	226 (91.9)		0.357

Abbreviations: sd = standard deviation, IQR = interquartile range, BMI = Body Mass Index (kg/m^2), ASA = American Society of Anesthesiologists, cT = clinical tumor stage, cN = clinical nodal stage, MRF = mesorectal fascia, TaTME = Transanal Total Mesorectal Excision, TAMIS TME = TransAnal Minimal Invasive Surgery - Total Mesorectal Excision, pT = pathological tumor stage, pN = pathological nodal stage

Table 4. Differences in postoperative outcomes between SCRT-delay and CRT in the matched cohort. Data are presented as numbers (percentage), unless stated otherwise.

	SCRT-delay n = 246	CRT n = 246	p-value
Postoperative complications < 30 days after surgery	102 (41.5)	93 (37.8)	0.461
Non-surgical complications < 30 days after surgery			
Pulmonic	18 (7.3)	10 (4.1)	0.173
Cardiologic	14 (5.7)	11 (4.5)	0.681
Infectious	25 (10.2)	16 (6.5)	0.192
Thrombotic	0	1 (0.4)	1.000
Neurological	0	0	1.000
Other	38 (15.4)	38 (15.4)	1.000
Surgical complications < 30 days after surgery	54 (22)	46 (18.7)	0.433
Re-intervention	22 (8.9)	25 (10.2)	0.759
Type of re-intervention			0.769
Radiologic	0	1 (0.4)	
Surgery, laparoscopic	3 (1.2)	2 (0.8)	
Surgery, open	13 (5.3)	13 (5.3)	
Other	6 (2.4)	9 (3.7)	
Reason re-intervention			0.504
Anastomotic failure [$]	3 of 57 (5.3)	2 of 73 (2.7)	
Abscess	7 (2.8)	8 (3.3)	
Bleeding	0	0	
Ileus	1 (0.4)	5 (2)	
Fascial dehiscence	2 (0.8)	1 (0.4)	
Iatrogenic bowel injury	0	2 (0.8)	
Ureter / urethra injury	1 (0.4)	0	
Other	7 (2.8)	6 (2.4)	
≥1 day on ICU	72 (29.3)	76 (30.9)	0.768
Hospital stay > 7 days	125 (50.8)	106 (43.1)	0.104
Readmission < 30 days after surgery	28 (11.4)	31 (12.6)	0.781
Death during or ≤ 30 days after surgery	2 (0.8)	4 (1.6)	0.434

Abbreviations: ICU = intensive care unit. [$] Data shown for patients that received a primary anastomosis

Discussion

In this nationwide, propensity score matched study we found no difference in the occurrence of surgical complications between patients who underwent SCRT-delay or CRT as neoadjuvant therapy for LARC. However, more pulmonic, cardiologic, infectious and neurologic complications in the pre-matching cohort in the SCRT-delay group. These differences diminished when patients were matched on age, gender, comorbidities, tumor characteristics and distance from the anus.

The pre-matching cohort represents daily clinical practice in the Netherlands. With the addition of SCRT-delay as regimen for LARC to the Dutch guidelines in 2014, more elderly patients are offered neoadjuvant treatment [25]. In our dataset, 642 of 2,926 (21.9%) patients who underwent surgery were aged ≥75 years. The percentage of old and frail patients was higher in the SCRT-delay group, but these patients were also represented in the CRT group, which underlines the heterogeneity of the elderly population and the differences in treatment choice due to lack of evidence-based data. Previous studies showed various results in incidence of postoperative morbidity and mortality in the elderly [2-5,26-28]. Taking into account that mortality increases when postoperative complications occur [5,27] and anastomotic leakage results in significantly more anorectal and urinary symptoms and higher Low Anterior Resection Syndrome (LARS) scores [29,30], the lower prevalence of primary anastomosis in the SCRT-delay group in the unmatched cohort might be a result of a defensive attitude towards primary anastomoses in frail and older patients. More postoperative complications were seen in this cohort after APR in the SCRT-delay group. However, the low prevalence indicates that conclusions should be drawn cautiously. Also, the rate of pulmonic, cardiologic, infectious and neurologic complications was higher in this group. However, this was not related to surgical procedure and can therefore most likely be explained by the frailty of the SCRT-delay population. The lack of differences in surgical complications can partly be explained by improved quality of care and better selection of patients [6,31]. Moreover, frail patients that did not undergo surgical resection were not included in this study.

In the pre-matching cohort we found a significant lower pCR rate in the SCRT-delay group (8.0% vs. 16.1%) after a median interval to surgery of 11 weeks. This is comparable with pCR rates of 4.4% to 25% in literature, with intervals to surgery varying from 4 to 19 weeks [9,32-40]. However, there were some differences in tumor characteristics between the groups in our dataset. Furthermore, we cannot relate these outcomes to local recurrence rates or survival. Three-years OS of 73-78%

vs. 65-82.4% and DFS of 53-59% vs. 52-75.1% have been previously described for SCRT-delay and CRT, respectively [9,32]. However, these studies included younger, WHO 0-1 patients. Differences in survival are partly determined by differences in patient selection for surgical treatment and choices in management of older patients with colorectal cancer might greatly affect population-based survival [41]. Also, the majority of these patients received adjuvant chemotherapy. This is not a part of routine care in the Netherlands.

The post-matching cohort represents a comparison of SCRT-delay and CRT in two groups with equivalent age and comorbidities. Here we did not find a difference in postoperative complications nor in pathological outcomes between SCRT-delay and CRT. These results are in line with 2 randomized trials comparing SCRT-delay +/- chemotherapy and CRT [9,32]. Also, in the Stockholm III trial the frequency of postoperative complications decreased by delaying surgery with 4-8 weeks after SCRT [8,12]. This indicates that, considering surgery-related complications and pathological outcomes, SCRT-delay could be a good alternative neoadjuvant treatment option for LARC patients who are unable to undergo CRT. However, information on treatment compliance is lacking from this study. In the Stockholm III trial, 7% of patients were hospitalized for radiation toxicity. Previous studies suggest that compliance and immediate toxicity are in favor of SCRT (compared to CRT) [1], but more data is needed. Furthermore, long-term outcomes on local recurrence and survival is needed.

This is the first observational study that compares complications after SCRT-delay and CRT in a large population. Since observational studies cannot determine treatment effects as accurately as randomized trials [22], this propensity score matched study may provide a useful estimation of the differences between SCRT-delay and CRT. Nonetheless, the results of this study should be interpreted carefully. Confounding bias is frequently seen in observational studies [42,43]. Patient and disease characteristics may have influenced the selection of patients for neoadjuvant and surgical treatment. Most likely, only well-conditioned patients are included in this database. The biggest pitfall of this study, however, is confounding by indication, since the selection of neoadjuvant treatment is confounded by patient factors, which are also related to the outcome [44,45]. Adjusting for confounding by indication using propensity score analysis is reliable when data on all factors associated with the intervention and the outcome is precise and can be accounted for [23,44]. However, unadjusted confounding may still exist if unmeasured factors influenced treatment selection. This may lead to biased results [22,23,46].

The aging population, the rising incidence and the improved prognosis of rectal cancer will increase the need for surgery in the elderly population in the future [4,26]. Successful treatment of elderly patients depends on whether it is done safely, allowing them to preserve good quality of life, and a life-expectancy that is not reduced by the treatment [2]. Regarding surgery-related complications, SCRT-delay is a good alternative neoadjuvant treatment option for frail LARC patients. However, information on treatment compliance and quality of life is needed. Secondly, before the indication for SCRT-delay can be expanded to intermediate risk rectal cancer or high risk rectal cancer in the general population, more data on long-term outcomes, such as local recurrence and survival, is needed.

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Supplementary Table 1. Patient, tumor and treatment characteristics in the non-imputed and imputed dataset. Data are presented as number (percentage) unless stated otherwise.

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Patient characteristics			
Gender			0.055
Male	161 (55.9)	1,661 (63.0)	
Female	127 (44.1)	975 (37.0)	
Missing	0 (0.0)	2 (0.1)	
Age (mean years (sd))	77.89 (8.76)	64.60 (10.25)	<0.001 *
BMI (mean kg/m² (sd))	25.49 (3.96)	26.26 (4.34)	0.005 *
Comorbidities			<0.001 *
No	38 (13.2)	871 (33.0)	
Yes	248 (86.1)	1,765 (66.9)	
Unknown	1 (0.3)	2 (0.1)	
Missing	1 (0.3)	0 (0.0)	
Charlson Comorbidity Index			<0.001 *
0	98 (34.0)	1,613 (61.1)	
1	80 (27.8)	529 (20.1)	
2	47 (16.3)	263 (10.0)	
3	30 (10.4)	102 (3.9)	
≥4	21 (7.3)	48 (1.8)	
Missing	12 (4.2)	83 (3.1)	
ASA score			<0.001 *
1	20 (6.9)	575 (21.8)	
2	157 (54.5)	1,685 (63.9)	
3	103 (35.8)	363 (13.8)	
4	8 (2.8)	14 (0.5)	
Missing	0 (0.0)	1 (0.0)	
Tumor characteristics			
Distance from anus at colonoscopy (mean cm (sd))	6.76 (4.58)	6.40 (4.42)	0.241
cT			0.425
2	8 (2.8)	95 (3.6)	
3	225 (78.1)	1,971 (74.7)	
4	55 (19.1)	572 (21.7)	

	Imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Patient characteristics			
Gender			0.021 *
Male	161 (55.9)	1,663 (63.0)	
Female	127 (44.1)	975 (37.0)	
Missing	-	-	
Age (mean years (sd))	77.89 (8.76)	64.59 (10.25)	<0.001 *
BMI (mean kg/m^2 (sd))	25.54 (3.94)	26.27 (4.35)	0.006 *
Comorbidities			<0.001 *
No	38 (13.2)	871 (33.0)	
Yes	249 (86.5)	1,765 (66.9)	
Unknown	1 (0.3)	2 (0.1)	
Missing	-	-	
Charlson Comorbidity Index			<0.001 *
0	103 (35.8)	1,645 (62.4)	
1	85 (29.5)	554 (21.0)	
2	48 (16.7)	277 (10.5)	
3	31 (10.8)	111 (4.2)	
≥4	21 (7.3)	51 (1.9)	
Missing	-	-	
ASA score			<0.001 *
1	20 (6.9)	575 (21.8)	
2	157 (54.5)	1,686 (63.9)	
3	103 (35.8)	363 (13.8)	
4	8 (2.8)	14 (0.5)	
Missing	-	-	
Tumor characteristics			
Distance from anus at colonoscopy (mean cm (sd))	6.69 (4.69)	6.36 (4.42)	0.224
cT			0.425
2	8 (2.8)	95 (3.6)	
3	225 (78.1)	1,971 (74.7)	
4	55 (19.1)	572 (21.7)	

Supplementary Table 1. Continued I

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Distance to MRF > 1mm			0.016 *
No	193 (67.0)	1,688 (64.0)	
Yes	57 (19.8)	702 (26.6)	
Unknown	22 (7.6)	121 (4.6)	
Missing	16 (5.6)	127 (4.8)	
cN			<0.001 *
0	77 (26.7)	346 (13.1)	
1	80 (27.8)	648 (24.6)	
2	129 (44.8)	1,637 (62.1)	
x	2 (0.7)	7 (0.3)	
Treatment characteristics			
Interval between end of neoadjuvant treatment and surgery (median weeks [IQR])	11.00 [9.00, 15.00]	11.00 [10.00, 13.00]	0.472
Surgical approach			<0.001 *
Transabdominal open	58 (20.1)	561 (21.3)	
Transabdominal scopic	204 (70.8)	1,930 (73.2)	
TaTME or TAMIS TME	18 (6.2)	134 (5.1)	
Minimally invasive	8 (2.8)	10 (0.4)	
Missing	0 (0.0)	3 (0.1)	
Surgical procedure			<0.001 *
Local excision	8 (2.8)	6 (0.2)	
(Low) Anterior Resection	155 (53.8)	1,606 (60.9)	
Abdominoperineal Resection	124 (43.1)	996 (37.8)	
Other	1 (0.3)	29 (1.1)	
Missing	0 (0.0)	1 (0.0)	
Conversion			0.813
No	152 (52.8)	1,355 (51.4)	
Yes	14 (4.9)	149 (5.6)	
Missing	122 (42.4)	1,134 (43.0)	
Reason conversion			0.730
Extensive tumor growth	2 (0.7)	20 (0.8)	

	Imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Distance to MRF > 1mm			0.003 *
No	206 (71.5)	1,788 (67.8)	
Yes	58 (20.1)	721 (27.3)	
Unknown	24 (8.3)	129 (4.9)	
Missing	-	-	
cN			<0.001 *
0	77 (26.7)	346 (13.1)	
1	80 (27.8)	648 (24.6)	
2	129 (44.8)	1,637 (62.1)	
x	2 (0.7)	7 (0.3)	
Treatment characteristics			
Interval between end of neoadjuvant treatment and surgery (median weeks [IQR])	11.00 [9.00, 15.00]	11.00 [10.00, 13.00]	0.472
Surgical approach			<0.001 *
Transabdominal open	58 (20.1)	561 (21.3)	
Transabdominal scopic	204 (70.8)	1,933 (73.3)	
TaTME or TAMIS TME	18 (6.2)	134 (5.1)	
Minimally invasive	8 (2.8)	10 (0.4)	
Missing	-	-	
Surgical procedure			<0.001 *
Local excision	8 (2.8)	6 (0.2)	
(Low) Anterior Resection	155 (53.8)	1,607 (60.9)	
Abdominoperineal Resection	124 (43.1)	996 (37.8)	
Other	1 (0.3)	29 (1.1)	
Missing	-	-	
Conversion			0.839
No	249 (86.5)	2,297 (87.1)	
Yes	39 (13.5)	341 (12.9)	
Missing	-	-	
Reason conversion			0.658
Extensive tumor growth	8 (2.8)	6 (0.2)	

Supplementary Table 1. Continued II

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Accessibility	12 (4.2)	106 (4.0)	
Intraoperative complication	0 (0.0)	17 (0.6)	
Missing	122 (42.4)	1,140 (43.2)	
Intraoperative complication			0.954
No	275 (95.5)	2,501 (94.8)	
Bleeding	1 (0.3)	17 (0.6)	
Spleen injury	0 (0.0)	1 (0.0)	
Bowel injury	3 (1.0)	19 (0.7)	
Ureter / urethra injury	4 (1.4)	26 (1.0)	
Bladder injury	1 (0.3)	17 (0.6)	
Vagina injury	1 (0.3)	12 (0.5)	
Other	3 (1.0)	40 (1.5)	
Missing	0 (0.0)	5 (0.2)	
Primary anastomosis			<0.001 *
No	217 (75.3)	1,376 (52.2)	
Yes	63 (21.9)	1,253 (47.5)	
Missing	8 (2.8)	9 (0.3)	
Ostomy			<0.001 *
No	57 (19.8)	521 (19.7)	
Diverting ileostomy	26 (9.0)	770 (29.2)	
Permanent ileostomy	1 (0.3)	29 (1.1)	
Diverting colostomy	7 (2.4)	125 (4.7)	
Permanent colostomy	188 (65.3)	1,182 (44.8)	
Stoma, unknown type	1 (0.3)	4 (0.2)	
Missing	8 (2.8)	7 (0.3)	
Pathological characteristics			
pT			0.003 *
0	27 (9.4)	510 (19.3)	
1	15 (5.2)	157 (6.0)	
2	74 (25.7)	622 (23.6)	
3	152 (52.8)	1,200 (45.5)	
4	19 (6.6)	125 (4.7)	
x	0 (0.0)	13 (0.5)	
Unknown	0 (0.0)	6 (0.2)	
Missing	1 (0.3)	5 (0.2)	

	Imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Accessibility	28 (9.7)	216 (8.2)	
Intraoperative complication	3 (1.0)	47 (1.8)	
Missing	-	-	
Intraoperative complication			0.954
No	275 (95.5)	2,506 (95.0)	
Bleeding	1 (0.3)	17 (0.6)	
Spleen injury	0 (0.0)	1 (0.0)	
Bowel injury	3 (1.0)	19 (0.7)	
Ureter / urethra injury	4 (1.4)	26 (1.0)	
Bladder injury	1 (0.3)	17 (0.6)	
Vagina injury	1 (0.3)	12 (0.5)	
Other	3 (1.0)	40 (1.5)	
Missing	-	-	
Primary anastomosis			<0.001 *
No	224 (77.8)	1,381 (52.4)	
Yes	64 (22.2)	1,257 (47.6)	
Missing	-	-	
Ostomy			<0.001 *
No	57 (19.8)	521 (19.7)	
Diverting ileostomy	27 (9.4)	773 (29.3)	
Permanent ileostomy	1 (0.3)	29 (1.1)	
Diverting colostomy	7 (2.4)	125 (4.7)	
Permanent colostomy	195 (67.7)	1,186 (45.0)	
Stoma, unknown type	1 (0.3)	4 (0.2)	
Missing	-	-	
Pathological characteristics			
pT			0.001 *
0	27 (9.4)	512 (19.4)	
1	15 (5.2)	157 (6.0)	
2	75 (26.0)	623 (23.6)	
3	152 (52.8)	1,202 (45.6)	
4	19 (6.6)	125 (4.7)	
x	0 (0.0)	13 (0.5)	
Unknown	0 (0.0)	6 (0.2)	
Missing	-	-	

Supplementary Table 1. Continued III

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
pN			0.001 *
0	178 (61.8)	1,766 (66.9)	
1	66 (22.9)	568 (21.5)	
2	35 (12.2)	282 (10.7)	
x	1 (0.3)	6 (0.2)	
Unknown	0 (0.0)	2 (0.1)	
Missing	8 (2.8)	14 (0.5)	
Pathological complete response, ypT0N0			0.001 *
No	265 (92.0)	2,210 (83.8)	
Yes	22 (7.6)	420 (15.9)	
Missing	1 (0.3)	8 (0.3)	
Radicality			<0.001 *
R0	258 (89.6)	2,499 (94.7)	
R1-2	21 (7.3)	126 (4.8)	
Missing	9 (3.1)	13 (0.5)	

Abbreviations: sd = standard deviation, IQR = interquartile range, BMI = Body Mass Index (kg/m2), ASA = American Society of Anesthesiologists, cT = clinical tumor stage, MRF = Mesorectal Fascia, cN = clinical nodal stage, TaTME = Transanal Total Mesorectal Excision, TAMIS TME = TransAnal Minimal Invasive Surgery - Total Mesorectal Excision, pT = pathological tumor stage, pN = pathological nodal stage.

	Imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
pN			0.831
0	185 (64.2)	1,776 (67.3)	
1	67 (23.3)	571 (21.6)	
2	35 (12.2)	282 (10.7)	
x	1 (0.3)	7 (0.3)	
Unknown	0 (0.0)	2 (0.1)	
Missing	-	-	
Pathological complete response, ypT0N0			<0.001 *
No	265 (92.0)	2,213 (83,9)	
Yes	23 (8.0)	425 (16.1)	
Missing	-	-	
Radicality			0.087
R0	267 (92.7)	2,512 (95.2)	
R1-2	21 (7.3)	126 (4.8)	
Missing	-	-	

Supplementary Table 2. Postoperative outcomes in the non-imputed and imputed dataset. Data are presented as number (percentage) unless stated otherwise.

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Postoperative outcomes			
Postoperative complications < 30 days after surgery			0.001 *
No	164 (56.9)	1,650 (62.5)	
Yes	121 (42.0)	985 (37.3)	
Missing	3 (1.0)	3 (0.1)	
Non-surgical complications < 30 days after surgery			
Pulmonic	22 (7.6)	93 (3.5)	0.001
Missing	6 (2.1)	23 (0.9)	
Cardiologic	16 (5.6)	69 (2.6)	0.007
Missing	7 (2.4)	22 (0.8)	
Infectious	30 (10.4)	134 (5.1)	0.000
Missing	7 (2.4)	22 (0.8)	
Thrombotic	0 (0.0)	13 (0.5)	0.474
Missing	7 (2.4)	24 (0.9)	
Neurological	12 (4,2)	35 (1.3)	0.001
Missing	6 (2.1)	23 (0.9)	
Other	45 (15.6)	354 (13.4)	0.307
Missing	5 (1.7)	17 (0.6)	
Surgical complications < 30 days after surgery			0.003 *
No	220 (76.4)	1,985 (75.2)	
Yes	65 (22.6)	650 (24.6)	
Missing	3 (1.0)	3 (0.1)	
Re-intervention			0.002 *
No	257 (89.2)	2,319 (87.9)	
Yes	28 (9.7)	316 (12.0)	
Missing	3 (1.0)	3 (0.1)	
Type of re-intervention			0.012 *
Radiologic	2 (0.7)	35 (1.3)	
Surgery, laparoscopic	3 (1.0)	68 (2.6)	
Surgery, open	16 (5.6)	137 (5.2)	
Other	7 (2.4)	76 (2.9)	
Missing	3 (1.0)	3 (0.1)	

	Imputed dataset		
	SCRT-delay n = 288	CRT n = 2,638	p-value
Postoperative outcomes			
Postoperative complications < 30 days after surgery			0.070
No	164 (56.9)	1,651 (62.6)	
Yes	124 (43.1)	987 (37.4)	
Missing	-	-	
Non-surgical complications < 30 days after surgery			
Pulmonic	25 (8.7)	102 (3.9)	<0.001 *
Missing	-	-	
Cardiologic	18 (6.2)	71 (2.7)	0.002 *
Missing	-	-	
Infectious	32 (11.1)	136 (5.2)	<0.001 *
Missing	-	-	
Thrombotic	1 (0.3)	14 (0.5)	1.000
Missing	-	-	
Neurological	12 (4.2)	38 (1.4)	0.002 *
Missing	-	-	
Other	48 (16.7)	360 (13.6)	0.188
Missing	-	-	
Surgical complications < 30 days after surgery			0.538
No	222 (77.1)	1,985 (75.2)	
Yes	66 (22.9)	653 (24.8)	
Missing	-	-	
Re-intervention			0.598
No	257 (89.2)	2,321 (88.0)	
Yes	31 (10.8)	317 (12.0)	
Missing	-	-	
Type of re-intervention			0.334
Radiologic	2 (0.7)	35 (1.3)	
Surgery, laparoscopic	3 (1.0)	69 (2.6)	
Surgery, open	19 (6.6)	138 (5.2)	
Other	7 (2.4)	76 (2.9)	
Missing	-	-	

Supplementary Table 2. Continued

	Non-imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Reason re-intervention			0.142
Anastomotic failure	4 (1.4)	100 (3.8)	
Abscess	9 (3.1)	74 (2.8)	
Bleeding	1 (0.3)	11 (0.4)	
Ileus	2 (0.7)	38 (1.4)	
Fascial dehiscence	2 (0.7)	16 (0.6)	
Iatrogenic bowel injury	0 (0.0)	5 (0.2)	
Ureter / urethra injury	2 (0.7)	5 (0.2)	
Other	8 (2.8)	61 (2.3)	
Missing	3 (1.0)	6 (0.2)	
≥1 day on ICU	98 (34.0)	712 (27.0)	0.014 *
Hospital stay ≥ 7 days	145 (50.3)	1,076 (40.8)	0.002 *
Readmission < 30 days after surgery			0.698
No	252 (87.5)	2,250 (85.3)	
Yes	36 (12.5)	383 (14.5)	
Unknown	0 (0.0)	2 (0.1)	
Missing	0 (0.0)	3 (0.1)	
Death during admission or <= days after surgery	288	2638	0.246
Yes	4 (1.4)	22 (0.8)	
No	282 (97.9)	2,612 (99.0)	
Unknown	1 (0.3)	2 (0.1)	
Missing	1 (0.3)	2 (0.1)	

Abbreviations: ICU = intensive care unit

	Imputed dataset		
	SCRT-delay n = 288	**CRT n = 2,638**	**p-value**
Reason re-intervention			0.572
Anastomotic failure	6 (2.1)	101 (3.8)	
Abscess	9 (3.1)	74 (2.8)	
Bleeding	1 (0.3)	11 (0.4)	
Ileus	2 (0.7)	40 (1.5)	
Fascial dehiscence	2 (0.7)	17 (0.6)	
Iatrogenic bowel injury	0 (0.0)	5 (0.2)	
Ureter / urethra injury	2 (0.7)	5 (0.2)	
Other	9 (3.1)	62 (2.4)	
Missing	-	-	
≥1 day on ICU	98 (34.0)	712 (27.0)	0.014 *
Hospital stay ≥ 7 days	145 (50.3)	1,076 (40.8)	0.002 *
Readmission < 30 days after surgery			0.539
No	252 (87.5)	2,251 (85.3)	
Yes	36 (12.5)	384 (14.6)	
Unknown	0 (0.0)	2 (0.1)	
Missing	-	-	
Death during admission or <= days after surgery			0.268
Yes	4 (1.4)	23 (0.9)	
No	283	2,613 (99.0)	
Unknown	1 (0.3)	2 (0.1)	
Missing	-	-	

Supplementary Table 3. Postoperative (surgical) complications in the pre-matching cohort, stratified for surgical procedure. Data are presented as number (percentage) unless stated otherwise.

	Pre-matching cohort		p-value
	SCRT-delay n = 288	CRT n = 2,638	
Postoperative complications < 30 days after surgery			
Local excision	4 / 8 (50.0)	1 / 6 (16.7)	0.198
Low Anterior Resection	58 / 155 (37.4)	583 / 1,607 (36.3)	0.778
Abdominoperineal resection	62 / 124 (50.0)	386 / 996 (38.8)	0.016 *
Other	0 / 1 (0.0)	17 / 29 (58.6)	0.245
Surgical complications < 30 days after surgery			
Local excision	2 / 8 (25.0)	0 / 6 (0.0)	0.186
Low Anterior Resection	28 / 155 (18.1)	391 / 1,607 (24.3)	0.080
Abdominoperineal resection	36 / 124 (29.0)	249 / 996 (25.0)	0.331
Other	0 / 1 (0.0)	13 / 29 (44.8)	0.374
Pulmonic complication			
Local excision	0 / 8 (0.0)	0 / 6 (0.0)	-
(Low) Anterior Resection	12 / 155 (7.7)	58 / 1,607 (3.6)	0.012 *
Abdominoperineal Resection	13 / 124 (10.5)	40 / 996 (4.0)	0.001 *
Other	0 / 1 (0.0)	4 / 29 (13.8)	0.690
Cardiologic complication			
Local excision	1 / 8 (12.5)	1 / 6 (16.7)	0.825
(Low) Anterior Resection	8 / 155 (5.2)	32 / 1,607 (2.0)	0.011 *
Abdominoperineal Resection	9 / 124 (7.3)	36 / 996 (3.6)	0.051
Other	0 / 1 (0.0)	2 / 29 (6.9)	0.786
Thrombotic complication			
Local excision	1 / 8 (12.5)	0 / 6 (0.0)	0.369
(Low) Anterior Resection	0 / 155 (0.0)	9 / 1,607 (0.6)	0.350
Abdominoperineal Resection	0 / 124 (0.0)	5 / 996 (0.5)	0.429
Other	0 / 1 (0.0)	0 / 29 (0.0)	-
Neurologic complication			
Local excision	0 / 8 (0.0)	0 / 6 (0.0)	-
(Low) Anterior Resection	6 / 155 (3.9)	24 / 1,607 (1.5)	0.029 *
Abdominoperineal Resection	6 / 124 (4.8)	13 / 996 (1.3)	0.004 *
Other	0 / 1 (0.0)	1 / 29 (3.4)	0.850
Infectious complication			
Local excision	0 / 8 (0.0)	0 / 6 (0.0)	-
(Low) Anterior Resection	18 / 155 (11.6)	77 / 1,607 (4.8)	<0.001 *
Abdominoperineal Resection	14 / 124 ((11.3)	59 / 996 (5.9)	0.022 *
Other	0 / 1 (0.0)	0 /29 (0.0)	-
Other complication			
Local excision	3 / 8 (37.5)	0 / 6 (0.0)	0.091
(Low) Anterior Resection	24 / 155 (15.5)	205 / 1,607 (12.8)	0.335
Abdominoperineal Resection	21 / 124 (16.9)	147 / 996 (14.8)	0.522
Other	0 / 1 (0.0)	8 / 29 (27.6)	0.540

Supplementary Table 4. Baseline characteristics used for propensity score matching. Data are presented as number (percentage), unless stated otherwise. An SMD <0.1 indicates a good balance.

	Pre-matching			Post-matching		
	SCRT-delay n = 288	**CRT n = 2,638**	**SMD**	**SCRT-delay n = 246**	**CRT n = 246**	**SMD**
Age (mean years (sd))	77.89 (8.76)	64.59 (10.25)	1.394	76.72 (8.86)	75.90 (8.39)	0.095
Charlson Comorbidity Index			0.585			0.200
0	104 (36.1)	1,643 (62.3)		97 (39.4)	83 (33.7)	
1	83 (28.8)	557 (21.1)		74 (30.1)	72 (29.3)	
2	49 (17)	278 (10.5)		38 (15.4)	47 (19.1)	
3	30 (10.4)	108 (4.1)		22 (8.9)	33 (13.4)	
≥4	22 (7.6)	52 (2)		15 (6.1)	11 (4.5)	
cT			0.083			0.079
2	8 (2.8)	95 (3.6)		7 (2.8)	9 (3.7)	
3	225 (78.1)	1,971 (74.7)		190 (77.2)	182 (74)	
4	55 (19.1)	572 (21.7)		49 (19.9)	55 (22.4)	
Surgical procedure			0.260			0.081
Local excision	8 (2.8)	6 (0.2)		2 (0.8)	4 (1.6)	
(Low) Anterior Resection	155 (53.8)	1,606 (60.9)		133 (54.1)	128 (52)	
Abdominoperineal Resection	124 (43.1)	997 (37.8)		110 (44.7)	123 (45.9)	
Other	1 (0.3)	29 (1.1)		1 (0.4)	1 (0.4)	

Abbreviations: cT = clinical tumor stage, sd = standard deviation, SMD = standardized mean difference

PART II

Diagnostic perspective

CHAPTER 5

The association between Tumor Infiltrating Lymphocytes (TILs) density in biopsies and response to dose-escalated neoadjuvant therapy in patients with locally advanced rectal cancer

Submitted

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ABSTRACT

Background

Complete response (CR) following neoadjuvant chemoradiation (CRT) in locally advanced rectal cancer (LARC) provides the opportunity for rectum-sparing treatment. Intensified neoadjuvant treatment, such as dose-escalated radiotherapy, is sometimes administered to increase the probability of patients' eligibility for rectum preservation. This study examined the association between tumor infiltrating lymphocytes (TILs) density in pretreatment biopsies and complete response after standard versus dose-escalated neoadjuvant treatment and aimed to clarify whether TILs counts can contribute to adequate patient selection for treatment intensification.

Methods

82 participants from the RECTAL-BOOST trial (NCT01951521) who received standard CRT (25x2Gy with capecitabine, n=52), or dose-escalated CRT (5x3Gy boost prior to standard CRT, n=30) were included. Pretreatment biopsies were stained with CD3+ and CD8+ antibodies. TILs density was calculated by automated analysis using QuPath. The association between TILs density and CR, defined as ypT0N0 or clinical complete response, and the association between different TILs subgroups and CR were analyzed with Mann-Whitney U test, Chi-square or Fisher's Exact test as appropriate. Analyses were stratified by treatment.

Results

CR was achieved in 32 (39%) of 82 patients. There were no differences in CD3+ or CD8+ density between patient with or without CR. In the 'CD3+ low' and 'CD8+ low' subgroups, patients receiving dose-escalated CRT more often had CR, but this was not significant. The absence of association between pretreatment TILs and CR was similar for patients treated with and without dose-escalated RT.

Conclusion

This was the first study to investigate the association between TILs density in pretreatment biopsies and CR in LARC patients receiving CRT compared to those receiving dose-escalated CRT. We did not find indications that TILs may be helpful in selecting patients who are likely to benefit from dose-escalation. The absence of association may be due to low power of the study.

Introduction

Locally advanced rectal cancer (LARC) is conventionally treated with neoadjuvant chemoradiation (CRT), consisting of 45-50Gy radiotherapy with concurrent fluoropyrimidine-based chemotherapy, followed by surgery [1]. Approximately 8-29% of patients show a pathological complete response (pCR) following this regimen [2,3]. When imaging and/or endoscopy indicates a complete response before surgery (clinical complete response, cCR), organ-sparing approaches, such as local excision or a watch-and-wait strategy, can be considered [4]. These strategies aim to preserve quality of life and functional status, without compromising oncological safety [5,6].

Dose-escalated radiotherapy potentially improves tumor response and may increase a patient's eligibility for rectum preservation [7]. However, the RECTAL BOOST trial, a randomized trial investigating the effect of an external radiation boost to the tumor prior to CRT, showed no difference in tumor response after dose-escalation [8]. Nonetheless, the study showed a unexpectedly high pCR rate of 36% in both groups. The question arises which patient requires dose-escalated therapy to achieve a complete response and which patient will have a complete response without dose-escalated therapy. However, at this moment in time, there are no biomarkers available to predict tumor response in the pre-treatment biopsy and to identify eligible patients for dose-escalated radiotherapy.

There is increasing evidence that tumor-infiltrating lymphocytes (TILs) are associated with tumor regression after neoadjuvant CRT in LARC [9,10]. Both radiotherapy and chemotherapy potentiate systemic antitumor immune effects by causing tumor cell death and inducing T-cell response [11,12]. Higher levels of TIL subsets, such as CD3+ (a general T-lymphocyte that plays an essential role in the adaptive immune response [13]) and CD8+ (a cytotoxic T-lymphocyte that promotes apoptosis of cancer cells [13]), have been associated with tumor regression after CRT [12,14-16]. This suggests that tumors that attract T-cells are more sensitive to CRT and are thus more likely to respond better to treatment.

In this study we investigated whether the CD3+ and CD8+ TILs density in pretreatment endoscopic biopsies was associated with tumor response in patients with LARC and whether it may help to determine which patients can benefit from dose-escalated radiotherapy in order to achieve a better tumor response.

Methods

This study was conducted using material from patients participating in the RECTAL-BOOST trial (NCT01951521) [17]. Trial details have been described previously [8,17]. In short, the RECTAL-BOOST trial was a multicenter, non-blinded, phase 2 randomized controlled trial within a prospective cohort (prospective Dutch colorectal cancer cohort, NCT02070146)[18], in which the effect of chemoradiation with a 15Gy radiation boost was compared to standard chemoradiation in patients with locally advanced rectal cancer. Ethical approval was obtained from the Ethical Research Board of UMC Utrecht. Endoscopic biopsies were part of the routine diagnostic work-up. Tissue samples were retrospectively collected from the referring hospitals (n = 6). 46 36%) of the 128 patients in the RECTAL-BOOST trial had to be excluded from the current study as biopsy material was not available (n = 39) or there was insufficient material left in the tissue block (n = 2) (**Supplementary Figure S1**). Clinicopathological data were available from the RECTAL-BOOST trial database.

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Fifty-three patients underwent standard treatment, i.e. chemoradiation (CRT) that involved 50Gy in 25 fractions of 2Gy with concurrent capecitabine 825mg/m^2 twice daily for 5 or 7 days per week (control group). Thirty patients received dose-escalated CRT (boost group) including a sequential, stereotactic radiation boost to the tumor of 15Gy in 5 fractions of 3Gy without concurrent chemotherapy one week prior to the start of chemoradiation. Sixty-five patients (42 (81%) in the control group and 23 (77%) in the boost group) underwent TME surgery 12 weeks after the end of chemoradiation. Seventeen patients with a cCR (10 (19%) in the control group and 7 (23%) in the boost group) preferred organ preservation and entered a watch-and-wait follow-up program.

Two 4um sections were cut from the endoscopic biopsy block from every patient and deparaffinized using a standard protocol. Slides were pretreated with cell conditioner (CC1) and subsequently stained with the anti CD3 (Dako AO452) or anti CD8 (Dako M7103) using the Ventana immunostainer (© Roche). The step-by-step staining protocol can be found in the supplement (**Supplementary File S2**). DNA Mismatch Repair (MMR) deficiency status was collected from the patient charts. If MMR status was unknown and enough material was available, four 4um sections were stained with the MMR proteins MSH2 (Ventana, clone G219-1129), MSH6 (Ventana, clone SP93), MLH1 (Roche, clone M1) and PMS2 (Ventana, clone A16-4) using the Ventana immunostainer (© Roche). Expression of MMR proteins was visually assessed by a specialized gastrointestinal pathologist. Patients were classified as MMR deficient if one or more MMR proteins was lost in the tumor cells.

Slides were scanned at 40x magnification using digital scanner (Hamamatsu Nano Zoomer XR, © Hamamatsu Photonics K.K).

The total number of CD3+ and CD8+ lymphocytes (TIL density) was calculated by automated analysis using QuPath (**Figure 1**), according to its instructions on according to Qupath instructions on https://qupath.github.io/ [19]. First, tumorous tissue was annotated by one of the authors (SH). The annotation was quality controlled and adjusted if needed by a dedicated pathologist (ML,GO) blinded for the outcome. The *"positive cell detection"* feature in QuPath was used to calculate the number of stained lymphocytes in the annotated areas. This option identifies individual lymphocytes by digitally separating stains using color deconvolution.

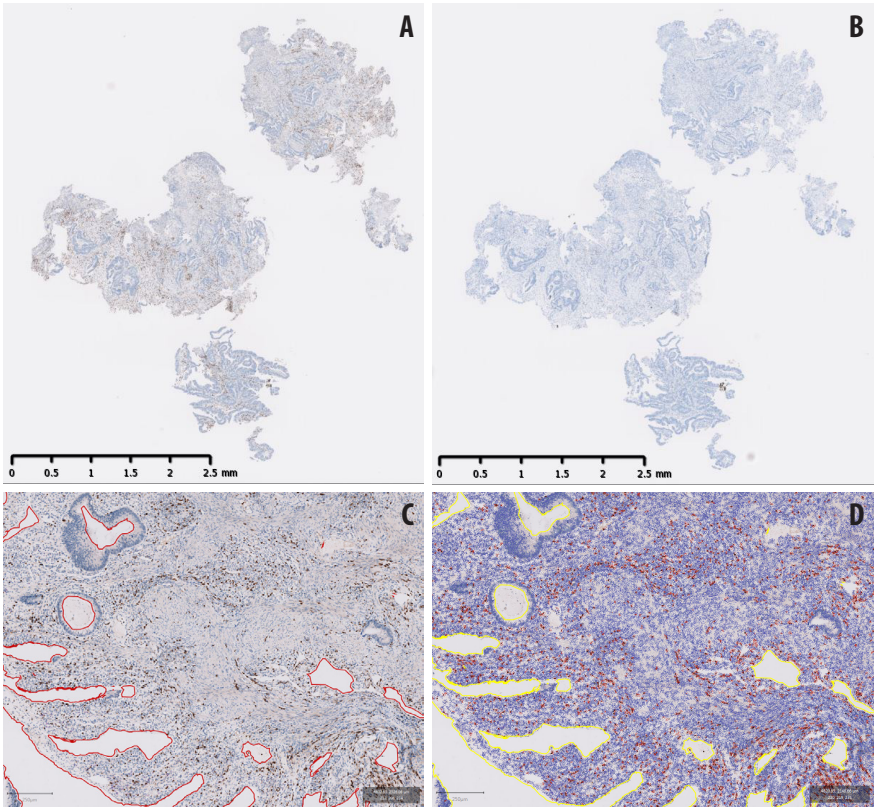

Figure 1. A CD3+ (**A**) CD8+ (**B**) stained rectum biopsy, an annotated biopsy (**C**) and positive cell detection (**D**) in Qupath.

The cell detection threshold whether a cell was classified as positive or negative was determined using stain vector estimation, a tool to identify and characterize the color of each stain, in order to enable color deconvolution (and herewith identification of lymphocytes). Markup images showing the detected cells were used for visual verification of the cell count. Cell detection parameters were optimized if needed. The number of positive cells and annotated surface area were used to calculate the number of positive cells per mm^2. For statistical analyses, the average TIL density across all biopsy fragments (average number of positive cells per mm^2) was used. Detailed information on parameter setting are provided in the supplement (**Supplementary File S2**).

Systematic review of literature

To compare the results of this study to previous literature, a Pubmed search was performed using the terms *"tumor infiltrating lymphocytes", "biopsy", "chemoradiation", "pathological complete response"* or *"tumor regression"* and *"rectal cancer"*, and all related terms. A cross-reference check of included manuscripts was performed to check for missed studies. Information on tumor characteristics, neoadjuvant treatment, pathological assessment, TIL analysis methods and associations between TILs, pathology and long-term outcomes were retrieved from the manuscripts. Results from the literature search are available in the Supplement.

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Statistical analysis

The primary outcome was a complete response (CR), i.e. a pathological complete response (pCR), defined as the absence of viable tumor cells in the resected specimen (ypT0N0) or a sustained clinical complete response (sustained cCR), reserved for patients in a watch-and-wait regimen without locoregional recurrence within 2 years after completing neoadjuvant therapy. Of the 17 patients who entered a watch-and-wait follow-up program, 5 (2 in the control group and 3 in the boost group) had a recurrence within 6 months and were therefore assigned to the no-CR group. Resected specimens were analyzed according to national pathology guidelines. Pathological reports were retrieved from the participating hospitals. Central pathology review was performed for all resection specimens classified as ypT0N0. The secondary outcome was a good response (GR), defined as tumor regression grade (TRG) 1 or 2 according to Mandard TRG scale [20], or sustained cCR. Only 1 patient had a MMR deficient tumor. Considering this low prevalence, no further analysis was performed. Baseline characteristics are presented as descriptive statistics. Differences in median TILs/mm^2 between patients with versus without CR or with versus without good response were analyzed using Mann-Whitney U test. CD3+ and CD8+ TIL values were dichotomized based on the median to classify patients as having high (> median) or low (≤ median) TIL density. To determine whether the association between TILs and (pathological)

tumor response was different in patients treated with dose-escalation, results were stratified for dose-escalated CRT (boost group) and standard CRT (control group). Differences in response were compared with Chi-square test or Fisher's exact test. The level of significance was defined as $p<0.05$. SPSS Statistics version 23 (© IBM) was used for data analysis.

Results

Gender, age, clinical lymph node stage (cN), Mesorectal Fascia (MRF) involvement and distance from the anus on sagittal MRI were well balanced between the control group and boost group (**Table 1**). There were more cT4-stage tumors in the control group compared to the boost group (31% versus 17%, respectively).

Table 1. Baseline characteristics of included patients. Numbers are presented as n (%), unless stated otherwise.

	Control group n = 52	Boost group n = 30
Gender		
Female	12 (23)	9 (30)
Male	40 (77)	21 (70)
Age (median years [IQR])	62.5 [57.0, 70.3]	65.5 [56.0, 69.8]
Clinical Tumor stage (cT)		
2	4 (8)	2 (7)
3	32 (62)	23 (77)
4	16 (31)	5 (17)
Clinical lymph node stage (cN)		
0	6 (12)	3 (10)
+	46 (88)	27 (90)
Mesorectal Fascia (MRF)		
Uninvolved	18 (35)	10 (33)
Involved	34 (65)	20 (67)
Distance from anus on sagittal MRI (median cm [IQR])	2.4 [1.0, 7.0]	3.8 [1.0, 7.5]
Complete response		
pCR or sustained cCR*	21 (40)	11 (37)
Good response		
TRG 1-2 or sustained cCR*	27 (52)	20 (67)

* Complete clinical response without locoregional recurrence within 2 years after completion of neoadjuvant therapy.

TIL density and tumor response

In the entire cohort, the median number of CD3+ cells/mm^2 was 1128.9 (IQR 766.3 - 1598.9) and the median number of CD8+ cells/mm^2 was 176.4 (IQR 90.9 - 273). Patients with ≤1128.9 CD3+ TILs/mm^2 were assigned to the 'CD3+ low' subgroup. The ones with ≤176.4 CD8+ TILs/mm^2 were assigned to the 'CD8+ low' subgroup. Median (range) of the size of tumor area per patient. Overall, CR (i.e. pCR or sustained cCR) was achieved in 32 of 82 patients (39%). There was no difference in the median number of CD3+ TILs/mm^2 in the pre-treatment biopsy between patients with CR compared to patients without CR (1330 (IQR 941.9 – 1886) vs. 1054 (IQR 728.3 – 1574), p = 0.12, **Figure 2a**). For CD8+ TILs/mm^2, medians were 181.2 (IQR 97.3 – 276.8) for CR patients and 170 (85.3 – 282.4) for non-CR patients (p 0.52, **Figure 2b**). Good response (GR: i.e. TRG 1-2 or sustained cCR) was achieved in 47 of 82 patients (57%). The median number of CD3+ TILs/mm^2 was 1247 (861.8 – 1887) in patients with GR and 1000 (676.7 – 1511) in patients with TRG 3-5 (p 0.06, **Figure 2c**). For CD8+ TILs/mm^2, these medians were 186.4 (90.8 – 294.9) for GR patients and 152.6 (85.9 – 250.1) for non-GR patients (p 0.36, **Figure 2d**).

Association with neoadjuvant treatment

CR was achieved in 21 (40%) of 52 patients in the control group and in 11 (37%) of 30 patients in the boost group (**Table 1**). GR was achieved in 27 (52%) of 52 patients in the control group and 20 (67%) of 30 patients in the boost group (**Table 1**).

Stratified analyses was performed to estimate the impact of dose-escalation in patients with high versus low TILs. In the 'CD3+ low' subgroup, patients in the boost-group had more often CR (26% vs. 39%). Also, GR was more often seen following dose-escalated therapy than after standard CRT (44% vs. 56%) in this subgroup, but these differences were not significant (**Table 2**). In the group of patients with a high CD3+ density, CR was more present following standard CRT (52% vs. 33%). On the contrary, this group more often showed GR following boost treatment (59% vs. 83%). These results were not significant (**Table 2**).

The same pattern was seen in the 'CD8+ low' and 'CD8+ high' subgroups. In the 'CD8+ low' subgroup more CR (36% vs. 46%) and GR (46% vs. 62%) was seen following boost treatment. In the 'CD8+ high subgroup a higher rate of CR following standard treatment was seen (46% vs. 29%), but these differences were not significant (**Table 2**).

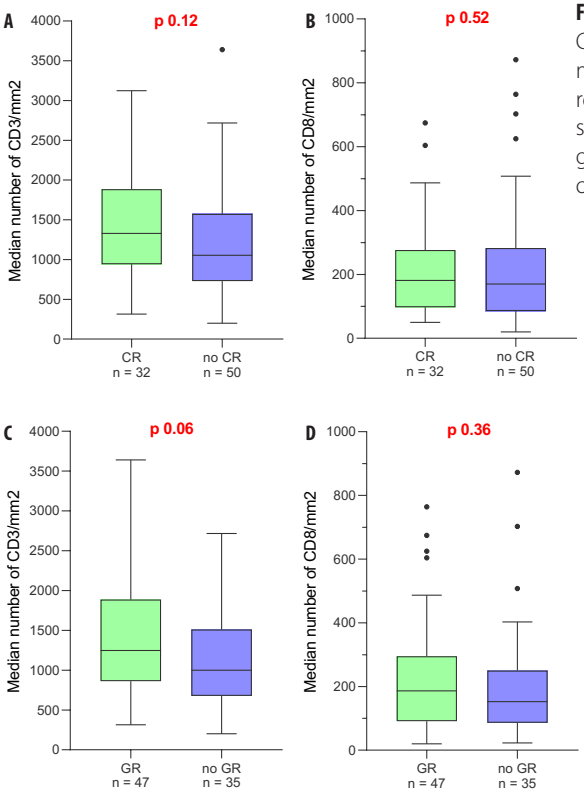

Figure 2. Boxplots of median CD3+ TILs/mm^2 and CD8+ TILs/mm^2, stratified for complete response (CR, i.e. pCR or sustained cCR, **A** and **B**) and good response (GR, i.e. TRG 1-2 or sustained cCR, **C** and **D**).

Table 2. (Pathological) response categories following neoadjuvant treatment for rectal cancer, stratified by dose-escalation strategy (Boost versus no Boost) and TILs subset. Numbers are presented as n (%), unless stated otherwise.

	CD3+ low (n = 41)			CD3+ high (n = 41)		
	CRT n = 23	**Boost n = 18**	**p-value**	**CRT n = 29**	**Boost n = 12**	**p-value**
Complete response			0.38			0.33
pCR or cCR	6 (26)	7 (39)		15 (52)	4 (33)	
Good response			0.44			0.13
TRG 1-2 or cCR	10 (44)	10 (56)		17 (59)	10 (83)	
	CD8+ low (n = 41)			**CD8+ high (n = 41)**		
	CRT n = 28	**Boost** n = 13	**p-value**	**CRT** n = 24	**Boost** n = 17	**p-value**
Complete response			0.52			0.29
pCR or cCR	10 (36)	6 (46)		11 (46)	5 (29)	
Good response			0.37			0.42
TRG 1-2 or cCR	13 (46)	8 (62)		14 (58)	12 (71)	

Discussion

This is the first study that looked at the association between TILs and the effect of dose-escalated CRT. No association between CD3+ and CD8+ density in pretreatment rectum biopsies and tumor response after CRT was found. Also, the association between TILs density and tumor response was not different between patients treated with or without dose-escalated radiotherapy.

The association between TILs density in rectal cancer pre-treatment biopsies and response to neoadjuvant treatment has been investigated in the past with inconsistent results among studies. This may be due to different counting techniques (automated or visually) and varying definitions of density, cut-off values and tumor regression [12,15,16,21-24]. In accordance with our findings, no associations between CD3+ and CD8+ density in biopsies and tumor regression were found in a study with automated cell detection [23]. Other studies with visual scoring of TILs showed that high CD3+ and CD8+ densities were associated with good response (i.e. Dworak TRG 3-4 or Mandard TRG 1-2), but not with complete response [12,21]. In our study, the median CD3+ and CD8+ density (cells/mm^2) was higher in patients with a good response (defined as TRG1-2 or cCR), but this difference was not significant. TRG 1-2 has shown to be associated with improved overall and disease-free survival and may indicate rectum-saving potential [25]. Nonetheless, the TRG definition of pathological complete response varies between approaches and the application of TRG is not recommended in the present TNM classification [26,27]. Furthermore, the inter-observer agreement for different TRG methods is very low [26]. Present study showed no significant difference in CD3+ or CD8+ density between patients with pCR (ypT0N0) versus without pCR, but these results cannot be compared to previous studies, because none of the studies defined pCR as ypT0N0.

This study found no association between TILs density in pretreatment rectum biopsies and tumor response. However, the small sample size diminishes the power to draw conclusions. Furthermore, the use of biopsies for analysis of lymphocyte density is controversial. The results may be distorted due to sampling. Previous studies showed that the majority of mutations detected in different fragments from rectal cancers are frequently unique to a single fragment, which suggests that pre-treatment biopsies might not be representative for mutations in the entirety of the tumor. This may limit the utility of the biological information provided by a single biopsy [28]. In addition, immune cells can be located in the core (the center) of the tumor, in the invasive margin or in the neighboring lymphoid structures [29].

Up to now, it remains unclear whether a biopsy is a good representation of the entire tumor microenvironment. In this study no distinction was made between stromal and intra-epithelial TILs. Previous studies suggest that TILs are more frequently expressed in the tumor epithelium [30,31], whereas another study found more CD8+ lymphocytes in stromal areas [32]. One study showed no association between CD8+ and recurrence-free survival in pre-treatment rectal cancer biopsies, for both stromal and epithelial TILs. The patients in this study that achieved pCR were categorized as CD8+/Foxp3 high in both stroma and epithelium [15]. A meta-analysis showed that the infiltration location of TILs was not a strong prognostic marker for colorectal cancer and that subsets of immune cells are distributed differently among different tumor types [10]. Since scientific proof for the value of TILs location is still lacking, combined with the fact that stromal changes can be enough for the diagnosis of cancer in a clinical setting, we argued that it was justified not to differentiate between stroma and epithelium.

It should be emphasized that the relationship between TILs and tumor response is more complex and might not only depend on infiltration of lymphocytes [33], but also on the interaction with the DNA mismatch repair (MMR) system [34]. MMR status is linked to prognosis, response to treatment and metastatic disease [35], but the prognostic value of MMR status appears to depend on the presence of TILs [36]. Higher levels of TILs are found in deficient MMR (dMMR) tumors, which suggests that some aspects of the tumor biology are involved in lymphocyte recruitment [34]. Conversely, the prevalence of dMMR tumors is low in rectal cancer [35]. In the present study only 1 patient had a dMMR tumor and no further analysis was performed. Although it is suggested that dMMR rectal tumors are resistant to neoadjuvant therapy [35], the relationship between TILs, MMR and tumor response has not been investigated yet.

Although this study focused on CD3+ and CD8+, other immune cells may play a role in the microenvironment. Immune infiltrates are heterogeneous between tumor types, and are very diverse between patients [29]. It is suggested that higher counts of CD4+ regulatory T-cells and the related transcription factor Foxp3 are associated with poorer clinical outcomes [23]. Contrarily, patients with low Foxp3+ density are more likely to achieve pCR [23,37]. However, the predictive role of Foxp3+ remains debatable [13,38]. This study primarily aimed to explore the association between TILs density and pCR. Since results of TILs are still contradictive in literature, we chose to focus on the most general (CD3+) and most investigated (CD8+) TILs.

Previous studies suggest that TILs are associated with tumor recurrence relapse and overall survival [13,39]. In particular the difference between pre- and post-treatment TILs might be associated with survival and recurrence [12]. Because present study lacks power to prove that patients with low CD3+ or low CD8+ density might benefit from dose-escalated radiotherapy, a subsequent trial with larger sample size is needed. Furthermore, investigation of TILs in resected specimen might clarify if dose-escalated chemoradiation changes the microenvironment of the tumor and whether this is associated with improved long-term outcomes, such as local recurrence and survival.

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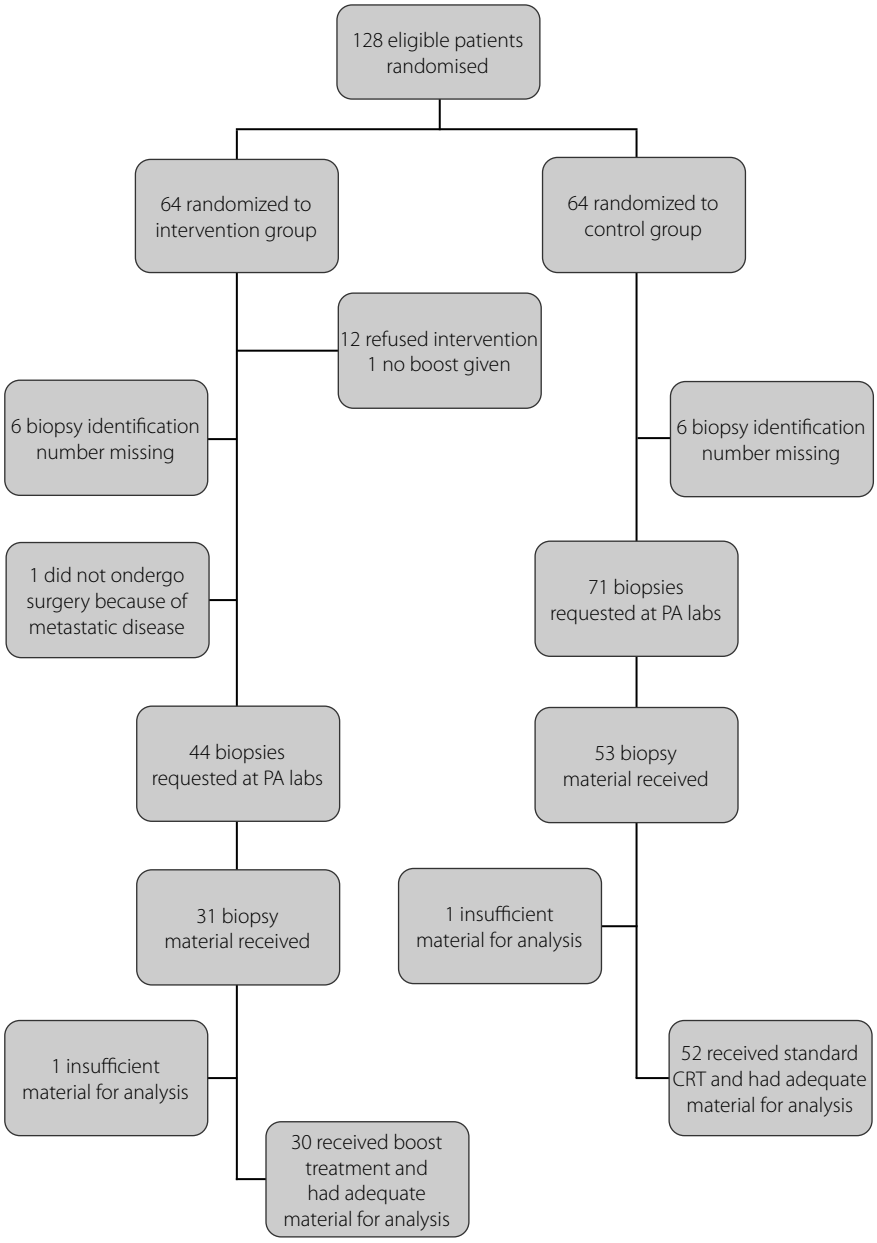
Supplementary material

Supplementary Figure S1. Flowchart of biopsy selection from RECTAL-BOOST trial



Supplementary File S2. Step-by-step staining details for CD3+ (**A**) and CD8+ (**B**).

Protocol Summary

Procedure: U OptiView DAB IHC v5 (v1.00.0117)

BenchMark ULTRA IHC/ISH Staining Module

UMCU, Afd Pathologie Utrecht

Protocol No	Protocol Name	Creation Date
30	CD3	02/09/2018

1	Paraffin [Selected]
2	Baking [Selected]
3	Warmup Slide to [75 Deg C], and Incubate for [8 Minutes] (Baking)
4	Deparaffinization [Selected]
5	Warmup Slide to [72 Deg C] from Medium Temperatures (Deparaffinization)
6	Cell Conditioning [Selected]
7	Ultra CC1 [Selected]
8	Warmup Slide to [100 Deg C], and Incubate for 4 Minutes (Cell Conditioner #1)
9	CC1 8 Min [Selected]
10	CC1 16 Min [Selected]
11	CC1 24 Min [Selected]
12	Pre Primary Peroxidase Inhibit. [Selected]
13	Primary Antibody [Selected]
14	Apply Coverslip, One Drop of [PREP KIT 14] (Antibody), and Incubate for [0 Hr 32 Min]
15	Counterstain [Selected]
16	Apply One Drop of [HEMATOXYLIN II] (Counterstain), Apply Coverslip, and Incubate for [12 Minutes]
17	Post Counterstain [Selected]
18	Apply One Drop of [BLUING REAGENT] (Post Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

Protocol Summary

Procedure: U OptiView DAB IHC v5 (v1.00.0117)

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9	CC1 8 Min [Selected]
10	CC1 16 Min [Selected]
11	CC1 24 Min [Selected]
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13	Primary Antibody [Selected]
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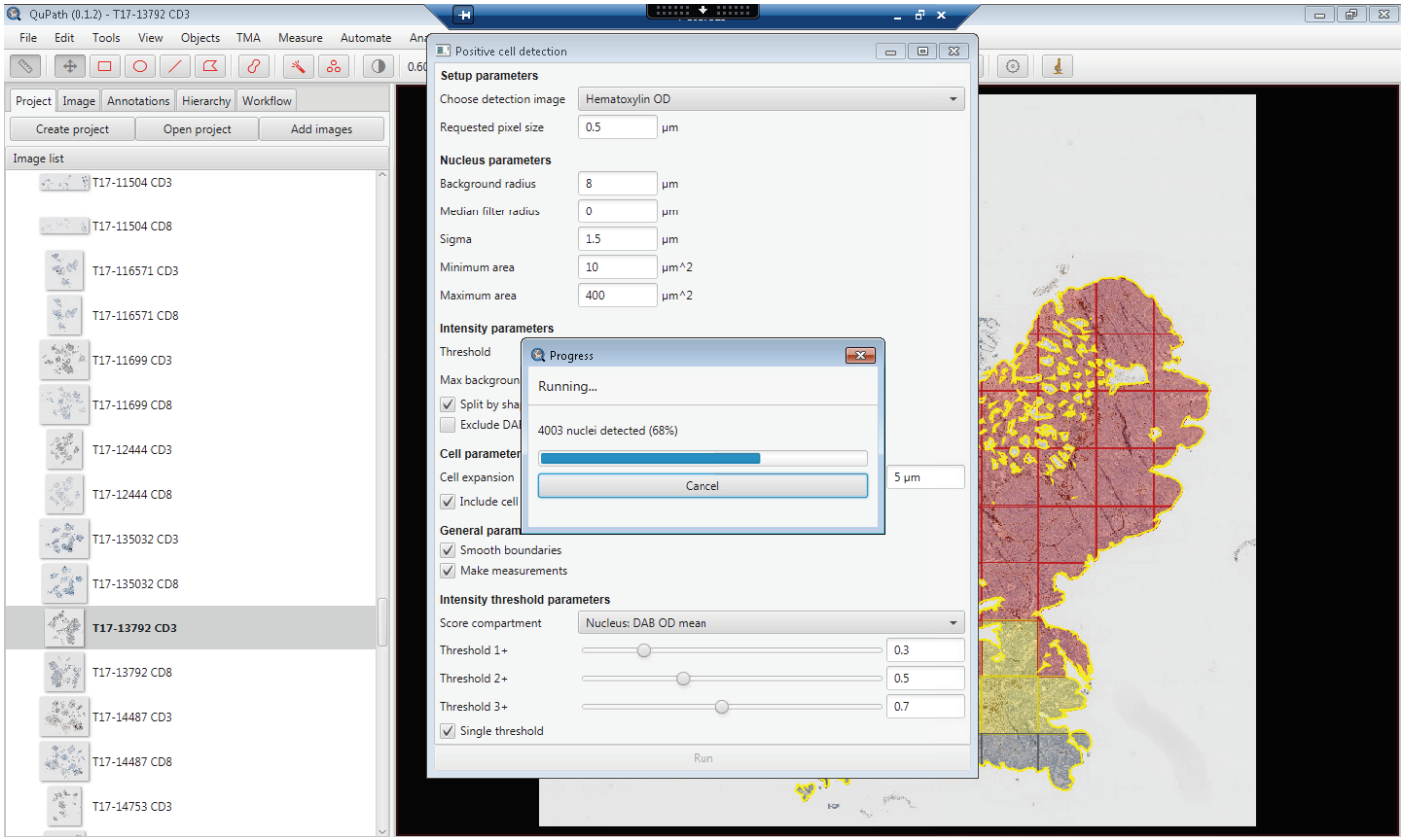
16	Apply One Drop of [HEMATOXYLIN II] (Counterstain), Apply Coverslip, and Incubate for [12 Minutes]
17	Post Counterstain [Selected]
18	Apply One Drop of [BLUING REAGENT] (Post Counterstain), Apply Coverslip, and Incubate for [4 Minutes]



Supplementary File S3. Positive cell detection in a CD3+ stained rectum biopsy in Qupath.

Supplementary Table 4. Overview of association between TILs in pre-treatment rectal cancer biopsies and response to neoadjuvant therapy, as described in previous literature.

Author Year	N	Stage	Treatment	pCR definition	TIL subsets
Teng [12] 2015	136 [§]	cT3-4 or cN+	40-45Gy RT with or without 5.4Gy boost in 25-28 fractions + single- or two-agent FU-based CT	Dworak TRG 4	CD3+; CD8+
McCoy [23] 2017	106	cT2-4, cN0-2, cM0-1	50.4 Gy RT in 28 fractions + FU-based CT	Dworak TRG 4	CD3+; CD8+; Foxp3+
Yasuda [16] 2011	48	cT2-4, cN0-2, cM0-1	50.4Gy RT + FU-based CT	JCCC grade 3	CD4+; CD8+
Matsutani [22] 2018	64 *	cT3-4 or cN+	50.4Gy RT in 28 fractions + FU-based CT with or without molecular-targeting drug	JCCC grade 3	CD4+; CD8+; T-bet+; GATA3+; RORγT+; Foxp3+
Xiao [24] 2017	92	cT3-4 or cN+	50Gy RT in 25 fractions + 1-4 cycles capecitabine + oxaliplatin	Mandard TRG 1	CD8+
Lim [21] 2014	52	cT3-4 or cN+, cM0	25Gy RT in 5 fractions or 45-50.5Gy RT in 28 fractions + FU-based CT	Mandard TRG 1	CD3+; CD4+; CD8+; CD56+CD57
Shinto [15] 2014	93	cT3-4	20Gy RT in 5 fractions + UFT 400mg/day	Dworak TRG 4	CD8+; Foxp3+
Huang [14] 2019	141	cT2-4, cN0-2	45-55Gy RT + FU-based CT	AJCC/UICC grade 0	CD4+; CD8+

§ 136 patients were included in the study, of which 63 received neoadjuvant; chemoradiation, 33 neoadjuvant chemotherapy and 40 neoadjuvant radiation; * 64 patients were included in the study, of which 31 received neoadjuvant chemoradiation and 33 neoadjuvant chemotherapy; # based on minimal p-value in pCR correlation analysis

Supplementary Table 4. Continued

Author Year	Counting method	Density definition	Cut-off value	Outcome
Teng [12] 2015	Visually	Percentage of tumor stroma containing TILs	Mean	High pre-treatment CD3+ and CD8+ density was associated with good response (TRG3-4).
				High CD3+ and CD8+ were associated with better 5-year DFS and OS.
McCoy [23] 2017	Automated (StrataQuest version 5)	cells/mm^2	Median	No association between TIL subsets and response to CRT.
				No difference in subsets between good responders (TRG 3-4) and poor responders (TRG 1-2).
				TILs were not associated with improved survival.
Yasuda [16] 2011	Visually, 3 different sections, 400-fold magnification	cells / field	NA	Higher numbers of CD4+ and CD8+ were associated with tumor regression. A higher number of CD8+ was associated with
				a complete response after CRT.
Matsutani [22] 2018	Visually, randomly selected field, 400-fold magnification	NS	Median	TILs showed no relationship with pCR or downstaging.
Xiao [24] 2017	Visually, 3 random fields within most strongly stained area	cells/mm^2	80/mm^2 #	No significant association between CD8+ density and pCR.
Lim [21] 2014	Visually	Cell count / TMA section	NA	No association between TIL subsets and pathological stage.
Shinto [15] 2014	Visually, counted in 3 different fields, 20-fold magnification	Count / field	Median	A high CD8+/Foxp3+ ratio was associated with favorable tumor regression.
				3 patients achieved pCR; they were categorized as CD8+/Foxp3 high (not tested).
Huang [14] 2019	Visually, in 3 fields with the greatest abundance of TILs, 400-fold magnification	Count / field	Maximum sensitivity and specificity for predicting TRG in ROC curve	CD4+ and CD8+TILs were distributed mainly in the stroma.
				CD8+ intra-epithelial TILs were significantly correlated with TRG 0-1 (P<0.01).
				No significant differences in the density of CD4+

Abbreviations: AJCC/UICC = American Joint Committee on Cancer and the Union for International Cancer Control, CRT = chemoradiation, CT = chemotherapy, DFS = disease free survival, FU = fluoropyrimidine, Gy = Gray, JCCC = Japanese Classification of Colorectal Carcinoma, NA = not applicable, NS = not specified, OS = overall survival, pCR = pathological complete response, ROC = receiver operating characteristic, RT = radiotherapy, TIL = tumor infiltrating lymphocyte, TMA = tissue microarray, TRG = tumor regression grade, UFT = tegafur/uracil.

CHAPTER 6

7 Tesla MRI for response assessment after neoadjuvant treatment for rectal cancer

Submitted

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ABSTRACT

Background

Organ-sparing approaches after neoadjuvant treatment for rectal cancer require reliable identification of a complete tumor response. Current standard measurements are insufficient to distinguish radiation-induced fibrosis from residual tumor. 7 Tesla (7T) MRI may improve response assessments given its higher signal-to-noise ratio (SNR). Also, the enhanced spectral resolution enables metabolic imaging techniques, such as chemical exchange saturation transfer (CEST) measurements. In this study, we aimed to develop a 7T MRI rectum protocol and investigate its added value compared to 3 Tesla (3T) MRI for tumor response assessment and residual tumor detection following neoadjuvant treatment in rectal cancer patients.

Methods

Nine rectal cancer patients and seven healthy volunteers were scanned on 7T MRI. Rectal cancer patients were also scanned on 3T MRI for diagnostic purposes according to treatment protocol. The 3T MRI protocol included T2 weighted (T2w), 3D T1 weighted (T1w) and diffusion weighted images (DWI). The 7T MRI protocol, included T2w, T1w DIXON and DWI. In addition, metabolic imaging in the form of CEST and proton magnetic resonance spectroscopic imaging (1H MRSI) were included. Two independent, dedicated GI-radiologists scored the quality of the scans on image quality, SNR, contrast-to-noise ratio (CNR), uniformity and chemical shift. Differences in scores between 3T and 7T MRI were assessed with Wilcoxon signed-rank test. For pathological confirmation, 7T MRI images of the resected rectum were compared to whole mount pathology images by eyeballing.

Results

Higher spatial resolutions were obtained at 7T, which led to more anatomic detail. Images acquired at 7T showed strong artifacts. At DWI, the 7T ADC maps did not show the complete anatomy due to B1+ inhomogeneity artifacts. The high spectral resolution of 7T MRI made amide- and amine-CEST measurements possible and showed elevated amide-CEST signals at the location of residual tumor. Image quality, CNR, uniformity, chemical shift and overall evaluation of region of interest were better at 3T MRI than 7T MRI.

Conclusion

High resolution 7 Tesla MRI for rectal cancer is feasible, but not superior to 3T for clinical use in rectal cancer patients, as strong field inhomogeneities strongly affect the image quality. However, when these inhomogeneities can be addressed, 7T MRI might improve the detection of complete responders after neoadjuvant treatment for rectal cancer, especially with the addition of metabolic imaging.

Introduction

Organ-sparing approaches are increasingly considered after neoadjuvant chemoradiation for rectal cancer. Several studies suggest that surgery can safely be omitted in patients with a clinical complete response (cCR) following neoadjuvant therapy [1,2]. cCR is defined as the absence of any residual scar, mass or ulcer at clinical and radiological assessment [3]. Organ preservation by a watch-and-wait approach or a local excision averts surgical morbidity while maintaining oncological safety and quality of life [4].

To justify the omission of surgery, accurate detection of cCR is essential. Currently 1.5 or 3 Tesla multimodal MRI followed by endoscopy is the preferred imaging strategy for the determination of residual tumor [5]. However, pre-operative identification of complete responders remains challenging. Radiation induced fibrosis is often misinterpreted as viable tumor, while undetected tumor residuals lead to incorrectly classifying patients as complete responders [6,7]. The negative and positive predictive values of T2 weighted (T2w) MRI at these magnetic field strengths vary from 35-92% and 23-94%, respectively [8]. Therefore, more accurate diagnostic tools are needed.

Ultra-high magnetic field strengths, such as 7 Tesla (7T), could improve the diagnostic accuracy in the detection of patients with a complete tumor response as it offers higher signal to noise ratio (SNR). This SNR increase can be traded for higher spatial or temporal resolutions and higher contrast-to-noise ratio (CNR). Moreover, the spectral resolution at this magnetic field strength is enhanced, which enables distinction of metabolites that overlap at lower field strengths [9]. Metabolic imaging with proton magnetic resonance spectroscopic imaging (1H MRSI) has the potential to differentiate between fibrosis and tumor tissue, as the fatty acid profiles differ between healthy, fibrotic and tumor tissue [10]. Due to the formation of connective tissue during fibrosis, a different metabolic profile can be expected. In addition, metabolites such as total choline, measured with 1H MRSI can be used as a biomarker to detect actively growing and aggressive tumors [11]. Furthermore, the enhanced spectral resolution available at 7T has an advantage for the measurement of chemical exchange saturation transfer (CEST) with better accuracy, when compared to lower magnetic field strengths. CEST quantifies chemical exchange processes between solute-bound protons and surrounding water (protons) molecules [12]. Amide-CEST has already proven to be useful in the differentiation of abnormal (e.g. tumors) from healthy tissue [13-15], making it a novel MR contrast mechanism that works without the use of an exogenous contrast

agent and can reach higher spatial resolutions than MR spectroscopy. This makes CEST an attractive technique for (small) residual tumor detection.

In this study, the feasibility of a multi-modal 7T MR protocol developed for in-vivo anatomical and metabolic imaging of rectal cancer was investigated. This protocol was developed in volunteers and tested in patients with rectal cancer after neoadjuvant therapy for the detection of residual tumor and healthy subjects. The images were compared to the standard clinical 3T MRI protocol.

Methods

Seven healthy volunteers and nine rectal cancer patients were included for in-vivo imaging. In addition, the resected rectum of five patients were scanned for ex-vivo purposes. All participants signed informed consent. The patient study was approved by the medical ethics committee of University Medical Center Utrecht.

7T MRI technique

Participants were scanned on a 7T whole body MR scanner (Philips, Cleveland, USA) in supine feet-first position. An 8-channel parallel-transmit system was used and interfaced to 8 fractionated dipole transceiver (TxRx) antennas [16], each equipped with 2 additional receive only (Rx) loop elements (24 Rx elements in total). Four of these antennas were positioned anteriorly and the other 4 posteriorly to the subject. Each Tx element was driven with a 2kW RF amplifier. The transmit part (8 TRx channels) of the coil setup used in the patient study was the same as for the volunteers. For the first 5 patients one of the 16 Rx loop elements was swapped by a Rx detunable endorectal monopole antenna for boosting the SNR [17], and investigate its contribution for this protocol. Volunteers were not scanned with the endorectal antenna.

7T MRI protocol

The 7T protocol included a proton density weighted (PDw) MRI that was first acquired for anatomy localization and planning of preparation phases. Preparation phases included a B1 map per channel which was obtained for B1 shimming purposes. An in-house developed MATLAB (The Mathworld, Inc) graphical user interphase was used to draw an ROI including the rectum to calculate the B1+ phase per element using a linear optimization algorithm, to obtain the maximum constructive RF interference in the ROI (i.e. rectum area). A B0 map was acquired

for Image based B0-shimming (IBS). IBS was performed in the complete anatomy for the imaging protocols and on a smaller ROI centered on the rectum for the metabolic protocols. After the preparation phases, high resolution transverse and oblique (perpendicular to the rectal axis) T2 weighted MRIs (MS TSE), T1 weighted multiple Dixon spoiled gradient echo (T1w mDixon SPGR) and diffusion weighted MRI (DWI) were acquired. DWI MRI was conducted with a MS TSE, with 3 to 5 b-values ranging from 0 to 1000s/mm^2 in three direction. The last part of the protocol included a DIXON-CEST protocol using 45 saturation offsets=-1500 to 1500Hz, 80 sinc pulses of 25ms and a saturation power of 1.3µT, 10ms spacing) and 1H-MRSI (2048 samples, 4 kHz read out bandwidth). Both metabolic protocols were performed on one slice with suspicion of residual tumor based on the available 3T MRI acquired prior to the neoadjuvant therapy.

3T MRI protocol

The clinical 3T MRI protocol includes a 3D T2wTSE MRI, 3D T1 weighted (T1w) MRI (spoiled gradient echo). Transverse DW SE single shot EPI (b-values=0, 150, 1000 s/mm^2, spectral pre-saturation with inversion recovery (SPIR) fat suppression).

DW, MRSI and CEST analysis

3T and 7T ADC maps were calculated with a mono-exponential fit using the scanner software. CEST datasets were analyzed in MATLAB using open source code (www.cest-sources.org). MRSI results were post-processed in MATLAB, using in-house built software where first all channels are combined using whitened singular value decomposition (WSVD). No zero-filling was performed, data was apodized with a Gaussian filter (15Hz) in the k-space domain and Fourier transformed to the spatial domain. Additional apodization (variable width Gaussian filter with depending on the case) was performed in the time domain prior to zero order phasing.

Comparison of MRI images and pathology

To confirm accurate imaging of anatomical structures on 7T MRI, ex vivo MRI images were compared to whole mount pathology. Following surgical resection of the rectum, the specimen was cleaned and inked on 4 sides for orientation. The ink was fixated with 5% acetic acid. The specimen was then placed for fixation in a specially designed MR-compatible tube, which was filled with formalin. The lumen of the specimen was extra filled with formalin to ensure optimal fixation and to prevent the formation of air bubbles in the tube. The rectum specimen was scanned at 7T MRI according to the aforementioned protocol. Images were processed and the tumor

was annotated in Radiant DICOM Viewer ©. Subsequently, the specimen went for further processing, after 48 hours of fixation in formalin. The rectum was dissected from top to bottom into 3 mm slices. The slices were numbered, photographed using a digital camera and the thickness of each slice was documented. Each slice was fully and intact paraffin-imbedded in a large container. Formalin-fixed paraffin-embedded (FFPE) large tissue blocks were then further processed according to standardized protocols of the pathology lab. The hematoxylin & eosin (H&E)-stained slides were digitalized using at 40x magnification using a digital scanner (Hamamatsu Nano Zoomer XR, © Hamamatsu Photonics K.K). The tumor regions were marked on the digital slides by a GI pathologist (GMR) using Automated Slide Analysis Platform (ASAP, Computation Pathology Group 2018 ©). Pathology reports were checked for tumor extent into different layers of the rectum specimen. These landmarks were localized in the HE-stained whole mount images as well as the MR images and were checked for correspondence by eyeballing.

3T and 7T quality assessment

All 3T and 7T MRI scans were anonymized and reviewed by two blinded, independent and dedicated GI-radiologists. The quality of the scans was subjectively scored on image quality, SNR, CNR, uniformity and chemical shift using a 3-point scale (**Table 2**). Differences in (averaged) scores between 3T and 7T MRI were assessed with Wilcoxon signed-rank test. Because of the small sample size and a consequential lack of power [18], no efforts to calculate Cohen's kappa to express the interrater reliability were made. Statistical analyses were performed using SPSS (Version 25, IBM ©).

Results

Three female and four male volunteers were scanned for protocol development purposes. Characteristics of included rectal cancer patients are shown in **Table 1**. Two out of nine included patients were female. Four patients underwent chemoradiation, three patients short-course radiotherapy and two patients did not receive neoadjuvant treatment.

7T MRI protocol

7T scanning was successful in all 8 volunteers and 8 out of 9 patients. Scanning with the endorectal monopole antenna at 7T (**Figure 1A**) showed an increase of the SNR as expected and as observed by the signal increase close to the antenna in the rectum. This signal enhancement was in most cases extreme as observed on the T2w MRIs (**Figure 1B-C**) resulting in local high intensities and signal voids that obscured the

Table 1. Baseline characteristics of included rectal cancer patients.

Gender	cTNM stage	Distance from anus [a]	Neoadjuvant treatment	Ex-vivo scan
Male	cT2N1 MRF- M0	10	None	No
Male	cT3N1 MRF+ M0	4	CRT	No
Male	cT4N2 MRF+ M1	15	SCRT + chemotherapy	No
Female	cT2N0 MRF- M0	9	None	No
Male	cT3N1 MRF+ M0	2	CRT	Yes
Male	cT2N1 MRF – M0	5	SCRT	Yes
Female	cT4N1 MRF+ M0	8	CRT	Yes
Male	cT3N1 MRF- M0	5	SCRT	Yes
Male	cT3N2 MRF + M0	1	CRT	Yes

CRT = chemoradiation, MRF = mesorectal fascia, NA = not applicable, SCRT = short-course radiotherapy; [a] Distance in cm, measured on sagittal 3T MRI; [b] MRI obtained before start of neoadjuvant treatment; [c] No 7T MRI obtained due to technical problems

Table 2. 3-point scale for quality scores of MR images.

Score	3	2	1
Image quality in region of interest	no artefacts	minor artefacts	major artefacts
SNR in region of interest	good SNR	minor noise	major noise
CNR in region of interest	good contrast	reasonable contrast	poor contrast
Uniformity in region of interest	good	fair	poor
Chemical shift	small	moderate	large
Overall evaluation of region of interest	good	fair	poor

CNR = contrast-to-noise ratio, SNR = signal-to-noise ratio

anatomy on T1w DIXON MRI (**Figure 1D**). Scanning without the endorectal antenna, improved image homogeneity and visibility of the rectal anatomy as seen from the T2w MRIs (**Figure 1E-F**) and the T1w DIXON water images (**Figure 1G-H**), which were obtained in volunteers. In all cases, (with and without the presence of the antenna), bands with signal voids were present elsewhere on the T2w MRI. Based on these results, scans were obtained without the endorectal antenna.

3T and 7T comparison

For 8 out of 9 patients clinical 3T MRI was available. **Figure 2** shows a comparison of T2w MRIs obtained at both field strengths in the same patient at approximately the

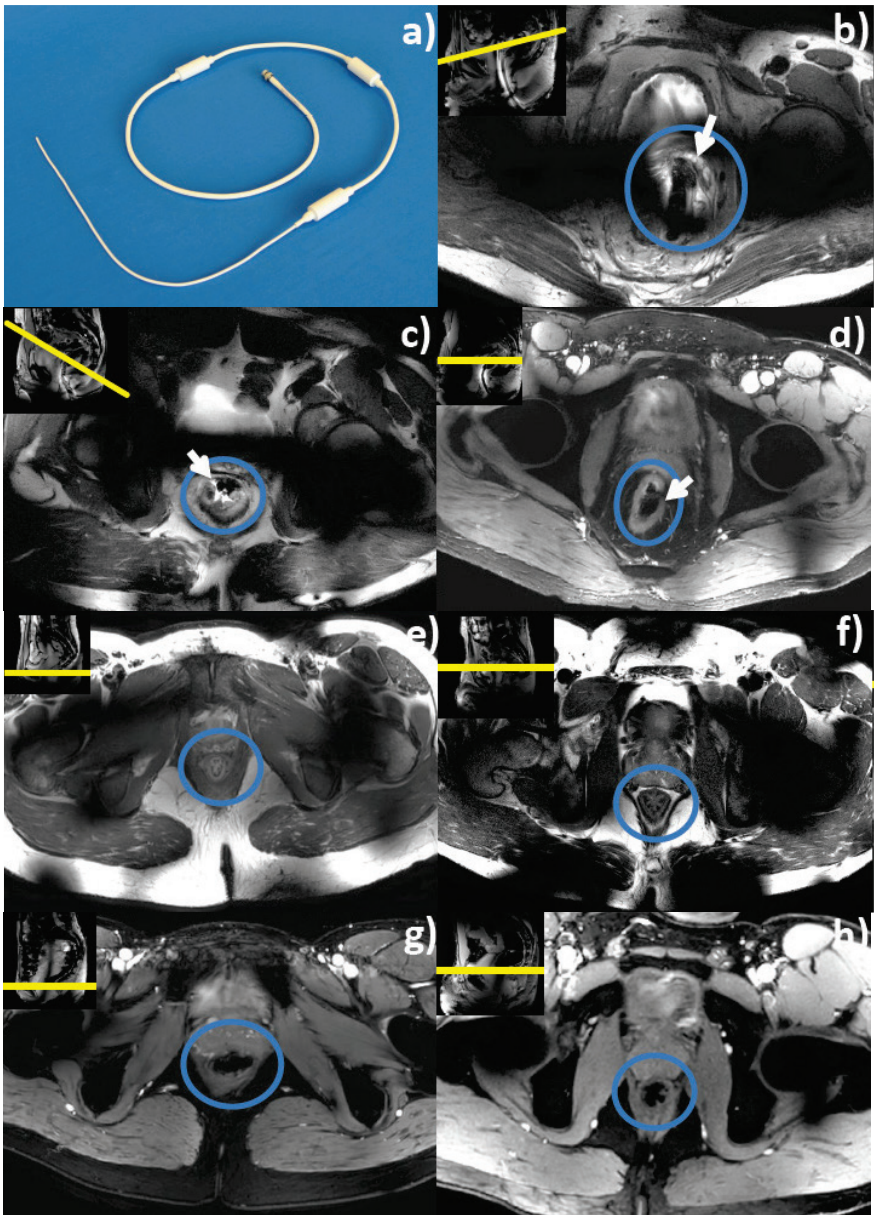

Figure 1. A. Endorectal antenna used for imaging the first 5 rectal cancer patients **B-C**. T2 weighted MRI of rectal cancer patients 6-9 weeks after chemoradiation, scanned with endorectal antenna. **D**. T1w DIXON MRI of rectal cancer patients 6-9 weeks after chemoradiation. The white arrows point to high intensities (**B**, **C**) on T2w MRIs or to a signal void (**D**) in a T1w DIXON water MRI that obscured anatomy. Images e-h show the equivalent T2w (**E**,**F**) and T1w DIXON MRI (**G**,**H**) acquired in different patients, without the use of the endorectal antenna.

same anatomic location. Higher spatial resolutions could be obtained at 7T, which led to higher anatomic detail (**Figure 2B**). However, the images acquired at 7T showed strong artifacts in the form of signal voids and field inhomogeneity (**Figure 2**).

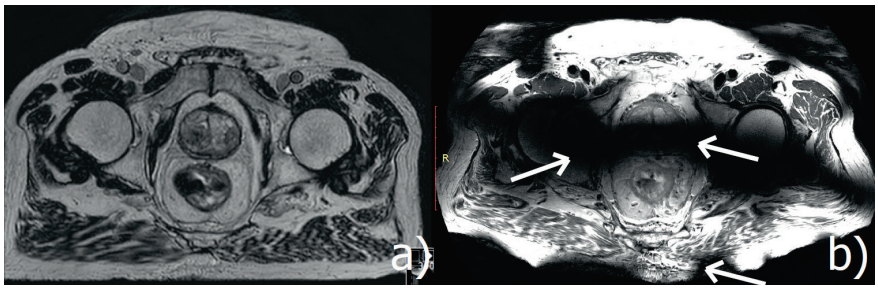

Figure 2. T2w MRI of a patient with rectal carcinoma 6 weeks after neoadjuvant chemoradiation acquired at 3T (a) and 7T (b). Tissue contrasts are different in T2w 3T or 7T MRI, due to intrinsic tissue characteristics at each field strength. Signal voids are present at 7T (white arrows) elsewhere.

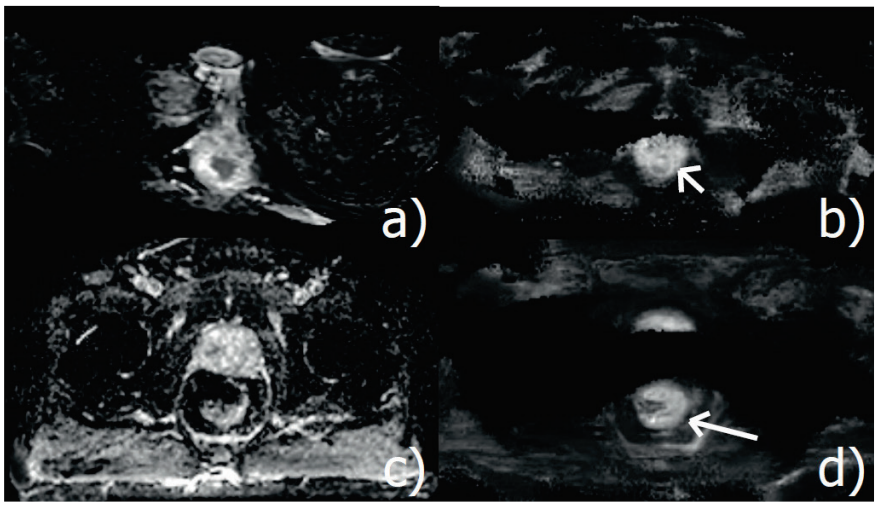

Figure 3. ADC maps obtained from DWI-MRI at 3T (a, c) and at 7T (b, d). Both patients had undergone neoadjuvant chemoradiotherapy prior to all MRIs. At 3T, better homogeneity is obtained, which is observed by the full FOV ADC map, whereas at 7T signal voids lead to missing anatomy ADC information. Despite the signal voids observed at 7T, anatomic detail in the rectum ADC is superior as can be observed by the distinction of the different rectal layers that have a different ADC.

Functional MRI in the form of DWI-MRI were also compared between field strengths. **Figure 3** shows ADC maps obtained for two different patients acquired at 3T and at 7T. **Figure 3** shows that 3T ADC maps have better diagnostic quality, given that the complete field of view ADC is available. The 7T ADC maps do not show the complete anatomy due to B1+ inhomogeneity artifacts. However, the 7T ADC maps can be obtained with a higher resolution, which is preferable for small lesion detection.

Figure 4 and **Figure 5** show examples of the acquired metabolic imaging at 7T. 1H-MRSI showed all peaks corresponding to a lipid spectral profile, which had 6 detectable fatty acid resonances (**Figure 4B**). **Figure 6A** shows an example of a successful CEST acquisition in the rectum of a patient with residual tumor. The high spectral resolution made it possible to fit individual amide- and amine-CEST effects to create voxel-wise maps (**Figure 5C-D**). Although artifacts and high signals are present elsewhere, the residual tumor location coincides with elevated amide-CEST signals (**Figure 5D**), where B1+ and B0 fields were optimized.

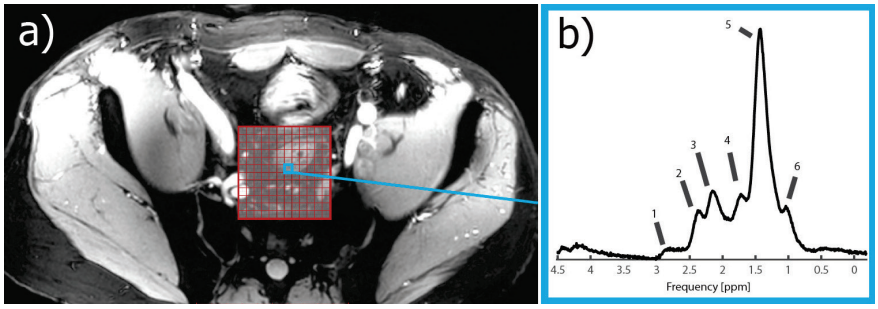

Figure 4. Example of 7T 1H-MRSI on a rectal cancer patient after neoadjuvant treatment. Figure a. shows the planning of the MRSI grid and figure b. shows a spectrum acquired with a STEAM sequence. The frequencies in b. correspond to a fatty acid spectral profile. No choline compounds are observed. 3T MRSI were not available.

Correlation of MRI images and pathology

7T MRI specimen images could be correlated with whole mount pathology in 2 patients (**Figure 6**). In the other 3 patients, MR images showed too much artifacts, mainly due to air pockets in the rectal lumen. Although 7T MRI resulted in high-detailed anatomical imaging, the tumor extent could be more accurately assessed with pathology images.

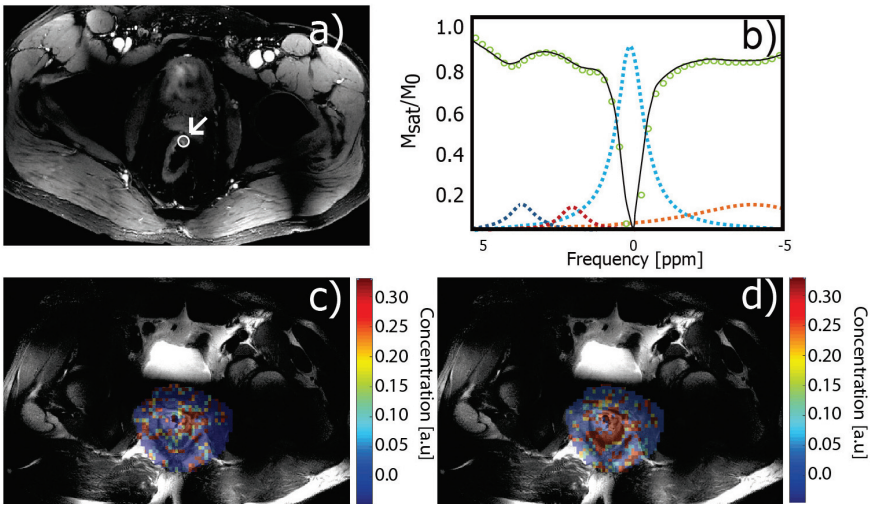

Figure 5. CEST acquisition in the rectum The increased spectral resolution available at 7 T makes it possible to differentiate between the amine-CEST (red fit in b) and the amide-CEST (blue fit in c) effects, enabling individual mapping of amine (c) and amide (d). 3T CEST were not available

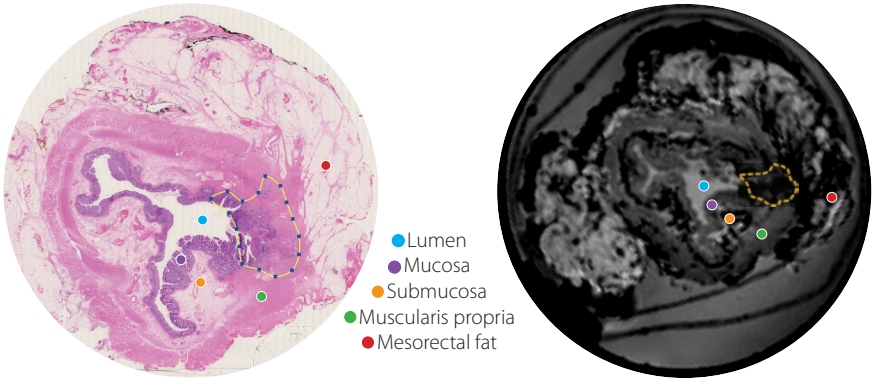

Figure 6. Example of correlation of 7T MR image and whole mount pathology in a patient that received neoadjuvant chemoradiotherapy. The microscopically assessed specimen showed tumor extent into the muscularis propria (yellow area).

Quality assessment

Outcomes of the quality assessment are shown in **Table 2**. Overall, the quality of 3T MRI was appreciated more than the quality of 7T MRI. According to the observers, the majority of 7T MRI scans had major artefacts, major noise, poor contrast and poor uniformity. Based on the Wilcoxon signed-rank test, 3T MRI scored statistically significant better on image quality, CNR, uniformity, chemical shift and overall evaluation of region of interest. (**Table 3**).

Table 3. Results from the quality assessment of 3T MRI and 7T MRI.

		3T MRI			7T MRI			p-value [a]
		Observer 1	Observer 2	Average	Observer 1	Observer 2	Average	
overall scores patient 1-8	Image quality in region of interest	2.0	1.9	1.9	1.1	1.0	1.1	0.02
	SNR in region of interest	2.4	2.0	2.2	1.9	1.3	1.6	0.34
	CNR in region of interest	2.4	2.6	2.5	1.3	1.0	1.1	0.02
	Uniformity in region of interest	2.7	2.3	2.5	1.0	1.0	1.0	0.02
	Chemical shift	2.7	2.6	2.6	1.0	1.0	1.0	0.02
	Overall evaluation of region of interest	2.3	2.3	2.3	1.0	1.0	1.0	0.02

3T = 3 Tesla, 7T = 7 Tesla, CNR = contrast-to-noise ratio, MRI = magnetic resonance imaging, SNR = signal-to-noise ratio; Based on Wilcoxon Signed Ranks Test.

Discussion

We were able to develop an adequate 7 Tesla MRI protocol for patients with rectal cancer that included clinically relevant T2w, T1w and DWI protocols. Furthermore, we showed the feasibility of performing metabolic imaging with 1H MRSI and CEST in the rectum. In addition, we showed that scanning without an endorectal coil is preferred to avoid local high intensities and signal voids that obscure rectal anatomy. At 7T the spatial resolution can be superior to the resolution obtained at 3T. However, 7T images suffer from B1+ field inhomogeneity resulting in signal voids, even after applying homogeneity corrections.

Previous studies have shown the limited accuracy of current MRI modalities in diagnosis of clinical complete response [7,8,19]. This can be explained by radiotherapy-induced changes, such as edema, fibrosis and necrosis, which are hard to differentiate from residual tumor. Furthermore, tumor characteristics, such as distance from the anal verge, diameter, and anterior tumor location can affect accuracy [7,8]. By using higher magnetic field strengths, such as 7T, we could benefit from the increased SNR, spatial and spectral resolution. The increased SNR made it possible to develop a rectal protocol without the need of an endorectal coil. The increased spatial resolution can be exploited to find small tumor residues. However, better transmit field (B1+) homogeneity is needed to make the rectal 7T protocol a step closer to clinical quality. In this study we have shown that even in the rectum, which is in close proximity to air pockets which causes large B0 inhomogeneities that lead to artifacts, 1H MRSI can be acquired with sufficient linewidth at 7T to distinguish individual resonances of the lipids. This can be useful to differentiate areas of fibrotic tissue from residual tumor tissue [20]. MRSI acquisitions at 3T are clinically not acquired. At 3T, the spectral resolution is lower than at 7T, which makes the separation of metabolites that resonate close to each other challenging. In addition, the need of a spectroscopy expert to process the images can be a hurdle when acquiring MRSI. Nevertheless, it remains challenging to acquire robust MRSI, even at 7T, due to the presence of large field inhomogeneities.

CEST imaging is a relatively new, promising metabolic imaging method. The amide proton transfer (APT) effect is particularly helpful in cancer imaging. Herewith, characteristics of the tissue microenvironment, such as temperature, pH, and metabolite concentration, can be assessed [13,21]. Amide-CEST has already proven to be useful in the differentiation of diseased tissue from healthy tissue at ultra-high magnetic field strengths [15,22]. In previous studies, CEST-MRI revealed metabolic differences in breast epithelial and breast cancer cell lines [13]. At 3T, studies claim that tissues can be differentiated between healthy tissue and tumor by looking at

the asymmetry profile [23]. In rectal cancer, heterogeneous intra-tumor response to neoadjuvant treatment results in unequally distributed residual tumor cells in different layers of the bowel wall [6]. Current MRI techniques cannot detect these small islands of tumor cells [19]. A mice breast cancer model showed that CEST-MRI can detect these local differences. Only the metabolically active part of the tumor showed a greater CEST effect [24]. Although preliminary, in this study we have also observed elevated amide-CEST signals that coincide with the location of residual tumor, from the 7T acquisitions.

Scanning patients at 7T may have advantages, but is associated with several limitations. Dedicated antennas are needed for body imaging, which are not widely available. The shortening of the wavelength at this field strength causes destructive interference, leading to heterogeneous signal voids. Therefore, B1+ shimming is crucial to have optimal image quality in the ROI, and shift the signal voids outside the ROI. This complicates assessment of possible metastatic lymph nodes. Techniques such as time interleaved acquisition of modes (TIAMO) [25], that adds two subsequently acquired images with different transmit phase settings can homogenize the B1+ field, to the cost of longer scan time. Special kt-points or tailored RF pulses are optimized for each case, compensating for each particular inhomogeneity [26,27]. These RF pulses however require additional software, computation time and an additional interface with the scanner software to import each new pulse shape. In addition to the field inhomogeneities, the intrinsic relaxation parameters of tissues are field dependent. This poses challenges to the radiologists when assessing 7T MRI as the contrast between tissues is different from 3T MRI. Furthermore, to analyze metabolic imaging from 1H MRSI and CEST, offline software needs to be used typically by expert MR physicists. Therefore, post-processing needs to be automatically implemented to have metabolic data readily available for the radiologist and oncologist.

Successful imaging of rectal cancer with ultra-high field MRI has been described before [28,29], but this is the first study to present an ultra-high field in-vivo MRI rectal cancer protocol in a clinical setting. Furthermore, comparison of a 3T and 7T protocol has not been described before. Although T1w, T2w and DWI MRI are possible at 7T, it is still not superior to 3T for clinical use in rectal cancer patients, as strong field inhomogeneities seriously affect the image quality. When these field inhomogeneities can be addressed, this protocol could have potential for the accurate discrimination of complete responders from non-responders after neoadjuvant treatment for rectal cancer, especially with the addition of metabolic imaging.

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PART III

Patients' perspective

CHAPTER 7

Impact of dose-escalated chemoradiotherapy on patient reported outcomes and disease free survival in patients with locally advanced rectal cancer: 2-year follow-up of the randomized RECTAL BOOST trial

Submitted

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ABSTRACT

Background

Dose–escalated chemoradiation (CRT) for locally advanced rectal cancer (LARC) aims to increase patients' eligibility for rectum-preserving approaches. This study compared patient reported outcomes (PROs), local recurrence (LR) and disease free survival (DFS) between patients that received dose-escalated CRT (boost group) or standard CRT (control group) within the randomized RECTAL BOOST trial (Clinicaltrials.gov NCT01951521).

Methods

Patients with LARC (n=128), participating in the BOOST trial, were included. Patients filled out EORTC QLQ-C30 and CR29 questionnaires at baseline, and at 3, 6, 12, 18 and 24 months following treatment. A linear mixed-effect model was applied to compare differences in functional domains. Symptoms were compared using Chi-square test or Fisher's exact test. DFS was estimated with Kaplan-Meier method and compared with log-rank test. Per protocol analysis was applied for 1PRO comparison; differences in LR and DFS were compared according to intention to treat analysis.

Results

At 3 and 6 months following start of treatment, patients treated with dose-escalation (n=51) experienced a significantly larger deterioration in global health and physical-, role- and social functioning than those treated with standard CRT (n=64). Patients in the boost group reported significantly more pain in the first 6 months following start of treatment. Symptoms of fatigue, sexual functioning and urinary complaints were similar. There were no differences in LR and DFS.

Conclusion

Dose-escalated radiotherapy is associated with more pain, and deterioration in most of the functioning domains up to 6 months following start of treatment. At two years follow up, there was no difference in quality of life or DFS between groups.

Introduction

Locally advanced rectal cancer (LARC) is treated with chemoradiation (CRT) followed by total mesorectal excision (TME) [1,2]. Neoadjuvant CRT, which entails radiotherapy of 45-50 Gray (Gy) in 25-28 fractions with concurrent fluoropyrimidine-based chemotherapy, is administered in order to facilitate surgery with a clear resection margin and improves local recurrence rates [2,3]. However, this multimodality approach is associated with side-effects, including bowel dysfunction, urinary incontinence, sexual complaints and stoma-related problems, and impaired long-term quality of life (QoL) [4-8]. As such, rectum-sparing treatments, including local excision and active surveillance, referred to as a watch-and-wait (WW) strategy, have gained interest in the past decade and might be feasible when neoadjuvant treatment results in a clinical complete response (cCR) [9]. Compared to surgery, WW was associated with better QoL in observational studies [10].

The randomized RECTAL BOOST trial was conducted to investigate whether an additional 15 Gy radiotherapy boost prior to CRT could improve pathological complete response (pCR) or sustained cCR compared with standard CRT [11,12]. Although this study showed no difference in the primary endpoint, dose-escalated radiotherapy did result in significantly more (near-)complete response (Mandard tumor regression grade 1-2) and sphincter preservation [12,13]. This suggests that there might be room for the use of dose-escalated radiotherapy to enable rectum-sparing treatment in selected patients.

After an intensified treatment regimen like dose-escalated radiotherapy, some patients might experience additional toxicity without the benefit of organ-preservation. Probability of complete response after different neoadjuvant strategies therefore need to be weighed against impact on QoL and oncologic outcomes. This study compares patient reported outcomes, 2-year local recurrence rates and disease free survival of locally advanced rectal cancer patients after dose-escalated versus standard CRT within the RECTAL BOOST trial.

Methods

The design of the RECTAL BOOST trial (Clinicaltrials.gov NCT01951521) has been described in detail before [11]. In short, the RECTAL BOOST trial was a non-blinded, phase II randomized controlled trial performed within a prospective cohort of colorectal cancer patients (Dutch Prospective Colorectal Cancer cohort, PLCRC) [14], according to the Trials within Cohorts design [15]. The RECTAL-BOOST trial was performed in the

UMC Utrecht and the MAASTRO clinic/MUMC+, the Netherlands. The Institutional Review Board of the UMC Utrecht and of the MAASTRO Clinic approved PLCRC and the RECTAL BOOST trial. Cohort participants with locally advanced tumors within 10 cm from the anorectal junction and WHO performance status 0-2, who consented to filling out patient reported outcome measures (PROMs) and broad consent for randomization to future intervention studies, were eligible. Exclusion criteria were presence of inflammatory bowel disease, prior pelvic radiotherapy, contra-indication for MRI or capecitabine, pregnancy within the last year and inadequate command of the Dutch language. Treatment details are available from prior publications [11,12]. Patients were allocated to either standard treatment, i.e. chemoradiation (CRT) that involved 50Gy in 25 fractions of 2Gy with concurrent capecitabine 825mg/m^2 twice daily for 5 or 7 days per week (control group), or dose-escalated CRT (boost-group) including a stereotactic radiation boost to the tumor of 15Gy in 5 fractions of 3Gy without concurrent chemotherapy in the week prior to the start of CRT.

Data acquisition

Baseline patient, disease and treatment characteristics, as well as clinical outcomes, including pathological and postoperative complications, were collected within PLCRC. QoL questionnaires were provided online or on paper and collected within the Patient Reported Outcomes Following Initial treatment and Long-term Evaluation of Survivorship (PROFILES) registry [16]. QoL was assessed before start of neoadjuvant therapy (baseline) and at 3, 6, 12, 18, and 24 months after the start of neoadjuvant therapy. Patients completed the EORTC core (EORTC QLQ-C30) [17] and colorectal cancer specific questionnaire (EORTC QLQ-CR29) [18]. EORTC QLQ-C30 includes 5 functional domains (physical, role, emotional, cognitive, and social functioning), a global health score, and 9 cancer-related symptoms [17]. The EORTC QLQ-CR29 contains colorectal cancer-specific domains and symptoms [18]. Information on disease recurrence and vital status was obtained from the electronic patient records up to July 2020.

Statistical analysis

Baseline patient, tumor and treatment characteristics and clinical outcomes were described using descriptive statistics. The QoL questionnaires were processed according to their manuals [18,19]. QoL outcomes were linearly transformed into scores between 0 and 100. A high score on global health or functional domains represents a high level of functioning or a high QoL, while a high score on symptom

scales represents a high level of complaints [19]. The QoL scores were derived from multilevel Likert scale answer options that were categorized as "no" (score 0), "mild" (score 1-49), "moderate" (score 50-99), and "severe" (score 100) complaints for symptoms and "not at all" (score 0), "a little" (score 1-49), "quite a bit" (score 50-99), and "very much" (score 100) for sexual interest. Differences in moderate/severe EORTC C30 and CR29 symptoms between groups at different time points were analyzed using Chi-square test or Fisher's exact test when prevalence was lower than 5. As most symptoms occurred within 1 year following neoadjuvant therapy, we only tested the differences at 3, 6 and 12 months.

A linear mixed-effects model was applied with a random intercept, time (as factor), the interaction between time and treatment and an autoregressive covariance structure of the first order (assuming that the correlation systematically decreases with increasing distance between time points [20]). The outcomes were presented as mean differences (MDs) between the treatment groups at each time point with 95% confidence intervals (CIs). Clinically relevant changes in QoL were determined based on the standardized effect size (ES), calculated as the MD divided by the pooled standard deviation of the baseline score. ES was categorized into "no change" (ES < 0.2), "small change" (ES, 0.2-0.4), "moderate change" (ES, 0.5-0.7), and "considerable change" (ES ≥ 0.8) [21].

All time-to-event data were calculated from start of radiotherapy until occurrence of the event, death, or July 2020, whichever came first. Crude incidence rates of events were calculated over the total follow up period. Two year survival and disease recurrence rates were estimated with the Kaplan-Meier method. Local recurrence rate was calculated excluding patients who had an irradical resection (n=4). The distant metastasis rate was calculated excluding patients who had metastatic disease at diagnosis (n=5). A new primary tumor (i.e. other than rectal origin) was not considered an event for DFS. Survival probabilities were compared using log rank test.

The impact of dose-escalated radiotherapy on patient reported outcomes was evaluated in the per protocol population, i.e. quality of life scores and symptoms were compared among the 64 patients who received standard CRT and the 51 patients who received dose escalation. Reason behind this approach is that dose-escalation was expected to unfavorably impact quality of life scores and symptoms. By including patients who did not undergo the boost intervention in the intervention arm, the real effect estimate would be diluted. These patients were therefore excluded from the PRO analysis.

The impact of dose-escalated radiotherapy on patient reported outcomes were analyzed in the per protocol population, because unfavorable impact of dose-escalation on QoL was expected. Intention-to-treat analyses (ITT) would dilute the real effect estimate probably underestimate the impact of dose-escalation on PROs. Survival data were analyzed as ITT. The level of significance was set at $p < 0.05$. Analyses were performed using SPSS Statistics version 25 (IBM ©) and RStudio Version 1.1.442 (© 2009-2018 RStudio, Inc.).

Results

Between September 2014 and July 2018, 128 patients were included in the RECTAL BOOST trial. Sixty-four patients were randomized to the control group, and all underwent standard CRT. A total of 51 of 64 (80%) patients who were randomized to the intervention group accepted and received the boost intervention. Thirteen patients refused the intervention and underwent standard CRT (**Supplementary Figure 1**). Patient, tumor and treatment characteristics of the per protocol analyzed patients are presented in **Table 1**. Gender was well balanced between groups and the majority of patients were male the boost group as well as the control group (75% vs. 74%). Median age was 64 and 62 years in boost vs. control group, respectively. Most tumors were located within 3.0 cm of the anus. The boost group included less cT4 tumors compared to the control group (18% vs. 31%). Patients in the boost group more often underwent low anterior resection (LAR) than patients in the control group (41% vs. 33%), and less often received a permanent colostomy (35% vs. 56%).

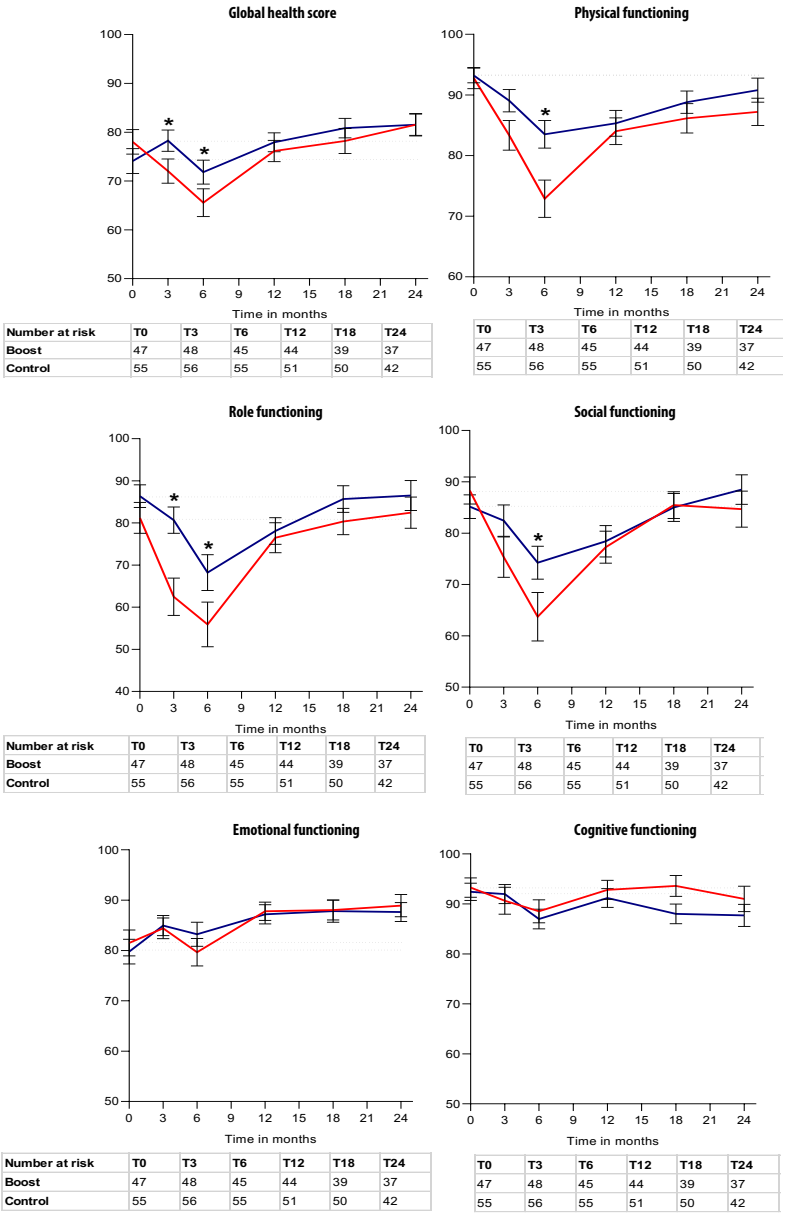
Patient-reported quality of life

Response rates for the QLQ-C30 and QLQ-CR29 questionnaires were 86% vs. 92% at baseline and 66% vs. 73% at 24 months for the control group and boost group, respectively (**Supplementary Table 1**). There was a larger decline in global health score, physical functioning, role functioning and social functioning in the boost group as compared to the control group in the first year after start of neoadjuvant treatment (**Figure 1**). Based on a linear mixed-effect model, this difference was statistically significant for global health and role functioning at 3 months (ES -0.4 and -0.7, respectively) and 6 months (ES -0.4 and -0.5, respectively), and for physical and social functioning at 6 months (ES -0.7 and -0.5, respectively, **Table 2**). All functioning scores, except for physical and cognitive functioning, returned to baseline level (or above) within 2 years following treatment.

Table 1. Patient, tumor and treatment of the per protocol study population. Data presented in number (%) unless stated otherwise

	Boost group N = 51	Control group N = 64
Age, median years (IQR)	64 [54 – 69]	62 [56 – 71]
Sex		
Male	38 (74.5)	47 (73.4)
Female	13 (25.5)	17 (26.6)
Comorbidities		
None	24 (47.1)	27 (42.2)
1 or more	27 (52.9)	37 (57.8)
Tumor height [a]		
≤3.0cm	27 (52.9)	36 (57.1)
3.1-5.0 cm	8 (15.7)	8 (12.7)
5.1-10.0cm	16 (31.4)	19 (30.2)
Clinical tumor stage		
cT2	2 (3.9)	5 (7.8)
cT3	40 (78.4)	39 (60.9)
cT4	9 (17.6)	20 (31.2)
Distance to the mesorectal fascia		
≤1 mm	33 (64.6)	46 (71.9)
>1 mm	18 (35.4)	18 (28.1)
Clinical nodal stage		
cN0	5 (9.8)	9 (14.1)
cN1	12 (23.5)	17 (26.6)
cN2	34 (66.7)	38 (59.4)
Clinical metastatic stage		
cM0	47 (92.2)	62 (96.9)
cM1	3 (5.9)	2 (3.1)
Mean dose to the tumor (Gy, SD) [b]	61.09 (5.82)	49.04 (0.37)
Treatment after chemoradiation		
Low anterior resection	21 (41.2)	21 (32.8)
Abdominoperineal resection	17 (33.3)	32 (50)
Local excision	1 (2)	0
Watch-and-wait	10 (19.6)	9 (14.1)
Palliative treatment	2 (3.9)	2 (3.1)
Ostomy		
Diverting ileostomy	17 (33.3)	14 (21.9)
Diverting colostomy	3 (5.9)	5 (7.8)
Permanent colostomy	18 (35.3)	36 (56.3)
Postoperative complications	20 (48.8)	20 (35.1)

[a]measured from the anorectal junction on sagittal MRI. [b]Mean dose (D95) to the planned target volume of the tumor (PTV tumor).



Number at risk	T0	T3	T6	T12	T18	T24
Boost	47	48	45	44	39	37
Control	55	56	55	51	50	42

Figure 1. QLQ-C30 outcomes for different functional quality of life domains, measured at baseline, and at 3, 6, 12, 18 and 24 months following start of radiotherapy, stratified by control group (blue) and boost group (red). Scores are presented as means with its standard error (SE). A higher score indicates better global health, better functioning or a higher level of symptoms. Baseline levels are indicated with a dotted line. Significant differences, based on a linear mixed effect model with the control group as reference, are marked with an asterisk (*).

Table 2. Mean differences (MD) in different QLQ C30 QoL domains between the boost and the control group (ref.) over time. Clinical relevance of the MD is expressed as the standardized effect size (ES), calculated as the MD divided by the pooled standard deviation of the mean QoL score per domain at baseline. Values marked with an asterisk (*) are significantly different from baseline, based on a linear mixed-effect model ($p < 0.05$).

		Baseline	3 months			6 months			12 months			18 months			24 months		
		Mean (SD)	MD	95% C.I	ES	MD	95% C.I	ES	MD	95% C.I	ES	MD	95% C.I	ES	MD	95% C.I	ES
Global health score	Control	74.2 (17.6)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	77.9 (17.0)	-6.7 *	-13.1 ; -0.4	-0.4	-6.8 *	-13.2 ; -0.3	-0.4	-2.5	-9.1 ; 4.0	-0.1	-3.6	-10.3 ; 3.2	-0.2	0.5	-6.5 ; 7.5	0.0
Physical functioning	Control	93.5 (15.6)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	92.1 (15.1)	-5.5	-11.1 ; 0.2	-0.4	-11.2 *	-16.9 ; -5.5	-0.7	-2.2	-8.0 ; 3.6	-0.1	-3.0	-9.0 ; 3.0	-0.2	-3.0	-9.2 ; 3.3	-0.2
Role functioning	Control	86.6 (27.3)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	80.2 (26.4)	-19.0 *	-28.8 ; -9.2	-0.7	-13.5 *	-23.5 ; -3.4	-0.5	-2.8	-7.4 ; 13.0	-0.1	-7.8	-2.8 ; 18.3	-0.3	-3.8	-7.2 ; 14.9	-0.1
Social functioning	Control	85.9 (23.6)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	87.1 (22.9)	-8.1	-16.6 ; 0.4	-0.4	-11.2 *	-19.8 ; -2.6	-0.5	-3.1	-11.9 ; 5.7	-0.1	-0.5	-9.5 ; 8.6	0.0	-2.6	-12.1 ; 6.9	-0.1
Emotional functioning	Control	79.8 (17.0)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	81.5 (16.4)	-0.5	-6.7 ; 5.6	0.0	-3.6	-9.8 ; 2.7	-0.2	0.6	-5.7 ; 6.9	0.0	0.2	-6.3 ; 6.7	0.0	1.3	-5.4 ; 8.0	0.1
Cognitive functioning	Control	92.2 (15.8)	Ref.			Ref.			Ref.			Ref.			Ref.		
	Boost	92.3 (15.3)	-2.2	-7.8 ; 3.5	-0.1	1.2	-4.6 ; 6.9	0.1	1.0	-4.9 ; 6.9	0.1	4.2	-1.8 ; 10.2	0.3	4.2	-2.1 ; 10.4	0.3

Moderate to severe pain as measured by EORTC-C30 was reported significantly more often in the boost group than in the control group at 3 and 6 months (31% vs. 9% and 42 vs. 16%, **Table 3**). In the male population, there were no differences in (a lack of) sexual interest. The occurrence of erectile dysfunction increased over time to a similar extent in both groups. In the female population, there was no to little sexual interest throughout the entire follow-up period.

Table 3. Comparison of severe/moderate symptoms as reported in the QLQ-C30 and QLQ-CR29 questionnaires, measured at baseline and at 3, 6 and12months.

		Control group N (%)	Boost group N (%)	p-value
Fatigue	Baseline	6 / 55 (11)	4 / 47 (9)	0.75
	3 months	8 / 56 (14)	12 / 48 (25)	0.17
	6 months	11 / 55 (20)	13 / 45 (29)	0.30
	12 months	8 / 51 (16)	4 / 44 (9)	0.37
Pain	Baseline	7 / 55 (13)	5 / 47 (11)	0.99
	3 months	5 / 56 (9)	15 / 48 (31)	0.01
	6 months	9 / 55 (16)	19 / 45 (42)	0.01
	12 months	5 / 51 (10)	4 / 44 (9)	1.00
Sexual interest men *	Baseline	26 / 35 (74)	28 / 34 (82)	0.42
	3 months	30 / 39 (77)	30 / 34 (88)	0.21
	6 months	33 / 37 (89)	31 / 33 (94)	0.48
	12 months	28 / 36 (78)	23 / 30 (77)	0.92
Erectile dysfunction	Baseline	6 / 26 (24)	4 / 22 (18)	0.74
	3 months	4 / 27 (15)	4 / 17 (24)	0.69
	6 months	7 / 22 (32)	4 / 17 (24)	0.73
	12 months	10 / 21 (48)	5 / 18 (28)	0.20
Sexual interest women *	Baseline	12 / 13 (92)	8 / 10 (80)	0.39
	3 months	12 / 12 (100)	8 / 9 (89)	0.24
	6 months	12 / 12 (100)	6 / 7 (86)	0.18
	12 months	11 / 11 (100)	9 / 9 (100)	-
Urinary frequency	Baseline	17 / 55 (31)	10 / 47 (21)	0.27
	3 months	13 / 56 (23)	17 / 48 (35)	0.17
	6 months	16 / 54 (30)	11 / 45 (24)	0.56
	12 months	11 / 51 (22)	11 / 43 (26)	0.65
Urinary incontinence	Baseline	1 / 55 (2)	0 / 47	1.00
	3 months	1 / 56 (2)	0 / 47	1.00
	6 months	3 / 54 (6)	0 / 45	0.25
	12 months	5 / 51 (10)	0 / 43	0.06
Blood or mucus in stool	Baseline	12 / 55 (22)	14 / 47 (30)	0.36
	3 months	2 / 56 (4)	5 / 48 (10)	0.24
	6 months	0 / 55	2 / 45 (4)	0.20
	12 months	0 / 51	6 / 43 (14)	0.01

* Outcomes for "not at all" and "a little" sexual interest

However, sample sizes were too small to draw any conclusions. In both groups, there a decrease in blood or mucus in the stool was reported, but remained present in the boost group. This difference was significant at 12 months (**Table 3**). There were no differences between groups in terms of urinary frequency or -incontinence.

Local recurrence and disease-free survival

Median follow up time was 33 months (IQR 23-42) in both groups. During this period, 4 patients died in both arms. Two patients in the boost group and 5 in the control group developed local recurrence (3% vs. 8%, p=0.2), and 11 vs. 12 patients developed distant metastasis (18% vs. 19% respectively, p=0.9, **Table 4**). Two out of 12 patients in the boost group and 2 out of 9 in the control group that entered a WW-strategy developed a local regrowth (**Table 4**).

Table 4. Incidence of mortality, local and distant disease recurrence and new primary tumors after dose-escalated chemoradiation (boost group) versus standard chemoradiation (control group) during total follow up time.

Number of events (%) or median time in months (IQR)	Boost group (n=64)	Control group (n=64)
Total follow up time	32 (22-41)	33 (23-42)
Deceased	4 (6.3)	4 (6.3)
Local recurrence	2 (3.2)	5 (8.1)
Distant metastasis	11 (18.0)	12 (19.4)
New primary tumor	2 (4.0)	2 (4.0)
	WW after Boost (n=13)	**WW control (n=9)**
Sustained cCR	9 (69.2)	5 (55.5)
Sustained cCR time (months,IQR)	39 (37-45)	39 (26-51)
Local regrowth	4 (30.8)	4 (44.4)
TEM	2	0
LAR	1	2
APR	1	2

APR: abomdinoperineal resection. cCR: clinical complete response. IQR: interquartile range. LAR: low anterior resection. TEM: transanal endoscopic microsurgery. WW: watch and wait strategy.

Discussion

Dose-escalated CRT was associated with a significant, moderate decrease in most functional QoL domains and more pain at three and six months following start of treatment. Two years after start of treatment, no differences in PROs or symptoms were observed between dose-escalated and standard CRT. Dose-escalated radiotherapy did not result in better 2-year disease free survival.

The RECTAL BOOST trial randomized LARC patients between dose-escalated CRT and standard CRT. The original intention-to-treat analysis evaluated the QLQ-C30 summary score in the first year and found a significantly lower score in the boost group at 3 months after randomization (MD with the control group -7.5, 95%CI 3.0-12.1; p=0.001). The summary score was comparable between the groups at baseline, 6 and 12 months. The present study further investigated the different functional domains and found a transient, significant deterioration in the global health score and physical-, role- and social functioning (with moderate effect sizes), as well as more pain 3 and 6 months following dose-escalated therapy compared to standard CRT. A previous study on QoL after rectal cancer treatment also described deterioration in most C30 and CR29 domains within 6 months following start of neoadjuvant treatment, with worse deterioration after (long course) CRT versus short course radiotherapy [22]. For lung and prostate cancer, radiotherapy dose-escalation was similarly negatively associated with QoL domains during the first half year after treatment, with restoration there onwards [23,24]. The relative decrease in QoL at 3 and 6 months after dose-escalation might partly be explained by the bigger rate of postoperative complications in the boost group. At 3 months after start of treatment, patients were still in their recovery time between CRT and surgery.

In line with our results, a randomized trial for dose-escalated radiotherapy versus multidrug CRT in intermediate risk rectal cancer reported comparable DFS (5-year DFS of 75% versus 74%, respectively) [25]. We also did not see improvement in DFS after dose-escalated CRT among LARC patients (2-year DFS of 77% in the boost vs. 80% in the control group). The original study purpose of the RECTAL BOOST trial was to increase pCR rate, as a surrogate marker for oncological outcome [11]. pCR is not only associated with lower local recurrence rates, but also with less distant metastasis rates [26]. Although no difference in pCR was found, the current 2-year follow-up period might be too short to show how the observed differences in tumor regression translate into survival variances.

This study investigated the effect of dose-escalated chemoradiation for LARC on quality of life, symptoms and oncological outcome in a randomized setting. Randomized trials might be susceptible to different types of bias. Low intervention acceptance rates can indicate selection bias, for instance when patients with worse health status are less likely to accept an intervention with possible health risks (self-selection bias) [27]. The acceptance rate of the intervention was 80% in the RECTAL BOOST trial, which is relatively high. In addition, this trial was designed according to the TwiCs design, so only patients in the intervention group were informed about the trial and were not blinded to the intervention [15]. Non-blinding of participants might cause information bias and subsequently influence PROMs [28,29]. However, control group participants of a TwiCs study are not aware that they take part in a clinical trial, in contrast to a classical randomized controlled trial. Therefore the PROMs of participants of the control group could not have been influenced by information bias.

The collection of PROMs through the infrastructure of PLCRC and PROFILES registry resulted in reasonably good questionnaire response rates. High response rates are important for both internal and external validity [30]. Missing data can be a problem in the analysis of PROMs, especially since these data are usually not missing at random (e.g. patients who have worse health fill out questionnaires less often) [31]. A linear mixed model, as applied in this study, takes the clustering of data on a patient level into account and allows some flexibility regarding the number of completed questionnaires per patient [30,32]. As a result, bias due to non-random missing data is minimized.

In conclusion, dose-escalated CRT is associated with a significant decrease in most functional QoL domains and more pain during the first half year following start of treatment. In the pursuit of rectum-sparing treatment, dose-escalated radiotherapy is one of many emerging neoadjuvant treatment options. Although many patients are willing to 'trade' some disease free survival time for rectum preservation or improvement in functional outcome [33], it must be emphasized that a reasonable part of patients would prefer surgery over a WW-strategy [34].This heterogeneity in patient preferences underlines the importance of a well-informed discussion about different treatment options with respect for QoL, functional outcome, disease recurrence and survival.

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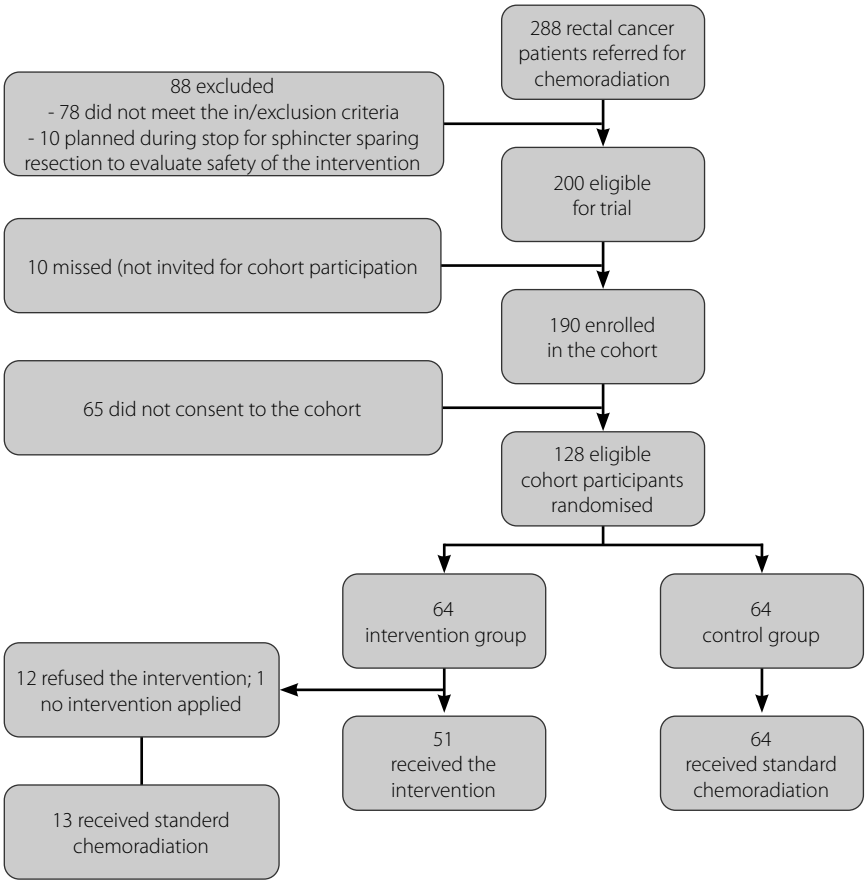
SUPPLEMENTARY DATA



Supplementary Figure 1. Flowchart of patient inclusion in the RECTAL BOOST trial (adjusted from Couwenberg et. Al, DOI:https://doi.org/10.1016/j.ijrobp.2020.06.013)

Supplementary Table 1. Per protocol response rates for the different questionnaires.

QLQ-C30		
	Boost (n = 51)	**Control (n = 64)**
T0	47 (92%)	55 (86%)
T3	48 (94%)	56 (88%)
T6	45 (88%)	55 (86%)
T12	44 (86%)	51 (80%)
T18	39 (76%)	50 (78%)
T24	37 (73%)	42 (66%)
QLQ-CR29		
	Boost (n = 51)	**Control (n = 64)**
T0	47 (92%)	55 (86%)
T3	48 (94%)	56 (88%)
T6	45 (88%	54 (84%)
T12	43 (84%)	51 (80%)
T18	39 (76%)	50 (78%)
T24	37 (73%)	42 (66%)
QLQ-CR29 Sexuality questionnaires		
	Boost (n = 51)	**Control (n = 64)**
Men		
T0	34 (89%)	35 (74%)
T3	34 (89%)	39 (83%)
T6	33 (87%)	38 (81%)
T12	30 (79%)	36 (77%)
T18	25 (66%)	30 (64%)
T24	26 (68%)	29 (62%)
Women		
T0	10 (77%)	13 (76%)
T3	9 (69%)	12 (71%)
T6	7 (54%)	12 (71%)
T12	9 (69%)	11 (65%)
T18	9 (69%)	10 (59%)
T24	8 (62%)	9 (53%)

CHAPTER 8

Patient Reported ACute Toxicity of short course radiotherapy with Interval to surgery for rectal CancEr: the PRACTICE study.

Preliminary results

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ABSTRACT

Background

Short-course radiotherapy with a prolonged interval to surgery (SCRT-delay) might be an adequate neoadjuvant treatment for intermediate to high risk rectal cancer, with decreased postoperative complications compared to SCRT and immediate surgery. Furthermore, it could enable organ-sparing treatment strategies if a clinical complete response (cCR) is achieved. However, an interval to surgery may also introduce radiotherapy-induced toxicity in the interval period. This study assessed the physician and patient reported acute toxicity of SCRT-delay in the waiting period before surgery for rectal cancer.

Methods

Rectal cancer patients referred for neoadjuvant SCRT-delay (5 times 5 Gy without immediate surgery) were asked to score low anterior resection syndrome (LARS) symptoms before, during and weekly after radiotherapy. In addition, toxicity (dermatitis, diarrhea, fatigue, cystitis and urine incontinence according to CTCAE 4.0) was assessed by the physician. Only descriptive statistics were applied.

Results

Twenty-one patients (9 female, 12 male) completed follow-up. Median age was 64 years (IQR 55 - 79). Fourteen (67%) patients received SCRT-delay for intermediate risk rectal cancer, 3 (14%) patients as an alternative for chemoradiation for locally advanced rectal cancer and 4 (19%) patients for oligometastatic disease. Median interval to surgery was 10 weeks (IQR 7 -12.5). A transient increase in CTCAE grade 2 or 3 diarrhea was seen 2-3 weeks after SCRT. The majority of patients did not experience dermatitis, fatigue, cystitis or urine incontinence during or after radiotherapy. Up to 85% of patients experienced major LARS within 2 weeks following radiotherapy. Most reported complaints were frequency, urgency and re-evacuation within one hour. Most patients were free of complaints at time of surgery. No hospital admissions were recorded during the study period.

Conclusion

Within 2-3 weeks following SCRT for rectal cancer, diarrhea and LARS symptoms are reported by the majority of patients These complaints recovered before surgery. No grade >3 toxicity was seen. These results indicate that the SCRT-delay strategy is safe and well tolerated by patients.

Introduction

As a result of the Dutch TME trial, which showed that a combination of radiotherapy and surgery reduced local recurrence rates [1], short-course radiotherapy (SCRT) was introduced as neoadjuvant treatment for intermediate risk rectal cancer. Subsequent trials suggested that prolonging the interval between SCRT and surgery (SCRT-delay) decreased postoperative complications compared to SCRT and immediate surgery [2]. There are several advantages of an extended interval to surgery. Due to its lower toxicity and shorter treatment time, SCRT-delay may be an adequate alternative for chemoradiation (CRT) in frail patients [3-5]. In addition, the introduction of an interval may promote downstaging of the tumor before surgery and herewith facilitate organ-sparing treatment strategies [4,6]. Lastly, an interval may be used for treatment of oligometastatic disease prior to rectum resection [7-9].

Conversely, a waiting period between radiotherapy and surgery may introduce radiotherapy-induced toxicity. The Stockholm III trial, a large randomized trial that compared SCRT with SCRT-delay and long-course radiotherapy, reported an increase in radiation toxicity-induced hospital admissions in the SCRT-delay group [2]. However, structured (patient reported) data on the toxicity during this interval is still lacking.

Several toxicity-scoring methods are available. A commonly used simple self-administered questionnaire that measures bowel dysfunction after rectal cancer surgery is the Low Anterior Resection Syndrome (LARS) [10]. The LARS score is an internationally validated questionnaire that contains 5 dimensions of bowel dysfunction, including incontinence for flatus or liquid stool, stool frequency, reevacuation and urgency [10,11]. A*nother* well-known toxicity score is the Common Terminology Criteria for Adverse Events (CTCAE), a descriptive terminology which can be utilized for Adverse Event (AE) reporting [12].

In this study we assessed the physician- and patient reported short-course neoadjuvant radiotherapy-induced acute toxicity in the waiting period before surgery for rectal cancer.

Methods

Patients referred to the Radiotherapy Department of University Medical Center Utrecht or Jeroen Bosch Ziekenhuis for SCRT-delay were eligible. Treatment indications included intermediate risk rectal cancer, frail patients with high risk

rectal cancer that could not receive chemoradiation or patients with metastatic disease that were scheduled for SCRT and systemic therapy prior to surgery. Patients that received a diverting ostomy prior to start of radiotherapy were excluded. The study was ethically approved by the Institutional Review Board (IRB) of University Medical Center Utrecht.

Patients underwent short-course radiotherapy, which entailed 5 fractions of 5 Gray on consecutive days. Patients were either treated with standard external beam radiation therapy (EBRT) or with online adaptive MR-guided external beam radiotherapy on a MR-linac [13,14]. Patients that received SCRT-delay for oligometastatic disease received systemic therapy in the interval to surgery. The interval between the end of radiotherapy and the start of systemic therapy, as well as the type of systemic therapy was left to the oncologist discretion. Surgery was planned in the referring hospital. The interval to surgery was determined by the referring surgeon.

Included patients were asked to keep a diary with LARS questionnaires at baseline, during treatment and 1, 2, 3, 4, 6 and 8 weeks following radiotherapy. During this period, the radiation oncologist or the clinical researcher reported the radiation toxicity, including diarrhea, dermatitis, fatigue, urine incontinence and cystitis according to the Common Terminology Criteria for Adverse Events (CTCAE v 4.0. **Supplementary Table 1**) following consultation by telephone. The scores for the different dimensions in the LARS questionnaire are derived from a weighted 3 or 4-level Likert scale (**Supplementary Figure 1**). The total LARS score is the sum of these different questions, ranging from 0 to 42 and interpreted as "no LARS" (total score 0-20), "minor LARS" (total score 21-29), or "major LARS" (total score 30-42).

Statistical analysis

Since this is an overview of preliminary results and only 21 patients were included, analysis was limited to descriptive statistics. Plots were created using RStudio (version 1.1.442 – © 2009-2018 RStudio, Inc., *"tableone"*, *"ggplot2"* and *"likert"* packages).

Results

Patient characteristics

Till day, twenty-one patients agreed to participate and completed the 8 weeks follow-up period. Baseline patient characteristics are presented in **Table 1**. The majority of patients was male (57.1%), had a cT3 tumor (71.4%) and lymph node

metastases (90.5%). Median age was 64 years (IQR 55 – 79). Fourteen patients (66.7%) received SCRT-delay according to the new guideline for intermediate risk rectal cancer. Three patients (14.3%) because of frailty, and four (19%) because of oligometastatic disease. One patient had cM1 disease, but received SCRT-delay because of advanced age; no chemotherapy was administered. Six patients did not proceed to TME surgery, four because of progression of distant metastases and two because of frailty. Median interval to surgery was 10 weeks (IQR 7 – 12.5).

Table 1. Baseline characteristics of patients included in the PRACTICE study.

	PRACTICE study n = 21
Gender	
Female	9 (42.9)
Male	12 (57.1)
Age (median, IQR)	64 [55, 79]
cT	
2	6 (28.6)
3	15 (71.4)
cN	
0	2 (9.5)
1-2	19 (90.5)
Involvement of MRF	3 (14.3)
cM1	5 (23.8)
Distance from anus at endoscopy (median cm, IQR)	4.0 [1.5, 10.0]
Reason for SCRT-delay	
Frailty	3 (14.3)
Oligometastatic disease	4 (19.0)
Intermediate risk rectal cancer	14 (66.7)
Surgery after SCRT	
Yes	15 (71.4)
No	6 (28.6)
Interval to surgery (median weeks, IQR)	10.0 [7.0, 12.5]

The weekly LARS scores, as well as its 5 dimensions, are presented in **Figure 1**. There was an increase in major LARS in week 1 to 3 following SCRT. Up to 85% of patients experienced major LARS within 2 weeks following radiotherapy. The LARS score restored to baseline levels at week 4. In addition, there was a transient increase at week 1 to 3 in stool frequency, liquid stool incontinence, reevacuation and urgency.

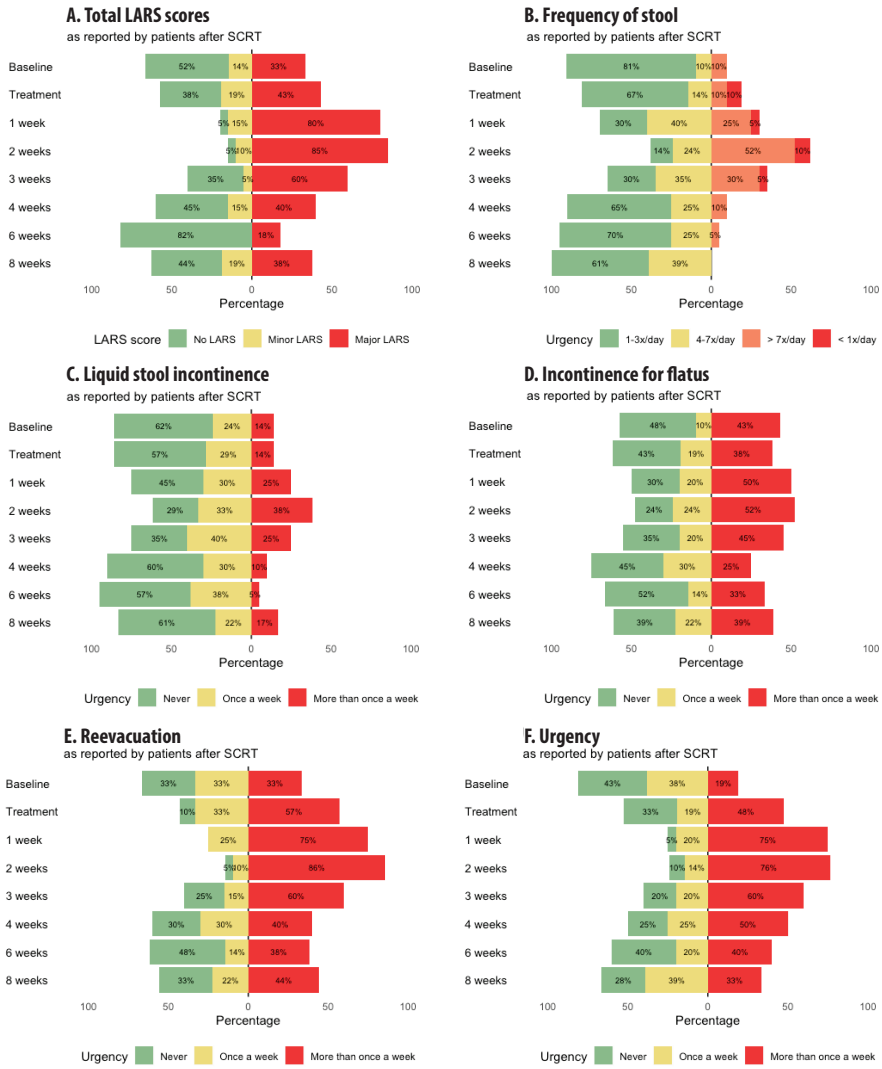

Figure 1. Low Anterior Resection Syndrome (LARS) scores at baseline, during treatment and 1-4, 6 and 8 weeks following short-course radiotherapy for rectal cancer (**A**), and the 5 dimensions from which the sum score was derived (**B-F**).

Clinician reported toxicity

CTCAE-scored radiation toxicity is presented in **Figure 2**. Throughout the 8 week follow-up period, no grade 4 toxicity was seen. Grade 2-3 diarrhea occurred in 5-19% of patients up to week 4 following radiotherapy, but diminished in the subsequent weeks. Cystitis grade 2 was reported in 5-11% of patients in weeks 1 to 3. Urinary incontinence did not occur throughout the whole follow-up period. A minor increase in fatigue complaints was seen up to week 3, but these complaints restored after this period. No hospital admissions in the waiting period before surgery were recorded during the study period.

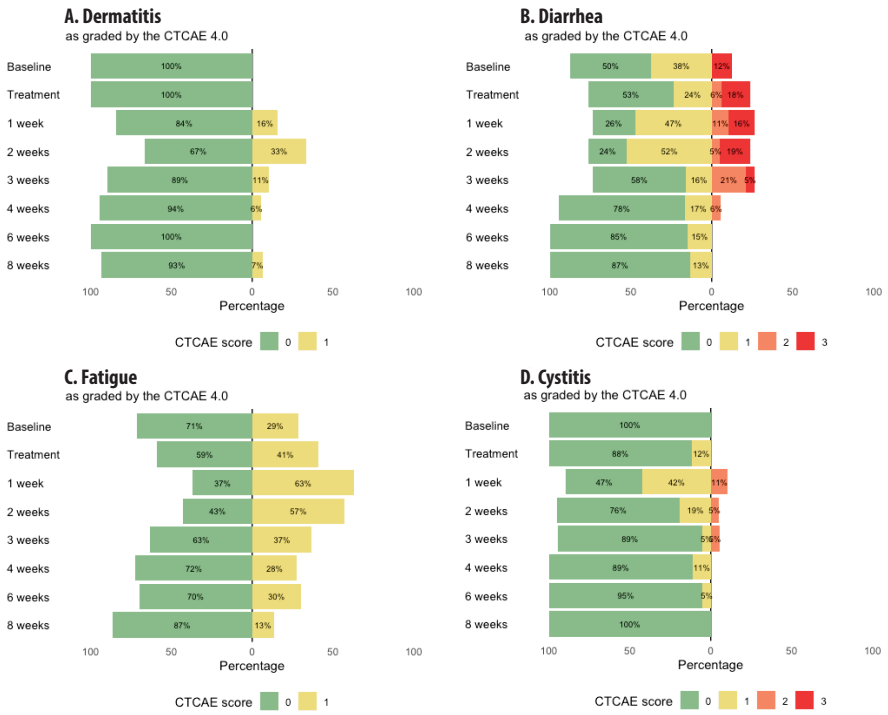

Figure 2. Radiation toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE v 4.0).

Discussion

These preliminary results show that clinician- and patient reported toxicity, particularly diarrhea and LARS symptoms, are most reported within 2-3 weeks following SCRT for rectal cancer, and that these complaints recover before surgery. No >grade 3 toxicity was seen.

SCRT was introduced in the Netherlands as a result of the Dutch TME trial, which showed that the addition of radiotherapy prior to surgery resulted in lower 5-year local recurrence rates compared to patients that received surgery alone (6% vs. 11%, respectively) [15]. Radiation-induced toxicity following SCRT, such as abdominal pain, urgency, and diarrhea, is reported in 27-41% of patients 3–7 days after the completion of radiotherapy [4,16-22]. This toxicity is mostly minor (grade 1-2) and resolves within a week [17]. However, in this treatment regimen surgery takes place before radiation toxicity can occur. More side effects are expected when surgery is delayed. The largest randomized trial that investigated the effect of SCRT-delay was the Swedish Stockholm III trial [2]. In this trial, 7 of 128 (6%) patients were admitted to the hospital due to radiation-induced (mostly diarrhea and abdominal pain) toxicity following SCRT-delay [2]. No hospital admission were reported in present study, but there might be (cultural) differences in treatment and hospital logistics. In addition, the results of the Stockholm III trial cannot be extrapolated due to lack of baseline tumor characteristics.

Patients with oligometastatic disease might benefit from SCRT-delay followed by systemic therapy. The rationale for this approach is that chemotherapy can be delivered in full doses, whereas, if traditionally combined simultaneously with long-course irradiation, the chemotherapy doses must be reduced [8,17,23]. The preliminary results of the international, multicenter RAPIDO trial, which randomly assigned LARC patients to SCRT with subsequent systemic therapy or CRT, show lower rates of distant metastases following SCRT and systemic therapy [9]. Other studies reported grade 3 gastro-intestinal toxicity or higher in 9-12% of patients following this regimen [8,23]. This is lower than in our cohort, but it is not clear when this toxicity was measured.

In specific patient populations (elderly or frail) or in some countries SCRT-delay is preferred over CRT because of its lower costs, better compliance and less demanding nature [24]. However, high-level evidence for efficacy and tolerability is limited. Toxicity is not separately reported for this subgroup in this preliminary analysis due to the low patient numbers.

The LARS score is a widely used method to evaluate bowel function following rectum resection [10,11]. Previous studies show that many of these patients suffer from major LARS, with a large impact on their quality of life [25-27]. Although data on LARS scores prior to surgery are scarce, it is well-known that similar symptoms are already present at time of diagnosis, possibly caused by the presence of the cancer and the patient's normal bowel function [28]. Furthermore, since major LARS is

common in the general population, it is unclear what proportion of observed LARS symptoms is caused by rectal cancer treatment, and what proportion is expected to be present before the cancer occurs [28]. Weekly LARS scores, measured prior to surgery, may help to estimate a patient's function before going into surgery and can therefore help to manage expectations regarding the functional outcome following resection.

This is the first study that looks at patient reported outcomes in the waiting period before surgery. As patients are still being recruited and more data is collected, future analyses using linear-effects mixed models can clarify whether the transient changes in toxicity are clinically significant and which patients are more susceptible to therapy-induced toxicity. In addition, these short-term data will be linked to long-term outcomes, to see whether toxicity in the interval to surgery influences postoperative outcomes. Lastly, as an increasing group of patients will be treated using the MR-linac, these data may support the hypothesis that radiation-induced toxicity is reduced by the higher accuracy of the MR-linac. Although a larger dataset and additional analyses are needed, these results suggest that the SCRT-delay strategy is safe and tolerated by patients.

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Supplementary Table 1. CTCAE version 4.0

	1	2	3	4	5
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death
Noninfective cystitis	Microscopic hematuria; minimal increase in frequency, urgency, dysuria, or nocturia; new onset of incontinence	Moderate hematuria; moderate increase infrequency, urgency, dysuria, nocturia or incontinence, CAD placement or bladder irrigation	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective intervention indicated	Life-threatening consequences; urgent radiologic or operative intervention indicated	Death
Urinary incontinence	Occasional (coughing, sneezing, etc.), pads not indicated	Spontaneous; pads indicated limiting instrumental ADL	Intervention indicated	-	-
Fatigue	Relieved by rest	Not relieved by rest, limiting instrumental ADL	Not relieved by rest, limiting self-care ADL	-	-
Dermatitis	Faint erythema or dry desquamation	Moderate to brisk erythema pathy moist desquamation, moderate edema	Moist desquamation, bleeding after minor trauma	Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated	Death

The aim of this questionnaire is to assess your bowel function. Please tick only one box for each question. It may be difficult to select only one answer, as we know that for some patient's symptoms vary from day to day. We would kindly ask you to choose one answer which best describes your daily life. If you have recently had an infection affecting your bowel function, please do not take this into account and focus on answering questions to reflect your usual daily bowel function.

Q.1 : Do you ever have occasions when you cannot control your flatus (wind)?

□	No, never	0
□	Yes, less than once per week	4
□	Yes, at least once per week	7

Q.2 : Do you ever have any accidental leakage of liquid stool?

□	No, never	0
□	Yes, less than once per week	3
□	Yes, at least once per week	3

Q.3 : How often do you open your bowels?

□	More than 7 times per day (24 hours)	4
□	4-7 times per day (24 hours)	2
□	1-3 times per day (24 hours)	0
□	Less than once per day (24 hours)	5

Q.4 : Do you ever have to open your bowels again within one hour of the last bowel opening?

□	No, never	0
□	Yes, less than once per week	9
□	Yes, at least once per week	11

Q.5 : Do you ever have such a strong urge to open your bowels that you have to rush to the toilet?

□	No, never	0
□	Yes, less than once per week	11
□	Yes, at least once per week	16

Add the scores from each of the five answers to one final score.

Interpretation: 0-20 = No LARS 21-29 = Minor LARS 30-42 = Major LARS

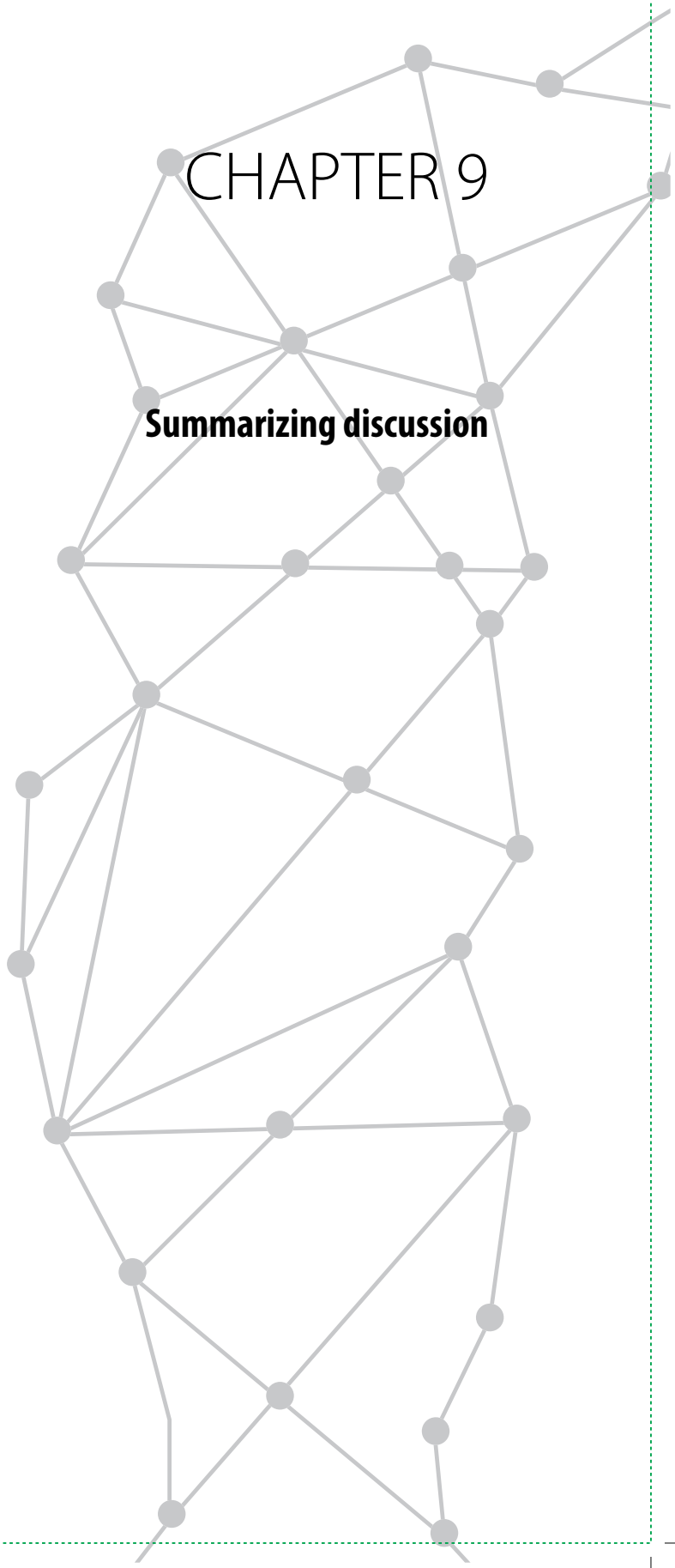
Supplementary Figure 1. The LARS questionnaire (Juul et al. International validation of the low anterior resection syndrome score. Annals of surgery. 2014;259(4):728-734).

PART IV

Summary

CHAPTER 9

Summarizing discussion



Treatment of rectal cancer patients has been subjected to a lot of changes in the past decades [1,2]. Advances in radiotherapy, chemotherapy, surgery and diagnostics resulted in an increase in treatment options and improved survival [2]. The observation of pathological complete response (pCR) after neoadjuvant treatment has led to a paradigm shift in rectal cancer management. A complete response may eliminate the necessity of subsequent surgery and thereby spare patients from surgery-associated morbidity and the associated quality of life impairment [3,4]. Therefore, rectum preservation, such as Dit is active surveillance (watch-and-wait) or local excision, has become an increasingly important endpoint over the past years [5]. In order to increase the number of eligible patients for this strategy, several (new) neoadjuvant therapies have been investigated. This thesis aimed to clarify the effect of these different neoadjuvant treatment strategies on oncological outcomes (**part I**), investigated how the response to these treatments can be predicted and assessed (**part II**) and how they influence quality of life (**part III**).

Part I: The oncological perspective

Pathological response to neoadjuvant treatment

pCR following neoadjuvant therapy may reflect the rectum-preserving potential of that particular treatment. **Chapter 2** describes the results of a meta-analysis, which investigated response rates after different neoadjuvant treatment strategies for locally advanced rectal cancer (LARC) that were compared to standard chemoradiation (CRT, i.e. long course radiotherapy and concurrent fluoropyrimidine-based chemotherapy) in randomized trials. These treatments involved intensification strategies (e.g. multi-agent CRT, targeted therapy or a combination of CRT and consolidation or induction chemotherapy) and short-course radiotherapy and delayed surgery (SCRT-delay). Only the addition of oxaliplatin to preoperative fluoropyrimidine-based CRT resulted in higher pCR rates. However, in line with previous studies, this regimen does not improve survival, including local recurrence (LR) and disease-free survival (DFS) or surgical outcomes and is associated with high toxicity rates [6-9]. Dose-escalated radiotherapy might also result in higher response rates [10]. However, dose-escalated radiotherapy was not analyzed in Chapter 2, since the results of the only randomized trial regarding this treatment (that met the inclusion criteria of the meta-analysis) were not available at the time. In contrast to the conclusion of a previous meta-analysis, which analyzed the pCR rate following dose-escalation in observational and single-arm trials [11], the recently published randomized RECTAL-BOOST trial showed no improvement in

pCR when CRT was preceded by a 15Gy radiation boost [12]. This was a contradictory finding compared to the dose response relationship described by Appelt et al. after a brachytherapy boost [10]. This may be caused by limited target coverage in the RECTAL BOOST trial. Large safety margins were used, because target and organ at risk visibility with cone-beam CT imaging during treatment was poor. The consequential organ constraints resulted in reduced coverage of the tumor and herewith probably less downsizing than was expected [12]. More accurate MR-guided delivery of the external beam irradiation dose may solve this issue. With this technique safety margins can be reduced, because tumor and organs at risk are visualized during treatment delivery and daily online plan adaptations can be made based on the actual on MRI visualized anatomy [13]. Next to these new external beam radiotherapy techniques the role of focused local treatment techniques, such as high dose rate (HDR) brachytherapy and low energy contact x-ray therapy, are currently investigated for their use in LARC [14,15]. Nevertheless, future studies should also focus on tumor characteristics and biomarkers to identify which patients are potential candidates for dose-escalated therapy to achieve a complete response and which patient will have a complete response without dose-escalated therapy.

The results of the large randomized Stockholm III trial, which showed that pCR rates increase from 2.1% to 11.8% when the interval between SCRT and surgery is prolonged [16,17], have led to an increased use of this regimen. Previous studies showed that SCRT-delay induces downsizing and may result in pCR in smaller tumors [16-21]. This regimen is less demanding than CRT, since chemotherapy is eliminated and treatment period is shorter (5 vs. 25 days). Furthermore, the interval to surgery may be used to timely address the risk of distant metastatic disease [22,23]. In the Netherlands, SCRT-delay is therefore considered for patients with intermediate risk rectal cancer, with oligometastatic disease or frail patients who are unable to receive CRT. In LARC patients, pCR rates are lower following SCRT-delay compared to CRT due to a lower biological effective radiation dose. This outcome was confirmed in **Chapter 3**, were pathological outcomes following SCRT-delay and CRT were compared in a nationwide analysis of LARC patients. This study showed that patients treated with SCRT-delay have a lower probability to become eligible for rectum-sparing approaches.

In conclusion, based on the available evidence, neoadjuvant treatment alteration is not recommended and should be reserved for a selected group of patients. However, increasing insight in tumor biology and advances in neoadjuvant therapies may influence future treatment. Optimized outcomes might be

accomplished through targeted therapy, based on individual tumor biology, and/or by more accurate treatment techniques like MR-guided radiotherapy, HDR brachytherapy or low energy contact X-ray therapy. In addition, new treatment combinations may improve treatment outcomes. Recent insights suggest that the combination of SCRT and chemotherapy in the waiting period before surgery might reduce the risk of distant metastases and improve survival in LARC patients. Although the addition of chemotherapy in the waiting period between SCRT and surgery is associated with considerable toxicity, no increase in postoperative complications were described in the initial results of the RAPIDO trial [24]. The results regarding the effect of this regimen on pCR rates, survival and quality of life are still awaited. The added value of these innovations have to be confirmed in larger, randomized studies.

Surgical outcomes

The aging population, the rising incidence and the improved prognosis of rectal cancer will increase the need for surgery in the elderly population [28,29]. Despite careful patient selection and improvements in surgical techniques and perioperative care [30], rectal surgery remains associated with substantial morbidity and decreased quality of life [4], especially in older patients that are more susceptible to treatment-related complications [29,31-34]. As postoperative complications have a large negative impact on their physical- and role functioning [35], rectum preservation might be a good solution for this particular patient category. However, omission of surgery is often infeasible as a result of less downstaging following moderated neoadjuvant treatment, as shown in **Chapter 2** and **3**.

Previous studies showed that the rate of postoperative complications can be reduced by delaying surgery following SCRT [36]. Postoperative outcomes following SCRT-delay were compared to outcomes following CRT in **Chapter 4**. No difference in the occurrence of surgical complications were found. This suggests that, considering surgery-related complications, SCRT-delay followed by surgery is a safe treatment option for LARC patients who are unable to undergo CRT.

The shifting focus towards rectum-preserving treatment raises the question whether there is a place for local excision following neoadjuvant treatment. Since accurate detection of a clinical complete response (cCR) remains challenging with the current imaging techniques [37,38], the ypT-stage, based on the surgical specimen obtained with local excision, may help to determine whether active surveillance is feasible. A large randomized trial for cT2-3N0 rectal cancer is still ongoing [39],

but positive results regarding quality of life, DFS and OS for local excision of low and intermediate rectal tumors have been described [40-46]. However, approximately 35% of patients still require completing TME surgery because of local recurrence or irradical local excision [46]. This completing surgery is associated with increased morbidity and side-effects, that compromises the potential advantages of local excision [44]. Evidence on local excision following neoadjuvant therapy in high risk tumors is scarce. A systematic review reported a higher local recurrence risk after local excision compared to TME surgery [47]. A randomized trial comparing local excision with TME surgery in LARC patients is not available yet; the results of the Spanish PRONAR trial (NCT03064646) and the Italian observational ReSARCh study are still awaited [48,49]. Lastly, it must be emphasized that local excision in an irradiated rectum may compromise tissue healing, leading to partial or complete dehiscence and subsequent morbidity, including anal pain or the need for a diverting ostomy [44,50]. For now, local excision should be reserved for selected patients, like those who are unfit for major surgery or who are treated with a palliative intent [50].

Part II: The diagnostic perspective

Outcome prediction before start of treatment

The observation that treatment intensification does not result in improved pCR rates in randomized trials might be a due to suboptimal patient selection. Since some patients show a complete response without dose-escalated therapy, the question is whether we can do better in selecting patients who will benefit from dose-escalated treatment, and those who don't need it. To prevent unnecessary exposure of patients to higher toxicity, it is essential to identify factors that can predict the efficacy of neoadjuvant treatment. Many clinical features, radiologic findings and molecular markers have been suggested to be related to response, but the clinical usefulness of these markers remain controversial [51].

Better understanding of the tumor micro-environment and molecular pathways through translational research may help to understand differences in treatment outcomes. There is growing evidence that the anti-tumor immune response plays an important role in the occurrence and progression of tumors [52,53]. As such, assessment of the immune response against tumor may be of prognostic value [54]. Potential systemic antitumor immune effects of both radiotherapy and chemotherapy, causing tumor cell death and inducing T-cell response, have been described before [55,56]. Higher levels of Tumor-infiltrating lymphocytes (TILs), such as CD3+ (a general T-lymphocyte that plays an essential role in the adaptive immune

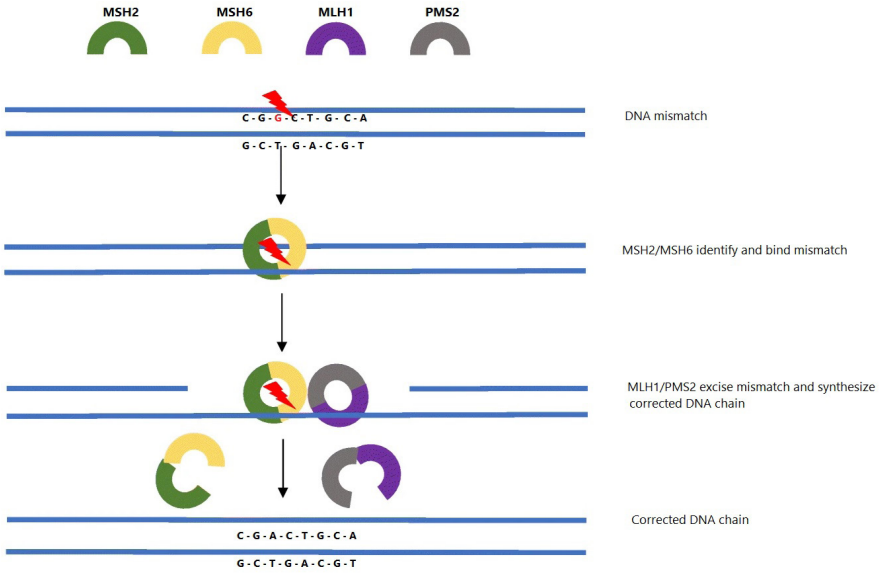


Figure 1. The process of DNA mismatch repair (adjusted from Zhao et al. [56]).

response [57]) and CD8+ (a cytotoxic T-lymphocyte that promotes apoptosis of cancer cells [57]), have been associated with tumor regression after CRT [51,56,58-61]. This suggests that tumors that attract T-cells are more sensitive to CRT and are thus more likely to respond better to treatment. Yet, the relationship between TILs and response to therapy remains unclear. The study described in **Chapter 5** investigated whether TILs density in pretreatment biopsies was associated with tumor response and aimed to find a TILs subset that may help to determine which patients can benefit from dose-escalated radiotherapy in order to achieve a better tumor response. No significant association was found, but this might be explained by the relatively small dataset.

Nonetheless, it should be emphasized that the relationship between TILs and tumor response is more complex and does not only depend on infiltration of lymphocytes [53]. The presence of TILs in the tumor environment might be related to the DNA mismatch repair (MMR) system [52]. This system plays a key role in the repair of errors that occur during DNA replication [62]. The MMR pathway usually depends on four proteins: MLH1, PMS2, MSH2 and MSH6 (**Figure 1**). When one or more proteins are not expressed or dysfunctional, this is referred to as deficient MMR (dMMR) [63]. MMR status is linked to prognosis, response to treatment and metastatic

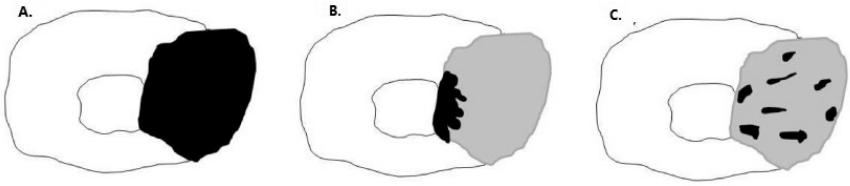


Figure 2. Response patterns after neoadjuvant therapy for rectal cancer. (adjusted from Nagtegaal et al. [62]).
A: locally advanced tumor. **B**: scenario in which neoadjuvant therapy results in fibrosis (gray) and the residual tumor shrinks in the direction of the mucosa. **C**: tumor fragmentation with scattered groups of tumor cells in the fibrotic area.

disease [64], but the prognostic value of dMMR status seems to be dependent on the presence of TILs [54]. Higher levels of TILs are found in dMMR tumors, which suggests that some aspects of the tumor biology are involved in lymphocyte recruitment [52]. Conversely, the prevalence of dMMR tumors is low in rectal cancer [64]. In Chapter 5, only 1 patient had a dMMR tumor and no further analysis was performed. Although it is suggested that dMMR rectal tumors are resistant to neoadjuvant therapy [64], the relationship between TILs, MMR and tumor response has not been investigated yet and should be validated in larger studies.

Outcome assessment

To justify the omission of surgery, accurate detection of cCR is essential. Currently, 1.5 or 3 Tesla multimodal MRI combined with digital rectal examination and endoscopy is the preferred strategy for the determination of residual tumor [65]. Mucosal features at endoscopy, such as the degree of ulcer healing, scarring and erythema of the rectal wall, are related to the likelihood of pCR, but accurate identification of complete responders remains difficult [1,66-68]. In addition, digital examination and endoscopy assume that when a tumor responds to treatment, it shrinks towards the lumen (in the direction of the mucosa). However, locally advanced tumors that have grown to a deeper layer of the bowel wall, may be destructed in a fragmented manner, resulting in the formation of small groups of tumor cells (**Figure 2**) [69]. This fragmentation is associated with more residual lymph node metastases, local regrowth in patients in a watch-and-wait follow-up program, and poorer outcome [69,70].

MRI-based restaging following neoadjuvant therapy also faces some challenges. Radiation induced fibrosis is often misinterpreted as viable tumor, while undetected

tumor residuals lead to incorrectly classifying patients as complete responders [37,38]. The negative and positive predictive values of T2 weighted (T2w) MRI at these magnetic field strengths vary from 35-92% and 23-94%, respectively [71]. Therefore, more accurate diagnostic tools are needed.

Chapter 6 investigated the added value of ultra-high magnetic field (7 Tesla) MRI for tumor response assessment following neoadjuvant treatment. 7 Tesla MRI was thought to improve response assessments given its higher signal-to-noise ratio (SNR) and the possibility for metabolic imaging techniques, such as chemical exchange saturation transfer (CEST) measurements, for residual tumor detection. Current MRI techniques cannot detect small tumor residuals that are a result of heterogeneous intra-tumor response to neoadjuvant treatment [37,72]. With metabolic imaging, characteristics of the tissue microenvironment, such as temperature, pH, and metabolite concentration, can be assessed [73,74]. CEST has already proved its usefulness in the differentiation of diseased tissue from healthy tissue [75,76]. In addition, a mice breast cancer model showed that CEST-MRI can detect small local differences. Only the metabolically active part of the tumor showed a greater CEST effect [77].

Successful imaging of rectal cancer with ultra-high field MRI has been described before [78,79], but this was the first study that attempted to use this diagnostic method in patients. Although ex-vivo images resulted in high resolution images with a strong correlation with pathology images, in-vivo images acquired at 7T showed strong artifacts and the overall evaluation of 3T MRI was better than 7T MRI. However, this study faced a lot of difficulties due to poor patient recruitment and technical issues. Patients refused participation because of the long scanning time, the general discomfort of MRI scanning or the inability to lay still for a long time because of radiation-induced diarrhea. Therefore, the results of this study are not representative. If the technical issues of 7T MRI can be addressed, it might improve the detection of complete responders after neoadjuvant treatment for rectal cancer, especially with the addition of metabolic imaging.

The patient perspective: effect of altered neoadjuvant treatment on quality of life

In the pursuit of rectum preservation or personalized treatment, deviation from the standard of care can be considered. In order to make a well-informed choice for a certain neoadjuvant treatment, information on the impact on quality of life (QoL) and oncologic outcomes of new treatment modalities is needed.

Differences in QoL as reported in the RECTAL-BOOST trial, in which patients were randomized between dose-escalated radiotherapy and standard CRT, were described in **Chapter 7**. This study showed a significant deterioration in QoL up to 6 months following dose-escalated radiotherapy. At 2-year follow up, there was no difference in QoL or disease free survival between groups. This information, in combination with predictive biomarkers for a complete response, will support a well-informed discussion about the choice for dose-escalated radiotherapy with respect for QoL, functional outcome, disease recurrence and survival.

The introduction of a waiting period between radiotherapy and surgery as a result of the Stockholm III trial, may contribute to radiotherapy-induced toxicity. The Stockholm III trial reported an increase in radiation toxicity-induced hospital admissions following SCRT-delay [17]. Minor (grade 1-2) radiation-induced toxicity following SCRT, such as abdominal pain, urgency, and diarrhea, is reported in 27-41% of patients 3–7 days after the completion of radiotherapy [16,18,19,80-84]. However, in this treatment regimen surgery takes place before radiation toxicity can occur. More side effects are expected when surgery is delayed. The first clinical study to investigate patient reported outcomes following SCRT-delay was initiated last year. Preliminary results were presented in **Chapter 8** and indicate a transient increase in bowel dysfunction and recovery of these complaints before surgery. Although data on bowel dysfunction prior to surgery are scarce, it is well-known that some symptoms are already present at time of diagnosis, possibly caused by the presence of the cancer and the patient's normal bowel function [85]. Furthermore, since bowel dysfunction is common in the general population, it is unclear what proportion of observed complaints is caused by rectal cancer treatment, and what proportion is expected to be present before the cancer occurs [85]. Weekly assessment of symptoms (prior to surgery) may help to estimate a patient's function before going into surgery and can therefore help to manage expectations regarding the functional outcome following resection. As patients are still being recruited and more data is collected, future analyses can hopefully clarify whether the transient changes in toxicity are clinically significant and which patients are more susceptible to therapy-induced toxicity.

Connecting the dots: final remarks

In the past decade, advances in rectal cancer treatment resulted in good survival outcomes, but at the cost of significant morbidity. Future challenges include the identification of treatment approaches that improve oncologic outcomes while

preserving quality of life. Also, it needs to be clarified whether every patient needs all components of multimodality treatment [2]. In the era of personalized treatment, more high-level evidence on tumor biology, (pre-)treatment response prediction, oncological outcomes and quality of life after different treatment modalities is needed to support optimal and individualized rectal cancer management. Hereto, as patients and clinicians may have conflicting preferences [86], it is important to keep in mind that one size does not fit all and that "the optimal outcome" may differ per person.

Previous surgical and oncological innovations have been implemented without accurate assessment of the outcome [87]. To address this issue, frameworks for structural evaluation of surgical and radiotherapy innovations (IDEAL and R-IDEAL, respectively) have been described before [87,88]. These frameworks recommend structural assessment of innovations and comprise proof of concept, feasibility and safety, clinical and patient reported outcomes, cost-effectiveness and long-term quality assurance. In addition to these frameworks, tumor biology should form the foundation on which outcome optimization depends. Till day, treatment is based on the TNM classification of the tumor at time of diagnosis. However, this cTNM stage provides no information on the biological characteristics of the tumor. It should be emphasized that favorable oncological outcomes of patients who reach pCR might be attributable to favorable tumor biology. Previous studies showed that local recurrence is generally observed in poor responders to neoadjuvant CRT and that survival is correlated to the pathological T-stage, rather than the clinical T-stage [50], which suggests that biological features of a tumor determine the effectiveness of a treatment. Therefore, better understanding of tumor biology may help to improve treatment outcomes.

To illustrate: in the R-stage of the R-IDEAL framework, the radiotherapy predicate studies, research identifies in which patient categories new treatment strategies are most promising (for example based on tumor biology) and can facilitate personalized treatment. Subsequently, trials can be conducted for alternative neoadjuvant treatment strategies, stratified for different subgroups. The advantage of this framework is that innovation and evaluation evolve together in an ordered manner from concept, through exploration, to validation by randomized trials [88]. Outcome assessment should include oncological outcomes as well as patient reported QoL, with room for investigation of new diagnostic modalities for response assessment. In case the treatment does not lead to better outcomes, the technique is taken back to the R-stage, in which the technique is adapted, or patient categories (based on tumor biology) in which the technique may be beneficial are identified.

Patients' preferences and patient-reported outcome measures are crucial elements of future studies. Although rectum-sparing treatment is by many perceived as the ideal option for patients, not all patients may agree, nor may it always be appropriate given a patient's circumstances [89]. Active surveillance requires frequent and thorough follow-up and is associated with uncertainty, especially in the first year following neoadjuvant therapy. A previous study showed that in hypothetical treatment-outcome scenarios, including SCRT or CRT followed by either abdominoperineal resection low anterior resection, local excision, or a wait-and-see approach, approximately 49% of patients prefer a surgical approach over a watch-and-wait scenario [90]. In addition, it is important to keep in mind that QoL is determined by more than rectum preservation, including the risk for recurrent or metastatic disease, neoadjuvant treatment and associated complications, surgical complications, functional status, and comorbidities [89].

Lastly, rectal cancer treatment is multidimensional and requires all disciplines to be involved in order to improve the outcome. Hereto, new efficient and innovative research infrastructures, such as large prospective cohorts in which trials can be conducted [91-94], supported by multidisciplinary and multiregional collaborations, are needed to deliver high-level evidence for the wide variety in treatment options. This will enable investigation of prognostic and predictive factors in large populations as well as in small subgroups of patients, and simultaneously provides the platform to conduct randomized trials that provide clinically relevant answers [92,93].

Key findings

Oncological perspective

- There is no high-level evidence to show that neoadjuvant treatment intensification improves pathological response nor survival.
- LARC patients that are offered SCRT-delay as an alternative for CRT are less likely to achieve a complete response, and are herewith less eligible for rectum-preserving treatment.
- Regarding treatment-related complications, SCRT-delay is a safe alternative neoadjuvant treatment option in LARC patients that are unable to undergo CRT.

Diagnostic perspective

- There is no indication that TILs may be helpful in selecting patients who are likely to benefit from dose-escalated radiotherapy.
- High resolution 7 Tesla MRI is not superior to 3 Tesla MRI for response assessment following neoadjuvant treatment for rectal cancer.

Patients' perspective

- Dose-escalated radiotherapy is associated with a deterioration in quality of life up to 6 months following start of treatment and does not result in improved disease-free survival.
- The introduction of a waiting period following SCRT causes diarrhea and LARS symptoms within 2-3 weeks following end of radiotherapy, but these complaints recover before surgery.

Recommendations

- Better understanding of tumor biology will help to improve treatment outcomes.
- Large multidisciplinary and multiregional collaborations, innovative research infrastructures and a structured research approach are required to deliver high-level evidence for the wide variety in treatment options for rectal cancer .

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Appendices

Summary in Dutch (Nederlandse samenvatting)

Authors and affiliations

Review committee

List of publications

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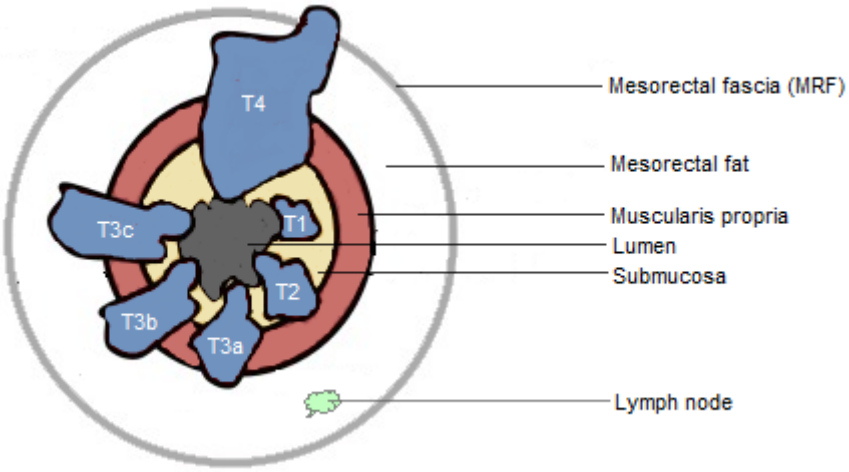
Curriculum vitae

Inleiding

Wereldwijd wordt bij ongeveer 1,8 miljoen mensen per jaar de diagnose darmkanker gesteld. Een derde van deze kankers bevindt zich in de endeldarm (het rectum). In Nederland resulteert dit in een jaarlijkse incidentie van ongeveer 4.000 rectumcarcinoom patiënten.

De diagnose rectumcarcinoom wordt het beste gesteld met behulp van MRI en CT, waarbij gelet wordt verschillende tumordimensies, zoals de tumorinvasie-diepte (**Figuur 1**), uitzaaiingen naar de lymfeklieren en de aanwezigheid van uitzaaiingen elders in het lichaam. Daarnaast is de relatie tot de mesorectale fascie (MRF) een belangrijk anatomisch herkenningspunt voor de beoordeling van de lokale tumoromvang. De MRF is het beste te omschrijven als een bindweefselmantel die het rectum en het perirectale vetweefsel omsluit, inclusief lymfeklieren en lymfevaten. Dit fungeert als een natuurlijke barrière voor tumorspreiding.



Figuur 1. Dwarsdoorsnede door het rectum met tumorinvasie-diepte

Neoadjuvante behandeling

De noodzaak van een neoadjuvante behandeling en het type behandeling wordt bepaald aan de hand van het risico op een lokaal recidief. Deze is gebaseerd op TNM-classificatie (**Tabel 1**) en de relatie tot de MRF. Een afstand van tumor tot MRF is <1 mm (betrokken MRF) kan radicale resectie van de tumor belemmeren en moet daarom worden behandeld met neoadjuvante therapie.

Patiënten met vroeg-stadium rectumcarcinoom hebben een laag risico op lokaal recidief (2% in 5 jaar) en worden daarom alleen met een operatie behandeld. Patiënten met intermediair-risico rectumcarcinoom ondergaan kort schema radiotherapie (short-course radiotherapy, SCRT), die bestaat uit 25 Gy in 5 fracties van 5 Gy, gevolgd door een operatie.

Tabel 1. Overzicht van risicoclassificatie volgens TNM-stadium en de aanbevolen behandeling.

TNM stadium	Recidief risico	Aanbevolen behandeling
cT1-2 N0 cT3a-bN0, afstand tot MRF >1 mm	Laag	Chirurgie of locale excisie voor laag-risico T1 tumoren
cT1-3c-d N1 cT3c-d N0, afstand tot MRF >1 mm	Intermediair	Kort schema radiotherapie (5x5) en chirurgie
cT4 cT3, afstand tot MRF ≤1 mm cN2 Aangedane lymfeklieren buiten MRF	Hoog	Chemoradiotherapie en chirurgie

Dit proefschrift richt zich voornamelijk op hoog-risico tumoren, hierna lokaal gevorderd rectumcarcinoom (locally advanced rectal cancer, LARC) genoemd. LARC wordt behandeld met neoadjuvante chemoradiatie (CRT). Dit is een combinatie van lang schema radiotherapie (50Gy in 25 dagen) en chemotherapie (bijv. Capecitabine of 5FU). Naast tumorverkleining en het hiermee mogelijk maken van radicale resectie, beoogt deze behandeling de overleving te verbeteren en lokaal recidief te voorkomen. Hierbij is aandacht voor behandeling-gerelateerde morbiditeit en wordt gestreefd naar het behoud van darm-, seksuele en urogenitale functie. SCRT wordt aanbevolen als alternatief voor CRT bij zwakke of oudere patiënten met comorbiditeiten, omdat zij een hoger risico lopen op complicaties.

Chirurgie

Tot op heden is chirurgie de hoeksteen van de behandeling van rectumcarcinoom. Hierbij wordt gekozen voor een low anterior resectie (LAR), waarbij een deel van het rectum blijft staan en een anatomose wordt gemaakt met het colon, of een abdominoperineale resectie (APR), waarbij naast het rectum ook de anus wordt verwijderd en een definitief colostoma wordt aangelegd. De keuze voor LAR of APR hangt af van de locatie van de tumor, aangezien er voldoende distale darmlengte

van de tumor nodig is om een colorectale anastomose te kunnen construeren. Daarnaast spelen ziektestadium, leeftijd, comorbiditeiten, cultuur en de voorkeur van patiënt en chirurg een rol bij deze beslissing.

Na chirurgische resectie wordt de endeldarm door de patholoog geanalyseerd. Hierbij wordt op onder meer gekeken naar de tumorrespons op neoadjuvante therapie. De ultieme respons is een complete pathologische respons (pCR), gedefinieerd als de afwezigheid van tumorcellen in het chirurgische verwijderde rectum. Patiënten met een pCR na neoadjuvante therapie hebben een betere overleving, een lager risico op lokaal recidief en afstandsmetastasen.

Rationale voor dit proefschrift

De behandeling van patiënten met endeldarmkanker is de afgelopen decennia aan veel veranderingen onderhevig geweest. Verbeteringen in radiotherapie, chemotherapie, chirurgie en diagnostiek hebben het aantal behandelingsmogelijkheden uitgebreid en de overleving verbeterd. Bovendien resulteerde de observatie van pCR in de introductie van rectumsparende behandelstrategieën, zoals active surveillance (ook wel watch-and-wait genoemd), of lokale excisie. Deze strategie kan chirurgie-gerelateerde morbiditeit en de daarmee samenhangende verslechtering van de kwaliteit van leven voorkomen. Als zodanig krijgen patiënten met een complete respons na neoadjuvante behandeling steeds vaker rectumsparende strategieën aangeboden.

Om geïndividualiseerde behandeling, inclusief rectumsparende strategieën, in de toekomst te ondersteunen, is meer informatie nodig over tumorkarakteristieken, oncologische uitkomsten, uitkomstpredictie en kwaliteit van leven na verschillende therapieën. Omdat verschillende medische disciplines betrokken zijn bij de behandeling van rectumcarcinoom, en elk discipline dus zou kunnen bijdragen aan een optimale en geïndividualiseerde behandeling, benadert dit proefschrift deze behandeling vanuit verschillende perspectieven.

Het oncologische perspectief

De oncoloog en de radiotherapeut kunnen de neoadjuvante behandeling wijzigen / intensiveren. De chirurg bepaalt of het rectum al dan niet kan worden behouden. Daarom richt **Deel I** van dit proefschrift zich op het oncologische perspectief, en onderzoekt hoe de oncoloog/radiotherapeut en de chirurg bijdragen aan de optimalisatie van de behandeling.

Respons op neoadjuvante behandeling

De huidige behandeling van LARC (lang schema radiotherapie met chemotherapie) resulteert in 15-27% van de patiënten in pCR. Om te onderzoeken of met een andere behandeling een betere respons bereikt kan worden, werden in **Hoofdstuk 2** pCR percentages na verschillende neoadjuvante behandelstrategieën, die waren onderzocht in gerandomiseerde studies, vergeleken. Dit waren zowel intensievere behandelingen (bijv. CRT met meerdere chemotherapeutica, of CRT met aanvullende consolidatie- of inductiechemotherapie) als minder intensieve strategieën, zoals SCRT. Deze studie toonde aan dat alleen de toevoeging van oxaliplatin aan CRT resulteerde in meer pCR. Zoals beschreven in eerdere studies, verbetert dit regime echter de overleving niet en gaat het gepaard met veel bijwerkingen.

De resultaten van de Zweedse gerandomiseerde Stockholm III-trial, die aantoonde dat de pCR-percentages stijgen van 2,1% tot 11,8% wanneer het interval tussen SCRT en operatie wordt verlengd, heeft in Nederland geleid tot de invoer van een wachttijd tot de operatie na SCRT (SCRT-delay). SCRT-delay kan in kleinere tumoren tot pCR leiden. Dit regime is minder zwaar dan CRT, omdat chemotherapie wordt geëlimineerd en de behandelingsperiode korter is (5 vs. 25 dagen). Bovendien kan het interval tot chirurgie worden gebruikt om afstandsmetastasen tijdig te behandelen. In Nederland is SCRT-delay daarom op dit moment aanbevolen voor intermediair risico rectumcarcinoom, bij oligometastatische ziekte of voor kwetsbare patiënten die geen CRT kunnen ondergaan. Bij LARC-patiënten zijn de pCR-percentages echter lager na SCRT-delay als gevolg van een lagere effectieve stralingsdosis in vergelijking met CRT. Deze uitkomst werd bevestigd in **Hoofdstuk 3**, waar met behulp van landelijke data van het Integraal Kankercentrum Nederland (IKNL) pathologische uitkomsten na SCRT-delay en CRT werden vergeleken in LARC-patiënten. Dit heeft tot geval dat patiënten die met SCRT-delay worden behandeld een kleinere kans om in aanmerking te komen voor rectumsparende behandeling.

Concluderend wordt op dit moment wijziging van de neoadjuvante behandeling niet aanbevolen en moet deze worden gereserveerd voor een geselecteerde groep patiënten.

Chirurgische resultaten

Door de vergrijzing, de toenemende incidentie en de verbeterde prognose van endeldarmkanker zal de noodzaak voor chirurgie bij ouderen toenemen. Ondanks

zorgvuldige selectie van patiënten en verbeteringen in chirurgische technieken en perioperatieve zorg, blijft rectumchirurgie geassocieerd met morbiditeit en verminderde kwaliteit van leven, vooral bij ouderen die gevoeliger zijn voor complicaties. Aangezien postoperatieve complicaties een grote negatieve impact hebben op hun functioneren, kan rectumsparende therapie een goede oplossing zijn voor deze specifieke patiëntencategorie.

Uit de eerder genoemde Stockholm III-trial bleek dat het aantal postoperatieve complicaties kan worden verminderd door de operatie na SCRT uit te stellen. Postoperatieve uitkomsten na SCRT-delay werden vergeleken met uitkomsten na CRT in **Hoofdstuk 4**. In patiënten met vergelijkbare leeftijd en comorbiditeit werd geen verschil gevonden in chirurgische complicaties. Dit suggereert dat, rekening houdend met het bestaan van patiëntenselectie in dit onderzoek, SCRT-delay een veilige optie is voor LARC-patiënten die geen CRT kunnen ondergaan.

Het diagnostisch perspectief

De respons op de behandeling zou kunnen verbeteren als er betrouwbare methoden beschikbaar komen om te voorspellen wie baat kan hebben bij intensievere neoadjuvante therapie en wie ondanks een dergelijke behandeling resttumor zal hebben. **Deel II** concentreerde zich op het diagnostisch perspectief. De patholoog kan voorafgaand aan de behandeling helpen individuele tumorkenmerken te identificeren. De radioloog kan ondersteunen bij het verbeteren van de beeldvorming ten behoeve van betere evaluatie van de respons op neoadjuvante behandeling.

Voorspelling van de respons voor aanvang van de behandeling

Dat intensievere behandeling in eerdere studies niet resulteerde in hogere pCR-percentages kan het gevolg zijn van patiëntenselectie. Dat wil zeggen, hoewel sommige patiënten een complete respons hebben zonder intensivering van neoadjuvante therapie, is de vraag of geselecteerde patiënten wel baat zullen hebben bij aangepaste behandeling. Het is daarom essentieel om factoren te identificeren die de effectiviteit van een behandeling kunnen voorspellen. Er zijn al veel voorspellende klinische factoren, radiologische bevindingen en moleculaire markers gesuggereerd, maar bewijs voor klinische toepasbaarheid van deze markers blijft tot op heden uit.

Kennis over de microscopische omgeving van de tumor kan helpen om verschillen in behandelresultaten te begrijpen. Er zijn steeds meer aanwijzingen dat de immuun-

respons een belangrijke rol speelt bij het ontstaan en uitbreiden van tumoren. De beoordeling van de immuunrespons tegen tumor zou daarom van prognostische waarde kunnen zijn. Het voorkomen van tumorinfiltrerende lymfocyten (TILs) wordt in verband gebracht met meer tumorregressie na CRT. Dit suggereert dat tumoren die T-cellen aantrekken, gevoeliger zijn voor CRT en dus beter reageren op behandeling. Het onderzoek in **Hoofdstuk 5** had als doel om te bepalen welke patiënten baat kunnen hebben bij een extra dosis radiotherapie om een betere tumorrespons te bereiken. Hiertoe werd bekeken of het aantal TILs in rectumbiopten geassocieerd was met tumorrespons na 2 verschillende behandelingen: standaard CRT en CRT met extra radiotherapie. Er werd geen verband aangetoond, maar dit zou verklaard kunnen worden door de relatief kleine dataset. Een laag aantal TILs zou geassocieerd kunnen zijn met een betere respons op intensievere radiotherapie, maar dit moet in een grotere studie worden gevalideerd.

Het beoordelen van respons

Om het weglaten van chirurgie te rechtvaardigen, is nauwkeurige detectie van een complete respons na neoadjuvante behandeling essentieel. Momenteel is MRI in combinatie met rectaal toucher en endoscopie de voorkeursstrategie. Nauwkeurige identificatie van complete respons met deze methoden blijft echter moeilijk. Bij onderzoek middels rectaal toucher en endoscopie kan een gefragmenteerde tumorrespons, waarbij tumorresten in groepjes in de darmwand zitten, gemist worden. Bij MRI wordt door straling geïnduceerde fibrose vaak ten onrechte aangezien voor resttumor, terwijl niet-gedetecteerde tumorresiduen ertoe leiden dat patiënten onjuist worden geclassificeerd als complete responders. De negatieve en positieve voorspellende waarden van huidige MRI modaliteiten lopen uiteen (respectievelijk 35-92% en 23-94%). Daarom zijn nauwkeurigere diagnostische hulpmiddelen nodig.

Hoofdstuk 6 onderzocht de toegevoegde waarde van ultrahoog magnetisch veld (7 Tesla) MRI voor het beoordelen van tumorrespons na neoadjuvante behandeling. Het idee was dat 7 Tesla MRI de beoordeling van de respons door een hogere resolutie zou kunnen verbeteren en dat de toevoeging van metabole metingen zouden ondersteunen bij het opsporen van resttumor. Dit was het eerste onderzoek dat probeerde deze diagnostische methode bij patiënten te gebruiken. Ten gevolge van sterke artefacten op de 7T MRI beelden werd de conventionele MRI beter beoordeeld. Het klinisch gebruik van 7 Tesla MRI is daarom niet aanbevolen bij rectumcarcinoom patiënten.

Het perspectief van de patiënt

Last but not least, benadrukt **deel III** het perspectief van de patiënt. Dit deel gaat in op de kwaliteit van leven na alternatieve neoadjuvante behandelingen, te weten CRT met extra dosis radiotherapie of SCRT-delay. Bij het streven naar gepersonaliseerde, inclusief rectumsparende, behandeling kan worden afgeweken van de standaard behandeling. Om een weloverwogen keuze te maken voor een bepaalde neoadjuvante behandeling is informatie nodig over de impact op kwaliteit van leven en oncologische uitkomsten.

Effect van gewijzigde neoadjuvante behandeling op kwaliteit van leven

In de RECTAL-BOOST-studie werden patiënten werden gerandomiseerd tussen dosis-geëscaleerde radiotherapie en standaard CRT. Het effect van beide behandeling op de kwaliteit van leven en overleving werden beschreven in **Hoofdstuk 7**. Deze studie toonde een significante verslechtering in kwaliteit van leven aan na dosis-geëscaleerde radiotherapie. Deze verslechtering houdt tot 6 maanden aan. Bij 2-jaar follow-up na was er geen verschil in kwaliteit van leven of overleving tussen de groepen.

De invoer van SCRT-delay kan bijdragen aan radiotherapie-geïnduceerde toxiciteit, met name darmfunctiestoornissen. Het is bekend dat dergelijke symptomen ook voorkomen in de algemene populatie, maar ze kunnen versterkt worden door de aanwezigheid van kanker in de darmwand. Het is nog onduidelijk welk deel van deze klachten wordt veroorzaakt door behandeling en welk deel al aanwezig zal zijn voordat de kanker optreedt. Wekelijkse beoordeling van symptomen (voorafgaand aan een operatie) kan helpen om de functie van een patiënt te schatten en kan daarom helpen om de verwachtingen met betrekking tot het functionele resultaat na chirurgie bij te stellen. In 2019 werd daarom een klinische studie gestart om de door patiënten gerapporteerde uitkomsten na SCRT-delay te onderzoeken. In **Hoofdstuk 8** werden de voorlopige resultaten gepresenteerd. In de eerste groep patiënten wordt een voorbijgaande toename van darmklachten gezien. Deze herstellen voordat patiënten een operatie ondergaan. Aangezien er nog steeds patiënten worden geworven en er meer gegevens worden verzameld, kunnen toekomstige analyses hopelijk duidelijk maken of deze voorbijgaande veranderingen in toxiciteit klinisch significant zijn en welke patiënten gevoeliger zijn voor functionele klachten.

Conclusie

De behandeling van rectumcarcinoom is in het afgelopen decennium verbeterd. Dit heeft geresulteerd in uitstekende overleving, maar gaat tot op de dag van vandaag ook gepaard met bijwerkingen en een negatief effect op de kwaliteit van leven. Uitdagingen voor de toekomst zijn onder meer de identificatie van gepersonaliseerde behandelmethoden die de oncologische resultaten verbeteren terwijl de kwaliteit van leven behouden blijft. Hierbij is het belangrijk om in gedachten te houden dat "het optimale resultaat" per persoon kan verschillen en dat er dus meerdere opties kunnen zijn.

De huidige behandeling is gebaseerd op de TNM-classificatie. Het TNM-stadium kan echter afhangen van het tijdstip van diagnose en bevat geen informatie over de biologische kenmerken van de tumor. Gunstige oncologische resultaten van patiënten die pCR bereiken zijn mogelijk te wijten aan een gunstige tumorbiologie. Daarom kan een beter begrip van de tumorbiologie helpen om de behandelresultaten te verbeteren.

Een structurele aanpak van onderzoek is nodig om de toegevoegde waarde van nieuwe behandelstrategieën en diagnostische middelen te beoordelen. Bij deze benadering zouden patiënt- en tumorkarakteristieken de basis moeten vormen vanuit waaruit verder onderzoek uitgebouwd wordt. Analyse van de uitkomst moet naast oncologische resultaten ook de door patiënt gerapporteerde kwaliteit van leven uitkomsten omvatten, met ruimte voor het vinden van nieuwe diagnostische modaliteiten. Daarbij moet worden aangemerkt dat rectumsparende behandeling misschien niet voor elke patiënt geschikt is, en dat kwaliteit van leven van meer afhankelijk is dan alleen het behoud van het rectum, zoals het risico op een recidief of metastasen, de toxiciteit van neoadjuvante behandeling, chirurgische complicaties en functionele status.

Tot slot, om betrouwbaar wetenschappelijk bewijs te leveren voor de grote verscheidenheid aan behandelingsopties zijn nieuwe, efficiënte en innovatieve onderzoeksinfrastructuren nodig. Hierbij kan gedacht worden aan grote prospectieve cohorten waarbinnen gerandomiseerde studies kunnen worden uitgevoerd. Dit maakt het mogelijk prognostische en predictieve factoren te onderzoeken in zowel grote populaties als kleine subgroepen. Omdat de behandeling van rectumcarcinoom multidimensionaal is, vereist dit ondersteuning van multidisciplinaire en multiregionale samenwerkingen om de uitkomst te optimaliseren.

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Appendices

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Appendices

Summary in Dutch (Nederlandse samenvatting)

Authors and affiliations

Review committee

List of publications

Acknowledgements (dankwoord)

Curriculum vitae

Sieske Hoendervangers was born on June 25th 1987 in Roosendaal (Noord-Brabant, the Netherlands). After graduating from Gymnasium at Norbertus College Roosendaal in 2005, she moved to Rotterdam to study Health Policy and Management at Erasmus University. She was admitted to Medical School (Erasmus Medical Center, Rotterdam) in 2006.

During her internships, she developed specific interest in Surgical Oncology. She completed her internships at the Department of Surgery in the IJsselland hospital (Capelle a/d IJssel, the Netherlands). In the following two years she worked as surgical resident not in training (ANIOS) in Havenziekenhuis (Rotterdam, the Netherlands) and University Medical Center Utrecht (Utrecht, the Netherlands). In November 2016, she started her PhD research project under supervision of prof. dr. H.M. Verkooijen, dr. M.P.W. Intven and dr. W.M.U. van Grevenstein in University Medical Center Utrecht. During three years, she worked on several studies on optimizing diagnosis and treatment of locally advanced rectal cancer. These studies were performed in collaboration with several hospitals in the Netherlands. In the second year of her PhD, she finished the post-graduate master Epidemiology.

Sieske continued her medical career in January 2020, and is currently a surgical resident not in training in Jeroen Bosch Ziekenhuis ('s Hertogenbosch, the Netherlands) under supervision of dr. W.J.A. Brokelman. She will start her surgical residency (in training) in January 2021. One of her future goals is to optimize health care outcomes and improve scientific output through (regional) multidisciplinary collaborations.

In her free time she enjoys road and indoor cycling, travelling, photography, cooking and spending time with family and friends.