Rectal cancer: steps towards tailored treatment

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Voor mijn vader

Contents

Chapter 1

Introduction

Colorectal cancer is a frequently diagnosed malignancy. Approximately 13,000 new cases are diagnosed in the Netherlands each year, which makes it the second most diagnosed malignancy after prostate cancer in men and breast cancer in women. Of these 13,000 colorectal tumors, 4,000 are located in the rectum. The incidence of rectal adenocarcinoma has risen from 2,400 new cases per year in 2001 to almost 4,000 in 2012 [1].

Over the past decades, the outcome of rectal cancer treatment has improved dramatically. Due to improvements in imaging technologies, surgical techniques and the increased use of radiotherapy, along with advances in knowledge about optimal timing of radiotherapy, locoregional recurrence rates have decreased from 25–30% in the seventies of the past century to less than 10%, and in the majority of patients even less than 5% nowadays. With these high control rates, one may wonder whether there is still room for improvement of local treatment.

The most pronounced improvement in the treatment of rectal cancer came from the introduction of the concept of Total Mesorectal Excision (TME) by Heald [2]. The change from a classical blunt dissection to the sharp dissection led to a decrease in local failures of about 15%. The Dutch TME trial showed that even with optimal surgical techniques short-course preoperative radiotherapy could halve the local recurrence rate [3]. Another important finding is the significance of the treatment sequence: preoperative radiotherapy has been proven superior to post-operative radiotherapy [4]. A British-Canadian group tested whether selective postoperative chemoradiation for patients with an involved circumferential resection margin (CRM) would have the same outcome as short-course (5x5 Gy) preoperative radiotherapy (SCRT) for all patients with a primary resectable rectal cancer. The local recurrence rate turned out to be higher in the postoperative group. Therefore, preoperative treatment remains the standard of care in rectal cancer treatment. A complicating factor of preoperative treatment is that decisions have to be based on clinical examination and imaging data, without knowing pathologic staging.

A more difficult question to answer is which preoperative treatment regimen should be chosen in which situation and if neo-adjuvant treatment can be

omitted in a subgroup of patients. SCRT followed by immediate resection results in a 50% decrease of the chance of local recurrence in patients with primary resectable tumors. However, the absolute benefit depends on the risk of the individual patient and varied from 2.6% to 12% in the Dutch TME trial [5]. The absolute reduction of local recurrences is most pronounced in patients with pathological stage III disease [5, 6]. In node negative patients with no or limited infiltration of the perirectal fat, pre-operative radiotherapy may be omitted. The prediction of nodal status remains a challenge, even in this era of rapidly developing imaging techniques. Until now, nodal staging is mainly based on size and morphological criteria. Although modern MRI scanners have a high spatial resolution, sensitivity and specificity remain low using these criteria [7, 8]. The use of special contrast agents can enhance the performance of MRI in nodal staging, but this technique is not widely available at this moment [9].

For locally advanced rectal cancer, defined as tumors with either a close relationship to the mesorectal fascia, invasion of surrounding organs or more than 3 positive lymph nodes, there is consensus that with the current evidence, chemoradiation is the treatment of choice [10], although some questions remain to be answered. One of these questions is whether the results of SCRT followed by surgery after an interval with or without systemic treatment are comparable to long course chemoradiation (CRT). Several trials are currently comparing these treatment options in a randomized setting. It is known that short course radiotherapy (SCRT) followed by an interval before surgery also leads to important downsizing [11-14]. The advantage of a treatment strategy with an interval of several weeks between radiotherapy and resection is that tumor downsizing can occur. This leads to a pathological complete response (pCR) in up to about 10% in patients treated with 5x5 Gy and resection after 6-8 weeks [11-14] and 15-20% after CRT [15]. This group of patients seems to have a better prognosis [15], although it is not clear whether this pCR per se leads to the improved prognosis or whether it is merely a reflection of favorable tumor biology.

The observation that patients who develop a pCR have a better prognosis led to thoughts about ways to increase the chance of a pCR. An additional advantage

of this approach is that more patients can be offered an organ sparing treatment. Although the omission of a surgical resection is still considered experimental, the first experiences in Brazil and in our own region are very promising [16-20]. Strategies to increase the response of tumors to radiotherapy include the administration of a higher dose to the tumor and the combination of radiotherapy with agents that make tumor cells more sensitive to radiation, so called radiosensitizers. It has indeed been shown that increasing the radiotherapy dose in rectal cancer increases the pCR rate [21-24]. The most used radiosensitizer in rectal cancer is 5-FU based chemotherapy. Combinations with classic forms of chemotherapy have been studied in phase II (5-FU + irinotecan) and phase III (5-FU + oxaliplatin) trials. This doublet chemotherapy led to an increase in toxicity without a significant increase in pathological response in the majority of trials [25-28], but the German CAO/ARO/AIO-04 trial found a modest but significant increase in pCR rate without a difference in toxicity [29]. Another approach is the combination of 5-FU based chemoradiation with new targeted drugs, like epidermal growth factor receptor (EGFR) antagonists and angiogenesis inhibitors. Until now, only small phase I and II trials have been published. Although preclinical data support the combination of these agents with neoadjuvant treatment, there is no evidence that response rates increase and some questions remain to be answered, like the optimal timing and the identification of patients who are going to benefit from the addition of a targeted agent to chemoradiation [30].

Although treatment results for rectal cancer have improved substantially, response to treatment is still very heterogeneous. In locally advanced rectal cancer treated with neoadjuvant chemoradiation, half of the patients do not show tumor down staging [31]. Predictive models may be helpful in choosing the right treatment approach for an individual patient. The advantage of these models is that they can include a wide range of prognostic and predictive factors, not only clinical, but also, for example, imaging and histological and molecular features [32]. As we learn more about molecular pathways that play a role in the development of rectal cancer and the response to treatment and the amount of imaging data from different modalities is increasing, it becomes

more and more difficult for physicians to integrate all these factors and make the right treatment choice [33].

The aim of improving treatment response and tailoring treatment was the basis of the research presented in this thesis (figure 1).

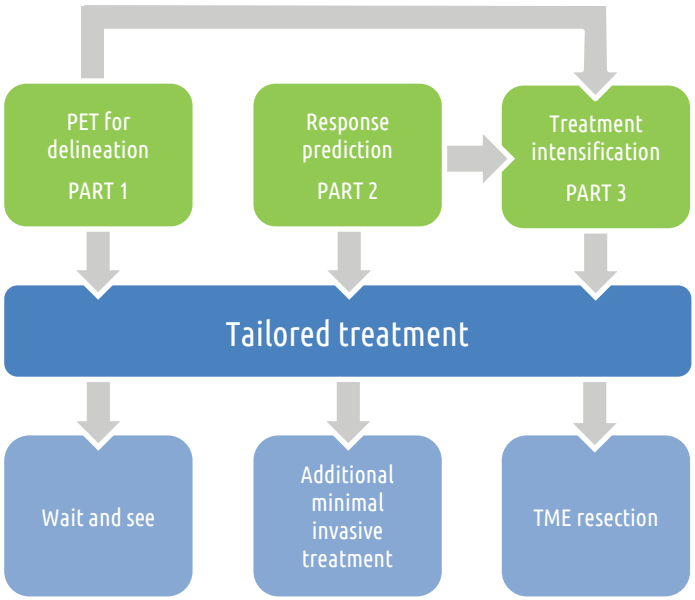

Figure 1 Schematic overview of the different topics discussed in this thesis and the treatment options for rectal cancer

Outline of this thesis

Three hypotheses formed the basis of this thesis:

1. PET-CT is a reliable tool for tumor delineation in rectal cancer treatment and diminishes interobserver variation.
2. Multimodality predictive models are able to predict response to neo-adjuvant treatment in rectal cancer.
3. The combination of AKT inhibition and radiotherapy and mTOR inhibition and radiotherapy leads to an increased tumor response in rectal cancer.

Studies related to the first hypothesis are presented in part I of this thesis. In chapter 2, we evaluated whether tumor dimensions in rectal cancers are adequately represented by the signal seen on a PET-scan. Chapter 3 describes the use of an automatic PET-based delineation algorithm and the influence on inter-observer variability in treatment volume definition.

The second hypothesis was the basis of part II of this thesis, which is dedicated to the role of different models for response prediction in rectal cancer. These models were developed to identify patients with a high chance of good response to neoadjuvant treatment. Chapter 4 describes a prediction model based on sequential PET-imaging before and during treatment. In chapter 5, the added value of blood biomarkers to the existing imaging based models is tested. Chapter 6 gives an overview of the literature on biochemical and molecular factors that predict response to treatment.

The third hypothesis is tested in two clinical trials presented in part III. These trials investigated the addition of a radiosensitizer to two different existing radiotherapy regimens in rectal cancer. In chapter 7, the results of a phase I trial testing the combination of nelfinavir and chemoradiation are described and in chapter 8 a phase I-II study on the combination of the mTOR inhibitor rapamycin with short-course hypofractionated radiotherapy is reported.

Finally, in chapter 9, the different subjects of this thesis are discussed and recommendations for future research and clinical practice are given.

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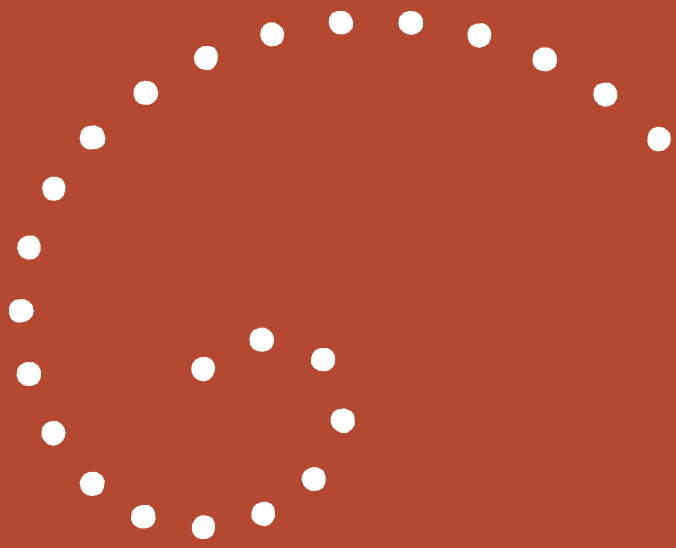
Part I
The role of PET-CT in tumor delineation



Chapter 2

FDG-PET provides the best correlation with the tumor specimen compared to MRI and CT

Jeroen Buijsen, Jørgen van den Bogaard, Marco H.M. Janssen, Frans C.H. Bakers, Stephanie Engelsman, Michel Öllers, Regina G.H. Beets-Tan, Marius Nap, Geerard L. Beets, Philippe Lambin, Guido Lammering

Radiotherapy and Oncology
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Abstract

Purpose

To compare CT-, MR- and PET-CT based tumor length measurements in rectal cancer with pathology.

Patients and Methods

Twenty six rectal cancer patients underwent both MR and PET-CT imaging followed by short-course radiotherapy (RT 5x5 Gy) and surgery within 3 days after RT. Tumor length was measured manually and independently by 2 observers on CT, MR and PET. PET-based tumor length measurements were also generated automatically using the signal-to-background-ratio (SBR) method. All measurements were correlated with the tumor length on the pathological specimen.

Results

CT-based measurements did not show a valuable correlation with pathology. MR-based measurements correlated only weakly, but still significantly (Pearson correlation=0.55 resp. 0.57; $p<0.001$). Manual PET measurements reached a good correlation with pathology, but less strong (Pearson correlation 0.72 and 0.76 for the two different observers) than automatic PET-CT based measurements, which provided the best correlation with pathology (Pearson correlation of 0.91 ($p<0.001$)). Bland-Altman analysis demonstrated in general an overestimation of the tumor diameter using manual measurements, while the agreement of automatic contours and pathology was within acceptable ranges. A direct comparison of the different modalities revealed a significant better precision for PET-based auto-contours as compared to all other measurements.

Conclusion

Automatically generated PET-CT based contours show the best correlation with the surgical specimen and thus provide a useful and powerful tool to accurately determine the largest tumor dimension in rectal cancer. This could be used as a quick and reliable tool for target delineation in radiotherapy. However, a 3D volume analysis is needed to confirm these results.

Introduction

Rectal cancer is a frequently occurring malignancy. Over the last two decades multimodality treatment has led to important improvements in the treatment of this disease. Preoperative RT, often combined with chemotherapy, followed by a Total Mesorectal Excision (TME) has become the standard of care for most patients. In this respect, modern imaging techniques are extremely important in the preoperative workup for treatment decision making. Endoscopic ultrasound (EUS) is most suitable for the evaluation of superficial tumors [1], whereas magnetic resonance (MR) imaging provides more accuracy in determining the margin between the tumor and the mesorectal fascia [2, 3].

While there is growing interest in the possible role of FDG-PET in response evaluation [4, 5], until now, the use of 18F-fluoro-2-deoxy-glucose Positron Emission Tomography (FDG-PET) imaging has no clearly defined role in local staging of rectal cancer. However, a unique advantage of FDG-PET-scanning is the ability to use the quantitative information of the glucose uptake within the tumor to automatically create a contour around the tumor. This auto-contouring process significantly reduces the interobserver variability in the interpretation of images [6, 7], as it eliminates the human factor, increases consistency, diminishes interobserver variability and also saves time. It is well known from other tumor sites that CT-based tumor delineations result in a substantial interobserver variation [7, 8]. Since there is a growing interest in the use of a boost in the treatment of rectal cancer, accurate delineation of the tumor volume becomes increasingly important [9, 10]. However, presently the evidence of the use of PET-CT in radiotherapy planning for rectal cancer is limited [11]. Therefore we need to compare this method with the pathological specimen, before we can use PET as a reliable method to define the Gross Tumor Volume (GTV) in rectal cancer. Thus, the aim of the present study was to evaluate the accuracy of FDG-PET-based auto-delineation in determining the maximal cranio-caudal diameter of rectal tumors and to compare it with CT and MR-based measurements.

Materials and methods

Since the purpose of this study was to directly correlate the initial tumor length measurements with pathology as gold standard, we only included patients, who were referred for a short-course of 5 fractions of 5 Gy preoperative RT, followed by a TME within 3 days after the last fraction of radiotherapy. This preoperative treatment is known to have no influence on the size of the tumor if there is only a short time-window between completion of radiotherapy and resection [12].
In total, 26 patients diagnosed with primary resectable rectal cancer (T2-early T3, N0-1, with a predicted circumferential resection margin on MR of more than 2 mm) were enrolled. As part of their preoperative workup, all patients had also been investigated with an MR scan.

PET-CT imaging
Before the start of radiotherapy, all patients underwent a PET-CT scan for treatment planning purposes using a PET-CT-simulator (Biograph, SOMATOM Sensation 16 with an integrated ECAT ACCEL PET scanner, Siemens, Knoxville, USA) with a spatial resolution of approximately 6.5 mm in the centre of the axial field of view (162 mm). Data acquisition was performed in 3D and the acquisition time per table position was 5 minutes. The acquired PET images were corrected for scatter and attenuation using a CT based attenuation map. Image reconstruction was performed using FORE (Fourier rebinning) and OSEM reconstruction (ordered subsets expectation maximization, 4 iterations, 8 subsets) with an inter-slice distance of 3.45 mm. The patients were instructed to fast for at least 6 hours prior to PET-imaging. The FDG was injected intravenously, with the activity based on the patient weight (weight [kg] * 4 + 20 [MBq]). PET-CT imaging was started approximately 60 minutes after FDG injection. The patient was scanned in supine position. At the start of the examination, a native CT scan was made from the top of the skull to the mid thigh. Scan parameters were 120 kV, 140 mA, a pitch of 1.25 and a 5 mm reconstructed section thickness. Immediately thereafter, a PET-scan was performed, covering the identical transverse field of view (500 mm).

Auto-delineation

For study purposes, the tumor was automatically delineated using dedicated software (Esoft 5.0, Siemens MI, Erlangen, Germany). An SBR method was used to find for each patient a percentage threshold of the maximal SUV within a user defined Volume of Interest (VOI) around the tumor as previously described [13, 14]. For each patient, the SBR was calculated, using the gluteus muscle as relevant background. From the SBR, the corresponding threshold percentage served as input for the SUV auto delineation algorithm in the Esoft software. The auto delineation algorithm is based on region growing [15]. Image voxels that are adjacent to one another in a certain neighborhood are clustered or connected.

Using this method, an auto-contour was created for each patient (figure 1) and the maximal cranio-caudal length of the contour was calculated automatically.

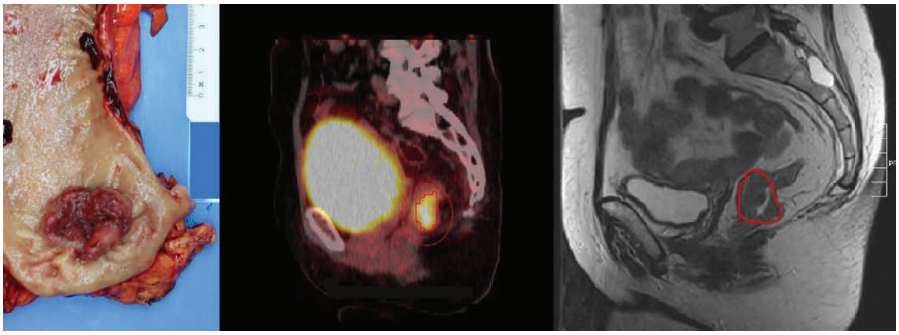

Figure 1 Images of respectively the surgical specimen, the corresponding CT-PET image and T2 weighted MR image of the same patient, with the tumor contour presented in red.

Manual PET and CT measurements

All PET-CT scans were imported in our treatment planning system (XiO/Focal 4.34.02, CMS Inc. Maryland Heights, MO, USA) and PET-scans were read by two radiation-oncologists with experience in the use of PET-CT for radiotherapy planning. A fixed window-level setting was used as well as standard color settings. Maximal tumor lengths were measured manually on a Focal workstation. At a second time point (at least 4 weeks after the PET-

measurements to ensure that PET-measurements did not influence CT-measurements), tumor lengths were measured by the same two radiation-oncologists on the CT-images, without knowledge of MR and PET images.

Magnetic Resonance (MR) imaging

All patients underwent MR-imaging of the pelvis as a part of their preoperative workup. These scans were performed at the university hospital and in the surrounding referring hospitals. The field strength of the MR machines was 1 and 1.5 T, and the protocol for rectal cancer MR imaging was regionally standardized, using T2W FSE sequences in 3 planes [16]. The maximum tumor length in cranio-caudal direction was measured manually on the PACS viewing system on the sagittal T2-weighted images, with the use of transversal and coronal projections when required. For this purpose the standard measurement tool in the viewing system was used. Measurements were performed independently by two experienced GI radiologists, blinded from any clinical information except for the distance from the anal verge to the caudal margin of the tumor on endoscopy and/or the distance to the tumor based on digital rectal examination.

Pathology

After resection, a standardized routine pathology examination was performed by a pathologist specialized in gastro-intestinal pathology using the protocol as described by Quirke et al [17]. The length of the tumor was carefully measured macroscopically with a ruler, before slicing was done. The pathologist was blinded from any information regarding the image-based tumor measurements.

Statistical analysis

SPSS® software (SPSS® for Windows, version 15.0, SPSS Inc, Chicago, IL), was used to perform statistical analysis. Tumor diameters as measured with the different imaging modalities were compared with pathology using linear regression, including the Pearson correlation coefficient. In addition to correlation analysis, Bland-Altman analyses were performed to evaluate the

absolute agreement of the measurements between the different modalities [18]. To directly compare the predictive performance of the various tests a method as described by Sheiner and Beal [19] was used. This method evaluates the predictive performance of a test by calculating the prediction error and the bias. The prediction error is an indicator for the precision of a test, while the bias gives information about the systematic component of the prediction error. In other words, the bias reflects an under- or overestimation of a test. Furthermore, this method gives the opportunity to compare the performance of the different imaging modalities.

Results

Tumor diameters varied from 1.6 to 8.0 cm on pathology examination (mean 4.2 cm, SD 1.4), from 2.9 to 10.8 cm on CT (mean 5.1 cm, SD 1.7 cm) from 3.1 to 7.6 cm on MR (mean 4.8 cm, SD 1.4) and from 2.2 to 8.1 cm on PET-scan (manual measurements, mean 5.0 cm, SD 1.7 cm) resp. 2.0 to 8.0 cm (automatic measurements, mean 4.2 cm, SD 1.4). Individual measurements are shown in table 1.

The results of the Pearson correlation analyses are shown in figure 2. Measurement of the tumor length on CT showed no correlation with pathology for one observer and only a very weak correlation for the second observer (Pearson correlation = 0.34 ($p=0.09$) resp. 0.50 ($p=0.01$)). Intra-observer correlation for CT-scan was the weakest of all modalities (Pearson correlation = 0.69; $p<0.001$). The correlation between MR and pathology was somewhat stronger than CT for both observers (Pearson correlation = 0.55 resp. 0.57; $p<0.001$). Intra-observer correlation between both MR observers was less strong than for PET scan, but still considerable (Pearson correlation = 0.78 ($p<0.001$)). The correlation between manual PET measurements and pathology was stronger than for MR, resulting in a correlation coefficient of 0.76 for observer 1 and 0.72 for observer 2 ($p<0.001$). Intra-observer correlation between the two PET-observers was very strong (Pearson correlation = 0.97, $p<0.001$). The best correlation was found between the automatic PET measurements and the

measured tumor length on pathology (Pearson correlation = 0.91; $p < 0.001$).

Table 1 Individual measurements of each modality in cm.

Patient	Pathology	CT_{obs1}	CT_{obs2}	MRI_{obs1}	MRI_{obs2}	PET_{man1}	PET_{man2}	PET_{auto}
1	6,0	5,8	5,8	6,9	8,2	6,8	6,6	4,8
2	4,5	5,6	7,0	3,7	5,7	5,5	6,3	5,0
3	4,0	4,0	3,2	3,4	2,8	4,2	4,0	3,8
4	8,0	8,0	9,0	7,3	7,3	7,8	8,1	8,0
5	3,0	4,0	3,5	3,5	3,2	3,9	4,3	3,0
6	4,0	4,3	4,2	4,4	4,2	5,0	4,9	4,2
7	2,9	4,3	3,8	3,9	3,7	5,6	5,4	3,2
8	5,0	6,0	5,7	4,1	5,5	6,2	6,4	5,2
9	4,0	4,6	5,8	5,4	4,6	4,3	3,7	3,7
10	3,5	3,5	2,2	3,6	4,8	3,8	2,9	3,0
11	4,5	6,3	4,3	4,8	4,3	6,6	6,5	4,8
12	5,5	7,3	6,2	4,3	3,5	8,2	7,7	7,0
13	4,0	5,5	4,8	4,7	4,7	6,0	5,8	4,9
14	4,0	4,0	4,3	6,4	6,5	7,4	7,5	4,5
15	5,0	7,1	6,2	6,3	4,8	6,6	6,3	5,7
16	3,0	4,6	1,6	3,2	3,6	3,0	2,2	2,0
17	3,0	4,5	3,2	2,5	3,7	2,6	3,0	3,0
18	6,0	5,0	4,4	5,5	5,1	6,8	6,4	6,5
19	4,2	3,9	4,5	4,5	3,8	3,2	3,0	3,6
20	3,5	12,4	9,1	6,9	7,1	5,4	6,0	4,5
21	3,2	6,1	1,8	3,9	3,4	4,2	3,9	2,8
22	3,0	3,6	5,3	3,1	3,0	2,8	3,2	2,9
23	1,6	4,5	3,9	3,6	3,4	3,1	3,5	2,4
24	3,8	4,7	5,3	5,9	4,3	4,2	3,8	4,0
25	4,0	6,3	5,0	6,7	4,5	4,4	4,2	4,1
26	3,8	7,2	4,4	7,1	7,0	4,0	3,9	3,6

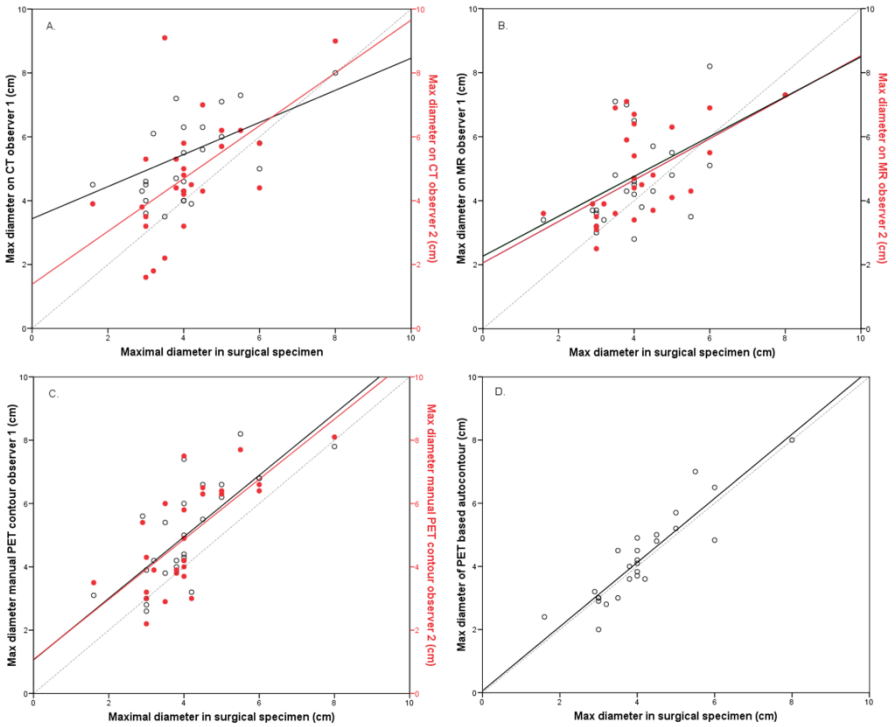

Figure 2 Linear regression curves for maximal tumor diameter resulting from CT-imaging (2 observers, red and black) (A), MR-imaging (2 observers) (B), manual PET-contours (2 observers) (C) and automatic PET-contours (D). The dotted line represents the ideal situation of absolute agreement between two modalities.

A comparative analysis using Bland-Altman plots (figure 3) shows that CT scans tend to overestimate tumor length, reflected by a mean difference of 1.03 with limits of agreement -1.99 and 4.04. This means that 95% of the diameters measured on CT lie within a range of -1.99 to +4.04 cm from the true diameter as measured in the pathology specimen. The agreement between MR and pathology proved to be better than CT but worse than automatic PET-measurements: the mean difference reached 0.66 cm (limits of agreement -1.69 and 3.01). For manual measurements on PET scan, differences were somewhat larger (mean difference 0.91, limits of agreement -1.23 and 3.05) than for automatically generated contours on PET scans, which showed the best

agreement with pathology. For the majority of patients the agreement between PET auto-contours and pathology was within 1 cm. The mean difference was 0.13 cm and the limits of agreement were -1.06 and 1.31.

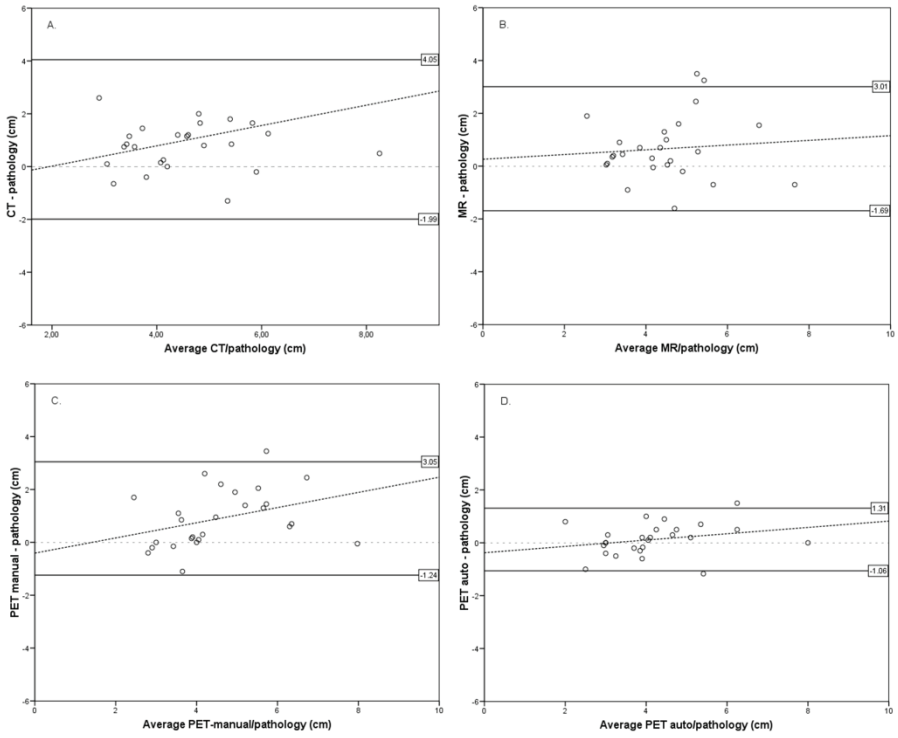

Figure 3 Bland-Altman plots showing the difference against mean for the three imaging modalities (A. CT, B. MR, C. manual PET contours, D. automatic PET contours). The thick lines represent the confidence intervals around the mean of difference, marking the level of agreement between the different imaging modalities.

The results of the analysis of precision and bias are shown in table 2. These results are in line with the observations in the Bland-Altman plots: PET-autocontours show the highest precision and the smallest bias and this precision was significantly better than any other measurement in this study, as can be gathered from the 95% confidence intervals.

Table 2 Predictive performance of the different modalities using the method as described by Sheiner and Beal[19].

	PET $_{auto}$	**PET $_{manual}$**	**MR**	**CT**	**Endoscopy**
Precision	0.37	1.97	1.82	3.35	3.82
Bias	0.13	0.91	0.66	1.03	0.46
Difference in precision (95% CI)[1]		-1.60 (-2.71 - -0.50)	-1.45 (-2.70 - -0.19)	-2.98 (-4.00 - -1.96)	-3.45 (-7.60 - 0.70)

[1] Difference as compared to the performance of PET auto-contour

Discussion

This is the first study demonstrating a strong correlation between the rectal tumor length measured in an automated way on PET-images and pathology as reference. These automated PET-based tumor measurements showed a stronger correlation with pathology than CT, MR and manual PET measurements.

PET-based auto delineation and correlation with pathology in rectal cancer has been described in one other study [20]. This study found only a modest correlation with pathology. However, these patients were treated with a long course of chemoradiotherapy, which certainly resulted in heterogeneous tumor downsizing, thus strongly influencing the interpretation of correlative studies between pre-treatment images and pathology. Furthermore, a different delineation algorithm was used. Our findings are in concordance with the paper of Van Baardwijk et al. and Daisne et al. who found that source-to-background ratio-based auto-delineation showed a good correlation with pathology in respectively lung and head and neck cancer [7, 21]. Daisne et al. compared FDG-PET with MR and CT and found PET to have the strongest correlation with pathology in pharyngeal and laryngeal cancer [21]. However, it should be mentioned that their results were based on a relatively small group of 12 patients. An advantage of their study is that they were able to make a reconstruction of the tumor volume. Unfortunately, we were unable to reliably

measure the 3D tumor volume in the surgical specimen. However, we made the assumption that the tumor length is indicative for the volume, as has been shown before for cervical cancer [22]. For future research we have planned to develop a method for accurate volume measurements. A possible limitation of our study is that the macroscopic tumor diameters may have been measured at different time points after resection. This may have led to different degrees of shrinkage of the surgical specimen. No conclusive data on tumor shrinkage has been reported for rectal cancer. However, in prostate cancer, linear correction factors for tumor shrinkage between 1.04 and 1.14 have been described [23], indicating that the influence of shrinkage might be rather low. Since the composition of the tumor stroma of colorectal tumors in general is even more compact than the fibromuscular stroma of the prostate, we assume that the shrinkage effect in colorectal tumors by formalin will be even less than in the prostate.

Another characteristic of our patient group is that they all have received short-course radiotherapy pre-operatively. In the Netherlands, pre-operative radiotherapy has become standard of care for the large majority of rectal cancer patients. Therefore it was for ethical reasons not feasible to include patients who would only undergo surgery. As mentioned earlier no downstaging has been observed in the Dutch TME trial after 5x5 Gy. However, they observed a small but statistically significant difference in tumor diameter between the irradiated and the non-irradiated group (4.5 vs 4.0 cm, $p<0.001$). They did not report on reliable tumor measurements at diagnosis, making it impossible to draw firm conclusions whether this difference in tumor size may have been caused by tumor shrinkage as a result of radiotherapy. In addition, a substantial part of the patient group in the TME trial (57%) was operated 4 or 5 days after completion of radiotherapy, while in our patient group all patients were operated within 3 days. However, in order to have more certainty that tumor shrinkage did not influence our results, we went on and analyzed the maximal diameters on PET-CT scans of 21 rectal cancer patients who had been imaged with PET-CT before and immediately after the fifth fraction of radiotherapy as part of a sequential PET-CT study [4]. The mean tumor diameters as measured

by auto-contouring were 5.1 vs 5.0 cm on pre- vs. post-radiotherapy scans (p=0.13). This to our opinion confirms that at least up until day 5 of radiotherapy no significant tumor shrinkage has taken place. It is rather unlikely that 3 further days at the time of surgery would still result in a measurable down-sizing.

For tumors with smaller volumes it is important to rule out the influence of partial volume effects (PVE) on PET-imaging [24]. PVE typically occur if the tumor size is smaller than three times the spatial resolution (that means 3 x 6.5 mm=1.95 cm). As the smallest maximal tumor diameter in this patient group as measured on PET-scan was 2 cm, we do not expect that PVE had an important influence on the data. Due to the limited spatial resolution of PET-imaging, one may not expect PET-scan to be able to detect microscopic tumor extension.

For the current radiotherapy treatment planning of rectal cancer, PET-imaging is only of limited value in the determination of the radiation treatment field borders, because the preoperative treatment of rectal cancer normally includes a locoregional treatment encompassing the whole posterior pelvis. However, new developments of intensified regimens aiming at completely eradicating the tumor might lead to the necessity of an accurate definition of the tumor volume in order to avoid geographical misses and to limit the volume of normal tissue being irradiated. So far, several groups studied the use of PET-imaging for the delineation of rectal tumors. Patel et al. found in a limited number of patients (n=6), that the use of PET-CT in radiotherapy planning for rectal cancer resulted in a decrease in the inter-observer variability in boost target volumes [6]. Bassi et al. recently reported an important increase in the target volume when using PET-CT data as compared to only CT [25]. Anderson et al showed a slight decrease in the delineated volume based on PET-CT as compared to CT. The mean overlap volume between the CT-based tumor volume and PET-based tumor volume was 46.7% [26]. Very recently, the group of Paskeviciute described the impact of FDG-PET-scan on planning of neoadjuvant radiotherapy in rectal cancer [27]. They also found a decrease in volumes defined on basis of PET information. However, their CT volume consisted of the entire rectal circumference at the level of the tumor, whereas the PET volume consisted of

the PET positive part of the rectal wall only with a 2 cm margin. They found geographical misses in 46% of the patients. The use of MR scans when delineating on CT is certainly helpful and is expected to diminish differences between contouring on CT only and PET-CT. Another important difference between our study and these two other studies is that they did not use automatic delineation software.

A disadvantage of the use of PET-imaging in colorectal cancer is the fact that PET has only a very low sensitivity for nodal disease [28]. For that reason PET-CT is not suitable for sub-boosting of pathological nodes. Furthermore, PET-CT cannot reliably predict the involvement of the mesorectal fascia [29]. Therefore MR certainly has an important role in decision making in rectal cancer. Another disadvantage of PET-scan is the low spatial resolution. Therefore, it is difficult to compare the inter-observer variabilities between the different imaging modalities found in this study. It cannot be entirely ruled out that the better agreement between the two observers on PET-scan is partially the result of the lower spatial resolution of PET as compared to CT and MRI.

As mentioned before, the use of auto-contours avoids interobserver differences [7]. Furthermore, it is well known that manual delineation is largely operator-dependent [30]. Our own group previously compared delineation on CT with auto-contours generated on PET-scans using static and dynamic PET-scans in rectal cancer patients [31]. We found that manually drawn contours on CT were significantly larger than contours based on dynamic PET-scans. In this cohort, no direct comparison was made between static PET-scan contours using the SBR method and manual contours.

In conclusion, this is, to our knowledge, the first study that confirms the accuracy of PET-CT-based tumor length measurements in rectal cancer by pathology validation. PET-CT-based measurements show a strong correlation with pathology compared to other frequently used imaging modalities and can therefore be defined as a useful tool for the GTV delineation in rectal cancer.

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Chapter 3

FDG-PET-CT reduces the interobserver variability in rectal tumor delineation

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Abstract

Background and purpose

Previously, we showed a good correlation between pathology and an automatically generated PET-contour in rectal cancer. This study analysed the effect of the use of PET-CT scan on the interobserver variation in GTV definition in rectal cancer and the influence of PET-CT on treatment volumes.

Materials and methods

42 patients diagnosed with rectal cancer underwent an FDG-PET-CT for radiotherapy planning. An automatic contour was created on PET-scan using the source-to-background ratio. The GTV was delineated by 5 observers in 3 rounds: using CT and MRI, using CT, MRI and PET and using CT, MRI and PET auto-contour. GTV volumes were compared and concordance indices (CI) were calculated. Since the GTV is only a small portion of the treatment volume in rectal cancer, a separate analysis was performed to evaluate the influence of PET on the definition of the CTV used in daily clinical practice and the caudal extension of the treatment volumes.

Results

GTV volumes based on PET were significantly smaller. CIs increased significantly using PET and the best interobserver agreement was observed using PET auto-contours. Furthermore, we found that in that in up to 29% of patients the CTV based on PET extended outside the CTV used in clinical practice. The caudal border of the treatment volume can be tailored using PET-scan in low seated tumors. Influence of PET on the position of the caudal border was most pronounced in low seated tumors.

Conclusion

PET-CT increases the interobserver agreement in the GTV definition in rectal cancer, helps to avoid the geographical misses and allows tailoring the caudal border of the treatment volume.

Introduction

Pre-operative radiotherapy has become an essential part of the treatment of most patients with rectal cancer, since it is very effective in reducing the risk of a locoregional recurrence [1]. Combining radiotherapy and chemotherapy results in downsizing and in up to 10-33% of patients pathological complete responses (pCR) have been reported [2-5]. Patients with a good clinical response may benefit from less invasive surgery, like sphincter-saving surgery or transanal endoscopic microsurgery (TEM). Even, in selected cases, a wait-and-see policy might be safe [6]. These innovative modified surgical approaches may lead to better quality of life. Thus, it would be attractive to further increase the probability of a good tumor response, e.g. by increasing the dose to the tumor [5, 7, 8]. To achieve higher doses to the tumor, a simultaneous integrated boost technique has been shown to be feasible [9-11]. In order to identify the boost volume easily and reliably, high quality imaging is important. MRI is considered the most accurate staging method for rectal cancer [12-14], but its role in a precise determination of the boost volume is unknown [15]. For PET-imaging it has been reported that it is reliable in defining tumor size in rectal cancer [16] and has the additional advantage that it can easily be acquired in treatment position simultaneously with a CT-scan, which is needed for treatment planning.

Another reason why it is important to define more precisely the primary tumor in rectal cancer is that it may help to reduce long term toxicity that is observed after radiotherapy for rectal cancer [17, 18], through a further reduction of treatment fields. As our group has shown before, 3-D conformal planning results in a better PTV coverage and dose homogeneity as compared to standard 3- or 4-field techniques based on bony anatomy [19]. However, the use of more conformal techniques, like IMRT, poses the risk of geographical misses. Furthermore individual delineation makes it possible to better spare normal tissues. A better identification of the primary tumor allows avoiding irradiation of the sphincter in selected cases and reducing the volume of small bowel in high seated tumors, resulting in less toxicity. It has been shown that positive

lymph nodes are most frequently located at the level of the tumor. The proximal spread of lymph nodes is limited to 5 cm from the distal margin of the tumor and the distance to the most distal nodes is 4 cm at maximum [20, 21]. Furthermore, an analysis of the Dutch TME trial showed that in primary resectable rectal cancer short course radiotherapy is especially effective in the prevention of anastomotic recurrences [22, 23]. This finding confirms that it is important to know the exact location of the tumor in order to safely reduce radiotherapy treatment fields.

For these reasons we hypothesized that the use PET-CT can help to define the GTV in rectal cancer more accurate leading to a better tailored definition of the treatment volume, that it would diminish interobserver variability and diminish the time needed to define the GTV. Furthermore we hypothesized that the influence of the use of PET-CT on treatment volume would be larger in low seated tumors as compared to high rectal tumors, because of the low soft tissue contrast on CT in the lower part of the pelvis.

Methods and materials

For this study 42 patients diagnosed with rectal cancer (cT2-4N0-2M0) were selected. Patients were scheduled to undergo a neo-adjuvant treatment consisting of chemoradiotherapy (28x1.8 Gy with concurrent capecitabine 825 mg/m2 bid). All patients underwent an FDG-PET-CT scan for radiotherapy planning on an integrated PET-CT scanner (Truepoint Biograph 40, Siemens Erlangen, Germany). The PET-scan protocol has been described in detail earlier [24]. PET-CT images were fused and an automatic contour around the primary tumor was created, using the Signal-to-Background-Ratio (SBR)-method as described earlier [25, 26] using dedicated software (Esoft 5.0, Siemens MI, Erlangen, Germany). This contouring method has been shown to have a good correlation with pathology in rectal cancer [16].

We balanced the number of patients with low- and high-seated tumors (22 high, 20 low). For this study high seated tumors had a caudal border ≥7 cm from the anal verge.

For delineation, fixed window/level settings were used (400/50 for CT and 30000/15000 for PET). In PET-scans not showing enough contrast using these settings, an adjusted W/L setting was used, identical for all 5 observers.

GTV delineation and interobserver variability

The GTV was delineated by 5 observers: 2 radiation-oncologists sub-specialized in gastro-intestinal (GI) tumors, 1 senior-resident, 1 radiation-technologist and 1 radiologist. At the time of delineation clinical details were available and presented in a standardized format to each observer including the findings on digital rectal examination and the endoscopy and pelvic MR-imaging reports.

Each study set was delineated 3 times by each observer in 3 consecutive rounds. During each delineation round observers had, in addition to the standardized clinical information, access to different imaging information, creating 3 sets of GTV contours per observer. In each round an MR-scan was available. MR and CT were not fused and MR was projected on a second screen. Round 1: MR- and CT-images only (CT-GTV (GTVCT)), round 2: MR-, CT- and PET-images (PET-GTV (GTVPET)) and in round 3 in addition to the MR-, CT- and PET-images the automatic generated contour on PET was provided (automatic GTV (GTVauto)). In the third round observers were asked to edit the provided contour, in such a way to obtain a clinically acceptable GTV. For each observer, the delineation rounds were spaced with a minimum interval of 4 weeks, to prevent bias from a preceding delineation round. Observers were blinded to each other's delineations.

Time needed for each delineation was registered by the observers. The volumes of the different GTVs were collected from the planning system and compared. Pairs of contours from the 3 different delineation methods were compared by calculating the concordance index (CI), defined as the ratio of the intersection and the union of the two volumes [27, 28].

$$CI = \frac{(A \cap B)}{(A \cup B)}$$

Differences in GTV delineation were analysed for the total patient group as well as for high- and low-seated tumors separately.

CTV delineation and interobserver variability

Since the GTV is only a small part of the clinical target volume in the current treatment of rectal cancer, 3 observers also delineated the complete CTV as used in daily clinical practice (CTV_{compl}), including regional lymph nodes, according to our local protocol as described earlier [19]. In brief, the CTV_{compl} included at least 3 cm of the rectal wall in the oral and aboral direction, to cover possible intramural tumor spread, the mesorectal subsite, posterior pelvic subsite, and the regional lymph nodes at risk, which were defined by contouring the internal iliac vessels with a margin of 5 mm and the obturator region for low seated tumors (<7 cm from the anal verge in this protocol). The obturator region was delineated as proposed by Roels et al [29]. The CTV of the primary tumor was obtained by circumferential expansion of the GTVs with 0.5 cm, resulting in CTV_{CT}, CTV_{PET} and CTV_{auto}. The percentages of the different CTVs located outside the CTV_{compl} were analysed.

We were particularly interested in the caudal tumor extension. There is evidence that the risk of microscopic disease in lymph nodes >4 cm caudal from the caudal border of the primary tumor is very limited [20, 21]. Reducing the caudal CTV extension could result in a reduced radiation dose delivered to the anal sphincter, decreasing the risk of sphincter dysfunction as described in patients undergoing pre-operative radiotherapy followed by sphincter sparing surgery [18, 30]. Therefore we also analyzed if the caudal border intramural margin differed between the CT- and PET-based delineations.

Statistics

SPSS 17.0 (SPSS, Chicago, IL) was used to perform statistical analysis. For the comparison of the differences in time needed to perform the delineations a paired-samples t-test was used. The volumes of the GTVs, CIs of both methods, as well as the percentages of CTV lying outside the CTV_{compl} and differences in caudal borders were compared using the Wilcoxon signed rank test, because

data did not follow a normal distribution. Two-sided p-values are provided; p-values <0.05 were considered significant.

Results

The availability of PET images resulted in about 40% decrease in time needed to complete GTV delineation (mean time GTV_{CT} 4.1 min, GTV_{PET} 2.5 min (p<.001) and GTV_{auto} 1.6 min (p<.001)).

The volumes for each observer are shown in table 1. GTV volumes were significantly smaller using PET-scan (mean GTV_{CT} 46.8 cm^3 vs. mean GTV_{PET} 28.8 cm^3 (p<0.001)). Editing automatically created contours resulted in the smallest volumes (mean GTV_{auto} 18.2 cm^3 (p<.001)).

Table 1 Mean GTV volumes for the 5 different observers using three different delineation methods.

Observer	CT-based			PET-based			PET-auto					
	Mean	Range		Mean	Range		p	Mean	Range		p[1]	p[2]
All	46.8	6.3	185.7	28.8	1.5	131.9	**<0.001**	23.6	2.4	96.3	**<0.001**	**<0,001**
1	41.2	6.3	159.3	26.0	1.5	107.3	**<0.001**	23.3	2.4	88.1	**<0.001**	0,27
2	52.7	11.6	168.5	26.2	3.8	115.3	**<0.001**	23.0	2.8	87.5	**<0.001**	0,24
3	48.8	9.6	185.7	37.2	7.7	131.9	**<0.001**	25.3	2.5	93.6	**<0.001**	**<0,001**
4	43.6	7.3	166.6	29.6	2.6	119.7	**<0.001**	23.6	2.7	89.7	**<0.001**	**<0,001**
5	47.4	7.9	167.5	25.2	4.5	128.7	**<0.001**	22.9	2.5	89.9	**<0.001**	0,21

[1] Compared with CT-based delineation

[2] Compared with PET-based delineation

An example of the delineation of 2 patients is depicted in figure 1. Conformity indices increased when PET information was added, reflecting a better agreement between observers. The mean conformity index (mean±SD) for the 5 observers was 0.79±0.17 (range: 0-0.98) using CT only in combination with MRI, 0.82±0.16 (range: 0.10-1.00, p=0.103) using PET-data without automatically created contours and 0.93±10.5 (range: 0.28-1.00, p<0.001) using PET auto-

contours (figure 2). Using CT-scans only, in 2 cases a complete disagreement between observers occurred (reflected by a CI of 0).
No differences were found between low- and high-seated tumors: 0.78 vs 0.79 (p=0.31) for CT-only, 0.82 vs. 0.82 (p=0.50) for PET-manual and 0.93 vs 0.92 (p=0.94) for PET with auto-contours (figure 2).

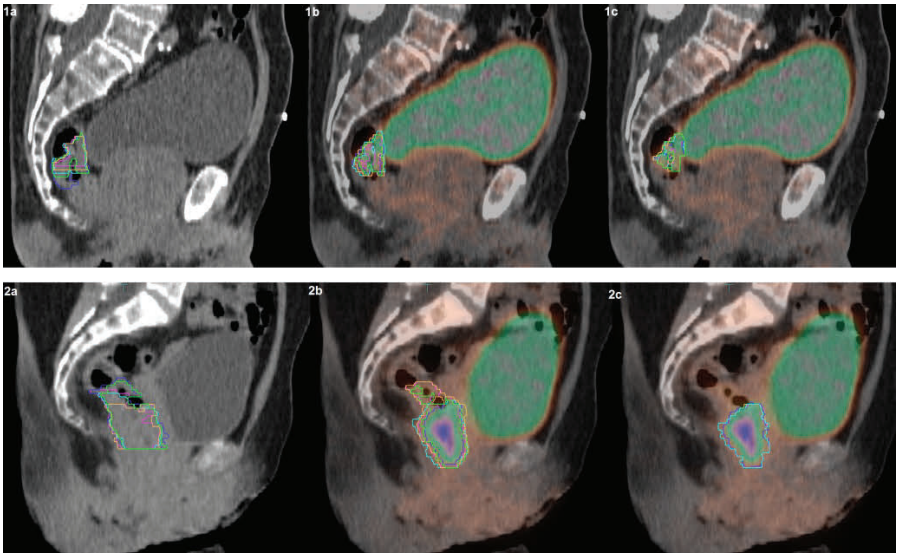

Figure 1 Example of the delineations by 5 observers in a patient with a high-seated (1) and a low-seated tumor (2), based on CT-only (a), PET-CT (b) and PET-CT with auto-contour (c).

The analysis of the CTVs showed that, with the addition of PET, in some patients a part of the tumor CTV was not covered by the CTV_{compl}. The mean CI for CTV_{PET} was 0.98 (range: 0.27-1.00) and for CTV_{auto} 0.98 (0.29-1.00). For CTV_{PET} the percentage of volume lying outside CTV_{compl} exceeded 5% in 8 cases (19%) (4 times in observer 1, 2 times in observer 2 and 2 times in observer 3). For CTV_{auto} this was the case in 12 delineations (29%) (6 times in observer 1, 3 times in observer 2 and 2 times in observer 3). In 1 patient more than 75% of the CTV_{PET} and CTV_{auto} was lying outside the CTV_{compl} in 2 observers. This was a high seated tumor which was not correctly delineated based on CT and MR only, but was correctly identified on PET.

On average, the caudal border of the intramural margin was located 0.6 cm more cranial if based on automatic PET-contours as compared to CT. In 7 patients (17%), the caudal border of the PET-based margin was located 1 cm or more caudal than the CT-based margin for at least one observer. Four of them had a high seated tumor, 3 had a low seated tumor. In 3 of these patients the intramural margin extended ≥1 cm caudally based on PET for all 3 observers. In 19 (45%) patients the caudal border of the PET-margin was located ≥1 cm higher than the CT-margin. In 6 patients this difference of ≥1 cm was observed in all 3 observers, in another 6 patients in 2 observers and in the remaining 7 patients in 1 observer. Sixteen of these tumors were located high and 3 were located low. On average the caudal border of PET-margin of high-seated tumors was located 1.1 cm more cranial than CT-margin. For low seated tumors the difference was negligible (figure 3). The difference between low- and high-seated tumors was statistically significant.

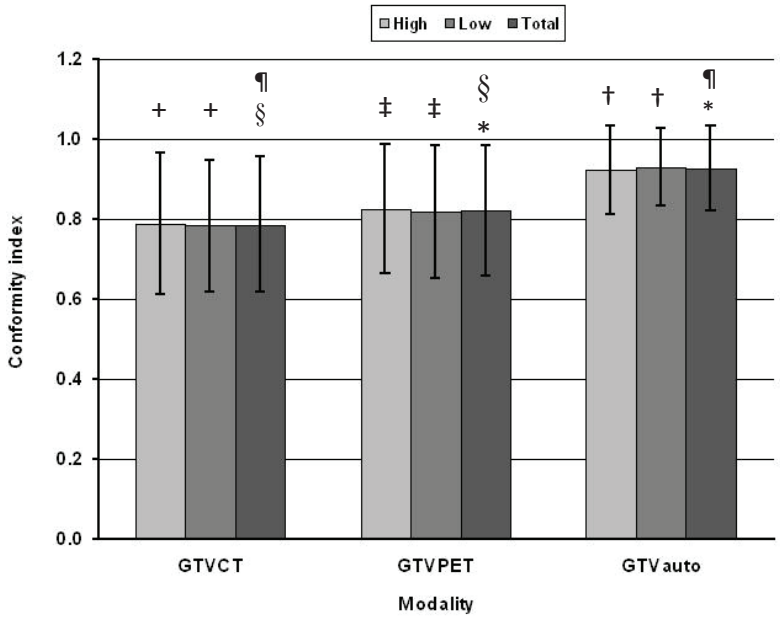

Figure 2 Concordance index according to delineation method (CT, PET-CT and PET-CT with autocontour) and divided in low seated (≤7 cm from the anal verge) and high seated tumors. +: p=0.31, ‡: p=0.50, †: p=0.94, §: p=0.103, ¶: p<0.001, *: p<0.001

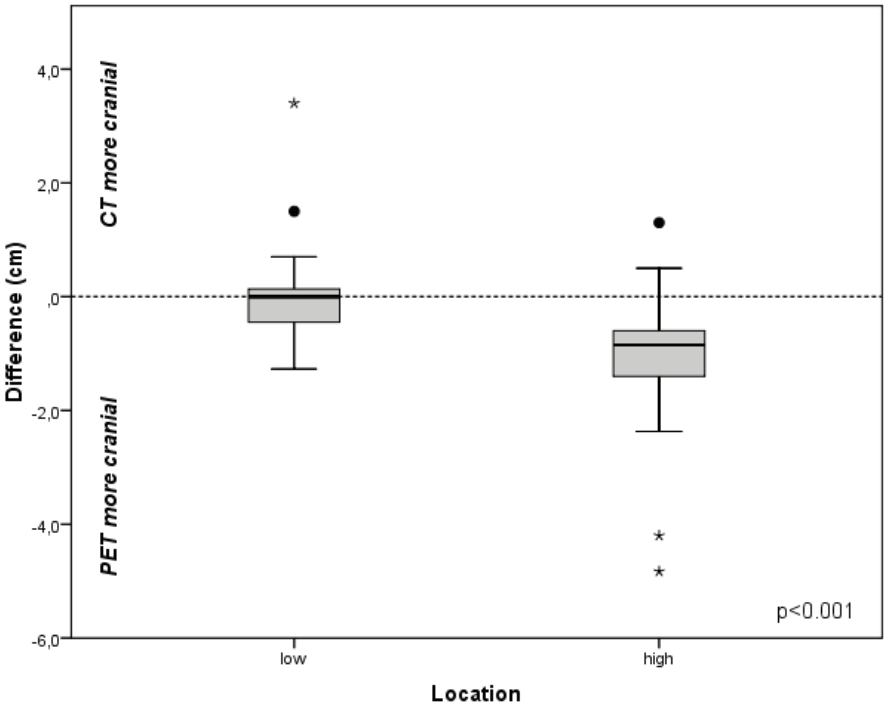

Figure 3 Mean difference ± SD in cm of the 3 observers who delineated the complete treatment volume between the most caudal extension of the intramural margin based on CT and the caudal boundary based on the automatic PET-based contour. A negative value means that the caudal border based on PET was located more cranial than the CT-based border (i.e. in that case the volume based on PET was shorter in caudal direction).

Discussion

To the best of our knowledge, this is the first study looking at the influence of the use of automatically created PET-contours on GTV delineation and interobserver variability in rectal cancer. As has been shown earlier, the SBR method results in contours with a very good correlation with pathology [16]. The smallest GTV volumes are created using PET-based autocontours as compared to CT-based contours and manual PET-based contours (23.6 cc vs. 28.8 cc vs. 46.9 cc). This study confirms that the use of PET-based autocontours leads to a very good interobserver agreement, reflected by a mean CI of 0.93 for

automatic PET-contours as compared to 0.82 (manual PET-contours) and 0.79 (CT-based contours). Furthermore, we found that PET-scan may help to avoid geographical misses in selected cases, especially in very low and very high located tumors and therefore may be helpful to define an adequate boost volume. In addition it could help to reduce treatment fields leading to a reduction of the amount of sphincter in the radiation volume, possibly leading to less late sphincter related toxicity and it allows for GTV-boosting in dose escalation trials.

A better interobserver agreement using PET-scan has been shown for the delineation of lung-, brain- and head and neck tumors [31-33]. Furthermore it has been demonstrated that the use of automatically created contours results in better agreement than visual interpretation of PET [34]. Obviously, visual interpretation depends on many factors, like window/level settings and the display mode of the PET information (for example grayscale or colormode) and experience of the observers. In this study 5 observers with different experience and background were asked to delineate the GTVs. Although it is difficult to draw firm conclusions, we observed that the differences in volumes differed statistically significant for all but 2 observer combinations when using CT data only. Differences in volume became smaller using PET-data and CIs decreased for all observers, indicating that PET is helpful for observers with different levels of experience in delineation of rectal tumors.

Other groups have looked at the influence of PET on target volume delineation before. Ciernik et al [35] was the first group that published about the use of PET-CT in delineation for rectal cancer using a growing region algorithm. They found a good correlation with GTVs created manually on CT, but did not analyse the influence of PET information on delineation decisions by the physician. No comparison was made between different observers. In a study of Bassi et al [36] tumor delineation was done by 2 radiation-oncologists together. They concluded that GTVs based on PET were significantly smaller than CT-based GTVs, which is in line with our findings. Patel et al compared delineations of tumor and lymph nodes in 6 rectal cancer patients who underwent an FDG-PET

as well as FLT-PET [37]. In contrast to our findings, they did not observe clear differences in GTV volumes, but the interobserver agreement was better using PET. No differences between FDG-PET and FLT-PET were seen and no SUV-based auto-contour was used.

Results of rectal cancer treatment have improved markedly in the last decades, due to better surgical techniques and the widespread use of radiotherapy [1]. However, the use of radiotherapy results in long-term toxicity in a substantial part of patients. Therefore, it is important to make a better patient selection on one hand and to tailor treatment fields as much as possible on the other hand. Irradiation of the sphincter may result in problems with fecal continence [38, 39]. In the Dutch TME trial 62% of patients without a stoma reported faecal incontinence or soiling in the radiotherapy and surgery arm versus 38% in the surgery only arm [18]. This study shows that the use of PET-scan can help to better tailor treatment fields. Especially the caudal border of the radiation fields can be limited in an important proportion of patients, resulting in a lower sphincter dose and a lower dose to the distal rectal wall in higher seated tumors, without the risk of geographical misses. Theoretically this could lead to less late toxicity. We hypothesized that the influence of PET on GTV volume and caudal extension of the treatment volume would be largest in lower seated tumors. This study showed that influence of PET was not different between low- and high-seated tumors. Therefore, the use of PET may be beneficial to all rectal cancer patients. When looking specifically at the influence of imaging modality on the position of the caudal border of the treatment volume (figure 3), it can be concluded that PET is helpful to individualize this border. In some patients this border will be located more cranial when based on PET, possibly resulting in a lower dose to the sphincter and distal rectal wall, while in others it will be located more distal. For the analysis of the caudal border we included a 3 cm margin in the course of the rectal wall in both directions. In our protocol we do not stop this margin at the border of the sphincter. For example: if the caudal border of the tumor is located 2 cm cranial to the sphincter, 1 cm of the anal canal was included in the intramural margin. It can be argued whether this is

really necessary or whether one could see the anorectal junction as an anatomical barrier. Of course this can influence the results of our analysis. Recent literature suggests that distal surgical margins as close as 1 cm may be safe, but this is based on surgical data of patients who have been treated with pre-operative radiotherapy in majority [40, 41]. Therefore, we do not feel comfortable at the moment to leave the complete sphincter out of the treatment volume in very low lying tumors and use the intramural margin based on PET-scan.

As stated in the introduction, adequate identification of the tumor is essential to create a reliable boost volume. This allows studying if a boost to the primary tumor results in more pathological complete responses and if this can lead to the use of less invasive surgery. This study shows that use of PET results in a good agreement between observers and our pathology validation study showed a very strong agreement between tumor length defined by automatic PET-contours and measured by the pathologist in the surgical specimen. If we assume that the representation of the position of the tumor and tumor edges is accurate, we can conclude that PET-CT makes it possible to define a reliable GTV in rectal cancer. Although we think that this assumption is very plausible, no analysis of the position of the tumor on PET-CT and in vivo has been performed. Therefore, in clinical practice this method should be used with caution and a clinical prospective evaluation is necessary.
A second problem that has to be solved to define an adequate boost volume is the internal organ motion, which can be quite substantial in the case of rectal cancer. It has been shown that especially in the cranial part of the mesorectum deformations can be quite substantial and that these deformations are caused mainly by differences in bowel filling [42].

Although a PET-scan adequately images the primary tumor and can be used for tumor delineation, it is not reliable for the distinction between benign and pathological lymph nodes [43]. The specificity is acceptable, but the sensitivity is rather low [44-46]. Therefore, additional imaging is strongly needed to

adequately identify positive nodes. MR in combination with special contrast agents seems to be a promising method [47]. For this study we did not compare different PET segmentation algorithms, because we found a good correlation between pathology and SBR-based PET-contours. However, the SBR-method has several disadvantages [48]. It is dependent on many parameters, so that each modification in the process makes it necessary to perform a new calibration and each scanner has to be calibrated separately. In addition this method does not perform well if the source-to-background ratio is low. In future projects we will compare the performance of other segmentation methods with the SBR method in rectal cancer. Apart from a useful tool in delineation, FDG-PET can also be helpful to get insight in tumor heterogeneity. Our group has shown that in NSCLC residual metabolic active areas after radiotherapy are the areas with the highest uptake before treatment [49, 50]. Recently we showed that this is also the case for rectal cancer [51]. Another step for the future could therefore be the development of sub-boosting techniques.

In conclusion, PET-CT reduces interobserver variation and volumes in GTV definition in rectal cancer, enables tailoring treatment fields, especially in cranio-caudal direction, and makes it possible to define volumes for boosting. The use of PET-CT makes it possible to create a reliable boost volume in the treatment of rectal cancer and is expected to reduce toxicity due to the tailoring of treatment fields.

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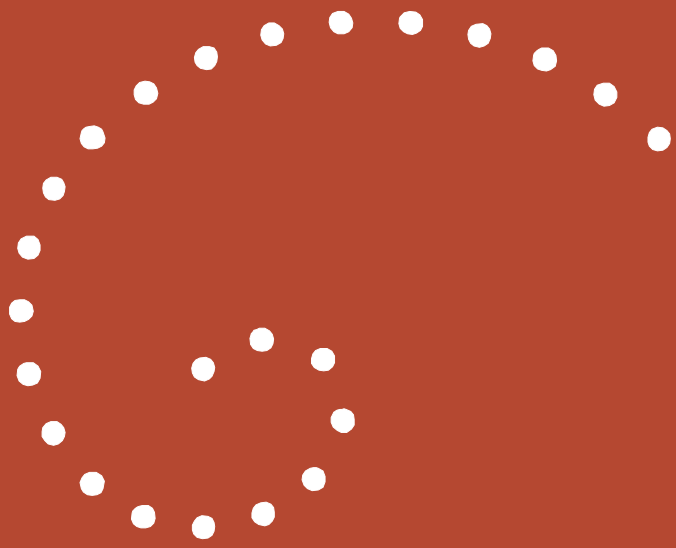
Part II
Response prediction in rectal cancer treatment



Chapter 4

Development and external validation of a predictive model for pathological complete response of rectal cancer patients including sequential PET-CT imaging

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Abstract

Purpose

To develop and validate an accurate predictive model and a nomogram for pathologic complete response (pCR) after chemoradiotherapy (CRT) for rectal cancer based on clinical and sequential PET-CT data. Accurate prediction could enable more individualised surgical approaches, including less extensive resection or even a wait-and-see policy.

Methods and materials

Population based databases from 953 patients were collected in four different institutes and divided into three groups: clinical factors (training: 677 patients, validation: 85 patients), pre-CRT PET-CT (training: 114 patients, validation: 37 patients) and post-CRT PET-CT (training: 107 patients, validation: 55 patients). A pCR was defined as ypT0N0 reported by pathology after surgery. The data were analysed using a linear multivariate classification model (support vector machine), and the model's performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve.

Results

The occurrence rate of pCR in the datasets was between 15% and 31%. The model based on clinical variables (AUC_{train}=0.61 ± 0.03, $AUC_{validation}$=0.69 ± 0.08) resulted in the following predictors: cT- and cN-stage, and tumor length. Addition of pre-CRT PET data did not result in a significantly higher performance (AUC_{train}=0.68 ± 0.08, $AUC_{validation}$=0.68 ± 0.10) and revealed maximal radioactive isotope uptake (SUV_{max}) and tumor location as extra predictors. The best model achieved was based on the addition of post-CRT PET-data (AUC_{train}=0.83 ± 0.05, $AUC_{validation}$=0.86 ± 0.05) and included the following predictors: tumor length, post-CRT SUV_{max} and relative change of SUV_{max}. This model performed significantly better than the clinical model (p_{train}<0.001, $p_{validation}$=0.056).

Conclusions

The model and the nomogram developed based on clinical and sequential PET-CT data can accurately predict pCR, and can be used as a decision support tool for surgery after prospective validation.

Introduction

Over the past decades, treatment outcomes for rectal cancer have changed dramatically. A better surgical technique, total mesorectal excision (TME), and the introduction of neoadjuvant treatments in locally advanced rectal cancer (LARC) have significantly decreased the risk of locoregional relapse [1, 2]. In the last nine years at least seven published phase III trials have evaluated the role of adjuvant radiotherapy in rectal cancer [3]. These have provided an evidence base demonstrating the efficacy of both preoperative radiotherapy and preoperative concurrent chemotherapy (CRT). CRT has been reported to induce significant tumor downsizing and downstaging [4-6], with a pathologic complete response (pCR) after CRT observed in 10% - 30% of patients [2, 4-8]. Although some studies showed no correlation [9], many others reported that patients showing a pCR following preoperative CRT have improved long-term outcomes including excellent local control rates and disease-free survival, regardless of their initial clinical T- and N-stages [10-13].

However, despite the often phenomenal downsizing and sometimes even complete pathological responses after CRT, these patients are still operated with a standard extended surgical procedure due to the lack of reliable accurate preoperative diagnostic tools. However, it may be questioned whether a standard resection is still necessary, considering the good outcome of these patients reported with less invasive treatments [14, 15]. If accurately selected, patients with a complete response (no residual tumor) may undergo a less extensive resection or even a so called ‘wait-and-see’ policy. Compared to standard surgery, the benefits of these treatments are reduced morbidity and mortality (e.g., anastomotic leakage, relaparotomy, wound and pelvic infection, abscess, colostomy, chronic wound healing disturbances, faecal or urinary incontinence and sexual dysfunction), improved quality of life and reduced treatment costs.

Thus, an accurate prediction of pCR can help in the selection of patients for more optimised treatment, sphincter-preserving surgery, less extensive resection, more intense radiation treatment, or even delayed surgery with a

wait-and-see policy [2, 3, 16]. These considerations led to the overall goal of this study: to develop an accurate, data-driven model to predict pathologic complete response for rectal cancer patients as decision support for more individualised treatment approaches in the future.

The clinical variables associated with a better response to preoperative CRT include circumferential tumor extent, tumor differentiation, preoperative classification, carcinoembryonic antigen (CEA) level, distance from anal verge, and time to surgery [6, 17, 18].Recently, it has also been suggested that PET imaging might be correlated with tumor response after CRT in locally advanced rectal cancer. However, the studies involved used only a small number of patients, which meant that contradictory results were found. Further, only semi-quantitative PET measurements were used and analysed with univariate statistics [4, 5, 7, 19-26]. Multivariate analysis was performed in only one study, whose results lacked statistical significance [27]. Notably, no studies verified and validated their results with external datasets, despite the fact that this represents an important prerequisite for the generalizability of prediction models for other institutes.

In the current study, population based data from four different institutes were collected and used to train and validate predictive models for pCR. We hypothesised that the addition of PET imaging data to clinical variables significantly increases the performance of prediction models for pCR after CRT as compared to models based on clinical data alone.

The study was performed within the framework of a decision support system based on centralised datasets. The increasing amount of available patient information requires automatic methods for model building and analysis. Machine learning methods can be used to update the models continuously by feeding them with information of new patients. The increasing complexity of prediction models, too, means that the representation and interpretation of the results also become more important. Tools to enhance interpretation for the

clinic include visualisation techniques such as nomograms and graphical networks. Nomograms are statistical tools that enable users to calculate the overall probability of a specific clinical outcome for an individual patient [28]. In this study, the nomogram with the highest accuracy for the prediction of pCR is provided.

Methods and materials

Study population

Six population based datasets were collected in four institutes: Maastro Clinic (GROW, MUMC, Maastricht, the Netherlands), Università Cattolica del S.Cuore (Rome, Italy), S. Maria della Misericordia Hospital (Rovigo, Italy) and University Hospital Gasthuisberg (Leuven, Belgium). In total, 953 patients met the criteria for inclusion: long-course RT with neoadjuvant chemotherapy and the availability of pathological outcome for pCR. Of these, 276 patients underwent a pre-CRT PET scan (one week before the start of CRT), and 169 patients had both pre- and post-CRT PET scans (one week before surgery, and six to eight weeks after the end of CRT). The sequential PET data from Rovigo have already been published as a prospective study[20], the Leuven data were collected prospectively for the BioCare project (LSHC-CT-2204-505785) and the rest of the data were gathered for a population-based study registered in the Dutch Trial Register (NTR2166). All compositions of the cohorts were approved by the local IRB committees. The patient characteristics are reported in table 1. The datasets were divided into three groups, based on PET data availability: 1. clinical variables only, 2. clinical variables with pre-CRT PET variables (PET-pre), 3. clinical variables with both pre- and post-CRT PET variables (PET-post). For each group, a training set and an external validation set were defined. The training sets were used to identify the pCR predictors, while the validation sets were used to test the performance of the models in other centres. Datasets from a single centre with the highest number of patients were used for training. A dataset was deemed not useful for external validation if it originated from the same centre as the corresponding training set. The definition of the different

combined training and validation sets is explained in table 2, based on the datasets in table 1.

The available clinical variables were age, gender (0: female, 1: male), clinical tumor (cT) and nodal (cN) stage, and two variables based on MRI (or endoscopy if MRI was unavailable): tumor location categorised in three levels (1: low, 0–5 cm from anal verge; 2: mid, 5–10 cm from anal verge; 3: high, >10 cm from anal verge) and tumor length (cm). For the patients who had PET-CT scans, the tumors were semi-automatically contoured at Maastro Clinic using dedicated software (TrueD, Siemens Medical, Erlangen, Germany). Standardised uptake-value (SUV) thresholding was based on the tumor-to-background signal ratio, with the gluteus muscle as reference background [29, 30]. From the resulting tumor contour, maximal tumor diameter (MaxD), gross tumor volume (GTV), and maximal and mean SUV values within the GTV were calculated. If the post-CRT PET-CT scan was available, the same variables were scored, and a response index (RI) for each variable was calculated. For variable X, the response index is the relative percent difference between the value of the post-CRT and pre-CRT and it was defined as $RI = (X_{pre} - X_{post}) / X_{pre} * 100\%$. Thus, six variables were evaluated for the clinical dataset, 10 for the PET-pre dataset and 18 for the PET-post dataset. From these sets, the models selected subgroups of variables with significant predictive value for pCR.

All patients underwent surgery. Pathological complete response was defined as ypT0N0, extracted from the pathologic reports of surgical specimens. All other cases (ypT+ and/or ypN+) were considered non-responders, making the pCR a binary outcome (0/1). The specimens were not re-evaluated centrally but the pathology protocols were very similar between institutes (3-5 mm slices of rectum tumor, intensified evaluation on several blocks of tissue at the tumor site, evaluation on 2-3 sublevels when no tumor tissue was found in initial block).

Statistical analysis

Missing values in the dataset were substituted by the mean [31]. This method

performed similarly to other, more complex substitution methods for small percentages of missing values (e.g., expectation-maximisation imputation, regression estimation). No variables in the datasets exceeded 5% of missing values. Patients who missed tumor location and length in the clinical datasets (Roma: n=132 and Maastricht: n=29) were excluded because of too large amounts of missing data for these variables. All patient numbers stated in this paper were extracted after the missing value procedure. To compare the weights of significance assigned to the variables by the model, all variables were normalised by subtracting the mean, and then divided by the standard deviation.

To classify the complete responders and non-responders, a linear multivariate method suitable for binary classification from the machine learning field was used: the support vector machine (SVM) [32].The SVM variant used (proximal SVM or pSVM) performs equally accurately but much faster than normal support vector machines [33]. The different datasets' performances in predicting pCR were evaluated by analysing the area under the curve (AUC) of the receiver operating characteristic (ROC) curve[34].The maximum value of the AUC is 1.0, indicating a perfect prediction model; a value of 0.5 indicates a random chance of correct prediction.

To select the variables that contribute to pCR prediction, an exhaustive feature search was performed, with all possible variable combinations used as input for the pSVM model. The set of variables resulting in the highest AUC was selected as the final predictive set. To avoid over-fitting of the model through selection of the highest AUC, the variable sets resulting in AUCs that deviated less than 5% from the maximal AUC were compared to the final variable set. If conflicts occurred or if variables did not contribute significantly, selected variables were interchanged by considering their prevalence in the highly predictive sets, the factor analysis and the Spearman correlation coefficient (i.e., highly correlated and dependent variables are not present in the same predictive set). Furthermore, an extra univariate analysis was performed using the Wilcoxon rank sum test.

Classification methods normally require at least several hundred cases. Because of the relatively small number of available patients, two extra evaluation methods were used. The first was leave-one-out (LOO) cross-validation, used to calculate an AUC for the training set. In LOO cross-validation, a single patient is selected from the original training dataset and used as the validation dataset, while the data from the remaining patients are used to train the model. This is repeated until all patients have been selected once for validation. However, no LOO cross-validation was used for the external dataset. The second evaluation method was bootstrapping, which results in a more accurate approximation of the real dataset distribution [35]. This means that 1000 datasets are generated from the original dataset containing *n* patients by selecting these *n* patients, but with resampling (i.e., patients can be present in the dataset more than once). For every bootstrapped dataset, an AUC was calculated. The mean AUC with the corresponding standard deviation was then calculated with size 1000. This non-parametric method allows comparison of the confidence intervals of the AUCs of different datasets without making assumptions about the AUC distributions [36]. The distribution of the difference in mean AUC (ΔAUC) between the datasets was tested by calculating the two-sided p-value, i.e., the fraction of ΔAUC samples smaller or larger than zero (depending on the dominant sign of ΔAUC).

Nomograms can reduce statistical predictive models to a single numerical estimate of the probability of an event, and visualise the effect of each selected variable on this probability [37]. The model output of the pSVM models consists of assigned weights for each variable and an offset. The probability of a patient having a pCR can be calculated using logistic regression on the pSVM output [38]. The complete procedure to convert SVM output to a nomogram is described in detail elsewhere [39]. Developing a nomogram requires threshold selection in the ROC curve. For response prediction specificity is most important, because it is not preferred to predict non-responders as responders, which would result in under-treatment. Therefore, the threshold was selected in such a way that at least 90% of non-responders were correctly predicted. Partial

ROC curve optimisation has been tested but it had no gain for specificity compared to overall AUC maximisation [40]. Calibration of the nomogram, i.e., the agreement between predicted probability of complete response and true probability in the population, was performed by an assessment of the overall agreement and the Hosmer-Lemeshow statistic in four subgroups of patients in the validation data. The nomogram algorithm was implemented in MATLAB (version 7.1, MathWorks Inc., Natick, MA), as were all algorithms described in this section.

Results

The occurrence of pCR in the patient population varied between 15% and 31% (mean: 21.8%, SD: 5.4%) depending on the dataset (Table 1). A first evaluation of CRT's effect on the tumor demonstrated significant downsizing of the tumor in the PET-CT, and a significant decrease in metabolic activity within the tumor (Figure 1). Both gross tumor volume and maximal SUV decreased significantly between the pre- and post-CRT PET-CT scans ($p<0.001$).

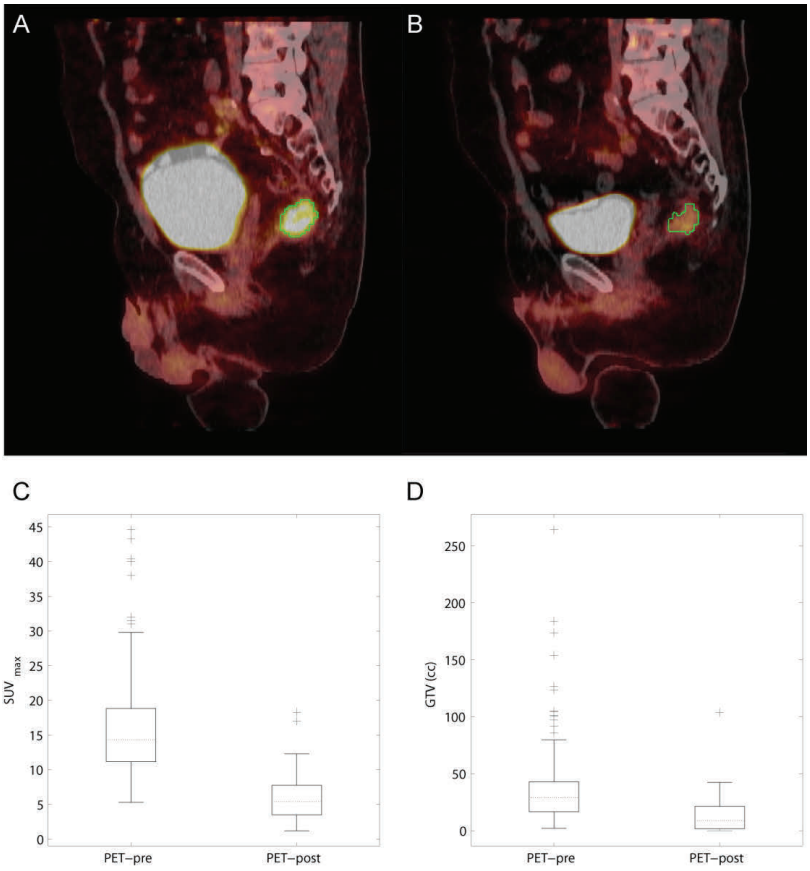

Figure 1 (A) Tumor contour in a fused FDG-PET-CT made pre-CRT. (B) Corresponding post-CRT FDG-PET-CT scan with tumor contour. (C) Boxplot of SUV_{max} on PET-scans made pre-CRT and post-CRT; significant decrease: $p<0.001$ (D) Boxplot of the GTV for the case of pre-CRT and post-CRT; significant decrease: $p<0.001$.

Table 1 Patient characteristics for six datasets from four different institutes. Clinical, PET-pre and PET-post groups are defined. Percentages of the total patient numbers are given for binary or ordinal variables. Mean and standard deviation (SD) are given for continuous variables. *x* denotes missing values. RT = Radiotherapy, PF = per fraction.

Center	Maastricht		Rome		Rovigo	Leuven
Dataset	M1	M2	R1	R2	C1	L1
Period	2004–2006	2004–2006	1984–2008	2007–2008	2003–2007	2005–2007
# Patients	114	21	677	18	107	16
Clinical	Validation	-	Training	-	-	-
PET-pre	Training	-	-	Validation	-	Validation
PET-post	-	Validation	-	Validation	Training	Validation
Gender (%)						
Male	63	67	63	83	74	81
Female	37	33	37	17	26	19
Age						
Mean	65.6	66.1	61.3	60.4	66.3	58.6
SD	10.0	10.6	10.2	7.1	10.8	10.1
cT (%)						
1	0	0	0	0	0	0
2	1	0	3	11	0	0
3	68	81	86	56	90	94
4	30	14	11	33	10	6
x	1	5	0	0	0	0
cN (%)						
0	25	38	23	17	51	0
1	48	48	45	33	38	62
2	26	10	30	50	10	38
x	1	4	2	0	1	0
cM (%)						
0	73	71	100	94	100	100
1	25	19	0	6	0	0
x	2	10	0	0	0	0
ypT0N0 (%)						
No	85	81	80	78	76	69
Yes	15	19	20	22	24	31
RT dose						
Mean	50.4	50.4	49.0	52.7	55.7	45.7
SD	0	0	5.5	3.3	3.1	1.8
RT dose PF	1.8	1.8	1.8	1.8	2.2	1.8
# Chemo Types	1	1	11	2	1	1

Table 2 Predictor selection and ROC analysis. Predictive variables are given with their corresponding assigned normalized weights from multivariate analysis (MVA). For each variable the p-value from univariate analysis (UVA) is given. Mean AUC and standard deviation (SD) are given for each variable set. RI = response index, SUV = standard uptake value, MaxD = maximal diameter (PETCT).

Variable set	Type	Size	Predictors (MVA)	Weights (MVA)	p-value (UVA)	AUC	SD
Clinical	Training (R1)	677	Tumor length	-0.085	<0.001	0.61	0.03
			cT-stage	-0.074	0.001		
			cN-stage	-0.060	0.001		
	Validation (M1)	85	-	-	-	0.69	0.08
Clinical + PET-pre	Training (M1)	114	$MaxD_{pre}$	-0.12	0.003	0.68	0.08
			cN-stage	-0.12	0.001		
			Tumor location	0.094	0.84		
			$SUV_{max\text{-}pre}$	-0.087	0.29		
	Validation (R2, L1)	34	-	-	-	0.68	0.10
Clinical + PET-pre + PET-post	Training (C)	107	RI_{SUVmax}	0.20	<0.001	0.83	0.05
			Tumor length	-0.20	<0.001		
			$SUV_{max\text{-}post}$	-0.14	<0.001		
	Validation (M2, R2, L1)	55	-	-	-	0.86	0.05

Table 2 shows the predictor selection results and the ROC curve analysis. For the clinical dataset, the univariate analysis reveals three variables significantly associated with pCR (95% confidence interval): tumor length ($p<0.001$), cN-stage ($p=0.001$), and cT-stage ($p=0.001$). These variables were also selected in the multivariate analysis. The normalised weights assigned to them by the pSVM model are tumor length (-0.085), cT-stage (-0.074), and cN-stage (-0.060). The selected variables were ranked in importance (i.e., weights). The sign of the weights can be interpreted by the effect on the probability of a pCR. For a negative sign, this probability decreases when the variable increases. For the clinical dataset, this means that the probability of a pCR increases for small tumor lengths and low cT- and cN-stages. The predictive performance of the clinical dataset for pCR, expressed by the AUC of the ROC curve, is 0.61 ± 0.03 (mean ± SD) for the training set and 0.69 ± 0.08 for the external validation set.

For the dataset with pre-CRT PET data, the multivariate analysis selected these variables (ranked by weight): maximal diameter (-0.12), cN-stage (-0.12), tumor location (0.094), and SUV_{max} (-0.087). This resulted in a high probability of pCR for patients with small maximal tumor diameters, low cN-stage, high tumor locations, and small maximal metabolic activity. Maximal diameter (p=0.003) and cN-stage (p=0.001) were selected by univariate analysis, while the other two variables were not. The AUCs for the training and validation set were both 0.68, but the SD differed (0.08 and 0.10 respectively).

The dataset including the post-CRT PET data resulted in the highest performance: AUC_{train} = 0.83 ± 0.05 and $AUC_{validation}$ = 0.86 ± 0.05. The response index for SUV_{max} (0.20), tumor length (-0.20), and the post-CRT SUV_{max} were found to be predictive for pCR and significantly associated with pCR in the univariate analysis (p<0.001).

In evaluating the predictive value of the additional PET data to the clinical data, only the AUCs of the post-CRT PET data differed significantly from the clinical dataset AUC (Figure 2). The p-value for the AUC difference for the training set was <0.001, while that for the validation sets was 0.056 (just outside the 95% confidence interval). When only post-CRT PET data were used for the models (i.e., no clinical variables), the significant difference between the AUCs and the clinical dataset was no longer observed (training: p=0.47, validation: p=0.58). This indicated that a combination of both clinical and PET data was required to reach a significantly higher performance when using PET as a predictive imaging modality.

The assigned weights for all the predictors formed the basis for the construction of the nomogram. The nomogram based on the post-CRT dataset is provided in Figure 3. The nomogram performs with a sensitivity of 0.62 and a specificity of 0.88 for the validation data. In the training phase these were respectively 0.65 and 0.90. The calibration of the nomogram (Figure 4) with the validation data reveals that the overall predicted and the actual probability are equal (23.6%,

OR=1.0). If the validation data are divided into four equally numbered groups, the Hosmer-Lemeshow test results in a p-value of 0.78, which means a good calibration in this test (p>0.05). The linear fit through these probabilities results in a slope of 1.02 with R^2 of 0.99, confirming a good balance between calibration and discrimination.

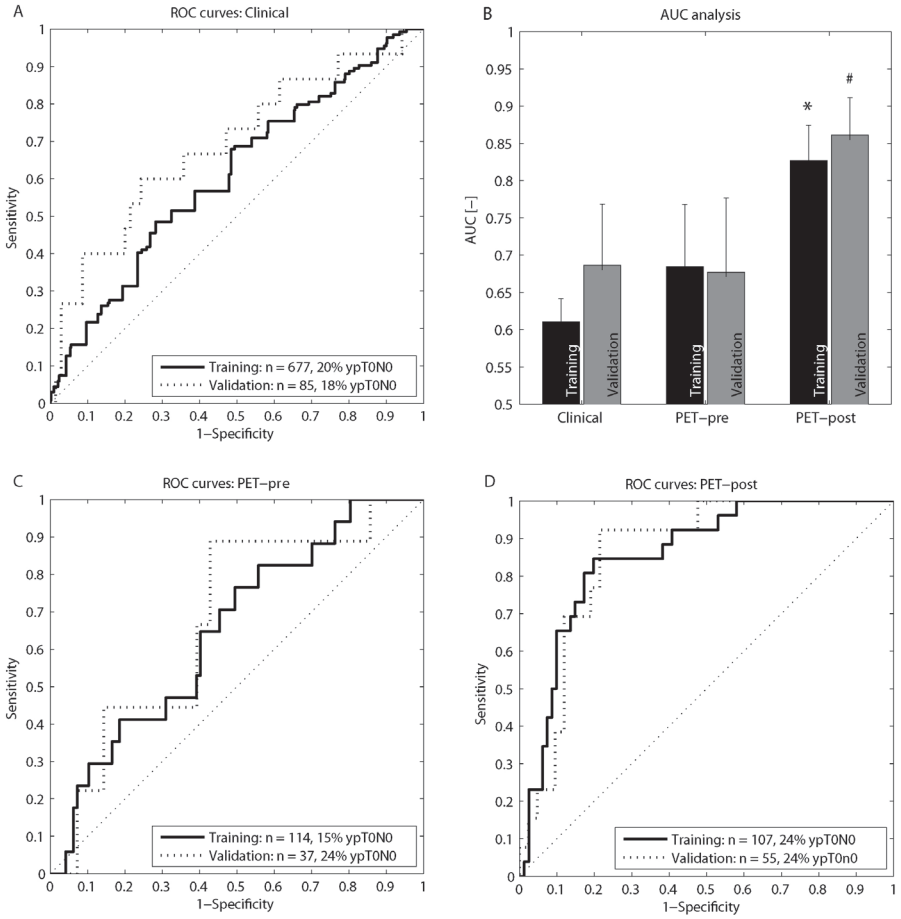

Figure 2 ROC curves of training and validation datasets for the clinical set (A), the PET pre-CRT set (C) and the PET post-CRT set (D). The straight dashed line represents a random prediction model. The bar plot (B) shows the corresponding mean AUC for each dataset and the standard deviation (error bars). There was a significant difference with clinical datasets of (*) p<0.05 and (#) p<0.06.

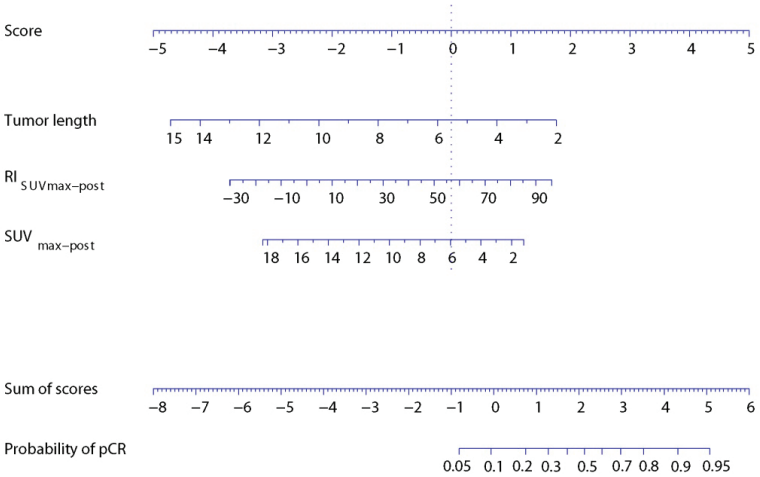

Figure 3 Nomogram for PET post-CRT dataset. A score for each predictor can be read out at the top scale (Score). All summed scores (Sum of scores scale) can be converted directly to the probability of responding with a pCR (ypT0N0). The probability scale is the only logarithmic scale.

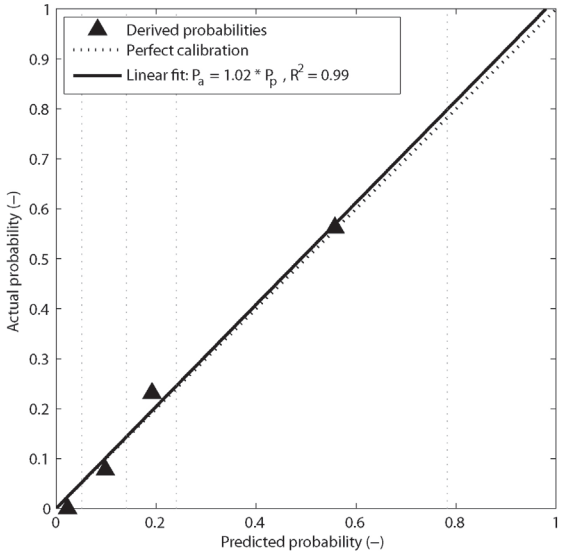

Figure 4 Calibration of the nomogram for the validation data. For the four equally numbered subgroups (vertical lined intervals in figure), the predicted probability of a pCR and the actual fraction in the population were evaluated. The dashed line represents perfect calibration and the solid line is the linear fit of the calibration data.

Discussion

We have developed predictive models based on clinical and PET-based data for pathologic complete response in patients diagnosed with rectal cancer. The performance of these models was externally validated using patient cohorts from different institutes treated with long-course preoperative chemoradiotherapy. The models showed that the accuracy of the predictions increased over time, i.e., when more information became available. Information from PET-CT scans significantly improved the performance of the models.

The significant difference in AUCs that we reported between the performance of the clinical model and the post-CRT PET data model reflects what others have found in their post-treatment PET analyses; like us, some have reported (significant) indications that the response index and post-treatment SUV_{max} are predictive for response, while the pre-treatment PET data do not provide enough predictive power [12, 19, 27]. However, our PET-based models also contain clinical variables, which appeared to be necessary to obtain the high performance provided in Table 2. The most important clinical variables were tumor length and maximal diameter, which were selected in the models and are significantly correlated (spearman $\rho = 0.55$, $p<0.001$). Overall, this means that the dominant tumor dimension in combination with (differences in) the maximal metabolic activity inside the tumor is the most predictive variable set for pCR, which was confirmed in the external datasets.

Whether the corresponding AUC of 0.86 is accurate enough for clinical practice depends on the choice of the threshold in the ROC curve. A high specificity is preferred over a high sensitivity to avoid possible under-treatment (less surgery when surgery is required) rather than over-treatment (standard treatment when less surgery could have been considered). The provided nomogram focuses on specificity (training: 0.90, validation 0.88). Selecting higher specificities results in fast decreasing sensitivities. Careful follow-up is therefore necessary for the patients selected for a ‘wait-and-see’ policy to detect any possible local

recurrences early on. To gain more specificity in the future, the addition of new variables and the other classification methods would have to be considered.

The nomogram performs well, i.e., the distribution of the probability of a pCR provided by the nomogram represents the true distribution in the data, confirmed by overall calibration, calibration of the slope and Hosmer-Lemeshow test (figure 4). Because of the number of events and the division of the patient cohorts into few probability intervals, the higher probabilities occur much less frequently and are thus the least accurate. Therefore, prospective validation of the model and the nomogram is required to ensure sufficient statistical power for clinical application of the models. Besides the number of patients to increase the models' accuracy, more predictors could be added to increase the models' performance, including biological variables such as gene signatures [41] and blood biomarkers, and also more imaging variables from (perfusion) CT and (diffusion) MRI. The first indications have also appeared that PET-CT data during CRT may be highly predictive for response [25, 26, 42]. This time point is more favourable than post-CRT because of the possibility of earlier treatment changes and the decreased presence of inflammatory rectum cases, potentially causing impaired evaluation of fused PET-CT scans. After prospective validation of the model, an intervention trial with less surgery for patients with a high probability for pCR will be performed.

The population based collected datasets date back five years, except for the clinical Roma database, which was collected from 1984 onward. Therefore, this dataset shows a higher variety in treatment schemes than the other datasets. This could explain the discrepancy of the higher prediction performance of the clinical validation set. On the other hand, the validation set is much smaller, implying that the distribution of data could not be representative of the true distribution. The consequence of population based data collection is that treatment protocols are not well tuned. This results in, for example, small differences in irradiation schemes and deviations in the evaluation of pathology outcome. Ideally, pathology is reviewed centrally to reduce the intra- and inter-

observer variability for the outcome measure. However, in this study the quality of pathology is acceptable because of the prospective nature of most datasets and because the outcome was limited to only complete response evaluation. Also, glucose correction for SUV values was not applied to all datasets. However, minor variation in treatment schemes can be seen as an advantage because it leads to higher generalizability for other centres. In other words, the model still performs well, despite the disparities mentioned here.

In conclusion, we have shown that sequential PET-CT data in combination with clinical variables significantly increase the performance of prediction models for pathologic complete response. So far, this is the largest study of its kind and the only one that used external datasets for validation. The dominant tumor dimension and the maximal uptake of radioactive isotopes in the tumor as well as its relative difference between PET scans were found to be the best predictors for pCR resulting in very good overall performance AUC's of 0.83 and 0.86 for training and validation, respectively. Including also biological and other imaging variables will probably further improve the performance. When prospectively validated, the model and the nomogram therefore provide a valuable decision support for more individualised treatment approaches in the future.

Note: The predictive models in this paper are published on the website http://www.predictcancer.org.

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Chapter 5

Blood biomarkers are helpful in the prediction of response to chemoradiation in rectal cancer: a prospective, hypothesis driven study on patients with locally advanced rectal cancer

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Abstract

Purpose/objective

Chemoradiation (CRT) has been shown to lead to downsizing in an important part of rectal cancers. In order to tailor treatment at an earlier stage during treatment, predictive models are being developed. Adding blood biomarkers may be attractive for prediction, as they can be collected very easily and determined with excellent reproducibility in clinical practice. The hypothesis of this study was that blood biomarkers related to tumor load, hypoxia and inflammation can help to predict response to CRT in rectal cancer.

Material/methods

295 patients with locally advanced rectal cancer who were planned to undergo CRT were prospectively entered into a biobank protocol (NCT01067872). Blood samples were drawn before start of CRT. Nine biomarkers were selected, based on a previously defined hypothesis, and measured in a standardized way by a certified lab: CEA, CA19-9, LDH, CRP, IL-6, IL-8, CA IX, osteopontin and 25-OH-vit D. Outcome was analyzed in two ways: pCR vs. non-pCR and responders (defined as ypT0-2N0) vs. non-responders (all other ypTN stages).

Results

276 patients could be analyzed. 20.7% developed a pCR and 47.1% were classified as responder. In univariate analysis CEA (p=0.001) and osteopontin (p=0.012) were significant predictors for pCR. Taking response as outcome CEA (p<0.001), IL-8 (p<0.001) and osteopontin (p=0.004) were significant predictors. In multivariate analysis CEA was the strongest predictor for pCR (OR0.92, p=0.019) and CEA and IL-8 predicted for response (OR 0.97, p=0.029 and OR 0.94, p=0.036). The model based on biomarkers only had an AUC of 0.65 for pCR and 0.68 for response; the strongest model included clinical data, PET-data and biomarkers and had an AUC of 0.81 for pCR and 0.78 for response.

Conclusion

CEA and IL-8 were identified as predictive biomarkers for tumor response and PCR after CRT in rectal cancer. Incorporation of these blood biomarkers leads to an additional accuracy of earlier developed prediction models using clinical variables and PET-information. The new model could help to an early adaptation of treatment in rectal cancer patients.

Introduction

Combined treatment is the cornerstone of rectal cancer treatment. In case of locally advanced rectal cancer, defined as a tumor with a predicted positive circumferential resection margin (CRM) or four or more positive lymph nodes, chemoradiotherapy (CRT) has become standard of care [1]. Pathological complete response (pCR) rates typically lie between 15 and 20% after CRT [2, 3] depending on the radiotherapy dose given and the interval between CRT and surgery. The group of patients that develop a pCR is of particular interest, because they have a better prognosis [2] and may be offered less invasive surgery [4] or surgery may be completely omitted [5, 6]. Therefore it would be an advantage if the pCR rate could be increased. There are several possible strategies, of which early response prediction during CRT, leading to treatment adaptation, is very attractive.

In the past, clinical parameters as well as information from PET-scans before and during treatment have been found to be predictive for treatment outcome [7-9]. A prediction model based on tumor length, cT- and cN-stage had a predictive performance of of 0.61 as expressed by the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. A second model including maximal standardized uptake value (SUV) of the tumor derived from a PET-scan before the start of treatment, maximal tumor diameter as measured on PET-scan, tumor location and cN-stage, resulted in an AUC of 0.68 [9]. PET-scan after 2 weeks of CRT has been shown to be very predictive for response (tumor regression grade (TRG) 1-2 vs TRG 3-5 according to Mandard) [8].

It is attractive to consider the addition of blood biomarkers to these predictors, since samples can be collected easily, are relatively cheap to measure, and they contain information about different aspects of tumor biology. Furthermore, they can be measured accurately and precisely using standardized methods. Reports on the predictive value of blood biomarkers are limited to studies mainly evaluating 1 or 2 biomarkers [7, 10-13]. The most studied biomarker for response to CRT in rectal cancer is carcinoembryonic antigen (CEA) and one study analyzed the predictive value of osteopontin and interleukin-6. Based on

these data combined with data of prognostic studies in colorectal cancer and our experience with a blood biomarker model in lung cancer [14], we decided to include 9 biomarkers. CEA and CA19-9 are related to tumor load, interleukin-6 and -8 (IL-6 and IL-8) and C-reactive protein (CRP) are markers of inflammation, lactate dehydrogenase (LDH) is a marker of cell death, carbonic anhydrase IX (CA IX) and osteopontin are hypoxia markers and 25-OH-vitamin D may induce growth arrest and apoptosis of tumor cells.
In this prospective study we tested the hypothesis that these biomarkers are helpful in the prediction of response to CRT in rectal cancer.

Methods and materials

Patient population
All patients who were treated according to the protocol "chemoradiation, rectal cancer, 50.4 Gy" between January 2005 and December 2009 and who gave written informed consent for inclusion in the biobank protocol (NCT01067872) and from whom blood samples were collected were included in this study. This query resulted in 295 patients. Nineteen patients were ineligible for different reasons (1 patient was treated with short course radiotherapy 5x5 Gy, 5 patients were not operated on and entered in a wait&see study so no ypTN stage could be determined, 2 patients underwent a TEM resection so no ypN stage was available, 6 patients had metastases and were treated with palliative intent, 1 patient died during treatment, in 1 patient all biobank material was hemolytic and in 3 patients the PA report could not be retrieved), resulting in 276 patients for analysis. In 9 patients there were technical problems with the biomarker measurements, so that 267 patients were available for the biomarker analysis. Locally advanced disease was defined as a distal T3 tumor and/or N2 status and/or a mid- or upper-rectal tumor with a predicted circumferential resection margin <2 mm, or any T4 tumor. Locoregional staging for clinical tumor and nodal stage was based on MRI. In 198 patients for whom biomarkers were available a PET-CT was made for radiotherapy planning. Treatment consisted of 28 fractions of 1.8 Gy on the primary tumor, mesorectum, presacral area and external iliac

lymph nodes in combination with capecitabine 825 mg/m^2 BID. Fourteen patients did not receive a dose of 50.4 Gy. In 12 patients treatment had to be discontinued because of toxicity and in 2 patients a lower total dose was prescribed because of radiotherapy in the past. Patients were operated on 8-10 weeks after the end of CRT. Pathology reports were collected from the referring hospitals.

Blood samples

Blood samples were collected before the start of treatment. Samples were processed and stored using a standard protocol. All biomarkers were measured in serum, except for osteopontin and CA IX, which were measured in EDTA plasma. Biomarker measurements were done in one single, certified laboratory, using commercially available kits. All samples were analyzed simultaneously. Measurements were performed using the following kits: CEA was measured using a solid-phase, two-site sequential chemoluminescent immunometric assay (Siemens Medical Solutions Diagnostics, LA, USA), IL-6 and IL-8 were determined with a solid phase, enzyme labeled, chemoluminescence sequential immunometric assay (Siemens Medical Solutions Diagnostics, LA, USA). (LDH (Beckman Coulter, Fullerton, CA), CRP (Beckman, Coulter Fullerton, CA) CA 19-9 has been determined on Brahms Kryptor (Brahms, ThermoFisher, Hennigsdorf, Germany) with a sandwich immuno-fluorescent assay. 25-OH-Vitamine-D was measured with a commercially available radioimmunoassay (IDS, Frankfurt am Main, Germany). CA IX is measured by an enzyme-linked immunosorbent assay (Wilex (OncogenScience), Cambridge, MA, USA), and OPN was measured by an enzyme-linked immunosorbent assay (Quantikine Human Osteopontin Immuno assay; R&D Systems, Minneapolis, MN, USA). OPN, 25-OH-vitamin-D and CA IX were measured using manual methods in duplicate. All other biomarkers were measured in singletons.

Statistical analysis

Primary endpoint in this study was pCR defined as the absence of any tumor cells in the operative pathologic specimen, at the primary site, or in lymph node regions. It was hypothesized that the addition of biomarkers would lead to an

increase of the AUC from 0.7 to 0.8. Assuming a correlation between the models of 0.6 a sample of 57 patients with pCR and 219 without pCR achieves a power of 0.74 to detect a difference of 0.1 between the model with and without biomarkers. An alternative endpoint was good responders, defined as ypT0-2N0, versus poor responders, defined as ypT3 and/or ypN1-2. Missing values were completed using expectation-maximation imputation. Correlations between biomarkers were analyzed by calculating Spearman's rho. Because the biomarkers showed a skewed distribution, the Mann-Whitney *U* test was used to determine significant univariate predictors of response. Logistic regression analysis was used to identify the significant multivariate predictors of response. The next step was to combine blood biomarkers with clinical data and PET parameters. For this analysis clinical and PET-variables were selected manually, based on an earlier predictive model for pCR [9]. The included clinical variables were tumor length, clinical T and N stage, all based on MRI. Included PET-features were maximum SUV, pretreatment metabolic volume and maximum diameter. The two latter variables were measured using a source-to-background ratio method as has been described earlier [15]. ROC curves were constructed and the AUC was calculated. In order to approximate the true AUC and calculate confidence intervals bootstrapping (n=1000) was used. A perfect prediction model results in an AUC of 1.0, while an AUC of 0.5 indicates that response is predicted correctly in 50% of cases (*i.e.* as good as chance). Statistical analyses were performed using Matlab, release 2010b (The MathWorks, Natick, MA).

Ethics

The biobank study was conducted according to the Dutch law and approved by the local medical ethics committee. All patients gave written informed consent before collection of the blood samples.

Results

The patient characteristics are shown in table 1. The majority of patients had a tumor penetrating through the bowel wall and predicted positive lymph nodes on MR. In the total database 20.7% of patients developed a pCR and 47.1% of patients were classified as responder. Table 2 shows the results of the biomarker measurements for the different outcome groups, as well as the PET-parameters that were included in the model. In general lower serum levels for blood biomarkers were seen in the poor responding groups (except for 25-OH-vitamin-D). Additional analysis (not shown in the table) revealed significant positive correlations between IL-6, IL-8, CRP and osteopontin and between CEA and CA19-9.

Table 1 Patient characteristics (N = 276)

Characteristic		N	[%]
Age [years]	median	65.8	
	range	23.0 - 92.2	
Gender	male	179	[64.9]
	female	97	[35.1]
Clinical tumor stage (cT)	2	26	[9.4]
	3	207	[75.0]
	4	43	[15.6]
Clinicalnodal stage (cN)	0	47	[17.0]
	1	114	[41.3]
	2	115	[41.7]
WHO performance index	0	218	[79.0]
	1	53	[19.2]
	2	5	[1.8]
Tumor length [cm]	median	5.0	
	range	2.0 - 13.0	
pCR (ypT0N0)	No	219	[79.3]
	Yes	57	[20.7]
Good response (ypT012N0)	No	146	[52.9]
	Yes	130	[47.1]

Univariate analysis

Univariate analysis indicated that CEA and osteopontin were significant predictors for pCR (p=0.001, p=0.012 respectively) and that CEA, IL-8 and

osteopontin were significant predictors for response (p<0.001, p<0.001, p=0.004 respectively) as shown in table 3. Lower serum levels of these markers correlated with a higher chance of response to chemoradiation. Of the clinical parameters clinical N-stage was predictive for pCR (p=0.026) and response (p=0.001) in univariate analysis and tumor length (p=0.02) and clinical T-stage (p=0.004) for response only. The pre-treatment metabolic volume and maximum diameter based on PET were predictive for both outcome measures (p=0.016 and 0.009 for pCR and p=0.006 and 0.005 for response respectively).

Table 2 Biomarker levels and PET parameters (average ± standard deviation) compared for the subpopulations of pCR vs no pCR and good response vs no good response

		pCR (ypT0N0)		**Good response (ypT012N0)**	
		no	*yes*	*no*	*yes*
Biomarkers	**CEA**	14.7 ± 28.4	8.7 ± 22.9	18.1 ± 33	8.5 ± 18.5
(N = 267)	**IL-6**	4.0 ± 8.4	2.9 ± 2.7	4.8 ± 10.1	2.7 ± 2.4
	IL-8	15.8 ± 9	14.0 ± 7.4	17.3 ± 10	13.4 ± 6.4
	LDH	181.3 ± 43.8	176.2 ± 33.9	181.9 ± 46.7	178.5 ± 36.1
	CRP	11.5 ± 24.5	7.8 ± 8	13.8 ± 29.1	7.5 ± 9.2
	CA 19-9	26.9 ± 29.1	25.1 ± 31.9	28.3 ± 28.8	24.6 ± 30.6
	vitD-25	53.2 ± 20.9	55.5 ± 18.8	52.6 ± 22.4	54.8 ± 18.2
	CA-9	282.5 ± 275	274.4 ± 302.9	282.0 ± 238.4	279.6 ± 320.9
	OPN	79.2 ± 28.9	68.2 ± 16	81.6 ± 30.9	72 ± 21.3
PET parameters	**SUV_{max}**	15.3 ± 6.3	13.6 ± 5.2	14.3 ± 5.6	15.6 ± 6.7
(N =198)	**SUV_{mean}**	8.2 ± 3.1	7.4 ± 2.7	7.8 ± 2.7	8.4 ± 3.3
	MTV [cc]	33.8 ± 33.5	25 ± 20.2	37.6 ± 39.1	25.7 ± 17.6
	Max diameter [cm]	6.5 ± 1.9	5.8 ± 1.6	6.7 ± 2.1	5.9 ± 1.4

Multivariate biomarker model

Table 3 shows the results of the multivariate analysis for the total set of parameters as well as a selection of biomarkers and clinical and PET-parameters. In the complete set of parameters IL-8 was the only significant predictor for response (p=0.05), while osteopontin was borderline significant for pCR prediction (p=0.056). As a next step a manual selection of the most promising predictors was made. Blood biomarkers that had a significant

Table 3 Odds ratios for the tested biomarkers, clinical factors and PET-based parameters

	Univariate		**Multivariate total**				**Multivariate selection**			
	pCR	Response	pCR		Response		pCR		Response	
	p	*p*	*OR [95% CI]*	*p*	*OR [95% CI]*	*p*	*OR [95% CI]*	*p*	*OR [95% CI]*	*p*
Age	.282	.775	1 [0.96-1.03]	.956	1.01 [0.98-1.04]	.395				
Sex	.224	.916	1.65 [0.84-3.26]	.147	1.18 [0.66-2.11]	.57				
WHO	.353	.168	0.95 [0.44-2.06]	.894	0.97 [0.52-1.78]	.913				
TumorLength	.404	.020*	1.01 [0.85-1.19]	.925	0.94 [0.82-1.08]	.388	1 [0.79-1.27]	.987	0.94 [0.78-1.14]	.525
cT	.096	.004*	0.69 [0.35-1.39]	.299	0.53 [0.29-0.96]	.035*	0.28 [0.1-0.78]	.015*	0.55 [0.26-1.14]	.109
cN	.026*	.001*	0.68 [0.44-1.04]	.076	0.58 [0.4-0.84]	.005*	0.43 [0.24-0.78]	.006*	0.46 [0.29-0.73]	.001*
CEA	.001*	<.001*	0.99 [0.97-1.01]	.323	0.99 [0.97-1]	.095	0.92 [0.85-0.99]	.019*	0.97 [0.95-1]	.029*
IL-6	.487	.119	1 [0.91-1.09]	.928	0.95 [0.87-1.03]	.196	0.99 [0.89-1.1]	.878	0.91 [0.82-1.01]	.073
IL-8	.107	<.001*	1 [0.96-1.05]	.991	0.96 [0.92-1]	.05*	1.01 [0.96-1.07]	.746	0.94 [0.89-1]	.036*
LDH	.637	.840	0.99 [0.98-1]	.162	1 [0.99-1]	.21				
CRP	.875	.291	1 [0.98-1.02]	.937	1 [0.98-1.02]	.75				
CA 19-9	.245	.103	1 [0.99-1.01]	.723	1 [0.99-1.01]	.998				
vitD-25	.348	.147	1.01 [0.99-1.02]	.523	1 [0.99-1.02]	.547				
CA-9	.564	.134	1 [1-1]	.570	1 [1-1]	.706				
OPN	.012*	.004*	0.98 [0.96-1]	.056	1 [0.98-1.01]	.433	0.98 [0.96-1.01]	.136	1.01 [0.99-1.03]	.218
SUVmax0	.335	.193	0.92 [0.64-1.33]	.665	1.02 [0.78-1.32]	.91	0.94 [0.87-1.02]	.141	1.08 [1.02-1.14]	.009*
SUVmean0	.316	.153	1.08 [0.52-2.21]	.844	1.09 [0.64-1.87]	.74				
GTV0	.016*	.006*	1 [0.97-1.03]	.965	0.98 [0.96-1.01]	.226				
MaxD0	.009*	.005*	0.99 [0.58-1.7]	.967	0.98 [0.65-1.5]	.94				

predictive value in either univariate or multivariate analysis were included and IL-6, although not significant, was included because it had a predictive value in a prognostic model for lung cancer [14]. In this selection of biomarkers consisting of CEA, IL-6, IL-8 and osteopontin, CEA was the only significant predictor of pCR in multivariate analysis (p=0.019) and CEA and IL-8 significantly predicted response (p=0.029, p=0.021 respectively). Including all biomarkers resulted in an AUC of 0.65 (95% CI 0.57-0.73) for pCR prediction and 0.68 (95% 0.61-0.75) for response prediction. Table 3 (supplementary data) shows the odds ratios for all tested biomarkers as well as the clinical and PET-based parameters.

Combination of blood biomarkers with clinical and PET data

The biomarker selection was then added to the parameters that were predictive for response in an externally validated prediction model based on clinical and PET-scan data [9]. The final model consisted of eight variables: tumor length, clinical T stage, clinical N stage, CEA, IL-6, IL-8, osteopontin, and maximal SUV on PET before start of treatment. In the current dataset tumor length was not a significant predictor for response to chemoradiation, but cT and cN were. Maximal SUV was only predictive for response, not for pCR.

In figure 1 the ROC curves for the combined models based on biomarkers and clinical data (figure 1A and 1a) and biomarkers, clinical data and PET information (figure 1B and 1b) are depicted, as well as the resulting AUCs (figure 1C and 1c). The model based on biomarkers only resulted in an AUC that was more or less comparable to the clinical model. The AUC of the clinical model was 0.64 (95% CI 0.56-0.71) for pCR and 0.66 (95% CI 0.60-0.72) for response.

Combining clinical parameters and biomarkers (AUC 0.69 (95% CI 0.62-0.77) for pCR and 0.73 (95% CI 0.65-0.79) for response) made the model stronger than the model based on biomarkers only or clinical data only. This effect was most pronounced for prediction of response. Addition of information from a PET-scan acquired before the start of treatment leads to the strongest models for the prediction of both pCR and response, resulting in AUCs of 0.81 (95% CI 0.73 - 0.88) for pCR and 0.78 (95% CI 0.71-0.85) for response.

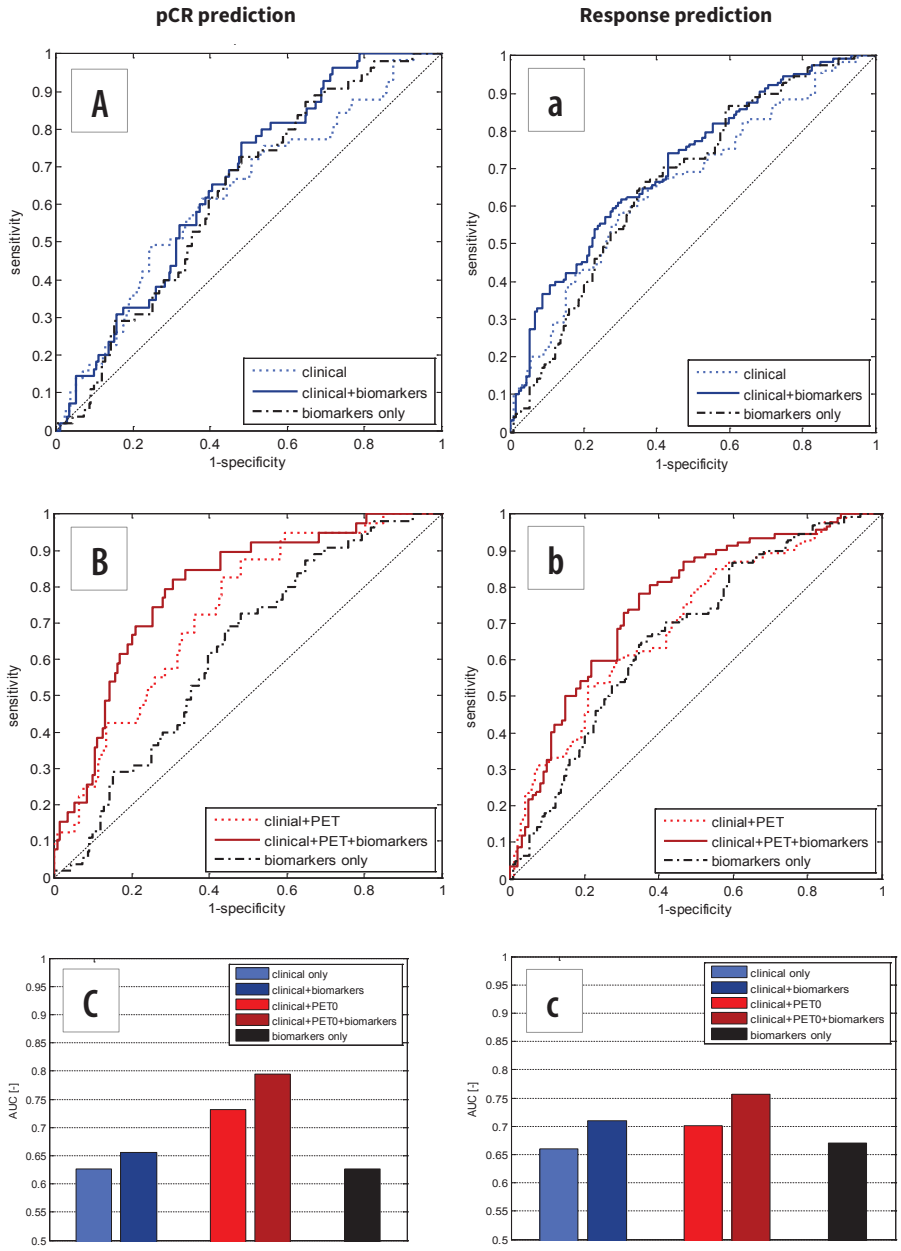

Figure 1 ROC curves for prediction models for pCR and response including biomarkers and clinical variables (A and a) and biomarkers, clinical variables and PET-data (B and b) and resulting AUC for the different prediction models (C and c)

The data of the combined prediction model was used to build a nomogram, which is depicted in figure 2. This nomogram can be used online at www.predictcancer.org. Because this nomogram has not yet been externally validated, it should be regarded as hypothesis generating.

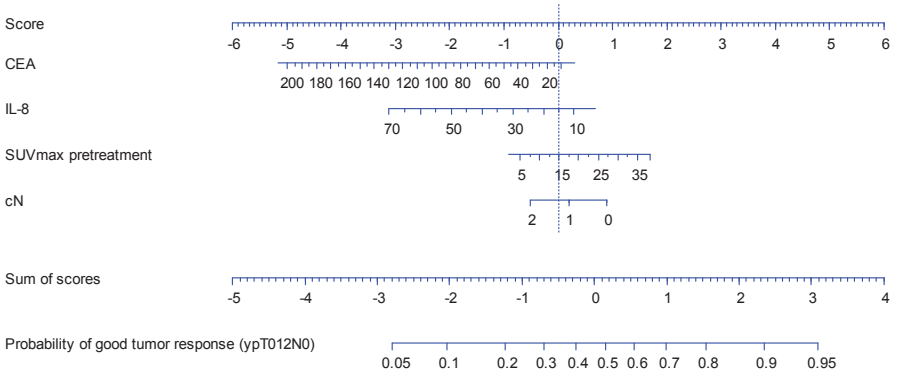

Figure 2 Nomogram for response prediction. A score for each predictor can be read out at the top scale (Score). All summed scores (Sum of scores scale) can be converted directly to the probability of response.

Discussion

To the best of our knowledge this is the first prospective study evaluating the predictive value of a broad range of blood biomarkers analyzed in a standardized way for response prediction to CRT in rectal cancer. CEA turned out to be a significant predictor for pCR and CEA and IL-8 were predictive for response. Including these blood biomarkers in models based on clinical parameters and PET-based parameters resulted in an increased performance of the prediction models.

Osteopontin is a glycophosphoprotein secreted by different cell types, that has been shown to be associated not only with malignancies but also with acute and chronic inflammatory processes. It influences adhesion, migration, invasion, chemotaxis and cell survival [16] and is a marker of tumor aggressiveness and early progression [17]. Although the role of osteopontin as a

prognostic factor has been studied extensively in a broad range of solid tumors, only one study looked at the predictive value of osteopontin levels and response to CRT in rectal cancer. Debucquoy *et al*. found an association between lower osteopontin levels and better response in 30 rectal cancer patients, which is in line with our findings, but this did not reach statistical significance [13]. In our patient group it was a significant predictor in univariate analysis, but it lost significance in multivariate analysis. This could be explained by the fact that osteopontin and IL-8 showed a positive correlation.

CEA is an antigen produced by the normal fetus and only in very low concentrations by normal cells of the adult body. In the tumor environment it plays a role in intercellular recognition and attachment. It has been shown to be of prognostic value in colorectal cancer independent of clinical stage and differentiation grade [18]. Although preoperative CEA levels cannot be used to make treatment decisions in colorectal cancer, they consistently have a prognostic value. Patients having a CEA level >5 µg/l have a significant worse disease free and overall survival than patients with lower CEA levels. Three studies looked at the predictive value of CEA levels for response to CRT in rectal cancer. Das *et al* studied a group of 562 rectal cancer patients treated with CRT [7]. In their patient group CEA was only predictive for pCR in univariate analysis. However, they chose to dichotomize CEA levels (below and above 2.5 µg/l), while in our model CEA was incorporated as a continuous variable. Yoon *et al* did an analysis in a group of 351 rectal cancer patients [12]. In multivariate analysis CEA levels ≤5 µg/l were predictive for downstaging and complete regression. Park *et al* did a retrospective analysis in 352 rectal cancer patients [11]. CEA was analyzed as a continuous variable and had a significant predictive value for responders vs. non-responders. A higher CEA was an independent predictor of poor response to CRT and a worse disease free survival.

IL-8 is a pro-inflammatory chemokine that plays a role in attracting neutrophils. Through the activation of phosphatidyl-inositol-3-kinase (PI3-K) and phospholipase C it can activate the Akt/mTOR and Raf/Mek/Erk pathways, leading to the promotion of angiogenesis, proliferation and survival and the migration of cancer cells [19]. Polymorphisms in IL-8 have been described to be

related to an increased risk of recurrence in rectal cancer [20] and the risk of nodal involvement [21], indicating a possible relationship with tumor biology of IL-8 in rectal cancer. However, until now there are no reports of a potential value of IL-8 in the prediction of response to CRT.

In this study response was measured in two ways: pCR and ypT0-2N0. Of these 2 endpoints pCR is the most robust, although the definition of pCR can be difficult. In this cohort routine pathological examination was performed and reported in a standardized way. A recent comparison between routine pathological examination and additional step sections in resection specimens showing no viable tumor cells at initial examination, showed no differences in outcome [22]. Furthermore, pooled analysis of a large series of patients included in different studies, showed a clear prognostic value of pCR after CRT for long-term outcome, even if pooled from different studies, indicating that pCR as scored in routine pathology procedures is a valuable endpoint [2]. The most frequently used method to distinguish responders from non-responders is by means of a tumor regression grade (TRG). However, for this cohort TRG was not scored routinely and we chose to use ypT0-2N0 as definition of response. It makes sense to predict the group of good responders, because they could be suitable for less invasive surgery, like transanal resection or TEM-surgery.

Blood biomarkers give information about tumor biology in an indirect way. A more direct insight can be gained from genetic alterations within the tumor. An overview of these molecular biomarkers is given in the review of Kuremsky *et al* [23]. A possible problem related to molecular biomarkers is the heterogeneity in tumors, making it necessary to collect a representative sampling of tumor material and the invasive procedure needed to collect material. Blood biomarkers have the advantage that they are easy to collect and that they provide information about the “average” tumor. This makes blood biomarkers useful for daily practice. However translation of the results of this study should be done cautiously, because all biomarkers in this study were measured in a standardized way in one single laboratory, using the same kits. Less thorough and sensitive procedures might influence negatively the added value of biomarkers.

Ideally response prediction takes place before the start of a treatment, so that a patient can be offered the treatment with the highest success rate. In the case of response prediction for rectal cancer, clinical factors and pre-treatment PET-scan have been shown to have predictive value before the start of treatment [9], but the performance of predictive models based exclusively on pre-treatment data typically lies between 0.65 and 0.70. The predictive value of biomarkers only is in the same range, but the combination of biomarkers and other pre-treatment data results in a stronger prediction model.

The data presented here can be seen as a proof of principle that biomarkers contain predictive information for rectal cancer, but external validation of this prediction model is necessary for a better estimation of the performance and reproducibility of the model [24]. For use in clinical practice a stronger performance is desirable. A possibility to strengthen the predictive model is to incorporate response data obtained early during CRT. If this time point lies early in the treatment course, it is still possible to modify treatment. For PET-CT it has already been shown that changes in glucose metabolism after 2 weeks of CRT resulted in a stronger prediction model [8]. An intriguing question is whether early changes in blood biomarker levels during CRT can further enhance the performance of this model. This question will be subject of future research.

In conclusion, pre-treatment CEA levels help to predict pCR after CRT for rectal cancer and CEA and IL-8 levels are helpful in the prediction of response to CRT. These blood biomarkers have added value to earlier published prediction models based on pre-treatment clinical- and PET-data and can be used in decision support systems for tailored therapy [24].

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Chapter 6

What biochemical and molecular biological factors have greater relevance to treatment decisions?

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Abstract

All current treatments for rectal cancer come with a price to pay, namely, the risk for over- and undertreatment based on current treatment decisions leading to sometimes even unnecessary toxicities. Thus, it would further improve the results of both treatment outcome and quality of life, if we could better tailor the treatment for rectal cancer based on validated biochemical and molecular biological factors. It is clear that several already known tumor biological features have an important impact on the individual tumor responses to certain treatments. Thus, detection and analysis of these biomarkers will ultimately lead to better treatment decisions in all parts of the current treatment algorithm. However, most studies so far have been rather limited in its attempts to identify biomarker based predictors for e.g. response to CRT, surgical decisions and systemic therapy. Six genetic biomarkers were identified with the potential of being predictive in the outcome after CRT, like p53, EGFR, TYMS, Ki-67, p21 and bax-bcl-2. TYMS expression measurements seem to be worthwhile to further study in future trials, while survivin showed mostly conflicting results and gene expression profiles with microarrays need further validation with larger patient groups. Nevertheless, there is great interest in the identification of biochemical and molecular biological factors in treatment decision making and many promising developments are on the way, like whole genome DNA, RNA analysis, multiple mutation testing, full sequencing and the association of genotype with phenotype. However, before the first successful biomarker development can been finalized and implemented clinically, several collaborations and consecutively external validations still need to be completed.

Introduction

The current standard therapy for rectal cancer in most countries around the world consists of a preoperative treatment either with long-term radiotherapy (RT) and a 5-FU based chemotherapy (CT) or a short course RT followed by surgery or surgery alone depending on the pretherapeutic staging resulting in a 5-year cumulative rate of local relapse of less than 10% and an incidence of distant metastases of about 35 % [1, 2]. The preoperative treatment has a major impact on local control, however with only minimal impact on survival and disease-free survival. So far, no clear evidence has been produced for the benefit of adjuvant chemotherapy, leading to different protocols in different countries and regions around the world. Generally, all parts of the current standard treatment of rectal cancer have its toxicity profiles. Preoperative chemoradiotherapy (CRT) can lead to diarrhea, radiation dermatitis and increased risk for anastomotic leakage and perineal wound complications [3] while short course radiotherapy generally increases the risk for fecal incontinence, anal blood loss, mucus loss and erectile dysfunction [4-6]. The surgical treatment itself is generally accompanied with an increased risk for urinary, bowel and erectile dysfunction, anastomic leakage, perineal wound complications and a mortality rate of 4 - 11% depending on age and co-morbidity [7-9]. Thus, all treatments come with a price to pay, namely the risk for over- or undertreatment based on the current imaging-related treatment decisions leading to probably unnecessary toxicities and subsequently impact on quality of life. However, rectal cancer is a biological tumor, characterized by several biological features, which are nowadays better and better understood. Dramatic technical improvements in detection assays have made it possible to characterize more and more biochemical and biological features and phenotypes related to individual rectal cancer patients. Thus, biological markers might be able to better tailor the treatment of rectal cancer, thereby minimizing the risk for over- and undertreatment with also an improvement in outcome and quality of life. However, this needs prospective evaluation and stringent validation. It is clear that there is a biological basis for the different responses of individual tumors to certain treatments, like CRT, RT, and CT. The

tumor microenvironment, e.g. angiogenesis and hypoxia, repopulation and the intrinsic radiosensitivity are currently the best known biological tumor phenotypes, which undoubtly have an important impact on the degree of response to anti-tumor treatments [10].

This chapter will focus on the question where we are now in the detection and analysis of biochemical and biological factors influencing treatment decisions and how far we have already come in our attempts to better individualize treatment based on biomarkers.

Biochemical and molecular biological factors for radiochemotherapy

The combination of chemotherapy and radiotherapy leads to some degree of pathological downstaging in approximately 40-60% of patients. The percentage of patients developing a pathological complete response (pCR) varies, but lies typically around 20%. In treatment decision making it would be very helpful to identify good and poor responders in order to give a tailored treatment. Until now the predictive value of several biomarkers has been studied. In these studies various endpoints have been used, but the most common endpoints are overall survival, tumor regression grade (TRG), pCR and downstaging. Kuremsky et al published a review evaluating the potential of genetic biomarkers in predicting the outcome of locally advanced rectal cancer patients treated with chemoradiation [11]. In their review they focused on gene products with more than 5 studies in the literature. The 6 biomarkers that met this criterion were p53, epidermal growth factor receptor (EGFR), thymidylate synthase (TYMS), Ki-67, p21 and bax/bcl-2.

The most studied marker is p53, a gene that plays a key role in apoptosis, tumorigenesis and sensitivity to chemotherapeutic agents. Twenty-one studies studying the predictive value of p53 status were identified. Seventeen of these studies could not identify a correlation between p53 expression and tumor response. Of the 4 studies that did show a relationship between p53 and outcome, 3 identified a p53 mutation as a predictor of poor response, whereas the other study found a positive predictive value for mutated p53. Therefore,

p53 does not seem to be a good predictor for tumor response to chemoradiation.

EGFR is important in the regulation of various cellular responses, like proliferation, apoptosis and differentiation. Overexpression of EGFR has been shown in 60-80% of all colorectal cancers and has been associated with a poor prognosis independent of lymph node status. Five studies have been published studying the value of EGFR expression as response predictor. These were studies looking at chemoradiation only, without addition of EGFR inhibitors. Although these studies looked at different end-points, the studies that found a correlation all point towards a better response to chemoradiation in tumors with low EGFR expression. It seems to be more important to quantify the level of EGFR expression rather than divide tumors into positive or negative. One study identified a single nucleotide polymorphism that may be useful as a biomarker for response.

Thymidylate synthase is an important enzyme in DNA synthesis and is the main target of 5-fluorouracil (5-FU). Overexpression of this enzyme leads to 5-FU resistance. Nine studies were identified in the Kuremsky review analyzing the influence of TYMS expression and SNPs in the TYMS enhancer region on tumor response and very recently 2 additional studies have been published. Four studies used immunohistochemical staining and 5 studies used PCR for genotyping. Three studies did not show a correlation between TYMS and tumor response. The remaining 6 studies found in general a better response to chemoradiation in tumors with low TYMS expression. Two studies also included a group of patients treated with radiotherapy only and as could be expected in this group no correlation between TYMS expression and response was observed. Only one study found an inverse correlation, however this was a small study with only 19 patients, who received both 5-FU and oxaliplatin, which could have confounded the results. Recently, however, two studies found a correlation between high expression of TYMS and better response after CRT for rectal cancer [12, 13]. Although there is not enough evidence to use TYMS in daily practice, incorporation of TYMS expression measurements in chemoradiotherapy trials would be worthwhile. Recently, the results of a phase II study have been

published in which patients were stratified in a good-risk group (no TYMS overexpression) and a poor-risk group (TYMS overexpression) [14]. The good-risk group was treated with standard chemoradiation consisting of radiotherapy and continuous 5-FU infusion, while the poor-risk patients received weekly irinotecan in addition to standard chemoradiation. A pCR and tumor downstaging was found in 18.9% and 64.4% of good-risk patients respectively and in 35.5% and 64.5% of poor-risk patients. However, the combination with irinotecan turned out to cause more toxicity. RFS and OS were comparable between both risk groups. This study is the first attempt to stratify the treatment of rectal cancer patients according to TYMS status. Although further phase III trials are needed, it was remarkable that the proportion of patients showing downstaging was comparable between the good- and poor-risk group and the percentage that developed a pCR was higher in the poor-risk group.

Ki-67 is a proliferation marker, which has not shown a clear relationship with chemo- or radiosensitivity. Thirteen articles have been published about the predictive value of Ki-67, of which only 2 showed a correlation. One study found a better response in tumors with high Ki-67 staining, the other showed an association between low Ki-67 expression and response. Therefore Ki-67 is not a good biological marker in rectal cancer.

The tumor suppression gene p21 is activated by DNA damage and causes cell cycle arrest. Four of 8 studies found a correlation between p21 expression and response to chemoradiation. However, the results of these 4 studies are conflicting. Two studies found better responses or survival in patients with low or negative p21 expression, while the other two studies found the opposite. Based on in vitro studies one would expect better results in tumors with low p21 expression, because p21 suppresses apoptosis in case of DNA damage. The two studies which showed better results in positive p21 tumors used other treatments in addition to radiotherapy and 5-FU which may have confounded the results.

The bax and Bcl-2 proteins are involved in apoptosis. Loss of bax function is correlated with chemoresistance in colorectal cancer and Bcl-2 overexpression has been linked with resistance to different chemotherapeutic agents and inhibition of radiation-induced apoptosis. Three studies evaluated the role of

bax expression in response to chemoradiation. Only one study found a significant correlation. In that study the percentage of bax-positive tumors was significantly higher in the complete-response group than in the partial-response group (54% vs. 29%). Bcl-2 was analyzed in 12 studies, but a correlation with response was found in only one study including only 17 patients.

Two rather small studies have tested the value of microarray in prediction of response. The first study tested 54 genes in 23 patients. A different expression pattern was found between responders and non-responders based on downstaging, but this difference was no longer significant if response evaluation was based on TRG. The negative predictive value (NPV) was 86% and the positive predictive value (PPV) 78%. The second study included 43 patients and found 42 genes that were differentially expressed between responders and non-responders based on TRG. The PPV using this gene set was 71% and the NPV 86%. Although these studies show for the first time that gene expression profiles may be helpful in response prediction, they certainly need further validation with larger patient groups.

Quite recently X-ray-repair-cross complementing 1 (XRCC1) has been identified in 3 studies as a potential useful marker for response prediction in rectal cancer [12, 13, 15]. XRCC1 plays an important role in DNA-repair as it is involved in the base excision repair (BER) pathway. One polymorphism, A399G, has been shown to have a predictive role in radiosensitivity of malignant tumors. The first study included 81 patients with locally advanced rectal cancer treated with chemoradiation. Of the patients with an AG phenotype 47% showed a major response, as compared to 22% in the AA and GG phenotype group. The second study analyzed 93 patients treated with chemoradiation for rectal cancer. Genotyping was done on peripheral blood monocytes. They found a better response in G/G carriers as compared with G/A carriers (OR 4.180; p=0.003). In the third study DNA from monocytes of 128 rectal cancer patients was analyzed. In this study no statistical significant association with tumor response was found. In conclusion, results of XRCC1 as a predictor of response in rectal cancer are conflicting.

Another protein that attracted interest in the past years is survivin, an inhibitor of apoptosis [16-18]. Again, results are conflicting. Two recent studies reported

a correlation between survivin expression and tumor response. Higher survivin expression correlated with worse response to chemoradiation. One of these studies compared survivin expression in pre-treatment biopsies and surgical resection specimens and found a worse survival in patients with tumors expressing high levels of survivin after chemoradiation. However, other studies could not find a correlation between survivin expression and clinical outcome, which underlines the need for further studies.

Biochemical and molecular biological factors for surgical decisions

The surgical treatment strategy for rectal cancer has dramatically changed over the last years. Coming from a standard radical surgical procedure performed in strictly defined TME protocols with only radical resection being the primary goal, it nowadays changes to a more tailored approach with also the aim to reduce toxicity with better quality of life. The surgical treatment usually takes place initially after diagnosis in early stage cancer or after a neoadjuvant treatment consisting of either radiotherapy alone (5x5 Gy) or RCT. In case of a neoadjuvant treatment, a short-course radiotherapy will usually be followed within 1 to 3 days by an immediate surgical resection of the TME, as has been published by the DUTCH TME trial [19]. Thus, the short-course radiotherapy does not induce any down-sizing or down-staging, since the timing of the followed surgical replacement of the rectum tumor does not allow any tumor shrinkage [4]. However, newer trials have suggested that the short-course preoperative radiotherapy indeed could be used to also downsize and downstage the tumor, if the surgical treatment is postponed by at least 6 to 8 weeks after radiotherapy [20, 21]. This strategy might increase the potential for better sphincter- and even organ- sparing surgical treatment techniques etc., as has been already proposed after neoadjuvant chemoradiotherapy in selected patients [22]. However, accurate tumor response assessment is crucial in these modern adaptive surgical strategies after neoadjuvant radio- or radiochemotherapy. Biomarkers could help in the treatment decisisons for less aggressive conservative surgical treatments, beside modern imaging and

clinical examinations. But this applies not only for preoperatively pretreated rectal cancer patients, but also for initially diagnosed patients, in which early stage disease is suggested. These patients could benefit from conservative surgery (e.g. TEM) instead of radical surgery, if accurate biomarkers associated with disease progression, particularly mesorectal nodal metastasis, would become available.

One recently published study compared patterns of gene-specific hypermethylations in radically excised rectal cancers with histopathological stage and came to the conclusion, that locus-specific hypermethylation was more prevalent in early- than late-stage disease and that the hypermethylation of two or more of a panel of five tumor suppressor genes was associated with localized disease [23]. Another study by Rasheed et al., assessed the microvessel density (MVD) and the CA9 expression in more than 100 rectal cancer specimen and came to the conclusion that the MVD was higher in more advanced T and N stages, whereas the CA9 expression was generally higher in earlier stages [24]. This however has not been validated yet and warrants further evaluation. Generally, the only molecular markers being currently prognostically relevant in rectal cancer are the Deficient Mismatch Repair and possibly the Kras mutation and the Braf mutations. All else, like expression assays, copy number variation tests and even proteomics is experimental and have not shown any validated correlation with prognosis yet. The recently published analysis of the QUASAR study, which mainly analyzed stage II cases showed MMR, Kras or Braf abnormalities in rectal cancer in 1-4%, 30-35% and 2-3% respectively [25]. All three abnormalities had prognostic impact at different levels, with MMR being the strongest marker for prognostic impact. The defective MMR genes were hMLH-1, hMSH-2 PMS-2 and hMSH-6, detected by either immunohistochemistry or microsatellite testing. However, up until now no biomakers have reached the level of clinical relevance which would allow inclusion in surgical decision making. Recently, the first cohort analysis of 20 patients selected for a non- surgical wait and see strategy after RCT has been published with very promising local and distant controls [22]. The decision making for such a non-surgical approach in patients with good to complete

clinical response after RCT, which is currently only based on clinical and imaging parameters due to the lack of sufficient biomarkers should be stepwise improved with also biochemical and molecular markers, as soon as they have been approved and validated.

Biochemical and molecular biological factors for adjuvant and systemic therapy

The value of adjuvant chemotherapy in node-positive colon cancer is clear. Since rectal tumors originate in an organ that is in anatomic continuity with the colon and tumors show a similar histology, it is often argued that results of adjuvant trials in colon cancer can be translated to rectal cancers. However, until now, randomized trials in rectal cancer have failed to show a clear benefit of adjuvant chemotherapy [26]. Especially in patients who have a good response after pre-operative chemoradiation the expected gain of adjuvant treatment is small. Recently, data of 5 large rectal cancer trials including chemoradiation have been pooled to build nomograms for the prediction of local and distal recurrence as well as survival. This analysis confirms that in patients showing a good response after chemoradiation the added benefit of adjuvant chemotherapy is small [27].

Biomarkers could be helpful to identify patients who are at higher risk for recurrent disease. When looking at the value of biomarkers, it is important to distinguish prognostic from predictive factors. A prognostic factor gives information about the risk of recurrence of disease irrespective of a certain treatment while a predictive factor predicts the chance that a patient will benefit from a treatment. Of course, a marker can be both prognostic and predictive.

In colorectal cancer, most evidence is available for the prognostic value of mismatch repair (MMR) gene status. Tumors that are MMR deficient have a better prognosis. There is some debate whether MMR status also has a predictive value. However, the proportion of rectal tumors that is MMR deficient is small (around 1%). Therefore, it is not a useful marker for rectal cancer.

The role of BRAF status seems to have a predictive value, but the debate is going

on about a possible predictive role. Because the proportion of rectal tumors carrying a BRAF mutation is very low, BRAF does not seem to be a very important biomarker in rectal cancer.

KRAS status also has been shown to be of prognostic value. In about 40% of all rectal tumors KRAS is mutated. KRAS mutant tumors have a poor prognosis and a higher chance of recurrence. In the QUASAR trial this difference was even more pronounced for tumors located in the rectum as compared to tumors in other parts of the colon. In this trial the reduced risk of recurrence with chemotherapy was comparable between KRAS wild type and KRAS mutated tumors. However, for the prediction of response to EGFR-inhibitors, KRAS status has a predictive value. This has been confirmed for metastatic colorectal cancer in a recent meta-analysis [28].

Conclusion and perspective of biomarkers for treatment decisisons

Biochemical and molecular biological factors could help in treatment decision making along the different treatment steps in the treatment of rectal cancer. This would allow more tailored treatment approaches thereby improving quality of life with even the benefit of more effective treatments. When analyzing the current value of these biomarkers in treatment decision-making, it is important to identify markers as being prognostic or predictive or even both. In rectal cancer, the only markers reaching clinical relevance in prognosis so far are deficient MMR, Kras and Braf, however its frequency in rectal cancer is rather low with only 1-4 % deficient MMR, 30-35% Kras mutation and 2-3 % Braf mutation [25]. The defective MMR genes hMLH-1, hMSH-2, PMS-2 and hMSH-6, detected by immunohistochemistry or by microsatellite testing showed a prognostic value in a meta-analysis [29], in the PETACC trial [30], the QUASAR and numerous single centre studies, which might lead to MMR related treatment decisions in the near future. Kras status has also been shown to be of prognostic value. Kras mutant tumors have a worse prognosis and a higher chance of recurrence. For EGFR-inhibitor therapy, the Kras status even reaches a predictive value.

With regard to prediction, 6 genetic biomarkers were identified with the potential of being predictive in the outcome of locally advanced rectal cancer after CRT. These were p53, EGFR, TYMS, Ki-67, p21 and bax-bcl-2. While EGFR expression levels seems to provide some prediction to CRT response, p53 does not seem to serve as a good predictor, neither does Ki-67 or p21 or bax and Bcl-2. TYMS expression measurements are worthwhile to further study in future trials, while survivin showed mostly conflicting results and gene expression profiles with microarrays need further validation with larger patient groups.

Taken together, the current value of biochemical and molecular biological factors in treatment decision-making is rather low, however with many promising developments in the pipeline. Especially whole genome DNA or RNA analysis with copy number variation, multiple mutation testing, full sequencing and the association of genotype with phenotype will ultimately lead to more biomarker based treatment decisions in rectal cancer. However, successful biomarker development needs collaboration, external validation and meta-analyses to reach the level of accuracy necessary to base treatment decisions on these markers.

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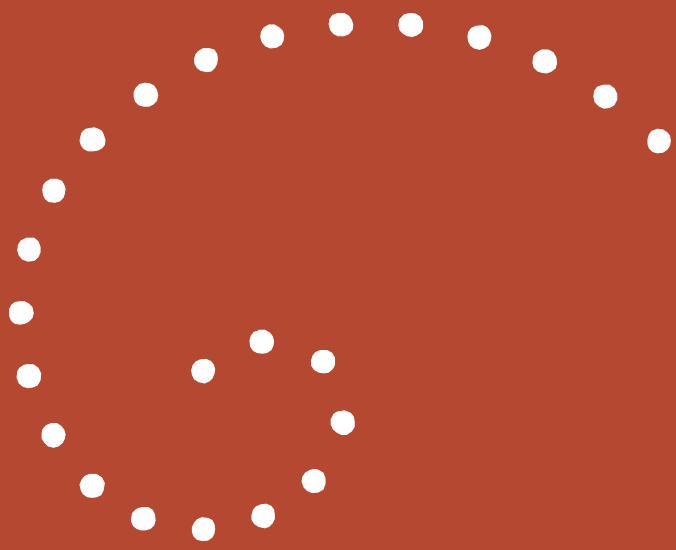
Part III
Combined treatment in rectal cancer



Chapter 7

Phase I trial of the combination of the Akt inhibitor nelfinavir and chemoradiation for locally advanced rectal cancer

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Abstract

Purpose

To investigate the toxicity of nelfinavir, administered during preoperative chemoradiotherapy (CRT) in patients with locally advanced rectal cancer.

Material and methods

Twelve patients were treated with chemoradiotherapy to 50.4 Gy combined with capecitabine 825 mg/m^2 BID. Three dose levels (DL) of nelfinavir were tested: 750 mg BID (DL1), 1250 mg BID (DL2) and an intermediate level of 1000 mg BID (DL3). Surgery was performed between 8 and 10 weeks after completion of CRT. Primary endpoint was dose-limiting toxicity (DLT), defined as any grade 3 or higher non-hematological or grade 4 or higher hematological toxicity

Results

Eleven patients could be analysed: 5 were treated in DL1, 3 in DL2 and 3 in DL3. The first 3 patients in DL1 did not develop a DLT. In DL2 one patient developed gr 3 diarrhea, 1 patient had gr 3 transaminase elevation and 1 patient had a gr 3 cholangitis with unknown cause. An intermediate dose level was tested in DL3. In this group 2 patients developed gr 3 diarrhea and 1 patient gr 3 transaminase elevation and gr 4 post-operative wound complication. Three patients achieved a pathological complete response (pCR).

Conclusions

Nelfinavir 750 mg BID was defined as the recommended phase II dose in combination with capecitabine and 50.4 Gy pre-operative radiotherapy in rectal cancer. First tumor response evaluations are promising, but a further phase II study is needed to get more information about efficacy of this treatment regimen.

Introduction

Pre-operative radiotherapy plays an important role in the multidisciplinary treatment of rectal cancer. It has been shown that pre-operative radiation improves local control and may increase the chance of sphincter-saving surgery [1, 2]. In case of locally advanced rectal cancer, radiotherapy is often combined with chemotherapy to enhance the effect of radiotherapy [3]. The most frequently used combination is 5-fluorouracil or capecitabine (an oral prodrug of 5-fluorouracil) and radiotherapy to doses around 45–50.4 Gy. This regimen results in a pathological complete response (pCR) rate of 10–33%, depending on radiation dose and the interval between completion of treatment and surgery [4-6]. Patients who develop a complete response have a better prognosis [7-8] and may be candidates for less invasive surgery or even a wait-and-see policy [9].

It has been shown that PI3Ks were genetically altered in 74 (32%) of 234 human colorectal cancers [10] and that the PI3K signalling pathway can be constitutively activated in colorectal cancer [11]. Furthermore, epidermal growth factor receptor (EGFR) expression in rectal cancer is known to be an indicator of poor response to radiotherapy and a poor disease-free survival [12, 13]. Laboratory data have shown that inhibition of Ras and/or the downstream PI3K-Akt pathway increases radiosensitivity in cells in which this pathway is activated, but does not affect cells without activation of this pathway, including normal tissues [14, 15]. As PI3K mutations seem to be common in colorectal cancer [10, 16-18], inhibition of the PI3K pathway may well be able to influence the response to radiation. In addition to PI3K mutations, this pathway may be upregulated without mutations or due to mutations of growth factor receptors (like EGFR or vascular endothelial cell growth factor receptor (VEGFR)) or downstream messengers. Furthermore, the PI3K/Akt pathway also seems to be involved in the radiosensitivity of endothelial cells. Several pre-clinical studies have demonstrated that inhibition of this pathwayincreases the radiosensitivity of tumor blood vessels even at lower fraction doses [5]. However, up until now, no clinically usable PI3K inhibitors have been available.

HIV protease inhibitors (HPIs) are a group of drugs that have been used for more than a decade in the treatment of HIV as part of highly active anti-retroviral therapy (HAART). More recently, HPIs have been shown to possess an anti-tumor activity that is independent of their anti-retroviral activity and to have a radiosensitizing effect through the inhibition of phosphorylated-Akt (pAKT) [14]. The inhibition of PI3K during radiotherapy is not expected to result in increased normal tissue toxicity because this pathway is not constitutively activated in normal cells. No specific pre-clinical data is available for colorectal cancer cells,but it has been shown in vitro that inhibition of the PI3K/Akt pathway leads to radiosensitization of different cell lines in vitro and in vivo, including colon cancer, bladder cancer and glioma cell lines [19-21]. Therefore, we hypothesize that the addition of an HPI to chemoradiation for rectal cancer will increase the effect of treatment, resulting in a higher percentage of pCR. Due to the extensive clinical experience with HPIs in HIV treatment, they form an interesting group of Akt inhibitors to test in clinical practice.The HPI nelfinavir was the most potent inhibitor of the PI3K-Akt pathway in pre-clinical studies. In HAART therapy it is prescribed in a dose of 1250 mg BID and the most commonly observed side-effects are diarrhea (>10%) and transaminase elevations (around 2%) and nausea. Since there is room for improvement in the number of pCRs and PI3K seems to be mutated in an important percentage of human colorectal cancers, we felt it was worthwhile to set up a phase I trial testing the combination of different nelfinavir dose levels with capecitabine and radiotherapy. The primary endpoint was to evaluate the toxicity of this combined modality treatment in order to find the recommended phase II dose (RP2D).

Patients, Materials, and Methods

Eligibility

Patients who were at least 18 years old and who had histologically proven adenocarcinoma of the rectum, cT3-4N0-2M0, were eligible for this study. Clinical staging was based on MRI. Additional inclusion criteria were a World Health Organization (WHO) performance status ≤2 and sufficient hematological,

renal and liver function. Concurrent medication known to be metabolized by the CYP3A4 isoenzyme was not allowed, including the use of St John's wort. Patients gave written informed consent prior to inclusion in the trial, according to the Dutch law. The study protocol was approved by the MAASTRO Institutional Review Board and the Medical Ethical Committee of Maastricht University Medical Centre. The trial was registered at ClinicalTrials.gov as NCT000704600.

Treatment

Oral nelfinavir was added to our standard chemoradiation (CRT) scheme for locally advanced rectal cancer, consisting of 28 fractions of 1.8 Gy concomitant with capecitabine 825 mg/m^2 BID (figure 1). A 3D-conformal treatment plan was made based on a PET-CT scan. The primary tumor, complete mesorectum, presacral area, internal iliac lymph nodes and obturator region (in case of low-seated tumors) were included in the treatment volume [22]. Three-dimensional treatment planning was used to obtain adequate coverage and a homogeneous dose distribution.

Two dose levels (DLs 1 and 2) of nelfinavir were planned to test with the possibility of exploring an intermediate DL3 if DL2 turned out to be too toxic. Dose levels were defined as 750 mg nelfinavir BID for DL1, 1250 mg BID for DL2 and 1000 mg BID for DL3. Nelfinavir was started seven days before the start of chemoradiotherapy and nelfinavir plasma levels were monitored in weeks two, four and six, using an HPLC-UV method. To correct for the different timepoints at which samples for drug level measurement were taken, the drug concentration ratio (CR) was calculated for each sample [23] by dividing the drug concentration measured in that sample by the time-adjusted value in the standardized pharmacokinetic curve.

Surgical resection (either a low anterior resection or abdominoperineal resection, left to the discretion of the surgeon) was performed preferably between eight to ten weeks after the end of CRT.

Patients who had node-positive disease, either on MRI or pathologic examination, received adjuvant chemotherapy (capacitabin/oxaliplatin). According to the protocol of the referring hospital and the condition of the

patient, 1 or 2 cycles could be administered in the interval between the completion of CRT and resection.

Toxicity evaluation

The National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE) was used to score acute toxic effects of chemoradiotherapy and post-operative toxicity [24]. Dose limiting toxicity was defined as any grade 3 or more non-hematologic toxicity, any grade 4 or more hematologic toxicity or any grade 4 or higher post-operative toxicity within 30 days post-surgery.

Toxicity was scored weekly during chemoradiation and one, two and four weeks after the end of CRT. Post-operative toxicity was scored weekly until four weeks after surgery. Nelfinavir doses were adjusted if CRs were lower than 0.90 or higher than 1.5.

Three patients were initially given DL1 (750 mg BID). If none of the three patients experienced a dose-limiting toxicity (DLT), the second dose level was investigated. If one-third of the patients had grade 4 or two-thirds had grade 3 toxicity, up to three additional patients were added; if none of the additional patients developed DLT, the second dose level was investigated. Otherwise the RP2D was exceeded in the first dose level.

Three patients were initially given DL2 (1250 mg BID). If one-third experienced DLT, then up to three additional patients were entered. If no more than one of the five patients developed DLT, then the second dose level was declared the RP2D. Otherwise, the RP2D was exceeded and the first dose level would be expanded to a total of five patients (if only three patients had been treated). Thereafter, an intermediate dose level of 1000 mg BID could be explored. If at least two of the three patients had DLT, the RP2D was exceeded and the first dose level would be declared the RP2D.

Response evaluation

All patients underwent a resection. Resection specimens were analyzed in a standardized way according to the national guidelines. Tumor regression grade

(TRG) was scored by one pathologist according to the Mandard scoring system [25]. In short, scores ranged from TRG5 (*no changes*) to TRG1 (*no viable tumor cells left*).

Statistical methods

The primary objective of this study was to evaluate the safety of nelfinavir, capecitabine and radiotherapy and to identify the RP2D of nelfinavir among patients with locally advanced rectal cancer. A standard 3+3 dose escalation design (modified Fibonacci design) was used [26]. Categorical variables were summarized using frequencies, whereas continuous variables were reported using the median (range).

Results

Patient characteristics

Twelve patients (five DL1, three DL2 and four DL3) were enrolled in the study between October 2008 and September 2010. One patient in DL3 had no measurable nelfinavir levels at three different time points. Although he affirmed that he took the medication as prescribed, we decided to exclude this patient from further analysis and include an extra patient in DL2 since we judged this patient to be unrepresentative for toxicity analysis. Therefore only 11 patients were analyzed. Patient characteristics are shown in table 1.

Toxicities

The first three DL1 patients did not experience any DLT. In DL2, one patient developed a grade 3 transaminase elevation (without clinical signs), defined as more than five times the upper limit of normal, and one patient had a grade 3 cholangitis for which no specific cause could be found. Therefore, a relationship with the treatment regimen could not be ruled out. In both patients, symptoms disappeared and lab values normalized after nelfinavir was stopped. Radiotherapy treatment could be completed and both events were graded as DLT. The third patient had grade 2 diarrhea and developed a grade 3 ileus

caused by excessive use of loperamide. This grade 3 toxicity was considered to be at most indirectly related to the use of nelfinavir. Radiotherapy had to be interrupted and stopped after 32.4 Gy in this case.

Table 1 Patient characteristics

Characteristic		No. of patients (n=11)	%
Age, years	Median	61	
	Range	51-72	
Sex	Male	10	91
	Female	1	9
cT stage	T2	2	18
	T3	8	73
	T4	1	9
cN stage	N0	2	18
	N1	4	36
	N2	5	46
Tumor position	Low	9	82
	Middle	2	8
	High	0	0

Because 2/3 patients developed DLT, DL2 was deemed too toxic and according to the definitions of the protocol DL1 was expanded with another two patients, who did not experience any toxicity ≥grade 3. Therefore an intermediate nelfinavir dose was explored in DL3. At this dose level two of the three patients were hospitalized for grade 3 diarrhea. Radiotherapy could be completed in both patients. The third patient showed a grade 3 transaminase elevation, again without clinical symptoms, which normalized after stopping nelfinavir and he developed a grade 4 wound complication of the abdominal wound for which surgical revision was necessary. Although a relationship between this wound complication and the study medication seems to be unlikely, it cannot be ruled out completely. Because DLT occurred in all three patients in DL3, DL1 was declared the MTD. An overview of all toxicities ≥grade 3 is shown in table 2. As can

be concluded from the overall treatment times (OTT) mentioned in this table, a treatment interruption of more than five days was necessary for one patient.

Table 2 Acute toxicity during chemoradiation until 4 weeks post-surgery

Patient no[1]	Dose level NFV	Toxicity ≧ gr 3	Dose adjustment NFV[2]	Description	Relation with study medication
1	750 mg BID	n	n		
2	750 mg BID	n	n		
3	750 mg BID	n	n		
7	750 mg BID	n	n		
8	750 mg BID	n	n		
9	1000 mg BID	y	y	grade 3 transaminase elevation grade 4 wound dehiscence	likely unlikely
10	1000 mg BID	y	y	grade 3 diarrhea	possible
11	1000 mg BID	y	y	grade 3 diarrhea	possible
4	1250 mg BID	y	n	grade 3 ileus	indirectly
5	1250 mg BID	y	n	grade 3 transaminase elevation	likely
6	1250 mg BID	y	y	grade 3 cholangitis	possible

[1] In chronological order of inclusion in the trial.

[2] Dose adjustment due to high plasma levels.

Nelfinavir plasma levels

Nelfinavir plasma levels turned out to be quite variable between patients. In our patient group we observed mainly high CRs: the median CRs were 1.7 (range 1.2–7.1), 1.2 (0.6–6.0) and 1.7 (1.1–4.1) respectively in weeks two, four and six of nelfinavir use. In four patients (three in DL2 and one in DL3), the prescribed nelfinavir dose was adapted because of CRs >1.5. All patients who needed a dose adjustment also developed a DLT. In total 4/6 patients with DLT also had a CR>1.5. No clear relationship between the height of CR and the occurrence of DLT was observed.

Table 3 Treatment details and response to therapy

Patient no	Dose level NFV	NFV completed	RT dose (Gy)	OTT (days)	Adj chemo[1]	Interval RT surgery (days)	Type of surgery	cTN stage	ypTN stage	TRG[2]
1	750 mg BID	y	50.4	37	n	58	APR	cT3N2	ypT1N0	2
2	750 mg BID	y	50.4	37	y, 2	76	LAR	cT3N2	ypT3N0	3
3	750 mg BID	y	50.4	37	y, 1	58	APR	cT2N2	ypT3N2	4
7	750 mg BID	y	50.4	39	n	49	APR	cT3N0	ypT3N0	3
8	750 mg BID	y	50.4	38	n	59	APR	cT3N1	ypT3N0	3
9	1000 mg BID	y	50.4	38	n	58	APR	cT3N1	ypT0N0	1
10	1000 mg BID	n	50.4	37	y, 2	71	LAR	cT4N2	ypT0N0	1
11	1000 mg BID	n	50.4	53	n	63	APR	cT2N1	ypT2N0	4
4	1250 mg BID	n	32.4	23	n	112	LAR	cT3N2	ypT3N0	5
5	1250 mg BID	n	50.4	37	n	63	LAR	cT3N1	ypT0N0	1
6	1250 mg BID	n	50.4	40	n	72	LAR	cT3N0	ypT2N0	3

[1] Adjuvant chemotherapy given before surgery and if yes number of cycles.

[2] TRG scores according to Mandard.

Resections, response and follow-up

All patients underwent a radical resection: an abdominoperineal resection in six cases and a low anterior resection in five cases. The mean interval between radiotherapy and surgery was 67 days (range: 49–112 days). Table 3 shows the responses of all patients. Three patients (27%) showed a pathological complete response. These pCRs were all observed in DL2 and 3. Taking TRG into account and considering TRG1 and 2 to be good responders, four patients had a good response (TRG1 and 2). T-downstaging was found in five (45%) patients and N-downstaging in eight (73%) patients. Surgery resulted in a radical resection in all patients. At the time of analysis, all patients were alive without local or distant recurrence with a follow-up between five and 27 months.

Discussion

This study demonstrates that the combination of capecitabine, radiotherapy and nelfinavir is feasible, although the RP2D in this combination turned out to be the lowest dose level tested (750 mg BID). This is the third phase I study evaluating the use of a protease inhibitor in combination with chemotherapy and radiotherapy and the first study in rectal cancer patients. The other studies evaluated pancreatic cancer and non-small cell lung cancer patients [27, 28]. In these studies, nelfinavir was added to radiotherapy combined with gemcitabine and cisplatin the pancreas trial and radiotherapy and cisplatinum and etoposide in the lung trial. In contrast to our observations, no DLT related to the study medication was observed in both studies. The reason for that may be the different chemotherapy used in both studies and the different toxicity profiles of radiation of the upper abdomen versus the pelvic region.

In this study design we chose to escalate the dose of nelfinavir because the combination of radiotherapy and capecitabine in a dose of 825 mg/m^2 is already well established and widely used. Diarrhea is a well-knownside effect of this treatment regimen. In a recent series of 147 patients treated with 25x2 Gy in combination with capecitabine, severe diarrhea (grade 3) was observed in 10.2% of patients [29]. Furthermore, diarrhea has been reported in up to 50% of patients treated with capecitabine monotherapy, of which 15% was classified as severe diarrhea (13% grade 3, 2% grade 4) [30]. Because diarrhea is also a frequently occurring side effect of nelfinavir [31], we decided to start with a relatively low dose (750 mg BID) of this drug. In pre-clinical studies, nelfinavir has been tested at concentrations normally achieved in HIV positive patients treated with HAART and it has been shown that higher doses only slightly increased radiation response. Therefore, the dose used in HIV treatment (1250 mg BID) was used as the highest dose level.

Diarrhea was observed frequently in this study: three patients developed grade 3 diarrhea for which they were hospitalized. Severe diarrhea (≥grade 3) was only observed in DL2 and DL3. Patients who have a deficiency in dihydropyrimidine dehydrogenase (DPD), an important enzyme in the catabolism of 5-FU of which

capecitabine is a prodrug, can develop severe toxicity during capecitabine use. This may have influenced the endpoint of this study. Although patients in this study were not tested for DPD deficiency, we had no clinical suspicion of such a deficiency as these patients often develop severe toxicity of multiple organ systems, including a very pronounced mucositis, early during treatment.

It was quite remarkable that two patients showed grade 3 transaminase elevations and one patient developed a grade 3 cholangitis. All patients in this study had normal liver function tests before start of treatment. In trials testing nelfinavir for treatment of HIV, the reported incidence of transaminase elevations was around 2%, thus it is not a frequently occurring side effect [32]. Although elevated liver enzymes are reported frequently (1–10%) during capecitabine monotherapy according to the product information, this side effect is often mild and seldom a reason for dose adjustments [30].

Hepatotoxicity has also been described with the use of HPIs, though the reported rate of severe hepatotoxicity differs between different HPIs. None of the patients in this study had a pre-existing liver disease. In a meta-analysis including four studies looking at hepatotoxicity, nelfinavir showed the lowest rate of transaminase elevations as compared to other HPIs [32]. No additive effect of liver toxicity between capecitabine and nelfinavir was described in the literature. It is not clear whether the case of cholangitis was treatment related, but no other clear cause could be found. Therefore a relationship with the study drug could not be ruled out.

Four out of six patients who developed DLT also needed a dose adjustment of NFV because high plasma levels. Although the most important P450 isoenzymes in the metabolism of nelfinavir are CYP3A and CYP2C19, CYP2C9 is also involved in the metabolism of nelfinavir. Capecitabine is an inhibitor of CYP2C9. This could be a possible explanation of the relative high number of patients showing high nelfinavir plasma levels requiring dose adjustments. In an important part of the Caucasian population polymorphisms of CYP2C19 and CYP2C9 have been described [33]. These polymorphisms can also lead to a different metabolism. Another possible explanation could be a different absorption of nelfinavir due to mucosa changes in the gastrointestinal tract caused by nelfinavir. From the

experience in HIV treatment no connection between high plasma levels and toxicity has been described. Although our observationsindicate a possible relationship between high NFV plasma levels and toxicity, the number of patients in this phase I trial is too small to draw conclusions.

Three of 11 patients (27%) had a pathological complete response and four of 11 patients (36%) had a major response taking TRG into account. Although the percentage is rather high compared to response rates reported in trials using a comparable chemoradiation regimen [7], no conclusions can be drawn about the additive effect of nelfinavir because of the small sample. All three complete responders were treated in dose level 2 or 3, but this could be by chance.

The radiosensitizing effects of HPIs have been attributed to several mechanisms. The first mechanism is the inhibition of Akt phosphorylation [14]. In pre-clinical studies, drug concentrations comparable with therapeutic levels achieved in HIV patients were shown to inhibit Akt. Apart from dephosphorylation of Akt, HPIs are thought to have direct anti-tumor effects through several other mechanisms. Amongst the mechanisms described in literature are proteasome inhibition, the inhibition of matrix metalloproteinases and immunomodulatory effects [34], as well as stimulation of apoptosis and autophagy [35] and improvement of vascular flow and decrease of hypoxia [36, 37]. In combination with radiation, HPIs have effects on endothelial cells, leading to increased apoptosis of endothelial cells and blockage of endothelial cell migration and organization [38]. It is important to note that these mechanisms occur at different nelfinavir concentrations in laboratory studies.

One of the downsides of nelfinavir is that plasma levels can vary importantly between patients and that there is an interaction with a broad group of drugs [39]. In the treatment of HIV patients, concentration ratios <0.90 are frequently observed [23]. In our patient group, we mainly observed CRs that were higher than expected: the dose adjustments were all results of too-high plasma levels. Three of the four patients needed a dose adjustment developed DLT, but it is not known whether there is a relationship between plasma levels and the chance of toxicity. For diarrhea, no clear relationship has been shown between dose levels and the chance of severe diarrhea [40] and no information is

available about dose levels and the chance of hepatotoxicity.

In conclusion, this study shows that the combination of nelfinavir with capecitabine-based chemoradiation in locally advanced rectal cancer is feasible, but the toxicity rates are rather high. From this phase I trial, nelfinavir 750 mg BID turned out to be the RP2D. A further phase II study is needed to learn more about the safety and efficacy of this combination treatment.

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Chapter 8

A phase I-II study on the combination of rapamycin and short course radiotherapy in rectal cancer

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Submitted

Abstract

Purpose

This phase I/II study sought to determine the safety and maximum tolerated dose (MTD) of the combination of rapamycin, an mTOR inhibitor, with short-course radiotherapy in rectal cancer patients. Antitumor activity, changes in metabolic activity and perfusion on imaging, and changes in phosphorylation status of the mTOR pathway were also assessed.

Experimental design

Patients with primary resectable rectal cancer were treated with short-course hypofractionated radiotherapy (5x5 Gy), followed by surgical resection.

Results

Thirteen patients were entered in phase I. One patient developed a dose-limiting toxicity, consisting of a grade 4 leak and grade 4 bleeding. Because of an unexpected high rate of grade 3 postoperative toxicity, it was decided to treat patients with delayed surgery in phase II. Thirty-one patients were treated with the MTD of 6 mg rapamycin daily. One patient (3%) developed a pathological complete response (pCR) and 3 patients (10%) had a ypT1N0 tumor at the time of resection. No change in tumor perfusion was observed on perfusion CT, but a significant decrease of metabolic activity was found on PET-scan.

Conclusion

The combination of short-course radiotherapy and rapamycin turned out to be feasible, provided that the interval between neo-adjuvant treatment and surgical resection is at least 6 weeks. Although from this cohort no clear increase in pCR could be observed, a clear metabolic response after rapamycin run-in was observed, indicating a biological activity of this drug in rectal cancer.

Introduction

Pre-operative radiotherapy has an important role in the treatment of rectal cancer. For primary resectable rectal cancer, short-course hypofractionated radiotherapy (5x5 Gy) results in a 50% reduction of local recurrence [1]. If resection takes place within a week after the completion of radiotherapy, no downsizing of the tumor will occur. However, after an interval of at least 6 week, substantial downsizing can be observed [2, 3]. As this short course schedule is primarily used for relatively small tumors, a part of the patients will develop a complete response. This opens the way for alternative organ preservation treatment strategies [4]. In view of the relatively limited toxicity observed with this treatment, there is room for treatment intensification.

In order to further increase the tumor response rates, radiotherapy can be combined with radiosensitizing drugs. The mammalian target of rapamycin (mTOR) pathway is attractive to exploit, because it plays a central role in survival strategies of tumor cells and influences angiogenesis [5]. Furthermore, it has been shown that mTOR has a direct link with the phosphatidylinositol-3'-kinase (PI3K)/PTEN-AKT survival pathway [6]. Preclinical evidence shows a radiosensitizing effect of mTOR inhibitors [7, 8]. Especially the link between mTOR inhibition and the inhibition of angiogenesis may be important for the radiosensitizing effects [9]. Neovasculature in tumors tends to be unstructured and leaky, leading to an inefficient blood flow and hypoxic areas. Anti-angiogenic treatments may result in normalization of this vasculature resulting in improved oxygen supply [10].

Rapamycin is the oldest mTOR inhibitor used for a long time as an immune suppressant. It was shown more than 20 years ago that rapamycin also had antiproliferative effects in tumor cell lines. During the last years analogues of rapamycin have been developed as anti-cancer drugs. The advantage of rapamycin is that there is extensive clinical experience with its use as immune suppressant after kidney transplantation and it is an oral drug.

The objective of this trial was to investigate the safety and the activity of rapamycin, administered before and during preoperative radiotherapy in

patients with resectable rectal cancer. The purpose of phase I was to determine the recommended phase II dose (RP2D). For phase II of this study, change in tumor perfusion was chosen as the primary endpoint. We believe this is an attractive surrogate endpoint predicting response, as it can be measured early after the start of treatment. Furthermore it gives insight in the mechanism of action of rapamycin in rectal cancer with and without radiation. It was hypothesized that rapamycin would lead to a decrease of the activation status of mTOR related and dependent molecules in the tumor reflected by a decrease in staining for phosphorylated mTOR (p-mTOR) and phosphorylated 4E-BP1in tumor biopsies. 4E-BP1 is a protein that is activated and dissociated from eIF4E after phosphorylation, leading to increased translation, and is downstream effector of mTOR complex 1 (mTORC1).

Methods and materials

This was an open-label, single-institution prospective phase I/II trial, approved by the local Medical Ethical Committee. Radiotherapy was performed at Maastro Clinic, surgery was done in the referring hospital. All patients gave written informed consent. The trial has been registered at clinicaltrials.cov (NCT00409994).

Patient eligibility and treatment

Patients with a primary resectable rectal cancer, defined as cT2-3 without involvement of the circumferential resection margin (CRM) as judged on MRI and cN0-1, for whom short course pre-operative radiotherapy was advised in the Tumor Board meeting, were eligible for inclusion in the trial. The most important exclusion criterion was the concurrent use of CYP3A4 inhibiting drugs.

An outline of the study is presented in figure 1. Radiotherapy treatment consisted of 5 fractions of 5 Gy given within an overall treatment time of 7 days. The treated volume consisted of the primary tumor, mesorectum, presacral area and internal iliac lymph nodes. Patients were treated using a 4-field 3D-

conformal technique or a VMAT technique. During phase I patients were operated within 3 days after the last fraction of radiotherapy. In phase II resection was performed preferably 7-8 weeks after completion of radiotherapy, but a minimum interval of 6 weeks was required.

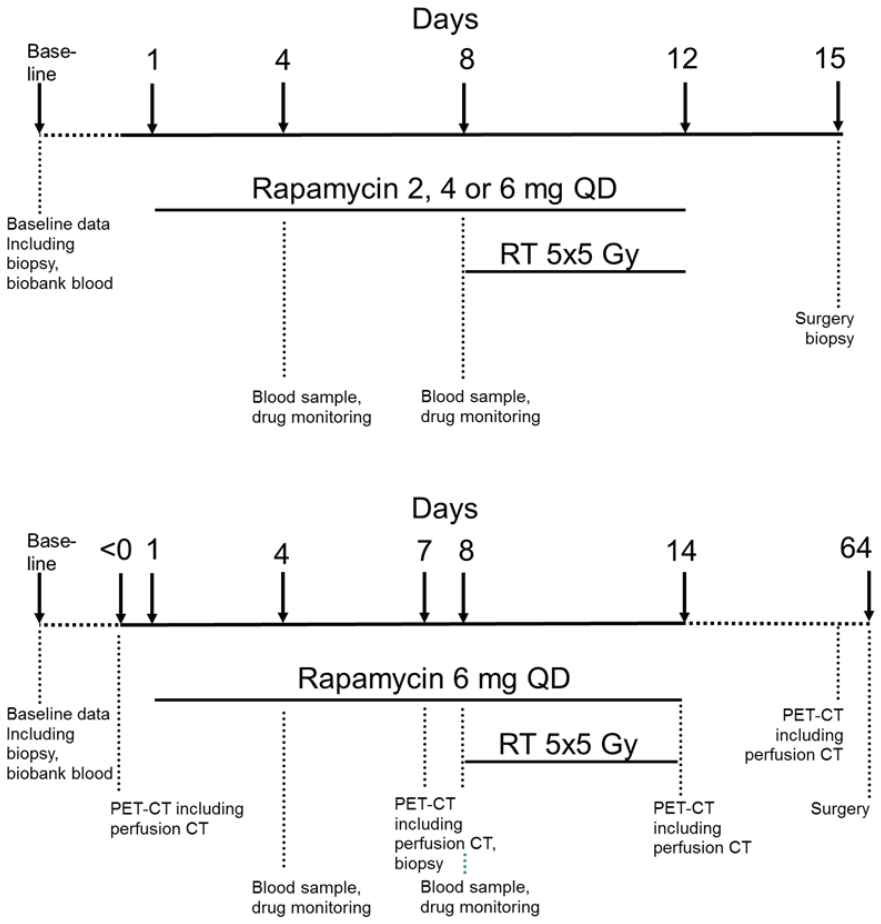

Figure 1 Outline of phase I (upper panel) and phase II (lower panel) of the study.

Rapamycin treatment was started 1 week before the start of radiotherapy. For phase I, three dose levels were defined: 2 mg, 4 mg and 6 mg daily respectively. Cohorts of 3–6 patients were treated. If no DLT occurred, then subsequent

patients were treated at the next dose level. In order to monitor rapamycin plasma levels, blood samples were taken on day 4, 8 and 15. Trough levels aimed at were 5-15 µg/l for dose level (DL) 1, 10-30 µg/l for DL2 and 15-45 µg/l for DL3. If plasma levels were lower than expected on day 4, patients were instructed to increase the rapamycin dose.
Plasma levels were then checked again on day 8 just before the start of radiotherapy. For phase II, the recommended phase II dose (RP2D) resulting from phase I was used.

Patient evaluation
During phase I acute toxicity was scored after 1 week of rapamycin single use, at the day of the last fraction of radiotherapy and weekly thereafter until 4 weeks post-operatively. Toxicity was graded according to the common toxicity criteria (NCI-CTCAE v3.0). Dose limiting toxicity (DLT) was defined as grade 4 or 5 postoperative toxicity. Secondary endpoints for phase I were incidence of other toxicity and activation status of mTOR related and dependent molecules in the tumor. During phase II, toxicity was evaluated at the same time points during treatment and every 2 weeks after treatment until resection. Secondary endpoints for phase II were tumor response, incidence of acute toxicity and changes in tumor metabolism as measured on PET scan.

Treatment evaluation
During phase II an FDG-PET-CT scan was performed before start of treatment, at day 8 after 1 week of rapamycin use only and on day 15, directly after completion of radiotherapy. A fourth scan was made around day 64. Changes in perfusion were measured by means of a perfusion CT-scan (pCT), which was made with the PET-CT scanner directly after the PET-scan. The volume for perfusion measurement was located within the area of high FDG-uptake, to ensure central location within the tumor. The trans- endothelial volume transfer constant K^{trans} was measured at all different time points. Technical details have been described previously [11]. PET-scans were used to analyze changes in metabolic activity.

Statistical analysis

Differences in perfusion and changes in metabolic activity were analyzed using the Wilcoxon signed rank test. Primary endpoint for phase I was incidence of severe postoperative complications (grade 4 or grade 5 toxicity). Primary endpoint for phase II was tumor blood flow (K^{trans}) assessed by perfusion CT. Secondary endpoints were incidence of acute toxicity, pathological response and metabolic response. It was hypothesized that tumor perfusion measured as K^{trans} would decrease with 40% [12] with a standard deviation of 26 [13]. A sample size of 44 patients was required for a beta error of 5% and an alpha error of 10%. The dropout rate was estimated to be 10%, and therefore the inclusion of 47 patients was aimed for.

Tissue correlative studies

In phase I extra tumor biopsies were taken after 1 week of rapamycin monotherapy and after completion of radiotherapy. Tumor biopsies were stained immunohistochemically, for phospho-mTOR (Bioke #2976) and phospho-4E-BP1 (Bioke #9451), according to the manufacturer's protocol. It was hypothesized that the use of rapamycin, an mTOR inhibitor, would result in a decrease of mTOR and 4E-BP1 phosphorylation. Sections were scored by two individual pathologists.

Results

Phase I – Patient characteristics

Thirteen patients were entered in phase I of the study, between November 2006 and March 2009. Three patients were treated in DL1, 4 in DL2 and 6 in DL3. Patient characteristics are shown in table 1. In DL1 no dose adjustments were necessary at day 4, in order to reach the planned plasma trough levels at the start of radiotherapy. In DL2 2 patients needed a dose adjustment to 6 mg rapamycin daily. In DL3 a dose adjustment was needed in 4 patients. In 2 patients the daily dose of rapamycin was increased to 10 mg and in 2 patients to 12 mg.

Table 1 Patient characteristics

Characteristic		Phase I (n=13)	Phase II (n=31)
Sex	Male	11 (85)	21 (68)
	Female	2 (15)	10 (32)
Age	Median	62 [49-82]	66 [44-82]
cT stage	T2	5 (38)	10 (32)
	T3	8 (62)	21 (68)
cN stage	N0	4 (31)	21 (68)
	N1	9 (69)	10 (32)
Type of surgery	LAR	10 (77)	24 (77)
	APR	3 (23)	2 (7)
	TEM	0	5 (16)
(y)pT stage	T0	0	1 (3)
	T1	1 (8)	3 (10)
	T2	6 (46)	17 (55)
	T3	5 (38)	10 (32)
	T4	1 (8)	0
(y)pN stage	N0	7 (54)	21 (68)
	N1	6 (46)	10 (32)

Phase I – Toxicities

During the preoperative treatment with rapamycin only and radiotherapy with rapamycin no toxicities greater than grade 2 were observed. The most reported mild side effects were headache, diarrhea and stomatitis. In the postoperative phase, 2 patients suffered from a grade 3 wound infection, one patient in DL1 and one in DL3. Four patients experienced a grade 3 ileus, which persisted for >1 week in 1 patient (1 patient in DL1, 2 patients in DL2 and 1 in DL3). Two patients developed a grade 3 leak (1 in DL1 and 1 in DL2) and 1 patient was faced with a grade 4 leak (treated in DL4). The patient who had a grade 4 leak, also had a grade 4 bleeding and grade 3 colitis. In total 7 patients developed grade 3 or higher toxicities (some patients developed >1 toxicity). A complete overview of all toxicities ≥ grade 3 is given in table 2.

Although only one case of DLT was observed and according to the protocol DL3 could be declared the RP2D, based on the clinical judgment of the investigators the percentage of postoperative grade 3 toxicities was deemed unacceptable compared to the published toxicity profile of 5x5 Gy without rapamycin. Because wound infections and leakage may have been influenced by the immunosuppressive, protein synthesis inhibiting and anti-angiogenetic effects of rapamycin [14], it was decided in accordance with the ethical committee to postpone the time of surgery for phase II to 7-8 weeks after the completion of radiotherapy. A daily dose of 6 mg rapamycin was declared RP2D.

Table 2 Overview of toxicities in phase I

Patient no	Dose level rapamycin	Dose adjustment	Adjusted dose	Toxicity ≥gr 3
1	2 mg	n		gr 3 woundinfection
2	2 mg	n		gr 3 ileus
3	2 mg	n		gr 3 GI leak
4	4 mg	y	6 mg	
5	4 mg	n		gr 3 ileus
6	4 mg	n		gr 3 ileus gr 3 GI leak gr 3 anorexia
7	4 mg	y	6 mg	
8	6 mg	y	10 mg	
9	6 mg	y	12 mg	gr 3 ileus gr 3 malabsorption gr 3 woundinfection
10	6 mg	n		gr 3 colitis gr 4 GI leak gr 4 hemorrhage
11	6 mg	n		
12	6 mg	y	10 mg	
13	6 mg	y	12 mg	

Phase I – Immunohistochemistry

Tumors were stained immunohistochemically for phosphorylation of mTOR and 4E-BP1 (figure 2). For the p-mTOR staining, results were available for 7 patients. In 1 patient p-mTOR was scored as 'strong' before treatment, 'moderate-strong' in 4 patients, 'moderate' in 1 patient and 'weak-moderate' in 1 patient.

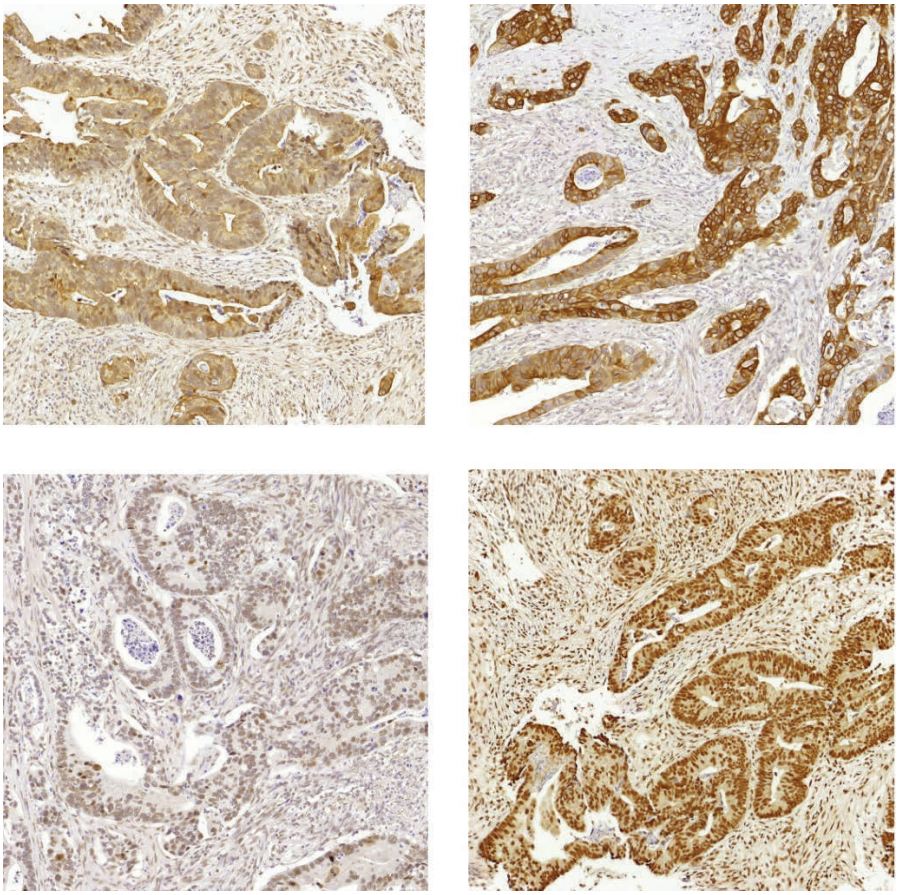

Figure 2 Immunohistochemical analysis of the phosphorylation status of mTOR (upper panels) and 4E-BP1 (lower panels) before treatment (left panels) and after treatment (right panels) on tumor biopsies of the same patient.

In 3 patients no change in phosphorylation status of mTOR was observed, in 3 patients there was a slight increase in p-mTOR and in one patient a decrease in

p-mTOR was found. Phosphorylated 4E-BP1 data were available for 8 patients. The pre-treatment score was 'strong' in 5 patients and 'moderate-strong' in 3 patients. In 6 patients a slight decrease in p4E-BP1 status was found, while 1 patient showed a slight increase and in 1 patient a phosphorylation status remained stable. No clear correlation between changes in p-mTOR staining and p-4E-BP1 staining was observed.

Phase II – Patient characteristics

In total, thirty-one patients were entered in phase II, between April 2010 and April 2013. Inclusion was stopped because the proportion of rectal cancer patients referred for short-course radiotherapy decreased due to a change in the national guidelines and because it became clear that the primary endpoint would not be reached. Patient characteristics are shown in table 1. All patients received 6 mg rapamycin daily. In 18 patients a dose adjustment was necessary on day 4, in order to reach the planned plasma trough levels at the start of radiotherapy. Six patients were switched to 8 mg rapamycin daily, 8 patients to 10 mg and 4 patients to 4 mg. The rapamycin plasma levels are depicted in figure 3.

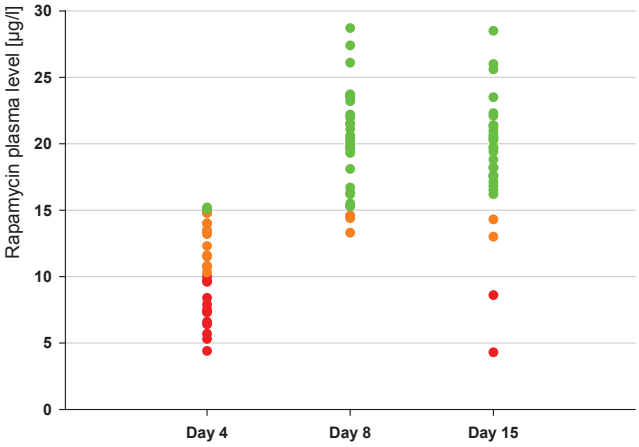

Figure 3 Rapamycin plasma trough levels measured at different time points. The level aimed at was >15 µg/l (green dots).

Phase II – Toxicities

During the first 2 weeks of treatment (rapamycin only and radiotherapy combined with rapamycin) no toxicities worse than grade 2 were reported. Two weeks after completion of radiotherapy one patient reported grade 3 diarrhea, nausea and vomiting. This was the only grade 3 or higher toxicity reported in the interval between radiotherapy and surgery and it was judged to be related to the chemotherapy that was started after completion of radiotherapy. The most frequently reported toxicities post-radiotherapy were proctitis (45% grade 1-2 on day 22 and 19% on day 36) and diarrhea (45% grade 1-2 on day 22 and 35% on day 36). No severe postoperative complications have been reported in phase II.

Table 3 PET and perfusion parameters

Day	**SUV_{max}**			**PET volume**			**K_{trans} mean**		
0	14.5	(5.2-27.9)		13.6	(2.10-30.2)		.55	(.31-.82)	
8	12.3	(4.2-21.0)	p=.033[1]	12.0	(3.1-27.9)	p=.019[1]	.54	(.30-.87)	p=.92[1]
15	11.8	(3.8-28.2)	p=.113[1]	9.9	(1.0-25.3)	p=.035[1]	.51	(.26-.83)	p=.26[1]
64	6.8	(0-15.8)	p<.001[1]	6.0	(0-24.8)	p<.001[1]	.50	(.40-.60)	p=.72[1]

[1]Wilcoxon signed rank test, SUV_{max}as compared to the scan one timepoint earlier

Phase II – Perfusion and PET-response

The results of the changes in PET-activity and tumor perfusion are summarized in table 3 and figure 4. Metabolic activity decreased significantly after 1 week of rapamycin only treatment (mean SUV_{max} 14.5 vs. 12.3 (p=.033)). No further significant decrease was observed at the last day of radiotherapy (mean SUV_{max} 12.3 vs. 11.8 (p=.113)). On day 64 a marked further decrease in FDG-uptake was found (mean SUV_{max} 6.8 (p<.001)). Tumor volume measured on PET-scan decreased gradually over time, as well after rapamycin treatment as after 5x5 Gy. Tumor perfusion, as reflected by K^{trans}, remained at a constant level during the entire treatment. No difference was observed in perfusion between patients who had tumor downstaging as compared to patients in whom no downstaging occurred.

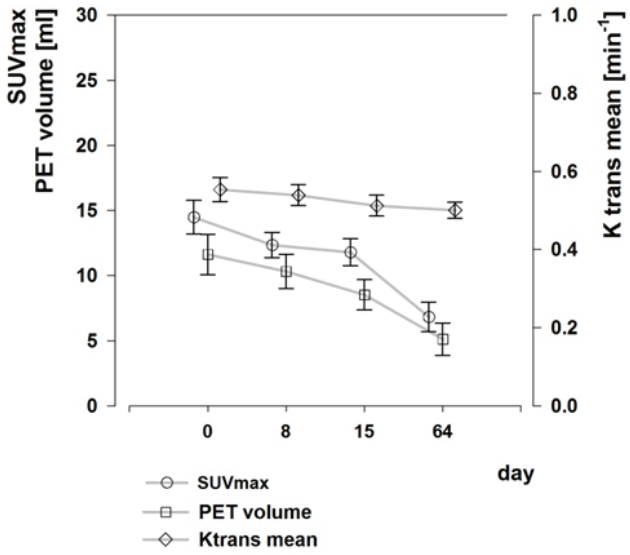

Figure 4 SUV_{max}, tumor volume and mean K_{trans}at different time points during treatment.

Phase II – Surgery, pathological response and local control

In 29 patients sphincter saving surgery was initially performed: a low anterior resection (LAR) in 24 patients and transanal endoscopic microsurgery (TEM) in 5 patients (table 1). In the remaining 2 patients an abdominoperineal resection (APR) was chosen. Two of the five patients who were initially treated by TEM were finally diagnosed with a ypT3 tumor, which consequently led to a LAR subsequently. One patient was diagnosed with a pCR in the resected specimen and 2 patients developed a local recurrence 5 resp. 12 months after TEM. One of these 2 patients was salvaged by chemoradiation followed by a pelvic exenteration and the second patient underwent a salvage APR. Unfortunately this last patient was recently diagnosed with a second local recurrence. Median time between end of radiotherapy and surgery was 65 days (range 45-235).

Apart from the patient with a pathological complete response in the TEM-specimen, in 3 patients the tumor was downsized to ypT1N0, resulting in a major response in 13% of patients (4 out of 31). Seventeen patients had a T2 tumor at the time of resection; in 4 (24%) of them positive nodes were found in

the resected specimen. Of the 10 patients who still had a T3 tumor after neoadjuvant treatment, 6 had positive nodes (60%). After a median follow-up of 39 months, 3 patients (7%) developed metastases. At the date of last follow up 2 patients had died of their metastases.

Discussion

This phase I/II trial used change in tumor perfusion as a surrogate endpoint in order to have a rapid endpoint of biological activity and to gain insight in a specific mechanism of action of rapamycin in combination with radiotherapy. This novel approach allows for a more rapid answer to specific research questions for a biological modifier, as compared to more classical endpoints such as local control and overall survival. In contrast to the hypothesis, no change in perfusion was seen, nor after rapamycin nor after combined rapamycin and radiotherapy. The combination of radiotherapy and rapamycin turned out to be safe, on the condition that surgery was delayed to 8 weeks after the end of radiotherapy.

Although the formal criteria for DLT were not met in phase I of this trial, the postoperative toxicity observed in phase I of the study was quite remarkable and deemed clinically unacceptable. Wound healing can be influenced by mTOR inhibitors by the inhibition of angiogenesis and fibroblast proliferation [14, 15]. There is some evidence from randomized trials using different immunosuppressive regimens in post-transplant patients that wound complications occur more often in patients using rapamycin as compared to patients using other types of immunosuppressants, especially in patients with high trough concentrations (15-20 ng/ml) [14, 15]. In our study, postoperative toxicities were observed at all dose levels. In phase II, after the introduction of delayed surgery, no severe postoperative toxicities were seen. Although the patient numbers do not allow firm conclusions, we think it is thus advisable to keep a gap between any neo-adjuvant treatment with mTOR inhibitors and surgery.

Treatment results of short-course pre-operative radiotherapy directly followed by TME surgery for primary resectable cancer are good. The 10-year cumulative

incidence of local recurrence for this treatment arm was 3% in the Dutch TME trial. Overall survival at ten years was similar for the irradiated and non-irradiated group (48 vs. 49%) [1]. Therefore it is not to be expected that addition of rapamycin leads to substantial improvement in treatment outcome when choosing this treatment approach. An alternative approach is to delay surgery to 6-8 weeks after completion of radiotherapy [2, 3]. This has the advantage that tumor downsizing can occur before surgery is performed and allows to choose less invasive treatment options in case of a good clinical response. Because the postoperative toxicity observed in phase I was not negligible and a relation with the study medication could not be ruled out, it was decided to treat patients in phase II with delayed surgery, in order to avoid any negative impact on wound healing. In addition, the effect of the combined treatment on tumor downsizing could be observed. Pathological complete response rates reported after 5x5 Gy and delayed surgery typically lie in the order of 8% [2, 3, 16, 17]. In this small patient group only 1 patient (3%) developed a pCR. However, a rather strict interval between the end of radiotherapy was used in this trial, while in the majority of the published reports the exact interval was not mentioned. This may influence the pCR rate quite substantially. In addition, inhibition of mTOR slows down proliferation and leads to cell cycle arrest [18]. A slower proliferation may result in a slower mitotic cell death after irradiation and thus a slower tumor response. Recently, a paper reviewing the negative feedback loops that become activated as a result of mTOR inhibition, has been published. Suppression of these feedback loops leads to overactivation of upstream pathways, including PI3K, AKT and ERK. This may counteract the antiproliferative effects of mTOR inhibitors [19].

Primary endpoint of the study was a decrease in tumor perfusion as reflected by K^{trans}. This endpoint was not met and actually no change in K^{trans} was found, neither after rapamycin run-in nor after rapamycin and radiotherapy. A possible explanation could be a mechanism that was observed after the development of this trial. K^{trans} values were measured in 23 patients treated with short-course radiotherapy only (5x5 Gy) [11]. Perfusion CT imaging was performed before the start of treatment and at the day of the last fraction of radiotherapy. A

significant increase in perfusion was observed as reflected by an increase in K^{trans} from 0.36±0.11 min^{-1}beforetreatment to 0.44±0.13min^{-1} at day 5 (p<0.001). However, in the present study K^{trans}remained unchanged during radiotherapy and the addition of rapamycin. It could therefore be hypothesized that the increase in K^{trans}that was observed after 5x5 Gy without rapamycin was counterbalanced by the decrease in K^{trans} expected to result from the anti-angiogenic effects of rapamycin. Recently published data using everolimus, another mTOR inhibitor, also did not observe a change in tumor perfusion measured with DCE-MRI after everolimus monotherapy [20]. The influence of rapamycin on tumor vasculature was studied in a rhabdomyosarcoma mouse model [8]. In this model, a decrease in tumor microvasculature was found and an increase in oxygenation was observed already after 5 doses of rapamycin, but tumor vessel permeability only minimally changed. This raises the question whether perfusion imaging is the most suitable method to evaluate vasculature changes caused by rapamycin. However, Willett *et al* found the same changes in microvessel density after the administration of bevacizumab, a VEGF antibody, but they noted a clear decrease in tumor perfusion on perfusion CT [12]. K^{trans} describes the transfer rate of contrast agent from the blood to the extravascular-extracellular space and is related to microvascular blood flow, vessel wall permeability and vessel density. This means that this constant is influenced by different aspects of tumor vasculature. A pre-clinical study looked at the specific anti-angiogenic mechanisms of an mTOR inhibitor (everolimus) as compared to a VEGFR tyrosine kinase inhibitor [21]. They described that alterations in tumor vascular biology were partly caused by comparable mechanisms but differences in the vascular response were also observed. In the mouse model of this study, K^{trans} as measured with DCE MRI remained constant during mTOR treatment, despite a clear tumor response, but a clear decrease in $K^{trans}$was seen after treatment with a VEGFR inhibitor. Apparently, different ways of anti-angiogenic treatment result in different types of vasculature changes and this translates into different changes on imaging. Furthermore, responses of tumor vasculature to mTOR inhibitors can be quite heterogeneous possibly depending on differences in the tumor microenvironment [22].

In contrast to the perfusion scans, a significant decrease in metabolic activity was seen on PET-CT. This observation is in line with the findings of Ciunci *et al* in their phase I trial with everolimus and cetuximab [20]. In the run-in phase of everolimus a mean decrease in SUV_{max} of 24% was seen. Other clinical and preclinical studies showed the same results. Recently a quick and clear decrease in FDG uptake was demonstrated under mTOR1/2 inhibition in a mouse glioma model [23]. In a preclinical model of cisplatin-resistant ovarian cancer, treatment with a dual PI3K/mTOR inhibitor resulted in a significant decrease of FDG-uptake, which correlated with a decrease in proliferation and inhibition of the PI3K/mTOR pathway [24]. In a mouse model in which colon tumors are initiated by a dominant active PI3K, a clear response was seen on PET-scan after treatment with rapamycin, but not after placebo treatment [25]. Honer *et al* used different cell lines in a mouse model: cell lines that were *in vitro* characterized as sensitive to everolimus and cell lines that were insensitive. The cell lines that were insensitive *in vitro* did show growth inhibition but no changes in FDG metabolism were observed, while in the sensitive tumor model a clear decrease of FDG-uptake was observed [26]. The authors hypothesize that this may be explained by a different mechanism of action, namely the anti-angiogenic action of everolimus, and that the change in FDG-metabolism is caused by other mechanisms. In a study with patients with metastatic renal cell cancer, a decrease in FDG-uptake was found after treatment with everolimus [27]. This early metabolic change was correlated with change in tumor burden.

In conclusion, rapamycin turned out to be safe in combination with short-course hypofractionated radiotherapy in rectal cancer treatment, but it is advisable to postpone surgical treatment until 8 weeks after combined treatment to avoid a possible increase in postoperative complications. Although rapamycin is thought to cause changes in tumor vasculature, this does not translate in changes in Ktrans in vivo. Rapamycin has a biological influence on rectal cancer as reflected by the changes in FDG-uptake. In this patient cohort no clear increase in tumor response was observed after combined radiotherapy and rapamycin.

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Chapter 9

General discussion and future perspectives

Results of rectal cancer treatment have improved substantially during the last 25 years. Patients diagnosed with rectal cancer in 1990 had a 5-year overall survival of approximately 50%, while patients who were treated in 2010 had a 65% chance of being alive 5 years later [1]. This improvement in treatment outcome can be ascribed mainly to improvements in surgical techniques. The most important contribution of preoperative radiotherapy is an improvement in local control. Although one would expect that better local control ultimately translates in better overall survival, randomized trials do not support this [2-5]. Considering the local recurrence rates of around 5%, it is not to be expected that these results can be improved. Although the relative risk reduction of pre-operative treatment is 50% in all subgroups, the estimated absolute reduction of local recurrence varies from 1 to 10% [5]. This suggests that the optimum in local treatment has been reached and that a risk of overtreatment is present.

This observation makes it more essential to take into account the toxicity caused by the different treatment components. Although short course preoperative radiotherapy followed by an immediate resection usually does not result in any acute toxicity, it has been shown to add toxicity compared to surgery only. In the Dutch TME trial, toxicity and quality of life have been evaluated carefully. Patients who were treated with radiotherapy and surgery had a higher defecation frequency, fecal soiling and more sexual problems, although this did not translate in a decrease in overall functioning [6, 7]. It is important to keep in mind that long term toxicities of surgery and radiotherapy are overlapping and that it is difficult to separate them. Hence, there are two strategies to minimize treatment related toxicity: 1. a stricter selection of patients who will benefit from pre-operative treatment and 2. the omission of surgery or the use of less invasive surgical techniques in selected patients. The work presented in this thesis can be seen as steps towards a better informed choice between those two strategies (part II of the thesis) and a higher chance to fit in the second strategy (part I and III).

Part 1, devoted to the role of PET-CT in the delineation of rectal tumors, describes methods that can be used to adequately identify the volume of the primary tumor, which makes it possible to give an integrated boost in order to

increase the chance of developing a complete response. The prediction models described in part 2 are a first step towards early selection of patients more likely to respond to neoadjuvant treatment. The clinical trials presented in part 3 were aimed at treatment intensification in order to increase the proportion of patients developing a complete response, making it possible to select them for a wait and see protocol without a surgical resection.

Organ preservation in rectal cancer

Before discussing the results presented in this thesis, it is necessary to discuss briefly the current status of organ preserving strategies in rectal cancer. Although organ preservation sounds as an attractive alternative to major surgery, many questions about oncological safety and functional outcome remain to be answered.

The only patient group that is suitable for organ preserving surgery according to the Dutch guidelines consists of patients with a good risk T1 tumor (T1, maximum diameter 4 cm, grade 1-2, no lymfangio-invasion). In all other patients, organ sparing approaches must be seen as experimental.

For patients who develop a clinical complete response (cCR) after neoadjuvant treatment, a wait and see approach could be an option. However, the definition of cCR is difficult and differs between the groups who reported on this strategy [8, 9]. The concordance rates between cCR as scored pre-operatively and pCR as described by the pathologist after resection are variable and disappointing in several studies [10]. Combination of different modalities (digital rectal examination, endoscopy and MR with diffusion weighted imaging) seems to be the best way to evaluate response to neoadjuvant treatment, but an experienced team is needed to interpret the findings of these examinations. The addition of imaging is very important, not only for the evaluation of the response of the primary tumor, but also for the evaluation of nodal status. An alternative for a watch and wait approach after preoperative treatment is to perform a TEM procedure, which can be seen as a diagnostic and therapeutic procedure. It allows adequate T-staging and removes residual tumor. Although

N-staging is not possible, the ypT-stage can be used to estimate the risk of remaining positive nodes, since there is a clear relationship between T-stage after neoadjuvant treatment and the risk of positive lymph nodes (ranging from <5% for ypT0 tumors to >20% in ypT3-4 tumors) [11-13]. A possible disadvantage of this approach is the concern that the results of completion surgery after TEM may be worse than the results of primary TME surgery [14] although other studies do not report a worse outcome [15-18]. An Italian trial randomized between TEM and TME after chemoradiation in patients with low-lying cT2N0 rectal tumors [19]. Local recurrence rates were not statistically significant between both arms (8% after TEM and 6% after TME). However, the recurrence rate in the TME arm was rather high, taking into account that only small tumors were included and treated pre-operatively with chemoradiation. In two other phase II trials [17, 20], the decision to perform a completion resection was based on predefined criteria. In general, patients who were treated according to the protocol (i.e. completion TME in case of adverse histopathological findings) had a good outcome, but the local recurrence rates were rather high in patients who did not undergo completion surgery. These findings confirm that local surgery after chemoradiation in high-risk patients is an inadequate treatment.

In case of a “watch and wait” approach, patients are followed with frequent imaging and endosocopy. The tumor is expected to recur in a proportion of patients, since it will be very difficult to develop a decision algorithm with 100% specificity for cCR. Therefore, local recurrence rate is not the best endpoint to evaluate organ sparing treatments. In the series of Habr Gama *et al* a 68% 5-year disease free survival was reported in a watch and wait protocol, but this number improved to 94% if the first recurrence was not take into account [21]. As long as salvage surgery is possible, a local recurrence can be seen as a calculated risk. However, it has to be proven that this approach, leaving residual tumor in place for a longer time after chemoradiation than in the case of immediate surgery, does not result in a higher chance of distant metastases. In the Habr-Gama series the occurrence of distant metastases did not differ significantly between patients who developed a local recurrence and who did

not [21]. This question will be very difficult to answer and ideally a randomized trial including radical surgery as a treatment arm should be performed.

PET-CT for tumor delineation in rectal cancer

PET-CT was hypothesized to be a reliable tool for tumor delineation in rectal cancer treatment, leading to a decrease in interobserver variation.

PET-CT scan has gained a lot of attention in the past decade among radiation-oncologists. Particularly ^{18}FDG-PET scans have been proven to be very useful not only for staging, but also for response prediction, treatment evaluation and tumor delineation [22, 23]. The use of PET-CT as a tool for tumor delineation has been studied principally in head and neck cancer [24] and lung cancer [25]. In these two sites, the benefit of PET-CT is more obvious than in rectal cancer, because radiotherapy is often used as a curative treatment in lung and head and neck cancer, whereas in rectal cancer, radiotherapy is considered an adjuvant treatment to surgery. Consequently, lower radiation doses are used for rectal cancer treatment and a large elective volume is treated, which makes it less critical to exactly define the GTV. However, for reasons stated above (limitation of toxicity, dose escalation), PET-CT could be of benefit for treatment planning in rectal cancer as well.

The study described in chapter 2 was essential to know whether PET-CT could be used as a reliable tool for delineation purposes in rectal cancer. The use of a source-to-background ratio-based (SBR) auto-delineation algorithm resulted in a very good correlation between tumor dimensions measured by PET-CT and measured by the pathologist in lung and head and neck cancer [26, 27]. A big advantage of automatic delineation algorithms is the absence of human influence, which introduces inter- and intraobserver variation. This has been confirmed in the work presented in chapter 2: the strongest correlation with pathology results was found for PET-based auto-contouring and the correlation was less strong when observers were involved. The use of PET-CT, nevertheless, has some potential pitfalls. The SBR method as used in chapter 2 depends on a correct calibration of the PET-scanner and this calibration has to be done

separately for each scanner. A second point of attention is the occurrence of peri-tumoral inflammation, which can influence the dimensions measured on PET-CT. Furthermore one has to realize that PET-CT, like any other imaging modality, is not able to detect any microscopic extension of the tumor. A specific caveat for rectal tumors when using PET-based automatic delineation has to do with the anatomy of the rectum, which lies in close proximity to the bladder. Due to the high concentration of FDG in urine it may be difficult to separate the tumor contour from the bladder and a manual check or pre-definition of a region of interest may be necessary.

Apart from the SBR method, other automatic delineation algorithms exist [28, 29]. Recently Withofs *et al* published the results of a comparison of 6 different PET-based GTVs [30]. Four GTVs were created with 2 different commercially available software systems and based on fixed thresholds (SUV 2.5 and 45% of SUVmax), one contour was based on the fuzzy locally adaptive Bayesian algorithm (FLAB) and for the sixth contour a watershed transform and cluster analysis was used. They concluded that contours produced by the different algorithms differed significantly from each other. Even contours created with the same method in another software package were not exactly equal. These findings illustrate that the results that are obtained from PET-based algorithms are helpful but should be interpreted with caution. Nevertheless, the reproducibility of an automated delineation algorithm appears to be much better than for manual PET-based delineation [31]. One should realize that the complete chain influences the outcome of the automatic contour: not only the scanner hardware, but also the reconstruction algorithms used for CT and PET, the delineation software and the transfer to the planning software. It is therefore very important to have a good quality assurance system before automatic delineation can be introduced clinically in a department.

The question is whether all these considerations and variations in PET-technique and delineation algorithms are of great importance in clinical practice for the treatment of rectal cancer. We have shown that GTVs and the intra-observer variation were significantly smaller with the use of PET (chapter 3). This gain in accuracy is partly lost by the wide margins that are needed to

compensate for the uncertainties when treating rectal tumors. As the rectum is a hollow organ and it lies in close proximity to the bladder, there is a large day to day variation in organ filling and organ position [32]. This makes the use of rather big PTV margins to account for this organ motion imperative and makes the potential role of PET different from its role in other tumor sites. However, what remains critical in tumor delineation is a reproducible and reliable identification of the primary tumor and this may even become more important in the context of dose escalation in selected patients.

The hypothesis that PET-CT is an adequate and useful method for tumor delineation in rectal cancer has been confirmed in these 2 studies.

Response prediction in rectal cancer

It was hypothesized that response prediction in rectal cancer treatment was possible by means of multimodality predictive modeling.

In current clinical practice most oncological treatments are based on national guidelines. This has the advantage that treatments are more or less comparable between different hospitals and that treatment choices are evidence based. A disadvantage is that treatment guidelines generally are not detailed enough to help with treatment individualization and that the process of writing and implementation is rather slow, so that it is difficult to introduce the most recent developments. Although in the most recent update of the Dutch guideline for rectal cancer treatment, a first step towards tailoring of treatment was attempted through the identification of three subgroups of rectal cancer patients who need different treatments, this is still a very rough division (figure 1). Moreover, this division is based on tumor extension in the surrounding tissues and nodal status. Especially the prediction of nodal status is still cumbersome.

The development of prediction models can help the clinician make better supported and individualized treatment choices. The first question is what the goal of the treatment should be. If radiotherapy is seen only as an adjunct to surgery in order to ameliorate local control (which is the more “classical”

approach), the prediction model should be aimed at the identification of patients who will benefit from pre-operative treatment versus patients who may go for immediate resection and the differentiation between patients who need neoadjuvant chemoradiation versus patients in whom short-course radiotherapy suffices. Another possible goal of response prediction is to enhance the chance of developing a pathological complete response, in order to maximize the chance of organ saving treatment. On the one hand this may help to identify patients who will benefit from treatment intensification, and on the other hand save patients who have a very low chance of developing a complete response from a potential toxic treatment. In order to be able to influence treatment decisions, these models need to be based on variables that are available at the time of diagnosis or early during treatment.

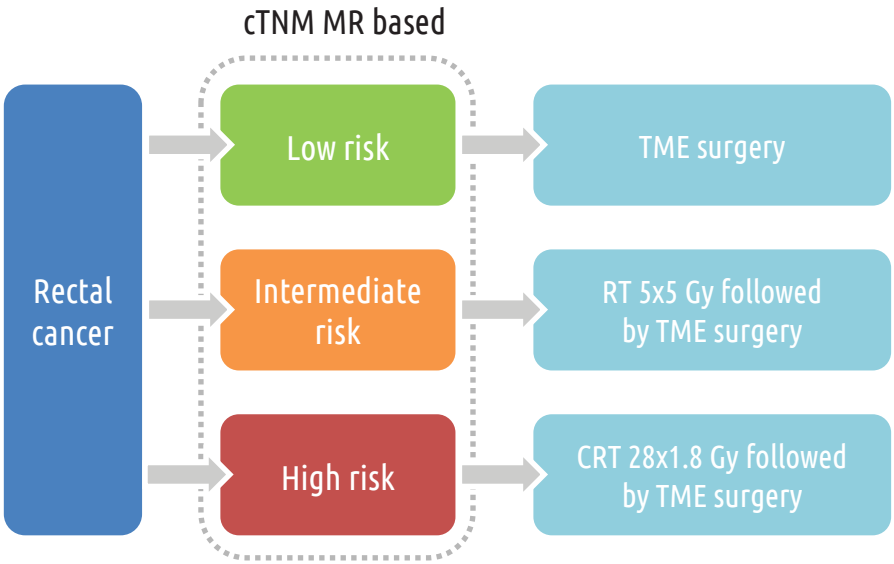

Figure 1 Treatment of rectal cancer according to the Dutch national guidelines.

Until now, most research has focused on PET-scan as a possible predictor. A recent meta-analysis identified 31 studies using PET-scan for response prediction in rectal cancer [33]. Early changes on scans made during treatment

as well as changes on scans made after treatment can both be useful in clinical practice. Early scans make it possible to adapt treatment (e.g. add a boost for patients in an intermediate risk group) and scans made after the completion of chemoradiation can help in the decision making for surgical treatment (e.g. proceed to TME surgery in case of a partial response, consider local excision for a near complete response and discuss a wait and see approach in clinical complete responders).

Chapter 4 describes the development of a prediction model incorporating PET information before and after treatment. What makes this model different from most other papers on PET and response prediction is the relatively large sample size, the multivariate methods used and the validation of the models. The model based on the maximal SUV before treatment, the relative change in SUV_{max} and tumor length proved to be the most predictive. Relative changes in SUV_{max} have been reported by other authors to be predictive for response. SUV_{max} as a single parameter is not strong enough to predict outcome [34-36]. Other imaging techniques are emerging rapidly, of which magnetic resonance imaging (MRI) is the most promising technique [37]. An advantage of MRI over PET is the better spatial resolution. Modern MRI techniques, like dynamic contrast enhanced MR (DCE-MR) and diffusion-weighted imaging (DWI), may significantly increase the predictive value of this imaging modality. While rather strong response predictions as well early during treatment as after treatment completion have been reported for DWI imaging [38-41], the results and added value of DCE-MR are more variable [42, 43]. One study reported on the combination of PET and MR data, showing that combining both modalities resulted in a stronger predictive value of response [44].

The use of other biological markers may have added value compared to prediction models based on imaging data. In chapter 5 a model that uses blood biomarkers is introduced. Blood biomarkers are potentially attractive because they can be collected easily, measurement is relatively cheap and easy and, in contrast to for example genetic biomarkers in tumor cells, they are less influenced by tumor heterogeneity. The study presented in this thesis is the first one that tested a panel of 9 blood biomarkers with potential predictive value.

CEA turned out to be a predictor for complete response and good response in this multivariate model. CEA has been shown in several other studies to be of predictive value for response to chemoradiation in rectal cancer [45-47]. Interleukin-8 was also predictive for response to chemoradiation. Although previous reports describe an association between IL-8 polymorphisms and nodal involvement and recurrence in rectal cancer, our work was the first to demonstrate an association with response to chemoradiation. Although this study is a proof of principle that blood biomarkers have additional value to clinical and imaging derived parameters, external validation is necessary before this model can be implemented in clinical practice. In this validation process, monitoring the effect of using blood sample analyses from different laboratories will be important, since the measurements for this study were all done in one laboratory, using the same technique for each biomarker.

While blood biomarkers give more global information about tumor biology, molecular markers can provide more detailed information. The review presented in chapter 6 summarizes the available evidence regarding biochemical and molecular biological factors that could help with treatment decision making in rectal cancer. Predictive as well as prognostic factors are described. A prognostic factor gives information about the risk of recurrence of disease irrespective of a certain treatment, while a predictive factor predicts the chance that a patient will benefit from a treatment. The general conclusion is that the value of this type of markers is currently limited. More recently several specific genetic alterations and microRNA expression patterns that have a predictive value for response in rectal cancer have been published [48-51]. Although this shows that very specific changes in rectal tumors may play a role in response prediction, the field of genomics in rectal cancer is still in its infancy. A difficulty of the genomic approach is the influence of tumor heterogeneity. Especially in advanced rectal tumors, a small biopsy, used for diagnosis, may not be representative for tumor biology. The biochemical and genetic factors that have been tested until now were mainly isolated features, not tested multivariately.

The hypothesis that multimodality models can predict response to neoadjuvant treatment holds true for PET-based information and blood biomarkers. Until

now, biochemical and genetic factors have limited value in response prediction, but there is certainly a need for further testing of this category of markers in a multivariate and multimodality setting.

Combined treatment in rectal cancer

For the clinical trials presented in this thesis we hypothesized that the combination of AKT inhibition and radiotherapy and mTOR inhibition and radiotherapy would lead to an increased tumor response in rectal cancer.

Combined modality treatment in rectal cancer has a long history. The first trials combining radiotherapy and chemotherapy date back to the 80's [52]. This combination has been proven to be more active than radiotherapy alone in advanced rectal cancer in terms of local control [53]. However, no significant difference in disease free and overall survival has been shown. The cornerstone of chemoradiation in the treatment of rectal cancer is fluorouracil. In the past years the addition of oxaliplatin to fluorouracil and radiotherapy has been tested. Although in one study an increase in response was found [54], two other studies reported comparable responses and an increase in toxicity in the oxaliplatin arm [55, 56]. The combination of fluoropyrimidine-based chemotherapy with irinotecan has been studied in several phase II studies [57-62]. Except for one study, pCR rates were not significantly higher than the rates reported after chemoradiation with 5-FU or capecitabine alone and acute toxicity rates, especially diarrhea, were generally higher.

More recently, targeted biological strategies have been tested in phase I and II trials. A potential advantage of adding targeted agents over adding another cytostatic drug, is the avoidance of overlapping toxicities. On the other hand, it may be a challenge to identify patients who will benefit from the addition of a targeted agent, because these drugs often only have effect when certain mutations are present or absent in a tumor. We know from treatment of colorectal cancer in the palliative setting for example, that patients who have a KRAS mutated tumor do not benefit from anti-EGFR treatment [63]. After the landmark phase III study showing an improved overall survival in head and neck

cancer patients for the combination of radiotherapy and the EGFR-inhibitor cetuximab as compared to radiotherapy alone [64], EGFR-inhibition was tested also in rectal cancer treatment [65-71]. The results of these trials are quite variable, with the majority of them showing a disappointing percentage of pCR. The predictive value of KRAS status was less unequivocal than in the studies combining chemotherapy and cetuximab in colon cancer. However, a pooled analysis of 4 phase II trials identified EGFR and VEGF mRNA expression levels and KRAS mutation status as predictive markers for response to cetuximab-based chemoradiation [72]. Thus, although the results of the addition of cetuximab are disappointing, it is possible that a subgroup of patients will benefit from it. It has also been suggested that timing of chemotherapy, radiotherapy and EGFR-inhibition may be critical [73]. A third possible explanation of a negative interaction between EGFR-inhibitors and chemoradiation is the slowing down of the cell cycle that is caused by EGFR inhibition, as the additive effect of 5-FU is cell cycle dependent [74].

Another interesting target in combined therapy for rectal cancer is vascular endothelial growth factor (VEGF). The most used agent is bevacizumab, a monoclonal antibody against VEGF. No phase III trials have been published, but multiple phase II trials have reported encouraging results with respect to tumor response [75-88]. Complete response rates vary from 13 to 37%, but the pooled pCR rate is 21%, which is comparable to conventional chemoradiation. Moreover, the reported postoperative toxicity rates are worrisome.

These two examples of targeted agents that are added to existing chemoradiation schedules, are an illustration of unexpected responses one may observe in the translation of new drugs from bench to bedside [89]. In this thesis, 2 early translational trials in rectal cancer are presented. A special characteristic of these phase I/II trials is the use of existing drugs that have been used clinically for other indications than cancer. Possible advantages of this ‘drug repurposing’ are the well-known toxicity profile and the relatively low costs as compared to newly developed targeted drugs. Nelfinavir was developed as a protease inhibitor for the treatment of the human immunodeficiency virus (HIV). Its potential role in the treatment of cancer was

discovered at the beginning of this century, when it was found that protease inhibitors could inhibit the PI3K/Akt pathway, which plays a central role in cell cycle survival [90]. PI3K is a downstream messenger of EGFR amongst others. Therefore, in theory nelfinavir could also work in EGFR-mutated tumors. The phase I trial testing the addition of nelfinavir to capecitabine-based chemoradiation (chapter 7) proved the feasibility of this combination. Yet, the toxicity profile of nelfinavir, with diarrhea as a frequent side effect, seemed to be less favorable for this specific combination. Furthermore, plasma levels of nelfinavir turned out to be quite variable in clinical practice, which makes it less attractive to use in daily clinical practice. The use of nelfinavir for HIV-treatment was discontinued after the completion of this phase I trial and the drug no longer available in the Netherlands. This made us decide to abolish phase II of this trial.

The second clinical trial described in this thesis, investigated the safety and efficacy of the addition of rapamycin, an inhibitor of mTOR, to short course radiotherapy (chapter 8). Rapamycin has been used for over 2 decades as an immunosuppressant in patients who underwent renal transplantation. The mTOR pathway plays a central role in cell survival and activation leads to pro-survival signals. It influences protein synthesis, lipid synthesis, autophagy and energy metabolism. It has been known for a long time that inhibition of mTOR inhibits tumor growth [91]. Inhibition of mTOR can overcome radioresistance [92, 93]. In phase I of the trial, surgery was planned immediately after the end of radiotherapy with rapamycin. Change in tumor perfusion was chosen as a "modern" alternative primary endpoint. The combination treatment was well tolerated and only one patient experienced a dose limiting toxicity in the post-operative period. However, a remarkable number of patients experienced grade 3 complications after surgery. The immunosuppressive action of rapamycin, which can lead to impaired wound healing, was suspected to be causing these complications. Therefore, surgery was postponed to 8 weeks after RT+rapamycin in phase II. The hypothesized decrease in tumor perfusion as a result of rapamycin was not confirmed: perfusion remained constant during the entire treatment period. However, on PET-scan a decrease in metabolic volume

as well as SUV_{max} was found after rapamycin treatment, indicating a biological effect of the drug in rectal cancer. Based on these data it cannot be ruled out that rapamycin is effective, but further studies are needed to investigate the most optimal timing and combination of mTOR inhibition, possible other drugs and radiation schemes.

The hypothesis as formulated prior to the start of these two clinical trials cannot be proven nor rejected on the basis of these results. The combination of radiotherapy, capecitabine and nelfinavir showed an unfavorable toxicity profile, therefore this trial was discontinued after phase I. Despite promising response rates, the patient group was too small to draw conclusions regarding efficacy. In the rapamycin trial, the primary endpoint was not reached and the response rate was not higher than in series using the same regimen without rapamycin. Imaging, however, demonstrated that rapamycin has biological activity in rectal cancer.

Future perspectives

Until recently, rectal cancer therapy was characterized by a tendency to intensify treatment. However, a shift towards individualization of treatment and less aggressive approaches can be observed during the last years. It has become clear that rectal cancer, as almost all other cancer types, is not “one disease” but a spectrum of diseases with large differences in biological behavior. In the evolution of the treatment of rectal cancer, one can draw a parallel with breast cancer treatment. William Halsted (1852-1922) introduced the principle of radical mastectomy, which led to a dramatic improvement in treatment results. This opened the way to more and more radical surgery, with the hypothesis that an aggressive surgical locoregional treatment would be able to remove all sources of metastases. In the second half of the 20th century there was a reversal, towards less radical and less mutilating treatment and it was shown that breast conserving treatment is a safe option for a large group of patients with equivalent outcome. Finally, in recent years it has become clear that an axillary lymph node dissection, which was standard for all patients with breast

cancer, could be safely omitted in patients with a negative sentinel node. The most recent insights show that it is even safe to leave the axilla untreated in some patients, even in case of a positive sentinel node.

In rectal cancer, as described in the introduction of this thesis, Bill Heald recognized the importance of good radical surgery and its impact on local control and survival. Several large trials then showed the added value of radiotherapy or chemoradiation to TME-surgery. Trials followed which investigated intensification of local treatment in order to maximize response. And since the beginning of this century it was noticed that in some cases that respond well to pre-operative treatment, surgery can be less invasive (e.g. TEM surgery) or can even be omitted in selected patients (the wait and see approach).

The challenge for the future will be an early identification of different biological groups within all rectal cancers. Clinically, four groups of tumors can be identified:

1. Early tumors: small (<3 cm), clinically node negative tumors
2. Tumors growing mainly locoregionally, responding very well to neoadjuvant treatment
3. Tumors growing mainly locoregionally, responding poorly to neoadjuvant treatment
4. Tumors with a strong propensity to metastasize rapidly, often responding minimally to local treatment

TME surgery will be overtreatment in the majority of patients from the first group (early tumors). A local treatment, e.g. TEM surgery [94], brachytherapy or contact radiotherapy [95-97] can be an organ sparing alternative in the case of negative lymph nodes. At present, TEM surgery is an accepted alternative for T1, grade 1-2 tumors without angioinvasion. The difficulty in clinical practice is the prediction of nodal status. With standard imaging techniques, this prediction is notoriously unreliable [98, 99]. Rapid developments in imaging techniques help to improve the predictive value, but rapid implementation of these techniques in clinical practice is hampered by the high technical demands and the fact that interpretation of these images is time consuming. Computer aided techniques

could be helpful in the future to solve the problem of increased workload and to diminish inter-observer variability [100, 101].

For the second group (locoregional growth, responding well to (chemo)radiation), an intensification of locoregional treatment is an attractive approach. Further studies are needed to identify the optimal strategy for treatment intensification in these patients, e.g. an increase of radiotherapy dose or a combination with different radiosensitizers. Maybe for this biologically favorable group, increasing the radiation dose can already result in a substantial increase in complete responses, opening the way to organ preservation.

The third group (poor response to chemoradiation) is expected to be more heterogeneous biologically. Just as for the second group, treatment intensification may lead to more complete responders. The difficulty will be that a single strategy will not fit all tumors in this group. Therefore, better predictive models are needed to stratify patients into different treatment regimens. Ideally, these models should incorporate information about different aspects of tumor biology: not only clinical information, but also imaging data, genetic alterations and blood biomarkers for example. It may finally turn out that a "pan-omics" approach will help us with the most optimal treatment choice [102]. However, it is important to keep in mind that more complete responses do not necessarily translate into better outcome. The biggest gain of developing a pCR, is the possibility of organ sparing treatment. It has still to be proven, however, that this approach is equivalent to standard neoadjuvant treatment and surgery in terms of oncological outcome.

The fourth group (rapid development of metastases) consists of tumors with a very aggressive biology. In these tumors intensification of local treatment is unlikely to result in better outcome. Short course radiotherapy followed by aggressive systemic therapy may be the most optimal approach in this patient group. It has already been shown in the metastasized setting that this treatment leads to a high rate of good local response [103].

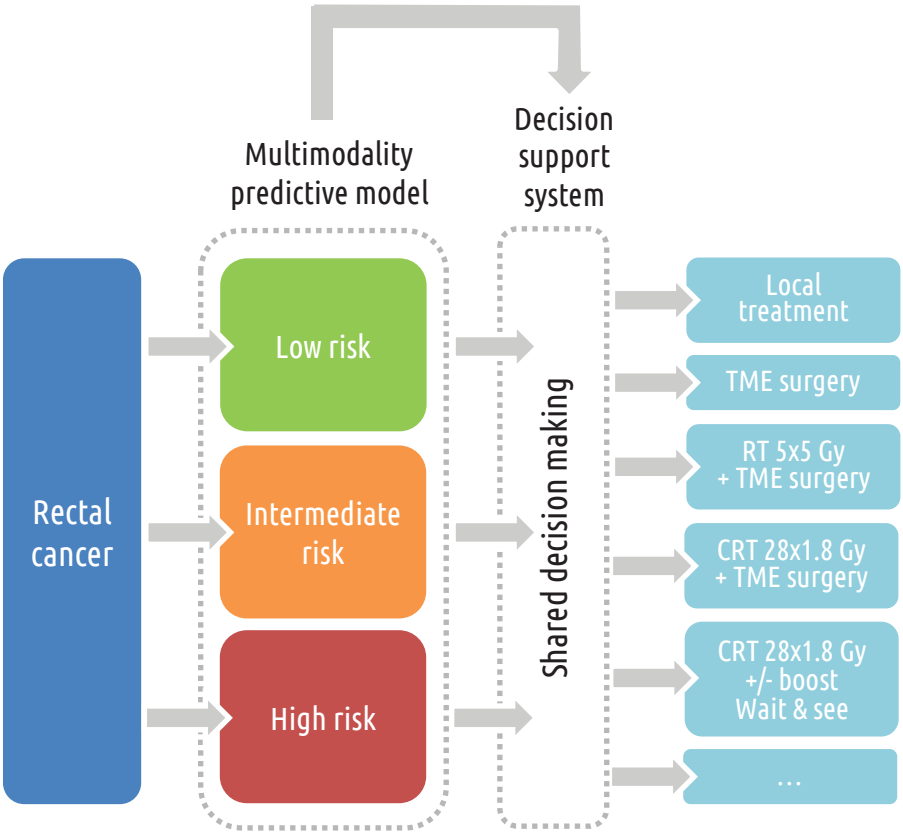

Figure 2 Possible treatment algorithm incorporating predictive models and shared decision making, leading to better patient tailored treatment.

The considerations for future developments in rectal cancer treatment mentioned above, all focus on the best outcome in terms of recurrence free and overall survival and do not take the functional outcome of the different treatment strategies into account. It is known from studies in prostate cancer that patients do not always choose the treatment with the highest chance of cure, if this more aggressive treatment has a higher chance of long term toxicity [104]. We therefore need to incorporate patient's preferences in the final treatment decision. However, this is only possible if patients are well informed about the benefits and harms of each treatment. Information given by

physicians about long term side effects is known to be very variable [105]. Decision aids are helpful in guiding patients and doctors through the complex process of shared decision making [106]. Thus, decision aids should be developed for rectal cancer and these should ideally be combined with accurate predictive models. This opens the way to real patient-oriented tailored treatment (figure 2).

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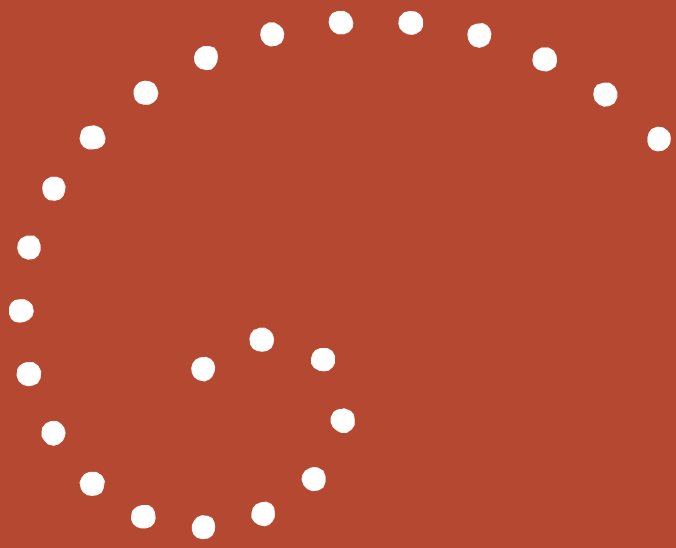
Summary and valorization



Chapter 10

Summary

In this thesis three different strategies that can lead to treatment individualization and optimization in rectal cancer are discussed. In the first part, the possible role of PET-CT in radiotherapy treatment planning for rectal tumors is investigated. Accurate tumor delineation is necessary to be able to implement boost techniques with the aim of dose escalation. The second part is devoted to response prediction in rectal cancer. Reliable response prediction is essential to tailor treatment and to give advice to patients about the optimal treatment. In the third part, two early phase clinical trials are presented testing the addition of an Akt inhibitor and an mTOR inhibitor to different neoadjuvant treatments in rectal cancer. The goal of these trials was to enhance tumor response to neo-adjuvant treatment.

Part I: The role of PET-CT in tumor delineation

In chapter 2, the correlation between tumor length measured in the pathology specimen and on different imaging modalities is investigated. Rectal tumors of 26 patients were measured manually on CT, MR and PET-scan. Furthermore an automated tumor length was derived from PET-scan, using the signal-to-background (SBR) method. Measurements based on PET-scan correlated better with tumor length in the surgical specimen than those based on CT or MR and automated PET-measurements performed better than manual measurements.
Chapter 3 discusses the results of a delineation study incorporating different imaging modalities. Tumors of 42 rectal cancer patients were delineated by 5 observers 3 times: using only CT and MR, using CT, MR and PET and using all three modalities including an automatic contour based on PET-scan. Tumor volumes delineated using PET information were significantly smaller than volumes determined on CT and MR and the smallest volumes were created using the automatic delineation method. With respect to interobserver variation, a significant increase of the conformity index was observed when PET-information was added to the delineation process, resulting in a mean conformity index of 0.93 using PET auto-contours. The caudal border of the treatment volume can be adapted using PET information.

Part II: Response prediction in rectal cancer treatment

Prediction of pathological complete response (pCR) after chemoradiation (CRT) using sequential PET-imaging is described in chapter 4. Large databases of 4 different institutes were used to build a prediction model. A PET-scan was made before the start of treatment and approximately 8 weeks after the end of chemoradiation. The strongest model for prediction of pCR was based on post-treatment PET-results and included tumor length, post-treatment SUV_{max} and relative change in SUV_{max}, resulting in an area under the ROC curve (AUC) of 0.83 for the validation set.

The value of blood biomarkers for response prediction after CRT was studied in chapter 5. Blood samples of 276 patients treated with CRT for locally advanced rectal cancer were analyzed for 9 serum biomarkers. The choice of these biomarkers was based on a pre-defined hypothesis. CEA turned out to be predictive for pCR, while CEA and interleukin-8 (IL-8) predicted a good response (ypT0-2N0). The addition of biomarker information to an existing prediction model based on clinical data and PET-information led to an increase of the performance of these models, indicating that blood biomarkers have added value.

In chapter 6, the existing literature on biochemical and molecular biological factors and their role in decisions in the treatment of rectal cancer is reviewed. Until now, the value of this type of predictive factors turns out to be limited. Genetic biomarkers showing a predictive value for response to chemoradiation still need external validation and prospective evaluation in larger patient groups.

Part III: Combined treatment in rectal cancer

Nelfinavir, an inhibitor of the PI3K/Akt pathway, is a potential radiosensitizing drug. Its incorporation in a neoadjuvant chemoradiation schedule with capecitabine for rectal cancer treatment is tested in chapter 7. Although the combination treatment was feasible, a high incidence of grade 3 toxicities was observed. Diarrhea and transaminase elevation were the most frequently occurring side effects, indicating possible overlapping toxicities between

capecitabine and nelfinavir. The recommended dose for a phase II trial was 750 mg BID, the lowest dose level tested in this trial.

The inihibition of mTOR, another possible attractive target to increase radiosensitivity, is tested in chapter 8. Rapamycin, an mTOR inhibitor, was added to 5x5 Gy pre-operative radiotherapy in a phase I/II trial. The combination turned out to be well tolerated, although a high number of postoperative complications was observed if the surgical resection was performed immediately after the end of radiotherapy and rapamycin. The primary endpoint, a decrease in tumor perfusion, was not reached. However, changes in metabolic activity reflected by changes in FDG-uptake on PET-scan, confirm the biological activity of rapamycin in rectal cancer.

In dit proefschrift worden drie verschillende strategieën besproken die kunnen leiden tot de individualisering en optimalisering van de behandeling van het rectumcarcinoom. In deel I wordt de mogelijke rol van PET-CT in de radiotherapie planning voor rectumtumoren onderzocht. Om boost technieken te kunnen invoeren met het oog op dosis escalatie, is een betrouwbare intekening van de tumor onmisbaar. Deel II is gewijd aan respons predictie voor endeldarmkanker. Betrouwbare respons predictie is essentieel om behandeling op maat te kunnen bieden en om patiënten advies te kunnen geven over de optimale behandeling. In deel III worden twee klinische trials besproken die het toevoegen van een Akt-remmer en een mTOR-remmer aan twee verschillende neo-adjuvante behandelingen voor rectumcarcinoom onderzoeken. Het doel van deze behandelingen was het versterken van de respons van de tumoren op de neo-adjuvante behandeling.

Deel I: De rol van PET-CT bij tumor delineatie

In hoofdstuk 2 wordt de correlatie tussen tumorlengte gemeten in het pathologie preparaat en op de verschillende beeldvormingsmodaliteiten onderzocht. Rectumtumoren van 26 patiënten werden handmatig gemeten op CT, MRI en PET-scan. Verder werd een automatisch gemeten tumorlengte verkregen door het creëren van een automatische contour op de PET-scan met behulp van de source-to-background ratio (SBR) methode. Metingen gedaan op de PET-scan correleerden beter met de tumor lengte in het chirurgisch preparaat dan metingen op CT of MRI en de geautomatiseerde PET-metingen waren beter dan de handmatige metingen.

Hoofdstuk 3 behandelt de resultaten van een intekenstudie waarin verschillende beeldvormingsmodaliteiten worden gebruikt. Tumoren van 42 rectumcarcinoom patiënten werden 3 maal ingetekend door 5 verschillende observatoren: de eerste keer gebruik makend van CT en MRI, de tweede keer van CT, MRI en PET en de derde keer alle modaliteiten inclusief een automatisch gegenereerde contour gebaseerd op de PET-scan. De tumor volumes die werden ingetekend op basis van de PET-scan waren significant kleiner dan de

volumes bepaald op grond van CT en MRI en de kleinste volumes werden verkregen met de automatische delineatie methode. Met betrekking tot de interobserver variatie werd een significante toename van de conformity index gezien als PET-informatie werd betrokken in het intekenproces, resulterend in een gemiddelde conformity index van 0,93 bij gebruik van automatische PET-contouren. De caudale begrenzing van het behandelvolume blijkt op grond van PET-informatie aangepast te kunnen worden.

Deel II: Response predictie voor de behandeling van rectum-carcinoom

In hoofdstuk 4 wordt de predictie van pathologisch complete respons (pCR) na chemoradiatie (CRT) met behulp van herhaalde PET-scans beschreven. Grote databases van 4 verschillende instituten werden gebruikt om een predictiemodel te bouwen. Voor de start van de behandeling en ongeveer 8 weken na het einde van de chemoradiatie werd een PET-scan gemaakt. Het sterkste predictiemodel voor pCR was gebaseerd op PET resultaten van na de behandeling en omvatte tumor lengte, de SUV_{max} na de behandeling en de relatieve verandering in SUV_{max}. Dit model resulteerde in een AUC van 0.83 voor de validatie set.

De waarde van bloed biomarkers voor respons predictie na CRT werd bestudeerd in hoofdstuk 5. Bloedmonsters van 276 patiënten behandeld met CRT voor lokaal uitgebreid rectumcarcinoom werden onderzocht op 9 serum biomarkers. De keuze voor de ze biomarkers was gebaseerd op een vooraf bepaalde hypothese. CEA bleek voorspellend te zijn voor pCR, terwijl CEA en interleukine-8 een goede respons (ypT0-2N0) konden voorspellen. De toevoeging van biomarker informatie aan een bestaand predictiemodel gebaseerd op klinische gegevens en PET-informatie leidde tot een sterkere predictie van deze modellen, hetgeen aantoont dat bloed biomarkers toegevoegde waarde heeft.

In hoofdstuk 6 wordt de literatuur over biochemische en moleculaire biologische factoren en hun rol in behandelbeslissingen voor het

rectumcarcinoom besproken. Tot op heden blijkt de rol van dit type predictieve factoren beperkt te zijn. Genetische biomarkers die predictieve waarde voor de respons op chemoradiatie moeten nog extern gevalideerd en prospectief onderzocht worden in grotere groepen patiënten.

Deel III: Combinatiebehandeling voor het rectumcarcinoom

Nelfinavir, een remmer van de PI3K/Akt signaleringsroute, kan mogelijk de stralingsgevoeligheid van tumoren doen toenemen. In hoofdstuk 7 wordt de toevoeging van nelfinavir aan een neo-adjuvant chemoradiatie schema met capecitabine onderzocht. Hoewel de behandelcombinatie klinisch uitvoerbaar bleek te zijn, werd wel een hoge incidentie van graad 3 toxiciteit gezien. Diarree en transaminase stijgingen waren de meest frequent optredende bijwerkingen, duidend op mogelijke overlap van toxiciteit veroorzaakt door capecitabine en nelfinavir. De aanbevolen dosis voor een fase II trial was tweemaal daags 750 mg, het laagste dosisniveau dat werd onderzocht in deze trial.

De remming van mTOR, een ander mogelijk aantrekkelijk doelwit om de stralingsgevoeligheid verhogen, wordt beschreven in hoofdstuk 8. Rapamycin, een mTOR inhibitor, werd toegevoegd aan 5x5 Gy pre-operatieve radiotherapie in een fase I/II studie. De combinatie bleek goed verdragen te worden, hoewel er relatief veel postoperatieve complicaties werden gezien als de resectie direct na beëindiging van de radiotherapie met rapamycin werd uitgevoerd. Het primaire eindpunt, een afname in tumorperfusie, werd niet gehaald. Veranderingen in metabole activiteit, af te lezen uit verandering in FDG-opname op de PET-scan, bevestigen een biologische activiteit van rapamycin bij het rectumcarcinoom.

Chapter 11

Valorization

Valorization

Cancer is the leading cause of death in the Netherlands and its incidence is expected to increase substantially in the coming years. This expected rise in cancer incidence is even more pronounced due to the increasing proportion of elderly in the population and the increased life expectancy. Colorectal cancer is the second most occurring cancer type in men and the third in women. This makes rectal cancer an important health care problem.

Although the results of rectal cancer treatment have been improved substantially in the last decades, there is certainly room for improvement. Traditionally, cancer treatment has been based on clinical staging, which is a rather rough classification and results in an important percentage of under- and overtreatment. Rectal cancer treatment has been characterized by a tendency to overtreatment in the Netherlands in the last years, resulting in an increased risk of long-term sequelae and higher costs. Furthermore, it is expected that the distribution of clinical stages at the time of diagnosis will shift to earlier stages in the coming years, due to the recent introduction of a national screening program for bowel cancer. The most important themes in Dutch healthcare at this moment are control of costs, improvement of quality and shared decision-making. These considerations all ask for soundly based individualization of treatment.

The main theme of this thesis is the development of more tailored treatment of rectal cancer. This tailoring of treatment can consist of an intensification of treatment for patients who will benefit from it and a de-escalation of pre-operative treatment in patients who have very sensitive tumors or who do not need neo-adjuvant treatment at all. To make it even more complex, treatment intensification can have two different aims: a better chance of locoregional control or a higher chance of complete response, opening the way to organ preservation. Although more information is needed about long-term outcome of oncological outcome of organ-preserving treatment, it has important advantages: post-operative complications are avoided, possible toxicity caused by the surgical intervention (fecal urge and incontinence, urinary incontinence,

sexual dysfunction) does not occur and a colostomy can be avoided. This is expected to lead to less healthcare costs and a better quality of life. Therefore, if oncological safety of the watch-and-wait approach in clinical complete responders can be confirmed, it may become the ultimate goal of (chemo)radiotherapy in rectal cancer treatment.

Relevance of the scientific results of this thesis

The results presented in the first part of this thesis can be seen as a step in the development of boost techniques for radiotherapy in rectal cancer. Dose escalation is one possibility to increase the chance of a complete response in radiotherapy. The studies presented in this thesis have proven that PET-CT is a reliable and reproducible method for adequate delineation of the primary tumor, a prerequisite for tumor boosting.

The second part of this thesis presents the first steps in the development of predictive models for rectal cancer treatment. PET-scan has been proven to be a strong instrument in response prediction in rectal cancer treatment. The work presented here was a first indication that PET-information has predictive value. The nomogram has been made available online (www.predictcancer.org). Based on these results, we developed a program aimed at the optimal use of PET-scan in predictive models for rectal cancer. In the meantime, our group has published more articles about the use of PET for early response evaluation and we have found that PET-scan after 2 weeks of treatment is able to separate good responders from bad responders. This concept has been validated in a prospective multicenter trial.

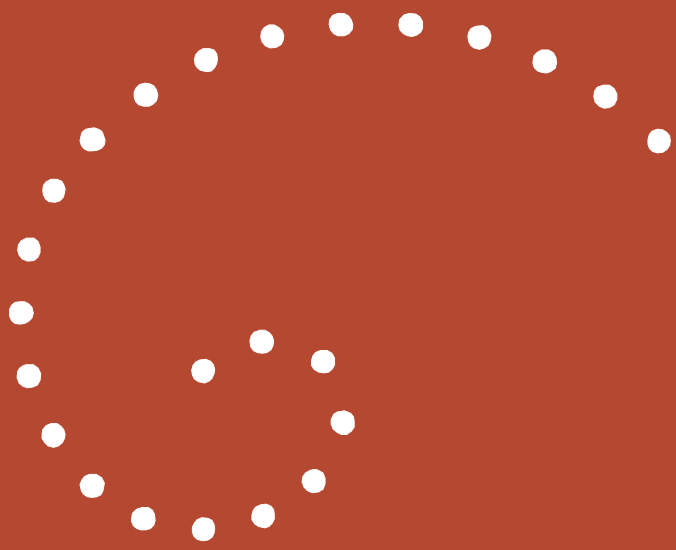
The studies discussed in the third and last part were aimed at treatment intensification. Both studies are examples of early translation of laboratory findings into clinical practice. The second particularity of these 2 studies is the use of drugs that were registered already for other indications. The advantage of this approach is not only that the side effects of these drugs are well known, but also that the costs are substantially lower than the costs of recently developed and patented drugs. Unfortunately the combination of nelfinavir and

chemoradiation turned out to have an unfavorable toxicity profile and the distribution of nelfinavir was stopped in the Netherlands, which made us decide not to go on to phase II. The combination of rapamycin and radiotherapy was well tolerated if surgery was delayed, but our hypothesis could not be confirmed. Although rapamycin had a clear biological activity in rectal cancer, as reflected by the SUV-changes on PET-scan, this did not translate into a substantial increase in response. Therefore, the scheme as tested in this phase I/II trial is not promising enough to continue to a phase III trial.

Innovation and future

The principle of response prediction has been shown to be feasible in clinical practice. We therefore want to further explore the possibilities in clinical practice by adapting treatment based on early response prediction to increase the response rates. The use of prediction models allows us to intensify treatment only in patients who have a high chance to benefit from it, which is a new approach as compared to the “one-size-fits-all” recommendations in guidelines. Another important goal for future projects is to incorporate patient preferences into treatment decisions. This process will also be supported by reliable response prediction.

Appendix



Appendix

Dankwoord

Na al die jaren werken aan dit proefschrift rest het schrijven van het eerst gelezen hoofdstuk van ieder “boekje”. Onderzoek doen is bij uitstek samenwerken en velen ben ik dan ook dank verschuldigd.

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Ik wil graag de leden van de beoordelingscommissie (Prof. dr. F. Mottaghy, Dr. M. Lahaye, Prof. dr. C. Marijnen, Prof. dr. H. Rutte, Prof. dr. V. Tjan-Heijnen) bedanken voor hun tijd en energie om mijn proefschrift te beoordelen.

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Appendix

Curriculum vitae

Jeroen Buijsen werd geboren op 30 juni 1974 te Halsteren en hij groeide op in Heerle. In 1992 behaalde hij het gymnasiumdiploma aan het Norbertuscollege te Roosendaal. Aansluitend startte hij met de opleiding geneeskunde aan de Universiteit Maastricht, waar hij in 1996 het doctoraaldiploma haalde (cum laude) en in 1998 zijn artsexamen deed (met genoegen). Zijn ervaringen tijdens de co-schappen legde hij vast in een serie columns voor het Algemeen Dagblad. Na zijn artsexamen werkte hij ruim 2 jaar als arts-onderzoeker bij de afdeling interne geneeskunde/endocrinologie van het azM, onder begeleiding van dr. B. Wolffenbuttel. In die tijd bleef hij een medische rubriek schrijven voor het Algemeen Dagblad. In 2001 besloot hij de overstap te maken naar de radiotherapie en begon hij aan de opleiding tot radiotherapeut-oncoloog bij het RTIL in Heerlen (het huidige Maastro) met als opleiders dr. J. Jager en prof. dr. P. Lambin. De opleiding werd voltooid in 2006, waarna hij werd aangesteld als staflid bij Maastro. In die tijd zijn ook de eerste onderzoeksactiviteiten gestart die hebben geleid tot dit proefschrift. Het onderzoek werd begeleid door prof. dr. P. Lambin, dr. G. Lammering en dr. M. Öllers. Zijn huidige aandachtsgebieden zijn gastro-intestinale tumoren, mammatumoren en radiotherapie voor benigne aandoeningen.

Appendix

List of publications

This thesis

Development and external validation of a predictive model for pathological complete response of rectal cancer patients including sequential PET-CT imaging | Van Stiphout RG, Lammering G, **Buijsen J**, Janssen MH, Gambacorta MA, Slagmolen P, Lambrecht M, Rubello D, Gava M, Giordano A, Postma EO, Haustermans K, Capirci C, Valentini V, Lambin P | *Radiother Oncol. 2011 Jan; 98(1):126-33.*

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FDG-PET-CT reduces the interobserver variability in rectal tumor delineation | **Buijsen J**, van den Bogaard J, van der Weide H, Engelsman S, van Stiphout R, Janssen M, Beets G, Beets-Tan R, Lambin P, Lammering G | *Radiother Oncol. 2012 Mar;102(3):371-6.*

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MRI and Diffusion-Weighted MRI Volumetry for Identification of Complete Tumor Responders After Preoperative Chemoradiotherapy in Patients With Rectal Cancer: A Bi-institutional Validation Study | Lambregts DM, Rao SX, Sassen S, Martens MH, Heijnen LA, **Buijsen J**, Sosef M, Beets GL, Vliegen RA, Beets-Tan RG | *Ann Surg. 2014 Sep 10. [Epub ahead of print]*

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