Clinical and Radiomics prediction of complete response in Rectal cancer.

A thesis submitted to The University of Manchester for the degree of Doctor of Medicine in the Faculty of Biology, Medicine and Health.

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Table of Contents

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List of figures

List of tables

Declaration

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright statement

Preface

This thesis takes the form of a literature review of rectal cancer in chapter 1 and then the two studies undertaken in chapters 2 and 3; both are presented in journal paper format, followed by a discussion, conclusion and future work planned in chapter 4. The study approvals and protocol are in chapter 7.

For the study in chapter 2, the author was responsible for all aspects of the work except the radiomics feature extraction performed by Dr Eliana Vasquez Osorio using open-source radiomics software. Dr Hitesh Mistry provided statistical guidance for all studies in this thesis. An abstract was accepted by the radiotherapy and oncology journal (Green journal) in 2021, and the author delivered a poster highlight oral presentation on this work at ESTRO 2021 Madrid. An invitation to submit to the full article was received from Physics and Imaging in Radiation Oncology (phiRO) journal. The full article of Chapter 2 has now been published. The full article of the study in chapter 3 was published in February 2022 in the cancer treatment and research communications journal.

The scope and duration of this study were affected by the COVID 19 pandemic. The suspension of all research work unrelated to COVID during the lockdown periods impacted this project most and caused significant delays.

The author of this work undertook a two-year clinical research fellowship funded by the Christie hospital Lower GI charity funds. He is a Consultant Clinical Oncologist at the Christie hospital in Manchester, specialising in colorectal and anal cancers. He graduated with honours in Medicine and Surgery (MBCHB) from the University of Liverpool in 2006. He obtained the Royal College of Physicians (MRCP) membership in 2011 and a Fellowship of the Royal College of Radiologists (FRCR) in 2017. His main research interest is in ways to enhance organ preservation treatment plans in rectal cancer.

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I would like to express my most profound appreciation to my family for their unrestricted support over the years.

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To
Mum and Dad

Abbreviations

ADC- Apparent diffusion coefficient.

APR- Abdominal perineal resection

AUC- Area under the curve

BD- Bis die- twice per day

BMI- Body mass index

Ca- Cancer

cCR- Clinical complete response

CERR- Computational environment for radiology research

Chemo- Chemotherapy

COX-2-Cyclooxygenase-2

CRC- Colorectal cancer

CRM- Circumferential resection margin

CRT- Chemo-radiotherapy

CSS- Cancer-specific survival.

CTV- Clinical target volume

DFS- Disease-free survival

DM- Disease mortality.

DRE- Digital rectal examination.

DTA – Distance to agreement.

DWI- Diffusion-weighted images

EGFR- Epidermal growth factor receptor.

EMVI- Extramural vascular invasion

ESMO- European society of medical Oncologists.

FAP- Familial adenomatous polyposis

FBC- Full blood count

FolFOX- Chemotherapy regime of folinic acid, 5 fluorouracil and oxaliplatin.

GTV- Gross tumour volume

Gy- Grays (unit of radiotherapy)

HNPCC- Hereditary non-polyposis colorectal cancer (Lynch syndrome)

IBSI- Image Biomarker Standardisation Initiative

ICC- Intra-class correlation

IMRT- Intensive modulated radiotherapy

LARC- Locally advanced rectal cancer

LR- Local recurrence

LCCT- Long course chemo-radiotherapy

M2- Metre square

Mg- Milligram

Mg/m2- Milligram per metre square.

MR – Magnetic resonance

MRI- Magnetic resonance image/s

MRF- Mesorectal fascia

MSF- Meso-rectal fascia

MSS- Micro-satellite stable

MSI –Micro-satellite instability

NICE- National Institute of Clinical Excellence.

NPV- Negative predictive value

NHS- National Health Service

NS- Not significant.

OnCoRe-Rectal Cancer Oncological Complete Response Database.

OAR- Organs at risk

OS- Overall survival

PCA- Principal component analysis

pCR- Pathological complete response

pPR - Pathological partial response

PPV- Positive predictive value

PTV- planning target volume

QOL- Quality of life.

RCT- Randomised control trial

ROC AUC-. The area under the curve of the Receiver operating characteristic curve

ROI- the region of interest

R0- Resection margin clear- no cancer cells seen microscopically within 1mm of resection margin.

SIB - Simultaneous integrated boost.

SCCT- Short course chemo-radiotherapy.

T1WI - T1 weighted images

T2WI – T2 weighted images

TEM- Trans-anal endoscopic microsurgery.

TME- Total mesorectal excision

TRG- Tumour regression grade

UK- United Kingdom

VEGF- Vascular endothelial growth factor

VMAT- Volumetric modulated arc therapy.

WaW- Watch and Wait

Abstract

Purpose: About 15% of patients post neoadjuvant chemoradiotherapy in rectal cancer achieve clinical complete response (cCR) and could avoid or defer surgery by entering a watch and wait surveillance treatment plan. Patients that achieve cCR have overall better outcomes. Prediction of complete response before treatment is essential for neoadjuvant treatment selection.

Method: Using the UK-based research **OnCoRe (The Rectal Cancer Oncological Complete Response Database)** database, we performed a propensity-score matched (1:1) case-control study of 322 patients (161 patients with cCR and 161 without cCR) who received neoadjuvant chemoradiotherapy. We collected pre-treatment MR images, demographics, clinical and blood parameters and radiotherapy-related characteristics. We segmented the gross tumour volume on the T2W MR Images and extracted 1430 stable radiomics features per patient. We wanted to compare the predictive power of clinical parameters and the radiomics variable in predicting complete response.

Results: Using Logistic regression analysis, the PCA-derived combined model (radiomics plus clinical variables) gave a ROC AUC of 0.76 in the training set and 0.68 in the validation set. The clinical-only model achieved an AUC of 0.73 and 0.62 in the training and validation set. The radiomics-only model had an AUC of 0.68 and 0.66 in the training and validation sets. Various clinical variables were associated with cCR. A nomogram using only routinely acquired clinical variables was developed with a resulting ROC AUC of 0.75.

Conclusion: The predictive abilities of clinical variables for cCR are better than radiomics variables. Combining clinical and radiomics variables improves predictability. However, their predictive

characteristics remain modest. The Nomogram of the clinical variable produced will need to be enhanced before prospective validation and clinical use.

Chapter 1: Introduction

1.1 Background on Rectal Cancer

The global incidence of lower GI cancers in 2020 was estimated at 1.9 million, with 935,000 deaths representing ten per cent (10%) of global cancer deaths[1]. Colorectal cancers are the fourth most common cancers in the UK[2]. There were nearly 30,000 new cases of bowel cancer diagnosed in 2019[2]. Bowel cancer mortality accounted for 10-12% of cancer deaths each year [3]. Rectal cancer is the most common anatomical site of bowel cancer. There are about 10,000 new cases of rectal cancer in the UK each year[4]. The two significant advances in the treatment of rectal cancer since the 1980s have been better-quality resective surgery with total mesorectal excision (TME) and neoadjuvant therapy with radiotherapy and chemotherapy. Both have significantly reduced local recurrence and possibly improved survival rates in rectal cancer[5][6]. Hence, the current standard of care treatment for locally advanced rectal cancer is neoadjuvant chemo-radiotherapy, followed by resective surgery either as a total mesorectal excision surgery (also termed anterior resection) or abdominoperineal resection [7]. Surgical resection is associated with considerable short and long-term morbidity, up to 3% risk of perioperative mortality, and up to 40% of these patients require a permanent stoma[2]. For two decades, pathological complete response (pCR) (the absence of microscopic disease post-resection) has been recognised in 15%-27% of patients who had resective surgery post chemoradiotherapy[8]. Neoadjuvant chemo-radiotherapy comes with a wide range of responses from clinical complete response (cCR) to no response at all. Clinical complete response is the absence of clinical and radiological detectable disease post-neo-adjuvant chemoradiotherapy before surgery. Clinical complete response is verified radiologically after chemo-radiotherapy with

investigations- such as pelvic MRI, and clinically with digital rectal examination and sigmoidoscopy. Pooled analysis of trials showed that pCR is associated with a good prognosis and an indicator of a biologically favourable tumour [8]. Achieving pCR can only be established after surgery. However ,patients with cCR have a comparable excellent long term outcome similar to those with pCR[9]. With increasing demand from patients to reduce the burden of their treatment toxicities on their life long after their treatment, the question of whether this group of patients with biologically favourable disease needs radial surgery. Patients who achieved cCR now have the opportunity to be monitored in a surveillance treatment plan pioneered by Prof Habr-Gama and her team from São Paulo, known as 'Watch and Wait' [10]. This organ preservation treatment plan offers this group of patients an opportunity to defer or avoid surgery (thereby avoiding the risks of surgery) seemingly without any detrimental effect on their clinical outcomes[11][9][12]. The concern about organ preservation is that deferring or omitting surgical treatment will negatively affect the patient's curative outcome. Therefore to minimise this concern and maximise the main benefit of this treatment strategy, there needs to be a robust way of predicting patients likely to have cCR before initiating treatment. However, there is no robust predictor of either pCR or cCR before neoadjuvant chemoradiotherapy. Ryan et al. reported a systematic review, including 85 studies, evaluating predictors (including biochemical, gene expression, mutational, and protein expression analyses) for pCR but concluded that there were 'no robust markers'[13]. A recent published systemic review[14] in May 2021 including 167 studies looking at clinical, biochemical and radiological predictors of pCR came to the same conclusion that our current ability to predict response in chemoradiotherapy in rectal cancer is very limited. This lack of robust predictive maker for either pCR or cCR has been one of the major drawbacks of the organ preservation treatment plan. NICE (National Institute of Clinical Excellence) in its 2020 guideline recognised that there are still some unanswered questions around organ preservation which will become clear through research. It recommended that all patients going into the watch and wait to be registered in a clinical trial or a national registry. NICE also

recommended a clear agreed definition for complete response and good evidence around factors that predict recurrence.

This project aims to provide evidence for predicting complete response using one of the largest research registries of patients with clinical complete response. The work will look into two research questions:

a) Can we predict clinical complete response with MR radiomics features using pre-treatment MR scans? (Chapter 2)
b) Can we predict clinical complete response using routinely acquired clinical variables on diagnosis? (Chapter 3)

The clinical implications of this project are that patients predicted to have cCR before their neoadjuvant chemo-radiotherapy in rectal cancer would have this treatment and will be followed up on the organ preservation clinical pathway. Those not anticipated to have this excellent outcome from neo-adjuvant chemo-radiotherapy will be treated with an intensified neo-adjuvant treatment plan to improve their outcome. This will be an essential strategy to limit the toxicity of neo-adjuvant treatment strategies to what is needed to achieve an excellent outcome and will lead to a more personalised treatment plan.

The introductory part (chapter 1) introduces rectal cancer, its epidemiology and risk factors, anatomy, diagnosis and treatment. The introduction ends with a description of radiomics and the steps involved in using radiomics as a biomarker. The second chapter focuses on using radiomics to predict clinical complete response in rectal cancer from the OnCoRe database. The third chapter similarly uses routine clinical variables to predict clinical complete response.

We hope that the results of this project will add to the available evidence in promoting the use of organ preservation treatment plans in the management of rectal cancer.

1.2: Epidemiology/Risk factors of Rectal Cancer

Various risk factors attribute to a rectal cancer diagnosis include age, obesity, smoking, red and processed meat, alcohol intake, family history, and other medical conditions such as inflammatory bowel disease. Like in most risk association studies, there have been some inconsistencies in the literature regarding the modifiable risk factors associated with cancer. In some cases, differences exist between bowel cancer and rectal cancer risk factors, and there could also be some gender variations. The lifetime risk of being diagnosed with colorectal cancer is 1 in 15 (7%) for males and 1 in 18 (6%) for females born after 1960 in the UK.[4][15] The additional modifiable risk is needed to be reduced to reduce the burden of the disease. It is estimated that 54% of all bowel cancer cases in the UK are preventable[16].

Cigarette smoking raises the risk of a wide variety of cancers ranging from respiratory cancers, head and neck cancers, gastrointestinal cancers, kidney, liver, urogenital, gynaecological cancers and some haematological malignancies[17]. It is a major modifiable risk factor for most solid tumours. It increases the risk of bowel cancers in general with higher risks in rectal cancers [18]. Like in many other solid cancers, the risk related to smoking follows a dose-response relationship in that the more you smoke, the higher your risk[19]. A large meta-analysis has shown that the risk of developing bowel cancer is about 17-25% higher in previous or current smokers compared with never smokers. [4][19][20]. It is estimated that 7% of bowel cancer cases in the UK are caused by smoking.[16] However, the evidence of smoking and increased risk of colorectal cancers is not consistent in all trials[21][22], but the balance of the more extensive systemic reviews show that cigarettes are a major risk factor for all colon cancers and especially for rectal cancer[19]

Overweight (BMI 25-29.9kg/m^2) and obesity (BMI of 30kg/m^2 or more) are significant risk factors for a large number of solid tumours [23]. A large meta-analysis has shown a 6% higher risk of rectal cancer

in overweight men and a 25% higher risk in men who are obese compared with men of average weight[24]. Still, there is no association between BMI and rectal cancer in women[24]. In another extensive umbrella review of 204 systemic reviews and meta-analyses involving adiposity and risk of developing cancer, rectal cancer risk increases by 9% in men per 5-units (i.e. 5kg/m^2) BMI increase. Still, there was no association between BMI and rectal cancer risk in women[25]. In general, the risk of colon cancer increases by 30% in men with a 5-unit increase in BMI but 12% in women with a similar increase in BMI[25]. Another large systemic review involving 221 studies[26] also showed an increase in risk with an increase of 5 units of BMI in both males and females with a higher risk in men. Based on these large convincing reviews, it is fair to conclude that obesity is not a risk factor for rectal cancer in women and a smaller risk factor in all colon cancer in women compared to men. It could well be that female hormones sway the mechanism of developing bowel cancer from being overweight. It is estimated that 11% of bowel cancer cases in the UK are caused by overweight and obesity.[16] The mechanism of the link between obesity and cancer remains unclear. One of the postulated mechanisms are that obesity increases inflammatory cytokines and insulin growth factor, which are linked with solid tumours[27]

Similarly, it has been shown that although physical inactivity increases the risk of all bowel cancers in general, the effect is larger in colon cancer compared to rectal cancer [18]. In a large umbrella review, colon cancer risk is 19% lower in individuals with the highest total physical activity level than individuals with the lowest level[4][28]. The effect is generally less pronounced in the rectal cancer cohort; the risk of rectal cancer is 6% higher in the most sedentary people compared to the active group in a large cohort study[29]. Low physical activity levels also increase the risk of colon cancers, diabetes and ischemia[30]. The possible explanation for this has been that physical activity improves insulin sensitivity, and the colon is more susceptible to insulin effects than the rectum hence the impact of physical inactivity on the risk of colon cancer[21].

There is enough evidence to say that a high intake of red meat increases the risk of colon cancer and to a lesser degree rectal cancer[31]. The general impression is that processed meat carry a higher risk than red meat. The currently available evidence supports limiting the intake of red and processed meat to reduce the risk of colon and rectal cancers.[4][32] Colon cancer risk is 17-30% higher per 100-120g/day of red meat intake.[31][32] The risk of processed meat increases by similar figures for a smaller average daily intake; 50g daily intake (half that of red meat)[4][31]. It is estimated that 13% of bowel cancer cases in the UK are caused by eating processed meat.[16] It is clear from the evidence that processed meat is one of the major modifiable risk factors for rectal and bowel cancer and can be argued to be on par with cigarette smoking.

Bowel cancer risk is 4% higher in people who drink up to 2 units of alcohol per day [4][33]. Meta-analyses have shown that the risk is estimated to be 10-17% higher in people who consume up to 6 units per day and 33% higher in those who consume more than six units of alcohol per day, compared with non or occasional drinkers [4][33]. It is estimated that bowel cancer risk increases by 7% per unit of alcohol taken per day[34]. Six per cent (6%) of bowel cancers in the UK are estimated to be caused by alcohol intake.[16]

Family history is also an important risk in developing colo-rectal cancer. Hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome and Familial adenomatous polyposis (FAP), are the two primary genetic linked conditions in colo-rectal cancers[35]. HNPCC carries a lifetime risk of 80% and FAP a lifetime risk of 100% and account for about 5% of all colo-rectal cancers[35]. The mutation resulting in these two conditions is located on either the adenomatous polyposis coli (APC) gene for FAP or the mismatch repair gene for HNPCC, which can be inherited or sporadic. The mismatch repair gene mutation is more common than the APC mutation. It mainly occurs sporadically, accounting for 15% of all colorectal cancers and can be tested by looking for this mutation in the tumour for MSI (microsatellite instability)[35]. Positive MSI mutation in the tumour with a patient less

than 50 years or with associated family history raises the suspicion of a germline inherited mutation of this gene. Numerous other inherited factors increase the predisposition of colorectal cancers, which are less well recognised but contribute to developing colo-rectal cancers. A systematic review[36] has shown that people with a first degree relative with colorectal cancer have a significantly increased risk of having colo-rectal cancer than those without a much higher risk if the relative is diagnosed young or when multiple relatives have the disease.

Other risk factors for colo-rectal cancers include associated diseases such as type 2 diabetes, which increased the risk of colo-rectal cancers by about 20%[37][38] and inflammatory bowel disease[39]. Drugs such as metformin in diabetes[40], aspirin[41] and combined oral contraceptives[42] have been shown to lower the risk of bowel cancers. Asprin has been shown to reduce the risk by as much as 17% in the population that has ever taken it [41]. Combined oral contraceptives also reduce the risk of colorectal cancer by as much as 19%, depending on the duration of its use[42][43].

Table 1.1 Bowel Cancer Risk Factors

Evidence	Increases risk	Decreases risk
'Sufficient' or 'convincing' evidence	• Alcoholic drinks • Tobacco smoking • X-radiation, gamma-radiation[a] • Processed meat • Body fatness • Adult attained height	• Physical activity[b]
'Limited' or 'probable' evidence	• Asbestos • *Schistosoma japonicum* • Red meat	• Wholegrains • Dietary fibre in foods • Dairy products • Calcium supplements • Oestrogen-progestogen contraceptives • Asprin

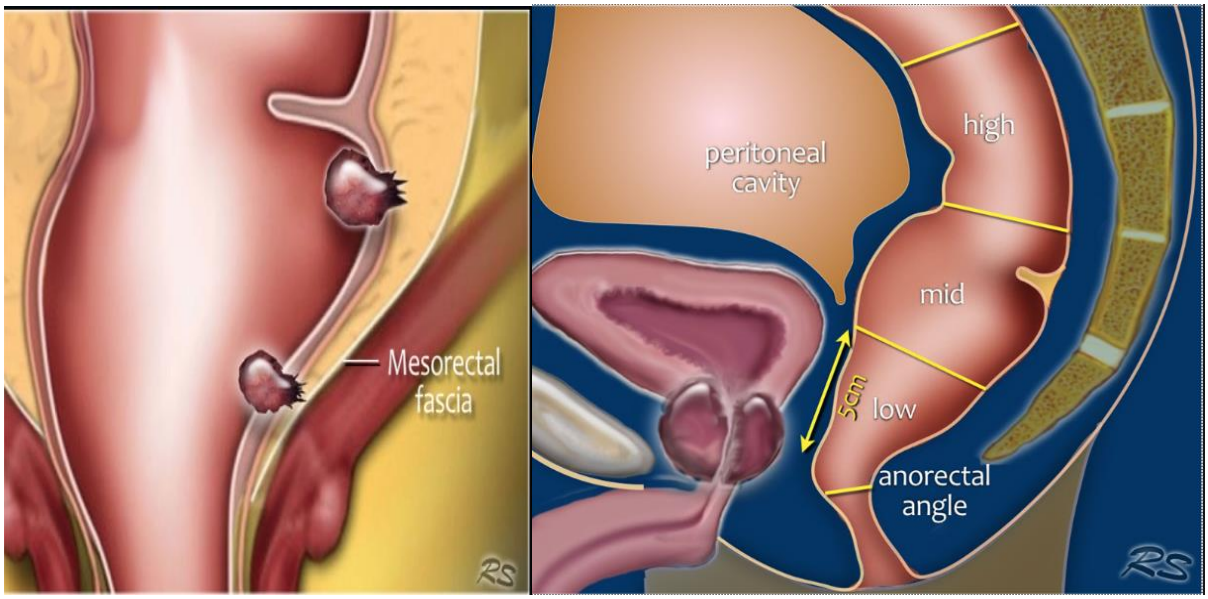
The table shows the risk factors of developing bowel cancer.
Source- International Agency for Research on Cancer (IARC) and World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) classifications.

1.3: Anatomy

The rectum measures 15cm from the anorectal junction, the dentate line to the recto-sigmoid proximally. It is divided into the upper, mid and lower rectal, with the most distal 5cm being the lower rectum. The rectum is typically located below the peritoneal reflection. The posterior wall is entirely extra-peritoneal. The blood supply enters the rectum posteriorly. The upper rectum receives its blood supply via the superior rectal artery (SRA), a branch of the inferior mesenteric artery (IMA). The middle and lower rectum are provided by the middle rectal artery and the inferior rectal artery, which branches from the anterior division of the internal iliac artery and the pudendal artery, respectively[44]. The lymphatic drainage of the upper two-thirds of the rectum is along the pathway of the superior haemorrhoidal vein, anterior to the inferior mesenteric nodes and the para-aortic nodes. The lymphatic drainage of the lower third of the rectum is anterior and lateral along the middle haemorrhoidal vessels to the internal iliac nodes[44]. The upper or high rectum is close anatomically to the sigmoid and closer to the small bowel, a significant dose-limiting organ for radiotherapy. Due to its anatomical position, the lower rectum has the highest recurrence rate as its proximity to the pelvic wall and mesorectal fascia (the layer of connective tissue enclosing the peri-rectal fat surrounding the rectum) makes it easier for tumours to invade surrounding structures. Patients with lower rectal tumours are also likely to end up with permanent stoma post surgery as diseases up to 5cm from the ano-rectal junction do in most cases require a permanent stoma.

Figure 1.1: Gross anatomy of the rectum

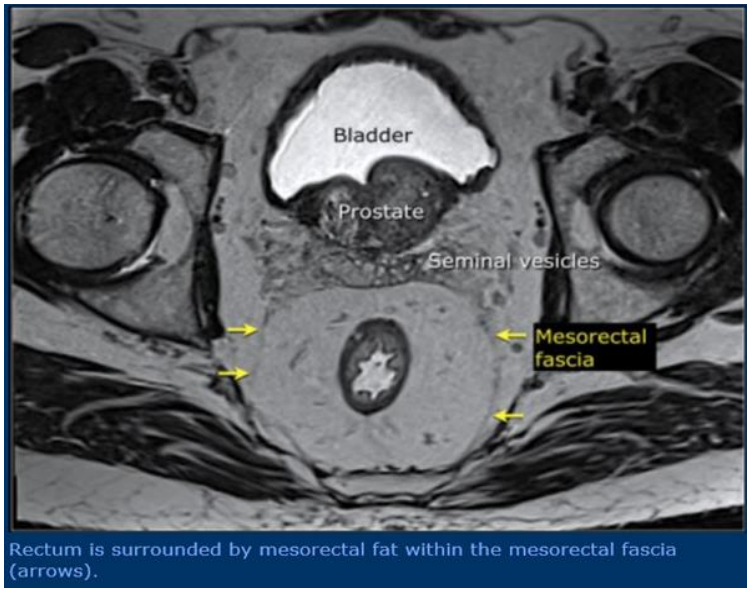
This shows the division of the rectum into high, mid and low rectum starting distally from the dentate line described here as the anorectal angle.



Source- http://www.radiologyassistant.nl

Figure 1.2: Axial cross-sectional image of the rectum

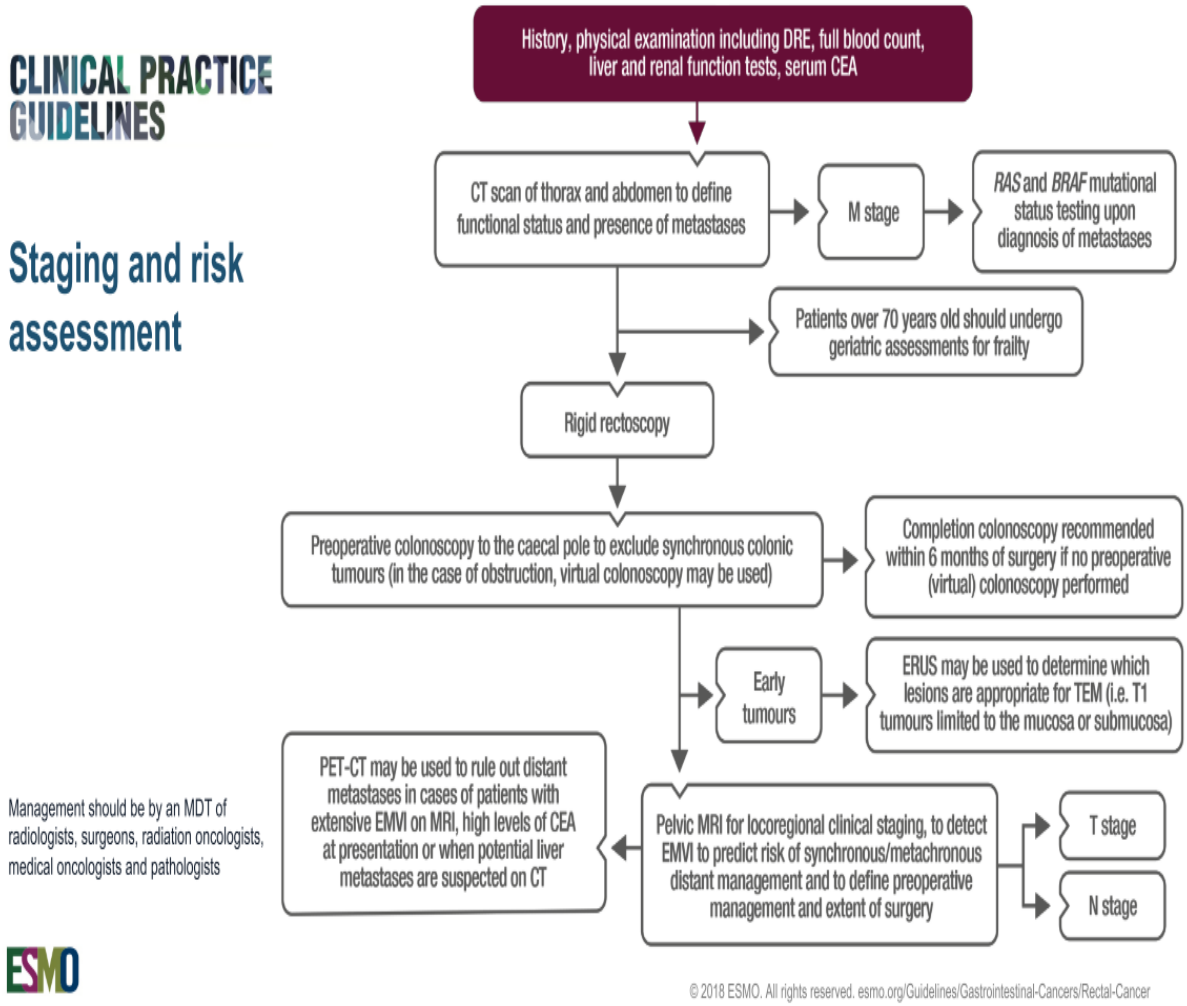
This shows the axial anatomy of the rectum and other surrounding organs, and it highlights the mesorectal fascia.



Source-http://www.radiologyassistant.nl

Figure 1.3: ESMO Rectal staging and risk assessment.

This figure showed the process of staging investigations in the diagnosis of rectal cancer.



ESMO guidelines rectal cancer 2001

1.4: Diagnosis and Staging of Rectal cancer

The first investigation in rectal cancer diagnosis is a clinical assessment with a digital rectal examination (DRE); this is used to assess the tumour's mobility or fixation to the pelvic wall and invasion onto the retro-vaginal septum **and the distance from the anal verge**. Blood tests generally include full blood count, liver and renal function tests to assess the patient's organ function.

The diagnosis of rectal cancer is made on tissue diagnosis and radiological investigations. Tissue biopsies are obtained via endoscopic investigations. The standard of care endoscopic investigation is colonoscopy. Colonoscopy has become the main route of getting tissue and assessing the extent of the disease with a direct view. Colonoscopy has become essential to ensure no synchronous disease in the large bowel; this is becoming more common. The incidence of synchronous cancer is thought to be around 4%[45]. CT colonoscopy is an alternative in the assessment of the disease. It has shown comparable sensitivity and specificity to conventional colonoscopy when done by radiological units with a good level of expertise[46]. CT colonoscopy is also an option in stenotic tumours where the scope could not pass the diseased area for a full assessment of the bowel.

Since the mid-2000s, magnetic resonance imaging (MRI) has been the standard of care for pre-treatment staging in rectal cancer patients[47]. MRI has shown good accuracy in determining the size and stage of the rectal tumours. It is also crucial in deciding invasion into the mesorectal fascia, which is essential in deciding if neo-adjuvant treatment is required[48]. Although trans-rectal endoscopic ultrasound (TEUS) has been seen as an alternative to pelvic MRI in the staging of rectal cancer, MRI is still considered as the standard, especially in looking at the circumferential resection margin (CRM) [7]. The distance of the tumour from CRM determines the likelihood of achieving a complete resection with surgery.

MRI staging is an essential diagnostic tool in determining if the mesorectal fascia (MSF) is involved or not. The mesorectal fascia is threatened if the tumour lies within 1mm of the fascia; the mesorectal fascia is also known as the circumferential resection margin (CRM). This is the point of resection during TME surgery. Patients with threatened or involved mesorectal fascia are highly likely to have residual tumour post-surgery, which puts them at high risk of local recurrence and worse prognosis; therefore, they need neo-adjuvant down-staging treatment before surgery. Optimal down-staging is obtained with chemo-radiotherapy, making pre-operative chemo-radiotherapy the standard of care for this group of patients. For a patient who does not have MRF involved or threatened, the decision on

whether they need short-course radiotherapy (25Gy/5 fractions) or up-front surgery depends on the location of their disease and the size of their tumour and the overall risk of recurrence of their disease. The site of the disease is essential in that the anatomy of the rectum is such that the more distal, the narrower and closer the lumen is to the CRM. The closer the tumour is to the CRM, the higher the risk of local recurrence.

CT images of the chest, abdomen, and pelvis assess any distant metastatic disease, particularly the liver, in the chest and abdomen. If there are any liver changes, the nature of those lesions, if uncertain, are verified with MR images of the **liver** in most cases. USS of the liver could be used to obtain a liver biopsy if indicated.

Once the rectal cancer diagnosis is made, the staging is done using the TNM stagging system, and the most recent is the eighth edition. The stage of the disease determines the suitable treatment option for the patient. The diagnosis and staging of rectal cancer should always be made after verifying the diagnosis investigations by the rectal cancer multi-disciplinary team (MDT).

Table 1.2: Staging of Rectal Cancer

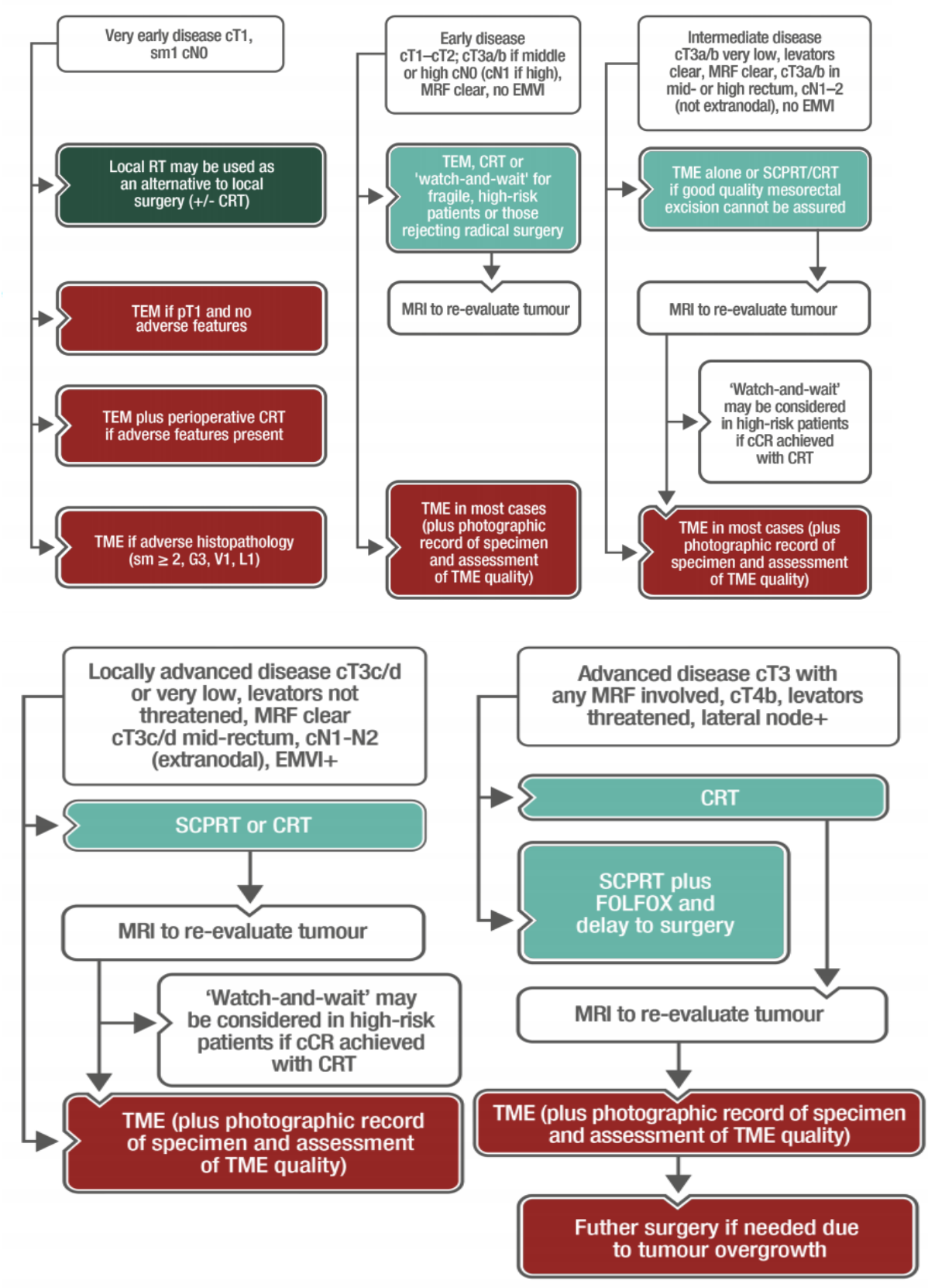
Primary tumour (T)	Regional lymph nodes (N)	Distant metastasis (M)
TX: primary tumour cannot be assessed	NX: Regional lymph nodes cannot be assessed	M0: no distant metastasis by imaging
T0: No evidence of primary tumour	N0: no regional lymph node metastasis	M1: distant metastasis M1a: metastasis confined to 1 organ or site without peritoneal metastasis M1b: metastasis to 2 or more sites or organs is identified without peritoneal metastasis M1c: metastasis to the peritoneal surface is identified alone or with other site or organ metastases
Tis: carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)	N1: metastasis in 1 - 3 regional lymph nodes	
T1: tumour invades submucosa propria)	N1a: metastasis in 1 regional lymph node N1b: metastasis in 2 - 3 regional lymph nodes. N1c: no regional lymph nodes are positive, but there are tumour deposits in the sub-serosa, mesentery or non-personalised peri-colic or perirectal/mesorectal tissues	

T2: tumour invades muscularis propria	N2: metastasis in 4 or more regional lymph nodes N2a: metastasis in 4 - 6 regional lymph nodes N2b: metastasis in 7 or more regional lymph nodes	
T3: tumour invades through the muscularis propria into the peri-colorectal tissues		
T4: **T4a: tumour invades through the visceral peritoneum (including gross perforation of the bowel through the tumour and continuous invasion of the tumour through areas of inflammation to the surface of the visceral peritoneum)** **T4b: tumour directly invades or adheres to other adjacent organs or structures.**		

This table shows the staging of rectal cancer as defined by AJCC 8th edition.
Source: The AJCC Cancer Staging Manual, Eighth Edition, 2017 [49].

Figure 1.4: ESMO Treatment Algorithm

The figure shows the ESMO algorithm in the management of Rectal cancer [7]



1.5: Neo-adjuvant treatment of Locally Advanced Rectal Cancer

Staging of rectal cancer is done clinically through digital rectal examination and flexible sigmoidoscopy and radiologically via MRI of the pelvis. For rectal cancer at moderate or high risk, neoadjuvant chemo-radiotherapy followed by resective surgery is the definitive treatment. Besides the stage of the disease, the mesorectal fascia (MRF) plays an essential role in determining the treatment of localised rectal cancer. The mesorectum is the fat plane that contains blood, lymphatics and nerve supply to the rectum. The surgical removal of the rectal tumour and the surrounding mesorectum along the mesorectal fascia is called total mesorectal excision (TME). Total mesorectal excision has remained the gold standard surgical treatment in rectal cancer since its introduction in the early '80s. The proximity of tumours to the mesorectal fascia became important because complete surgical resection depended on this area being free of tumour. The two major advances in the treatment of rectal cancer since the 1980s have been total mesorectal excision (TME) and neoadjuvant radiotherapy. Both significantly changed the local recurrence and survival rates in rectal cancer[5][50]. Before these two changes were introduced, rectal cancer had frequent pelvis recurrences and significant morbidity. These treatments revolutionised rectal cancer treatment in that surgery was optimised by TME, and chemotherapy and radiotherapy were used to shrink the disease before surgery to ensure good resection and reduce the risk of cancer recurrence.

1.5.1: Radiotherapy in Rectal cancer

One of the two early trials that showed significant benefits of radiotherapy in rectal cancer was the MRC CR02[51] and CR03[52] trials that randomised patients to either upfront surgery or pre and postoperative radiotherapy, respectively. At the time of recruitment of these two UK trials, total mesorectal excision (TME) was not yet the standard of care surgical treatment and radiotherapy delivery is different compared to today. CR02 trial showed a 10% reduction in local reoccurrence at five years (p=0.04) using pre-operative radiotherapy [51], and the CR03[52] showed a 13% reduction (p=0.001) favouring the radiotherapy group. CR02 gave radiotherapy peri-operatively, and CR03 did it post-operatively. The Swedish study[53] was a larger study that randomly assigned 1,168 patients between 1987 and 1990; 908 had curative surgery; 454 of these patients had surgery alone, and 454 were administered preoperative radiotherapy (25 Gy in 5 days) followed by surgery within one week. After median follow-up time 13 years. The overall survival rate in the radiotherapy group was 38% v 30% in the surgery alone group (P =0.008). The cancer-specific survival rate in the radiotherapy group was 72% v 62% in the surgery alone group (P =0.03) and the local recurrence rate was 9% v 27% (P < .001) respectively. This represents a two-thirds reduction in local recurrence rate with radiotherapy

and an 8% absolute reduction in mortality with the addition of radiotherapy. It is important to note that patients on the Swedish study did not all have TME operation as their standard of care; most were recruited before 1990s when TME was fully adopted. The Dutch trial [11] was done between 1996-1999 when TME was the generally accepted standard of care. The evidence from the Dutch trial showed a 5yr reduction in local recurrence with pre-operative radiotherapy from 12% to 6% for patients who have an upfront resectable disease. There was no significant overall survival benefit at ten years in the Dutch trial.

Looking at the four major trials that defined radiotherapy in rectal cancer, the balance of evidence shows that the rate of local recurrence has moved from about 50% in the early '80s with CR02 and CR03 trials to 6-7% in the Dutch trial[50] in more recent times due to the introduction of TME and radiotherapy to rectal cancer treatment. Radiotherapy reduces the local recurrence rate by 50% [50] [53]. It is likely that with the introduction of TME, the significant overall survival seen with the addition of radiotherapy in the earlier trials became insignificant.

Table 1.3: Evidence For Radiotherapy in Rectal Cancer.

Trial	N=	Patients	Method	Results	Interpretation	Points
CRO2 [51]	279	140 patients had surgery alone. 139 patients pre-op RT then surgery.	40/20# pre-op RT then surgery vs surgery alone	5yr LR 36%vs 46%	XRT lowers LR	Enrolled in the UK from 1981-1989 with 5 yrs follow-up. Pre TME
CRO3 [52]	469	235 in post-op RT vs 234 surgery alone	Surgery alone vs surgery plus 40/20# RT post-op	5yr LR 21%VS34%	XRT lowers LR	Enrolled in UK and Ireland between 1984-1989 with 5yrs follow-up. Pre TME.
Swedish[53]	1168	Resectable rectal ca	25/5# RT then surgery vs surgery alone	5yr LR 11%vs 27% 5yr OS 58%vs 48% 13yr OS 38%vs 30% 13yr LR 9% vs 27% 12yr CSS 72%vs 62%	Pre-op RT improves os and LR	Not all had TME. Recruited 1987-1990
Dutch[50]	1861	Resectable rectal cancer	25/5# RT then TME vs TME alone	5yr LR 6% VS 12% 10yr LR 5% VS11%. 10yr LR for stage 3-9%vs19% 10yr os ~48% no change.	Pre-op RT reduces LR post-surgery.	TME was within 2-3 weeks post-RT. They were recruited between 1996-1999.

The table shows randomised trials that defined radiotherapy treatment in rectal cancer

1.5.2 Choice of systemic radiosensitiser.

The choice of systemic therapy use in concurrent chemoradiotherapy has evolved to be either capecitabine or infusional 5fu. However, the question has remained whether these represent the optimal agents. A randomised phase III German trial[54]comparing capecitabine with infusional 5fu concurrent with radiotherapy in locally advanced rectal cancer found capecitabine non-inferior to 5fu. As expected, both have slight differences in toxicities. Although the local recurrence rate was very similar (6 versus 7 per-cent with 5fu), the distant metastasis rate was better with capecitabine which is interesting (19 versus 28 per cent) [54]. One could therefore say that capecitabine is the more comfortable choice compared to 5fu given its convenience, lower cost as an oral drug and lower distant metastasis.

In concurrent chemoradiotherapy, the additions of oxaliplatin to capecitabine have been investigated to see if this will improve outcomes. The Italian STAR-01[55] trial tested this in a randomised trial with 742 patients randomised to either concurrent treatment with 5fu and radiotherapy or 5fu with a weekly infusion of oxaliplatin and radiotherapy[55]. The results showed that oxaliplatin increased toxicity with no added benefit to clinical outcomes. The pCR rate in both arms of the study was 16%, with three times more grade 3/4 toxicities in the oxaliplatin group (24% v 8% of treated patients; P < .001). Other large randomised trials have shown similar results [56][57][58][59]. In a recently published randomised phase III study, the FOWARC trial; [60][61] 495 patients were randomised to FOLFOX (folinic acid, fluorouracil, and oxaliplatin chemotherapy) alone, FOLFOX with radiotherapy or capecitabine with radiotherapy. This trial showed an increased pCR with concurrent FOLFOX and radiotherapy compared with standard capecitabine and radiotherapy (27.5% vs 14%). The neo-adjuvant chemotherapy group had a pCR rate of 6.5% after 4-6 cycles of FOLFOX and surgery. Patients were all planned to receive adjuvant chemotherapy. There was no improvement in DFS (disease free survival) or recurrence with the addition of oxaliplatin in this study, even though the pCR rates were

encouraging. Although the primary outcome of this study, which is three years DFS, was similar in each group, we need to wait longer to see if there are sustained long term differences.

Nevertheless, compared with the standard, the significant pCR rate with FOLFOX radiotherapy remains attractive in the era of organ preservation. The similar R0 (Resection margin clear) rates and DFS in all three groups suggest that FOLFOX alone could be an alternative downstaging treatment if radiotherapy is not given. Although these early results might be positive regarding oxaliplatin and 5FU used in concurrent chemoradiotherapy, the balance of evidence is still against the addition of oxaliplatin in concurrent treatment due to lack of improved outcome with increased toxicity.

Another possible chemotherapy to be used in combination with 5fu/capecitabine in concurrent chemoradiotherapy is irinotecan, as tested in the ARISTOTLE trial. Aristotle is a phase 3 randomised trial that recruited patients with locally advanced rectal cancer into two arms; the standard aim is chemoradiotherapy using capecitabine. The comparative arm is chemo-radiotherapy with capecitabine and irinotecan chemotherapy[62]. The preliminary results presented at ASCO 2020 and abstract produced showed that the pCR rate(available from > 95% of patients recruited) is 20.2% (46/228) for the Irinotecan-CRT group vs 17.4%(40/230) for CRT (p=0.45) [62]. There is a similar R0 resection rate of >84% in both groups. The grade 3-4 gastrointestinal adverse event rate was 21% (58/276) with Irinotecan-CRT and 12% (34/283) with CRT (p = 0.004). Patients receiving Irinotecan-CRT had significantly more diarrhoea as expected; 13.8% vs 3.5% (p<0.001) and neutropenia; 9.8% vs 1.1% (p < 0.001)[62]. The conclusion is that the addition of irinotecan did not significantly improve the clinical outcomes of these patients when compared with the standard chemo-radiotherapy regimen. It was also associated with decreased radiotherapy compliance and a higher rate of adverse events.

The RTOG 00-12 trial [63] randomised patients to concurrent chemo-radiotherapy with 5fu (and radiation intensification dose of 55-60Gy) vs 5fu and irinotecan (using the standard dose of 50-54Gy) in patients with distal rectal tumours. In this trial, The pCR rate was higher than that seen in most

other studies (i.e. 15-20%), where a standard dose of radiation (45-50.4 Gy) was used, but there was no significant difference in pCR between the two groups (26% vs 30%, 5fu CRT and Iri+5fu CRT respectively). Other oncological outcomes were better than expected but did not differ between the two arms. It is also noteworthy that the patients that received irinotecan had higher than expected second primary cancers, which were unusual. The results suggest that the higher than anticipated pCR rates are primarily due to the higher radiotherapy doses and not due the radiosensitisers.

In concurrent chemoradiotherapy, molecularly targeted drugs can be added to capecitabine/5fu to improve clinical outcomes. The DREAM therapy trial[64] evaluated the addition of cediranib or selumetinib to preoperative chemoradiotherapy for locally advanced rectal cancer in a phase 1 trial. Cediranib and selumetinib are tyrosine kinase inhibitors, with cediranib targeting the vascular endothelial growth factor (VEGF) receptor and selumetinib targeting the Mitogen-activated enzyme protein kinase (MEK). Patients in this trial [64] received the standard chemoradiotherapy with capecitabine with the addition of either cediranib or selumetinib. The combination with cediranib (at a recommended cediranib dose of 20 mg/day) was well-tolerated and efficacious, 41% of patients achieved a clinical or pathological complete response(7/17), and 53% (9/17) had an excellent clinical or pathological response (ECPR)[64]. These results look promising but need to be investigated in a larger cohort of patients.

Preoperative Radiotherapy and E7046 in Rectum Cancer (PRAER 1 trial) is a multi-centre, open-label, phase 1b study in patients with locally advanced rectal cancer currently testing the drug; E7046 both concurrently and sequentially over ten weeks period, with either short-course radiotherapy (25Gy in 5 daily fractions over one week) or long-course chemoradiotherapy (45Gy in 25 daily fractions over five weeks), followed by surgery at week 14-16. Two dose levels are being tested with this drug. In the abstract submitted to ESMO in 2019[65], safety has been established in 14 patients treated at the dose of 250mg and recruitment to the higher dose of 500 mg is ongoing. Initial analysis of the response

rate in the first 13 patients shows a reasonable clinical complete response rate of 5/13 patients (38%)[65].

Another targetted radiosentitiser is AN0025 (previously E7046) which is an inhibitor of the EP4 receptor and targets macrophages and immunosuppressive cells of myeloid lineage in the tumour microenvironment. Pre-clinical/ animal study has shown this drug to be effective in combination with radiotherapy [66].

The PRIME RT trial [67] offers one of the first use of immunotherapy in the neoadjuvant treatment setting in colorectal cancer. PDL1 inhibitor Durvalumab is combined with pre-operative chemotherapy, chemoradiotherapy, or short-course radiotherapy. Locally advanced rectal cancer patients on this trial will be randomised between short-course radiotherapy then six, two weekly cycles of oxaliplatin-5fu doublet chemotherapy versus long-course chemo-radiotherapy then four, two weekly cycles of oxaliplatin-5fu doublet chemotherapy. Durvalumab will be given to the patients from the commencement of radiotherapy for 17 weeks. The trial aims to determine which arm has the highest complete response rates with acceptable toxicity.

Another exciting phase 1 trial in this setting is the CEDAR trial, a cancer research UK-funded trial that uses the drug; Enadenotucirev. This drug will be added to the standard chemotherapy used in the chemoradiotherapy regime. Enadenotucirev is an oncolytic adenovirus. The rationale behind using this drug is to create a viral-mediated oncolysis on the rectal cancer cells. This will be achieved by inhibiting the process of DNA repair in the rectal cancer cells, making them more susceptible to chemoradiotherapy. This investigators hope to see both a local effect on the rectum and a systemic impact on lowering the metastatic rate. This drug was developed via a process of bio-selection using the human HT-29 colorectal cancer cells[68]

1.5.3: Chemo-radiotherapy vs radiotherapy alone

The evidence for chemo-radiotherapy, as opposed to radiotherapy alone, is based on two essential trials; EORTC 22921[69], used four arms to examine both the benefit of concurrent chemo-radiotherapy (using a five-day bolus FU and LV regimen during weeks 1 and 5 of RT) and radiotherapy alone treatment in pre-operative setting and postoperative adjuvant chemotherapy in resect-able locally advanced disease. The preoperative chemo-radiotherapy group had a significantly higher pCR rate (14 versus 5 per cent) than the radiotherapy alone group. The local relapse rate was also better in the groups that received chemo-radiotherapy. Five years relapse rate was 17.1% in the radiotherapy alone group compared with 8.7% in the CRT group[69]. The addition of postoperative adjuvant chemotherapy did not improve the outcome of this study.

A Cochrane review of six randomised trials (two comparing short course with long course CRT) has also shown that the addition of concurrent chemotherapy to neoadjuvant RT improved local control (odds ratio [OR] for local recurrence 0.56, 95% CI 0.42-0.75) with a higher rate of acute grade 3 or 4 treatment-related toxicity in chemoradiotherapy patients (OR 3.96, 95% CI 3.03-5.17). There was no significant impact on rates of sphincter preservation or overall survival.[70] Given the evidence from EORTC 22921 and the meta-analysis together with the higher PCR in CRT, the standard of care for neo-adjuvant down staging where MSF is involved or threatened has become chemo-radiotherapy. Pre-operative chemo-radiotherapy using concurrent capecitabine combined with radiotherapy followed by TME is also recommended by NICE in this group of patients.[71] About 65% of patients with localised rectal cancer currently recieve pre-operative chemo-radiotherapy.

Table 1.4: EORTC 22921.

(N=1011. T3 and T4 disease)	Preop RT (45/25#)	Preop RT + adjuvant chemo	Preop CRT + adjuvant chemo	Preop CRT
Number of patients	252	253	253	253
Treatment-related grade ≥2 diarrhea (%)	17.3	17.3	37.6	37.6
Sphincter-preserving surgery (%)	50.5	50.5	52.8 (p=0.47)	52.8 (p=0.47)
Overall survival rate at 5yr (%)	63.2	67.2 (p=0.12)	67.2 (p=0.12)	63.2
5-year cumulative rate of local relapse (%)	17.1	9.6	7.6	8.7
pCR	5%			14%

Table 1.4: This table shows the results of the EORTC 22921 trial.

Data from: Bosset JF, Collette L, Calais G, et al. chemotherapy with preoperative radiotherapy in rectal cancer. N Engl J Med 2006; 355:1114.

1.5.4: Pre-operative Chemo radiotherapy vs Postoperative Chemoradiotherapy.

The German rectal trial [72] randomised 823 patients with clinically staged T3/4 or node-positive rectal cancer to the same chemo-radiotherapy regimen administered either preoperatively or post-operatively; 50.4 Gy in 28 daily fractions to the tumour and pelvic lymph nodes concurrent with infusional 5FU. At 46 months, preoperative chemo-radiotherapy was associated with a significantly lower pelvic relapse rate (6 vs 13 per cent with postoperative). At ten years, the differences were persistent (7 versus 10 per cent). Overall survival rates were similar; 76% vs 74% in the two groups, respectively. The CR07[73] trial randomised 1350 patients' with operable adenocarcinoma of the rectum from 80 centres in four countries to short-course preoperative radiotherapy (25 Gy in five fractions; n=674) or to initial surgery (n=676) with selective postoperative chemo-radiotherapy (45 Gy in 25 fractions with concurrent 5-fluorouracil) restricted to patients with involved margin (n=77,12% of the immediate surgery group). They showed a reduction of 61% in the relative risk of local recurrence for patients receiving preoperative radiotherapy (hazard ratio [HR] 0·39, 95% CI 0·27–0·58,

p<0·0001), and an absolute difference at three years of 6·2% (95% CI 5·3–7·1) (4·4% preoperative radiotherapy vs 10·6% selective postoperative chemo-radiotherapy). A relative improvement in disease-free survival of 24% for patients receiving preoperative radiotherapy (HR 0·76, 95% CI 0·62–0·94, p=0·013), and an absolute difference at three years of 6·0% (95% CI 5·3–6·8) (77·5% vs 71·5%)[73]. Together with other similar randomized trials, these studies support the practice of neo-adjuvant radiotherapy rather than postoperative treatment. It will be reasonable to consider postoperative radiotherapy in a patient who did not receive pre-operative radiotherapy and is likely to benefit from one, i.e. those under staged at the time of their diagnosis or those with a positive margin at significant risk of recurrent disease. This risk will have to be carefully balanced against the risk of increased toxicity associated with post-operative radiotherapy.

Table 1.5: Evidence for Pre-operative chemo-radiotherapy vs Post-operative.

Trial	N=	Patients	Method	Results	Interpretation
German trial[25]	823	T3 to T4 or N+	50.4/28# with 5fu then surgery vs surgery then 50.4/28#+boost with 5fu	5yr LR 6% VS 13% Compliance better pre-op. Sphincter preservation 39% vs 19%. G3/4 acute toxicity 27% vs 40%. G3/4 late toxicity 14% vs 24%. 5yr os 76% vs 74 %(NS) 10yr os 60%vs60%.	Better toxicity, compliance and LR with pre-op RT
CR07 [26]	1350	Resectable disease	25/5# RT(n=674) pre surgery vs surgery upfront (n=676) if positive margin(n=77) post op chemo-RT 45/25# with 5fu	3yrs RR of LR 4.4% vs 10.6%. 3yrs DFS 77.5% vs 71.5%. No OS difference.	Better LR and DFS with Pre-operative RT
NSABP R03[74]	267	T3-T4 or N+ (130 pre-op,137 post op)	45/25# with 5fu then surgery vs surgery then 45/25#+boost with 5fu	5yr DFS 64.7% vs 53.4%. 5yr OS 75% vs 66%(p=0.065)	Better DFS with pre-operative RT.
Colorectal Cancer Group. [75]	8507	22 RCT comparing no/ pre/post-operative radiotherapy	Meta-analysis	OS at 5yrs 63% xrt vs 62% surgery alone (p=0.06). The annual risk of local recurrence was 46% vs 37% in favour of pre-op. There were fewer deaths from rectal cancer in preoperative radiotherapy than post-op (45% vs 50%, p=0.0003.	Preoperative radiotherapy (at biologically effective doses ≥30 Gy) reduces the risk of LR and death from rectal cancer and produces a better outcome than post-op RT.

The table shows the randomised trials that define pre-operative radiotherapy over post-operative radiotherapy.

1.5.5: The time between Radiotherapy and Surgery

The time interval between radiotherapy and surgery is one of the long-standing controversial questions in rectal cancer neo-adjuvant treatment. Most believe it should not be less than six weeks, but the optimum time remains unknown. Some studies have shown an increased rate of pCR with a longer time interval beyond eight weeks, while others did not. The concern expressed is that longer time interval means increased postoperative complication, but this has not been heavily supported with evidence[76][77].

A Dutch retrospective study[77] of 475 rectal cancer patients from 71 centres who received preoperative chemo-radiotherapy looked at routine practice around these centres. Surgery done before or after 14 weeks from chemo-radiotherapy did not differ between short- and long-term clinical outcomes. Circumferential resection margin involvement was 9.7% vs 15.9 %(p = 0.145) between ≤ 14 weeks or ≥ 14 weeks, thirty-day surgical complications were similar (20.1% vs 23.1%, p = 0.943), and no significant differences were found for local and distant recurrence rates, disease-free survival, and overall survival. [77]

A meta-analysis[78] that included thirteen trials, making up 3584 patients, has shown a higher pCR rate with a time interval to surgery more than the standard 6-8 weeks post-neo-adjuvant chemoradiotherapy. 13.7 % (in the 6-8 weeks group) vs 19.5% in the longer interval group (RR = 1.42, 95% confidence interval: 1.19–1.68; P < 0.0001). Although the pCR rate was increased by about 6% for an interval period more than 6-8 weeks post CRT, there was no improvement in other clinical outcomes such as DFS, OS, R0 rate and complication rates[78]. A systematic review including 13 studies with a total of 19,652 patients concluded that an interval of ≥ 8 weeks from the end of chemoradiotherapy is safe and efficacious because of higher pCR rates, without increasing complication rates or affecting survival rates. The study demonstrated that pCR was significantly increased in patients with locally advanced rectal cancer on a time interval of ≥ 8 weeks compared to patients with a waiting interval of < 8 weeks after chemoradiotherapy (RR = 1.24; 95% CI, 1.14-1.35; P

< .0001)[79]. The united states' OSTRiCh[80] group looked at 17,255 patients treated between 2006 to 2011 on the National Cancer Data Base with neoadjuvant chemo-radiotherapy. A multivariable logistic regression analysis was used to assess the association between the interval period (less than six weeks, 6 to 8 weeks, >8 weeks) and the odds of pCR, surgical morbidity, and tumour down staging. The results showed that pCR peaked at a waiting interval between 10 and 11 weeks. Interval of more than eight weeks was associated with higher odds of pCR (OR- 1.12, 95% CI 1.01 to 1.25) and tumour down-staging (OR 1.11, 95% CI 1.02 to 1.25). The longer time delay was also associated with lower odds of 30-day readmission (OR 0.82, 95% CI 0.70 to 0.92). There was no evidence of associated increased surgical complications with the increase in interval time[80].

A multi-centre retrospective cohort study[81] of locally advanced rectal cancer patients from 21 Italian radiotherapy institutions looked at the difference in pathological complete response (pCR) according to the time interval between chemoradiotherapy (CRT) and total mesorectal excision (TME) have shown higher pCR rate with a longer time interval. In this study of 2094 patients, 300 patients underwent TME within six weeks, the 2nd group- 1598 patients had TME in 7-12 weeks and the 3rd group- 196 patients had TME in 13 or more weeks after CRT. Overall, pCR was 22.3% (N = 468 patients). The proportion of patients achieving pCR to their time intervals was as follows: 12.6% (1st group; within six weeks), 23% (2nd group; 7-12 weeks) and 31.1% (3rd group;13 or more weeks) ($p < 0.001$)[81]. Kwak et al.[82] reported that tumour response was at its peak when the waiting interval between preoperative chemoradiotherapy and surgery was 7 to 10 weeks.

On the other hand, the GRECCAR-6 trial [83], a multi-centre randomised controlled trial of 265 patients, showed that waiting for 11 weeks after CRT did not significantly improve the pCR rate. The pCR rate in patients who had surgery in the seven-week arm was 15% compared to pCR of 17.4% ($p = 0.5983$) in patients in the 11-week arm. The quality of TME (78.7 vs 90%, $p = 0.0156$) was worse in the 11 weeks group but not significant, which may be due to the small size of the study.

In a short report in 2017 by the National Bowel cancer Organization[84], they investigated the impact of time to surgery after chemoradiotherapy on circumferential margin status, tumour downstaging, complete response rate, 18-month stoma presence and 24-month mortality in patients with rectal cancer. Four thousand one hundred sixty-four (4,164) patients with rectal cancer who completed chemo-radiotherapy between 28-182 days (4-26 weeks) before surgery were included. The median time interval was 12 weeks. There was no evidence that time to surgery affected mortality at 24 months after starting chemo-radiotherapy. The best tumour response appears to occur between 10-14 weeks. A longer delay to surgery is associated with an increased risk of having a stoma 18 months after surgery[84]. It could well be that as the delay beyond 14 weeks is unusual, the reasons for such delay are long-term stoma contributors.

It is acceptable on the available evidence that the ideal interval time should be > 8 ≤14 weeks unless patients received consolidation chemotherapy treatment after chemoradiotherapy, making the interval longer.

1.6: Clinical complete response (cCR) and watch and wait for rectal cancer.

Patients with locally advanced rectal cancer are assessed radiologically with a CT imaging of their thorax, abdomen and pelvis MRI imaging of their pelvis at 8-10 weeks post-chemo-radiotherapy (pre-surgery). Broadly, patients' outcomes from this investigation could be a complete clinical response (cCR), partial response, stable disease or disease progression. If a complete response is suspected, clinical investigations such as digital rectal examination and endoscopy could be used to confirm the absence of disease. A patient who achieves this post-chemo-radiotherapy can be closely monitored in a surveillance protocol called 'watch and wait'. This treatment pathway aims to avoid or defer surgery and its related complications. Surgery will only be indicated if their tumour regrows. About 25-30% will have a local tumour regrowth on the 'watch and wait' surveillance, mainly in the first 2-3 years

after treatment. This group of patients will be salvaged successfully in greater than 90% of cases with surgery[85].

A pathological complete response (pCR) is the absence of a viable tumour in the surgical specimen post-surgery. In a systemic review, pCR has been shown to equate to excellent oncological outcomes[86]. Patients with pCR in this review were nearly four times less likely to develop local failure compared with others without cCR (OR 0·25, 0·10 to 0·59; P = 0·002), 8·7 per cent of patients had distant metastasis at a median follow-up of 56months. Furthermore, a pCR was associated with a more than a four times decrease in the likelihood of developing distant failure (OR 0·23, 0·11 to 0·47; P < 0·001)[86]. Complete clinical response (cCR) has become a surrogate for a complete pathological response for patients who do not have immediate surgery as outcomes in the two patients are very similar. The growing evidence on 'watch and wait' for organ preservation treatment plans in rectal cancer has been pioneered by Prof Habr-Gama and her team from São Paulo in Brazil. This group holds the world's largest series of patients treated on rectal organ preservation strategy in rectal cancer. In 2004, Habr- Gama [87] et al. published their outcome of the non-surgical treatment strategy, which is now known as 'watch and wait'. They were able to show that the five years overall survival and disease-free survival for these groups of patients who had cCR post CRT and monitored on this treatment strategy were 100% and 92%, respectively. [87] The excellent clinical outcome in patients with cCR mirrors that of pCR.

Surgery has always carried a 3% perioperative mortality, life-threatening complications, such as anastomotic leak and lifelong complications such as bowel, bladder, and sexual dysfunction[88][89]. The concepts of avoiding these risks in patients who do not need surgery have never been more critical. Surgery also carries a risk of colostomy and stoma. Prospective health-related quality of life studies have shown stomas associated with persistently low social role, body image, and high defecation scores after rectal cancer surgery [90][91]. About 84% of all patients with rectal cancer surgery have a stoma[92]. Most are reversed after 12-18months, but about half will have the stoma

life long, and it cannot be reversed [92]. The organ preservation concept in rectal cancer aims to eliminate or at least defer the risks associated with surgery in patients with excellent clinical outcomes irrespective of resective surgery.

In the UK, Manchester is a significant contributor to the International' watch and wait' database (IWWD) and holds the third largest series of patients with complete clinical responses in the world through the OnCoRe database. Papers published through the OnCoRe database have been able to quantify rates of local regrowth and oncological safety[11][93][94]. In a 2015 paper published through the OnCoRe database, a propensity-score matched cohort analysis was used to compare clinical outcomes in patients with cCR that had immediate surgery and those managed on the 'watch and wait' surveillance protocol. There was no significant difference in 3yrs clinical outcome in the two groups except for a significantly better colostomy-free survival in the 'watch and wait' group; 74% [95% CI 64–82] vs 47% [37–57] HR 0·445, 95% CI 0·31–0·63; p<0·0001[11]. The International Watch and Wait Database (IWWD)[95] has the most extensive series of patients with cCR managed on the W&W strategy from participating centres across 15 countries. In 2018, the international Watch and wait database published its report on patients on the 'watch and wait' plan. Their results showed a local regrowth rate of 25%, in which 88% occurred within the first two years of follow-up. The overall 5-year survival is 85% in all patients. Local regrowth was located endoluminal in 97%.[9] The latest publication from the IWWD in 2021[85] showed that the risk of local regrowth after three years of cCR is a maximum of 5%. The risk of developing distance metastasis after one year of cCR (without metastasis) is also lower than 5%, suggesting that the intensity of the surveillance after three years could be safely reduced.

The Maastricht group[96][97] have provided encouraging evidence for organ preservation in rectal cancer. Their publications showed a regrowth rate of 14%, 3yr overall survival of 97% and distant metastasis-free survival of 97% in patients with cCR on' watch and wait' surveillance. Regrowth in all patients occurred within 25 months and was surgically salvageable with resective surgery. Their 2018

publication[96] had 102 patients with cCR at first assessment. Also, it included an additional 68 patients who had a near-complete response at 8-10 weeks staging post CRT (early review) whom 90% then preceded to a full clinical complete response on re-assessment 6–12 weeks later. In the 68 patients, 19 patients underwent trans-anal endoscopic microsurgery, and 49 patients opted for a second re-staging, of which 90% (44/49) showed a cCR at the second re-staging. The remaining five patients with clinical residual tumour underwent TME, with a ypT0N0 (ie pCR, no tumour in the resection specimen) in one Patient, ypT1N0 in two patients, and ypT3N1 in the remaining patients. In the trans-anal endoscopic microsurgery cohort, 10/19 patients achieved pCR, 3/19 had ypT1, and 6/19 had ypT2. The group with cCR at the first assessment had a 2-year local regrowth free rate of 84% and 2-year overall survival of 99%, while patients who achieved cCR at the second assessment had a 2-year local regrowth free rate of 73% and OS of 98% ($p > 0.05$). This result makes a good case for re-assessment of patients with a near-complete response as the majority of them (90% in this case) could progress to complete response and then benefit from organ preservation. A systematic review[12] of 15 studies identified all relevant publications between January 2004 and December 2016 with cCR patients showed a local regrowth rate of 21% at a mean follow-up of 15.6 months, of which 93% were surgically salvageable. The colostomy rate was 12%, disease-free survival (DFS) was 83%, and OS 92%. The study looked at 920 patients data, with 575 (62.5%) having organ preservation treatment plan.

The overall evidence from these studies is that patients monitored closely on the non-surgical pathway after cCR did not suffer any worse oncological outcome compared to those with immediate surgery. This evidence from the Maastricht, Sao Paulo, OnCoRe and the IWWD strongly supports ‘watch and wait’ oncological safety. The most robust evidence would be an RCT comparing ‘watch and wait’ and immediate surgery, but this is not feasible. Nevertheless, extensive comparative analyses have helped provide evidence to support this treatment strategy.

Table 1.6: Evidence for watch and wait treatment plan.

Trial	Number (N=)	Patients	Method	Results
Van der Valk et al., International watch and wait database (IWWD)[9]	1009 **880 with cCR (From 47 institutions in 15 countries)**	Patients on W&W from databases worldwide.	Analysis for local regrowth, survival and metastatic risk	87% have cCR 2yr local regrowth of 25% 88% of recurrence occurred within 2 yrs. 97% of regrowth occur in the bowel wall. Distant Mets rate 8%, 5yr OS 85%
Sammour et al. [12]	920 **575 cCR with nonoperative management (NOM). 345 cCR had surgery upfront.**	Review of 15 studies of patients with cCR. Those that went into the watch and wait vs upfront surgery.	Analysis of outcome comparing NOM and surgical group.	Mean follow-up 39.4 NOM and 39.8 surgery group. NOM group: The regrowth rate in the NOM group was 21.3% at 15.6 months. OS 91.7%, while disease-free survival was 82.7%. overall colostomy rate 12% Surgery group: Local recurrence rate is 8.4, OS 92.4, and disease-free survival of 87.5%. overall colostomy rate of 36.1%
Renehan et al. OnCoRe database[11]**.** **(Manchester, England)**	259 **129 cCR on W&W.**	109 surgical resection vs 109 on W&W matched.	Analysis of the outcome of the two groups	34% local regrowth in W&W: There is no significant difference in OS between the two groups. 3yrs colostomy free survival significantly better in W&W (74% vs 47%, p<0.0001)
Habr-Gama et al. 2014[98] **(Sao Paulo, Brazil)**	183 **90/183 had cCR.**	183 patients with distal rectal cancer were recruited pre CRT.	Analysis of outcomes.	49% cCR Local recurrence rate 31%. Salvage rate with TME 93% 5yrs OS 91%

Hupkens et al. Beets' group[96] **(Maastricht, Netherland)**	170 **102 with cCR at first assessment (WW1), 68 near cCR (WW2)**	Included both patients that achieved cCR in first re-staging and those with a near-complete response.	Analysis of outcomes- comparing WW1 to WW2.	For the WW1 group- 2 yr local regrowth free rate of 84%, OS at 2yrs 99%. For WW2 group- 2yr local regrowth free rate of 73%, OS at 2yrs 98% No significant difference in the outcome of the two groups, p=0.0237
Martens et al. **Beet's group** [97]	100 **61 with cCR at first assessment, 39 near cCR**	Includes both complete and near-complete response patients	Analysis of outcomes	3yr local regrowth free rate of 84.6%. 3yr OS 96.6%, Colostomy-free survival was 94.8%. Disease-free survival was 80.6%.

The table above shows the major RCT on the outcome of patients with clinical complete response on watch and wait treatment plan.

1.7: Patient's view on watch and wait

There has always been a question regarding the Patient's view on the 'watch and wait' process. A paper published in 2017[99] compared patients' quality of life in 'watch and wait' with a matched group of patients who had surgery. Quality of life was measured in this study using the validated European Organization for Research and Treatment of Cancer (EORTC) questionnaire. The results show that patients on watch-and-wait have a better physical, cognitive, and emotional function with enhanced global health status than the mesorectal excision group. The watch-and-wait patients also showed fewer problems with defecation, sexual and urinary tract functions [99]. A more recent prospective study[100] looking at the expressed views of 49 patients with locally advanced rectal cancer collected information before patients had CRT and showed that a three-monthly follow-up investigation and a 25% local regrowth rate are considered acceptable by 95% and 94% of these patients respectively. 83% of patients would consider the deferral of surgery in the case of cCR [100]. 17% are either undecided or would like surgery in the case of cCR [100]. This is not surprising as there remains a small subset of patients who are psychologically happier when their tumour is physically removed, since surgery is the only form of treatment that can achieve this. The vast majority of patients choose treatments that leaves them with less side effects balanced against clinical outcomes.

1.8: How do we increase the number having organ preservation?

Based on the evidence from a meta-analysis[78] and a systemic review [79] and the Bowel cancer UK retrospective audit study[84], increasing the time interval between completion of CRT increases pCR and, as such, may increase the cCR and the number having organ preservation. There is also direct evidence to suggest that extending the restaging time post-CRT could increase the number of patients going into 'watch and wait' by pushing the near-complete responders to cCR by 43%[44]. In this study, patients with a near-complete response at 8-10 weeks were restaged 6-12 weeks later, and those who achieved cCR went into a watch and wait treatment plan. The study showed that this patient group

had a non-significant increase in local regrowth rate but no impact on the oncological outcome (16% vs 27% local regrowth at 2 yrs, 99% vs 98% OS at 2yrs)[101]. The current practice is to restage at 6-8 weeks post-radiotherapy in most protocols in the country. Moving this to 10 weeks with the plan for surgery by 14 weeks could increase the number of patients being monitored on the watch and wait surveillance protocol and increase organ preservation.

The current practice of immediate surgery for a patient who receives short-course radiotherapy should also be changed (based on the evidence from the Stockholm trial[102]) to restaging at 8-10 weeks and surgery to follow if no cCR as there is increasing evidence that delaying surgery will increase the number of complete responders in this group of patients with no detrimental effect on their oncological outcome.

Intensifying chemo-radiotherapy is very likely to increase the number of organ preservation patients. Two meta-analyses; Hartley et al. 2005[103] and Sanghera et al. 2008[104], have shown that significant clinical factors associated with pCR were the use of doublet drugs, the method of fluoropyrimidine administration (with continuous intravenous 5-fluorouracil or oral capecitabine being the most effective) and a higher radiotherapy dose of 45gy or more. Although the use of two chemotherapy drugs was associated with a higher rate of pCR, no single schedule seemed to be more effective. Radiotherapy dose escalation[105] remains of interest, and further trials addressing this approach are warranted. The primary dose-limiting organ in rectal radiotherapy is the dose to the small bowel, so a trial that will either reduce the volume treated while maintaining the same effectiveness of the treatment or safer dose escalation via better image guidance will be needed. This could be achieved through adaptive radiotherapy such as offered by MR linac. The use of combination chemotherapy drugs either concurrent with radiotherapy or in the form of total neo-adjuvant therapy will also be essential. New agents, including immunotherapy in combination with standard chemoradiotherapy, will be tested in a new trial; ARTEMIS trial.

The ARTEMIS trial is a phase 2 randomised controlled trial with a multi-arm multi-stage format aimed at answering the question of the best systemic agent for rectal chemo-radiotherapy. It will be the first major rectal cancer trial with organ preservation as its primary end-point.

1.9: Total Neo-adjuvant Therapy (TNT)

Total neo-adjuvant therapy is another approach that has been suggested as a way to achieve a higher complete response rate than the standard chemoradiotherapy regime. Total neo-adjuvant treatment is when radiotherapy is combined with doublet or triplet chemotherapy before patients have surgery. The doublet chemotherapy primarily used here is oxaliplatin with either capecitabine or 5fu. The chemotherapy is given either before chemoradiotherapy (induction chemotherapy) or after chemoradiotherapy (consolidation chemotherapy) followed by surgery.

1.9.1 Induction vs Consolidation TNT

Various trials, mostly in phase 2, have looked at induction chemotherapy in locally advanced rectal cancer to improve clinical outcomes. These include oxaliplatin and 5fu[106], capecitabine and oxaliplatin (the EXPERT trial[107] and GCR-3 trial[108]) and capecitabine, oxaliplatin and cetuximab (the EXPERT-C trial[108]). The results from these trials suggest that although this is a tolerable route of delivering doublet chemotherapy, the clinical outcomes, especially the pCR rate, have not been improved significantly above the standard chemoradiotherapy regimen [109].

The German rectal cancer study group trial; CAO/ARO/AIO-12 trial[110] randomised 311 stage II or III rectal cancer patients into two arms; group A for induction chemotherapy using three cycles of fluorouracil and oxaliplatin before fluorouracil/oxaliplatin chemoradiotherapy (50.4 Gy) or to group B for consolidation chemotherapy after CRT. The results reported a pCR rate of 17% for the induction group and a significantly higher pCR rate of 25% for the consolidation group. The time interval between completion of chemoradiotherapy and surgery in the consolidation group is a median of 90 days v 45 days in the induction group, which did not increase surgical morbidity [110]. The double

interval time in the consolidation group could have contributed to the difference in the pCR rate as we know that a longer time interval between radiotherapy and surgery means a higher pCR rate. However, long term data is needed to show if the higher pCR rate equates to a significantly improved outcome.

The OPRA trial [111] randomised 324 patients with stage 2 and 3 rectal adenocarcinomas to two aims; 4 months of oxaliplatin plus 5fu or capecitabine before (Induction) or after (Consolidation) fluorouracil or capecitabine based chemo-radiotherapy. Patients were then re-staged 8-12 weeks after finishing total neo-adjuvant therapy with digital rectal examination, sigmoidoscopy and MRI imaging. Patients with complete or near-complete clinical responses were offered watch and wait while others had total mesorectal excision. At 3 years, with a median follow-up of 2.1 years, the disease-free survival between the induction and consolidation group was 78% vs 77% (p= 0.90), distance metastasis-free survival was 81% vs 83% (p=0.86), organ preservation was 43% vs 58% (p= 0.01)[111] favouring consolidation.

Consolidation TNT has shown a higher pCR rate than induction TNT. However, there is generally a prolonged period between chemoradiotherapy and surgery in consolidation TNT than induction TNT. Post chemoradiotherapy, there is a time interval before surgery, so utilising this period to augment the local and systemic treatment in rectal cancer is desirable, especially if it means a better outcome making consolidation more favourable than induction.

1.9.2 Consolidation TNT

The Polish colorectal group did one of the first large consolidation TNT RCT trials in locally advanced rectal cancer. They compared short-course radiotherapy (25Gy/5fractions in a week) followed by six weeks of oxaliplatin plus 5fu chemotherapy (3 cycles) then surgery with standard chemoradiotherapy followed by surgery in 515 patients. R0 resection rate was the primary endpoint, and there was no significant difference in the two groups; 77% versus 71 %(consolidation vs standard) P = 0.07. The pCR

rates in both groups were 16% versus 12%, P = 0.17. At three years, the rates of overall survival and disease-free survival in the two groups were 73% versus 65%, P = 0.046, favouring the consolidation group. [108] The more mature data presented by this group at ASCO 2019[112] with a median follow-up of seven years showed that the improved outcomes had disappeared. The cumulative incidences of local failure and distant metastases did not differ significantly between the consolidation and the chemo-radiotherapy group; 35.0% vs 31.9% respectively, (relative risk [RR] =1.08, 95% CI 0.70 - 1.23), p=0.59 and 35.5% vs. 33.3%, (RR=1.11, 95% CI 0.67 - 1.21), p=0.49, respectively. The rate of late complications was 21.5% vs 21.2% respectively, p=0.58 [112]

The question arising from the Polish trial was whether three cycles of chemotherapy were enough to change the long-term clinical outcome. Garcia-Aguilar et al. [113], in a non-randomised phase 2 study, looked at consolidation TNT in four groups of patients. The four groups were selected depending on the number of cycles of their doublet oxaliplatin regime. The results showed that the group (group 4) with the highest number of consolidation chemotherapy cycles (i.e. six cycles) had a significantly higher pCR rate of 38% (N=65, p=0·0036). Other groups, group 3 (4 cycles, N=67), had a pCR rate of 30%, group 2 (2 cycles, N=67) had a pCR rate of 25%, and group 1 without consolidation chemotherapy had a pCR of 18% (N=60). These findings indicate that the number of cycles of chemotherapy does matter, and maybe the Polish trial did not give enough chemotherapy to change long term outcomes.

The PRODIGE trial[114] randomised 461 patients with locally advanced rectal adenocarcinoma to two arms; 'Arm A' patients received standard neo-adjuvant chemoradiotherapy (50 Gy in 25 fractions + capecitabine) then surgery and adjuvant chemotherapy for six months. 'Arm B' patients received six cycles of mFOLFIRINOX (oxaliplatin 85 mg/m², leucovorin 400 mg/m², irinotecan 180 mg/m² D1, and 5-FU 2.4 g/m² over 46 h) every 14 days, followed by chemoradiotherapy then surgery and three months of adjuvant chemotherapy. Depending on the centre's choice, adjuvant chemotherapy consisted of mFOLFOX6 or capecitabine. The 3 years disease-free survival was significantly increased in arm B, 75.7% (CI: 69.4-80.8) (HR 0.69, p=0.034) vs 68.5% (CI: 61.9-74.2) in arm A. A higher pCR rate

was also achieved in arm B; 27.5% vs 11.7 (p<0.001). Three-year metastatic free survival was also significantly higher in arm B, 78.8% (HR 0.64, CI 0.44-0.93, p<0.02) compared to arm A, 71.7%. Compliance with chemoradiotherapy and adjuvant chemotherapy was not hampered by neo-adjuvant chemotherapy. Surgical morbidity was similar between the two arms.[114]

The RAPIDO[115] trial randomised 920 rectal cancer patients with MRI diagnosed either T4a/b, extramural vascular invasion, N2, involved mesorectal fascia or enlarged lateral lymph nodes to two arms of treatment; short course (5x5 Gy) followed by six cycles of capecitabine plus oxaliplatin (CAPOX) chemotherapy or nine cycles of FOLFOX followed by total mesorectal excision (TME) (experimental arm) or, capecitabine-based chemoradiotherapy (25-28 fractions x 2.0-1.8 Gy fractions) followed by TME and optional, postoperative eight cycles of CAPOX or twelve cycles of FOLFOX4 (standard arm). Higher pCR was achieved in the experimental arm; 27.7% vs 13.8% (p< 0.001). Probability at three years of distant metastasis and loco-regional failure were, 19.8% vs 26.6% (p = 0.004)and 8.7% vs 6.0% (p = 0.10) in the experimental and standard arms respectively[115]

Looking at these three trials presented at ASCO 2020 (OPRA, PRODIGE and RAPIDO), the primary objective of the OPRA trial is to answer the question of induction chemotherapy vs consolidation chemotherapy in total neo-adjuvant therapy. The proponents of induction chemotherapy will argue that the priority should be addressing micro-metastatic disease first with induction chemotherapy than a local disease with chemo-radiotherapy, especially in advanced disease. There were also concerns that more extended periods between radiotherapy in consolidation chemotherapy could mean higher surgical morbidity. Although these are valid points, the balance of evidence favours consolidation chemotherapy over induction chemotherapy. The prolonged time between radiotherapy and surgery has not increased surgical morbidity but somewhat produced higher pCR rates. Long term data will be needed to see if the more extended period between radiotherapy and surgery produces increased late toxicity and if the higher pCR rate returns significantly more sustained long term clinical outcomes.

The PRODIGE trial looked into a more dense chemotherapy regime of oxaliplatin, irinotecan and 5fu together in total neo-adjuvant therapy. The results showed a higher pCR rate of 27.5% with this regimen ($p<0.001$)[114]. This result means higher organ preservation in this group of patients and possibly better long term outcomes. The slight issue with this regime will be patients' tolerance of triplet chemotherapy which may not be evident in carefully selected patients that we see in clinical trials but will become an issue in real-world oncology practice. Also, patients with advanced disease are at risk of recurrent disease and suppose these patients recur and require systemic therapy. In that case, it is uncertain if exposing them upfront with both their first and second-line treatment options will affect the long term control of their metastatic disease. Adopting this treatment strategy will require compelling long term clinical outcome data.

The most interesting of the three trials is the RAPIDO trial, which recruited many patients (N=920). These patients are also of high-risk T4a/b or N2 disease, extramural vascular invasion, involved mesorectal fascia, or enlarged lateral lymph nodes. Nevertheless, they achieved a pCR rate of nearly 28% with the experimental arm of short-course radiotherapy followed by consolidation chemotherapy. The RAPIDO provided evidence that short-course radiotherapy with consolidation chemotherapy provided an even better downstaging than the long course. As this trial did not compare long course chemoradiotherapy with consolidation vs short- course with consolidation, it failed to fully answer the question of the optimum radiotherapy regime in total neo-adjuvant therapy. Although short-course radiotherapy may offer the advantage of improving the compliance with consolidation chemotherapy as the period of radiotherapy treatment is shortened, there is always that theoretical risk of increasing acute toxicity with a higher dose per fraction of radiotherapy, which may invariably become higher long term toxicity. However, the Polish trial[112] mentioned above did not show any difference in the long term toxicity between short and long course radiotherapy.

Total neo-adjuvant therapy offers high pCR rates, which regularly means higher rates of organ preservation in rectal cancer through the 'watch and wait' surveillance. With an increased need to

deliver this in more patients, total neo-adjuvant treatment could become the standard of care for locally advanced rectal cancer, mainly if the long term data in a few years retains the advantage seen in recent studies. The Polish trial[112] did not show significant outcome benefits in the long term data. Still, the more recent trials presented in 2020 (RAPIDO/OPAR/PRODIGE) gave at least double the chemotherapy cycles than the Polish trial. With the evidence from Garcia-Aguilar et al.[113] that the number of cycles matters, there is a reason to be optimistic. The optimum systemic therapy in total neo-adjuvant treatment will continue to evolve. There is also a need to run a randomised trial comparing long course consolidation with short course consolidation chemotherapy to answer the question of the optimum radiotherapy regime.

1.10: Biomarkers and radiomics

In oncological treatment, one of our many problems has been that cancers behave differently in different patients, and their response to treatment varies. This problem has given birth to the concept of 'biomarkers'. Biomarkers are traits in patients that determine the biological differences in cancer cells which predicts their growth pathway and how they behave and, therefore, how they respond to treatment. This concept is the most important aspect of personalised medicine, which in simple terms is giving patients the treatment most beneficial in treating their cancer based on their tumour biomarkers. The way we determine these biomarkers has been generally through biopsies. Biopsies are samples of tissue taken from the site of the tumours usually used to diagnose cancer. These specimens are sent to pathology laboratories for pathological and genetic testing to determine biomarkers predictive for targeted treatment. This method of predicting treatment response has been used in selecting treatment in oncology for many years. For example, we use KRAS status in metastatic colo-rectal cancer to determine if a patient will be suitable for EGFR targeted therapy and MSI status in determining if immunotherapy will be appropriate in patients. The use of biopsy in making these important treatment decisions comes with some notable drawbacks. The first disadvantage of

biopsies is that it remains an invasive procedure; patients often require anaesthetics for this procedure. This carries both mortality and morbidity risks, and, as such, assessing via biopsy route in multiple time points during the patient's treatment journey becomes difficult. Assessment at multiple times has become necessary as we know that one of the main hallmarks of a malignant cell is its ability to adapt and change over time using different mechanisms to survive. The other issue with biopsy-generated predictive models is the heterogeneity of cancer tumours. Therefore a biopsy from one point cannot claim to be a complete representation of the whole tumour. A study examining intra-tumour heterogeneity in renal cell primary and metastatic lesions from the same primary[116] showed that a single tumour-biopsy sample reveals only a small number of genetic aberrations (including mutations, allelic imbalance, and ploidy) that make up an entire tumour. This supports the evidence that the tumour environment contains differential mutations and genes, so targeted therapies sometimes only kill some part of the tumour, leaving the rest to proliferate, which is one of the consequences of relying on tissue biopsy for our predictive model. This issue has led to investigations using other predictive models to resolve this problem.

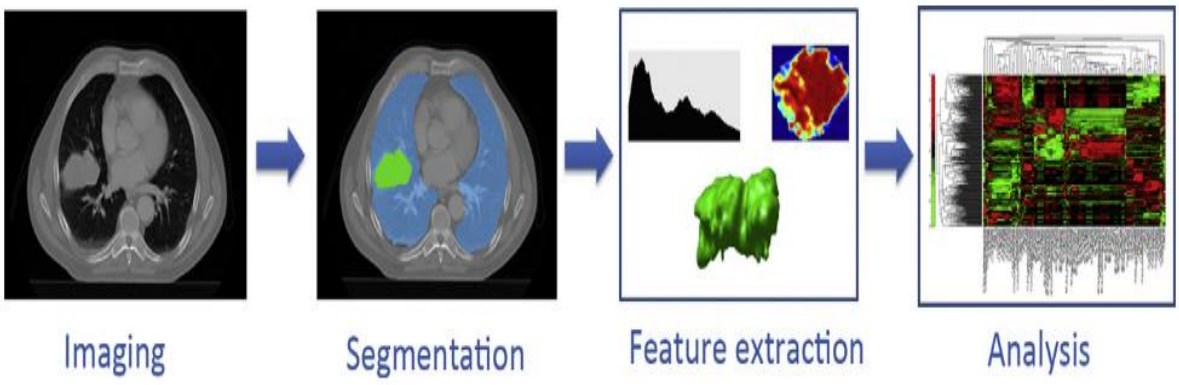
Image-based biomarkers seem to be a good candidate for improving our predictive models. They are non-invasive, representative of the whole tumour, and repeated at different time points. There is growing evidence on the addition of doublet chemotherapy in neoadjuvant treatment to augment radiotherapy in rectal cancer. Therefore, there is a research interest in predicting patients who will respond excellently to standard chemo-radiotherapy and do not need the addition of doublet chemotherapy to improve their outcome. Patients who are predicted to have an excellent response to radiotherapy can also qualify for dose de-escalation in other to reduce their toxicity.

Radiomics is the study of the extraction of quantitative image features from routine images such as CT, PETCT or MR images using a statistical model; these features can be used to predict treatment responses, prognosis and clinical outcomes. There are three main groups of features extracted; first-order radiomics, which are histogram-based features that take into account the pixel values

individually without considering their spatial relationship. The second order is textural features that look at two pixels' spatial relationships. Higher-order radiomics takes into account the relationship between 3 or more pixels. Radiomics workflow involves image acquisition, image normalisation, tumour segmentation, feature extraction, predictive modelling and model validation[116]. We will discuss these steps below in more details.

Figure 1.5: Radiomics Work Flow.

The figure below demonstrates the standard radiomics workflow



Radiomics workflow (Radiomics: Extracting more information from medical images using advanced feature analysis. Lambin et al,European Journal of Cancer 2012: 48;441-446.

1.10.1: Image acquisition/Normalisation

Image acquisition involves acquiring the images of the scans and the sequences required for the imaging study. Due to varying radiology protocols in different parts of the country, there is a lack of normalisation of acquired images that impact the image features extracted. Normalizing the images acquired in the image database remains essential to eliminate this issue. A large patient cohort is necessary to develop and validate high-quality radiomics work; therefore, acquiring images from one scanner using the same protocol for a large cohort of patients is not feasible. As variations in the imaging protocol are common in our radiology departments and are likely to cause differences in the textural features unrelated to biological changes and consequently impair the algorithm's accuracy, normalisation protocols will be needed to ensure consistency in feature extraction[117][118]. The process of normalisation is done either pre-extraction of features or standardisation of the features post-extraction. Normalisation is particularly important in MR images as different intensities are dependent on scanner, time points and protocols.

The three primary forms of intensity normalisation are (a) intensity normalisation of the region of interest of all images in a database to a set value of intensity, (b) adjusting each image to the mean intensity of the whole database and (c) Matching the images to the same histogram intensity based on a reference image[119]. Mean and histogram normalisation has been shown to increase radiomics features reproducibility in a recent work looking at normalisation effects on the reproducibility of radiomics features relating to T2WI of the pelvis [119]. The histogram matching intensity normalisation was proposed by Nyul et al. [120]. This matches the intensity value at each reference point of the intensity histogram to the same intensity at the same reference points on the reference image, thereby normalising the dataset to a uniform intensity.

1.10.2: Tumour Segmentation

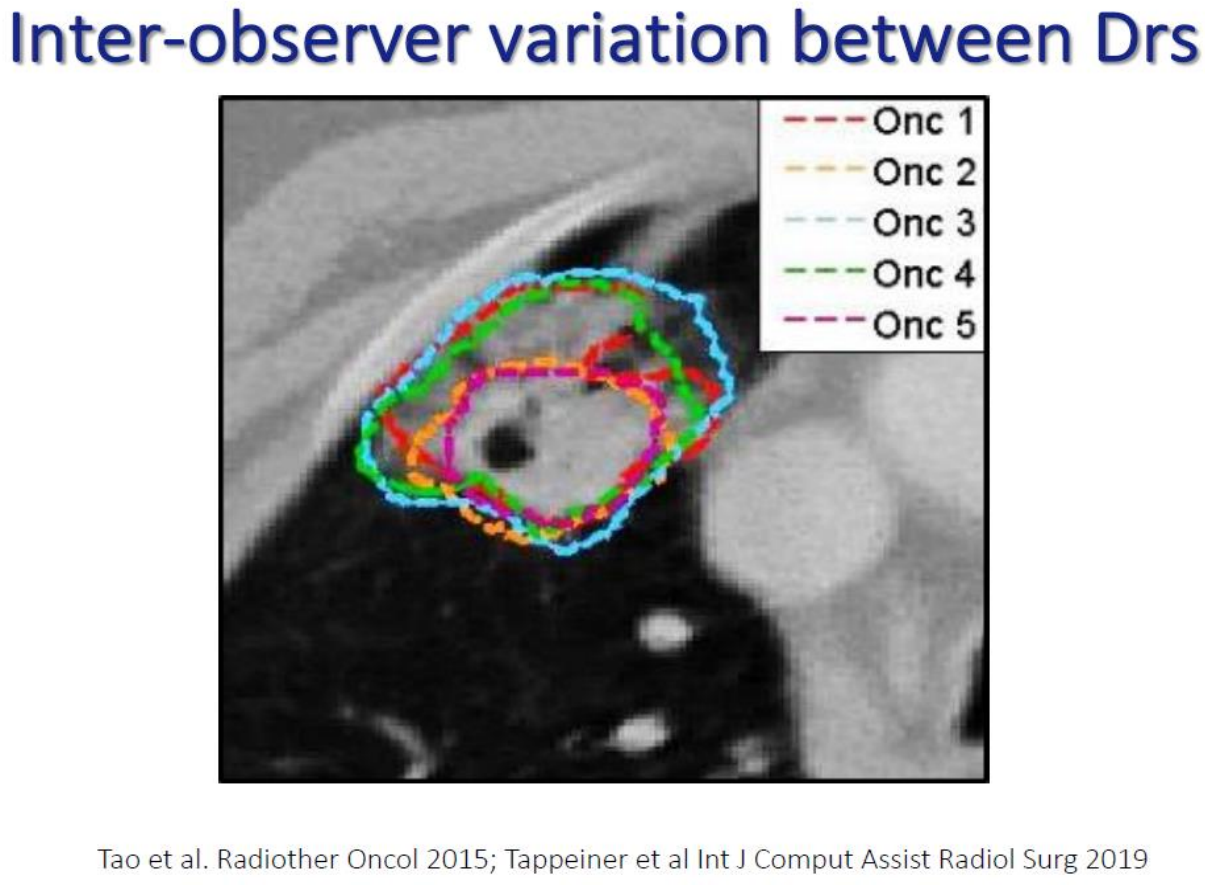
Tumour segmentation involves contouring or making out the region of interest (ROI) which in most cases in cancer-related radiomics, is the GTV (gross tumour volume). The GTV is the area covering the detectable volume of the tumour, excluding the surrounding normal tissue. Radiomics feature calculations are derived from the GTV. This makes segmentation a very essential part of the imaging study. Segmentation can be done either manually, semi-automatic or automatic. As with all manual contouring in oncology, oncologists have significant variations on what they contour as the GTV. This brings about inter and intra observer variation, affecting the radiomics features generated [121]. Manual delineation is also time-consuming and labour intensive in large studies. Automated contouring systems have been used to reduce the variations in contouring and save time spent in segmentation [121]. A fully automated segmentation can remove the intra and inter observation variations that affect radiomics work; however, the intricacies of MR images, especially in the pelvis and the differences in intensity of MR images, makes the development and accuracy of these systems difficult. In recent times, deep learning has been shown to offer an improved automated system for MR contouring through its pattern recognition algorithms[122].

Semi-automatic systems allow the input of clinicians to achieve a satisfactory segmentation in a reasonable time. The 3D-slicer is a semi-automatic volumetric segmentation that combines the delineations done into a uniform contour. Radiomics features extracted from 3D-Slicer segmentations had significantly higher reproducibility (avg. of two 3D-Slicer segmentation sets ICC = 0.85±0.15) as compared to the features extracted from the manual segmentations (ICC = 0.77±0.17)(p = 0.0009)[123]. Automated segmentation has generally been shown to reduce the contouring uncertainties of manual contouring and produce more consistent radiomics features[123]. It is essential to note that higher reproducibility does not always mean higher or improved accuracy. The aim with semi-automated and automated systems should always be accuracy before consistency as a system could be consistently wrong. The most common method of segmentation remains manual

segmentation. This could be improved by involving different clinicians to produce an average contour which will be a good representation of the tumour if the clinicians are all experts. Other ways of mitigating these inconsistences in segmentation are generating sets of features based on different contours of the same tumour and eliminating those features with poor consistence in the cohort by using the interclass correlation coefficient (ICC) and distance to point measurements. This is likely to produce stable features if trained clinicians do the contours.

Figure 1.6: Inter observer variations in tumour segmentation.

The figure shows various contours by different clinicians of the same lung lesion.



Tao et al. Radiother Oncol 2015; Tappeiner et al Int J Comput Assist Radiol Surg 2019

1.10.3: Feature extraction and selection

Feature extraction is done by uploading segmented image files to specific software that extracts quantitative parameters from the segmented area of interest. A high number of features is extracted and categorised as morphological or textural. Morphological features relate to the shape, size and location of the segmented area of interest. Textural features evaluate the distribution and pattern of the pixels or voxels[118]. These are then assessed in three different ways; (a) statistically, which looks at the distribution of the grayscale values. (b) Model-based, which evaluates the irregularity of the area and (c) transform-based, which transforms spatial information into frequency [124]. The number of stages required to reach a relationship is defined as the 'order' in the statistical method.

Zero-order features are features obtained directly from one or more sequences of the image dataset. They are a largely geometric description of the ROI such as shape, diameter, volume, surface area, sphericity and compactness. They are collectively described as shape features[125].

First-order features are based on the intensity histogram directly related to a pixel value without considering the relationship with the pixel values. First-order parameters are mean, median, voxel intensity, standard deviation, Skewness, Kurtosis, entropy and uniformity[125],

Second-order features consider the spatial relationship between 2 pixels [118]. The second-order statistical features are classified into 3 classes (a) grey level co- occurrence matrix (GLCM), (b) run-length matrix (RLM), and (c) grey level size zone matrix (GLSZM)[118][125]. GLCM takes into account the frequency of specific gray values along a distance or direction, RLM takes into account the length of consecutive pixels or voxels with the same grey values in a specific direction, and GLSZM takes into account the length of consecutive pixels or voxels with the same grey values in all directions[118].

Higher-order statistical features are achieved by applying filters or mathematical transformations of the images. Its analysis considers the neighbourhood gray difference matrices and the relationship

between 3 or more pixels[118]. Laplacian transforms of Gaussian-filtered images, fractal analysis, wavelet transformation, and Minkowski functionals are all part of this order[125].

Due to the use of different filers available in routinely used radiomics extraction software, huge numbers of calculated radiomics features can be extracted. These features could range into thousands per patient, and against a much lower number of patients in a given study can lead to overfitting. To remove unstable features, feature selection is usually needed after extraction to remove inconsistent features and increase the reliability and reproducibility of radiomics work. Inter-observer variation in segmentation is an error that could affect the repeatability and reproducibility of radiomics work. To mitigate this, radiomics work embarks on an inter-observer agreement together with a feature agreement. First, to demonstrate inter-observer agreement, the contours independently done by the observers are compared using overlapping metrics such as Dice and Jaccard coefficients, surface distance measures such as distance to an agreement, Hausdorff distance, mean, median and standard deviation between surfaces and volume measurements with ICC and volume similarity calculations[126]. After ensuring inter-observer agreement, features will be extracted from the sets of images independently segmented by the observers; given an expected level of agreement between these segmentations, the features with high consistence between the observers are considered stable features. The consistency between the features among the observers is evaluated using the intra-class correlation coefficient (ICC). The features that show poor correlation are unstable and removed from the analysis. This is generally known as feature selection. Feature selection ensures the repeatability of radiomics work, which is essential if outcomes of these work at used clinically in the management of patients.

1.10.4: Feature reduction, modelling and validation

After the extraction and selection of the features, the next step is to make sense of the features collected. This is done by using a suitable statistical model to answer the question. In most cases, this will start by removing features which correlate strongly with each other and will not be part of the

model. This is generally known as dimensionality reduction/feature reduction. Dimensionality reduction is made either by using a supervised or unsupervised approach. The unsupervised approach is used in dimensionality reduction of radiomics work as its more robust against over-fitting than supervised.[127] The most commonly used unsupervised approaches are the principal component analysis (PCA) and cluster analysis[125][128]. PCA works by generating a reduced set of uncorrelated variables from a large set of variables, removing variables that do not explain the total variation in the data set[125]. The principal components generated are new variables created as direct combinations or mixtures of the initial variables. These combinations are designed to make the new variables (i.e. Principal components) uncorrelated. Most of the information in the original variables are packed into the first components, then the maximum remaining information in the second then third etc. Cluster analysis aims to group similar features in a cluster and remove features with high correlation. After the unsupervised approach, a supervised approach is used to build a mathematic model to predict the outcome or the clinical question needed to be answered from the study. The supervised approach used in radiomics work is usually in the form of univariate and multivariate analysis. The type of this analysis used is generally very dependent on the purpose of the study.

Validation is an essential aspect of radiomics work. With validation comes reproducibility. Validation looks at whether the observed relationship is still valid in similar cohorts. Validation can be done using internal, external and prospective cohorts. The most robust way to validate a model will be to test the predictive model in a prospective cohort. An external independent cohort is the next best form of validation and can show the reproducibility of a radiomics model. Non-reproducible work cannot successfully be used in clinical practice.

Another important standardisation in radiomics is ensuring the features produced are similar using the various software available for feature extraction. The image biomarker standardisation initiative (IBSI)[129] is an independent collaboration to standardise the image biomarker work in radiomics. The main aim of the IBSI is to improve the validation and reproducibility of radiomics features generated

from the radiomics software, both commercial and open-source, so that work on radiomics can be reproduced provided the software used is IBSI compliant. It is worth noting that not all radiomics extracting software are IBSI compliant. Even among the IBSI complaint software, some significant variations in the values of the features produced affect studies' reliability [130].

Another important aspect of radiomics work is how the studies are reported. Predictive models are essential in health care to assess the likelihood of an outcome. Many studies on model prediction have been poorly reported leading to the development of the TRIPOD statement[131]. This statement is a 22 question item essential in transparent reporting of the predictive model study. This forms a checklist of items needed to report a predictive model accurately and has been well adopted in academic articles.

1.10.5: Limitations of Clinical use of radiomics features

The clinical use of radiomics faces numerous limitations hindering its use in a routine standard of care practice. The first issue is the differences in the scanning protocols between and even within centres. Standardisation of protocols used in obtaining the images will need to be done satisfactorily for clinically relevant radiomics work to be achieved. This applies to all image modalities used. This will involve communication and agreement between radiology units and can prove difficult outside a trial. Although the differences in the segmentation of images between individuals will always exist, the wider use of semi-automatic image segmentation will be needed for standardisation of segmentation to reduce intra observer variation in defining the ROI. The methodology of features extraction will also need to be standardised in published data and does require agreement in order to fully validate other studies done, which will allow more consensus in the evidence produced. Lastly, the features extracted are numerous, with very few patients involved in most studies making it more or less like a 'fishing expedition' and reducing the validity of the evidence produced. The possible way to improve this is by all radiomics studies clearly stating the features and their significance so that feature work

will test the validity of these features rather than generating more features using a non-standardised methodology and scanning protocol. Another way is to use statistical analyses, which are robust against over-fitting; in doing so, the results will be more reliable in a clinical setting.

The key to the clinical use of radiomics is collaboration to produce a large cohort of patients and validate produced features. This will test the reproducibility of work and will make the work useable in routine clinical practice.

1.10.6: Predicting complete response in rectal cancer with radiomics

Image base prediction of clinical outcomes is on the rise with the growing use of computer-based images in diagnosis and treatment assessment of oncology patients. Studies using radiomics in rectal cancer cohort have looked at the prediction of pCR in patients' post CRT as a way of determining whether patients can safely go into 'watch and wait' or surgery. Although this remains a worthwhile clinical question, endoscopic and digital rectal examination has been shown to effectively assess patients before going into 'watch and wait' and are very reliable even though they are more invasive[132]. Although the scope of DRE and endoscopy is also only limited to a luminal assessment, an MR image does look beyond this. Using radiomics features to replace the endoscopic assessment and MRI scans to replace post-treatment assessment in this scenario will be a tall order clinically. All surveillance protocols used in 'Watch and Wait' have adopted endoscopic follow-up with imaging, and I don't foresee radiomics features replacing endoscopic examination anytime soon.

However, the benefits of radiomics are in predicting patients who are likely to achieve excellent clinical outcomes before the treatment is started, which is making use of diagnostic pre-treatment images to pre-select patients for organ preservation treatment plan, which is the future of personalised medicine delivery in rectal cancer.

Horvat et al. [133] looked at retrospective MR images of 114 patients with CRT for rectal cancer.18% (n=21) had pathological compete response. The segmentation was done manually using post-treatment T2 weighted MR images. 14/34 features were significantly different between pCR and pPR (pathological partial response). The random forest classifier used in this study achieved an area under the curve of 93% (95% confidence interval [CI]: 0.87, 0.97) for differentiating pCR from pPR with a sensitivity of 100% (95% CI: 0.84, 1), specificity of 91% (95% CI: 0.84, 0.96), PPV of 72% (95% CI: 0.53, 0.87), and NPV of 100% (95% CI: 0.96, 1)[133] The clinical question in this study was to use radiomics features to differentiate between pathologic complete and pathologic partial response in patients with locally advanced rectal cancer after chemotherapy–radiation therapy. The main limitations of this study are clear, the number of patients who have had pCR is very low, just 21 patients, which significantly affects the robustness of this predictive model. In addition, no validation cohort is used or proposed to ensure that this work is reproducible. The processes and methods used in this study are well designed. Still, given that the images were from different scanners, there was no mention of any intensity normalisation process used to standardise the images.

Cusumano et al. in 2018 evaluated retrospectively pre-treatment MR scans of 198 patients who had CRT for locally advanced rectal cancer. The patients were treated in either of the two participating centres at Rome or Maastricht. The images used were T2 weighted MR images with manual segmentation. Their results showed that the fractal parameters relating to tumour sub-population have the highest performance in predicting pCR. Fractal features are parameters quantifying tumour heterogeneity. Their predictive model had an area under the curve (AUC) equal to 0.77 ± 0.07. The validation set confirmed the model reliability (AUC = 0.79 ± 0.09). Pathological complete response is the whole database was 54 (47 patients of Rome (pCR occurrence rate equal to 27%) and in 7 patients of Maastricht (pCR occurrence rate equal to 28%)[134]. This model relies on the theory that a sub-region of the ROI with a normalised intensity higher than 40% determines response to CRT. The features with the highest significance were entropy (p = 0.048) and skewness (p = 0.006). Invariably,

the overall tumour aggressiveness and response are determined by the characteristics of a sub-region in the tumour. This study also had about 20% pCR in the whole dataset. The two centres where the patients were recruited had differences in the radiotherapy dose. Rome has more than 80% of patients who had 55gy treatment. Although this will not affect the radiomics in a pre-treatment MRI but could affect the clinical outcome as we know that higher doses could increase the number of patients who have cCR. The author did say there was no significant relationship between radiotherapy dose and pCR probability here, but no evidence was provided.

Cui et al. did a retrospective study of 186 patients with pre-operative MR using T2W1, T1W1, ADC map sequences in patients with LARC (locally advanced rectal cancer) who had CRT. The segmentation was done manually, and the patient group was split into 131 training datasets and 55 validation datasets by random selection. 31 out of 186 (16.7%) patients achieved pCR. In total, 1,188 imaging features were extracted from the three examined modalities (T2-w, cT1-w, ADC) for each patient. Twelve features were selected into the radiomics score calculation formula due to their significance; this includes 5, 4, and 3 features derived from ADC, T2-w, and cT1-w images, respectively. The radiomics signature from the set yielded an AUC of 0.940 (95%CI, 0.892–0.987) and 0.944 (95% CI, 0.880–1) in the training and validation dataset, respectively, suggesting that the radiomics signatures from joint T2-w, ADC and cT1-w images achieved better predictive efficacy than the radiomics signature from any of them alone [135]. The main limitation of this study is the sample size of the patient who had pCR, which is low (n=31). The idea of splitting the data into a training and validation cohort is good, but this leaves a relatively small validation size in a retrospective study and will require a further valuation, ideally done prospectively. The paper also did not specify the various contributions of the different features to their predictive model and also either did not normalise the images or failure to mention the type of normalisation used because it is unlikely that all 186 patients were scanned on the same scanner using the same protocol and even so intensity normalisation is needed for MR scans used in a meaningful radiomics work.

Liu et al. studied 222 patients (152 in the primary cohort and 70 in the validation cohort) with LARC who received chemoradiotherapy before surgery. Pre and post-T2WI and DWI were used in this study. The segmentation was done manually. There were no significant differences between the two cohorts regarding pCR prevalence (17.11% and 17.14% in the primary and validation cohorts, respectively, P= 0.567). A total of 2,252 radiomics features per patient were calculated. It was reduced to 30 significant features that discriminated between those that had pCR and those that did not. The results show significant differences in radiomics scores between pCR and non-pCR patients in the primary cohort ($P < 0.01$), the same was true in the validation cohort ($P < 0.01$). The signatures achieved a PPV of 86.96% (95% CI, 84.84–90.40%) in the primary cohort and 90.00% (95% CI, 89.60–99.40%) in the validation cohort [136]. Like the previous study, this cohort of patients has a relatively low sample size of patients with pCR (~17%); due to fibrosis and tumour changes post-radiation treatment, delineation of post-treatment images will have more uncertainties in the contouring of the ROI, which will affect the features extracted from the post-treatment scan. It is also expected that the features generated in post-treatment MR images will be different due to the effects of radiotherapy and shrinkage of tumour, necrosis and fibrosis. Apart from this, we are almost certain on what the response to CRT is in a post-treatment MR at eight weeks, which defects its use for predicting pCR or cCR as the scan is already very informative.

In a smaller study of 15 patients using pre and post MR T2WI, Six patients showed pathological complete response (pCR), and four patients, partial response (PR). Five patients were classified as non-responders (NRs). Pre-treatment medium texture-scale quantified as kurtosis (a measure of the heterogeneity of tumour) was significantly lower in the pCR subgroup in comparison with the PR+NR subgroup (P= 0.01)[137]. This study also showed the changes in features generated pre and post-treatment, confirming that radiotherapy changes the features generated in post-treatment images. The results also show that patients with partial and no response have a higher kurtosis than pCR patients. These findings align with the observation made in lung cancer that tumours with higher

aggressiveness and poorer outcome have higher heterogeneity[138]. Heterogeneous texture features showing high entropy and low uniformity were associated with poorer overall survival in the oesophagus and squamous cell carcinoma of the head and neck.[139][140]

Many studies have looked at MR radiomics to predict complete response in rectal cancer but what has been lacking in the literature is a 1:1 case-control study between those that achieved complete response and those that didn't. All studies so far have used a database with proportionally fewer patients with complete response than those without complete response making their results prone to over-fitting the performance of the radiomics features used in the models. There is also a need to have a larger cohort of patients currently available in the literature to enhance further the reliability of the work produced for a clinically relevant result. A clinical predictive model, in addition, needs to recruit the group of patients who are the target group for the organ preservation treatment plan in rectal cancer treatment. All studies on this subject have attempted to predict pCR from a locally advanced rectal cancer patients database. As pCR is a surgical outcome, only achieved after patients have had surgery, it does, however, make sense that studies that predict patients for organ preservation recruit the right target patients in this setting, which are patients with complete clinical response (cCR) rather than those with a complete pathological response (pCR). Our work in the prediction of cCR using radiomics variables aims to bridge this gap in the literature to fully assess the benefits of using radiomics features in predicting complete response.

1.11.0 Clinical prediction of complete response

Table 1.7: Clinical variables associated with pCR in literature

Clinical factors	Association with pCR
CEA	Low pre-treatment CEA levels[141][142][143][144][145]
T and N stage	Lower clinical T and N stage[146]
Circumferential extent	Smaller circumferential tumour extent[145] [147]
Tumour size	Lower tumour size[148][149]
Nodal stage	Low nodal stage[148]
Time to surgery	Delaying surgery by more than eight weeks post chemoradiotherapy[146][78]
Tumour grade	A lower tumour grade[146]
Distance to the anal verge	A shorter distance from the anal verge[147][149]
Radiotherapy dose	An increase in radiotherapy dose[150][151]
Haemoglobin level	Anaemia [152][153] is negatively associated with pCR
Neutrophil to Lymphocyte ratio (NLR)	The higher the NLR, the lower the rate of pCR [154][155]
Neutrophil to albumin ratio (NAR)	neutrophil to albumin ratio[156] shows a negative relationship with pCR
Platelet to Lymphocyte ratio (PLR)	A negative relationship with pCR[155]
Lymphocyte	Lymphopenia reduces pCR rate[157]
prognostic nutritional index (PNI)	Higher PNI, higher likelihood of pCR[158]

This table shows the clinical factors associated with complete response in rectal cancer.

Various clinical factors have been associated with complete response in rectal cancer, but none have been predictive. One of the most studied clinical variables is the carcinoembryonic antigen (CEA) which has shown a negative association with complete response. However, the main difficulty with CEA use is that many rectal cancers do not excrete CEA. Elevated CEA is found in about 47% of colorectal cancers [159]. It has been reported that CEA ≥ 5 µg/L at diagnosis, tumour size ≥ 3 cm,

tumour distance from anal verge ≥ 3 cm, clinically node-positive disease are predictors of incomplete response [149] [160]. A study in Korea have shown that patients with elevated CEA (>5 ng/mL) at diagnosis have a poorer outcome in rectal cancer than those without(⩽5 ng/mL) even after control for other co-variables, such as stage of disease making elevated CEA a prognostic variable [161] Other clinical factors (see table 1.7) such as tumour size, TNM staging, distance to the anal verge, radiotherapy dose and tumour grade have shown some association to pCR and, as such, expected to show some association with cCR

Blood parameters such as haemoglobin have shown a positive association with radiotherapy outcomes likely due to the link between oxidation and increased radiotherapy effect[162]. A study looking at the influence of anaemia in tumour response in rectal cancer has shown a significantly better outcome in patients with pre-treatment Hb levels > 9 compared with those below 9 ($p < 0.001$) [152]. In addition, there were no differences in tumour response between the non-transfusion and transfusion groups of patients with Hb levels > or 9.0 g/dl suggesting a cut off Hb of 9 [152].

1.12.0 Aim of the thesis

With an increasing need for treatment selection in rectal cancer management, there is an equally vital need to predict which treatment is most suitable at every treatment stage of the patient's treatment journey. The better the predictability of clinical outcomes before patients are exposed to the side-effects of various treatment options, the better our overall management. In the last 50 years, solid tumours treatment has evolved from the days when we were solely focused on delivering effective therapy to now when we have to think about the long-term toxicities of the treatments we provide. Localised rectal cancer treatment has moved from a local recurrence rate of nearly 50% after surgery in the 1980s [51] to a recurrence rate of 5-6% in recent days [50]. More patients are now surviving longer with the long term effects of their treatment which was not the case two decades ago. With this comes a demand to limit the toxicity of rectal cancer treatments by selecting the treatment that

provides patients with the best overall clinical outcome. This will mean balancing overall survival (which essentially is determined by the effectiveness of treatments) and quality of life (which is largely dependent on the toxicity of treatment). We now have to ask patients where the balance lie in our joint decisions about their management plan. The reality of this is that treatment given in today's oncology world has to be more measured, focusing on limiting toxicity while still providing effective treatment. Only through this can we say that we are meeting the standard needed in delivering the best quality of oncological care in today's world.

Our focus on organ preservation is patient-driven; it is clear from our day-to-day care that many patients prefer this treatment plan. This is also clearly shown in patient-centred studies [99][100]**.** Most patients prefer the organ preservation treatment plan because of their dislike of a long-term stoma that affects their day-to-day lives. Stomas affect both the physical and the psychosocial wellbeing of our patients. They affect how patients see themselves and how they interact with others in their day to day living. It also comes with its morbidities to add to its psychosocial effects. Surgery also comes with some risks, with a 3% risk of perioperative mortality [2] and numerous long term risks; no wonder patients generally opt to reduce these risks by deferring or avoiding surgery altogether when possible.

Neo-adjuvant chemotherapy has provided alternative down-staging properties as radiotherapy[60][61]. Therefore, providing an alternative treatment to a patient who may be predicted not to be most suitable for neo-adjuvant radiotherapy. There is also increasing evidence of doublet chemotherapy in the neo-adjuvant rectal cancer treatment setting through the TNT trials. [114] [115] Doublet chemotherapy also carries its own toxicities. There is a need for treatment selection between patients that should have just the standard chemo-radiotherapy, neo-adjuvant chemotherapy alone or TNT. Since the addition of doublet chemotherapy to radiotherapy carries an additional toxicity risk, there is a case to be made that if a patient is predicted to have a complete response with standard chemo-radiotherapy, then they should have this treatment; otherwise, they could have TNT to improve their chances of organ preservation or in a case where radiotherapy is contra-indicated just neo-adjuvant doublet chemotherapy.

Thus if patients prefer the organ preservation treatment plan and we have alternative strategies that can increase the likelihood of organ preservation through treatment selection, one sees no clinical reasons not to encourage this form of treatment selection to increase the number of patients going into the organ preservation treatment pathway. Therefore, we must develop treatment selection in rectal cancer that can satisfactorily predict treatment outcomes before patients have treatment. It is also vital to learn more about the characteristics that could define their superior prognostic properties by studying this group of patients with complete responses. This could help understand how we can improve the outcome in other rectal cancer patients

This thesis aims to investigate the predictability of clinical complete response in rectal cancer by using radiomics and clinical variables. The work will use pre-treatment parameters to see if we can successfully predict complete response, and 1:1 matching with propensity score will be used to reduce selection bias. It will provide one of the most extensive studies of radiomics and clinical variables in patients with complete responses. The clinical implications of this study will be that if we could predict complete response pre-treatment, patients who are predicted to have a complete response with chemoradiotherapy will have this treatment; those not predicted to have this outcome would have an intensified neoadjuvant treatment plan. Studying the clinical variables linked with good clinical outcomes will also help our understanding of the complete response. It will aid in studying interventions that can help improve clinical outcomes in rectal cancer.

Chapter 2: Prediction of cCR using Radiomics features.

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Main author's contribution:

This project looks at the prediction of complete response using radiomics and clinical variables. For this work, I sort ethical approval to enable it. The recruitment of patients for this work was done by me with some help from Lee Malcomson, the data manager of the ONCORE database. I did all Contouring of patients, clinical data collection, planning and methodology. For feature selection and control of inter-observer variation, Dr Joe Mercer, Consultant radiologist, double-contoured 21 patients from the database. Image transfer was done with help from Dr Gareth Price. Dr Eliana Vasquez Osorio did the complex image pre-processing and feature extraction. A significant part of the advanced statistics work needed for this project was done and supervised by Dr Hitesh Mistry. I wrote up the manuscript.

2.1 Abstract

Objective - Patients with rectal cancer could go into the 'watch and wait' treatment plan if they achieve complete clinical response (cCR) post neoadjuvant chemoradiotherapy. This treatment plan provides the opportunity to avoid major surgery and stoma formation. To improve neoadjuvant treatment selection, we retrospectively investigated patients with cCR using their pre-treatment clinical and radiomics variables to formulate a predictive model for cCR.

Methods - Using the OnCoRe (The Rectal Cancer Oncological Complete Response Database) database, we recruited a matched case-control study of 304 patients (152 with cCR; 152 without cCR, See figure

2.1 page 80) deriving training (N=200) and validation (N=104) sets. We collected pre-treatment MR images, demographics (age, gender, T-stage, N-stage tumour diameter) and blood parameters (haemoglobin, neutrophil, lymphocyte, alkaline phosphate and albumin). We segmented the gross tumour volume on T2W MR Images and extracted 1430 stable radiomics features per patient. We used principal component analysis to reduce dimensionality. The ROC AUC was used to evaluate the discriminative power of the model achieved.

Results - Using Logistic regression analysis, PCA-derived combined model (radiomics plus clinical variables) gave a ROC AUC of 0.76 (95% CI: 0.69-0.83) in the training set and 0.68 (95% CI 0.57-0.79) in the validation sets. The clinical only model achieved an AUC of 0.73 (95% CI 0.66-0.80) and 0.62 (95% CI 0.51-0.74) in the training and validation set, respectively. The radiomics model had an AUC of 0.68 (95% CI 0.61-0.75) and 0.66 (95% CI 0.56-0.77) in the training and validation sets.

Conclusion- The predictive characteristics of radiomics can be improved with clinical variables. However, their predictive characteristics remain modest, and there is a need to explore other approaches to predict complete response.

2.2: Introduction

For patients with locally advanced rectal cancer, the standard of care treatment is neo-adjuvant chemoradiotherapy followed by resective surgery either as a total mesorectal excision surgery or abdominoperineal resection [7]. Surgical resection is associated with considerable short and long-term morbidity, up to 3% risk of perioperative mortality and 40% risk of requiring a permanent stoma[2]. For two decades, pathological complete response (pCR) (the absence of microscopic disease post-resection) has been recognised in 15%-27% of patients who had resective surgery post-chemoradiotherapy[8]. Clinical complete response (cCR) is the absence of clinically and radiological detectable disease post-neo-adjuvant chemoradiotherapy and pre resective surgery. The

identification of cCR followed by a decision between the patient and oncologist to actively monitor or 'watch and wait (W&W)', pioneered by Habr-Gama et al.[10], has become a novel management strategy to reduce surgery-related morbidity, mortality and permanent stoma. Patients with cCR on watch and wait are carefully monitored on a surveillance follow-up plan, and surgery is only needed in the event of a disease re-growth. About 25-30% of patients on this surveillance treatment plan will require surgery within the first three years after neoadjuvant treatment, while the rest will be managed without the need for surgery[9][85]. The growing evidence of organ preservation in rectal cancer has shown this management plan to be valid and safe without any decline in clinical outcome[9][11][12][101]. Pooled analysis of trials showed that pCR is associated with a good prognosis and an indicator of a biologically favourable tumour [8]. Patients with cCR also have a comparable excellent long term outcome similar to those with pCR[9]. The clinical complete response has become a surrogate for the pathological complete response for selecting patients who may not require surgery. Therefore it remains essential to predict patients likely to follow this treatment plan from the onset of their diagnosis in the era of personalised medicine.

However, there is no robust predictor of either pCR or cCR before neo-adjuvant chemoradiotherapy. Ryan et al. reported a systematic review, including 85 studies, evaluating predictors (including biochemical, gene expression, mutational, and protein expression analyses) for pCR but concluded that there were 'no robust markers'[13]. Since the mid-2000s, magnetic resonance imaging (MRI) has been the standard of care for pre-treatment staging in rectal cancer patients[47]. MRI has shown good accuracy in determining the size and stage of the rectal tumours. It is also crucial in deciding invasion into the mesorectal fascia, which is essential in determining if neo-adjuvant treatment is required[48]. However, the review by Ryan et al. concluded that volumetric measurement on standard pre-treatment MRI had not been shown accurately to predict the response[13].

MRI radiomics is an alternative dimension beyond standard clinical MR imaging, predicting complete response. Radiomics is the mining and analysis of large amounts of advanced quantitative imaging

features from routinely performed radiological investigations [163][164], from which statistical modelling can be used to predict treatment outcomes. Thus, radiomics analysis can potentially be a biomarker in treatment selection. Many studies[134][135][165][166] have evaluated radiomics features' predictive abilities for pCR using pre-treatment MRI scans alone. Notably, the end-points of these studies were either pCR or pathological tumour regression, which requires surgical resection to assess. In addition, the number of complete response cases was relatively small, which may lead to over-fitting radiomics features' contributions in the prediction models. As an alternative, here, we performed a 1:1 matched case-control study enhancing the number of cCR cases to 152 to create a predictive model for cCR combining radiomics features, clinical and routinely collected laboratory parameters.

Currently, different treatment options are available in neo-adjuvant rectal cancer treatment with different toxicities and it is important to select the treatment with the best outcome for a patient. This model aims at facilitating treatment selection in rectal cancer. Patients predicted to have cCR with chemoradiotherapy could have this treatment while others could have an alternative neoadjuvant plan such as total neoadjuvant therapy or intensive doublet chemotherapy in other to improve their overall treatment outcome.

2.3 Methods

2.3.1 Study population and selection

All appropriate research governance and ethics approval was obtained before starting this study (IRAS 265989). All patients recruited received their treatment either at The Christie NHS Foundation Trust or the Lancashire University Teaching Hospital, both cancer centres in the north of England, UK. We recruited patients from the OnCoRe database (The Rectal Cancer Oncological Complete Response database). The OnCoRe is a research database of patients who achieved clinical complete response.

All patients selected had locally advanced rectal adenocarcinoma and underwent neo-adjuvant long course chemoradiotherapy between 2008 and 2019. Three hundred ninety-five patients (395) were selected consecutively from the database - 165 patients with cCR and 230 patients without cCR, non-clinical complete response (NcCR). From these, MR images for four patients with cCR were not available. Propensity score matching of 0.1 callipers based on T-stage, age, N-Stage and performance status was used to select 161 out of 230 patients without complete response. Propensity score matching was used in this study to ensure that patients in both cohorts have similar baseline characteristics. A propensity matching of 0.1 resulted in the lowest bias in a study comparing different propensity widths [167] After segmentation, 9 and 4 out of 161 patients belonging to the cCR and NcCR groups were removed due to either low-quality MR images or incomplete tumour coverage in the required MR sequence. After segmentation, a re-run of the propensity matching was done to select an equal number of patients in both cohorts. Finally, 152 patients from both groups (304 patients) were enrolled in this study. **(See Fig 2.1).** Patients were then split into two groups; a training group and a validation group at the ratio of 2:1. In line with prospective studies to limit the selection bias of retrospective studies, allocation to the training and validation cohort was done using the patient's date of diagnosis rather than random assignment. Thus, the first 100 patients of the cCR and NcCR group were placed in the training cohort and the last 52 patients of the cCR and NcCR group in the validation cohort.

Figure 2.1: Patient flow.

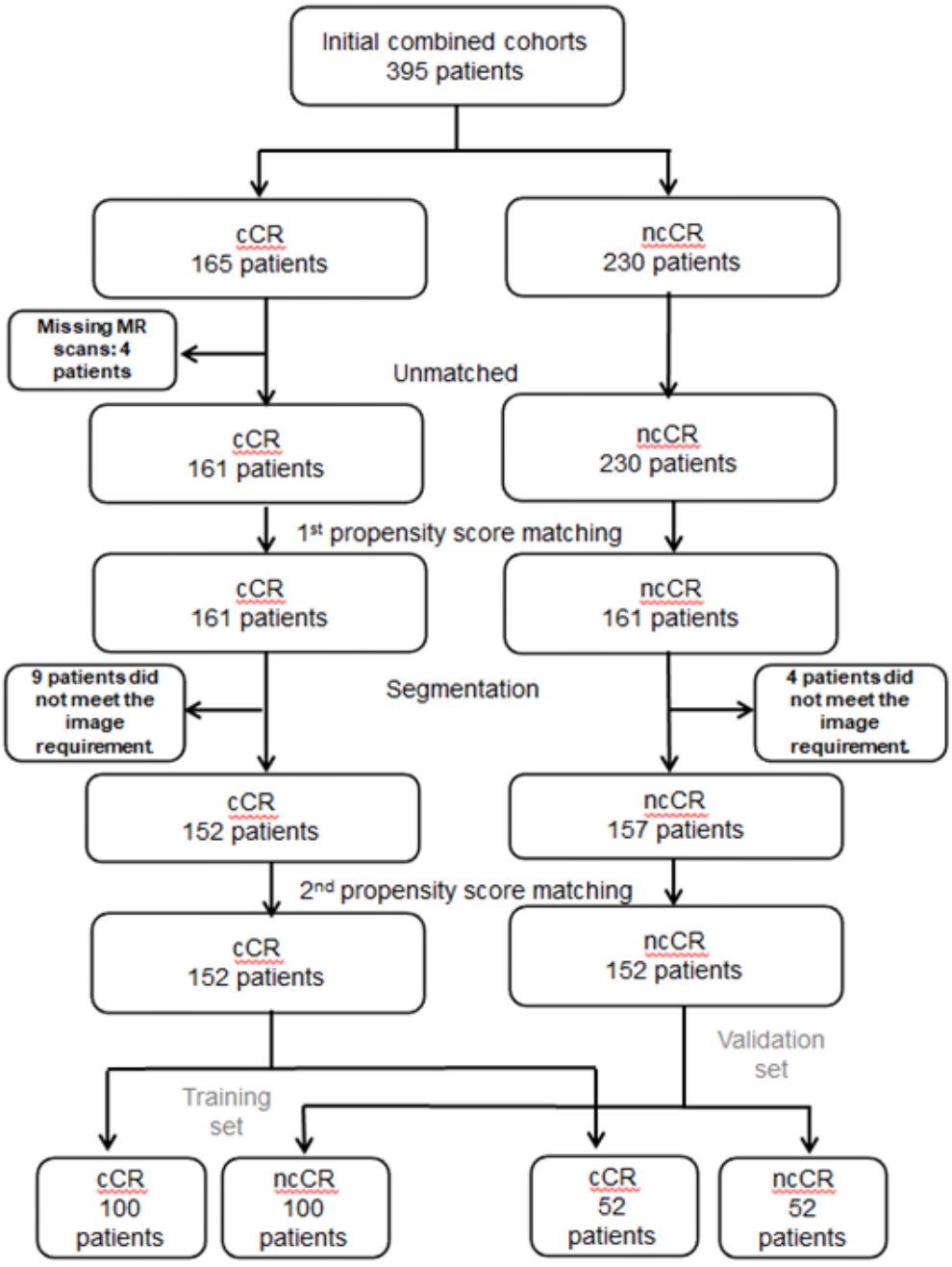


Figure 1: Flow diagram of matching and segmentation to final analysis. A total of 304 patients (matched 152 cCR and 152 ncCR) were included in the analysis from an initial group of 395.

2.3.2: Clinical variables

Clinical variables of each patient: demographics (age, gender, T-stage, N-stage tumour diameter) and blood parameters (haemoglobin, neutrophil, lymphocyte, alkaline phosphate and albumin) were obtained from the clinical records held at the treating institution.

2.3.3: Neo-adjuvant Chemo-radiotherapy treatment and assessment

All patients were aged 18 and over and underwent conformal planned pelvic radiotherapy, concurrent with capecitabine 825mg/m2 twice daily during treatment. They all received a prescribed dose of 45 Gy in 25 fractions of pelvic radiotherapy. They were all restaged with a pelvic MRI and CT imaging at 8-10 weeks post-radiotherapy. Patients that did not have a viable radiological tumour on this imaging were further investigated with a digital rectal examination (DRE) and colonoscopy. The multi-disciplinary team meeting independently verified the investigations. The absence of residue disease in all three investigatory modalities is defined as a clinical complete response. Patients with cCR were offered 'watch and wait' surveillance. The patient's population characteristics are summarised in Table 2.1

Table 2.1: Demographic table

Characteristics		cCR group (n=152)	NcCR group (n=152)
Age (Mean and range)		66.3 (41-86)	66.8 (31-89yrs)
Gender (Male: Female)		111 (73%) Male 41 (27%) Female	99 (65%) Male 53 (35%) Female
T staging	**T2**	31 (20%)	10 (7%)
	T3	108 (71%)	125 (82%)
	T4	13 (9%)	17 (11%)
N staging	**N0**	39 (26%)	35 (23%)
	N1	65 (43%)	66 (43%)
	N2	48 (31%)	47 (31%)
	N3	0	4 (3%)
Tumour diameter* (cm) **(Mean / range)**		4.8cm (2-10cm)	5.5cm (2-10cm)
Height above anal margin (cm)** **(Mean/range)**		5.9cm (0-15cm)	6.2cm (0-18cm)

Table 2.1 shows the baseline characteristics of the two groups.

*Tumour diameter is the maximum craino-caudal length of the tumour measured on the sagittal MRI planes.

**Height above the anal margin is the length from the most distal part of the tumour to the anal verge measured on a sagittal MR image plane

2.3.4: MRI and Segmentation

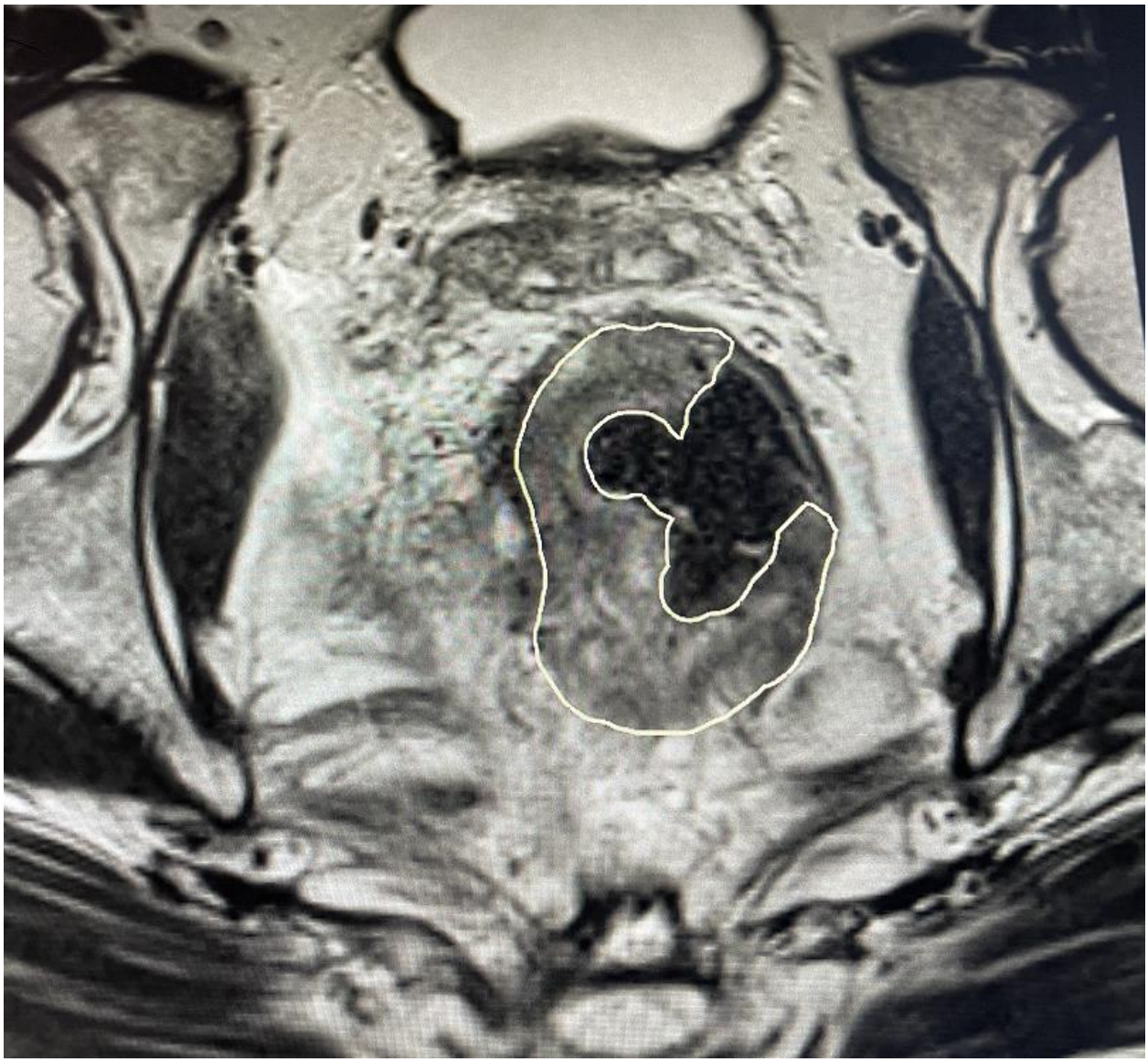
Retrospective pre-treatment MR pelvis sequences of recruited patients were acquired. All images were scanned on a 1.5T diagnostic MR with a 24cm field of view, 3-mm-thick sections and no intersection gap. Transverse T2-weighted (T2W) high-resolution axial MR images was the selected sequence. The T2W fast spin-echo sequence images were acquired in a plane orthogonal to the longitudinal tumour axis. No contrast was given during image acquisition. T2WI sequence is chosen to reflect the most commonly used sequence in previous published MR radiomics work in rectal cancer[118]. The images were segmented in the contouring software RayStation v6.99.

A clinical oncologist and a radiologist, both with expertise in lower GI malignancies, performed image segmentation. Only the tumour volume was segmented as the region of interest (ROI) in this study. (A representation of a segmented slide is seen in Figure 2.2).

Twenty-one patients were randomly selected and independently segmented by the clinical oncologist and the radiologist to investigate inter-observer variations. The two volumes were assessed for consistency, using volumetric differences, dice coefficient, distance to agreement, Hausdorff distance and intra-class correlation (ICC). (See table 2.2)

Figure 2.2 segmented MR slide

Figure 2.2 shows an example of a Segmented T2 MR plane slice. The gross tumour is outlined in white.



2.3.5 Feature Extraction and Image Normalisation

DICOM files containing the MR image segmentation were exported from RayStation. Nifti files for the MR and the rasterised delineations (masks) were then created from the Dicom files using in-house software. The images were then normalised before extraction of features using histogram intensity normalisation. Histogram normalisation has been shown to increase radiomics features

reproducibility in a recent work looking at normalisation effects on the reproducibility of radiomics features relating to T2WI of the pelvis [119]. MR images intensity were normalised in this study by applying histogram intensity matching using an arbitrary MR image as the reference (first image in the folder) [120].

As the images were acquired with different angles, we followed the recommendation of IBSI[129] to resample the images to an isotropic resolution. In addition, we used Fixed Bin Size (FBS) as recommended in several reports[119] [168] on MR feature reproducibility. All features available in pyradiomics v 3.0 were calculated (except for 2D specific features) on the original image and the following filtered images: Laplacian of Gaussian (LOG, for edge detection, using sigma 3 and 5), Wavelet, Square, SquareRoot, Logarithm, Exponential, Gradient and local binary pattern (LBP). A total of 1781 radiomics features were extracted per patient, and the quantitative values of these features were tableted for feature selection and statistical analysis.

2.3.6 Feature selection

The features extracted from the twenty-one doubly contoured patients were also used to determine stable features. Features were extracted from both sets of images independently segmented by two observers. Using the intra-class correlation coefficient (ICC), the features with excellent correlations in the two cohorts were selected as stable. An ICC greater than 0.90 suggests excellent reliability[169]. We accepted features with an ICC of more than 0.9 to be stable features. 1430 out of 1781 features were selected for analysis as stable features.

2.3.7 Principal component analysis (PCA)

Feature reduction is achieved through two forms of dimensionality reduction process; supervised or unsupervised. We choose to use unsupervised feature reduction in this study due to its beneficial characteristics over supervised feature reduction, which are prone to overfitting [170]. Unsupervised dimensional reduction is robust against overfitting and, therefore, more suitable[127]. The most

commonly used unsupervised approaches in radiomics work are cluster analysis and PCA[125]. PCA creates new variables from the existing ones which are uncorrelated and which maximise the variance captured in the data-set. PCA has returned the highest predictive performance in radiomics studies [125][171]. We performed principal components analysis on the dataset and clustered the observations using hierarchical clustering on the factor map.

2.3.8 Multivariate analysis

First, PCA was applied to the radiomics features as above mentioned. Then the PCA generated variables were clustered using hierarchical clustering. The clusters were assessed for variation in tumour diameter and volume to evaluate whether the variations captured by PCA is only representing differences in tumour size or diameter. Next, we pooled the first two principal components, which accounts for most of the variation in the data, PC1 and PC2, as explanatory variables to construct three logistic regression models; combined radiomics and clinical model, clinical only model, and a radiomics model. These models were built on the training cohort, assessed with the validation cohort using ROC AUC.

2.4 Results

2.4.1 Interobserver analysis and feature selection

Contours between the clinical oncologist and the radiologist were consistent. The average dice coefficient was 0.85 (range 0.78 to 0.92), average mean distance-to-agreement was 0.08 cm (range 0.05 to 0.15). The average Hausdorff distance was 0.55 cm (range 0.25 to 1.26 cm). The ICC of the volumes generated was 0.998 (CI 0.995-0.999), showing excellent consistency. (See table 2.2)

Table 2.2: Inter-observer volumes

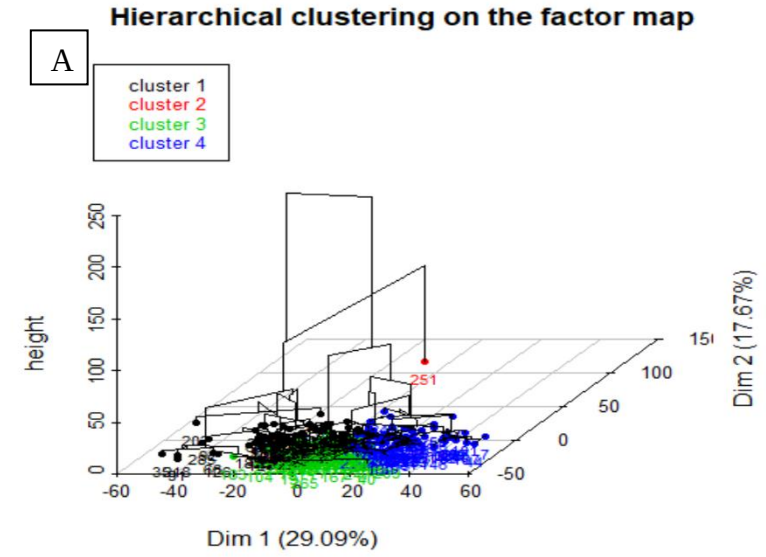
Contouring volumes

Patient	Volume_cm3 (Oncologist)	Volume_cm3 (Radiologist)	Dice	meanDTA_cm	maxDTA_cm
A	15.39304	13.57186	0.872946	0.065056	0.437554
B	40.6307	36.65081	0.896644	0.091529	0.860581
C	49.35989	48.77381	0.87909	0.079794	0.513548
D	17.95674	17.42393	0.789287	0.097939	0.516155
E	33.24779	29.74766	0.877722	0.070626	0.462093
F	7.249623	5.54623	0.808605	0.085972	0.432481
G	9.200744	7.286482	0.803542	0.075692	0.378237
H	6.706856	5.083187	0.792471	0.058761	0.353527
I	14.62942	12.00896	0.824089	0.082445	0.520337
J	49.68653	48.39394	0.884383	0.073275	0.6
K	20.80991	19.79021	0.89432	0.065795	0.720916
L	59.43295	55.73913	0.896231	0.068389	0.365507
M	96.11771	90.40692	0.892133	0.093406	0.898104
N	16.84945	14.55832	0.854024	0.069289	0.370401
O	59.42919	51.79663	0.850829	0.144743	1.26281
P	2.701361	1.922275	0.781196	0.055809	0.250457
Q	11.92443	8.982119	0.765001	0.079688	0.499444
R	28.71494	27.37065	0.867004	0.069622	0.356555
S	28.67871	27.3988	0.921683	0.052065	0.396013
T	22.21612	23.5726	0.897471	0.052602	0.269997
U	43.93989	41.10475	0.862193	0.13026	1.016658

Table 2.2: Inter-observer volumes

Figure 2.3: Hierarchical clustering/PCA

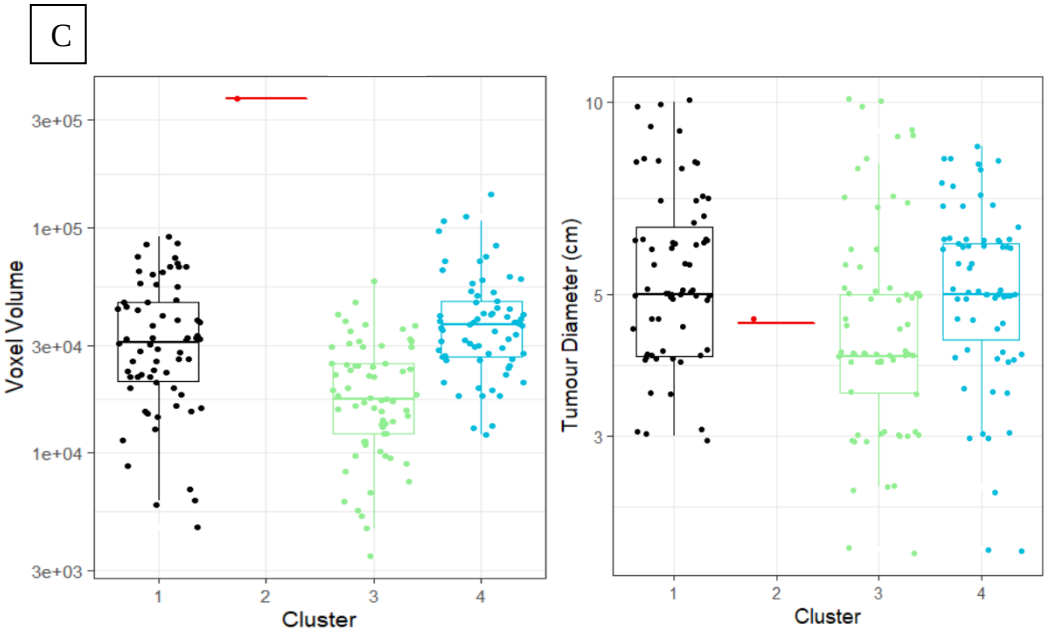
Figure 2.3: Hierarchical clustering using the leading principal components and Voxel Volume/Diameter distribution across the groups found. Cluster 2 is an outlier in our database.



B

Correlation between clusters and cCR

Cluster	cCR – N (%)
1	23 (35)
3	35 (53)
4	42 (63)



2.4.2: Principal component analysis/ Hierarchical Clustering

Figure 2.3A shows the clusters found by applying PCA hierarchical clustering to the selected 1430 radiomics features. Using cluster values as an explanatory variable within a logistic regression analysis, four cluster groups were identified. We found that the probability of cCR correlates with the cluster

groups (see figure 2.3B) (likelihood ratio-test p-value= 0.007). The odds ratio for cluster 4 vs 1 is 3.14 (95% CI: 1.56-6.46) and for cluster 3 vs 1 it is 2.11 (95% CI: 1.05-4.29).

Figure 2.3C shows the distribution of tumour volume and diameter within the PCA derived clusters; there were no differences in volume/diameter between the groups. Moreover, we found that PC1 and PC2 remained correlated to cCR after adjusting for tumour diameter within a logistic regression analysis. Multivariable analysis of the two principal components and tumour diameter against cCR showed that PC1 has an odd ratio of 1.26 (95% CI 1.12-1.42) and a p-value of < 0.001. PC2 had an odds ratio of 0.92 (95% CI 0.84-1.00), p-value of 0.062 and the tumour diameter 0.85 (95% CI 0.71-1.03) and a p-value of 0.094. Indicating that variations represented by PC1 and PC2 are independent of volumetric tumour measurements.

Table 2.3: Multivariable clinical/radiomics logistic regression analysis – training set

	ROCAUC-0.76 (95% CI: 0.69-0.83)	
	OR (95% CI)	p-value
PC1/10	**1.23 (1.07-1.41)**	**0.004**
PC2/10	0.90 (0.80-1.01)	0.061
Diameter (cm)	0.89 (0.72-1.11)	0.309
Age/10	0.86 (0.62-1.20)	0.375
Sex Female v Male	 0.86 (0.40-1.84)	 0.691
T-Stage 3 v 2 4 v 2	 0.41 (0.14-1.24) **0.21 (0.05-0.96)**	 0.115 **0.044**
N-Stage 1 v 0 2/3 v 0	 0.93 (0.40-2.16) 0.75 (0.30-1.89)	 0.869 0.545
Hb/10 (g/L)	**1.27 (1.00-1.60)**	**0.047**
Neutrophils (x10^9/L)	1.01 (0.83-1.22)	0.945
Lymphocytes (x10^9/L)	1.27 (0.86-1.88)	0.232
log(Alkalinephosphatase (log iu/L)	**0.23 (0.06-0.83)**	**0.024**
Albumin (g/L)	0.92 (0.82-1.04)	0.196

Hb- Haemoglobin, g/L- grams per litre, iu/L- units per litre, cm-centimetre.

Hightlighted variables have a p value < 0.05

Table 2.4: Comparing the models

	ROC AUC (95% CI)	
	Training	Validation
Clinical alone	0.73 (0.66-0.80)	0.62 (0.51-0.74)
Radiomics alone	0.68 (0.61-0.75)	0.66 (0.56-0.77)
Clinical and Radiomics	0.76(0.69-0.83)	0.68 (0.57-0.79)

Table is comparing the AUC value between the training and validation cohort of each model. The models with clinical variables have notable differences in AUC.

2.4.3: Multivariable logistic regression models

Table 2.3 shows the multivariable logistic regression of the combined, clinical, and radiomics models. Clinical and radiomics only models were also generated. Comparing model likelihoods, we found that the inclusion of the radiomics variable does improve the model fit of the combined model, p = 0.006.

Table 2.5: Patient's characteristics of the two cohorts.

	Training (N=200)	Test (N=104)
PC1 median (range)	 -6.8 (-53.2, 95.0)	 -2.1 (-48.1, 86.6)
PC2 median (range)	 -7.9 (-65.6, 513.2)	 -8.6 (-60.1, 144.7)
Diameter (cm) median (range)	 5 (2, 10)	 5 (2, 9)
Age median (range)	 66 (31, 89)	 68 (36, 90)
Sex – N (%) Female Male	 62 (31) 138 (69)	 31 (30) 73 (70)
T-Stage – N (%) 2 3 4	 24 (12) 155 (78) 21 (10)	 16 (15) 79 (76) 9 (9)
N-Stage 0 1 2 3	 49 (25) 86 (43) 61 (31) 4 (2)	 24 (23) 45 (43) 35 (34) 0 (0)
Hb (g/L) median (range)	 13.4 (7.7, 16.6)	 13.5 (8.7, 16.9)
Neutrophils (x10^9/L) median (range)	 4.7 (1.7, 12.4)	 5.0 (1.9, 11.4)
Lymphocytes (x10^9/L)		

median (range)	1.7 (0.3, 6.1)	1.7 (0.4, 4.7)
Alkaline Phosphatase(iu/L) median (range)	80 (40, 155)	83 (42, 158)
Albumin (g/L) median (range)	44 (24, 51)	44 (31, 49)

Hb- Haemoglobin, g/L- grams per litre, iu/L-units per litre, cm-centimetre

The training and validation cohort show similar characteristics.

Table 2.6: Multivariable logistic regression analysis in the training and validation cohort.

	Training (N=200)		Test (N=104)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
PC1/10	1.23 (1.07-1.41)	0.004	1.23 (0.98-1.54)	0.078
PC2/10	0.90 (0.80-1.01)	0.061	1.02 (0.86-1.20)	0.853
Diameter (cm)	**0.89 (0.72-1.11)**	**0.309**	**0.58 (0.38-0.88)**	**0.012**
Age/10	0.86 (0.62-1.20)	0.375	1.30 (0.73-2.31)	0.377
Sex Female v Male	0.86 (0.40-1.84)	0.691	1.13 (0.29-4.42)	0.856
T-Stage 3 v 2 4 v 2	0.41 (0.14-1.24) 0.21 (0.05-0.96)	0.115 0.044	0.07 (0.01-0.48) 0.35 (0.02-0.52)	0.007 0.447
N-Stage 1 v 0 **2 v 0**	0.93 (0.40-2.16) **0.75 (0.30-1.89)**	0.869 **0.545**	0.95 (0.22-4.20) **5.86 (1.16-29.7)**	0.947 **0.033**
Hb/10 (g/L)	1.27 (1.00-1.60)	0.047	1.14 (0.76-1.69)	0.531
Neutrophils ($x10^9$/L)	1.01 (0.83-1.22)	0.945	0.77 (0.54-1.09)	0.144
Lymphocytes ($x10^9$/L)	**1.27 (0.86-1.88)**	**0.232**	**0.56 (0.21-1.50)**	**0.250**
log(Alkaline Phosphatase) (log iu/L)	0.23 (0.06-0.83)	0.024	0.85 (0.09-7.74)	0.887
Albumin (g/L)	0.92 (0.82-1.04)	0.196	0.91 (0.74-1.12)	0.381

Table 2.6: Highlighted variables show a significant difference between the two cohorts. Hb- Haemoglobin, g/L- grams per litre, iu/L- units per litre, cm-centimetre.

This table shows the odd-ratios and p values of the variables. Highlighted variables show a major difference in odd ratios between the two cohorts.

2.4.4: Evaluation of the Model

Table 2.4 gives the evaluation of the three models in both cohorts. The AUC values have dropped significantly in the clinical variables models, and the radiomics only model has a similar AUC between the training and validation models.

The drop in ROC AUC between the validation and training cohorts was not due to differences in patient demographics (see table 2.5). We performed a multivariable logistic regression analysis on the validation cohort and compared the results to the training cohort (see table 2.6) to assess why such a significant drop was seen when using clinical variables.

Looking at the clinical variables and comparing the odd ratios of individual variables in the training and validation cohorts, it is clear that the main clinical drivers of cCR have changed significantly, with shifts in some of the clinical variables which may have affected the validation of the models containing the clinical variables (highlighted variables in table 2.6).

2.5 Discussion

2.5.1 Main findings

Our study compared the discriminative characteristics performance of a combined radiomics and clinical model with a clinical or radiomics only model. We found that the clinical variables on their own (based on ROC AUC) are potentially a better predictor of cCR than radiomics variables alone. However, the models containing the clinical variables failed to validate successfully. Even though the study was designed to minimise its risk, overfitting could cause this discrepancy. Another possible reason is calibration drift. With calibration drift[172][173], the expected predictive model is no longer binding as the observed and predicted outcomes differs over time. A recent paper[174] recommended that clinical prediction models be continuously updated and monitored to remain relevant over time. A dynamic prediction model approach[175], whereby a model consecutively adjusts to changes in population demographics, disease incidence, and clinical practice over time, has been proposed as a potential solution to this problem. A notable example of a clinical prediction model updated yearly and revised to include additional predictors is the QRISK[176]. Our results showed that even though the predictability of the radiomics only model is lower at ROC AUC of 0.68, it is unaffected by calibration drift and was validated successfully.

2.5.2: In context to the rest of the literature

Very few studies have used mono sequence pre-treatment images to predict complete response. A similar study[177] used an intensity histogram to predict pCR with external validation, and their results showed an AUC of 0.73 and 0.75 on external validation. Although this study demonstrated good predictability of the radiomics variable, the proportion of patients with pCR in the whole database is less than 30% which could skew the results. Patients in this study were also not matched to ensure a reduction in selection bias.

2.5.3: Strengths and limitations

This study represents the most extensive MR radiomics work with patients who had chemoradiotherapy in rectal cancer to the best of our knowledge. It also recruited patients with clinical complete response (cCR), the target group for organ preservation treatment pathway, unlike other studies that predominantly used patients with pCR. We used a large 1:1 matched cohort of patients (Ccr; N=152) and those without; N=152), representing the largest proportion of patients with complete response in any rectal cancer radiomics work.

This study has some limitations; firstly, all patients received their radiotherapy treatment in either two hospitals in the same region. A more diverse database would have been preferable to reduce selection bias inherent in a few centre studies. This bias could be said to have been reduced by the use of propensity matching. The validation cohort in this study was chosen internally even though the recruited patients were treated in two institutions, an external validation cohort from a different regional hospital may have provided extra validity. The analysis of this study assumed cCR to be a binary response. The reality is that patients without cCR have a wide variety of responses; near-complete, partial, stable, and no response, so a future radiomics study should look at predictors of good response to neo-adjuvant treatment by combining preferred clinical outcomes in one group. Inter-observer variation in the segmentation of radiomics work has been a source of bias. This study reduced this bias by ensuring consistency between the two clinicians involved in the segmentation. For sizeable radiomics work in the future, there is a need to develop automatic contouring software to allow radiomics in day-to-day clinical practice. In the future, it will be expected that contouring of ROI in radiomics will be done by automated delineation tools[178]. The most frequently used MR radiomics sequence is the T2WI; this was used as the protocol sequence in this work. It could be that combining different sequences might improve the predictability of radiomics features. A study[135] using MR radiomics in rectal cancer to predict pCR, showed that combining different image sequences

performed better than using one sequence. Although this study showed an improved radiomics model with multiple sequences, it had only 31 patients with pCR out of 186 patients recruited, which is a significant drawback.

2.5.4 Future research and Conclusion

The predictive abilities of our clinical variables, with or without radiomics, are modest, as demonstrated in this study. The predictive capabilities of the radiomics variables for cCR are improved by adding the clinical variables, but the absolute gains remain low. New approaches are essential to improve the predictability of cCR for neoadjuvant treatment selection in rectal cancer. Future approaches could investigate the addition of radiotherapy biomarkers such as hypoxia, gene expression signatures and deep learning techniques. Molecular markers could also be a valuable addition to a clinical model to improve the model's predictability.

Chapter 3: Prediction of cCR using clinical variables

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Main author's contribution:

This project looks at the prediction of complete response using clinical and radiotherapy variables. For this work, I sort ethical approval to enable it. I recruited patients for this work with some help from Lee Malcomson, the data manager of the ONCORE database. I did all clinical data collection and measurements, planning and methodology. A significant part of the advanced statistics work needed for this project was done and supervised by Dr Hitesh Mistry. I wrote up the manuscript.

3.1 Abstract

Purpose: In patients with rectal cancer who achieve a clinical complete response (cCR) after neoadjuvant chemoradiotherapy, watch and wait (W&W) offers a novel management strategy to avoid major surgery. Prediction of complete response before treatment will aid neoadjuvant treatment selection. Patients predicted to have complete response could have chemo-radiotherapy, and others could have additional doublet chemotherapy at this stage of their treatment to improve their overall outcome. This work investigates the role of clinical variables in predicting clinical complete response.

Method: Using the UK-based OnCoRe database (2008 to 2019), we performed a propensity-score matched (1:1) case-control study of 322 patients who received neoadjuvant chemoradiotherapy. We collected pre-treatment clinicopathological, inflammatory and radiotherapy-related characteristics.

We determined the odds for the occurrence of cCR using conditional logistic regression models. We derived the post-model Area under the Curve (AUC) as an indicator of discrimination performance. We stated a priori that an AUC greater than 0.75 was required for potential clinical utility.

Results: Pre-treatment tumour diameter, mrT-stage, haemoglobin, alkaline phosphate and total radiotherapy depths were associated with cCR on univariable and multivariable analysis. Additionally, neutrophil to lymphocyte ratio (NLR), neutrophil-monocyte to lymphocyte ratio (NMLR), lymphocyte count and albumin were all significantly associated with cCR on multivariable analysis. A nomogram using the above parameters was developed with a resulting ROC AUC of 0.75.

Conclusion: We identified routine clinic-pathological, inflammatory and radiotherapy-related variables independently associated with cCR. A nomogram was developed to predict cCR, and this model's performance characteristics were on our prior clinical utility threshold. Additional research is required to better select patients with rectal cancer undergoing chemoradiotherapy who may benefit from pursuing a W&W strategy.

3.2 Introduction

The mainstay of treatment in locally advanced rectal cancer patients is neoadjuvant chemoradiotherapy (nCRT) followed by surgical resection. Resective surgery here follows the principles of total mesorectal excision (either as an anterior resection or abdominoperineal resection) [5][6]. Surgical resection is associated with a 3% risk of peri-operative mortality; life-threatening early complications such as anastomotic leak; long-term bowel, balder and sexual dysfunction; permanent colostomy and risk of local pelvic recurrence. A permanent colostomy may be required in this patient group in up to 40% of patients. Neo-adjuvant chemoradiotherapy before surgery improves locoregional control and downstages the tumour before surgery to improve surgical resection.

In patients undergoing nCRT, 15% to 27% of patients may have a pathological complete response (pCR: no viable tumour on comprehensive histological examination)[179] in their resection specimen.

Achieving pCR is associated with improved long term prognosis [179][86]. Higher rates of pCR are also achievable after short-course radiotherapy, post-radiotherapy chemotherapy and delayed surgery[115]. The clinical equivalent of pCR is clinical complete response (cCR), the absence of clinical disease post-nCRT and before surgery, verified radiologically with an MRI, and clinically with digital rectal examination and sigmoidoscopy. In patients who achieve a cCR, watch and wait (W&W) offers a novel management strategy to avoid major surgery and its risks. High accuracy to predict cCR before nCRT could provide an advantage to pre-select patients likely to benefit from W&W. Similarly, those anticipated to have an incomplete response could have an alternative neoadjuvant plan such as TNT, intensive doublet chemotherapy, other targeted therapies or intensified chemoradiotherapy [114][115][111]. Since the response to neo-adjuvant treatment is associated with survival outcomes[180][181][182] and differing treatment regimens results in differing outcomes[183], The ability to predict response for treatment selection could also be an essential tool in improving survival.

However, predictors of cCR are poorly defined. A systematic review published in 2016[184] of 85 studies evaluating predictors of pCR (including clinicopathological variables, radiological, gene expression, mutational, and protein expression analyses) concluded that there were 'no robust markers'. In a 2007 large retrospective review (23,747 patients) [146], the significant clinical variables associated with pCR were; lower tumour grade, lower clinical T and N stage, higher radiation dose, and delaying surgery post neoadjuvant treatment by more than 6–8 weeks, though selection biases hindered concluding whether these associations were causal. Increasingly, it is recognised that the immune status is relevant to radiotherapy response, but this is understudied regarding rectal cancer. Additionally, radiotherapy-related parameters beyond dose (for example, depth) have been understudied.

Here, we performed a propensity-score matched (1:1) case-control study of patients who received nCRT and related pre-treatment clinic-pathological, serum inflammatory (as surrogates of immune status), and radiotherapy-related characteristics with the occurrence of cCR. We derived the post-

model receiving operator characteristics (ROC) Area under the Curve (AUC) as an indicator of discrimination performance.

3.3 Method

3.3.1 Patient Population

We performed a propensity-score matched (1: 1) case-control study. We identified cases (cCR) from the OnCoRe (The Rectal Cancer Oncological Complete Response database) between 2008 and 2019. We limited patients to those treated at The Christie NHS Foundation Trust and Lancashire University Teaching Hospital to capture detailed information on radiotherapy depths. We identified controls (non-clinical complete response: NcCR) from an audit cohort of patients undergoing nCRT at the Christie NHS Foundation Trust between 2011 and 2013 and subsequently treated by resection surgery (standard pathway). Ethics approval was obtained before starting this study (IRAS 265989).

3.3.2 Treatment

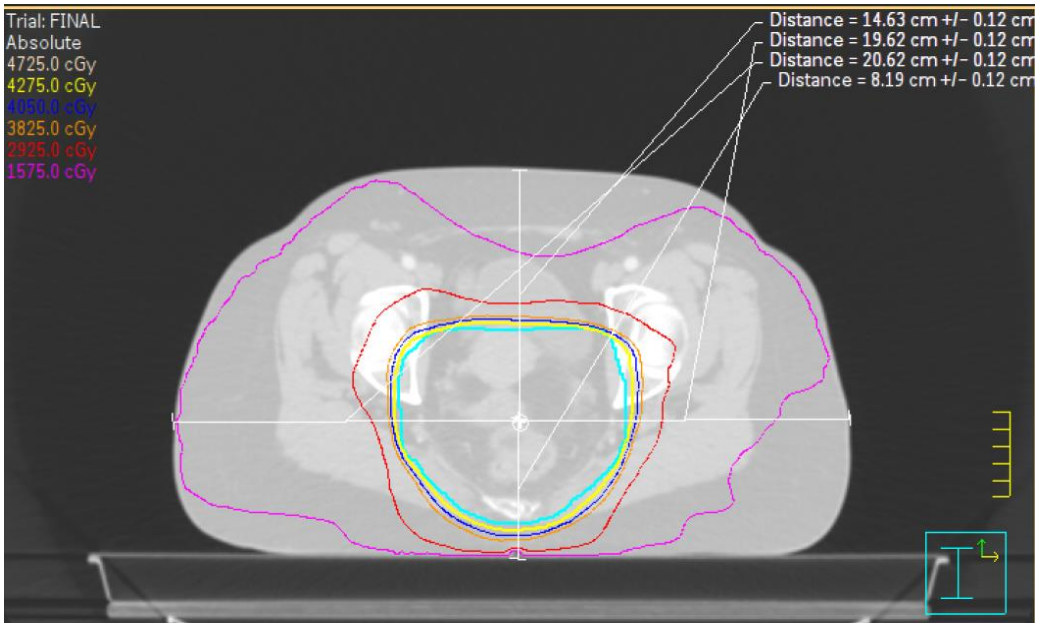
Cases and controls had locally advanced rectal adenocarcinoma treated by nCRT and received 45 Gy in 25 fractions of conformal three-dimensional pelvic radiotherapy with concurrent capecitabine 825mg/m^2 twice daily during treatment. All patients had a pre-treatment CT planning scan. The radiotherapy was delivered with 6-10MV photon beams: the superior border did not go above the L5-S1 vertebrae junction; the inferior border was at least 3cm below the lowest extent of the tumour. The gross tumour volume (GTV) compromised the visible tumour, while the clinical target volume (CTV) contains the mesorectum, pre-sacral space, regional lymphatics combined with a 2cm expansion of the GTV. The PTV (planning target volume) comprises the CTV plus an expansion of 1cm. The radiotherapy dose was prescribed to the iso-centre, and 1.8Gy was delivered daily, five days a week for five weeks.

3.3.3 Predictors

Clinical variables were obtained from the clinical records, including age, tumour diameter (i.e. the greatest tumour length), gender, mrT-stage and mrN-stage and body mass index (BMI). Pre-treatment blood test values included: serum haemoglobin, neutrophils, lymphocytes, monocytes, alkaline phosphate, and albumin. Serum inflammatory indices were derived as follow: neutrophil to lymphocyte ratio (NLR) by dividing the neutrophil count by the lymphocyte count; the monocyte to lymphocyte ratio by dividing the monocyte count by the lymphocyte count; and the neutrophil-monocyte to lymphocyte ratio as (neutrophils + monocytes)/lymphocytes ratio (NMLR).

We calculated beam depths of the radiotherapy plan characteristics as an indication of the radiotherapy integral dose relationship with body size. This is a routinely available variable**.** The beam depths were obtained from the radiotherapy treatment plan of all patients in four vertical dimensions-anterior, posterior, left lateral and right lateral at the level of the isocentre **(Figure 3.1)**. In effect, we measured the distance between the isocentre, i.e. the focus point of the treatment, and the skin in the main axis of the beam. The distance was calculated as the difference between the focus to source distance (FSD, set at 100 cm) and the radiotherapy plan's source to surface distance (SSD). In arithmetic terms, it is: Depth (cm) = FSD-SSD = 100-SSD. The depth variables were listed as anterior, posterior, anterior-posterior (AP), the arithmetic sum of anterior and posterior, right lateral, left lateral, total lateral (arithmetic sum of left and right lateral) and total depth, which is the sum of AP and total lateral.

Figure 3.1: Example of measured radiotherapy depth.



Anterior = 14.63cm, Posterior = 8.19cm, AP= 22.82cm (14.63+8.19), R LAT = 20.62cm, Left LAT= 19.62cm, Total LAT = 40.24cm (20.62+19.62), Total Depth= 63.06cm (40.24 + 22.82)

3.3.4 Follow-up and determination of cCR

Post radiotherapy, patients were restaged with a pelvic MRI imaging (typically) at 8-10 weeks after the end of chemoradiotherapy. Those with no radiological intra-luminal disease were further assessed clinically with endoscopy and digital rectal examination (DRE). We defined a cCR using international criteria as proposed by Habr-Gama et al [185] which requires absence of residual ulceration, stenosis, or mass within the rectum during the digital rectal and endoscopic examinations. Classification of cCR required an absence of malignant disease radiological examination of the mesorectum and pelvis. Those with a cCR were offered 'watch and wait' surveillance[11].

3.3.5 Matching and statistical analysis

To address the imbalance of potential confounders between the W&W and surgical resection groups, we matched treatment groups using propensity scores, similar to how we described elsewhere[11]. The propensity score model included mrT stage, mrN stage, age, and performance status (ordinal term). We then formed matched pairs between patients managed by W&W and those who had

surgical resection using a one-to-one nearest neighbour calliper of width 0・1 (maximum allowable difference in propensity scores). Only patients matched with propensity scores were included in the analysis. We compared matched characteristics using standard tests for continuous variables (Wilcoxon signed-rank test) and categorical variables (McNemar test). We determined the odds for the occurrence of cCR using conditional logistic regression models. We derived the post-model receiving operator characteristics (ROC) Area under the Curve (AUC) as an indicator of discrimination performance. We stated a priori that an AUC greater than 0.75 was required for potential clinical utility [186].

Table 3.1: Baseline characteristics by cases and controls after matching.

Characteristics		**cCR group (n=161)**	**NcCR group (n=161)**
Mean age (range)		66.5 (41-90)	66.6 (31-89yrs)
Gender	**Male** **Female**	118 (73%) 43 (27%)	108 (67%) 53 (33%)
T staging	**T2** **T3** **T4**	34 (21.1%) 113 (70.2%) 14 (8.7%)	10 (6.2%) 130 (80.8%) 21 (13%)
N staging	**N0** **N1** **N2** **N3**	43 (26.7%) 67 (41.6%) 51 (31.7%) 0	35 (21.7%) 70 (43.5%) 52 (32.3%) 4 (2.5%)
Mean tumour diameter* (cm) (range)		4.8cm (2-10cm)	5.5cm (2-10cm)
Blood parameters			
Mean Haemoglobin (range) (g/l)		135.59 (78-169)	129.47 (77-172)
Mean Neutrophil (range) (x10 g/l)		4.98 (1.86-12.37)	5.49 (1.7-12.3)
Mean Lymphocytes (range) (x10g/l)		1.81 (0.26-5.15)	1.82 (0.3-6.1)
Mean Alkaline phosphate (range) (iu/l)		79.81 (41-158)	87.08 (40-165)
Mean Albumin (range) (g/l)		43.44 (24-51)	43.34 (31-49)

Radiotherapy depth		
Anterior (cm)	13.5 (9.4-19.8)	13.3 (9.6-21.5)
Posterior (cm)	8.4 (5.1-13.7)	8.1 (3.2-11.9)
Right lateral (cm)	18.3 (12.7-25.9)	17.7 (8-23.5)
Left lateral (cm)	18.2 (10.8-26.6)	17.8 (10.9-23.3)
Ant-post (cm)	21.9 (16.2-31.2)	21.4 (16.8-30.2)
Total lateral (cm)	36.5 (23.5-52.5)	35.5 (24.6-46.8)
Total depth (cm)	58.3 (42.7-82.8)	56.9 (41.7-74.5)
Mean BMI (range) (kg/m^2)	27.78 (17.31-57.98)	26.58 (16.67-41.55)

*Tumour diameter is the maximum craniocaudal length of the tumour measured on the sagittal MRI planes.

3.4 Results

3.4.1: Matched groups

Initially, there were three hundred ninety-five (395) patients: 165 patients with cCR; 230 patients without clinical complete response (NcCR). Using propensity score matching, we derive a well-matched case-control pair of groups of 161 patients each **(Table 3.1)**.

3.4.2: Predictors of cCR (uni-variable modelling)

We performed a univariable analysis of all variables (**Table 3.2)**. This showed Significant associations: tumour diameter, mrT-stage, pre-treatment haemoglobin, neutrophil, alkaline phosphates and lateral and total beam depths. The analysis showed that the lower the tumour diameter, neutrophil or alkaline phosphate, the higher likelihood of a complete response. The higher the haemoglobin level, the more chance of a complete response. mrT staging also showed a significant association with a complete response with the odds ratio of 0.18 when comparing patients with mrT4 and mrT2—indicating that a patient is more than four times more likely to have a complete response if they are mrT2 compared to mrT4. The lateral beam depth shows a stronger association with complete response than the anterior and posterior depth.

Table 3.2: Univariable and multivariable logistic regression analysis.

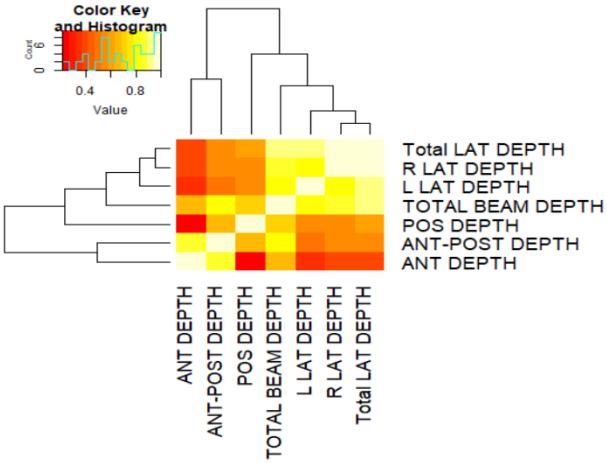
Variables	**Univariable analysis**		**Multivariable analysis ROC AUC of 0.79 (95%CI: 0.73-0.84)**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Tumour Diameter	**0.78 (0.68-0.89)**	**<0.001**	**0.75 (0.62-0.91)**	**0.004**
Age/10	0.99 (0.81-1.22)	0.942	1.00 (0.74-1.34)	0.999
Gender F v M	0.72 (0.45-1.17)	0.182	0.49 (0.23-1.05)	0.065
T Stage **3 v 2** **4 v 2**	0.25 (0.12-0.52) 0.18 (0.07-0.48	**<0.001** **<0.001**	**0.26 (0.09-0.70)** 0.28 (0.07-1.04)	**0.008** 0.058
N Stage 1 v 0 2 v 0	0.78 (0.45-1.36) 0.74 (0.41-1.33)	0.380 0.316	0.96 (0.45-2.03) 1.43 (0.61-3.33)	0.910 0.408
Hb*	**1.24 (1.08-1.43)**	**0.002**	**1.29 (1.06-1.58)**	**0.012**
Neutrophils	**0.86 (0.76-0.97)**	**0.018**	0.96 (0.78-1.20)	0.742
Lymphocytes	0.98 (0.74-1.30)	0.889	**2.48 (1.03-6.00)**	**0.043**
log(Alkaline Phosphatase)	**0.31 (0.13-0.74)**	**0.008**	**0.31 (0.10-0.95)**	**0.040**
Albumin	1.01 (0.94-1.09)	0.776	**0.86 (0.77-0.97)**	**0.011**
Monocyte	1.74 (0.57-5.29)	0.331	1.86 (0.08-4.19)	0.695
NLR	0.86 (0.95-1.06)	0.392	**0.65 (0.46-0.92)**	**0.016**
LMR	0.93 (0.82-1.06)	0.281	0.73 (0.51-1.05)	0.087
NMLR	0.99 (0.95-1.03)	0.751	**1.23 (1.07-1.41)**	**0.003**
BMI	1.05 (1.00-1.10)	0.052	0.93 (0.81-1.06)	0.285
Ant. Depth(cm)	1.07 (0.99-1.17)	0.103	0.85 (0.65-1.11)	0.234
Pos. Depth(cm)	1.16 (0.98-1.38)	0.093	0.93 (0.63-1.36)	0.708
Total Beam Depth (cm)	**1.05 (1.01-1.09)**	**0.023**	**1.20 (1.04-1.38)**	**0.010**

***Units of HB are g/dL. Highlighted variables are variables with significant p-values either in univariable or multivariable analysis**.

3.4.3 Checking for correlations

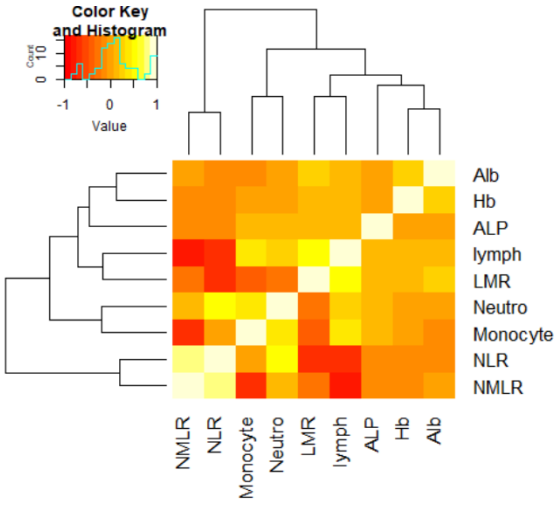
Before multivariable modelling, we tested for co-linearity by correlating variables, particularly assessing correlations between the beam depth variables. To reduce the dimensionality of the data-set, we did a spearman's rank correlation matrix presented as heat maps. **Figure 3.2** shows that the total Lateral depth, right Lateral depth, left lateral depth, and total depth correlate strongly (minimum Spearman's Rho between those four is 0.83, the highest being 0.97). The anterior depth and AP depth are also highly correlated, Spearman's Rho = 0.86. The posterior depth does not have a strong correlation; therefore, we argued that we could reduce the radiotherapy depth variables from seven to three groups.

Figure 3.2: Rank correlation heat-map of depth variables



We additionally assessed correlations among blood parameters **(Figure 3.3).** The strongest correlation is between NMLR and Lymphocytes (Rho = -0.79). We argued that these correlations were not exceedingly high, and therefore, we entered all blood parameters into the multivariable model.

Figure 3.3: Rank correlation heat-map of lab variables



3.4.4 Multivariable modelling

The multivariable analysis is shown in **Table 3.2**. In addition to the findings from the univariable analysis, we noted that NLR, NMLR, lymphocyte count and albumin were all significantly associated with cCR.

3.4.5 Clinical utility

A nomogram using the above parameters was developed with a resulting ROC AUC of 0.75 (**Figure 3.4)**. The clinical utility of this nomogram is in deciding the likelihood of cCR from routine clinical variables collected in the diagnosis of a patient. An excel sheet of the nomogram can be made to be used in multi-disciplinary meetings during discussions of patient's treatment options which, when prospectively validated through its use, will serve as a guild to treatment selection. **Table 3.3** shows examples of its use

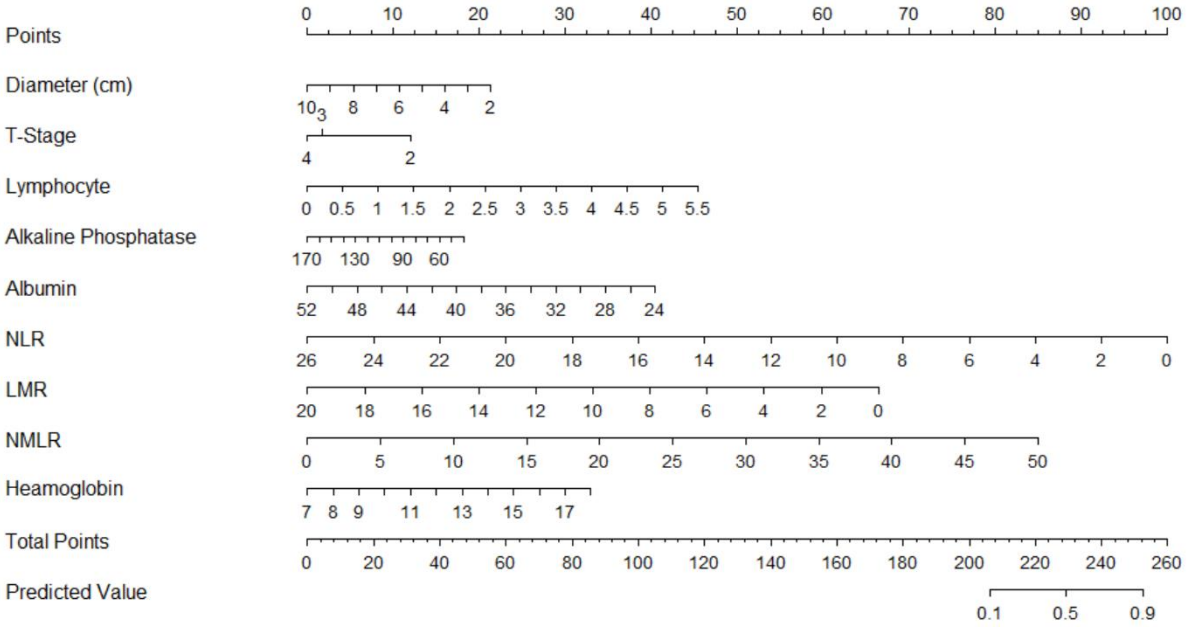
Table 3.3: clinical utility of nomogram.

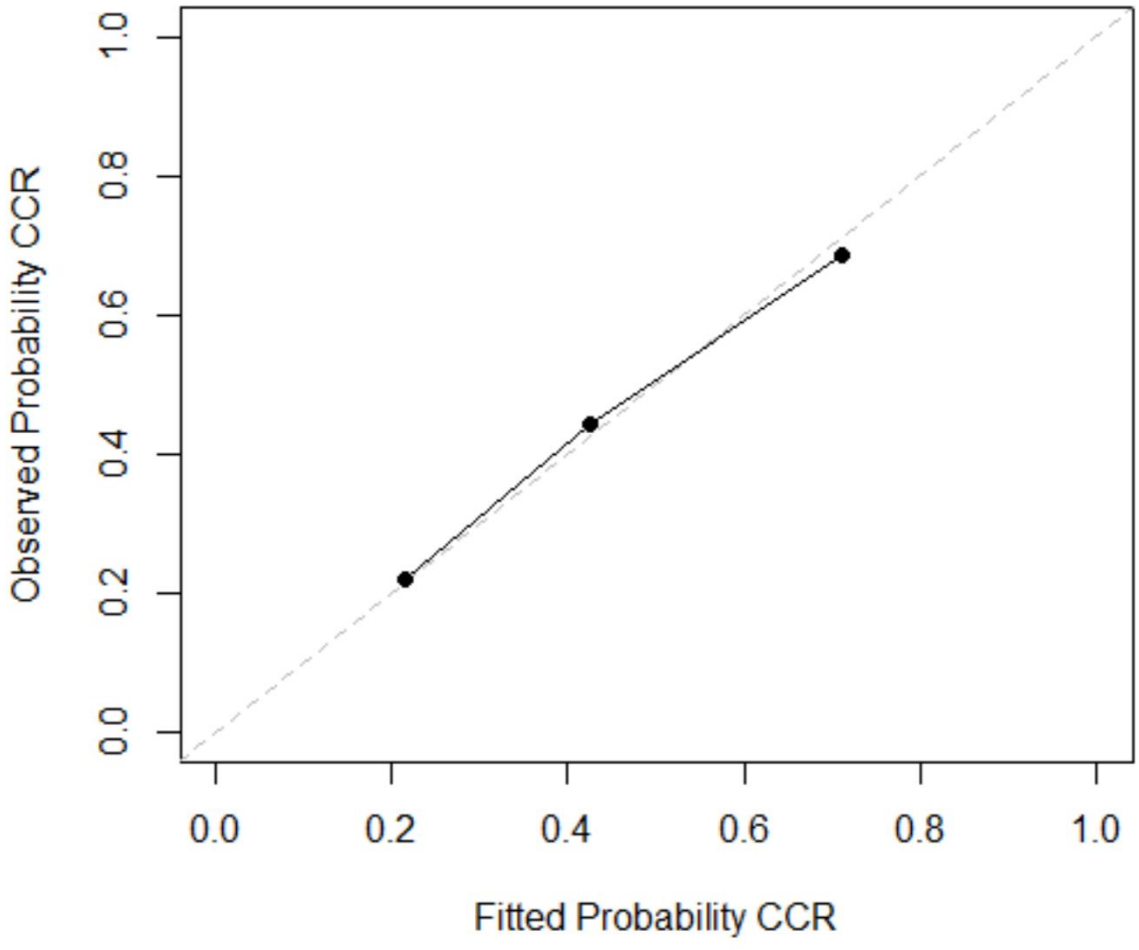
Patient	Tumour Diameter (cm)	T-stage	Lymphocyte (x10g/l)	ALP	Haemoglobin (g/dl)	Albumin (g/l)	Neutrophil (X10g/l)	Monocyte (x10g/l)	Chance of cCR*
A	2	T2	1.5	80	13.4	37	2.3	0.4	0.95
B	5	T2	1.5	80	13.4	37	2.3	0.4	0.90
C	5	T3	1.5	80	13.4	40	2.3	0.4	0.75
D	5	T3	1.5	80	10.4	40	2.3	0.4	0.5
E	10	T3	1.5	80	10.4	40	2.3	0.4	0.22
F	10	T3	1.5	80	10.4	40	8.5	0.4	0.11

Monocyte is used in the calculation of LMR and NMLR. Neutrophils are used in the calculation of NLR and NMLR

***The chance of cCR is related to the cohort used in this work and may not represent the chances of cCR in the general patient population.**

Figure 3.4: Nomogram on prediction of cCR. ROC AUC = 0.75 (0.70-0.81).





3.5 Discussion

3.5.1 Main findings

We found on multivariable analysis that tumour diameter, mrT-stage, serum haemoglobin level, serum alkaline phosphate, total radiotherapy depths, NLR, NMLR, albumin, and lymphocyte count are all significantly associated with clinical complete response. These findings are consistent with retrospective studies that used pCR as endpoints (Table S1). This is the first study to show serum alkaline phosphate (ALP), NMLR and radiotherapy depths significantly associated with complete response. This is also the first study to produce a nomogram to predict cCR. We carefully used only routinely available clinical parameters in the nomogram. This nomogram will, over time require the substitution of some of its variables with more strongly associated routine variables before prospective validation in other to improve its predictability.

3.5.2 In context with the rest of the literature

Although ALP has never until now been shown to be associated with response to chemoradiotherapy in rectal cancer, there is good evidence to show that elevated alkaline phosphate is a prognostic marker in colorectal cancer due to its association with liver metastasis[187]and its link with undetectable occult metastasis in the liver or bone[188]. Interpreting our result with respect to this, it could be that those with more elevated ALP have biologically more advanced disease at the start of their treatment than others, which may not have been fully reflected in their stagging investigations. Our univariable and multivariable analysis showed a positive association between the total radiotherapy depths and cCR. This relationship is not fully understood, and it could reflect the nutritional state of patients as their disease advances. Cancer is associated with weight loss and muscle loss, and it is clear that those losses increase as the disease progresses [189]. Two Systematic reviews[190][191] have shown that sarcopenia (i.e. loss of muscle bulk which occurs in malignancies)

is an adverse prognostic marker for survival in patients with colorectal cancer. The association between sarcopenia and the outcome of chemoradiotherapy in rectal cancer remains unclear. A small study of 61 patients showed that sarcopenia is a negative marker of pCR following chemoradiotherapy in locally advanced rectal cancer[192]. However, more studies are needed here, given that only seven out of 61 patients on this small study had pCR reducing the reliability of their results.

Neutrophils, lymphocytes, monocytes, platelets are all markers of inflammation. Systemic inflammation plays a significant part in cancer cell proliferation and the formation of metastasis[193]. Neutrophil, monocyte and platelets are all believed to promote cancer cell proliferation by their inflammatory activities; neutrophil stimulates circulating vascular endothelial growth factor[194], monocyte provides trophic factors for cancer growth proliferation[195], and platelets provide growth factor that aid cancer growth[196]. Lymphocytes, in contrast, have tumour suppressive properties by inducing cytokines that inhibit cancer cell proliferation[157]. So high neutrophils, monocyte and platelets favour cancer proliferation, but the opposite is the case for lymphocytes. High NLR and PLR due to lymphopenia are therefore expected to be a marker of poor prognosis, but the results have been conflicting; Kim et al. [155] have shown NLR and PLR as both a prognostic and predictive marker for pCR, while the most extensive reported study looking at the prognostic and predictive impact of NLR and PLR in rectal cancer failed to replicate this association with pCR[197]. Our results showed that cCR has a negative association with NLR, similar to Kim et al.[155] Albumin showed an unexpected effect in the multivariable analysis with a negative association with cCR. When the radiotherapy depth variables were added, the odds ratio with albumin moved to less than one on the MVA. This could suggest some unexplained link between the depth variables and albumin. It is reasonable to postulate that patients with wider separations are more likely to have normal or near-normal albumin levels, given that albumin is a nutritive maker hence the result.

Few studies have investigated building a predictive model using routine clinical variables[198][199]. Zhang et al.[198]used a cohort of patients who had neoadjuvant chemotherapy alone (without

radiotherapy), which is not a standard treatment in rectal cancer. Only ten (10) out of 137 of the patients had a complete response, which reduced the reliability of this work. Sun et al. [199] developed a nomogram predicting pathological complete response with a C-index of 0.81 and a drop off to 0.75 on the internal validation cohort. It recruited 85 out of 522 patients with pCR, only 16% of the whole database.

3.5.3 Strengths and limitations

This study is uniquely designed; it recruited a 1:1 matched cohort of patients with cCR and those without, which is lacking in the literature. It also recruited patients with a clinical complete response, the target patient group for the organ preservation treatment pathway, unlike other studies that used pCR, a surgical outcome. This study is the first to investigate the relationship between achieving complete response and radiotherapy treatment depth to the best of our knowledge. As radiotherapy parameters are likely to influence treatment outcome significantly, the investigation of radiotherapy parameters other than the dose is expected to be important in predicting complete response.

This study has notable limitations; firstly, all patients were treated in two cancer centres in the same region. A more diverse patient group would be expected to reduce selection bias. These biases were mitigated using propensity matching to produce a homogenous patient group in the two comparative cohorts. This study investigated routine variables with a strong association with cCR to create a nomogram for cCR; it does lack an external validation cohort. Even though a few of the clinical variables investigated have similar results with previous external studies, external validation would have improved its reliability. This study also recruited patients that were treated over 11 years ago period. During this period, variations in patient selection for treatment, treatment modalities, and changes in the laboratory variables' assay could have affected this study's results.

3.5.4 Unanswered questions and future research

One group of markers that could improve a predictive model is hypoxia biomarkers. A study(58) investigated vascular endothelial growth factor (VEGF) and Bcl-2 apoptosis regulator from pre-treatment biopsies in predicting response to neoadjuvant chemoradiotherapy. It was shown that the presence of VEGF indicates reduced radiotherapy response. This finding is not surprising, given that sustained hypoxia within the tumour leads to VEGF production[200]. These markers could be added to other clinical makers associated with cCR in a model to improve the predictability of complete response.

The predictive abilities of the clinical variables used in our nomogram will need to be improved. Thus, it is necessary to identify more variables significantly associated with cCR to form a robust nomogram that will be prospectively validated before its routine use in treatment selection.

Chapter 4: Discussion

4.1 Rectal cancer treatment

The first chapter of this thesis looked primarily at various evidence that defined the treatment of locally advanced rectal cancer. The epidemiology and the risk of rectal cancer show slight differences between the factors that increase the risk of colon cancer and rectal cancer. It is clear from the evidence that excessive alcohol intake, tobacco smoking, processed meat and obesity are all factors that can increase the risk of rectal cancer. The diagnosis and staging of rectal cancer have evolved through endoscopy for visual clinical examination and biopsy for definitive diagnosis. The introduction of MR pelvic images for the staging of localised rectal cancer has been critical in defining who needs neo-adjuvant chemoradiotherapy, neo-adjuvant radiotherapy alone and which patients should go straight to surgery. It also helps to accurately assess the circumferential resection margin (CRM), the resection point at radical surgery. The current optimal neoadjuvant treatment in locally advanced rectal cancer with threatened CRM has been established as chemoradiotherapy with 5fu or capecitabine and pelvic radiotherapy mainly due to the results of the EORTC 22921 study[69]. Neo-radiotherapy alone (without concurrent chemotherapy) reduces the risk of local recurrence of disease after surgery. It is clear from the results of the German trial [25] and similar trials (see table 1.5) that chemoradiotherapy is best delivered pre-operatively than post-operatively. This is primarily due to the increased toxicity associated with postoperative radiotherapy. Patients who achieve cCR have been shown to achieve excellent clinical outcomes and better prognoses than those that don't [86]. The results from trials comparing patients on the watch and wait treatment plan with a similar cohort of patients have shown that this treatment plan is safe. There are no detrimental effects on clinical outcomes in deferring or avoiding surgery (Table 1.6: Evidence for watch and wait treatment plan.). Patient-centred questionnaire studies have revealed that patients prefer the organ preservation plan and, in most cases, will prefer not to have surgery or at least defer it if this does not mean a detrimental effect on their oncological outcomes [99][100]. Early

diagnosis of rectal cancer is also being achieved by the introduction of the UK bowel cancer surveillance programme, which has seen asymptomatic diagnosis of patients and will invariably mean a better prognosis overall.

Increasing the proportion of patients with complete responses has to be the target of rectal cancer treatment. Just with establishing an organ preservation plan as the standard of care in anal squamous cell cancer, rectal cancer will likely follow. Achieving an increased ratio of complete response will increase the number of patients undergoing organ preservation and improve the clinical outcomes of patients. In the future, it will mean fewer stomas for patients with localised disease. The routes to achieving this through neoadjuvant radiotherapy are either via intensifying the standard chemoradiotherapy or using an alternative radiosensitiser that is much more effective than 5fu or capecitabine. Total neoadjuvant therapy (TNT) is a treatment plan to boost chemoradiotherapy with more doublet chemotherapy. TNT has been shown to doubly increase pCR rates above the standard ratio [115], but the question of what is our best radiotherapy schedule in TNT remains unanswered. Is it long course chemoradiotherapy followed by doublet chemotherapy and surgery or short-course radiotherapy followed by chemotherapy, then surgery? Only time and more studies to answer these questions will help define the best strategy.

Neo-adjuvant treatment in rectal cancer still has several unanswered questions: whether we have reached our optimal radiotherapy dose at our current standard dose fractionations is an open debate. This debate will continue, especially on the back of increasing evidence that higher doses could improve outcomes[103][104][105]. *Do we know what our optimal radio sensitiser is?* This will be the question expected to be answered with the much-awaited trials such as the PRAER 1, [65] PRIME RT, [67] and Artemis trials. *Do we know the best time interval between Chemo-radiotherapy and restaging before surgery?* The current evidence supports anything between 8 and 14 weeks[84] [77]. *Is there any merit to patients having neo-adjuvant radiotherapy followed with immediate surgery, which has been the current practice in intermediate-risk rectal cancer, or can the surgery be delayed safely here to increase the number of patients going into the organ preservation pathway?*

Is there a way of predicting the treatment outcome before they are delivered? So that we can choose the most effective neo-adjuvant treatment plan. This remains an essential scientific question in rectal cancer, given the trend towards increased organ preservation and individualised treatment. This project was aimed at contributing toward an answer to this question.

4.2 This project

Patients with clinical complete response post-neo-adjuvant treatment have been shown to have the best clinical outcome in the management of locally advanced rectal cancer [86]. There are currently three possible neo-adjuvant treatment options for managing locally advanced rectal cancer; chemoradiotherapy, doublet chemotherapy, and total neo-adjuvant therapy. To reduce the overall risk and toxicity associated with neoadjuvant treatment, selecting the treatment that offers a patient the best clinical outcome is essential. To achieve this, prediction of the clinical outcome before treatment is needed. By successfully predicting the possible outcome pre-treatment, patients can be offered the most suitable treatment to improve their overall outcomes and reduce toxicity.
This project aimed to assess the predictability of complete response in rectal cancer using pre-treatment clinical and radiomics variables in patients with chemoradiotherapy. Patients predicted to have a clinical complete response with chemoradiotherapy could be selected for this treatment. Those expected not to achieve clinical complete response can have an intensified neoadjuvant treatment options or be entered into a clinical trial.
To achieve this aim, I recruited patients from the OnCoRE database, one of the largest research databases with patients that achieved complete response post-neo-adjuvant chemoradiotherapy. To reduce selection bias, a propensity-score matched (1: 1) case-control study was designed to investigate two matched groups of patients; one group with a complete response and the other without a complete response. The objective is to investigate the predictability of clinical and

radiomics variables to cCR. The number of patients recruited was uniquely large (161 per group) to further enhance the robustness of the work. This is particularly important in radiomics work, which is prone to overfitting. I investigated both radiomics and clinical variables. I found that clinical variables are better predictors of clinical complete response from our work in chapter 2, and I developed a nomogram using clinical variables in chapter 3. Radiomics predictability was also shown to have been significantly enhanced by adding clinical variables. Radiomics model processes carry a lot of uncertainties ranging from complex image processing to differences in imaging protocols and difficulties in the explain ability of radiomics analyses, which undermines confidence in these model features, especially in their ability to be reproducible. As a result, chapter 3 of this work looked to enhance clinical-only variables predictability of Ccr without adding radiomics variables. In other to produce a more reliable and reproducible model.

Although this thesis attained modest predictability with radiomics and clinical variables, I achieved the aim of this study by showing that the prediction of complete response is achievable with routinely available variables. It is also clearly illustrated from the results of this project that radiomics variables should be used in combination with clinical variables in predictive models. This project produced the first nomogram to predict complete response using the target patients for organ preservation treatment. With an AUC of 0.75, this nomogram will need improvement and prospective validation before full clinical utility. The nomogram produced a probability of Ccr in table 3.3; this chance of Ccr is likely related to the cohort of patients in this project. This could be higher than expected in the general patient population due to the 1:1 matching of patients in this project. This study is the first to investigate the relationship between achieving complete response and radiotherapy. This thesis shows a significant link between radiotherapy depth and the probability of cCR. This finding will likely lead to future studies investigating the links between radiotherapy parameters and clinical outcomes of the patients treated. The results also highlighted the strong association between inflammatory clinical markers and response to radiotherapy. Inflammatory

markers such as neutrophils, lymphocytes and monocytes were strongly associated with cCR. With the growing work on immune response in cancer treatment and the current widespread use of immunotherapy, this work offers a data on the role of inflammatory markers and response to therapy in rectal cancer. The use of immunotherapy in the neoadjuvant treatment of rectal cancer remains in its trial stages. The results of this thesis could be an early indication of the possible outcome of these trials and aid in their design.

4.3 What we already know

Various studies have predicted clinical outcomes post chemoradiotherapy in rectal cancer using both radiomics and clinical variables. The focus of these studies has been on predicting pCR either pre or post neoadjuvant chemoradiotherapy. After chemoradiotherapy, complete response is assessed with clinical investigations, endoscopy and digital rectal examination, and radiological imaging, including MR images. These clinical and radiological assessments to confirm complete response are very reliable [132] and are unlikely to be replaced by a predictive model of variables. The prediction of treatment outcomes pre-treatment enables treatment selection in neo-adjuvant rectal cancer management.

Various major studies[134] [135] [136] [137][165][166] have looked at the prediction of complete response using pre-treatment MR radiomics features. These studies developed predictive models for pathological complete response based on radiomics features. They all primarily recruited patients with pCR as their target patient group and compared them with those without pCR. Therefore, the main limitation of these studies is that the target group of patients has been those with pCR which requires surgical resection to assess. Another major limitation of the current literature is that the proportion of patients recruited in these studies with complete responses is relatively small (all less than or equal to 20% of the whole database). This imbalance may lead to over-fitting the radiomics contributions in the predictive models.

This project is the most extensive radiomics work in rectal cancer that recruited a 1:1 matched group (cCRr; N=152 and those without; N=152) of patients with cCR, the target patient group for the organ preservation treatment plan. Using propensity matching, this project reduced the risk of selection bias. This method is unique compared to other radiomics work in the current literature. Our validation cohort was recruited using the date of diagnosis of patients to mimic prospective studies and further eliminate potential selection bias. The work of this project represents the most robust use of radiomics to predict complete response in rectal cancer due to its unique methodology.
The association between clinical variables and the pathological complete response has been investigated in various trials (see table 1.7: Clinical variables associated with pCR in literature). Although different clinical variables are associated with pathological response in rectal cancer, none of these variables has been individually predictive. None of the work listed in table 1.7 has recruited a 1:1 matched group of patients to investigate the association between complete response and clinical variables. Other than radiotherapy dose, no other radiotherapy parameters have been studied for association with complete response.

To improve the current literature using clinical variables to predict complete response, I use the same 1:1 matched cohort of patients. This project is the largest to investigate clinical variables' association with cCR, unlike other studies that used patients with pCR. This is also the first to investigate the relationship between achieving complete response and radiotherapy treatment depth. It is expected that more studies will take the lead from this work in investigating radiotherapy parameters' association with clinical outcomes.
This project was designed to enhance the current literature in predicting complete response with radiomics and clinical variables using patients with cCR. Its methodology was designed to be robust. After its validation, the nomogram of clinical variables is expected to help with treatment selection in neo-adjuvant management of rectal cancer.

4.4 Limitations

The main limitation of the work done in chapters 2 and 3 is the lack of external validation. When successfully validated, the results will be of greater clinical use. The results of this work were positive in identifying clinical variables associated with a complete response and have shone more light on the association of radiotherapy variables to treatment response which will need further investigations in the future. Another limitation is that this work relied on a retrospective cohort of patients. A prospective cohort would increase the robustness of a study in ensuring that the potential biases present in a retrospective work, such as selection bias, are reduced to the minimum. Despite its limitations, this work provides the first step in using routinely acquired clinical variables to predict complete response utilising the group of patients already in an organ preservation treatment plan.

The Clinical variables used in this project are a better predictor of cCR than radiomics variables based on the ROC and AUC of the two sets of models. Although clinical variables are more predictive than radiomics variables, clinical variables are prone to calibration drift. They will need to be constantly evaluated and revised over time for the model to remain relevant.

It is clear from the results presented in chapter 2 that radiomics variables can be used to predict complete response, but the predictive ability on their own is modest. MR-based radiomics has the disadvantage of requiring advanced imaging processing, and there are many uncertainties regarding its reproducibility and reliability due to differing image protocols and scanning variations.

Therefore, based on this study, it is fair to say that its role in routine clinical practice for the prediction of cCR is likely to remain limited. It is important to note that this project used only one image sequence in its predictive model. It could be that the predictive power of the radiomics variable could be improved by using multiple image sequences, just like is used in the clinical, radiological interpretation of images. The difficulty with using numerous sequence radiomics work is that it multiples the uncertainties and complexities involved in advanced image processing, limiting its use in routine day-

to-day clinical decision-making. It could be that radiomics variables will continue to be used as part of radiological diagnosis in cancer and less as the primary tool for predicting treatment response in rectal cancer. Advanced deep learning algorithms with better predictability and reproducibility could replace radiomics variables in the future [201].

4.5 Future work

Colorectal cancer is a highly heterogeneous disease[202]. Studies have demonstrated high intra-tumour heterogeneity [193], making it challenging to produce the high level of sensitivity and specificity needed to achieve sufficient predictability using one or two variables. The answer will be to study the association between different variables and cCR to appreciate their performance individually and in a group, formulate a model based on significant variables, validate it, and review it over time. Therefore, future should investigate other routinely acquired clinical variables against their association with cCR. These variables can help to develop a more predictive nomogram. This nomogram will then need to be prospective validated and updated over time. The follow-up work can build on the cohort used for this study. Still, it will require collecting more available clinical variables, investigating their links with a complete response and adding them to improve the nomogram before prospective validation.

Chapter 3 showed that routine clinical variables could predict complete response. A nomogram was produced with a ROC AUC of 0.75. The variables used were tumour diameter, T-stage, lymphocyte count, alkaline phosphate, haemoglobin, albumin, neutrophil and monocyte. These variables can easily be obtained as part of a patient's diagnosis. It is worth mentioning that Balachandran et al.; 'Nomograms in Oncology – More than Meets the Eye' [203], in its analysis of 19 nomograms in 8 reports in The Lancet Oncology and The Journal of Clinical Oncology in 2012-2013, revealed a median AUC was 0.74. This showed that the nomogram produced from this work could become a valuable tool in rectal cancer neoadjuvant treatment selection.

The clinical utility of this nomogram is that it can be used during MDT meetings to decide on which neoadjuvant treatment options patients are more suited to. It can also be used in an app form for easy accessibility in clinical decision making. Chapter 3 of this thesis also highlights that radiotherapy depth could be a variable of interest in predicting radiotherapy outcomes. We calculated beam depths from the radiotherapy plan to indicate the integral radiotherapy dose. Although this was not used as part of the nomogram being a non-routine clinical variable, it can be a focus on further studies. It can be incorporated into radiotherapy treatment planning. Radiotherapy depth could also represent the nutritional status of patients, which will mean that the higher the horizontal depth, the more likely the patient has less advanced disease and, therefore, the more likelihood of cCR. More studies will need to be done to establish this link more accurately.

4.7 Conclusions

This thesis shows that although a radiomics model can predict clinical outcomes in rectal cancer pre chemoradiotherapy, its predictive power is modest. Alternatively, clinical variables associated with complete response have higher predictive power for cCR than radiomics. Radiomics' predictive power for cCR could be improved by adding clinical variables but radiomics involves complex image processing and issues with reproducibility. When prospectively validated, the clinical variable based nomogram produced in this project can then be used to aid treatment selection in rectal cancer neoadjuvant treatment.

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Chapter 6: Appendx 1: Publications/Presentations

The following below relays the work presented in this thesis. Abstracts or full articles publicatioare appended where available.

6.1 Publications:

Clinico-pathological predictors of clinical complete response in rectal cancer

P. Mbanu , E. Vasquez Osorio , H. Mistry , L. Malcomson, S. Yousif, M. Aznar, R. Kochhar, M. Van Herk, A.G. Renehan, M.P. Saunders. Cancer Treat Res Commun. 2022; 31: 100540.

doi: 10.1016/j.ctarc.2022.100540.

Prediction of clinical complete response in rectal cancer using clinical and radiomics features.

P Mbanu, Eliana Vasquez Osorio, Hitesh Mistry, Joseph Mercer, Lee Malcomson, Rohit Kochhar, Andrew Renehan, Marcel van Herk, Mark Saunders. Radiotherapy and Oncology. 2021;161:S73-S4.

6.2 Presentations:

Prediction of clinical complete response in rectal cancer using clinical and radiomics features

Peter Mbanu, Eliana Vasquez Osorio, Hitesh Mistry, Joseph Mercer, Lee Malcomson, Rohit Kochhar, Andrew Renehan, Marcel van Herk, Mark Saunders

Oral poster highlight presentation at ESTRO 2021.

6.3 Posters

Clinical and radomics prediction of clinical complete response after chemoradiotherapy in rectal cancer.

Peter Mbanu, Eliana Vasquez Osorio, Hitesh Mistry, Joseph Mercer, Lee Malcomson, Rohit Kochhar, Andrew Renehan, Marcel van Herk, Mark Saunders.

The Association of Coloproctology of Great Britain and Ireland (ACPGBI) conference 2021

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Clinico-pathological predictors of clinical complete response in rectal cancer

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ABSTRACT

Purpose: Prediction of clinical complete response in rectal cancer before neoadjuvant chemo-radiotherapy treatment enables treatment selection. Patients predicted to have complete response could have chemo-radiotherapy, and others could have additional doublet chemotherapy at this stage of their treatment to improve their overall outcome. This work investigates the role of clinical variables in predicting clinical complete response.

Method: Using the UK-based OnCoRe database (2008 to 2019), we performed a propensity-score matched study of 322 patients who received neoadjuvant chemoradiotherapy. We collected pre-treatment clinic-pathological, inflammatory and radiotherapy-related characteristics. We determined the odds for the occurrence of cCR using conditional logistic regression models. We derived the post-model Area under the Curve (AUC) as an indicator of discrimination performance and stated a priori that an AUC of 0.75 or greater was required for potential clinical utility.

Results: Pre-treatment tumour diameter, mrT-stage, haemoglobin, alkaline phosphate and total radiotherapy depths were associated with cCR on univariable and multivariable analysis. Additionally, neutrophil to lymphocyte ratio (NLR), neutrophil-monocyte to lymphocyte ratio (NMLR), lymphocyte count and albumin were all significantly associated with cCR on multivariable analysis. A nomogram using the above parameters was developed with a resulting ROC AUC of 0.75.

Conclusion: We identified routine clinic-pathological, inflammatory and radiotherapy-related variables which are independently associated with cCR. A nomogram was developed to predict cCR. The performance characteristics from this model were on the prior clinical utility threshold. Additional research is required to develop more associated variables to better select patients with rectal cancer undergoing chemoradiotherapy who may benefit from pursuing a W&W strategy.

Introduction

The mainstay of treatment in patients with locally advanced rectal cancer is neoadjuvant chemoradiotherapy (nCRT) followed by surgical resection. Resective surgery here follows the principles of total meso-rectal excision (either as an anterior resection or abdomino-perineal resection) [1] [2]. Surgical resection is associated with a 3% risk of peri-operative mortality; life-threatening early complications such as anastomotic leak; long-term bowel, balder and sexual dysfunction; permanent colostomy and risk of local pelvic recurrence. In this patient group, permanent colostomy may be required in up to 40% of patients. Neo-adjuvant chemoradiotherapy (nCRT) before surgery improves

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loco-regional control and downstages the tumour before surgery to improve surgical resection.

In patients undergoing nCRT, 15% to 27% of patients may have a pathological complete response (pCR: no viable tumour on comprehensive histological examination)[3] in their resection specimen. Achieving pCR is associated with improved long term prognosis [3] [4]. Higher rates of pCR are also achievable after short-course radiotherapy followed by post-radiotherapy chemotherapy and delayed surgery[5]. This is known as total neoadjuvant therapy (TNT). The clinical equivalent of pCR is clinical complete response (cCR), the absence of clinical disease post-nCRT and before surgery, verified radiologically with a MRI, and clinically with digital rectal examination and sigmoidoscopy. In patients who achieve a cCR, watch and wait (W&W) offers a novel management strategy to avoid major surgery and its risks. High accuracy to predict cCR before nCRT could offer the advantage to pre-select patients likely to benefit from organ preservation plan; 'watch and wait' (W&W). Similarly, those anticipated to have an incomplete response could have an alternative neoadjuvant plan such as TNT, intensive doublet chemotherapy, other targeted therapies or intensified chemo-radiotherapy to improve their outcome [6] [5] [7].

However, predictors of cCR are poorly defined. A systematic review published in 2016[8] (and Table S1) of 85 studies evaluating predictors of pCR (including clinicopathological variables, radiological, gene expression, mutational, and protein expression analyses) concluded that there were 'no robust markers'. In a 2007 large retrospective review (23, 747 patients) [9], the significant clinical variables associated with pCR were; lower tumour grade, lower clinical T and N stage, higher radiation dose, and delaying surgery post neoadjuvant treatment by more than 6–8 weeks, though selection biases hindered concluding whether these associations were causal. Increasingly it is recognise that the immune status is relevant to radiotherapy response, but this is understudied with regards to rectal cancer. Additionally, radiotherapy-related parameters beyond dose (for example, depth) have been understudied.

Here, we performed a propensity-score matched (1:1) study of patients who received nCRT and related pre-treatment clinic-pathological, serum inflammatory (as surrogates of immune status), and radiotherapy-related characteristics with the occurrence of cCR. We derived the post-model receiving operator characteristics (ROC) Area under the Curve (AUC) as an indicator of discrimination performance.

Method

Patient population

We performed a propensity-score matched (1: 1) study. We identified cases (all patients with cCR post neoadjuvant treatment assessment on 'watch and wait' surveillance) from the OnCoRe (The Rectal Cancer-Oncological Complete Response Database) database, between 2008 and 2019. To capture detailed information on radiotherapy depths, we limited patients to those treated at the Christie NHS Foundation Trust and Lancashire University Teaching Hospital. We identified controls (non-clinical complete response: NcCR) from an audit cohort of patients undergoing nCRT at the Christie NHS Foundation Trust between 2011 and 2013, and who were subsequently treated by resection surgery (standard pathway). Ethics approval was obtained before starting this study (IRAS 265989).

Treatment

All patients had locally advanced rectal adenocarcinoma treated by nCRT and received 45 Gy in 25 fractions of conformal three-dimensional pelvic radiotherapy with concurrent capecitabine 825 mg/m^2 twice daily during treatment. All patients had a pre-treatment CT planning scan. The radiotherapy was delivered with 6–10MV photon beams: the superior border did not go above the L5-S1 vertebrae junction; the inferior border was at least 3 cm below the lowest extent of the tumour. The gross tumour volume (GTV) compromised the visible tumour, while the clinical target volume (CTV) contains the mesorectum, pre-sacral space, regional lymphatics combined with a 2 cm expansion of the GTV. The PTV (planning target volume) comprises the CTV plus an expansion of 1 cm. The radiotherapy dose was prescribed to the isocentre, and 1.8 Gy was delivered daily, five days a week for five weeks.

Predictors

Clinical variables were obtained from the clinical records including: age, tumour diameter (i.e. the greatest tumour length), gender, mrT-stage and mrN-stage and body mass index (BMI). Pre-treatment blood test values included: serum haemoglobin, neutrophils, lymphocytes, monocytes, alkaline phosphate, and albumin. Serum inflammatory indices were derived as follow: neutrophil to lymphocyte ratio (NLR) by dividing the neutrophil count by the lymphocyte count; the monocyte to lymphocyte ratio by dividing the monocyte count by the lymphocyte count; and the neutrophil-monocyte to lymphocyte ratio as (neutrophils + monocytes)/lymphocytes ratio (NMLR).

We calculated beam depths of the radiotherapy plan characteristics as an indication of radiotherapy integral dose. The beam depths were obtained from the radiotherapy treatment plan of all patients in four vertical dimensions- anterior, posterior, left lateral and right lateral at the level of the isocentre (Fig. 1). In effect, we measured the distance between the isocentre, i.e. the focus point of the treatment, and the skin in the main axis of the beam. The distance was calculated as the difference between the focus to source distance (FSD set at 100 cm) and the source to surface distance (SSD) on the radiotherapy plan. In arithmetic terms, it is: Depth (cm) = FSD-SSD = 100-SSD. The depth variables were listed as anterior, posterior, anterior-posterior (AP), the arithmetic sum of anterior and posterior, right lateral, left lateral, total lateral (arithmetic sum of left and right lateral) and total depth, which is the sum of AP and total lateral.

Follow-up and determination of cCR

Post radiotherapy, patients were restaged with a pelvic MRI imaging (typically) at 8–10 weeks after the end of chemoradiotherapy. Those with no radiological intra-luminal disease were further assessed clinically with endoscopy and digital rectal examination (DRE). We defined a cCR using international criteria as proposed by Habr-Gama et al. [10] which requires absence of residual ulceration, stenosis, or mass within the rectum during digital rectal examination and endoscopic examination. Classification of cCR also requires a normal radiological examination of the mesorectum and pelvis. Those with a cCR were offered 'watch and wait' surveillance[11].

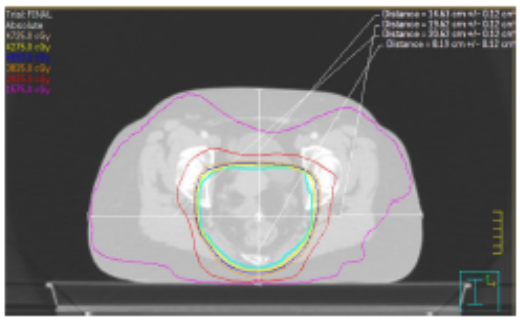

Fig. 1. Example of measured radiotherapy depth. Anterior – 14.63 cm, Posterior – 8.19 cm, AP– 22.82 cm (14.63+8.19), R LAT – 20.62 cm, Left LAT– 19.62 cm, Total LAT – 40.24 cm (20.62+19.62), Total Depth– 63.06 cm (40.24 + 22.82).

Matching and statistical analysis

To address the imbalance of potential confounders between the W&W and surgical resection groups, we matched treatment groups using propensity scores, similar to how we described elsewhere[11]. The propensity score model included mrT stage, mrN stage, age, and performance status (ordinal term). We then formed matched pairs between patients managed by W&W and those who had surgical resection using a one-to-one nearest neighbour calliper of width 0 · 1 (maximum allowable difference in propensity scores). Only patients matched with propensity scores were included in the analysis. We compared matched characteristics using standard tests for continuous variables (Wilcoxon signed-rank test) and categorical variables (McNemar test). We determined the odds for the occurrence of cCR using conditional logistic regression models. We derived the post-model receiving operator characteristics (ROC) Area under the Curve (AUC) as an indicator of discrimination performance. We stated a priori that an AUC greater than 0.75 was required for potential clinical utility [12].

Results

Matched groups

Initially, there were three hundred ninety-five (395) patients: 165 patients with cCR; 230 patients without clinical complete response (NcCR). Using propensity score matching, we derive a well-matched case-control pair of groups of 161 patients each (Table 1).

Predictors of cCR (univariable modelling)

We performed a univariable analysis of all variables (Table 2). This showed Significant associations as follows: tumour diameter, mrT-stage, pre-treatment haemoglobin, neutrophil, alkaline phosphates and lateral and total beam depths. The analysis showed that the lower the tumour diameter, neutrophil or alkaline phosphate, the higher likelihood of a complete response. The higher the haemoglobin level, the more chance of a complete response. mrT staging also showed a significant association with a complete response with the odds ratio of 0.18 when comparing patients with mrT4 and mrT2—indicating that a patient is more than four times more likely to have a complete response if they are mrT2 compared to mrT4. The lateral beam depth shows a stronger association with complete response compared with the anterior and posterior depth.

Table 1
Baseline characteristics by cases and controls after matching.

Characteristics	cCR group (n = 161)	NcCR group (n = 161)
Mean age (range)	66.5 (41–90)	66.6 (31–89yrs)
Gender Male	118 (73%) 43	108 (67%) 53 (33%)
Female	(27%)	
T staging (Clinical) T2	34 (21.1%)	10 (6.2%)
T3	113 (70.2%)	130 (80.8%)
T4	14 (8.7%)	21 (13%)
N staging N0	43 (26.7%)	35 (21.7%)
N1	67 (41.6%)	70 (43.5%)
N2	51 (31.7%)	52 (32.3%)
N3	0	4 (2.5%)
Mean tumour diameter* (cm)		
(range)	4.8 cm (2–10 cm)	5.5 cm (2–10 cm)
Blood parameters		
Mean Haemoglobin (range) (g/l)	135.59 (78–169)	129.47 (77–172)
Mean Neutrophil (range) (x10 g/l)	4.98 (1.86–12.37)	5.49 (1.7–12.3)
Mean Lymphocytes (range) (x10g/l)	1.81 (0.26–5.15)	1.82 (0.3–6.1)
	79.81 (41–158)	87.08 (40–165)
Mean Alkaline phosphate (range) (iu/l)	43.44 (24–51)	43.34 (31–49)
Mean Albumin (range) (g/l)	0.54 (0.19–1.38)	0.51 (0.10–1.40)
Mean Monocyte (range) (x10g/l)		
Radiotherapy depth		
Anterior (cm)	13.5 (9.4–19.8)	13.3 (9.6–21.5)
Posterior (cm)	8.4 (5.1–13.7)	8.1 (3.2–11.9)
Right lateral (cm)	18.3 (12.7–25.9)	17.7 (8–23.5)
Left lateral (cm)	18.2 (10.8–26.6)	17.8 (10.9–23.3)
Ant-post (cm)	21.9 (16.2–31.2)	21.4 (16.8–30.2)
Total lateral (cm)	36.5 (23.5–52.5)	35.5 (24.6–46.8)
Total depth (cm)	58.3 (42.7–82.8)	56.9 (41.7–74.5)
Mean BMI (range) (kg/m^2)	27.78 (17.31–57.98)	26.58 (16.67–41.55)

*Tumour diameter is the maximum cranio-caudal length of the tumour measured on the sagittal MRI planes.

Table 2
Univariable and multivariable logistic regression analysis.

Variables	Univariable analysis		Multivariable analysisROC AUC of 0.79 (95%CI: 0.73–0.84)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Tumour Diameter	0.78 (0.68–0.89)	<0.001	0.75 (0.62–0.91)	0.004
Age/10	0.99 (0.81–1.22)	0.942	1.00 (0.74–1.34)	0.999
Gender				
F v M	0.72 (0.45–1.17)	0.182	0.49 (0.23–1.05)	0.065
T Stage				
3 v 2	0.25	<0.001	0.26	0.008
4 v 2	(0.12–0.52)	<0.001	(0.09–0.70)	0.058
	0.18		0.28	
	(0.07–0.48		(0.07–1.04)	
N Stage				
1 v 0	0.78	0.380	0.96	0.910
2 v 0	(0.45–1.36)	0.316	(0.45–2.03)	0.408
	0.74		1.43	
	(0.41–1.33)		(0.61–3.33)	
Hb*	1.24	0.002	1.29	0.012
	(1.08–1.43)		(1.06–1.58)	
Neutrophils	0.86	0.018	0.96	0.742
	(0.76–0.97)		(0.78–1.20)	
Lymphocytes	0.98	0.889	2.48	0.043
	(0.74–1.30)		(1.03–6.00)	
log(Alkaline Phosphatase)	0.31	0.008	0.31	0.040
	(0.13–0.74)		(0.10–0.95)	
Albumin	1.01	0.776	0.86	0.011
	(0.94–1.09)		(0.77–0.97)	
Monocyte	1.74	0.331	1.86	0.695
	(0.57–5.29)		(0.08–4.19)	
NLR	0.86	0.392	0.65	0.016
	(0.95–1.06)		(0.46–0.92)	
LMR	0.93	0.281	0.73	0.087
	(0.82–1.06)		(0.51–1.05)	
NMLR	0.99	0.751	1.23	0.003
	(0.95–1.03)		(1.07–1.41)	
BMI	1.05	0.052	0.93	0.285
	(1.00–1.10)		(0.81–1.06)	
Ant. Depth(cm)	1.07	0.103	0.85	0.234
	(0.99–1.17)		(0.65–1.11)	
Pos. Depth(cm)	1.16	0.093	0.93	0.708
	(0.98–1.38)		(0.63–1.36)	
Total Beam Depth (cm)	1.05	0.023	1.20	0.010
	(1.01–1.09)		(1.04–1.38)	

*Units of HB are g/dl.. Highlighted variables are variables with significant p-values either in univariable or multivariable analysis.

Checking for correlations

Prior to multivariable modelling, we test for co-linearity by correlating variable, and in particular, assessing correlations between the beam depth variables. To reduce the dimensionality of the data-set, we did a spearman's rank correlation matrix presented as heat maps. Figure S1 shows that the total Lateral depth, right Lateral depth, left lateral depth, and total depth all correlate strongly with each other

(minimum Spearman's Rho between those 4 is 0.83, the highest being 0.97). The anterior depth and AP depth are also highly correlated, Spearman's Rho = 0.86. The posterior depth does not have a strong correlation; therefore, we argued that we can reduce the radiotherapy depth variables from seven to three groups.

We additionally assessed correlations amongst blood parameters (Figure S2). The strongest correlation is between NMLR and Lymphocytes (Rho = -0.79). We argued that these correlations were not exceedingly high, and therefore, we entered all blood parameters into the multivariable model.

Multivariable modelling

The multivariable analysis is shown in Table 2. In addition to the findings from the univariable analysis, we noted that NLR, NMLR, lymphocyte count and albumin were all significantly associated with cCR.

Clinical utility

A nomogram using the above parameters was developed with a resulting ROC AUC of 0.75 (Fig. 2). The clinical utility of this nomogram is in deciding the likelihood of cCR from routine clinical variables collected at diagnosis for aid in treatment selection as demonstrated in Table 3. Patients with higher predicted chances of cCR will have neoadjuvant chemo-radiotherapy and those with a lower predicted chance of cCR from radiotherapy will have a more chemotherapy intensified neoadjuvant therapy in other to improve their outcomes.

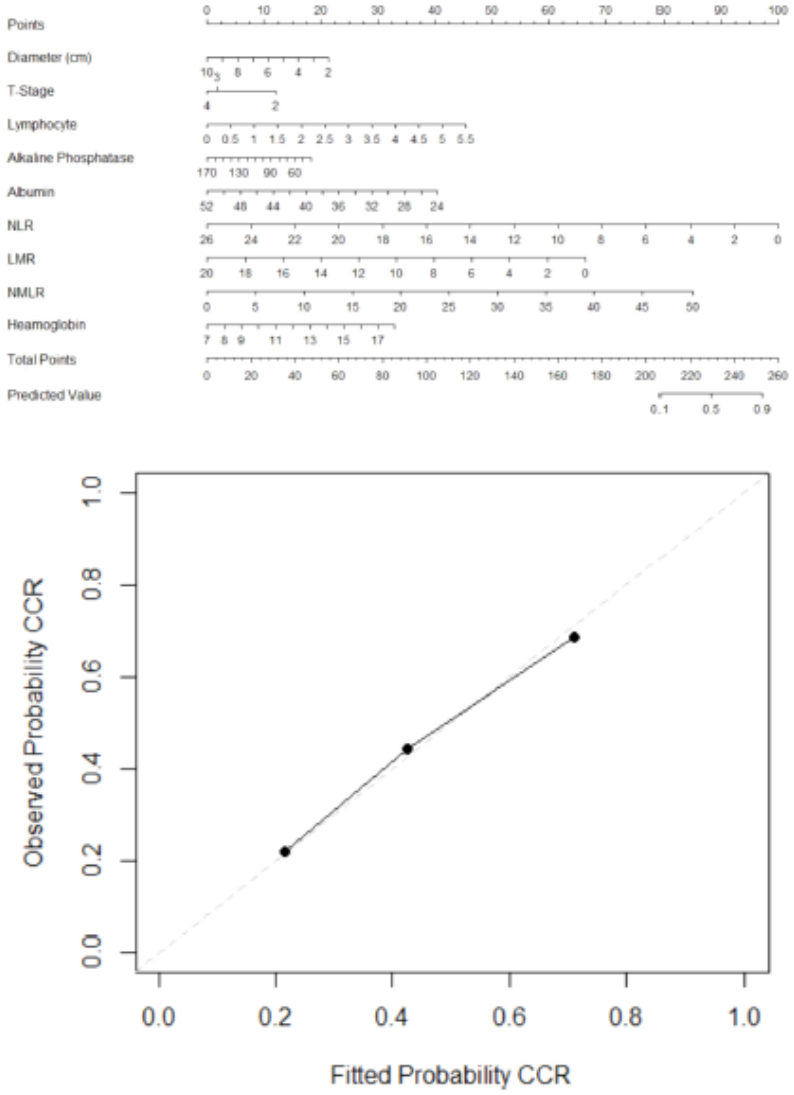

Fig. 2. Nomogram on prediction of cCR. ROC AUC = 0.75 (0.70–0.81).

Table 3
Clinical utility of nomogram.

Patient	Tumour Diameter (cm)	T-stage	Lymphocyte (x10g/l)	ALP (iu/l)	Haemoglobin (g/dl)	Albumin (g/l)	Neutrophil (X10g/l)	Monocyte (x10g/l)	Chance of cCR
A	2	T2	1.5	80	13.4	37	2.3	0.4	0.95
B	5	T2	1.5	80	13.4	37	2.3	0.4	0.90
C	5	T3	1.5	80	13.4	40	2.3	0.4	0.75
D	5	T3	1.5	80	10.4	40	2.3	0.4	0.5
E	10	T3	1.5	80	10.4	40	2.3	0.4	0.22
F	10	T3	1.5	80	10.4	40	8.5	0.4	0.11

Monocyte are used in the calculation of LMR and NMLR, neutrophil are used in the calculation of NLR and NMLR.

Discussion

Main findings

We found on multivariable analysis that tumour diameter, mrT-stage, serum haemoglobin level, serum alkaline phosphate, total radiotherapy depths, NLR, NMLR, albumin, and lymphocyte count are all significantly associated with clinical complete response. Some of these findings are consistent with retrospective studies that used pCR as its endpoints (Table S1). This is the first study to show serum alkaline phosphate (ALP), NMLR and radiotherapy depths have a significant association with complete response. This is also the first study to produce a nomogram for the prediction of cCR. We carefully used only routinely available clinical parameters in the nomogram. This nomogram will, over time require the substitution of some of its variables with more strongly associated routine variables before prospective validation in other to improve its predictability.

In context with rest of literature

Alkaline phosphate until now have not shown an association with response to treatment in rectal cancer, but there is evidence to show that elevated alkaline phosphate is a prognostic marker in colorectal cancer due to its association with liver metastasis[13]and its link with undetectable occult metastasis in the liver or bone[14]. Interpreting our result with respect to this, it could be that those with more elevated ALP have biologically more advanced disease at the start of their treatment which are unreflected in their stagging investigations. Our univariable and multivariable analysis showed a positive association between the total radiotherapy depths and cCR which is the first in litrature. This relationship is not fully understood. It could reflect the nutritional state of patients as their disease advances. Cancer is associated with weight loss and muscle loss, and it is clear that those losses increases as the disease progresses [15]. Two Systematic reviews[16] [17] have shown that sarcopenia (ie loss of muscle bulk which occurs in malignacies) is an adverse prognostic marker for survival in patients with colorectal cancer. The association between sarcopenia and the outcome of chemoradiotherapy in rectal cancer remains unclear. A small study of 61 patients showed that sarcopenia is a negative marker of pCR following chemoradiotherapy in locally advanced rectal cancer[18]. More studies are needed here, given that only seven patients out of 61 patients on this small study had pCR.

Neutrophils, lymphocytes, monocytes, platelets are all markers of inflammation. Systemic inflammation plays a significant part in cancer cell proliferation and the formation of metastasis[19]. Neutrophil, monocyte and platelets are all believed to promote cancer cell proliferation by their inflammatory activities; neutrophil stimulates circulating vascular endothelial growth factor[20], monocyte provides trophic factors for cancer growth proliferation[21], and platelets provide growth factor that aid cancer growth[22]. Lymphocytes, in contrast, have tumour suppressive properties by inducing cytokines that inhibit cancer cell proliferation[23]. So high neutrophils, monocyte and platelets favour cancer proliferation, but the opposite is the case for lymphocytes. High NLR and PLR due to lymphopenia are therefore expected to be a marker of poor prognosis but the results have been conflicting; Kim et al [24]. have shown NLR and PLR as both a prognostic and predictive marker for pCR, while the most extensive reported study looking at the prognostic and predictive impact of NLR and PLR in rectal cancer failed to replicate this association with pCR[25]. Our results showed that cCR has a negative association with NLR, which is similar to the results from Kim et al. [24].

Few studies have investigated building a predictive model using routine clinical variables[26] [27]. Zhang et al. [26].used a cohort of patients that had neoadjuvant chemotherapy alone (without radiotherapy), which is not the standard neoadjuvant treatment in rectal cancer and only a small proportion of patients (10/137) had a complete response. Sun et al [27]. developed a nomogram predicting pCR with a C-index of 0.81 and a drop off to 0.75 on validation. It also recruited a low proportion of patients with pCR which is only 16% of the database.

Strengths and limitations

This study recruited a 1:1 matched cohort of patients with cCR and those without which is lacking from the literature. It also recruited patients who had a clinical complete response, the target patient group for the organ preservation treatment pathway unlike other studies that used pCR which is a surgical outcome. This study is the first to investigate the relationship between achieving complete response and radiotherapy treatment depth to the best of our knowledge. As radiotherapy parameters are likely to influence treatment outcome significantly, the investigation of radiotherapy parameters other than the dose is expected to be important in predicting complete response.

There are notable limitations to this study; firstly, all patients in this study were treated in two cancer centres in the same region. A more diverse patient group would be expected to reduce selection bias. These biases were mitigated by the use of propensity matching to produce a homogenous patient group in the two comparative cohorts. This study investigated routine variables with a strong association with cCR to create a nomogram for cCR; it does lack an external validation cohort. Even though a few of the clinical variables investigated have similar results with previous external studies, external validation would have improved its reliability. This study also recruited patients that were treated over eleven year period. Over this decade the envitable variations in treatment selections and changes in the assay of the laboratory variables could have affected the results of this study.

Future research

The predictive abilities of the clinical variables used in our nomogram although it meets our pre-set target for clinical utility may need to be improved . Thus, there is a need to identify more routinely avalible variables significantly associated with cCR to form a more robust nomogram before a prospective validation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2022.100540.

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Chapter 7: Appendix 2; Protocol and approvals

7.1 Study protocol

Study Protocol

Optimisation of **R**adiotherapy to Achieve Increased Organ Preservation in **Re**ctal **C**ancer.

(ORREC)

Version 2.0

12th July 2019

Optimisation of Radiotherapy to Achieve Increased Organ Preservation in Rectal Cancer.

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List of abbreviations

BD- bis die (twice per day)

Ca- cancer

CCR- complete clinical response

Chemo- chemotherapy

CRC- Colo-rectal cancer

CRM- Circumferential resection margin

CRT- chemo-radiotherapy.

Gy- grays (unit of radiotherapy)

M^2- metre square

Mg- milligram

Mg/m2- milligram per metre square.

MR – Magnetic resonance

MRI- Magnetic resonance image/s

NHS- National Health Service

OnCoRe- Oncological Outcomes after Clinical Complete Response in Patients with Rectal Cancer) registry

PCR- Pathological compete response

QOL- Quality of life.

UK- United Kingdom

WaW- Watch and Wait

XRT- radiotherapy

XRT- radiotherapy.

7.1.1 Protocol summary

Title	Optimisation of radiotherapy to achieve increased organ preservation in rectal cancer.
Background	Radiotherapy has been used for the cure of cancer for many years. The current standard of care for anal cancer is chemo-radiotherapy following work pioneered by Nigro and his team in the 1970's[1]. Following this, patients with anal cancer have benefitted from organ preservation treatment strategy for many years. The standard of care for rectal cancer remains surgery mostly after either radiotherapy or chemo-radiotherapy. Surgery carries a risk of perioperative mortality, life-threatening early complications, such as anastomotic leak, long-term bowel, bladder, and sexual dysfunction. As a result, there is a drive both by patients and doctors involved in rectal cancer treatment towards organ preservation. The growing evidence on organ preservation in rectal cancer has been pioneered by Prof Habr-Gama and her team, from São Paulo in Brazil. In 2004, Habr- Gama[2] et al published their outcome of the non-surgical treatment strategy which is now termed 'Watch and wait'. They were able to show that the 5 years overall survival and disease free survival for these groups of patients who had cCR post chemo-radiotherapy and monitored on this treatment strategy were 100% and 92% respectively[2]. This was shown to be very similar to patients who had immediate surgery post radiotherapy treatment. With this evidence, it became important to increase the number of patients who are cured with chemo-radiotherapy treatment alone. Recent publication looking at 49 patients with locally advanced rectal cancer, 83% of patients would consider the deferral of surgery in the case of cCR[8]. 17% are either undecided or would like surgery in the case of cCR. This is not surprising as there remains a small subset of patients we see whom are psychologically happier when their tumour is removed and thrown away and as surgery is the only form of treatment that can achieve this; they will always prefer some surgery irrespective of the clinical evidence. Manchester has globally led in this research space through the OnCoRe project. Papers published through the OnCoRe database have been able to quantifying rates of local regrowth and oncological safety[3-6]. The overall evidence is that those patients who are monitored closely on the non-surgical pathway after cCR did not suffer any worsening oncological outcome compared to those that have immediate surgery. This has confirmed the oncological safety of the organ preservation strategy. Patients have been able to avoid or delay radical surgery which does come with multiple short and long term mortality and morbidity. This evidence from the OnCoRe database has given clinicians the confidence of using this approach. The most robust evidence would be to have a RCT comparing 'watch and wait' and immediate surgery but this is not feasible, large comparative analysis such as in the OnCoRe publications have helped in providing good evidence for this treatment strategy.

	There are about 9,000 new cases of rectal cancer per annum in the UK[11]. Patients undergo MRI of the pelvis to risk stratify their local treatment. For rectal cancer at high-risk of pelvic recurrence, neoadjuvant chemo-radiotherapy (CRT) 45Gy/25 fractions using a concurrent fluoropyrimidine chemotherapy (usually capecitabine 825mg/m2 BD daily dose) is recommended by NICE as standard of care[7]. These patients are assessed for response at 8 to 10 weeks after CRT with imaging including MR, and proceed to radical surgery at 10 to 12 weeks. For those that have cCR there is an option of the non-surgical pathway of 'watch and wait', if a regrowth occurs then they will have radical surgery. For Patients with intermediate risk of local recurrence they usually have short course radiotherapy (25Gy/5 fractions) followed by immediate surgery within 14 days of completion of their radiotherapy. Patient with low risk go straight to radical surgery. Broadly, patients can follow five types of response to CRT. They can have a cCR and not re-grow, a cCR and then regrows on follow-up, a pathological CR (pCR) if tumours are excised post treatment and no disease found, some incomplete response requiring surgery or no response at all. About 15-20% of patients that have chemo-radiotherapy end up with no residue disease (cCR) after their neo-adjuvant treatment and can be followed up without having to have surgery. These patients are monitored for at least five years in a surveillance protocol called 'Watch and Wait'. 30% of this group of patients will have regrowth of their tumour and require surgery within 2 years but the rest are cured with chemo-radiotherapy alone. About 84% of all patients who have rectal cancer surgery end up with a stoma, most are reversed after 12-18months but half will have the stoma life long and it cannot be reversed[11]. Patients don't like treatment that leaves them with long term side-effects and patients particularly don't like stomas as it affects their life both physically and emotionally. It is a constant reminder of their cancer diagnosis and it makes them withdrawn from their friends and society. Apart from the psychological effect of having a bag with their faeces on them, stomas usually have other issues such as occasional accidents in public places which can be a source of embarrassment for the patient. Part of our job as doctors is to constantly improve the treatment we give to our patients and provide our patients with the best possible clinical outcomes based on balanced clinical evidence. This study is designed to investigate how we can improve radiotherapy delivery in other to improve treatment response and increase organ preservation. This will be done through the understanding of how much the processes of radiotherapy account for the varying responses post treatment. Through the OnCoRe database, we have the largest UK database of patients who had a complete clinical response after chemo-radiotherapy. Interrogating this database will be beneficial in understanding why patients have better responses. We generally use re-growth to describe patient that had cCR and their disease returned and recurrence for patients that had surgery and their disease comes back locally. Pathological complete responses (pCR) are situations in which patients had surgery post neo-adjuvant radiotherapy +/- chemotherapy and histological examination of the surgical specimen shows no viable tumour.

Design	This will be an observational cohort study on retrospective data of patients with rectal cancer who received radiotherapy (+/- chemotherapy) treatment. The study will be using radiological images already acquired as part of diagnosis and follow up of patients together with planning images used for their treatment to investigate differences in disease activities between different cohorts of patients.
Objectives	- To investigate the relationship between dose and volume to treatment response and recurrences in rectal cancer. - To interrogate radiology images using MR scans pre and post radiotherapy to look at features that may predict treatment response and relapse in rectal cancer. This includes radiomics. We will use the OnCoRe database together with other series of patient treated with rectal radiotherapy to achieve these objectives.
Eligibility	Inclusion Criteria - Histologically confirmed rectal adenocarcinoma. - Received pelvic radiotherapy (+/- chemotherapy) as neo-adjuvant treatment - Age 18 and above - All patient's data and images will be imported through the Christie/NHS research and clinical databases. Exclusion Criteria - Other rectal pathologies.
Study Methods	The first step in this study will be to identify patients listed on the database who are eligible based on their patient identifier and available data regarding their disease. This will make use of the OnCoRe database and other clinical and research databases at the Christie NHS Foundation Trust. After identification of patients, the next step will be to acquire images, both their radiotherapy planning images and their pre and post radiotherapy clinical scans;

	A collective pool of images used for diagnosis and follow-up of all eligible patients on the database who meets the inclusion criteria will be collected from their various NHS imaging systems. The images will be transferred into the research system in a pseudonymised form and held in an encrypted password protected file, the transferred will either be electronically or via CD. This form of data transfer will allow identification of patients by a unique identifier number without personal details. Only routinely performed images which have been obtained for diagnosis and follow-up care of these patients will be used. A second collective pool is to obtain the radiotherapy plans of all eligible patients from their various radiotherapy centres. These planning images will have a record of the location and shape of the treated volume, as well as their planned dose distributions. Information on compliance with treatment and number of treatment fractions actually received will also be obtained. Patients will then be categorized using the clinical information held in the database according to their risk profile. The next step after this will be registration of the images into specially designed software which allows co-matching of the two sets of images. The software also allows contouring the areas of regrowth or recurrence and comparison with the area of the initial disease. The volume treated will also be measured. Using the co-registration, information on the dose gradient, distances and dose to different structures in the pelvis can be calculated. The relationship between the radiotherapy parameters in the areas of regrowth will be analysed using statistical tools to see if the regrowth is associated to differences in the treatment and delivery of radiotherapy. The significance of doses to different areas in the pelvis can also be analysed to see if this is related to disease activity in rectal cancer. The relationship between radiotherapy received in different groups of patients can also be compared. Analysis of the MRI images will also be undertaken to investigate imaging biomarkers that will be predictive of treatment response in rectal cancer. This will take the form of using specialised software for radiomics to identify these features. As part of the process of continuing to develop the work on imaging biomarker and disease activities, the following process will be undertaken: 1. To test the feasibility of using the OnCoRe database to set up a retrospective MR imaging based study, capturing routinely performed MR scans across many centres, establishing a central repository, with clinical complete response and local regrowth after watch and wait management as the central clinical setting of interest. 2. To develop and validate operating protocols to capture radiomics data from routinely performed MR images in patients with rectal adenocarcinoma, undergoing chemo-radiotherapy, with and without clinical complete response, and with and without local regrowth after watch and wait management 3. Once no. 1 and no. 2 are established, to then quantify the performance characteristics of the captured radiomics data from routinely performed MR images for the endpoint of clinical complete response and local regrowth after watch and wait management

	4. To then re-test the quality of the data from the OnCoRe database, and evaluate whether the signatures identified in no. 3 are confounded by known clinical and treatment factor like tumour size and radiotherapy dose.
Rationale	The ultimate rationale to this study is the need to increase the number of patients achieving cCR significantly to save patients from the toxicity of surgery and the psychological effects of having a stoma bag. We will also aim to predict using routinely acquired radiological images through radiomics the likelihood of response to treatment which will enable us to select patients for appropriate neo-adjuvant treatment and consider intensifying treatment for the good responders. The likely implications would be that patients more likely to have response to radiotherapy treatment would have radiotherapy (+/- chemotherapy); patients who are at high risk of not responding to radiotherapy will then have doublet neo-adjuvant chemotherapy for their down staging treatment. Through this study, looking at the relationship between radiotherapy planning and response to radiotherapy treatment, we will be able to add some evidence to the improvement of our planning protocols for better delivery of radiotherapy. Two meta-analysis of chemo-radiotherapy in rectal cancer have shown that increased radiotherapy dose or the use of additional chemotherapy were associated with higher rate of pCR[9-10]. With dose escalation, reduction of volume is important in other to be able to safely increase the dose without impacting on toxicity. Reduction of volume can only be achieved with knowledge of the areas of recurrences and the areas with the least risk of recurrence. This study will be able to provide this knowledge base to enable hypothesis of dose escalation. Through the use of radiomics, this study will also aim to provide sensitive signatures that predict cCR as well as signatures that will predict non-response to radiotherapy treatment using routinely acquired images. This will provide a good knowledge base in selecting appropriate treatment for patients.
Study Duration	20 months
Sample Size	1500
Funding	Funding will be from the lower GI Christie hospital charitable fund.

7.1.2 Imaging

Quality of MR Images

1.5 or more tesla MR scan with optimum pelvic images will be used. The preferred sequences of images will be obtained, most likely T2 weighted sequences, and with slice thickness ≤4mm. The field of view in the scans must extend to cover at least 2cm above and below the tumour in all planes to allow complete tumour assessment. The series of the images should overlap sufficiently for a complete assessment of the whole tumour area. The image interpretation will be as reported by the reporting radiologist. Any disagreement with interpretation will be verified by Dr Rohit Kochhar, consultant radiologist who has many years' of experience in pelvic image interpretation and is a co-investigator of this study. Poor quality images will be excluded.

Image Registration

This area of research will be carried out in collaboration with Professor Marcel van Herk, Professor of Radiotherapy Physics, and his team at the radiotherapy related research, at the University of Manchester. Prof van Herk and Dr Vasquez Osorio are both co-investigator of this study.

This step in the study will make use of specialised software which is developed in this field. The first step will be to register the acquired images into the software. The next step will be delineations from the planning CT images of the treatment volume and copying in the dose distribution from patient's planning images. Before this, the dose distribution will need to be adjusted to account for treatment fractions not completed during the patient's treatment. A 3D mapping using anatomical landmarks will be used to allow analysis based on distances and directions from the target areas. A template will be made based on one of the patients to allow anatomical mapping of the areas of recurrences or regrowth of different patients into one image. The points on the mapping will represent the central point of the area of the disease. Mapping will allow the calculation of the average dose distribution and standard deviations to the point of interest. Calculation of treatment volumes will also be done using specialised software designed for this application. Volume calculations will allow the analysis to see the effect of treated volume to outcomes.

Image registration and mapping systems allows analysis to establish the predictor of outcomes in rectal radiotherapy based on the patient scans and their radiotherapy planning dose distributions.

Radiomics

With the move from printed radiological films to computerised images, radiomics and radiogenomics has become an important area in oncology. Radiomics is the study of various imaging features and characteristics which can be used to predict response to treatment and clinical outcomes. There has been studies done on the use of radiomics in predicting response post chemo-radiotherapy but the main issues are the reproducibility and consistency of these studies and the signatures found. The development of a consistent and highly selective signature in this group of patients will go a long way in this era of personalised medicine. The ability to select the more appropriate neo-adjuvant treatment will spare patients from toxicities of treatments that they do not need.

Currently chemo-radiotherapy is given to locally advanced rectal cancer patients with CRM positive disease. About 70-75% of patients have a successful down staging with varying degree of responses to treatment, the other 25-30% whom critically needs down-staging before surgery but have not

responded to radiotherapy will have neo-adjuvant chemotherapy treatment if they are fit to try to achieve a successful down staging of their disease. Radiotherapy treatment carries various acute and long-time toxicities. If we can predict those that won't response, then we could spare them the toxicities of radiotherapy and go straight to neo-adjuvant chemotherapy. Alternatively we could reduce their risk of radiotherapy by the use of shorter and lower dose of radiotherapy with a longer course of chemotherapy as their neo-adjuvant treatment. We are also aware based on the available data that about 30% of patients who have cCR will have a regrowth in the first 2-3yrs post treatment. Being able to predict which patients most are at risk of regrowth will mean more intense surveillance for this group of patients and considerations regarding possible local excision or more intense radiotherapy could be made. The clinical implications of a consistent and reproducible radiomics trial will help to make these decisions.

The OnCoRe database and other series of patients who had rectal radiotherapy will be used to select patients who have achieved clinical complete response, those that have had regrowth post treatment as well as patients who have not responded to radiotherapy treatment. Using the T2 weighted MR images and DWI images acquired from diagnosis and imported into specialised software for mapping the area of disease, analysis of the qualitative features of the tumour could help develop different signatures which can predict both responses to treatment and risk of regrowth. This will enhance personalised treatment and will hopefully improve survival in rectal cancer.

7.1.3 Functionality Outcome

One of the important outcomes in cancer treatment relates to the toxicity that patient are left with post treatment. Radiotherapy and surgery comes with risk to local organs which could be acute or long term. Long term toxicities carry a significant effect to patient's quality of life long after their treatment. It has been known that doses to different structures in the pelvis are related to differing toxicities. Toxicities relating to radiotherapy and surgery include urinary symptoms, bowel and sexual symptoms. These symptoms vary from mild urinary and bowel symptoms to significant incontinence having significant effect to quality of life (QOL). Good knowledge of how to quantify the radiotherapy contributions to these risks based on doses to different structure in the pelvis will be very important. Patients mentioned on the watch and wait approach who have only had radiotherapy (+/- chemotherapy) as their only treatment for their rectal cancer, offers an opportunity to be able to quantify the contribution of radiotherapy to overall toxicity level without the confounder of surgical intervention. The availability of patients' planning scans and the knowledge of dose gradient through image registration will allow the analysis of doses to different parts of the pelvis compared with the toxicities reported by patients.

To achieve accurate analysis of long term toxicity in patients, these toxicities will need to have been measured pre-treatment and years after treatment. In order to do this, this study will look at patients treated on a standard of care phase 1 trial- DREAM trial and other series of patients treated with radiotherapy (+/- chemotherapy) for rectal cancer where the pre and post- treatment toxicity records are available. The toxicity records will be compared to the dose gradient from their radiotherapy plans to evaluate threshold doses that equate to differing toxicities.

7.1.4 Risks, burdens and benefits

As the study will use retrospective data and images acquired as standard of care for diagnoses and follow-up of patients without any direct patient intervention, the risk to patient is very low. We appreciate the importance of confidentiality and the possible distress to patients that could occur if there are breeches so will therefore take every step to minimise this. The risk of breach of confidentiality is also very low in this study as patient's data are pseudonymised with no direct personal details before transfer to the research database.

There is no direct benefit to patients whose data will be included but the possible increased in the proportion of patients not requiring surgery for their rectal cancer will improve both the quality of care we deliver as well as the quality of life for patients with rectal cancer in the future.

7.1.5 Confidentiality and Consent

This research is focused on using data information and scans which have already been taken for the diagnosis and follow-up of patients as per standard of care so no intervention and change in standard treatment will be required. This will include data that has been collected routinely as part of standard clinical care, clinical audit or held on the OnCoRe database as per the ethics approval of the database.

We will be looking at approximately 1500 patients in total. Obtaining consent for these patients will be in-practical as patients were treated in different locations in the North West over many years. They are also followed up at different locations in the country and most would have been discharged from routine follow-up. Having to consent them will require obtaining information such as addresses and personal details which will increase the risk of breech in confidentiality which we will like to minimise as much as possible. Also most of the patients that undergo chemo-radiotherapy will be patients with stage 3 rectal disease. The 5yrs survival for stage 3 rectal cancer patients will be around 60-65% therefore, a significant amount of patient will be decreased. There is also always a few weeks lag between patient's death and the record being updated in the hospital systems to show that a patient is deceased, which means that contact could be made to family members of patients whom are recently deceased by mistake, this will leave patient's relatives distressed at a difficult period of time.

Therefore based on the above reasons, we believe that trying to obtain consent for this retrospective non-interventional study on balance will not be practicable and could increase the risk of the trial.

7.1.6 Data Management

Radiology images that will be used will be pseudonymised before transfer outside the clinical database. The images transferred will be secured by an encryption with a password. The data will be held on an encrypted password secured file on the Christie hospital/ Manchester university network system. The data held will only be identifiable by a unique identifier which will not be NHS or

hospital number. The unique identifier will be needed for analysis of that patient's data without any link to personal information. The hospital number corresponding to the unique identifiers will be stored in a different file which will be encrypted with a password held in the Christie hospital system and only accessible by the chief investigator. In accordance with data protection policies of the University of Manchester, the data will be securely stored for a period of 10 years and destroyed. The data generated by the study will be analysed by the core research team. No identifiable personal data will be present during analysis.

7.1.7 Statistical analysis

Continuous data as appropriate will be summarized with mean, medians, standard deviations, interquartile ranges and ranges. Categorical data will be analyzed with frequency of occurrence and associated percentages. Both data will be analyzed using chi-squared tests, logistic regression, T-test, Mann-Whitney tests or other statistical tests as appropriate.

Oncological outcome will be presented with time-to-event statistics, Kaplan-Meier curves, log-rank testing and Cox regression analysis. Relative risk analyses can be achieved using Cox regression and absolute risk with Nelson-Aalen estimates.

Consideration will be made to the use of statistical strong matching techniques such as propensity score and case matching if appropriate.

7.1.8 Statements

Publication Policy

The results of this study will form part of MD thesis and will also be submitted to peer review journals for publication and will be presented at national / international conferences.

Statement of Indemnity

The University of Manchester will arrange insurance for this research. The insurance cover is available for research sponsored, managed, designed or conducted by, or on behalf of, subject to policy terms and conditions.

Peer Review

The study was approved for funding by the Christie NHS Foundation Trust charitable group following presentation of the study plan. It was also reviewed and approved by the Manchester cancer research (MCRC) educational committee.

7.1.9 References

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7.2 HRA Approvals

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

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19 September 2019

Dear Dr Mbanu

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Optimisation of Radiotherapy to Achieve Increased Organ Preservation in Rectal Cancer. (ORREC)**
IRAS project ID:	**265989**
Protocol number:	**n/a**
REC reference:	**19/NW/0539**
Sponsor	**The University of Manchester**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **265989**. Please quote this on all correspondence.

Yours sincerely,
Anna Bannister

Approvals Specialist

Email: hra.approval@nhs.net

Copy to: *Ms Lynne Macrae*

Notification of Non-Substantial/Minor Amendments(s) for NHS Studies

This template **must only** be used to notify NHS/HSC R&D office(s) of amendments, which are **NOT** categorised as Substantial Amendments.
If you need to notify a Substantial Amendment to your study then you MUST use the appropriate Substantial Amendment form in IRAS.

Instructions for using this template

- For guidance on amendments refer to http://www.hra.nhs.uk/research-community/during-your-research-project/amendments/
- This template should be completed by the CI and optionally authorised by Sponsor, if required by sponsor guidelines.
- This form should be submitted according to the instructions provided for NHS/HSC R&D at http://www.hra.nhs.uk/research-community/during-your-research-project/amendments/which-review-bodies-need-to-approve-or-be-notified-of-which-types-of-amendments/ . If you do not submit your notification in accordance with these instructions then processing of your submission may be significantly delayed.

1. Study Information

Full title of study:	*Optimisation of Radiotherapy to Achieve Increased Organ Preservation in Rectal Cancer. (ORREC)*
IRAS Project ID:	*265989*
Sponsor Amendment Notification number:	
Sponsor Amendment Notification date:	
Details of Chief Investigator:	
Name [first name and surname]	Dr Peter I Mbanu
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Details of Lead Sponsor:	
Name:	The University of Manchester
Contact email address:	fbmhethics@manchester.ac.uk
Details of Lead Nation:	England
Name of lead nation *delete as appropriate*	*England*
If England led is the study going through CSP? *delete as appropriate*	*If your study is CRN eligible (and you have received confirmation of this) please add details here.* *N/A*
Name of lead R&D office:	

Research and Development Team
The Oglesby Cancer Research Building
The Christie NHS Foundation Trust
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M20 4QL

Summary of amendment(s)

This template **must only** be used to notify NHS/HSC R&D office(s) of amendments, which are **NOT** categorised as Substantial Amendments.

If you need to notify a Substantial Amendment to your study then you MUST use the appropriate Substantial Amendment form in IRAS.

2. Summary of amendment(s)
This template must only be used to notify NHS/HSC R&D office(s) of amendments, which are NOT categorised as Substantial Amendments.
If you need to notify a Substantial Amendment to your study then you MUST use the appropriate Substantial Amendment form in IRAS.

No.	Brief description of amendment *(please enter each separate amendment in a new row)*	Amendment applies to *(delete/ list as appropriate)*		List relevant supporting document(s), including version numbers *(please ensure all referenced supporting documents are submitted with this form)*		R&D category of amendment *(category A, B, C) For office use only*
		Nation	Sites	Document	Version	
1	*Amended needed to add two additional NHS hospitals as site in the study.* *a)Lancashire Teaching Hospitals NHS Foundation Trust.* *Sharoe Green Lane* *Fulwood* *Preston* *PR2 9HT* b)Clatterbridge Cancer Centre – Wirral: Clatterbridge Road, Bebington, Wirral, CH63 4JY	England	Original site- Christie Hospital Additional sites via this amendment- Lancashire Teaching Hospital Clatterbridge Cancer Centre	*Please list the document title here*	*Please give the version number and date here*	
2		*Add detail here, as above in this column, and in every row that you add*				
3						
4						
5						

[Add further rows as required]

1. Declaration(s)

Declaration by Chief Investigator

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendment(s) to be implemented.

Signature of Chief Investigator: 

Print name: Peter Mbanu

Date: 06/12/2019