# Radiological Approaches to Personalise Neoadjuvant Therapies in Colon Cancer

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Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

School of Medicine

May 2026

# Intellectual Property and Publication Statements

I confirm that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others.

An alternative format thesis was chosen due to the successful publication of research outputs prior to thesis production. The manuscripts each represent self-contained pieces of academic work that stand alone but effectively combine into a coherent thesis under an overarching theme.

The thesis comprises an introduction, four manuscripts (chapters) containing original research, and a critical discussion. Supplementary material is provided for the corresponding manuscripts as appendices at the end of the thesis. The included manuscripts are detailed below, with contributions described according to the Contributor Roles Taxonomy (CRediT).

1. Title: CT Staging Performance in an International Trial of Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer

   Authors: James R Platt, Faye Elliott, Kelly Handley, Laura Magill, Philip Quirke, Matthew T Seymour, Nicholas P West, Dion Morton, Jenny Seligmann, Damian JM Tolan

   Date: 23rd August 2025

   Journal: British Journal of Radiology

   Contribution: James R Platt - conceptualization, data curation, investigation, methodology, project administration, visualization, writing - original draft preparation, writing - review & editing.

   Contribution of other authors: Faye Elliott - data curation, formal analysis, writing - review & editing; Kelly Handley - writing - review & editing; Laura Magill - writing - review & editing; Philip Quirke - writing - review & editing; Matthew T Seymour - writing - review & editing; Nicholas P West - writing - review & editing; Dion Morton - writing - review & editing; Jenny F Seligmann - supervision, writing - review & editing; Damian JM Tolan - supervision, writing - review & editing.

2. Title: Baseline Clinicopathological and Imaging Predictors of Disease Recurrence in Locally Advanced Colon Cancer: a Post Hoc Analysis of the FOxTROT Trial

   Authors: James R Platt, Faye Elliott, Russell Frood, Andrew F Scarsbrook, Jenny F Seligmann, Damian JM Tolan

   Date: Not applicable (draft manuscript pending submission)

   Journal: Not applicable (draft manuscript pending submission)

   Contribution: James R Platt - conceptualization, data curation, investigation, methodology, project administration, visualization, writing - original draft preparation, writing - review & editing.

   Contribution of other authors: Faye Elliott - data curation, formal analysis, visualization, writing - review & editing; Russell Frood - supervision, writing - review & editing; Andrew F Scarsbrook - supervision, writing - review & editing; Jenny F Seligmann - supervision, writing - review & editing; Damian JM Tolan - supervision, writing - review & editing.

3. Title: Characterising Radiological Response to Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer in the International FOxTROT Trial

   Authors: James R Platt, Faye Elliott, Russell Frood, Andrew F Scarsbrook, Jenny F Seligmann, Damian JM Tolan

   Date: Not applicable (draft manuscript pending submission)

   Journal: Not applicable (draft manuscript pending submission)

   Contribution: James R Platt - conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualization, writing - original draft preparation, writing - review & editing.

   Contribution of other authors: Faye Elliott - data curation; Russell Frood - supervision, writing - review & editing; Andrew F Scarsbrook - supervision, writing - review & editing; Jenny F Seligmann - supervision, writing - review & editing; Damian JM Tolan - investigation, validation, supervision, writing - review & editing.

4. Title: Risk of Bowel Obstruction in Patients with Colon Cancer Responding to Immunotherapy: an International Case Series

   Authors: James R Platt, Joel Allotey, Emily Alouani, James Glasbey, Rossana Intini, Sara Lonardi, Giacomo Mazzoli, Anna Maria Militello, Dominik P Modest, Juliette Palle, Filippo Pietrantonio, Kallingal Riyad, Leslie Samuel, Anne V Schulze, Kai-Keen Shiu, Julien Taieb, Damian JM Tolan, Nicholas P West, Alice C Westwood, Christopher J M Williams, Jenny F Seligmann

   Date: 5th September 2024

   Journal: ESMO Open

   Contribution: James R Platt - conceptualization, data curation, investigation, methodology, project administration, visualization, writing - original draft preparation, writing - review & editing.

   Contribution of other authors: Joel Allotey - writing - review & editing; Emily Alouani - writing - review & editing; James Glasbey - writing - review & editing; Rossana Intini - writing - review & editing; Sara Lonardi - writing - review & editing; Giacomo Mazzoli - writing - review & editing; Anna Maria Militello - writing - review & editing; Dominik P Modest - writing - review & editing; Juliette Palle - writing - review & editing; Filippo Pietrantonio - writing - review & editing; Kallingal Riyad - writing - review & editing; Leslie Samuel - writing - review & editing; Anne V Schulze - writing - review & editing; Kai-Keen Shiu - writing - review & editing; Julien Taieb - writing - review & editing; Damian JM Tolan - supervision, writing - review & editing; Nicholas P West - writing - review & editing; Alice C Westwood - writing - review & editing; Christopher J M Williams - writing - review & editing; Jenny F Seligmann - supervision, writing - review & editing.

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# Acknowledgements

I would like to thank my supervisors Professor Jenny Seligmann, Dr Damian Tolan, Professor Andrew Scarsbrook and Dr Russell Frood for their unwavering support throughout the last three years. Their insights and encouragement inspired me to overcome numerous challenges and have motivated me to pursue a future career in colon cancer research. While their academic supervision has been invaluable to this thesis, I am even more grateful to have them as personal mentors.

I am also grateful to Ms Faye Elliott for dependable statistical support, and the wider FOxTROT trial management group for their consistent encouragement of my research.

This work would not have been possible without funding from Yorkshire Cancer Research, which continues to support vital clinical trials for patients affected by colon cancer. Likewise, thank you to the patients who participated in the FOxTROT trial, who have helped re-define the treatment of colon cancer for future generations.

I must also thank Dr Dan Lee and Dr Jane Hook, my former and current Training Programme Directors for Medical Oncology in West Yorkshire, for their frictionless support in taking time out of training to pursue this PhD.

Finally, I must thank my partner, Dr Rosie McNeillis, for her resolute support, encouragement and patience, which she gave unconditionally through challenging periods of her own. I would not be where I am today, nor have completed this PhD without you. Thank you for keeping me well-fed, providing much-needed distractions and offering your excellent proofreading skills when it mattered most.

I acknowledge the use of NotebookLM (Google, https://notebooklm.google/) to proofread my final draft.

# Abstract

Colon cancer (CC) is the fourth most common cancer type worldwide, with over one million new diagnoses each year. Although localised disease can be cured with a combination of surgery and adjuvant chemotherapy, up to 30% of patients experience disease recurrence, with unacceptable levels of subsequent mortality. Neoadjuvant therapies represent a much-needed breakthrough in CC treatment, exemplified by the recent FOxTROT trial which has established neoadjuvant chemotherapy (NAC) as a new treatment option for locally advanced disease.

The use of NAC in CC represents a major shift in practice with several challenges to wider clinical implementation. This transition has introduced several potential new roles for radiology in prognostication, patient stratification and response evaluation. Yet, the evidence supporting these applications is underdeveloped, particularly as CC enters an era of precision oncology. The aims of this thesis are to explore the roles of radiology in the neoadjuvant setting, identify opportunities to personalise treatment and provide clinically relevant data to inform future clinical practice and trial design.

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# Abbreviations

| | |
|---|---|
| 5FU | 5-fluorouracil |
| AC | Adjuvant chemotherapy |
| ACPGBI | Association of Coloproctology of Great Britain and Ireland |
| AI | Artificial intelligence |
| AJCC | American Joint Committee on Cancer |
| CAPOX | Capecitabine & oxaliplatin |
| CC | Colon cancer |
| CEA | Carcinoembryonic antigen |
| CI | Confidence intervals |
| CMS | Consensus molecular subtypes |
| CRC | Colorectal cancer |
| CRF | Case report form |
| CT | Computed tomography |
| CTC | Computed tomography colonography |
| ctDNA | Circulating tumour deoxyribose nucleic acid |
| DFS | Disease-free survival |
| dMMR | Deficient mismatch repair |
| DNA | Deoxyribose nucleic acid |
| ECOG | Eastern Cooperative Oncology Group |
| EGFR | Epidermal growth factor receptor |
| EMVI | Extramural venous invasion |
| ERK | Extracellular signal-regulated kinase |
| ESGAR | European Society of Gastrointestinal and Abdominal Radiology |
| ESMO | European Society of Medical Oncology |

| | |
|---|---|
| FDG | Fluorodeoxyglucose F18 |
| FIT | Faecal immunohistochemical testing |
| FOLFOX | 5-fluorouracil & oxaliplatin |
| FOxTROT | Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy |
| HER | Human epidermal growth factor receptor |
| HR | Hazard ratio |
| HU | Hounsfield units |
| IQR | Interquartile range |
| MAPK | Mitogen-activated protein kinase |
| mCRC | Metastatic colorectal cancer |
| MDT | Multidisciplinary team |
| MMR | Mismatch repair |
| MPR | Multiplanar reconstruction |
| MRI | Magnetic resonance imaging |
| MSI | Microsatellite instability |
| NAC | Neoadjuvant chemotherapy |
| NAI | Neoadjuvant immunotherapy |
| NAT | Neoadjuvant therapy |
| NCCN | National Comprehensive Cancer Network |
| NICE | National Institute for Health and Care Excellence |
| NPV | Negative predictive value |
| OS | Overall survival |
| PD-L1 | Programmed cell death-ligand 1 |
| pCR | Pathological complete response |
| PET | Positron emission tomography |

| | |
|---|---|
| pMMR | Proficient mismatch repair |
| PPV | Positive predictive value |
| PS | Performance status |
| RADS | Reporting and data system |
| RCR | Royal College of Radiologists |
| RCT | Randomised controlled trial |
| RECIST | Response Evaluation Criteria in Solid Tumours |
| SD | Standard deviation |
| SIGN | Scottish Intercollegiate Guidelines Network |
| STS | Straight-to-surgery |
| SUV | Standardised uptake value |
| TD | Tumour deposit |
| TNM | Tumour, node, metastasis |
| TRG | Tumour regression grade |
| TTR | Time to recurrence |
| UICC | Union for International Cancer Control |
| UK | United Kingdom |

# 1 Introduction

Modern healthcare is characterised by increasingly complex diseases that require inter-disciplinary collaboration, coordination and care to ensure safety and wellbeing in evolving patient populations. Colon cancer (CC) exemplifies this premise, presenting a major global health issue with impact and scale beyond most other malignancies. Through advancements in treatment, technology and healthcare policy, CC is a treatable, and in many cases curable, disease. Yet, morbidity and mortality related to CC remain unacceptably high. Multidisciplinary team (MDT) engagement is pivotal and continues to drive innovation and practice-changing research in this field. The interface between oncology and radiology is a particularly exciting cross-specialty venture with considerable research potential to improve outcomes for patients with CC as we enter an era of neoadjuvant treatment.

## 1.1 Localised Colon Cancer

### 1.1.1 Epidemiology & Aetiology

As the most common abdominal malignancy and fourth most common cancer type worldwide, CC accounts for over one million new diagnoses and half a million deaths each year.[1] The disease is more common in men and in older age, with most diagnoses made in adults over 70 years old.[2] Other risk factors for CC include obesity, alcohol excess, a family history of CC, inflammatory bowel disease, hereditary colorectal cancer (CRC) syndromes (e.g. familial adenomatous polyposis and Lynch syndrome), smoking and red meat consumption, with around half of cases considered preventable.[3, 4]

While CC incidence has remained stable over recent decades, declining rates amongst older adults are counterbalanced by an alarming rise in younger adults, with CC now the leading cause of cancer death in those under 50.[1, 5, 6] Often termed early-onset CC, various underlying factors have been suggested with increasing evidence that it represents a clinically and biologically distinct disease where new therapeutic and public health strategies may be required.[7]

### 1.1.2 Biology Relevant to Treatment

#### 1.1.2.1 Carcinogenesis & Genomic Pathways

The development of CC is characterised by the adenoma-carcinoma sequence, first described by Fearon and Vogelstein in 1990, where the stepwise accumulation of genetic or epigenetic changes causes normal epithelium to transform into a benign polyp followed by invasive carcinoma (Figure 1.1).[8, 9] The understanding of this process has evolved over time such that the genomic instability is now thought to be driven by one of three, albeit not mutually exclusive, pathways: chromosomal instability (CIN), microsatellite instability (MSI) or serrated neoplasia.[10-12]

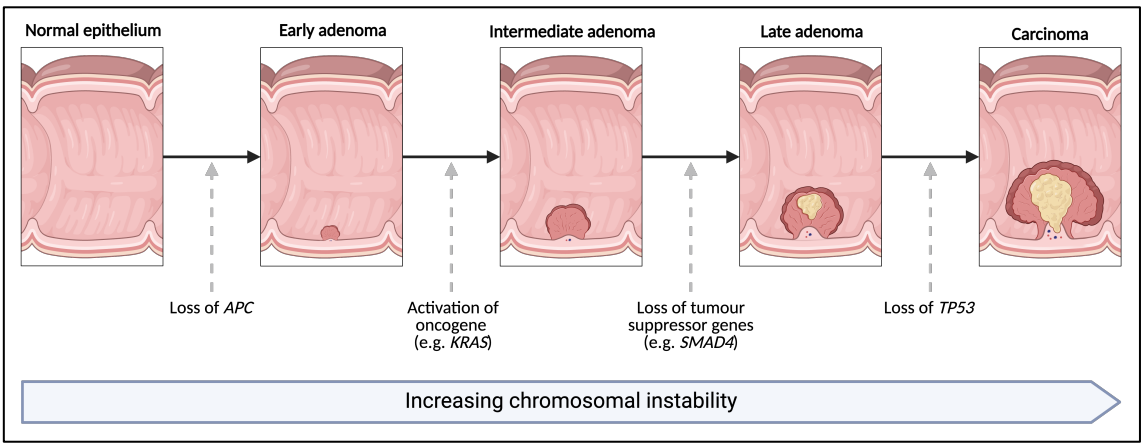



**Figure 1.1 Adenoma-Carcinoma Sequence**
Colon cancer carcinogenesis is characterised by the adenoma-carcinoma sequence, where a series of genetic 'hits' transform normal colonic epithelium to a benign adenoma followed by invasive carcinoma. Typical genetic events are shown in the figure. The process of chromosomal instability, which accounts for most colon cancer diagnoses, is closely aligned with the adenoma-carcinoma sequence. The microsatellite and serrated neoplasia pathways follow different multistep sequences. Created in https://BioRender.com.

Most CCs exhibit chromosomal instability, which involves changes in the number and structure of whole chromosomes.[13] This pathway is closely related to the classic adenoma-carcinoma sequence, where a characteristic series of mutations affect key tumour suppressor genes and oncogenes (Figure 1.1). Inactivation of the *APC* tumour suppressor gene is usually the initiating event which is followed by an activating mutation of a key oncogene (e.g. *KRAS*) and loss of tumour suppressor genes (e.g. *SMAD4* and *TP53*) for complete carcinogenesis.

Deficiency in mismatch repair (MMR), a physiological deoxyribose nucleic acid (DNA) repair mechanism, arises due to germline or somatic alterations affecting one or more of the MMR

proteins: MLH1, MSH2, MSH6 and PMS2.[14] The resulting accumulation of mutations within short sections of repeated DNA, known as microsatellites, leads to the development of the molecular phenotype MSI.

Around 15% of colorectal cancers develop via the serrated neoplasia pathway.[10] Serrated polyps are a distinct set of precancerous lesions with saw-toothed architecture and are often challenging to detect due to their flat and sessile morphology. CCs arising from the serrated neoplasia pathway are characterised by *BRAF V600E* mutations and high levels of CpG island methylation phenotype. Subsequently, these tumours either converge with the MSI pathway due to hypermethylation and silencing of tumour suppressor genes (e.g. *MLH1*) or acquire a *TP53* mutation and activate further oncogenic pathways.

Recent advances have highlighted the importance of gut microbiome, which has numerous physiological roles in supporting nutrient metabolism, pathogen eradication and immune priming, for CC development.[15] Disruption of the gut microbiome ('dysbiosis') is now widely accepted as a strong driver of CC carcinogenesis through the creation of an inflammatory microenvironment, immune dysregulation, direct genotoxic effects, and an imbalanced production of harmful and protective metabolites.[16] Western diet and lifestyle factors are heavily implicated in gut dysbiosis, which may contribute to disparate CC incidence between nations.[17] Compared to those without, patients with CRC have a distinct microbiome, suggesting potential diagnostic utility.[18] Accumulating evidence demonstrates the impact of gut microbiome on treatment efficacy, particularly immunotherapy, and that optimising this microenvironment (e.g. through dietary interventions) has the potential to enhance treatment effects.[19] While the relationship between CC development and the gut microbiome is becoming clearer, there is a need to move towards more mechanistic and translational research to identify effective opportunities for patient benefit.

### 1.1.3 Molecular Classification

Genetic alterations during carcinogenesis have direct effects on cellular behaviour and therapeutic vulnerabilities, for which molecular biomarker testing is now routine. The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, a series of intracellular proteins connecting the cell surface and nucleus to support physiological cell proliferation, growth and survival, is often impacted by these events (Figure 1.2).[20] Activation of the MAPK/ERK pathway occurs through ligation of the epidermal growth factor receptor (EGFR), which is part of the human epidermal growth factor receptor (HER)

family. Mutations affecting MAPK/ERK proteins are found in a range of malignancies, including CC, and cause constitutive activation of the pathway and subsequent tumour-promoting processes.[20] Of clinical relevance in CC, the RAS and RAF protein families are often affected, with implications for the use of targeted therapies.[21]

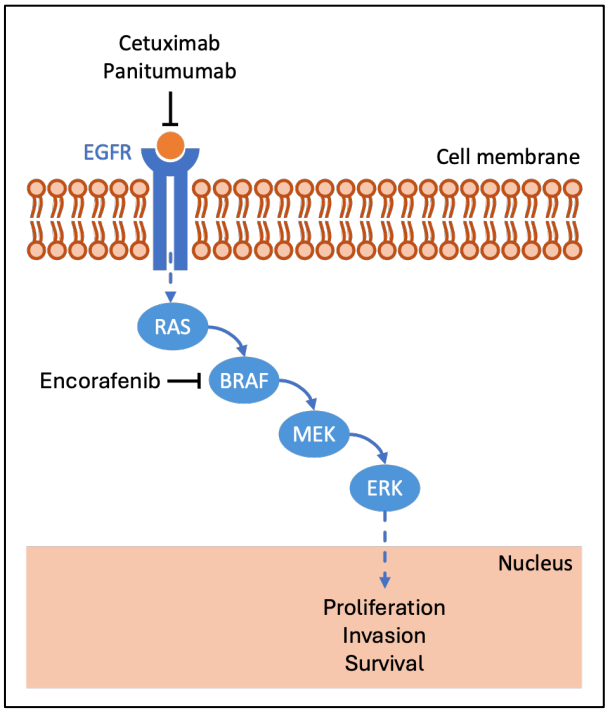



**Figure 1.2 Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Pathway**
The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway is a cascade of intracellular proteins which regulates normal cellular processes. Genetic alterations can affect different parts of the pathway and may cause constitutive downstream activation of these processes to facilitate tumour development. EGFR = epidermal growth factor receptor.

#### 1.1.3.1 RAS

The RAS proteins are encoded by the correspondingly named *RAS* oncogenes (*KRAS*, *NRAS* and *HRAS*) and occupy a major step in the MAPK/ERK pathway (Figure 1.2). *KRAS* and *NRAS* mutations are found in 40-50% and 1-4% of CRCs, respectively, with *HRAS* mutations extremely rare.[22] *RAS* mutations result in constitutive activation of the MAPK/ERK pathway, loss of EGFR regulation and propagation of cellular processes that support tumour development. They are more common in proficient MMR (pMMR) than deficient MMR (dMMR) CC, and in the former, are associated with a poorer prognosis compared to *RAS* wild-type tumours.[23]

Unlike in the metastatic setting, where anti-EGFR therapy use is dependent on *RAS* wild-type status, there is currently no established role for testing in the localised setting.[24]

#### 1.1.3.2 BRAF

The BRAF protein is a member of the RAF family encoded by the *BRAF* oncogene and occupies the next step in the MAPK/ERK pathway (Figure 1.2). Mutant *BRAF* is found in around 10% of CCs and considered mutually exclusive from *RAS* mutations.[25] Owing to the relationship between the MSI and serrated neoplasia pathways, many tumours are both dMMR and *BRAF*-mutant. Over 90% of *BRAF* mutations in CC are the *V600E* alteration, which is associated with older age, female sex, right-sided tumour location, mucinous and poorly differentiated histology, serrated tumour architecture and high rates of peritoneal and distant lymph node metastases.[25] These mutations are also associated with a poorer prognosis, compared to wild-type tumours.[23] The much rarer non-*V600E* mutations cause a distinct CC phenotype with contrasting clinicopathological associations and prognosis.[25] *BRAF* testing in localised CC has no therapeutic role but may be used to help differentiate tumours with sporadic MMR deficiency from those potentially caused by Lynch syndrome.[24]

#### 1.1.3.3 HER2

HER2 overexpression is most associated with breast and gastro-oesophageal cancers but is also estimated to affect 4% of CRCs, most commonly in tumours of the distal colon and those which are *RAS* and *BRAF* wild-type.[26] Like other members of the HER family, HER2 is a transmembrane receptor, but is instead activated by dimerisation with other receptors rather than ligation.[27] HER2 activation induces downstream signalling intracellular cascades, including the MAPK/ERK pathway, resulting in greater cell proliferation and inhibition of apoptosis, amongst other effects.[27] While the prognostic and therapeutic implications of HER2 status in CC are not fully known, molecular testing and targeted treatment options are starting to be recognised in the metastatic setting.[21]

#### 1.1.3.4 Mismatch Repair

Around 20% of dMMR CCs are hereditary, most commonly due to Lynch syndrome.[14] Tumours with MMR deficiency are more frequent in localised disease but overall account for around 15% of all CCs. Compared to pMMR, dMMR tumours have a distinct phenotype,

including a predominance for older adults, female sex, right-sided tumour location and mucinous differentiation, and greater immunotherapy sensitivity.[14] For localised CC, testing for MMR deficiency or microsatellite instability (usually considered interchangeable according to local policy and resources) is recommended in international guidelines, primarily to identify patients with undiagnosed Lynch syndrome, which may influence extent of surgical resection, long-term surveillance and genetic testing for relatives.[14]

#### 1.1.3.5 Consensus Molecular Subtypes

The consensus molecular subtypes (CMS) represent an alternative and more recently developed molecular classification system that captures the heterogeneity and complexity of CC biology. Four distinct CMS groups (CMS1 – MSI immune, CMS2 – canonical, CMS3 – metabolic, CMS4 - mesenchymal) were devised using gene-expression profiling with prognostic and predictive value beyond that provided by traditional staging and molecular biomarker testing.[28] However, the CMS system has not been widely adopted in routine practice due to the lack of a standardised and validated framework for assessment, intra-tumoural heterogeneity consistent with more than one CMS subtype and practical challenges related to technical infrastructure, expertise and cost.[29]

### 1.1.4 Histopathological Classification

Adenocarcinoma is the predominant CC histology, which, according to the World Health Organization classification system, can be further divided into subtypes, including medullary, micropapillary, mucinous and signet ring cell.[30] Other types of CC histology include adenosquamous, spindle cell and undifferentiated carcinoma. The extent of gland formation allows adenocarcinomas to be classified as well, moderate or poorly differentiated, which is recognised as an independent prognostic factor but limited by a lack of standardisation and inter-observer variation.[31] Various immunohistochemical markers, such as cytokeratin 20, cytokeratin 7 and homeobox protein CDX-2, are often detected in CC and may aid diagnosis.[31]

### 1.1.5 Clinical Presentation

The heterogeneous nature of CC includes clinical presentation, which ranges from asymptomatic detection via screening to emergency presentations like bowel obstruction. For

progressive or persistent symptoms, the nature of these will depend on tumour location and stage but may include change in bowel habit, rectal bleeding, abdominal pain, bloating, an abdominal mass, loss of appetite, fatigue and weight loss.[32]

### 1.1.6 Investigation & Diagnosis

Suspected CC is typically first investigated with stool testing, where faecal immunohistochemical testing (FIT) has superseded faecal occult blood testing.[33] Colonoscopy is subsequently used to examine the whole colon and biopsy areas concerning for cancer. If histological evaluation confirms the presence of invasive cancer, molecular biomarker testing for MMR/microsatellite status is routinely performed in all patients plus *RAS* and *BRAF* profiling in those with suspected metastatic disease.[21]

Radiological evaluation with computed tomography (CT) of the chest, abdomen and pelvis usually follows histological diagnosis to provide CC staging according to the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumour, node, metastasis (TNM) system (Table 1.1). CT colonography (CTC; also known as 'virtual colonoscopy') represents an alternative initial test for patients unsuitable for colonoscopy or those in whom proximal endoscopic evaluation was not achieved.[34] If a suspicious lesion is identified on CTC, endoscopic evaluation is still required for direct visualisation and biopsy, followed by completion imaging of the chest if a histological diagnosis of CC is confirmed.

Table 1.1 UICC TNM Version 9 for Colon Cancer

| | | | |
|---|---|---|---|
| T stage | TX | | Cannot be assessed |
| | T0 | | No evidence of primary tumour |
| | Tis | | Carcinoma in situ [a] |
| | T1 | | Invades submucosa |
| | T2 | | Invades muscularis propria |
| | T3 | | Invades subserosa or non-peritonealised pericolic tissues |
| | T4 | | Perforates visceral peritoneum and/or invades other organs or structures |
| | | T4a | Perforates visceral peritoneum |
| | | T4b | Invades other organs or structures |
| N stage | NX | | Cannot be assessed |
| | N0 | | No regional lymph node metastasis or tumour deposit |
| | N1 | | Metastases in 1 to 3 regional lymph nodes or tumour deposit(s) with 0 to 3 lymph node metastases |
| | | N1a | Metastasis in 1 regional lymph node |
| | | N1b | Metastases in 2 to 3 regional lymph nodes |
| | | N1c | Tumour deposit(s) in the subserosa or non-peritonealised pericolic tissue without regional lymph node metastases [b] |
| | N2 | | Metastases in ≥4 regional lymph nodes |
| | | N2a | Metastases in 4 to 6 regional lymph nodes |
| | | N2b | Metastases in ≥7 regional lymph nodes |
| M stage | M0 | | No distant metastasis |
| | M1 | | Metastasis in a distant site |
| | | M1a | Metastasis confined to one organ (including non-regional lymph nodes) without peritoneal metastases |
| | | M1b | Metastasis in more than one organ without peritoneal metastases |
| | | M1c | Metastasis in the peritoneum, with or without involvement of other organs |
| Staging groups | I | T1-2 N0 M0 | |
| | II | T3-4 N0 M0 | |
| | III | T1-4 N1-2 M0 | |
| | IV | T1-4 N0-2 M1 | |

TNM = tumour, node, metastasis; UICC = Union for International Cancer Control.

[a] Presence of intramucosal tumour cells without extension to the submucosa

[b] Tumour deposits are discrete nodules of cancer which are found within the adjacent lymphatic drainage area of, but discontinuous from, the primary tumour. They may contain residual foci of extramural venous invasion and perineural invasion.[35]

### 1.1.7 Systemic Therapies for Localised Colon Cancer

The treatment of CC requires input from across the MDT, with individual cases usually discussed at specialist colorectal MDT meetings. Surgical resection remains the cornerstone of treatment for localised CC but, despite improvements in surgical practice, is insufficient to achieve cure in a proportion of patients. Systemic therapies are increasingly used alongside surgery to improve survival outcomes and prevent recurrence in patients with high-risk disease.[24, 36]

#### 1.1.7.1 Adjuvant Therapies

##### 1.1.7.1.1 Chemotherapy

Adjuvant chemotherapy (AC) is used in patients with pathologically confirmed stage II or III CC (Figure 1.5).[24] In stage III CC, the risk of recurrence following surgery is around 50%.[37] Three to six months of combination AC with a fluoropyrimidine (5-fluorouracil [5FU] or capecitabine) plus oxaliplatin is the standard of care and reduces recurrence risk to around 30% (Figure 1.3).[24] The role of AC is less clear for stage II CC, a heterogeneous disease with a more limited evidence-base, where recurrence risk ranges from 10% to 40%. Therefore, AC is usually given according to individual risk: low-risk patients may not require AC; intermediate-risk patients may be treated with three to six months of 5FU or capecitabine monotherapy; and high-risk patients are often treated similarly to stage III CC with combination AC.[24]

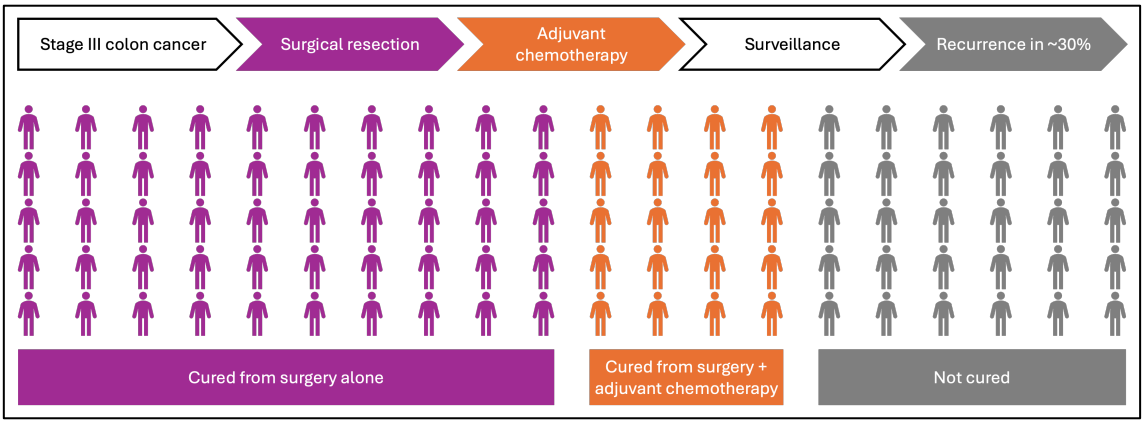



**Figure 1.3 Survival and Importance of Adjuvant Chemotherapy in Stage III Colon Cancer**
The standard treatment for stage III colon cancer is surgical resection followed by three to six months of combination adjuvant chemotherapy. Around half of patients are cured from surgery alone and a further 20% with adjuvant chemotherapy. Around 30% of patients with stage III colon cancer suffer disease recurrence despite optimal treatment.

#### 1.1.7.1.2 Other Therapies

Patients with dMMR CC derive less benefit from AC, compared to those with pMMR tumours, and are therefore treated more selectively.[38, 39] As dMMR tumours are highly sensitive to immunotherapy, which has become the standard of care for metastatic or unresectable CC, its role in localised disease is being increasingly explored.[21, 40, 41]

ATOMIC was the first phase III randomised controlled trial (RCT) to test adjuvant immunotherapy in stage III dMMR CC. Adding the anti-programmed cell death-ligand 1 (PD-L1) immune checkpoint inhibitor atezolizumab to 5FU and oxaliplatin significantly improved 3-year disease-free survival (DFS), compared to chemotherapy alone (86.4% vs. 76.6%; hazard ratio [HR] 0.5, $P < 0.001$).[42] Six months of chemotherapy was given in both arms but, in the experimental arm, was followed by a further six months of atezolizumab, totalling one year of systemic treatment. While the final trial analyses were only recently published, this combination of adjuvant chemotherapy and immunotherapy is likely to become a new standard of care (Figure 1.5).[42]

Beyond pharmacological interventions, the recent CHALLENGE trial showed that provision of health education materials and a 3-year structured exercise programme significantly improved DFS, compared to educational materials alone, in patients with stage III or high-risk stage II CC (HR 0.72, $P = 0.02$).[43] While there are clear issues around generalisability and implementation, the findings have already been adopted into international guidelines (Figure 1.5).[44]

Other systemic therapies used in metastatic CC, such as irinotecan, the anti-EGFR therapy cetuximab and the anti-vascular endothelial growth factor therapy bevacizumab, do not improve survival outcomes in the adjuvant setting.[45-50]

### 1.1.7.2 Neoadjuvant Therapies

#### 1.1.7.2.1 Rationale

The use of AC in stage III and high-risk stage II CC is a well-established standard of care internationally.[24] However, despite widespread adoption, disease recurrence still affects a substantial number of patients with drastic implications for prognosis.[37] Neoadjuvant therapies have proven effective across other cancer types with several potential advantages that may translate to CC: early treatment of micrometastatic disease, downstaging to improve complete resection rates, ensuring all patients receive systemic treatment regardless of

surgical recovery, splitting systemic treatment into smaller and likely less toxic components and an indication of treatment sensitivity to guide adjuvant decision-making.[51, 52] Testing neoadjuvant therapies in CC has therefore become an area of major research activity.

#### 1.1.7.2.2 Chemotherapy

Neoadjuvant chemotherapy (NAC) for locally advanced CC has been tested in three phase III RCTs (Table 1.2). The first and largest of these was the Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT) trial, which showed that six weeks of neoadjuvant fluoropyrimidine (5FU or capecitabine) plus oxaliplatin was associated with a significant reduction in the risk of residual disease or recurrence within two years, compared to upfront surgery (16.9% vs. 21.5%; rate ratio 0.72, P = 0.037) in patients radiologically staged T3-4 N0-2 M0 (Figure 1.4).[53] Significant reductions in pathological staging, extramural venous invasion (EMVI) and rates of incomplete surgical resection and peri-operative complications were also seen.

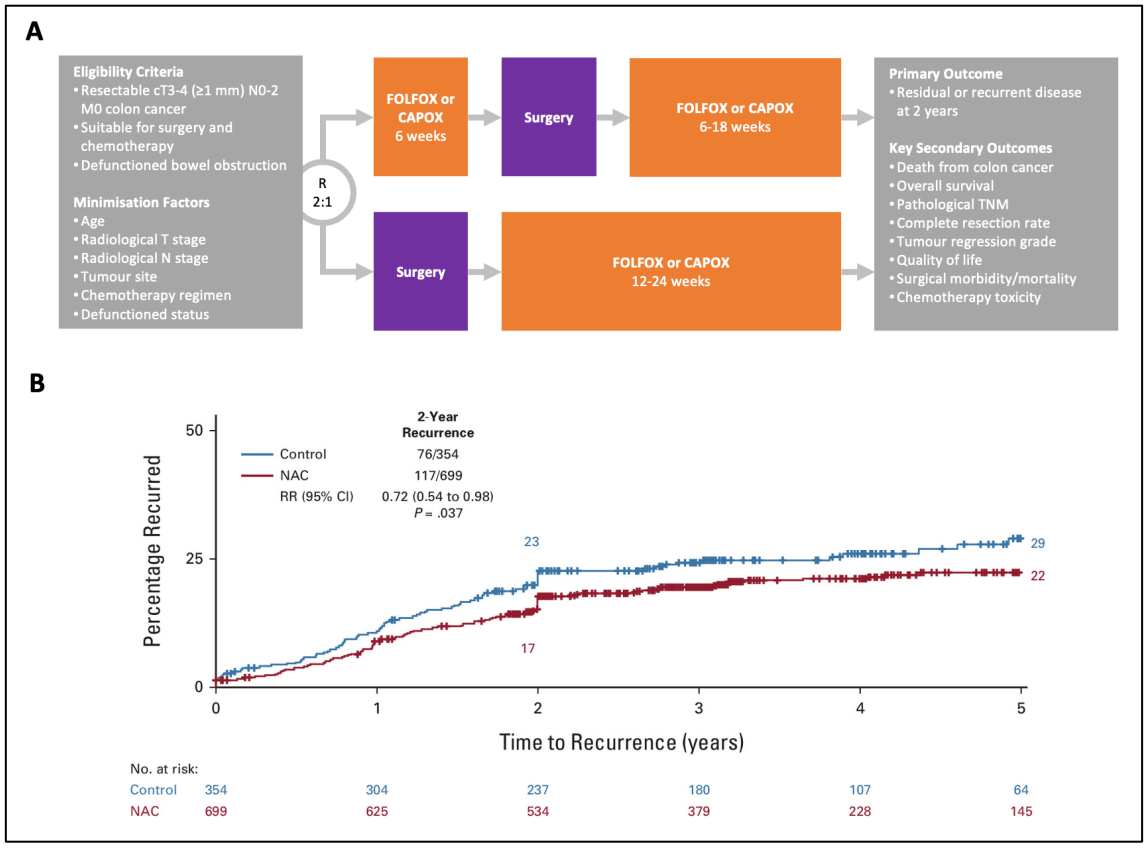



**Figure 1.4 The FOxTROT Trial**
A) Schematic for the FOxTROT trial. B) Incidence of recurrence or residual disease in all participants according to treatment arm. CAPOX = capecitabine plus oxaliplatin; FOLFOX = 5-fluorouracil plus oxaliplatin; stage; R = randomised; TNM = tumour, node, metastasis.

The OPTICAL trial aimed to select a higher-risk cohort than FOxTROT, requiring T3 with at least 5 mm extramural extension or T4 staging on CT.[54] While OPTICAL failed to meet its primary endpoint of improved 3-year DFS (82.1% vs. 77.5% for NAC vs. upfront surgery, respectively; HR 0.74, P = 0.07), an overall survival (OS) benefit was detected (95.1% vs. 89.6%, respectively; HR 0.44, P = 0.01). The use of a potentially inappropriate expected benefit in the power calculation and including patients with dMMR tumours, who have since been shown to not benefit from NAC, may have resulted in the trial being under-powered to reach its primary endpoint. This is worthy of consideration given the absolute risk reduction with NAC was comparable to FOxTROT.[53]

The recently published NeoCol trial also failed to show an improvement in DFS with NAC, compared to upfront surgery, but has several major limitations.[55] First, the expected 10% improvement in DFS used in the power calculation appears overly optimistic based the results of this trial and others, likely under-powering the study. Second, unlike FOxTROT and OPTICAL which protocolised peri-operative (both neoadjuvant and adjuvant) chemotherapy using baseline radiological staging, NeoCol used post-operative pathological staging to guide AC decisions. This approach could be considered inappropriate given the downstaging effects of NAC, as participants with high-risk disease who happened to respond well to NAC may only have received half a course of treatment, regardless of baseline risk. It could be argued that the opposite approach should be taken, whereby a good response to NAC (whether through apparent downstaging or assessment of pathological regression) indicates treatment sensitivity and a greater likelihood of benefit from AC. The investigators prioritised both treatment escalation (by using NAC in high-risk patients) and de-escalation (by limiting AC to only those with high-risk post-operative pathology) but may not have effectively achieved either. Finally, NeoCol was similarly limited by the inclusion of participants with dMMR tumours, including an unusually high proportion of the NAC arm (27%). Ultimately, these limitations prevent clinically meaningful conclusions being drawn from the trial.

A recent meta-analysis including FOxTROT, OPTICAL and NeoCol provides clarity on these inconsistent results, showing improvements in DFS and OS with NAC compared to upfront surgery.[56] Although these data support NAC as a new treatment option in locally advanced CC, adoption of this approach across international guidelines remains inconsistent (Figure 1.5).[24, 36]

Table 1.2 Randomised Trials of Neoadjuvant Chemotherapy in Locally Advanced Colon Cancer

| | Design | Population [a] | Patients | Neoadjuvant Treatment | Treatment Duration [b] | Primary Outcome | Main Result |
|---|---|---|---|---|---|---|---|
| PRODIGE 22 [58] | Phase II | cT3 (with ≥5 mm extramural extension or cN2) or cT4 | 120 | FOLFOX +/- cetuximab | NAC: 8 weeks AC: 16 weeks | MPR rate [c] | MPR in 8% (below pre-specified threshold of 10%) |
| FOxTROT [13] | Phase III | cT3 (with ≥1 mm extramural extension) or cT4 | 1,053 | FOLFOX or CAPOX +/- panitumumab | NAC: 6 weeks AC: 6-18 weeks | Residual disease or recurrence within 2 years | Residual disease or recurrence within 2 years 16.9% vs. 21.5% [d]<br>RR 0.72; P = 0.037 |
| OPTICAL [54] | Phase III | cT3 (with ≥5 mm extramural extension) or cT4 | 744 | FOLFOX or CAPOX | NAC: 12 weeks AC: 12 weeks | 3-year DFS | 3-year DFS 82.1% vs. 77.5% [d]<br>HR 0.74; P = 0.07 |
| NeoCol [55] | Phase III | cT3 (with ≥5 mm extramural extension) or cT4 | 250 | CAPOX | NAC: 9 weeks AC: Up to 15 weeks | 3-year DFS | 3-year DFS 83% vs. 87% [d]<br>P = 0.36 |

AC = adjuvant chemotherapy; c = clinical; CAPOX = capecitabine and oxaliplatin; DFS = disease-free survival; FOLFOX = 5-fluorouracil and oxaliplatin; HR = hazard ratio; MPR = major pathological response; NAC = neoadjuvant chemotherapy; RR = rate ratio.

[a] Represents the main inclusion criteria within the localised CC population.

[b] Experimental arm.

[c] According to Ryan et al.[57]

[d] NAC vs. upfront surgery, respectively.

#### 1.1.7.2.3 Other Therapies

The FOxTROT trial confirmed that, overall, patients with dMMR CC do not benefit from NAC.[53] Following the success of treating these tumours with immunotherapy in the metastatic setting, several early phase trials have demonstrated the safety, feasibility and efficacy of neoadjuvant immunotherapy (NAI) in localised dMMR CC (Table 1.3).[59-78] NICHE-2 is the largest of these trials and delivered particularly impressive results, where neoadjuvant ipilimumab plus nivolumab induced a pathological response in 99% of participants, including major and complete pathological response rates of 95% and 67%, respectively, with a 3-year DFS of 100%.[68, 79] While efficacy varies across this heterogeneous group of trials, and the lack of long-term survival data from randomised trials limits evaluations of clinical benefit, NAI is likely to be introduced in some form as a standard of care soon (Figure 1.5). High pathological complete response (pCR) rates also suggest that some patients may be cured from NAI alone and can pursue organ preservation. Although the efficacy of NAI is most convincing in dMMR CC, some studies have shown pathological responses in pMMR tumours either when used alone or in combination with chemotherapy.[59-61, 63, 64, 66]

The treatment of metastatic colorectal cancer (mCRC) is increasingly biomarker-led and personalised. As such, there is enthusiasm for translating these practices to the neoadjuvant setting using existing biomarkers, with preliminary data already suggesting potential benefit for the anti-EGFR therapy panitumumab alongside NAC in patients with *KRAS* wild-type tumours.[80, 81]

Table 1.3 Trials of Neoadjuvant Immunotherapy in Locally Advanced Colon Cancer

| | Design | Population [a] | Patients [b] | Neoadjuvant Therapy | Neoadjuvant Therapy Duration | Surgery | Adjuvant Therapy [c] | Primary Outcome(s) | Main Result(s) [d] |
|---|---|---|---|---|---|---|---|---|---|
| NICHE [61, 82] | Phase II Single arm | Any non-metastatic | 62 | Ipilimumab & nivolumab (+/- celecoxib in pMMR group) | 4 weeks | Yes | No | Safety Feasibility | All underwent surgery without delay. G3+ immune-related AE in 12%. |
| NICOLE [e] [59] | Phase II Single arm | cT3-4 | 22 | Nivolumab | 4 weeks | Yes | No | Safety Feasibility MPR rate | All underwent surgery without delay. MPR in 16% pMMR and 0% dMMR. |
| PICC [62] | Phase II Randomised | dMMR cT3-4 or cN+ | 34 (30 CC) | Toripalimab +/- celecoxib | 12 weeks | Yes | Optional | pCR rate | pCR in 76%. |
| Ludford et al. [65] | Phase II Single arm | dMMR locally advanced | 35 (19 CC) | Pembrolizumab | 24-48 weeks | Optional | No | Safety pCR rate | G3+ immune-related AE in 6%. pCR in 79%. |
| NEST-1 [e] [63] | Phase II Single arm | Any non-metastatic | 12 | Botensilimab & balstilimab | 2 weeks | Yes | No | Safety Pathological ORR | All underwent surgery without delay. Full response data awaited. |
| Cai et al. [e] [60] | Phase II Randomised | cT3-4 or cN+ | 22 | FOLFOX & toripalimab or FOLFOX | 12 weeks | Yes | Yes | pCR rate | pCR rate 31% vs. 0%. |
| Lin et al. [e] [64] | Phase II Single arm | pMMR cT3-4 or cN+ | 27 | Tislelizumab & CAPOX | 6-12 weeks | Yes | Yes | pCR rate | pCR in 52%. |
| NICHE2 [68, 69] | Phase II Single arm | dMMR cT3-4 or cN+ | 115 [f] | Ipilimumab & nivolumab | 4 weeks | Yes | No | Safety 3-year DFS | Delayed surgery in 3%. G3+ immune-related AE in 3%. Path response in 99% (pCR in 67%). 3-year DFS 100%. |

Table 1.3 (Continued)

| | Design | Population [a] | Patients [b] | Neoadjuvant Therapy | Neoadjuvant Therapy Duration | Surgery | Adjuvant Therapy [c] | Primary Outcome(s) | Main Result(s) [d] |
|---|---|---|---|---|---|---|---|---|---|
| NEOPRISM [e] [74] | Phase II Single arm | dMMR cT3 (with ≥5 mm extramural extension or EMVI) or cT4 or cN+ | 32 (Unknown CC) | Pembrolizumab | 3-9 weeks | Yes | No | pCR rate | pCR in 53%. |
| NEOCAP [77] | Phase II Single arm | dMMR cT3 (with ≥5 mm extramural extension) or cT4 | 52 (46 CC) | Camrelizumab & apatinib | 12-24 weeks | Optional | Optional | pCR/cCR rate | pCR/cCR in 73%. |
| IMHOTEP [e] [71] | Phase II Single arm | dMMR cT3-4 or cN+ | 87 (72 CC) | Pembrolizumab | 6-12 weeks | Yes | Yes | pCR rate | pCR in 55%. |
| NICHE3 [70] | Phase II Single arm | dMMR cT3-4 or cN+ | 59 | Nivolumab & relatlimab | 8 weeks | Yes | No | PR rate | PR in 97%.<br>MPR in 92%.<br>pCR in 68%. |
| RESET-C [e] [73] | Phase II Single arm | dMMR any non-metastatic | 85 | Pembrolizumab | 3 weeks | Yes | No | pCR rate | pCR in 44%. |
| Cercek et al. [67] | Phase II Single arm | dMMR any non-metastatic | 117 (33 CC) | Dostarlimab | 6 months | Optional | No | cCR rate | cCR in 91%. |
| Wang et al. [76] | Phase Ib/II Single arm | dMMR cT4 | 22 (14 CC) | Toripalimab, irinotecan & bevacizumab | 6 weeks | Yes | Yes | pCR rate | pCR in 57.1%. |
| Wang et al. [75] | Phase Ib Randomised | dMMR any non-metastatic | 101 | IBI310 & sintilimab or sintilimab | 6 weeks | Yes | No | pCR rate | pCR rate 78% vs. 47%.<br>P = 0.0015. |

Table 1.3 (Continued)

| | Design | Population [a] | Patients [b] | Neoadjuvant Therapy | Neoadjuvant Therapy Duration | Surgery | Adjuvant Therapy [c] | Primary Outcome(s) | Main Result(s) [d] |
|---|---|---|---|---|---|---|---|---|---|
| Kuznetsova et al. [72] | Phase II Single arm | dMMR cT2-4 (with cN+) or cT3 (with ≥5 mm extramural extension) | 30 (24 CC) | Prolgolimab | 24 weeks-1 year | Optional | No | pCR/cCR rate | pCR/cCR in 57%. |
| Zhang et al. [e] [78] | Phase II Single arm | dMMR (cohort A) or pMMR (cohort B) cT3-4 or cN+ | 63 | Envafolimab (cohort A) or envafolimab & CAPOX (cohort B) | 12 weeks | Yes | No | pCR rate | Cohort A: pCR in 52.4%. Cohort B: pCR in 16%. |

AE = adverse event; CAPOX = capecitabine & oxaliplatin; CC = colon cancer; cCR = clinical complete response; DFS = disease-free survival; dMMR = mismatch repair deficient; EMVI = extramural venous invasion; FOLFOX = 5FU & oxaliplatin; G = grade; MPR = major pathological response; ORR = overall response rate; pCR = pathological complete response; PR = pathological response; pMMR = mismatch repair proficient.

[a] Main criteria for eligibility. Specifically refers to colon cancer for studies that included other tumour sites.

[b] For trials not limited to colon cancer, number of participants with colon cancer given in brackets, where known.

[c] Protocolised peri-operative use of the study intervention, not including routine adjuvant therapy.

[d] In trials not limited to colon cancer, results given for colon cancer specifically, where known.

[e] Preliminary results from conference abstract only.

[f] Includes 32 patients with dMMR tumours from the NICHE study.

'pMMR'/'dMMR' used in place of 'MSS'/MSI-h' in some cases for brevity.

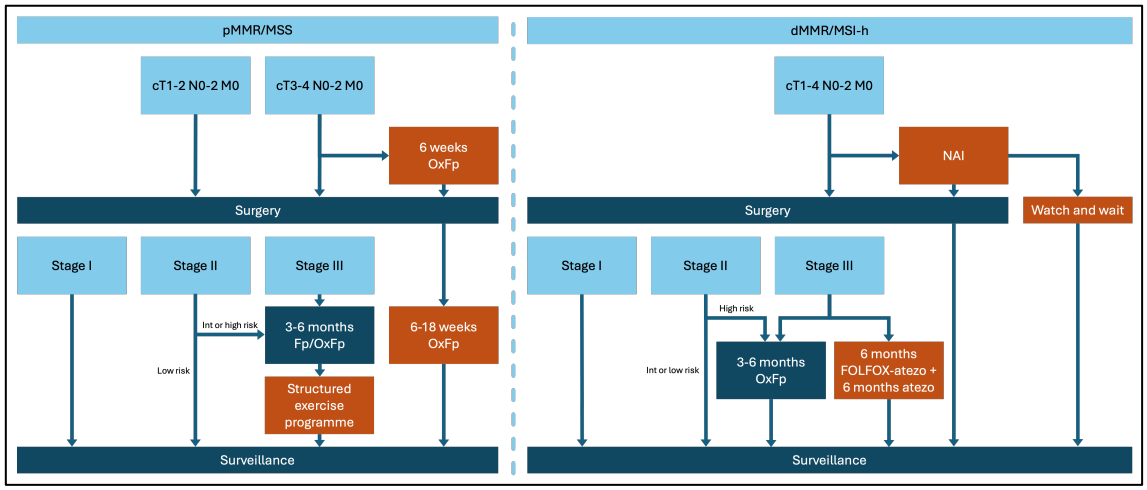



**Figure 1.5 Established and Emerging Treatment Pathways in Localised Colon Cancer**
Localised colon cancer treatment is determined by staging (radiological and pathological) and MMR status. This schematic provides an overview of the key steps in different treatment pathways but is not exhaustive. Established interventions are coloured dark blue, whereas emerging interventions are coloured orange. Atezo = atezolizumab; dMMR = deficient mismatch repair; FOLFOX = 5-fluorouracil & oxaliplatin; Fp = fluoropyrimidine; Int = intermediate; MMR = mismatch repair; MSI-h = microsatellite instability-high; MSS = microsatellite stable; NAI = neoadjuvant immunotherapy; OxFp = oxaliplatin & fluoropyrimidine; pMMR = proficient mismatch repair.

### 1.1.8 Survival

The 5-year survival for CC is heavily dependent on disease staging, with rates of 90.9%, 84.3%, 65.0% and 10.5% for stages I, II, III and IV, respectively.[4] Survival is linked to socioeconomic deprivation, with a 5-year survival of 61.5% in England's least deprived areas, compared to 52.6% for the most deprived.[4] Improvements in education, screening and diagnostic investigations have resulted in a stage shift over time, with stage IV accounting for 26.6% of diagnoses in 2013, compared to 21.5% in 2022.[83] Together, this shift and therapeutic advances have improved 5-year survival from 44.0% in 1995 to 59.0% in 2014.[1]

## 1.2 Colon Cancer Imaging

As the global standard for radiological assessment in CC, CT is widely used across screening, staging and follow-up. Alternative imaging modalities, such as magnetic resonance imaging (MRI) and positron-emission tomography (PET)-CT are used sparingly in CC. For the purposes of this thesis, unless otherwise specified, radiological assessments will refer to the use of CT.

### 1.2.1 Established Applications

Imaging has several established roles in CC which are mainly found in the pre- and post-treatment phases.

#### 1.2.1.1 Screening

Bowel cancer screening programmes exist internationally and are designed to detect asymptomatic CC at an earlier stage, where outcomes from curative treatment are better.[84, 85] In the United Kingdom (UK), the National Health Service Bowel Cancer Screening Programme invites adults between the ages of 50 and 74 to undergo FIT.[86] Those with a positive FIT result are offered a colonoscopy for full colonic examination and sampling of any suspicious lesions. The investigation of symptoms concerning for colorectal cancer often mirrors screening; depending on clinical concern, symptomatic adults may be offered FIT or proceed directly to colonoscopy.[87, 88]

If a person is unsuitable for colonoscopy (e.g. due to complex co-morbidities, mobility or intolerance of an initial attempt) or stenosis prevents full endoscopic assessment, CTC may be offered to assess for colorectal cancer or advanced polyps.[89] Air insufflation is used to distend the large bowel during CTC to improve the delineation of any detected lesions and allow complete evaluation of the proximal colon (Figure 1.6).[34] While CTC is effective for colorectal cancer screening and diagnosis, there is marked geographical variation in CTC provision and uptake.[90]

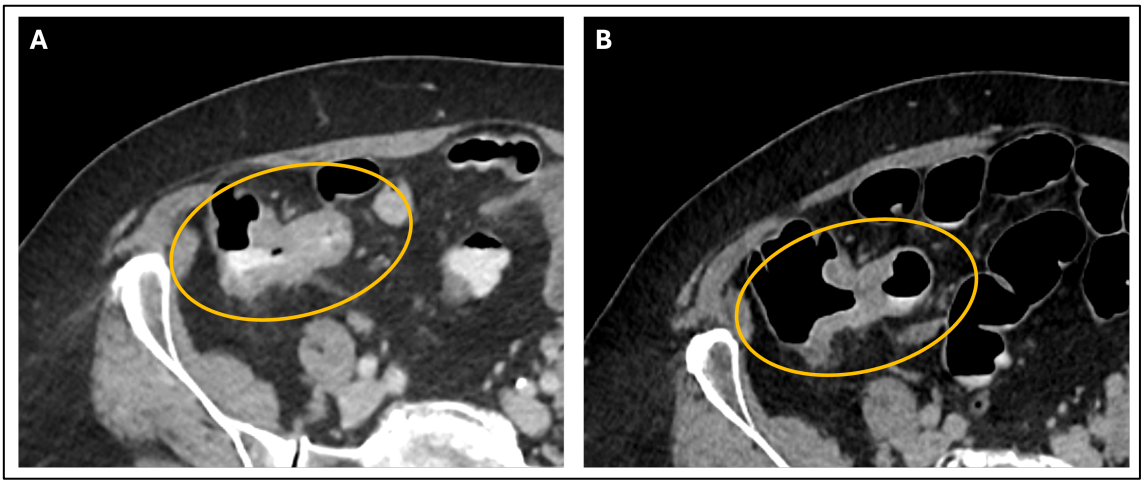



**Figure 1.6 Standard CT vs. CT Colonography**
Post-treatment images of a patient with colon cancer. Gas insufflation for CT colonography distends the colon and improves delineation of primary tumours. A) Caecum cancer which is difficult to delineate on standard CT. B) CT colonography distends the affected segment making the tumour morphology clearer. Yellow rings placed to indicate the area of the primary tumour. CT = computed tomography.

#### 1.2.1.2 Staging

Following a histological diagnosis of CC, CT of the chest, abdomen and pelvis is performed to provide radiological staging, according to the AJCC/UICC TNM system (Table 1.1).[35] Radiological staging using whole body MRI has been investigated but not widely implemented in routine clinical practice.[91]

##### 1.2.1.2.1 Tumour Location

Accurate localisation of the primary tumour is critical to guide surgical planning and intervention. Location also has oncological implications as tumour biology, and thus treatment efficacy, is known to differ throughout the colon.[92, 93] Colonoscopy is considered the gold-standard for determining the affected colonic segment but is hindered by a lack of reliable intra-luminal landmarks, anatomical variation, and until recently, no standardised definitions for these segments.[94]

As part of a wider assessment of the primary tumour, imaging can predict the affected colonic segment, providing a viewpoint distinct from colonoscopy. There are fewer data on the accuracy of CT for tumour localisation than colonoscopy but ranges from 43.0% to 96.8% in in the literature.[95-103] When compared directly in a prospective multicentre study, there was no significant difference in the accuracy of each modality.[100] However, CT was able to

correctly identify tumour location in 40% of those incorrectly located on colonoscopy, highlighting potential additive value. Conversely, around 10% of tumours were evaluable on colonoscopy despite not being visible at all on CT. Together, these data indicate that colonoscopy and CT are imperfect approaches to tumour localisation but may complement each other in baseline assessment.

#### 1.2.1.2.2 Depth of Invasion

Imaging is the primary component of clinical staging in CC, which is denoted in the TNM system by the prefix 'c'; pathological staging uses the prefix 'p'.[35] Primary tumours are assigned a cT stage based on the depth of invasion and involvement of other structures, ranging from cT1 to cT4 (Table 1.1).

As CT is unable to differentiate individual bowel wall layers due to limited soft tissue contrast, pT1 and pT2 tumours are usually indistinguishable and therefore grouped together (cT1-2).[104] These tumours are characterised by a smooth interface between the bowel wall and pericolic fat, reflecting the lack of penetration by the tumour through the muscularis propria (Figure 1.7). Due to limited spatial resolution and partial voluming effects, it also may not be possible to differentiate pT1-2 from 'early' pT3 (i.e. <1 mm extramural extension) tumours.[105] Furthermore, extramural venous, neural and lymphatic invasion, desmoplasia and peri-colic inflammation on CT could be mistaken for tumour invasion beyond the muscularis propria.[106]

The radiological appearances of cT3 tumours are heterogeneous, ranging from subtle irregularities or haziness at the tumour border to gross protrusion into the pericolic fat (Figure 1.7).[105] While the TNM system does not subcategorise cT3 tumours by the degree of extramural invasion, it is possible to measure this radiologically and has been used throughout the radiological literature and in neoadjuvant trials.[54, 55, 107]

Tumours which appear to be invading adjacent peritoneum or other organs are staged cT4a and cT4b, respectively (Figure 1.7).[35] Accurately predicting peritoneal involvement can be challenging, particularly for intra-peritoneal colonic segments where minimal extramural extension may breach the visceral peritoneum. It may also be difficult to determine whether an advanced tumour is invading, or simply abutting, an adjacent structure. Identifying pT4 tumours is clinically important as involvement of other structures may influence the extent or feasibility of surgical resection.

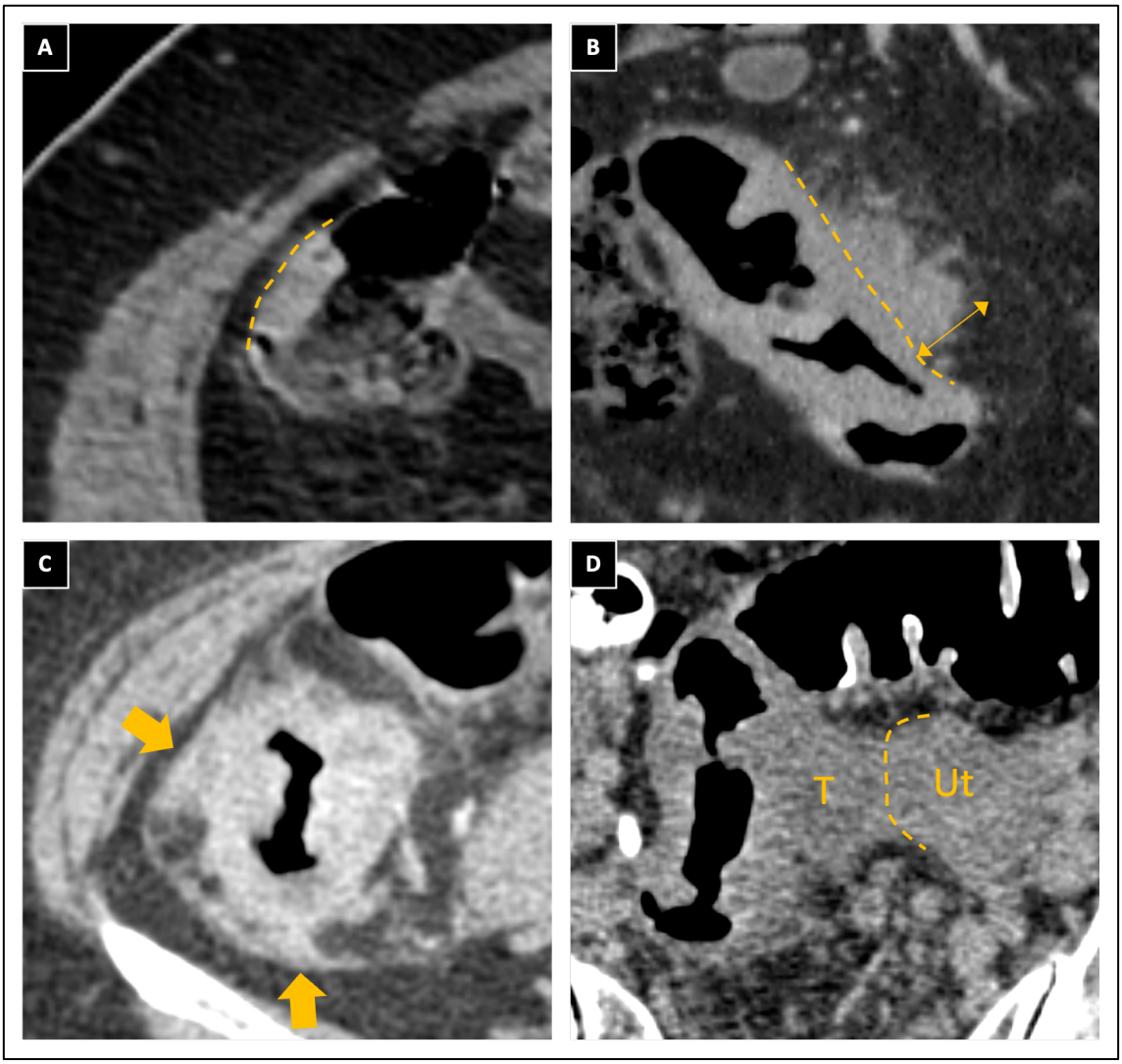



**Figure 1.7 Radiological T Staging**
Primary tumours are assigned a T stage according to depth of invasion and involvement of other structures. A) cT1-2: Ascending colon tumour with no visible extension beyond the muscularis propria. Dashed line indicates likely position of the in-tact bowel wall. B) cT3: The interface between sigmoid tumour and fat is no longer smooth with clear extension of tumour beyond the muscularis propria. Dashed line indicates likely position of the original bowel wall; arrow demonstrates depth of extension. C) cT4a: Caecum tumour invading adjacent peritoneum. Arrows indicate areas of peritoneal invasion. D) cT4b: Sigmoid tumour invading the uterus. Dashed line indicates estimated position of original interface between tumour and uterus. T = tumour; Ut = uterus.

The concordance between radiological and pathological TNM staging on CT has been comprehensively evaluated, including a series of meta-analyses.[107-111] The most recent of these, comprising 44 studies, demonstrated a pooled sensitivity and specificity of 83% and 70%, respectively, for differentiating pT1-2 and pT3-4 tumours.[107] Adjusting the dichotomy to pT1-3ab versus pT3cd-4, representing 5 mm extramural tumour extension, the same pooled values were 67% and 88%, respectively. Determining whether a tumour is invading, as

opposed to abutting, another structure is challenging, with CT therefore having limited accuracy for the identification of pT4 tumours.[112]

#### 1.2.1.2.3 Lymph Node Metastases

The presence and number of suspected lymph node metastases dictates radiological N staging (Table 1.1).[35] Crucially, unaffected lymph nodes are often visible on CT, indicating the need for reliable criteria to identify metastatic nodes. Yet, such criteria do not exist despite extensive research into different radiological characteristics.

Lymph node short axis ≥1 cm and clustering are two features frequently used in the literature but still achieve limited accuracy (Figure 1.8).[105] The Node Reporting and Data System (RADS) 1.0 provides a tumour agnostic approach to lymph node assessment on CT and MRI with specific criteria to improve consistency in reporting.[113] However, diagnostic accuracy with Node-RADS 1.0 appears similar to studies using alternative criteria. Ultimately, the presence of micrometastases in nodes which appear unremarkable and inflammatory lymphadenopathy in non-metastatic nodes are two major challenges that remain unresolved. The recent meta-analysis of CT staging accuracy included data for N status (N0 vs. N1-2) with a pooled sensitivity of 64% and specificity of 67%.[107]

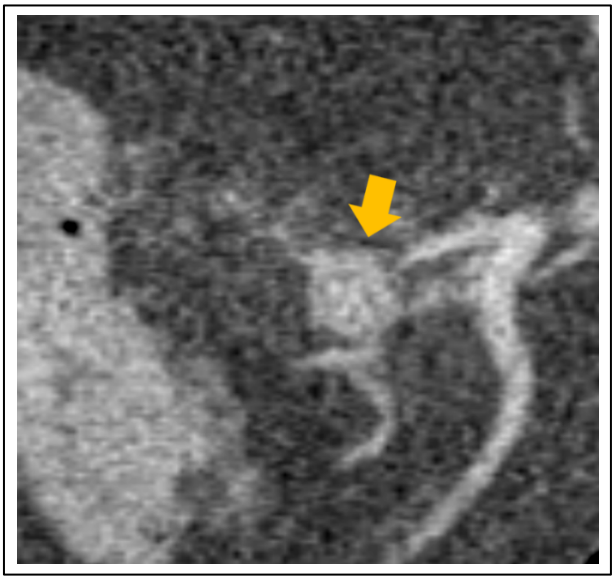


**Figure 1.8 Radiological N Staging**
Example of a regional lymph node adjacent to an ascending colon tumour with features concerning for metastatic involvement. The lymph node measures 12 mm in short axis and has heterogeneous enhancement. Yellow arrow indicates the location of the concerning lymph node.

#### 1.2.1.2.4 Extramural Venous Invasion

Although EMVI is not included in CC TNM staging, it is routinely reported on both radiological and pathological assessments. The importance of radiological EMVI is more established in rectal cancer when using MRI for patient stratification.[51] However, EMVI may still be visible on CT with a range of appearances, including clear tumour thrombus, serpiginous extension of the tumour into a vessel, expansion, irregularity and/or distortion of a vein, and the density of the vessel being similar to the primary tumour (Figure 1.9).[114, 115]

The radiological assessment of EMVI is limited by a lack of standardised criteria, reviewer subjectivity, small vessel involvement unlikely to be visible, and a series of mimics, including invasion of non-vascular structures, focal protrusion of the primary tumour into pericolic fat and peri-tumoural inflammation. Therefore, in the recent meta-analysis of CT accuracy, the pooled sensitivity and specificity for identifying EMVI was 49% and 77%, respectively.[107]

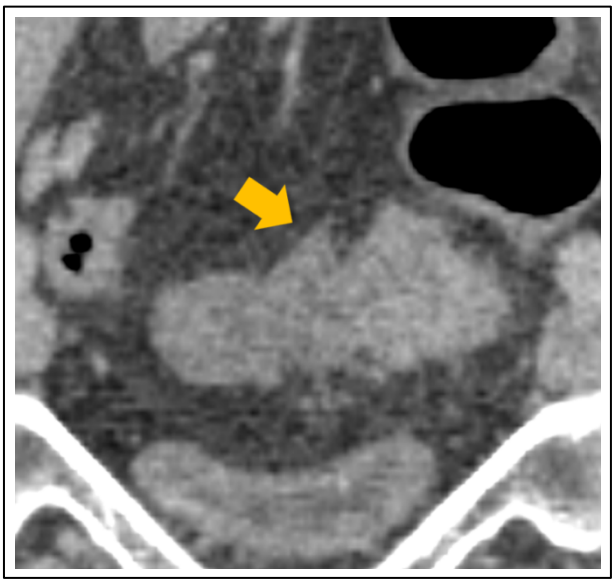


**Figure 1.9 Radiological Extramural Venous Invasion**
A sigmoid tumour with gross extension into an expanded blood vessel, suggestive of EMVI. Yellow arrow indicates area of suspected EMVI. EMVI = extramural venous invasion.

#### 1.2.1.2.5 Tumour Deposits

Tumour deposits (TDs) are discrete lesions of cancer discontinuous from the primary tumour within the regional lymphatic drainage area. Depending on the origin of a TD, there may be residual foci of EMVI or perineural invasion. According to the UICC TNM version 9, TDs are only considered part of staging in the absence of lymph node metastases (N1c), despite additional adverse prognostic value.[35, 116] In previous versions of the TNM system, TDs were included

in T staging as they were considered most related to the primary tumour. Like EMVI, suspected TDs are now sometimes reported during baseline radiological assessment, albeit not consistently and without standardised criteria. The accuracy of CT for predicting tumour deposits has not been well explored; however, one study reported a sensitivity and specificity of 46.7% and 89.3%, respectively, when compared to pathological evaluation.[117]

#### 1.2.1.2.6 Detection of Distant Metastases

The primary role of baseline radiological assessment is to determine whether the disease is localised or metastatic, which in most cases, would equate to whether curative or palliative treatment is pursued.[24] A CT of the chest, abdomen and pelvis covers the most common sites of metastasis, including the liver, lungs, and peritoneum.[118] Indeterminate lesions may be further investigated with MRI and PET-CT to confirm a treatment strategy.

#### 1.2.1.2.7 Other Imaging Modalities

Given the limitations of CT for accurately predicting pathological tumour characteristics, some studies have explored the utility of MRI and fluorodeoxyglucose F18 (FDG) PET-CT. However, despite these modalities utilising different criteria to CT, similarly limited diagnostic performance is seen. In a meta-analysis, the pooled sensitivity and specificity of MRI for differentiating pT1-2 vs. pT3-4 tumours were 81.4% and 78.5%, respectively.[119] For differentiating N0 and N1-2, these values were 61.5% and 70.3%, respectively, for MRI and 55.1% and 87.1%, respectively, for FDG PET-CT. While not directly compared, these values are similar to CT and do not address existing issues related to under- and over-staging. While fewer data are available for EMVI detection, MRI appears more sensitive than CT but remains limited by a lack of standardised criteria and consistency between radiologists.[120]

### 1.2.1.3 Assessment of Complications

Radiological investigations are often required to investigate and diagnose complications of CC and its treatment. Pre-treatment complications may include bowel obstruction or perforation.[121] Surgical complications, such as anastomotic leak, are well recognised.[122] Chemotherapy is associated with various toxicities which may require radiological investigation, including the localisation of chemotherapy-associated infection; diagnosis or exclusion of complications arising from fluoropyrimidine-induced enterocolitis; and

assessment of suspected venous thromboembolism, whether related to chemotherapy or underlying cancer.[123, 124] As new treatments emerge, additional complications must be considered. For example, immunotherapy may become a standard treatment for localised dMMR CC, which is associated with a distinct set of immune-mediated toxicities.[125]

#### 1.2.1.4 Follow-up & Surveillance

Surveillance following curative treatment for localised CC is recommended to identify pre-malignant polyps for removal and detect disease recurrence at an earlier stage, where salvage treatment may be considered. While current guidelines do not provide comprehensive recommendations, colonoscopy is advised at one and three years, and carcinoembryonic antigen (CEA) and CT surveillance for at least three years, post-resection.[126, 127] As disease recurrence most commonly occurs extra-luminally in the liver, peritoneum and lungs, imaging plays an important role alongside colonoscopy as part of long-term surveillance.[128]

### 1.2.2 Defining Image Quality and Influencing Factors

Images are created with key basic properties that must be defined. Spatial resolution refers to how well two adjacent structures with different densities can be differentiated from one another.[129] Image contrast is the difference in displayed intensity between adjacent structures.[129] Images are acquired on CT as voxels, volume elements which represent a 3-dimensional matrix to allocate density in an isotropic cube that is subsequently represented as a pixel on a screen.[130] When evaluating images in datasets, several factors influence quality. Radiology research should account for these variations, which are broadly categorised into patient-related, scan-related and reconstruction-related. An overview of each will be provided in turn.

#### 1.2.2.1 Patient-Related

##### 1.2.2.1.1 Body Habitus

Larger body habitus, in particular abdominal girth, can present both practical and image-related challenges. With increasing fat thickness, greater beam attenuation occurs, which results in lower contrast to noise ratio and a degradation in image quality.[131] Greater noise and image degradation may impede the evaluation of inconspicuous CC tumours and result in missed diagnoses or suboptimal baseline evaluations. The impact of body habitus can be

compensated for by an increase in radiation dose but this comes at the expense of additional radiation-related risks.[132] Conversely, visceral adipose tissue provides contrast with the colonic wall and therefore may improve the identification and accuracy of primary tumour and lymph node staging.[133, 134]

#### 1.2.2.1.2 Comorbidities

As a disease of older age, many patients with CC have other comorbidities which may impact scan quality. Chronic renal impairment may preclude or alter the use of intravenous contrast, potentially limiting the visible contrast between colonic tumour and normal soft tissue.[135] Respiratory disease may affect a patient's ability to breath hold during image acquisition; while this is less of a problem for modern multidetector CT scanners (because of rapid scan acquisition), it may still generate motion artefact.[136] Finally, any foreign bodies, such as metallic hip replacements, may cause beam hardening and streak artefact in the abdomen and pelvis, although new CT systems mitigate this risk by routinely deploying metal artefact reduction as part of image reconstruction.[137]

### 1.2.2.2 Scan-Related

Modern multidetector CT scanners rapidly acquire imaging volumes of patients rather than traditional single slices.[138] The interplay between scan acquisition time, radiation dose and image noise and contrast is increasingly complex, and is modified by many automated processes within scanner protocols. The use of advanced, iterative reconstruction techniques to enhance image quality with lower radiation doses, and more recent introduction of photon counting CT technology, reflect an increasing complexity of the physics and engineering processes behind CT.[139] However, the fundamental principles of CT and scan-related factors for image quality remain the same and are outlined below.

#### 1.2.2.2.1 Slice Thickness

Images on CT are formed from voxels which represent the calculated x-ray attenuation within a computed volume of the patient. Voxel size is determined by the design of the scanner detector and the reconstruction matrix size (e.g. 256 x 256, 512 x 512 or 1024 x 1024), which is then represented as CT image.

Acquired slice thickness on CT typically ranges from 0.625 mm to 10 mm, and is determined by collimators and scan protocols.[140] Thinner slices reduce the impact of partial voluming at the expense of greater image noise. Conversely, thicker slices reduce noise but with poorer spatial resolution. Hence, the balance between slice thickness and diagnostic performance must be considered within the specific clinical context and not as a one-size-fits-all approach.[141]

Detection of metastases is a key part of baseline radiological staging and studies have shown that using thinner slices is superior to thicker ones for the detection of small liver metastases.[142] For primary tumour and lymph node evaluation, the sensitivity and specificity of CT for differentiating T1-2 vs. T3-4 and N0 vs. N1-2 status are greater when using slices less than 5 mm thick, compared to 5 mm or more.[111]

#### 1.2.2.2.2 Contrast

Iodine-based intravenous contrast agents are used to help differentiate similar looking structures and provide image contrast for target tissues against adjacent backgrounds.[143] Spectral imaging techniques can boost the image contrast from iodine in an organ or blood vessel by preferentially sampling scan data from lower kilovolt x-rays. The difference in attenuation between tissues with and without iodine is therefore accentuated, allowing either a lower dose of iodine to achieve the same image contrast or greater image contrast with the same dose of iodine.

Timing and speed of contrast administration is critical to produce images within a particular phase of enhancement, where the target organ has taken up iodine.[144] The pharmacodynamics of this process are relatively simple, since iodinated contrast agents are readily absorbed and taken up by organs such as the liver and colon. The optimal timing of scan acquisition after contrast administration is categorised into ‘phases’, which are selected based on the suspected pathology and body part being imaged. Most typically, the thorax is imaged in either an arterial or portal venous phase, while the abdomen and pelvis are imaged in a portal venous phase for CC evaluation. While individual patient factors influence contrast timing and volume, including size, weight, the presence of vascular disease and cardiac function, these variations are accounted for using weight-based protocols and contrast triggering techniques. In CC, intravenous contrast improves visualisation of the primary tumour, lymph nodes and metastatic disease.[145] Oral iodinated contrast can delineate

primary tumours and differentiate the bowel from surrounding structures, with an established role in CTC. Water is now more commonly used in routine CT staging for CC.[146]

#### 1.2.2.3 Reconstruction-Related

##### 1.2.2.3.1 Reconstruction Algorithms & Kernel

For contemporary CT imaging, iterative reconstruction algorithms are now commonplace, maintaining image quality with less noise and at a lower radiation dose.[147] The reconstruction kernel is applied to the raw data to produce reconstructions that adjust the balance between spatial resolution and noise.[148] Sharp kernels accentuate differences between structures by highlighting edges and fine details, used for example in lung CT, but generate images with greater noise. Conversely, smooth kernels, which are used as standard in abdominal CT, suppress high-frequency image data, limiting noise and improving low contrast resolution.

##### 1.2.2.3.2 Multiplanar Reconstruction

Modern multidetector CT scanners provide such high-quality source images that they can effectively be reformatted into other planes. This multiplanar reconstruction (MPR) allows tumours to not only be viewed in the coronal and sagittal planes, but also in adjustable oblique planes.[149] Such versatility is helpful for CC evaluation where both primary tumours and the normal colon can have unusual and tortuous morphologies. Using MPR allows the plane to be set according to the axis of the primary tumour, providing an optimised orientation to support radiological staging.[149] Furthermore, if measurements of tumour length and thickness become routine for baseline evaluation or response assessment, MPR will allow these parameters to be accurately and consistently reported. While MPR does not directly affect image quality, its use is dependent on the quality and slice thickness of the acquired images. While additional, thinner slice reconstructions can be generated from raw data on the CT scanner, it is not possible to retrospectively reconstruct thinner image slices from acquired datasets. For this reason, it is essential that optimal slice thickness is generated routinely at the time of the scan.

### 1.2.3 Emerging Applications

#### 1.2.3.1 Precision Imaging

As CC is no longer seen as a single disease, instead representing a spectrum of distinct entities consistent with modern, precision oncology, opportunities are readily available to personalise care and ensure each patient receives the right treatment. While this concept is traditionally linked to treatment, such precision extends to imaging and the different radiological phenotypes observed between CC subgroups.

The impact of MMR status on clinicopathological features in CC is well established.[14] It is therefore unsurprising that imaging is also affected and is increasingly recognised. Hong et al. demonstrated that dMMR pT3 tumours are significantly longer and thicker than pMMR pT3 tumours.[150] Several studies have since shown that MMR status impacts staging accuracy, with over-staging of benign lymph nodes, likely due to greater systemic inflammation, a frequently reported issue in dMMR CC.[151-155] The radiological features most predictive of lymph node metastases also differ by MMR status; in the study from Hong et al., largest short axis diameter and internal heterogeneity were the features most predictive of lymph node involvement in dMMR and pMMR tumours, respectively.[150] While the relevance of *BRAF* status to imaging has been less explored, one study demonstrated that *BRAF* mutant tumours were significantly more likely to have shorter length, polypoid or 'mass-like' morphology and heterogeneous enhancement, compared to *BRAF* wild-type tumours.[156] As personalised neoadjuvant treatments are developed, understanding the differences in radiological phenotypes across molecularly and clinically defined populations must be considered a research priority.

#### 1.2.3.2 Patient Stratification

The advent of neoadjuvant therapies has placed radiology at the centre of patient stratification, complemented by existing clinical factors, such as age, frailty and comorbidities. Using imaging to select patients for systemic therapies represents a major shift in practice and is something MDTs may be less confident or experienced in implementing. Existing practices from other tumour sites, such as rectal cancer may serve as a guide for this transition and exemplify how imaging can be used to evaluate patient risk and select treatment strategies accordingly.[51]

The FOxTROT trial represents the strongest data for NAC in locally advanced CC, selecting patients with cT3-4 (minimum ≥1 mm extramural extension) N0-2 M0 staging.[53] The

OPTICAL and NeoCol trials required at least 5 mm extramural tumour extension on CT, with the aim of selecting a higher-risk cohort.[54, 55] While FOxTROT remains the only positive of these trials, suggesting a superior approach to patient selection, there are potential reasons for the negative findings seen elsewhere, which are discussed earlier in this thesis.

For NAI trials, selection criteria are more varied with many including cN stage, despite the myriad issues affecting lymph node evaluation in patients with dMMR tumours.[60, 62, 69-72, 74, 78] The inconsistencies in patient selection likely reflect a lack of confidence in radiological staging accuracy, but more importantly, the paucity of high-quality evidence to guide the identification of high-risk patients or those most likely to benefit from specific neoadjuvant treatments. To support the adoption of neoadjuvant therapies and radiological patient stratification in routine practice, high-quality clinical trial data should be leveraged to identify criteria that reliably meet the following conditions: (1) high-risk pathological features with strong radiology-pathology concordance; or (2) high-risk radiological features which either lack a pathological counterpart or confer adverse prognostic value, regardless of subsequent pathology.

#### 1.2.3.3 Prognostication

Pathological TNM staging was historically used to provide distinct prognostic groups. More recently, pathological evaluation became the gold standard for stratifying patients to, and predicting benefit from, AC.[24] While there is strong evidence for the prognostic value of pathological characteristics, both within TNM and beyond, the corresponding value of these features on imaging is poorly understood. A small number of studies have explored the prognostic effects of these radiological features and provided meaningful contributions to the literature, but major limitations hinder clinical translation.

These studies have focused on radiological T stage, N stage/status and EMVI. T stage has been evaluated as individual stages and dichotomised into either T1-2 versus T3-4 or by depth of extramural tumour extension (T1-3ab vs. T3cd-4).[114, 157-160] Regardless of how it is categorised, greater radiological T stage has been shown to be associated with a poorer prognosis. The strongest data for individual T stage comes from Huh et al. where greater radiological T stage was associated with significant reductions in DFS and OS in a multivariable analysis.[158] Similarly, D'Souza et al. showed that patients with T3cd-4 staging had significantly worse DFS than those with T1-3ab staging, and that recurrence risk was similar between high-risk T3 (T3cd) and T4 disease.[157] A further study explored 'cancer-induced

spiculation', defined as "reticular or linear opacification of pericolorectal fat tissues", which was associated with a significant reduction in recurrence-free survival; however, it is possible that the conceptualised spiculation is simply an alternative description for T3 staging.[161]

The prognostic value of lymph node assessment has been explored in different ways. Huh et al. and D'Souza et al. both found greater radiological N stage to be associated with poorer prognosis.[157, 158] One study explored the prognostic value of lymph node size in stage II (i.e. node-negative) CC, showing that the largest lymph node short axis was independently associated with poorer relapse-free survival. While tumour deposits are not equivalent to lymph nodes, they are still considered part of N staging; D'Souza et al. investigated the importance of radiologically identified TDs and, while not a standard CT evaluation, found an adverse prognostic relationship.[157, 158]

Radiological EMVI is the other main category to be investigated across multiple studies. While adverse prognostic value has been demonstrated, the subjectivity of criteria for CT-based EMVI presents a major challenge. For example, in the study by Kato et al., just 9.2% of tumours were categorised as EMVI positive radiologically, compared to 66.4% pathologically.[162] Although the aim of this study was not to achieve strong radiology-pathology correlation, this substantial discrepancy and the small number of patients who were considered EMVI positive suggests that only gross EMVI was identified. Unlike primary tumour or lymph node measurements, it is challenging to employ standardised criteria for EMVI assessment and achieve reliable interpretation on a large, multicentre scale.

Finally, two studies have developed novel combined scoring systems, both of which were shown to be prognostic. Smith et al., who published the first prognostic evaluation of radiological features, also evaluated a 'prognostic score' (good vs. bad), comprising T stage, N stage and EMVI.[160] D'Souza et al. combined T stage, EMVI and TDs into the 'TDV score', which also achieved strong prognostic value.[157]

While these studies indicate potential utility for baseline radiological features to evaluate recurrence risk and stratify patients, there are several major limitations to these data: small sample sizes, single-centre design, retrospective reporting from one or a small number of non-representative expert radiologists, and varied, poorly defined criteria. Contemporary neoadjuvant trials are well placed to provide high-quality analyses on baseline radiological data and address this critical weakness in the literature.

#### 1.2.3.4 Treatment Response Assessment

Pathological regression following neoadjuvant treatment is associated with survival outcomes across different tumour types, including locally advanced CC.[53, 163-166] Imaging represents an alternative approach to evaluating treatment response with the advantage of offering an *in vivo* and whole-tumour assessment of treatment sensitivity prior to surgical resection. Similar to pathological regression, radiological response to neoadjuvant treatment is associated with prognosis across tumour types, but its value in CC is unknown.[167-169] The relationship between radiological and pathological response to neoadjuvant therapies in locally advanced CC is also unclear. Potential applications of radiological response assessment in CC include prognostication, response-adapted treatment, guiding adjuvant decision-making and, in the case of neoadjuvant immunotherapy for localised dMMR CC, identifying patients suitable for organ preservation (Figure 1.10).

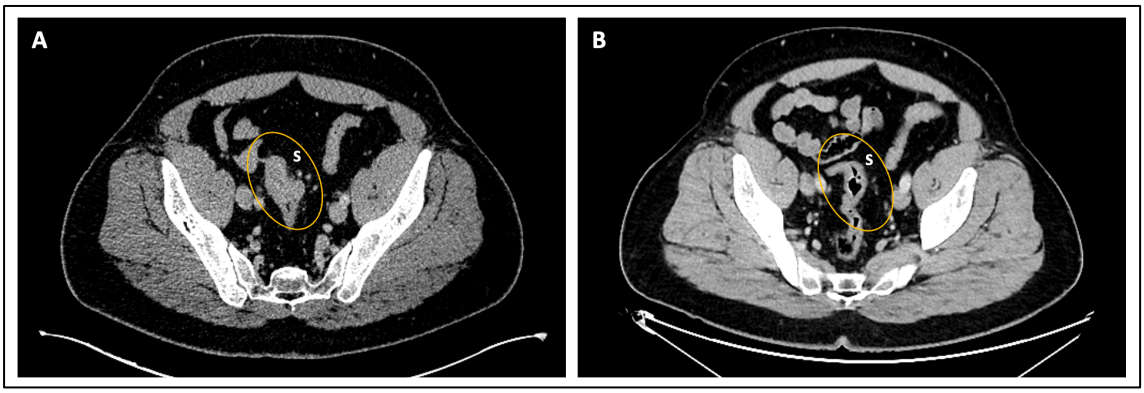



**Figure 1.10 Radiological Response to Neoadjuvant Chemotherapy**
A) Baseline axial image showing a T3 distal sigmoid tumour (yellow ring). B) Axial image showing a major reduction in tumour volume following 6 weeks of neoadjuvant chemotherapy. S = sigmoid.

##### 1.2.3.4.1 Neoadjuvant Chemotherapy

Radiological response to NAC in CC has only been explored in a few studies, some of which are focused imaging analyses, and others clinical trials with brief comments on imaging (Table 1.4).[170-175] While these studies vary in aim, the type and length of NAC, radiological features assessed and outcome measures, the data suggest that radiological response may be measurable following NAC. Tumour volume, depth of extramural extension, tumour thickness, tumour length, number of lymph nodes ≥5 mm, and length and width of the largest node have each been assessed, with some evidence of change following NAC. As metrics like tumour

thickness and length are not standardised or widely defined, studies presenting such data must be transparent regarding methodology to ensure reproducibility.

Two studies utilised the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria, which are commonly used for clinical trials in the metastatic setting.[173, 175, 176] However, this framework is unlikely to be suitable for evaluating response in single colonic tumours with complex morphology, luminal structure and variable bowel content. A further study also detected a significant metabolic response in a subset of participants who underwent an additional FDG PET-CT.[170] Je at al. developed a novel CT-based tumour regression grade (TRG) system with a hierarchical composition of unidimensional longest length, wall layer pattern, wall surface and wall thickness, showing a moderate correlation with pathological TRG.[172]

Importantly, there are clear limitations to each of these studies, including small patient numbers, single site design, variation in patient population and treatment regimen, poorly defined and/or chosen radiological measures, and a lack of correlation with meaningful pathological and survival outcomes. Further work is required to comprehensively characterise the radiological response to NAC in locally advanced CC, understand the impact of different NAC regimens, confirm its clinical relevance and establish a validated system for response assessment. Any system must be developed within a specific clinical context as tumour biology, drug type and treatment duration are likely to influence radiological appearances before and after NAC and the temporal changes that occur.

Table 1.4 Literature on Radiological Response to Neoadjuvant Chemotherapy in Localised Colon Cancer

| | Publication Type | Population | Patients | NAC Regimen | NAC Duration | Response Measures [a] | Key Results | Additional Comments |
|---|---|---|---|---|---|---|---|---|
| Dam et al.[171] | Focused imaging analysis of ongoing trial | cT3 (with ≥5 mm extramural extension) or cT4 | 67 | CAPOX +/- panitumumab | 9 weeks | Axial diameter<br>Largest dimension<br>Extension of extramural invasion<br>Number of LN >5 mm<br>Length of largest LN<br>Width of largest LN | Significant reductions in all measures | |
| Liu et al.[173] | Clinical trial | cT4 or cN1-2 | 47 | CAPOX | 6-12 weeks | Largest diameter (RECIST 1.1) | CR in 1/47 (2%)<br>PR in 31/47 (66%)<br>SD in 15/47 (32%)<br>PD in 0/47 (0%) | CT performed after 6 weeks treatment. Further 3-6 weeks given if PR/CR. |
| Zhou et al.[174] | Clinical trial | cT4 N2 | 23 | FOLFOXIRI | 2-8 weeks | Volume | Median reduction = 59.1% | |
| Arredondo et al.[170] | Retrospective analysis of routine data | cT3 (with ≥ 5mm extramural extension) or cT4 or cN1-2 | 65 (13 with FDG PET-CT) | CAPOX or FOLFOX | 8-12 weeks | Volume<br>SUVmax | Significant reductions in volume and SUVmax | |
| Je et al.[172] | Retrospective analysis of routine data | cT3-4 (with either ≥5 mm extramural extension or length ≥4 cm) | 53 | FOLFOX | 8 weeks | Unidimensional longest length<br>Volume<br>ctTRG | Non-significant reductions in length and volume<br><br>ctTRG and pTRG Spearman's rho = 0.54 | Novel ctTRG comprises hierarchical combination of unidimensional longest length, wall layer pattern, wall surface and wall thickness. |
| Zhou et al.[175] | Clinical trial | cT4 or cN2 or 'fused LNs' | 69 | FOLFOXIRI | 8 weeks | Largest diameter (RECIST 1.1) | CR in 1/69 (2%)<br>PR in 27/69 (39%)<br>SD in 37/69 (54%)<br>PD = 4 (6%) | |

CAPOX = capecitabine & oxaliplatin; CR = complete response; CT = computed tomography; ctTRG = computed tomography tumour regression grade; FDG = fluorodeoxyglucose F18; FOLFOX = 5-fluorouracil & oxaliplatin; LN = lymph node; FOLFOXIRI = 5-fluorouracil, oxaliplatin & irinotecan; NAC = neoadjuvant chemotherapy; PD = progressive disease; PET = positron-emission tomography; PR = partial response; pTRG = pathological tumour regression grade; RECIST = Response Evaluation Criteria in Solid Tumours; SD = stable disease; SUV = standardised uptake value.

[a] Referring to primary tumour unless otherwise specified.

#### 1.2.3.4.2 Neoadjuvant Immunotherapy

Due to the rapid expansion of early phase trials, there is more literature on radiological response to NAI than NAC, yet is similarly limited in scope and quality.[61, 67, 69, 72, 76, 77, 177-186] While the potential applications of NAI response are similar to NAC, there is greater focus on how effectively imaging can predict pCR and, as such, patients who may be suitable for organ preservation. Most existing literature comprises brief comments on radiological response within larger clinical trials or case series publications. As these are early phase trials, the sample sizes were usually small and taken from a single or limited number of centres. Furthermore, the methodologies used for response assessment were mostly confined to RECIST 1.1, or related systems like immune RECIST and Positron Emission Tomography Response Criteria in Solid Tumours, which are not specifically designed for evaluating complex luminal tumours. This heterogeneous literature is also limited by variation in the immunotherapy used, additional non-immunotherapy agents, duration of treatment and definitions of clinical complete response.

Collectively, these studies detailing radiological response to NAI demonstrate common themes: (1) limited concordance between radiological, endoscopic and pathological response, including in those with confirmed pCR; (2) radiological changes typically lag behind those seen on endoscopy, where residual tumour is visible on imaging despite a fully healed or scarred mucosa endoscopically; and (3) organ preservation appears feasible for some patients with good long-term outcomes.

The strongest data on NAI response comes from a post-hoc analysis of imaging data from the PICC trial, which included traditional primary tumour characteristics (EMVI), simple quantitative features (maximum tumour thickness, longitudinal diameter), less commonly used but recognised features (calcification, fat infiltration, fistulae and necrosis) and novel patterns (‘vascular sign’, ‘nodular sign’ and ‘extramural enhancement sign’).[183] In this analysis, significant differences in the percentage changes of maximum tumour thickness and longitudinal diameter were seen between those with pCR and those without. Additionally, the three novel patterns were all significantly less likely to be present in those with pCR.

As the relationship between radiological and pathological response to NAI appears inconsistent, new tools and systems must be developed to reliably identify tumours with pCR. A recent study proposed a model integrating dynamic changes in tumour diameter and thickness, endoscopic response and endoscopic biopsy results with strong predictive value for

pCR to NAI.[186] While further validation of this model is required, it highlights a potential direction of travel utilising multimodal data for outcome prediction.

#### 1.2.3.5 Experimental Approaches

##### 1.2.3.5.1 Advanced CT Techniques

As the role of CT imaging grows to facilitate the use of neoadjuvant therapies, advancements and adjuncts to existing techniques should be considered to address emerging challenges.

Spectral CT, including photon-counting CT, utilises detectors that can switch on and off more rapidly, and is therefore far more efficient than standard CT.[139] As such, lower radiation doses can be used to achieve the same image or ultrahigh-resolution images can be produced with the same radiation dose. Multi-energy technology is used to show how individual tissues interact with x-rays of different energies.[139, 187] Potential applications include diagnosis, staging and the prediction of histopathological features. As an emerging field, there are several challenges facing spectral CT, including the need for advanced infrastructure to view and manipulate images, variation in acquisition and measurement techniques, and current evidence still being limited to experimental settings.

Perfusion CT provides information on tumour vasculature, which may have utility for prognostication and treatment response assessment.[188] There is some evidence that measurable alterations in blood flow occur in rectal cancer following neoadjuvant treatment, but this has limited concordance with pCR.[189] In CC, the recent PROSPECT trial showed that perfusion CT metrics did not improve the prognostic value of an existing clinicopathological model.[190]

##### 1.2.3.5.2 Body Composition

Body composition is recognised as an important individual characteristic with implications for clinical outcomes across different cancer types.[191] Assessing muscle and fat distribution on routine CT imaging represents a simple surrogate for whole-body composition. The quality (using radiodensity) and quantity of skeletal muscle, visceral fat and subcutaneous fat are common measures, which may be further assessed as ratios (e.g. visceral to subcutaneous fat ratio) or converted to indices to normalise according to body stature. A single slice at the inferior border of the L3 vertebrae is often used as values at this level correlate strongly with whole body values.[191] As CC is predominantly a disease of older adults, many of whom have

co-existing frailty, the importance of body composition may exceed that seen in other tumour types.[1, 192]

There is extensive literature on the associations between body composition and clinical outcomes in CC, including surgical complications and recovery, chemotherapy-toxicity, and survival.[193-200] However, many of these studies are limited by heterogeneous design and inconsistent definitions for body composition measures. These issues have impeded the translation of body composition assessments into routine clinical practice.

The importance of body composition in the neoadjuvant setting for CC has not been investigated. Here, there is a different balance of risks and benefits, which have implications for clinical outcomes. For example, maintaining dose-intensity throughout the neoadjuvant period is considered crucial for tumour downstaging over a short treatment period. Therefore, being able to better predict treatment-toxicity (and thus potential for treatment delays) or identify patients that are too frail for systemic therapy at all would be useful. Furthermore, there is some evidence in rectal cancer that a higher level of visceral fat is associated with poorer responses to neoadjuvant chemoradiotherapy.[201] Understanding the relevance of body composition in the neoadjuvant setting for locally advanced CC is therefore an important avenue of research.

#### 1.2.3.5.3 Radiomics

Radiomics describes the use of computational image analysis to extract quantitative data from medical images beyond what is seen on normal visual inspection.[202] By shifting image interpretation from a qualitative to quantitative process, radiomics provides a non-invasive approach for biomarker detection. The concept of radiomics was introduced over a decade ago and comprises a standard sequence of steps: image acquisition (with or without additional reconstruction), delineation of the region of interest, feature extraction, feature selection, development of a radiomics signature and validation.[202]

In CC, various applications of radiomics have been explored, including the prediction of disease staging, pathological characteristics, and recurrence risk.[203-208] There is also growing interest in the use of radiomics as part of larger multi-omics platforms.[209] However, there are several recognised limitations of radiomics, including study heterogeneity, lack of standardisation across imaging protocols, poor reproducibility and validation, and a paucity of prospective studies. Furthermore, radiomics research within gastrointestinal oncology has

often focused on rectal cancer and other imaging modalities, such as MRI.[210] The role of radiomics in the neoadjuvant setting for locally advanced CC is unclear, but a non-invasive radiomics signature could provide valuable information for patient stratification, where current decision-making already utilises baseline CT imaging.

#### 1.2.3.5.4 Radiogenomics

Using radiomics for genomic characterisation is often termed 'radiogenomics', and the molecular heterogeneity of CC makes it an ideal candidate for such approaches.[211] Previous studies have suggested that it may be possible to use radiomic features to predict *KRAS*, *NRAS*, *BRAF* and microsatellite statuses.[212] However, these data come from individual, small studies focusing on a single molecular marker and therefore require validation to demonstrate wider utility for radiogenomics in this setting. Furthermore, the broader challenges of implementing radiomics remain.

#### 1.2.3.5.5 Multimodal Artificial Intelligence

Artificial intelligence (AI) is gaining traction across healthcare and has numerous potential applications in CC radiology, including, but not limited to, optimising scanning protocols, enhancing image quality, image harmonisation, tumour detection, automated segmentation, prognostication and treatment response.[213] To support neoadjuvant therapy delivery, AI is likely to play a significant role in biomarker development and the integration of data from other modalities, including digital pathology, circulating tumour DNA and other -omics domains.

## 1.3 Key Guidelines and Consensus Statements

In the following sections, recommendations related to the use of neoadjuvant therapies and imaging in localised CC from key national and international guidelines and consensus statements are summarised. Specific technical aspects of imaging are beyond the scope of this overview and are therefore not included. The level of detail presented reflects that provided in the source guidelines.

### 1.3.1 Association of Coloproctology of Great Britain and Ireland

The Association of Coloproctology of Great Britain and Ireland (ACPGBI) have published guidance on the management of CC, divided into individual sections. Information from the sections on 'diagnosis, investigations and screening' and 'follow up, lifestyle and survivorship' are summarised below.[214, 215]

#### 1.3.1.1 Neoadjuvant therapies

- None

#### 1.3.1.2 Imaging

- Colonoscopy or CTC should be offered to patients with suspected CC.
- CTC should now be performed in place of barium enema.
- CTC should be considered for patients in whom full colonoscopic evaluation was not possible.
- Radiological staging using a CT of the chest, abdomen and pelvis should be performed in all patients with suspected CC, including assessments of:
    - Extent of local invasion, including involvement of other structures.
    - Metastatic disease.
    - Synchronous colonic disease.
- MRI and FDG PET-CT may be used to investigate lesions indeterminate on CT.
- At least two CT scans of the chest, abdomen and pelvis are recommended within three years following surgical resection.
- CTC may be used as an alternative to colonoscopy for assessment of the "clean colon" at one year and five years post-resection.

### 1.3.2 European Society of Gastrointestinal and Abdominal Radiology

In conjunction with other professional societies, the European Society of Gastrointestinal and Abdominal Radiology (ESGAR) have provided guidance and recommendations in the form of a general educational 'essentials' piece (with the European Society of Radiology) and another focusing on the use of imaging over colonoscopy (with the European Society of Gastrointestinal Endoscopy).[216, 217]

#### 1.3.2.1 Neoadjuvant Therapies

- None.

#### 1.3.2.2 Imaging

- CTC is an established screening test for CRC and may be considered as the initial test where FIT-based programmes are not available.
- CTC should be performed in patients with positive FIT results where colonoscopy was incomplete or unfeasible.
- CTC should ideally be performed the same or next day following incomplete colonoscopy.
- CTC is the radiological examination of choice for the diagnosis of colorectal cancer.
- Baseline CT reports must include evaluations of:
    - Wall infiltration, including identification of high-risk T staging (i.e. cT3cd or cT4ab).
    - EMVI status.
    - Retroperitoneal margin (for ascending and descending colon cancers).
- MRI and FDG PET-CT should be used as “problem-solving” techniques.
- CTC should be considered for surveillance in patients where colonoscopy is contra-indicated or unfeasible.

### 1.3.3 European Society of Medical Oncology

As part of the Clinical Practice Guidelines range, the European Society of Medical Oncology (ESMO) have published recommendations specifically for localised CC, including on diagnostic workup.[24]

#### 1.3.3.1 Neoadjuvant therapies

- None.

#### 1.3.3.2 Imaging

- For diagnosis and the exclusion of synchronous tumours, CTC may be used as an alternative to colonoscopy if full colonoscopy is not possible.

- Intravenous contrast-enhanced CT of the chest, abdomen and pelvis is the preferred radiological method for assessing the presence of distant metastases, locoregional tumour extension and primary tumour complications (e.g. obstruction, perforation, fistula or abscess).
- MRI may be used to better evaluate the relationship between primary tumours and surrounding structures, characterise liver lesions indeterminate on CT and as an alternative to other CT-based assessments in the case of contrast allergies or renal impairment.
- FDG PET-CT is not recommended for routine radiological staging but may assist in the characterisation of lesions indeterminate on CT.
- Following surgical resection, CT of the chest and abdomen should be performed every six to twelve months for three years in high-risk patients, according to the TNM classification.

### 1.3.4 National Comprehensive Cancer Network

The United States-based National Comprehensive Cancer Network (NCCN) has similarly produced clinical guidelines specifically for CC. While guidelines from ESMO and the National Institute for Health and Care Excellence (NICE) are those primarily followed in the UK, the NCCN guidelines are often updated more frequently and seen as alternative best-practice recommendations.[36]

#### 1.3.4.1 Neoadjuvant Therapies

- NAC (5FU & oxaliplatin [FOLFOX] or capecitabine plus oxaliplatin [CAPOX]) may be considered for localised pMMR tumours that are staged cT4b or have “bulky nodal disease”.
- NAI may be considered for localised dMMR tumours staged cT4.
- NAI or NAC may be considered for localised dMMR (or *POLE*/*POLD1* mutant with hypermutated phenotype) tumours that are staged cT4b or have “bulky nodal disease”.

#### 1.3.4.2 Imaging

- Intravenous and oral contrast-enhanced CT of the chest, abdomen and pelvis should form part of the workup for localised CC appropriate for resection with the following aims:
    - Evaluate primary tumour invasion and involvement of other structures.
    - Confirm the presence or absence of distant metastatic disease.
- MRI of the abdomen and pelvis may be considered to aid the differentiation of distal sigmoid and upper rectal cancer or as an alternative to CT in patients with significant contrast allergy or chronic renal impairment.
- FDG PET-CT is only indicated in the initial diagnostic workup when both CT and MRI are contra-indicated (e.g. due to contrast allergy) or to evaluate lesions indeterminate on CT and/or MRI.
- CT of the chest, abdomen and pelvis should be performed prior to adjuvant therapy to assess response to primary therapy or resection.
- CT of the chest, abdomen and pelvis should be performed every six to twelve months for five years following surgery for stage II and III CC.

### 1.3.5 National Institute for Health and Care Excellence

The NICE guidelines for CRC provide a foundation for practice in the UK and includes sections specific to colon and rectal cancers.[127]

#### 1.3.5.1 Neoadjuvant therapies

- Neoadjuvant systemic anti-cancer therapy may be considered for cT4 CC.

#### 1.3.5.2 Imaging

- Staging for localised CRC uses the UICC TNM system.
- CT of the chest, abdomen and pelvis should form part of a surveillance plan for the first three years following surgery for localised CC.

### 1.3.6 Royal College of Radiologists

The Royal College of Radiologists (RCR) have produced three relevant guidance documents on the use of cross-sectional imaging of the lower gastrointestinal tract, CTC for suspected CRC and indications for PET-CT, which includes a section on CRC.[218-220]

#### 1.3.6.1 Neoadjuvant therapies

- None.

#### 1.3.6.2 Imaging

- CTC is indicated for the detection of CRCs where colonoscopy is contra-indicated, incomplete or has failed.
- Barium enema should no longer be performed as an alternative to colonoscopy.
- CTC should not be performed in patients with inflammatory bowel disease or a hereditary CC syndrome.
- Minimal preparation CTC may be considered for patients in whom fitness for treatment is uncertain.
- Probable CRCs identified on CTC should be followed by full staging, including a CT chest.
- All patients with newly diagnosed CC should undergo CT of the chest, abdomen and pelvis to provide staging with the following objectives:
    - Identify surgically challenging cases.
    - Assess the size of the primary tumour and extent of extramural invasion.
    - Identify involvement of adjacent structures.
    - Identify complications related to the primary tumour.
    - Assess for lymph node involvement, EMVI status, peritoneal involvement, and distant metastases (including retroperitoneal, pelvic and inguinal lymph nodes).
    - Evaluate size and distribution of distant metastases.
- FDG PET-CT is indicated for the following:
    - To support staging of patients with equivocal findings on other imaging (e.g. lung or liver lesions).
    - To detect recurrence in patients with rising tumour markers and/or clinical concerns with normal or equivocal findings on other imaging.

### 1.3.7 Scottish Intercollegiate Guidelines Network

Guidance for the diagnosis and management of CRC has been produced by the Scottish Intercollegiate Guidelines Network (SIGN).[88]

#### 1.3.7.1 Neoadjuvant therapies

- None.

#### 1.3.7.2 Imaging

- CTC can be used as an alternative diagnostic investigation to colonoscopy to provide complete colonic examination.
- In older adults or those with frailty, minimal preparation CTC may be considered as an alternative to standard CTC.
- Contrast-enhanced CT of the chest, abdomen and pelvis is the standard investigation for radiological staging.
- MRI and ultrasound may be used to characterise liver lesions indeterminate on CT.
- FDG PET-CT may be used to investigate patients with:
    - Raised CEA level and negative standard imaging investigations.
    - Concerns for pelvic recurrence and a pre-sacral mass.
- Follow-up after curative CC resection should include interval CT scanning.

### 1.3.8 Critical Evaluation

As a common and multidisciplinary disease, several expert guidelines and recommendations have been developed for CC from oncological, radiological and surgical professional societies. While these publications provide a foundation for clinical practice, there are important limitations and inconsistencies to consider.

Firstly, several of these guidelines are outdated given the speed at which new evidence is being generated and the complexity of the field. For example, UK oncology practice primarily follows the NICE and ESMO guidelines, which were both published in 2020. Likewise, the SIGN and ACPGBI guidelines were published in 2011 and 2017, respectively.

Neoadjuvant therapies have not been widely adopted in these guidelines. This absence may, in part, reflect the timescales of publication, where the first evidence for neoadjuvant therapies

came after guideline publication. However, these guidelines are often updated iteratively in response to new scientific findings. For example, the ESMO guideline for localised CC does not mention neoadjuvant therapies in any capacity, yet the 'living guideline' version integrated findings from the CHALLENGE trial into the recommended treatment algorithm earlier this year. Findings from the FOxTROT and OPTICAL trials have not been included in the same way. NICE also updates guidelines intermittently but has not done so since the publication of either trial.

The NCCN are the only organisation to split their guidance according to MMR status, which is appropriate and consistent with contemporary evidence. Unlike others, the NCCN recommends the use of NAC and NAI in specific situations, although these are not strongly evidence-based. First, they recommend considering NAC for cT4 tumours or those with "bulky nodal disease". While the subgroup analyses in FOxTROT and OPTICAL suggest those with cT4 tumours derive greater benefit from NAC than cT3, this was not definitive, and this hypothesis has not been tested in a clinical trial setting. FOxTROT was also a positive trial where cT3-4 staging predicted benefit from NAC and thus represents the strongest data available. The terminology "bulky nodal disease" is not defined, nor evidence-based, and introduces significant ambiguity alongside the myriad issues which already affect radiological lymph node assessment.

The NCCN guidance for NAI is similarly limited but is attempting to provide recommendations in a more challenging space. Typically, the existing trials of NAI would not be sufficient to change routine clinical practice due to the lack of phase III, randomised and long-term follow-up data. However, the remarkable efficacy seen with NAI in these trials, in addition to some long-term follow-up data from NICHE-2, has led some to believe that expedited approvals for routine use is appropriate.

The radiological guidance provided by ESGAR, RCR and the other multidisciplinary publications is more extensive. There is clear consistency between organisations that CTC has a role in screening and diagnostic settings, with detailed information and guidance to support this practice. Likewise, there is general agreement regarding the role of CT for baseline staging, albeit without explicit use of the AJCC/UICC TNM system. Subdividing cT3 staging according to depth of extramural extension (i.e. cT3a-d) is recommended by ESGAR despite not being a formal part of the TNM system or included in in other CC guidelines. The roles of MRI and FDG PET-CT are also viewed consistently, with most bodies recommending selective use to characterise indeterminate lesions. All the guidelines suffer from limited clarity on radiological

surveillance, reflecting an unclear evidence-base, which therefore represents an area for future research.

Although there is greater detail and consistency for imaging recommendations than the use of neoadjuvant therapies, it is notable that the radiology guidelines do not discuss the new roles of imaging in the neoadjuvant setting. Radiological patient selection, prognostication and response assessment are not mentioned, highlighting the lack of evidence in these areas. There is clearly a need to develop the imaging evidence base alongside novel therapeutic approaches to ensure both fields progress together and complement each other.

## 1.4 Outstanding Questions and Areas for Research

The development of neoadjuvant treatments in localised CC represents a major research success in a field that, for many years, had limited therapeutic advancements. This progress provides opportunities to undertake clinically impactful translational research across disciplines, including radiology, to refine treatment delivery and improve patient outcomes. In this emerging field, using high-quality data from clinical trials to address key outstanding questions is a priority. The research presented in this thesis utilises data from the FOxTROT trial to provide much-needed evidence on the role of imaging across three key components of neoadjuvant treatment delivery: patient selection, risk stratification and prognostication, and response assessment.

Selecting patients with localised CC for systemic anti-cancer therapy using imaging represents a major shift in practice. As such, it is crucial to examine the accuracy of radiological staging within this context and the impact it has on patient selection. As neoadjuvant therapies become more personalised, understanding whether staging accuracy differs between patient cohorts also represents an important question.

To refine patient selection beyond current approaches, relevant datasets should be evaluated to determine how baseline data can identify patients at greatest risk of disease recurrence, who should therefore be considered for neoadjuvant therapies. Currently there is limited evidence to inform this process with established biomarkers mostly restricted to data from post-operative evaluations. Furthermore, patient stratification remains focused on the TNM system which has major limitations across both radiology and pathology. Efforts to improve baseline patient stratification must look beyond TNM to identify more effective and reproducible biomarkers.

For patients who receive neoadjuvant therapies, there is limited evidence to show how radiology influences subsequent phases of the patient journey. Radiology is well placed to provide a comprehensive evaluation of treatment response with several potential applications. However, due to this lack of evidence, there is currently no established framework for pre-operative assessment of treatment response. To address this critical evidence gap, neoadjuvant trial data must be leveraged to: (1) characterise the radiological changes seen with different neoadjuvant treatment regimens; (2) explore how reliable these measures are between radiologists; (3) understand the relationship between radiological response, pathological response and other modalities; and (4) evaluate how different response metrics inform relevant clinical outcomes.

These opportunities for research have clear potential for clinical impact and will be explored in the chapters of this thesis.

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# 2 CT Staging Performance in an International Trial of Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer

## 2.1 Abstract


### 2.1.1 Objectives

In the Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT) trial, neoadjuvant chemotherapy (NAC) significantly reduced recurrence risk, compared to upfront surgery, in locally advanced colon cancer. This analysis evaluates the correlation between radiological and pathological staging within the trial to support the adoption of CT-based patient selection.

### 2.1.2 Methods

In this preplanned analysis of prospectively collected data, local radiological and pathological staging were compared in upfront surgery participants. T stage, N stage, and extramural venous invasion (EMVI) status were evaluated using overall agreement, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Subgroup analyses explored the impact of mismatch repair status and tumour side.

### 2.1.3 Results

A total of 354 participants were included. T stage agreement was 63.0%; T3 and T4 tumours were correctly identified in 78.9% and 41.1% of participants, respectively. The PPV for T3-4 status was 94.5%. N stage agreement was 39.8%; for N status (positive vs. negative), overall agreement, sensitivity, specificity, PPV, and NPV were 54.1%, 81.1%, 26.0%, 53.2%, and 57.1%, respectively. For EMVI, these values were 54.9%, 71.0%, 41.2%, 50.7%, and 62.5%, respectively. Accuracy metrics did not differ significantly by tumour side or mismatch repair status.

### 2.1.4 Conclusions

CT effectively predicted T3-4 status with minimal overstaging, but performed poorly for individual T stage, N stage, and EMVI. We propose radiological T3-4 status should be adopted as the primary biomarker for neoadjuvant patient selection, with molecular biomarkers to guide treatment choice.

### 2.1.5 Advances in Knowledge

In this multicentre trial, local radiologists accurately identified T3-4 status to select participants for NAC, indicating utility for future neoadjuvant trials and clinical practice.

## 2.2 Introduction

The international Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT; NCT00647530) study was the first phase III, randomised controlled trial to evaluate neoadjuvant chemotherapy (NAC) in locally advanced but resectable colon cancer (CC).[1, 2]

In contrast to adjuvant chemotherapy, where pathological staging guides treatment decisions, patient selection for NAC is based upon radiological (clinical) tumour, node, metastasis (TNM) staging using CT.[2, 3] FOxTROT selected patients with radiological T3-4 N0-2 M0 staging and determined that 6 weeks of NAC significantly reduced the risk of recurrence at 2 years, compared to immediate surgery.[2] Furthermore, NAC was associated with lower pathological T and N stages, the absence of extramural venous invasion (EMVI) and lower rates of incomplete surgical resection and post-operative complications. Therefore, NAC can be considered a safe and effective treatment for locally advanced CC.

The limitations of matching radiological and pathological staging are well-recognised and the selection of patients with CC for NAC using CT represents a major shift in practice.[4, 5] In addition, the recent emergence of neoadjuvant immunotherapy for patients with mismatch repair (MMR) deficient (dMMR) CC indicates radiological patient stratification will become increasingly important.[6] CT is the gold standard investigation for radiological evaluation with similar diagnostic accuracy compared to MRI and PET-CT.[7] However, as the neoadjuvant treatment landscape evolves, alternatives to CT may provide effective imaging biomarkers for patient selection and treatment-response assessment. While radiological staging performance in CC has been comprehensively evaluated, some studies are limited by small sample size, retrospective data, or single-centre design, and others present data where staging

assessments are unlikely to have been performed with patient selection for NAC in mind.[4, 5, 8-17] Understanding the correlation between radiological and pathological assessment of T stage, N stage, and EMVI status within a multicentre trial context could accelerate moving CT stratification for personalised CC neoadjuvant therapies into routine clinical practice.

## 2.3 Methods

The FOxTROT study protocol was granted ethical approval (MREC: 07/S0703/57), including analysis of prospectively collected CT scan data, with all participants providing written consent. The main trial results have already been published.[2]

### 2.3.1 Study Design

A preplanned analysis to correlate radiological and pathological staging was performed in participants randomised to the straight-to-surgery (STS) arm, who therefore had not received NAC. Participants randomised to the NAC arm were not included in this analysis due to the downstaging effect of NAC, following which pre-treatment radiological and post-treatment pathological staging are not directly comparable.

Participants had radiologically staged T3 (with ≥1 mm extension beyond the muscularis propria) or T4 non-metastatic CC on baseline CT. While the degree of extension beyond the muscularis propria, N stage, and EMVI status were also predicted and recorded by local site radiologists, they were not considered for trial eligibility. Patients with bowel obstruction were eligible, providing the obstruction was first relieved with a defunctioning stoma. Eligible patients were identified during local multidisciplinary team (MDT) meetings across 85 sites in the United Kingdom, Denmark, and Sweden between May 15, 2008 and December 23, 2016.

### 2.3.2 Pathological Staging

The reference standard was pathological staging of the surgical specimen, according to TNM version 5, which was recorded on a detailed pathology proforma supported by a written protocol.[18] In addition to pathological T and N stages, the depth of extramural extension (for T3 and T4 tumours) and presence or absence of EMVI were also recorded. A Lead Pathologist at each trial site attended in-depth face-to-face pathology training. Pathologists were not specifically blinded to results from pre-treatment scans.

### 2.3.3 Radiological Staging

Radiological staging (including T stage, depth of extramural extension, N stage and EMVI status) was the index test, which was performed at baseline using a portal venous phase, contrast-enhanced, spiral/multidetector CT of the thorax, abdomen, and pelvis with maximum 5 mm slice thickness. Oral contrast was permitted according to radiologist preference. A Lead Radiologist at each trial site attended in-depth face-to-face radiology training (led by a specialist gastrointestinal radiologist with over 10 years' experience) on image interpretation and tumour analysis to guide reporting using TNM version 5.[18] At least 148 radiologists (unnamed for 115/1052 [10.9%] CT scans) from 85 sites reported CT scans in the trial. Initial MDT review of radiological staging was used to confirm trial eligibility (as reported in the main trial publication).[2] A subsequent detailed review of the tumour was provided by the trained Lead Radiologist; differences between these reviews are detailed in Table 7.1. The local Lead Radiologist review is considered the final local centre radiology opinion for clinical staging for this analysis and subsequent publications for this trial.

### 2.3.4 Data Collection

Local radiological staging data were submitted by the Lead Radiologist using a detailed case report form (CRF). Local pathological staging data were also submitted using a CRF. MMR status was retrospectively determined using immunohistochemistry for MLH1, PMS2, MSH2, and MSH6 at the central trial laboratory on either resection or biopsy specimens.

### 2.3.5 Statistical Analysis

T stage, N stage, and EMVI status were assessed for radiological and pathological agreement. T stage and N stage were further evaluated as dichotomous outcomes known as T status (T1-2 vs. T3-4) and N status (N0 vs. N1-2[+]), which are more clinically relevant for treatment decisions. Staging subgroups (e.g. T4a vs. T4b) were not considered. However, since other studies have defined T3 with ≥5 mm extramural extension as a high-risk group of interest, we performed a further assessment of T status using this threshold.[19]

Staging assessments were evaluated against MMR status and primary tumour side (right vs. left colon), which have been reported to influence radiological staging accuracy.[20, 21] For this analysis, right-sided tumours were defined as those proximal to the splenic flexure and left-sided tumours those at, or distal to, the splenic flexure.

Overall agreement was calculated for each staging outcome. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CT were calculated for each dichotomous outcome (T status limited to PPV only) and reported with 95% CIs. To evaluate the alternative dichotomy of T3 with ≥5 mm extramural extension, the PPV of CT to predict the equivalent extent of pathological invasion (i.e. pT3 ≥5 mm) was calculated. Overall agreement was calculated as the number of participants with concordant radiological and pathological staging divided by the total number of participants with complete data. The other metrics were calculated according to standard definitions.[22] The 95% CIs for overall agreement were calculated using MedCalc (MedCalc Software Ltd. Diagnostic test evaluation calculator Version 22.023, Ostend, Belgium). STATA software was used for all other analyses (StataCorp, Stata Statistical Software: Release 16, College Station, TX, United States: StataCorp LLC). Illustrations were prepared in Microsoft Powerpoint (Microsoft, Version 16.97, Redmond, WA, United States) and Python (Python Software Foundation, Version 3.11.5, Wilmington, DE, United States) using the Matplotlib (Version 3.7.2) and Seaborn (Version 0.12.2) libraries.

## 2.4 Results

### 2.4.1 Participant Characteristics

A total of 354 participants were randomised to the STS arm and therefore included in this analysis, comprising 225 men and 129 women (mean age 63.2 years). Baseline clinical and staging characteristics for trial participants have been reported previously (Table 7.2).[2] Some differences were observed for tumour location, T stage, N stage, and extramural extension between initial MDT review and trained Lead Radiologist review, but crucially, only 2/1052 (0.2%) were revised from T3 to T2 staging (Table 7.1). Figure 2.1 illustrates the selection of participants for each component of the analysis.

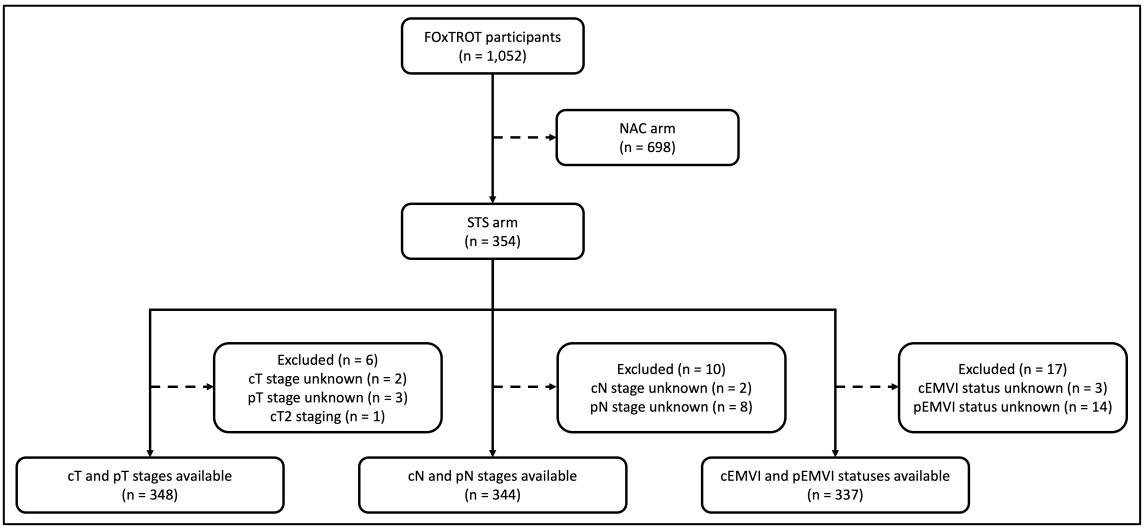



**Figure 2.1 Selection of Participants**
Assessments of radiological and pathological correlation were performed for participants in the STS arm. For each part of the analysis, participants were excluded if either of the corresponding radiological or pathological data were unknown. Abbreviations: c = radiological; EMVI = extramural venous invasion; N = lymph node; NAC = neoadjuvant chemotherapy; p = pathological; STS = straight-to-surgery; T = primary tumour.

### 2.4.2 T Staging

Both radiological and pathological T stage data were available in 98.5% (349/354) of participants (Figure 2.1).

The overall agreement for T stage was 63.0% (220/349), with T3 and T4 tumours correctly identified in 78.9% (176/223) and 41.1% (44/107) of participants, respectively (Table 2.1; Figure 2.2). Calculation of the sensitivity, specificity, and NPV of CT for predicting T3-4 status was not possible due to the exclusion of patients radiologically staged T1-2 from the trial. However, the PPV for predicting T3-4 status was 94.5% (329/348) (Tables 2.1 and 2.2; Figure 2.4). With the T status threshold adjusted to T3 (≥5 mm extramural extension)-T4 staging, the PPV was 68.7% (173/252) (Table 7.3). Of the 20 participants with pathologically staged T1-2 tumours, 8 had an alternative high-risk pathological feature (N+ or EMVI).

Table 2.1 Radiological and Pathological Correlation for T Stage, N Stage and EMVI Status

| | pT1 | pT2 | pT3 | pT4 | Total |
|---|---|---|---|---|---|
| cT1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| cT2 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.3) | 1 (0.3) |
| cT3 | 1 (0.3) | 15 (4.3) | 176 (50.4) | 62 (17.8) | 254 (72.8) |
| cT4 | 0 (0.0) | 3 (0.9) | 47 (13.5) | 44 (12.6) | 94 (26.9) |
| Total | 1 (0.3) | 18 (5.2) | 223 (63.9) | 107 (30.7) | 349 |

| | pT1-2 | pT3-4 | Total |
|---|---|---|---|
| cT1-2 | 0 (0.0) | 1 (0.3) | 1 (0.3) |
| cT3-4 | 19 (5.4) | 329 (94.3) | 348 (99.7) |
| Total | 19 (5.4) | 330 (94.6) | 349 |

| | pN0 | pN1 | pN2 | Total |
|---|---|---|---|---|
| cN0 | 44 (12.8) | 19 (5.5) | 14 (4.1) | 77 (22.4) |
| cN1 | 82 (23.8) | 51 (14.8) | 34 (9.9) | 167 (48.5) |
| cN2 | 43 (12.5) | 15 (4.4) | 42 (12.2) | 100 (29.1) |
| Total | 169 (49.1) | 85 (24.7) | 90 (26.2) | 344 |

| | pN0 | pN+ | Total |
|---|---|---|---|
| cN0 | 44 (12.8) | 33 (9.6) | 77 (22.4) |
| cN+ | 125 (36.3) | 142 (41.3) | 267 (77.6) |
| Total | 169 (49.1) | 175 (50.9) | 344 |

| | pEMVI present | pEMVI absent | Total |
|---|---|---|---|
| cEMVI present | 110 (32.6) | 107 (31.8) | 217 (64.4) |
| cEMVI absent | 45 (13.4) | 75 (22.3) | 120 (35.6) |
| Total | 155 (46.0) | 182 (54.0) | 337 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage/status; p = pathological; T = primary tumour stage. Green shading represents radiological and pathological concordance which was used to calculate overall agreement. Numbers in brackets represent staging combination as a percentage of the overall cohort.

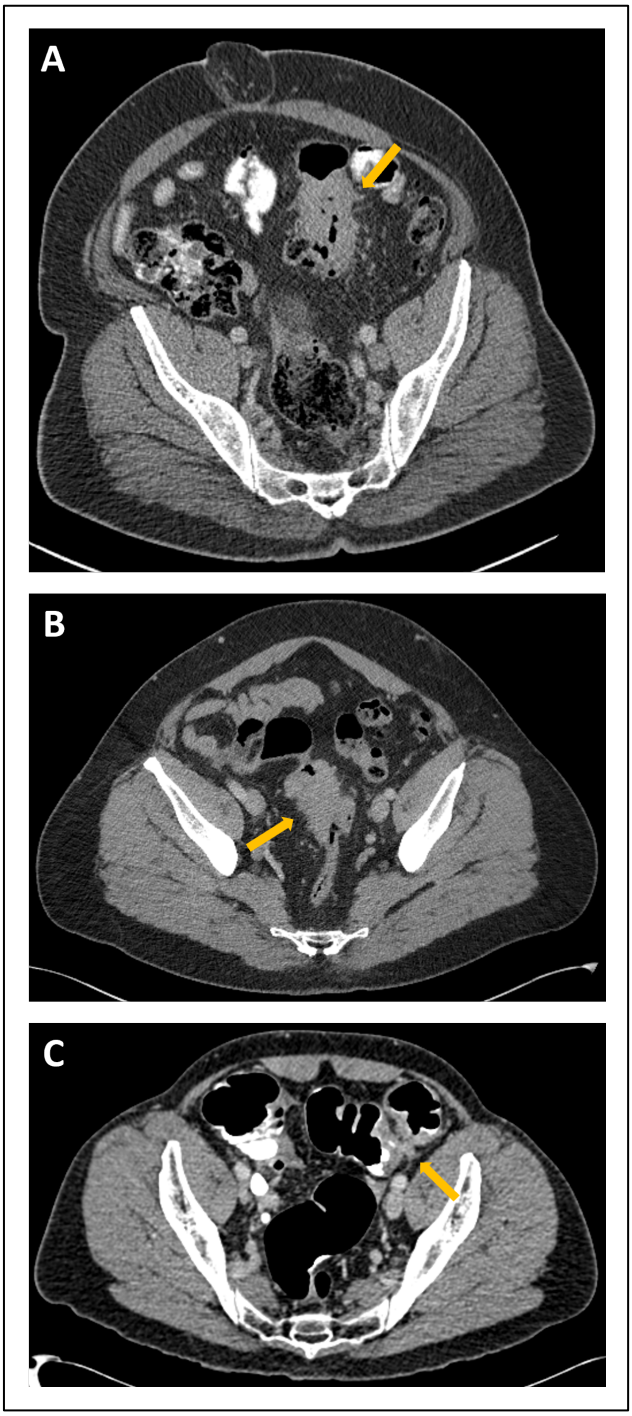



**Figure 2.2 Example T Staging**
Baseline axial images of participants in the straight-to-surgery arm. (A) Correctly staged T3 sigmoid tumour. Yellow arrow indicates area of extension beyond the muscularis propria. (B) pT2 sigmoid tumour that was over-staged as cT3. Yellow arrow indicates the primary tumour. (C) pT3 sigmoid tumour that was over-staged as cT4 with predicted involvement of peritoneum. Yellow arrow indicates area of suspected peritoneal invasion.

### 2.4.3 N Staging

Both radiological and pathological N stage data were available in 97.2% (344/354) of participants (Figure 2.1).

Overall N stage agreement was 39.8% (137/344) (Table 2.1). Pathological N0, N1, and N2 staging were correctly predicted in 26.0% (44/169), 60.0% (51/85), and 46.7% (42/90) of participants, respectively (Table 2.1; Figure 2.3). The overall agreement, sensitivity, specificity, PPV, and NPV of CT for predicting N status were 54.1% (186/344), 81.1% (142/175), 26.0% (44/169), 53.2% (142/267), and 57.1% (44/77), respectively (Tables 2.1 and 2.2; Figure 2.4).

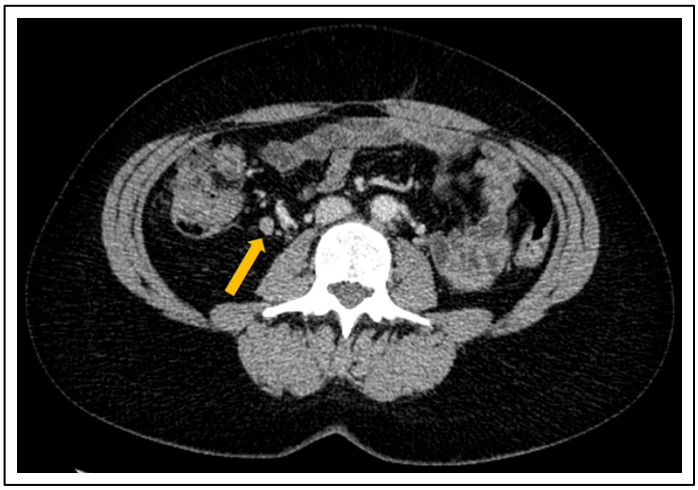


**Figure 2.3 Example of Incorrect N Staging**
Baseline axial image of a participant in the straight-to-surgery arm with an ascending colon tumour. This case was radiologically staged cN2 and pathologically staged pN0. Yellow arrow indicates a regional lymph node suspicious for malignant involvement.

### 2.4.4 EMVI Status

Both radiological and pathological EMVI status data were available in 95.2% (337/354) of participants (Figure 2.1).

The overall agreement, sensitivity, specificity, PPV, and NPV of CT for predicting EMVI status were 54.9% (185/337), 71.0% (110/155), 41.2% (75/182), 50.7% (110/217), and 62.5% (75/120), respectively (Tables 2.1 and 2.2; Figure 2.4).

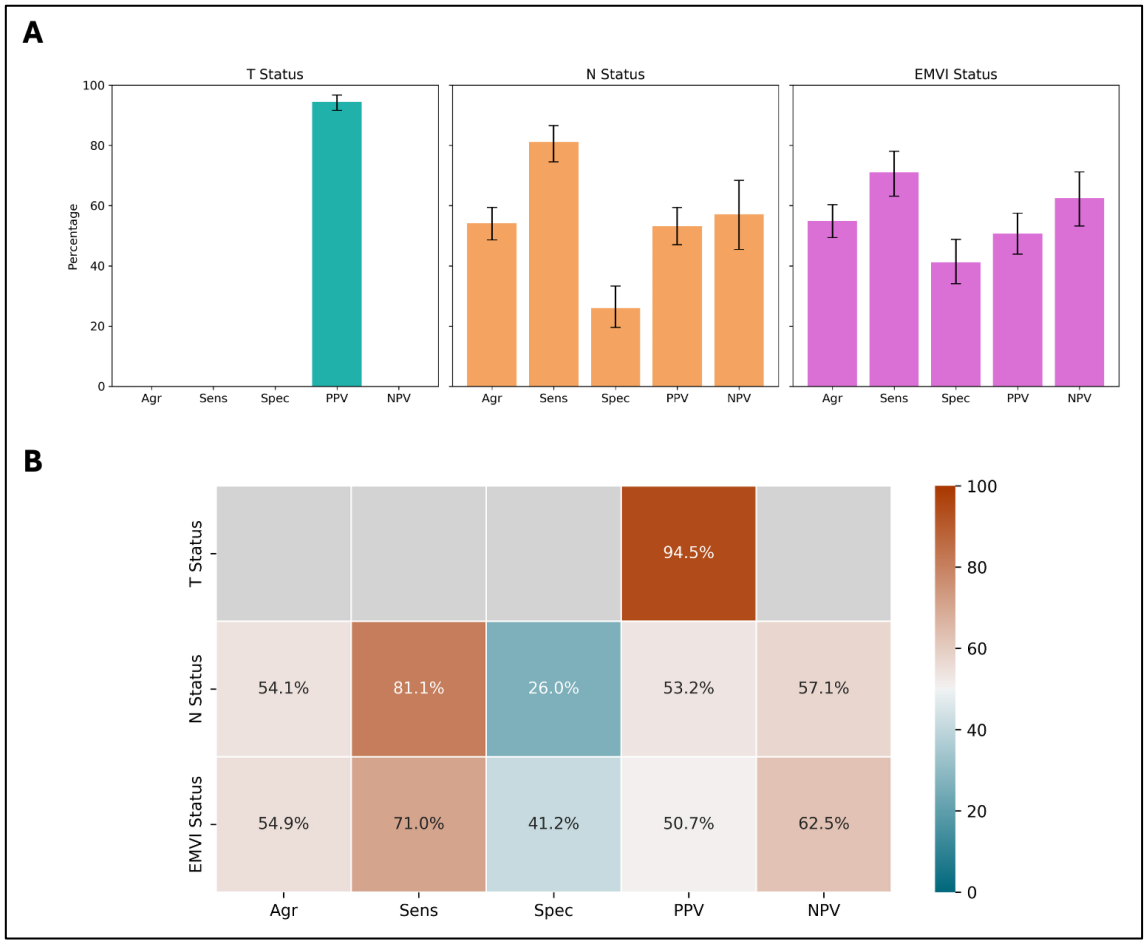



**Figure 2.4 Radiological Staging Performance in All Participants**
The overall agreement, sensitivity, specificity, PPV and NPV of CT for predicting T, N, and EMVI statuses shown as (A) individual bar charts and (B) a combined heatmap. Only PPV could be calculated for T status due to the exclusion of earlier stages of disease from the trial. Error bars represent 95% CIs. Agr = overall agreement; EMVI = extramural venous invasion; N = lymph node; NPV = negative predictive value; PPV = positive predictive value; Sens = sensitivity; Spec = specificity; T = primary tumour.

### 2.4.5 Impact of Mismatch Repair Status and Primary Tumour Side

Table 2.2 summarises the impact of MMR status and primary tumour side on staging concordance. Of the 248 participants with MMR proficient (pMMR) tumours, pathological T stage was T1 in a single participant, T2 in 16 participants, T3 in 159 participants, and T4 in 72 participants; 133 participants were pathologically staged N+ (N1 = 66 and N2 = 67) and 118 participants were found to have EMVI (Table 7.4). For the 68 participants with dMMR tumours, pathological T stage was T2 in 2 participants, T3 in 42 participants, and T4 in 24 participants; no participants had a T1 tumour; 28 participants were pathologically staged N+ (N1 = 11 and N2 = 17) and 27 were found to have EMVI (Table 7.5).

Table 2.2 Radiological Staging Performance According to MMR Status and Primary Tumour Side

| | | Overall agreement, (% [95% CI]) | Sensitivity, (% [95% CI]) | Specificity, (% [95% CI]) | PPV, (% [95% CI]) | NPV, (% [95% CI]) |
|---|---|---|---|---|---|---|
| All (n = 354) | T stage/status [a] | 220/349 (63.0 [57.7-68.1]) | | | 329/348 (94.5 [91.6-96.7]) | |
| | N status [b] | 186/344 (54.1 [48.6-59.4]) | 142/175 (81.1 [74.5-86.6]) | 44/169 (26.0 [19.6-33.3]) | 142/267 (53.2 [47.0-59.3]) | 44/77 (57.1 [45.4-68.4]) |
| | EMVI status | 185/337 (54.9 [49.4-60.3]) | 110/155 (71.0 [63.1-78.0]) | 75/182 (41.2 [34.0-48.7]) | 110/217 (50.7 [43.8-57.5]) | 75/120 (62.5 [53.2-71.2]) |
| pMMR [c, d] (n = 248) | T stage/status [a] | 153/247 (61.9 [55.6-68.0]) | | | 230/246 (93.5 [90.5-96.4]) | |
| | N status [b] | 136/245 (55.5 [49.0-61.8]) | 106/133 (79.7 [71.9-86.2]) | 30/112 (26.8 [18.9-36.0]) | 106/188 (56.4 [49.0-63.6]) | 30/57 (52.6 [39.0-66.0]) |
| | EMVI status | 140/242 (57.9 [51.4-64.2]) | 83/117 (70.9 [61.8-79.0]) | 57/125 (45.6 [36.7-54.7]) | 83/151 (55.0 [46.7-63.1]) | 57/91 (62.6 [51.9-72.6]) |
| dMMR [c, d] (n = 68) | T stage/status [a] | 42/67 (62.7 [50.0-74.2]) | | | 65/67 (97.0 [89.6-99.6]) | |
| | N status [b] | 36/68 (52.9 [40.5-65.2]) | 25/28 (89.3 [71.8-97.7]) | 11/40 (27.5 [14.6-43.9]) | 25/54 (46.3 [32.6-60.4]) | 11/14 (78.6 [49.2-95.3]) |
| | EMVI status | 30/65 (46.2 [33.7-59.0]) | 19/27 (70.4 [49.8-86.2]) | 11/38 (28.9 [15.4-45.9]) | 19/46 (41.3 [27.0-56.8]) | 11/19 (57.9 [33.5-79.7]) |
| Right side [e, f] (n = 175) | T stage/status [a] | 103/173 (59.5 [51.8-66.9]) | | | 166/172 (96.5 [92.5-98.7]) | |
| | N status [b] | 89/171 (52.0 [44.3-59.7]) | 74/86 (86.0 [76.9-92.6]) | 15/85 (17.6 [10.2-27.4]) | 74/144 (51.4 [42.9-59.8]) | 15/27 (55.6 [35.3-74.5]) |
| | EMVI status | 90/166 (54.2 [46.3-61.2]) | 52/70 (74.3 [62.4-84.0]) | 38/96 (39.6 [29.7-50.1]) | 52/110 (47.3 [37.7-57.0]) | 38/56 (67.9 [54.0-79.7]) |
| Left side [f, g] (n = 175) | T stage/status [a] | 117/174 (67.2 [59.7-74.2]) | | | 161/174 (92.5 [87.8-96.1]) | |
| | N status [b] | 96/170 (56.5 [48.7-64.0]) | 68/88 (77.3 [67.1-85.5]) | 28/82 (34.1 [24.0-45.4]) | 68/122 (55.7 [46.5-64.7]) | 28/48 (58.3 [43.2-72.4]) |
| | EMVI status | 93/168 (55.4 [47.5-63.0]) | 58/84 (69.0 [58.0-78.7]) | 35/84 (41.7 [31.0-52.9]) | 58/107 (54.2 [44.3-63.9]) | 35/61 (57.4 [44.1-70.0]) |

dMMR = mismatch repair deficient; EMVI = extramural venous invasion; MMR = mismatch repair; NPV = negative predictive value; pMMR = mismatch repair proficient; PPV = positive predictive value.

[a] Individual T stage used for overall agreement. Categorised as T-2 or T3-4 for calculation of PPV.

[b] N stage categorised as N0 or N+.

[c] According to immunohistochemistry.

[d] MMR status unknown for 38 participants.

[e] Tumours proximal to the splenic flexure.

[f] Tumour side unknown for 4 participants.

[g] Tumours at or distal to the splenic flexure.

A total of 175 participants had a right-sided tumour, where pathological T stage was T2 in 6 participants, T3 in 109 participants, and T4 in 58 participants; no participants had a T1 tumour; 86 participants were pathologically staged N+ (N1 = 41 and N2 = 45) and 70 had EMVI (Table 7.6). Likewise, 175 participants had a left-sided tumour, where pathological T stage was T1 in a single participant, T2 in 12 participants, T3 in 114 participants, and T4 in 47 participants. Eighty-nine participants were pathologically staged N+ (N1 = 45 and N2 = 44) and 85 had EMVI (Table 7.7).

Numerical differences in staging correlation were observed according to MMR status and tumour side but overlapping CIs indicate that these may not be significant (Table 2.2). While dMMR tumours are more common in the right colon and therefore represent a potential confounder, similar differences were seen according to tumour side when limited to just pMMR tumours: 57.9% (66/114) vs. 67.5% (112/166) for T stage agreement; 14.0% (7/50) vs. 32.1% (25/78) for N status specificity; and 72.5% (29/40) vs. 57.6% (34/59) for EMVI NPV in those with right, compared to left, sided tumours (Tables 7.8 and 7.9).

## 2.5 Discussion

FOxTROT was the first phase III, randomised controlled trial to show NAC significantly reduces the risk of recurrence in patients with locally advanced CC. Our analysis of prospective radiological staging by trained radiologists shows a combined T3-4 CT staging group is a reliable biomarker for neoadjuvant therapy patient selection with minimal overstaging of T1-2 tumours against final pathology. However, we also determined that CT cannot reliably select patients in the higher-risk T3 (≥5 mm extramural extension)-T4 or T4 alone groups and reaffirmed the limitations of CT for predicting N stage and EMVI status.[4, 5, 12, 23] In our opinion, these features, which are more challenging to identify, should be avoided or used with caution for patient selection in other neoadjuvant trials. These data underscore the role of simplified radiological T staging (T3-4) as the key biomarker for patient selection, bringing CC in line with other gastrointestinal malignancies.[24, 25]

The PPV of CT for predicting T3-4 tumours was 94.5%, which is similar to other studies (90%-92%).[14, 15, 20] At least 1 mm extramural extension on CT was required for trial eligibility because microscopic extension beyond the muscularis propria is unlikely to be visible within the limitations of CT contrast and spatial resolution. While the assessment of minimal tumour extension on CT is challenging and represents an area of uncertainty with regards to emerging neoadjuvant therapies, it is important to acknowledge that measurements on CT and pathology are not necessarily comparable; CT offers an assessment of the whole tumour *in vivo*, whereas pathological assessment may alter as a consequence of fixation and involves evaluation of select slices, rather than the whole specimen. Nevertheless, tumours with <1 mm extension are not a focus for neoadjuvant therapy, since they confer a lower-risk of disease recurrence and the benefit of systemic therapy is likely to be negligible.[26] Consistent with our pilot data, CT struggled to differentiate T3 and T4 tumours, which may reflect the difficulty posed by T4 tumours with microscopic extension through the peritoneum.[23]

Limiting T3 tumours to those with ≥5 mm extramural extension to explore an alternative dichotomy resulted in a PPV of 68.7%, suggesting this high-risk cohort may be challenging to reliably identify on CT. The choice of any threshold for patient selection represents a trade-off between those with high-risk tumours who are missed and others with low-risk tumours who are over-treated according to the target population. In the trial, 5.6% of participants had pT1-2 tumours who could be considered incorrect inclusions. When using the T3 ≥5 mm threshold, 4.4% of participants had pT1-2 tumours, suggesting that this approach may slightly reduce the proportion of early-stage tumours, but at the expense of losing 27.8% of the original trial population, most of whom had pT3-4 tumours. Criteria for patient selection in both clinical trials and routine practice must reflect the principles of this trade-off and any proposed treatment.

Only 20 (5.6%) participants had T1-2 tumours that were radiologically overstaged. However, other high-risk features (N+ and/or EMVI) were present in 8 of these participants. Considering established approaches to adjuvant treatment, it is possible that such patients may also benefit from NAC.[3]

Whilst we could only calculate the PPV for T status, other studies consistently report limited NPV (38%-51%).[4, 5, 9, 11, 14, 15, 20, 27, 28] This may lead to understaging and reduced NAC provision, but these patients would still undergo surgery with or without adjuvant chemotherapy, which remains an effective treatment option.[3]

T stage agreement was numerically greater in left-sided than right-sided tumours (67.2% vs. 59.5%, respectively) in both the whole STS arm and just those with pMMR tumours. To the best of our knowledge, this finding is not described elsewhere, may reflect the biological, morphological, and anatomical differences between the right and left colon, and should be a subject for further research.[29]

The limitations of radiological N staging due to micrometastases, small vessel EMVI, and reactive lymphadenopathy are well-recognised. N stage agreement was present in only 39.8% of participants, increasing to 54.1% when categorised as N status. The sensitivity and specificity of CT for predicting N status contrasts with wider literature (81.1% and 26.0% vs. 55%-71% and 66%-78%, respectively).[4, 5, 14, 15, 20] This distinction may reflect the inclusion of exclusively advanced tumours, which may bias reporting to incorrectly predict lymph node metastases.

A numerically lower PPV and specificity for predicting N status in dMMR (vs. pMMR) and right (vs. left)-sided tumours, respectively, are consistent with a Danish population-level study.[20] Lower prevalence of lymph node metastases and larger size of reactive nodes in dMMR than pMMR CC may explain the higher observed NPV.[30] These data further reinforce the need to consider MMR status when radiologically staging CC.[20, 21, 31, 32]

While MRI assessment of EMVI in rectal cancer is well-established, the evidence for CT-detected EMVI in CC is limited.[33] In this analysis, the sensitivity and specificity of CT for detecting EMVI is disappointing and differs from other reports (71.0% and 41.2% vs. 35%-63% and 67%-94%, respectively).[11, 12, 34-36] Again, we suspect that this may reflect the inclusion of exclusively advanced disease, where greater extramural extension, perineural or lymphatic invasion, and peritumoural inflammation may be mistaken for EMVI on CT. Furthermore, CT assessment of EMVI may be limited by the involvement of small vessels which are unlikely to be visible. The inflammatory nature of dMMR CC may also explain particularly poor agreement in this subgroup, where subtle peritumoural inflammation could be misinterpreted as EMVI.[30]

Macroscopic and microscopic pathological examination has long been established as the gold standard for guiding decisions on chemotherapy and predicting prognosis in CC.[3] In the era of precision oncology, the adoption of routine molecular biomarkers, such as MMR status, complements pathological staging to enhance the selection of systemic therapies. It is clear that complete matching of radiological and pathological TNM staging is imprecise and appears to be a futile endeavour. However, neoadjuvant treatment requires *in vivo* assessment of colon tumours. While our data show that CT is able to accurately select patients with T3 tumours and above, the limited prediction of other features indicates that we must move beyond the paradigm of “pathology matching” and towards “radiological phenotyping.”

Current radiology staging assessments are confined to the TNM system, which neglects features that may be effective radiological biomarkers. With the rapid expansion of neoadjuvant trials in CC, there is a need to optimise and harmonise radiological assessment, while identifying independent imaging biomarkers and those which contribute to a broader multimodal approach.[37, 38] Recent studies have identified additional biomarkers which support TNM staging, including a specific vascular pattern to differentiate T3 and T4a tumours (“small arteriole sign”), and systemic inflammatory markers, lymph node irregularity, and lymph node heterogeneity to predict N status.[21, 39-41] However, these studies remain

focused on TNM staging and the prediction of pathology over clinical outcomes, and are limited by smaller size, retrospective design, and a lack of validation.

Some studies have explored the performance of MRI and PET-CT, aiming to overcome the limitations of CT lymph node and EMVI assessment. Neither demonstrate superior performance for N staging, and while there may be some improvement in EMVI detection with MRI, this is not conclusive.[42] Furthermore, these data often comprise expert reviews which may not reflect real-world performance.[11, 12, 43] Increasingly, research is utilising radiomics and artificial intelligence applications to support radiological staging, but this remains experimental. CT, PET-CT, and MRI radiomics have all shown promise for predicting N stage, which may have clinical utility for patient selection in the era of neoadjuvant therapies. Such approaches may also deliver novel imaging biomarkers to improve patient risk assessment and further support treatment stratification.[44, 45] The value of radiological response to NAC has not been comprehensively evaluated and represents a new avenue for imaging research.

Radiologists and cancer specialists must recognise the importance of training to deliver accurate CC staging in routine practice.[46, 47] This trial successfully delivered a bespoke radiology training programme to local radiologists, resulting in strong performance for T stage assessment. Other benefits of this training may become clear once radiology-pathology correlation data is published for other neoadjuvant trials.[19] Analyses of trial datasets should be used to identify areas of unmet need to inform the development of training resources. We identified lymph node and EMVI assessment as clear weaknesses; however, in the context of such poor performance, it is unlikely that further training alone will produce substantial improvements. As novel features and biomarkers are developed and validated, training will be critical to ensure wider radiological practice advances efficiently. We recommend that proformas and formal training are considered a key component of any future trials in this setting to ensure consistency and generalisability. Template reporting of CC CT may also support the description and standardisation of key imaging biomarkers in routine, multidisciplinary practice, as is the case in rectal cancer.[48]

This analysis is limited by the inclusion of exclusively advanced disease, which may influence several aspects of staging performance and affect the generalisability of findings to radiological evaluation in the wider CC population. The results may also be limited by differences between TNM version 5 and the contemporary version 8. For example, tumour deposits <3 mm were previously considered discontinuous tumour extension as part of T staging, as opposed to N staging in version 8 (N1c in the absence of lymph node metastases).[49] The exclusion of

patients with <1 mm extramural extension on CT may also enhance performance by excluding challenging, borderline cases. Interobserver variability could not be assessed due to the design of the trial. However, the delivery of bespoke radiological training and use of established radiological features ensure a degree of standardisation. While the lack of central review will introduce heterogeneity to the data and could be considered a limitation, we believe that focusing on staging performance in real-world, multicentre practice, which delivered a positive trial, to be major strength. This evaluation of prospective, local staging performance in a large, international trial indicates broader applicability of this approach in routine clinical practice.

We have shown that CT reliably identifies a combined T3-4 staging group with minimal overstaging versus final pathology. However, limited performance was seen for assessing tumour extension, individual T stage, N stage, or EMVI status. We propose that T3-4 staging on CT should be considered the standard biomarker to select patients with CC for neoadjuvant therapies. Radiological phenotyping through the identification of additional imaging biomarkers is a priority to effectively predict benefit from neoadjuvant therapies to build multimodal approaches for patient selection.

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# 3 Baseline Clinicopathological and Imaging Predictors of Disease Recurrence in Locally Advanced Colon Cancer: a Post Hoc Analysis of the FOxTROT Trial

## 3.1 Abstract


### 3.1.1 Background

Neoadjuvant therapies are increasingly used in locally advanced colon cancer (CC) with patient selection predominantly based on radiological staging. Yet, the prognostic value of baseline radiological features in CC is poorly understood and limits how effectively high-risk patients can be identified. Here, baseline data from the FOxTROT trial, with a focus on imaging variables, were evaluated for prognostic associations.

### 3.1.2 Patients and methods

In FOxTROT, neoadjuvant chemotherapy (NAC) significantly reduced recurrence risk, compared to upfront surgery, in patients with radiologically staged T3-4 N0-2 M0 CC. In this retrospective analysis of prospectively collected trial data, the following radiological variables were explored for associations with disease-free survival (DFS), time to recurrence (TTR) and overall survival (OS) using Cox proportional hazards models: tumour side (right vs. left), T stage, extramural tumour extension, maximum tumour thickness, N stage, N status (N0 vs. N1-2), a lymph node ≥10 mm in short axis and extramural venous invasion (EMVI). Routinely available clinicopathological variables were also explored. Analyses were repeated according to mismatch repair (MMR) status.

### 3.1.3 Results

The whole trial population of 1,052 participants (672 men; mean age 63 $\pm$ 9.7) were included. Of those with known MMR status, 729 were pMMR and 185 were dMMR. Female sex (P = 0.03), performance status 1-2 (P = 0.003), poor tumour differentiation (P < 0.001), T4 stage (P = 0.008), extramural extension >7 mm (P < 0.001), maximum tumour thickness >22 mm (P = 0.005), lymph node ≥10 mm in short axis (P = 0.02) and EMVI (P = 0.02) were significantly associated with DFS. Significant prognostic factors differed according to MMR status.

Participants with a greater number of risk factors had significantly worse DFS than those with fewer risk factors.

### 3.1.4 Conclusions

Several variables routinely available at baseline are associated with prognosis in locally advanced CC, indicating that greater risk stratification is possible to support patient selection for neoadjuvant therapies.

## 3.2 Introduction

The Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT) trial established neoadjuvant chemotherapy (NAC) as safe and effective for locally advanced colon cancer (CC).[1] Compared to upfront surgery, NAC significantly reduced residual disease or recurrence within 2 years, pathological staging and rates of peri-operative complications. These benefits have since been reinforced by the OPTICAL trial and a recent meta-analysis.[2, 3] Both trials included participants with mismatch repair (MMR) deficient (dMMR) tumours, who are now known benefit less from NAC. As such, the benefit of NAC is enhanced for the refined MMR proficient (pMMR) population and now represents a standard treatment option.[3-6]

Metastatic colorectal cancer treatment is increasingly personalised due to the availability and utility of molecular biomarkers to identify patients with specific therapeutic vulnerabilities.[7] Immunotherapy is the standard first-line treatment for metastatic or unresectable dMMR CC.[7] As NAC is ineffective for locally advanced dMMR CC, neoadjuvant immunotherapy (NAI) has been investigated in multiple phase II trials, with impressive rates of pathological regression and long-term survival.[8-15] Recent studies also report immunotherapy responses for some in the pMMR population, previously considered resistant to such treatment.[16, 17] Other targeted agents from the metastatic setting are likewise being tested in neoadjuvant trials, with promising data already highlighting the potential of anti-epidermal growth factor receptor (EGFR) therapies.[18, 19] The rapid pace of change in this field indicates the need for high-quality data to guide patient selection and stratification in the neoadjuvant era.

Despite clear momentum for neoadjuvant therapy (NAT) in CC, cancer multidisciplinary teams (MDTs) often lack confidence in current patient selection criteria without insights from post-operative pathological evaluation. While pathological tumour, node, metastasis (TNM) staging

is the current gold-standard for guiding adjuvant treatment decisions, it remains an imperfect system with limited prognostic and predictive value.[20] Implementing NATs in routine practice requires a shift in decision-making to the pre-operative setting and represents a major challenge. Established approaches to patient stratification in rectal cancer may exemplify how high-risk imaging features can address this challenge.[21]

Radiological staging of CC using computed tomography (CT) utilises the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM system, and was traditionally used to differentiate localised and metastatic disease to guide surgical decision making.[22] However, the same radiological evaluation is now used to select patients for NAT. FOxTROT included patients with radiological T3-4 N0-2 M0 staging, whereas OPTICAL selected a more advanced population, requiring T3 tumours to have at least 5 mm extramural extension.[1, 3] Other criteria have been used in NAI trials, including positive lymph node staging, highlighting uncertainty in patient selection on top of the inconsistencies and subjectivity which already limit radiological assessment.[8-12]

The purpose of this analysis is to further understand whether patients with locally advanced CC at high-risk of recurrence can be identified at baseline, similar to established approaches in rectal cancer.[21] Generating data to support this goal would provide confidence and refinement to patient selection for neoadjuvant trials and standard of care treatment. Radiological criteria for neoadjuvant trials have largely been extrapolated from pathological data, where deeper extramural extension and lymph node metastases indicate greater recurrence risk.[23, 24] However, there are limited data on the prognostic value of these features and others on imaging. Radiological and pathological staging have limited concordance and should therefore be considered distinct for risk assessment.[25, 26] Here, the prognostic value of baseline characteristics in the FOxTROT trial are evaluated to determine whether they can predict disease recurrence.

## 3.3 Methods

Ethical approval was granted for the FOxTROT trial, including analyses of clinical and radiological data (MREC: 07/S0703/57; ISRCTN87163246). Written informed consent was obtained from all participants.

### 3.3.1 Trial Design

FOxTROT was an international, phase III randomised controlled trial for patients with locally advanced but resectable CC (T3-4 N0-2 M0 with ≥1 mm extramural extension), who were randomised 2:1 to NAC or upfront surgery. Both groups received post-operative chemotherapy with the total treatment duration equivalent between arms. The full trial results are published separately.[1]

### 3.3.2 Radiological Assessment

A Lead Radiologist was designated at each trial site, who attended training on tumour evaluation and staging (led by an expert gastrointestinal radiologist with more than a decade of experience in colorectal cancer imaging). Baseline radiological staging was performed for all participants using a portal venous phase, contrast-enhanced, spiral/multidetector CT of the chest, abdomen and pelvis, with maximum 5 mm slices, according to TNM version 5.[27] The use of oral contrast was permitted according to local practice.

Baseline imaging data were submitted to a central trials unit using a case report form (CRF). Eligibility was dependent on radiological T stage; other variables were collected to support subsequent analyses. At least 148 radiologists (for 115/1,052 [10.9%] participants, the reporting radiologist was not stated) contributed to baseline scan reporting. Radiological staging was initially reviewed by the local MDT to confirm eligibility and subsequently by the Lead Radiologist, as previously described.[28]

### 3.3.3 Pathological Assessment

A Lead Pathologist was also designated at each trial site. Tumour differentiation was assessed locally on haematoxylin & eosin-stained slides from the resection specimen but included as a baseline variable for the purposes of this analysis due to potential availability on diagnostic biopsies. *RAS* and MMR statuses were both confirmed at a central trial laboratory on biopsy and either biopsy or resection specimens, respectively.

### 3.3.4 Statistical Analysis

This exploratory analysis assessed the prognostic value of imaging and other clinicopathological characteristics at baseline across the entire trial population. Participants

from both arms were analysed together as the same total duration of chemotherapy was received and to maximise statistical power.

The following imaging variables were included: tumour location (colonic segment), T stage, N stage, depth of extramural extension (mm), maximum tumour thickness (mm), the presence of a lymph node ≥10 mm in short axis and evidence of extramural venous invasion (EMVI). These data were taken from CRFs submitted during the trial, rather than re-evaluation through central scan review. The non-imaging variables included were: age at randomisation (years), sex, performance status, tumour differentiation, *RAS* status and MMR status.

Age was categorised as <50, 50-59, 60-69 or ≥70. Tumour location was categorised by tumour side, with right-sided tumours defined as those proximal to the splenic flexure and left-sided tumours those at, or distal to, the splenic flexure. Depth of extramural extension and maximum tumour thickness were analysed both as continuous variables and dichotomised groups, with optimal cut-offs determined using Youden's J statistic. Individual N stage and dichotomised N status (N0 vs. N1-2) were both analysed. Performance status was categorised as 0 or 1-2.

Descriptive statistics were generated for each characteristic in the whole cohort and by treatment arm. The associations between individual characteristics and disease-free survival (DFS; time from randomisation to recurrence or death from any cause), time to recurrence (TTR; time from randomisation to disease recurrence) and overall survival (OS; time from randomisation to death from any cause) were evaluated using Cox proportional hazards models on complete follow-up data, with missing values excluded. Hazard ratios (HRs), 95% confidence intervals (CI) and P values were reported, with $P < 0.05$ considered statistically significant. Kaplan-Meier curves were used to illustrate recurrence risk between variable groups. Additional analyses were performed to evaluate prognostic factors according to MMR status and explore treatment effects across subgroups, illustrated using a forest plot. The treatment effect analysis was limited to those with proficient/unknown MMR status to reflect contemporary approaches to patient stratification.

Analyses were performed using STATA software (StataCorp. Stata Statistical Software: Release 16. College Station, Texas, USA: StataCorp LLC).

## 3.4 Results

### 3.4.1 Study Population

Baseline clinicopathological and imaging characteristics were well balanced between treatment groups, with 698 participants randomised to NAC and 354 to upfront surgery (Table 3.1). The study population included 672 (63.9%) men and 380 (36.1%) women, with a mean age of 63.0 (standard deviation [SD] 9.7). Of the 914 (86.9%) participants with known tumour MMR status, 729 (79.8%) were pMMR and 185 (20.2%) dMMR. In the overall population, the median follow-up was 37.2 months (interquartile range 29.4-58.7) with 210 (20.0%) experiencing disease recurrence and 168 (16.0%) having died from any cause.

Table 3.1 Baseline Clinicopathological and Imaging Characteristics by Treatment Arm

| | NAC (n = 698) | Upfront surgery (n = 354) |
|---|---|---|
| Age (years) | | |
| Mean (SD) | 63.0 (9.8) | 63.2 (9.4) |
| <50, n (%) | 66 (9.5) | 32 (9.0) |
| 50-59 | 149 (21.3) | 78 (22.0) |
| 60-69 | 288 (41.3) | 147 (41.5) |
| ≥70 | 195 (27.9) | 97 (27.4) |
| Sex | | |
| Male, n (%) | 447 (64.0) | 225 (63.6) |
| Female | 251 (36.0) | 129 (36.4) |
| Performance Status | | |
| 0, n (%) | 530 (75.9) | 274 (77.4) |
| 1-2 | 168 (24.1) | 80 (22.6) |
| Tumour differentiation | | |
| Well/moderate, n (%) | 559 (80.1) | 283 (79.9) |
| Poor | 93 (13.3) | 57 (16.1) |
| Unknown | 46 (6.6) | 14 (4.0) |
| *RAS* status | | |
| Wild type, n (%) | 302 (43.3) | 149 (42.1) |
| Mutant | 180 (25.8) | 88 (24.9) |
| Unknown | 216 (30.9) | 117 (33.0) |
| MMR status | | |
| Proficient, n (%) | 481 (68.9) | 248 (70.1) |
| Deficient | 117 (16.8) | 68 (19.2) |
| Unknown | 100 (14.3) | 38 (10.7) |
| Radiological Tumour side [a] | | |
| Right, n (%) | 333 (47.7) | 175 (49.4) |
| Left | 359 (51.4) | 175 (49.4) |
| Unknown | 6 (0.9) | 4 (1.1) |
| Radiological T stage [b] | | |
| 2, n (%) | 1 (0.1) | 1 (0.3) |
| 3 | 523 (74.9) | 256 (72.3) |
| 4 | 169 (24.2) | 95 (26.8) |
| Unknown | 5 (0.7) | 2 (0.6) |
| Extramural tumour extension (mm) | | |
| Median (IQR) | 7 (4-12) | 7 (4-12) |
| Unknown, n (%) | 2 (0.3) | 1 (0.3) |
| Maximum tumour thickness (mm) | | |
| Median (IQR) | 17 (13-25) | 19 (14-26) |
| Unknown, n (%) | 12 (1.7) | 5 (1.4) |
| Radiological N stage [b] | | |
| 0, n (%) | 169 (24.2) | 81 (22.9) |
| 1 | 328 (47.0) | 169 (47.7) |
| 2 | 196 (28.1) | 102 (28.8) |
| Unknown | 5 (0.7) | 2 (0.6) |
| Lymph node ≥10 mm short axis | | |
| Present, n (%) | 212 (30.4) | 112 (31.6) |
| Absent | 476 (68.2) | 239 (67.5) |
| Unknown | 10 (1.4) | 3 (0.9) |
| Radiological EMVI | | |
| Present, n (%) | 408 (58.5) | 226 (63.8) |
| Absent | 280 (40.1) | 125 (35.3) |
| Unknown | 10 (1.4) | 3 (0.9) |

EMVI = extramural venous invasion; IQR = interquartile range; MMR = mismatch repair; N = lymph node stage; NAC = neoadjuvant chemotherapy; SD = standard deviation; T = primary tumour stage.

[a] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[b] According to TNM version 5.

Percentages represent columns.

### 3.4.2 Prognostic Factors in All Participants

DFS events were observed in 24.0% (253/1,052) of participants with a 3-year DFS rate of 79.0% (831/1,052). Thresholds of 7 mm and 22 mm were selected for extramural tumour extension and maximum tumour thickness, respectively, based on the Youden's J statistic to balance sensitivity and specificity (Tables 7.10 & 7.11; Figure 7.1). Depth of extramural extension was ≤7 mm and >7 mm in 555 (52.9%) and 494 (47.1%) participants, respectively. Maximum tumour thickness was ≤22 mm and >22 mm in 695 (67.1%) and 340 (32.9%) participants, respectively.

Of the baseline clinicopathological variables included, univariable analyses showed that female sex (HR 0.73, 95% CI 0.56-0.96; P = 0.03), performance status 1-2 (HR 1.50, 95% CI 1.15-1.96; P = 0.003) and poor tumour differentiation (HR 1.89, 95% CI 1.39-2.56; P < .001) were significantly associated with DFS (Table 3.2). T4 stage (HR 1.44, 95% CI 1.10-1.88; P = 0.008), extramural tumour extension >7 mm (HR 1.72, 95% CI 1.34-2.22; P < 0.001), maximum tumour thickness >22 mm (HR 1.43, 95% CI 1.11-1.85; P = 0.005), a lymph node ≥10 mm in short axis (HR 1.36, 95% CI 1.05-1.75; P = 0.02) and EMVI (HR 1.37, 95% CI 1.05-1.78; P = 0.02) were the baseline radiological features with significant DFS associations (Table 3.2; Figure 3.1). Other than female sex, the same variables were significantly associated with TTR (Table 7.12).

The 5-year OS rate was 85.6% (900/1,052). Performance status 1-2 (HR 1.85, 95% CI 1.35-2.54; P < 0.001) and poor tumour differentiation (HR 2.32, 95% CI 1.61-3.33; P < 0.001) were the clinicopathological variables associated with a significantly worse OS (Table 7.14). Of the imaging variables, left-sided tumour location (HR 0.66, 95% CI 0.49-0.90; P = 0.009), T4 stage (HR 1.66, 95% CI 1.21-2.27; P = 0.002), extramural tumour extension >7 mm (HR 1.95, 95% CI 1.41-2.69; P < 0.001), maximum tumour thickness >22 mm (HR 1.41, 95% CI 1.03-1.92; P = 0.03) and a lymph node ≥10 mm in short axis (HR 1.50, 95% CI 1.10-2.05; P = 0.01) had significant OS associations. Age, *RAS* status, MMR status, N stage and N status were not significantly associated with DFS, TTR or OS.

Table 3.2 Univariable Analysis of Disease-Free Survival in All Participants

| | No DFS Event (n = 799) | DFS Event (n = 253) | HR (95% CI) | P value [a] |
|---|---|---|---|---|
| Age (years) | | | | |
| <50, n (%) | 79 (80.6) | 19 (19.4) | 1.0 | |
| 50-59 | 180 (79.3) | 47 (20.7) | 1.08 (0.63-1.84) | 0.79 |
| 60-69 | 325 (74.7) | 110 (25.3) | 1.28 (0.78-2.08) | 0.33 |
| ≥70 | 215 (73.6) | 77 (26.4) | 1.38 (0.84-2.28) | 0.21 |
| Sex | | | | |
| Male, n (%) | 493 (73.4) | 179 (26.6) | 1.0 | |
| Female | 306 (80.5) | 74 (19.5) | 0.73 (0.56-0.96) | 0.03 |
| Performance Status | | | | |
| 0, n (%) | 627 (78.0) | 177 (22.0) | 1.0 | |
| 1-2 | 172 (69.4) | 76 (30.6) | 1.50 (1.15-1.96) | 0.003 |
| Tumour differentiation | | | | |
| Well/moderate, n (%) | 658 (78.1) | 184 (21.9) | 1.0 | |
| Poor | 97 (64.7) | 53 (35.3) | 1.89 (1.39-2.56) | < 0.001 |
| *RAS* status | | | | |
| Wild type, n (%) | 332 (73.6) | 119 (26.4) | 1.0 | |
| Mutant | 196 (73.1) | 72 (26.9) | 1.01 (0.75-1.35) | 0.96 |
| MMR status | | | | |
| Proficient, n (%) | 557 (76.4) | 172 (23.6) | 1.0 | |
| Deficient | 144 (77.8) | 41 (22.2) | 0.95 (0.67-1.33) | 0.76 |
| Radiological Tumour side [b] | | | | |
| Right, n (%) | 379 (74.6) | 129 (25.4) | 1.0 | |
| Left | 413 (77.3) | 121 (22.7) | 0.89 (0.69-1.14) | 0.34 |
| Radiological T stage [c] | | | | |
| 3, n (%) | 607 (77.9) | 172 (22.1) | 1.0 | |
| 4 | 185 (70.1) | 79 (29.9) | 1.44 (1.10-1.88) | 0.008 |
| Extramural tumour extension, mm | | | | |
| Continuous, median (range) | 7 (0-50) | 9 (0-50) | 1.03 (1.02-1.04) | < 0.001 |
| ≤7, n (%) | 455 (82.0) | 100 (18.0) | 1.0 | |
| >7 | 341 (69.0) | 153 (31.0) | 1.72 (1.34-2.22) | < 0.001 |
| Maximum tumour thickness, mm | | | | |
| Continuous, median (range) | 17 (1-96) | 19 (4-88) | 1.15 (1.05-1.25) [d] | 0.002 |
| ≤22, n (%) | 549 (79.0) | 146 (21.0) | 1.0 | |
| >22 | 237 (69.7) | 103 (30.3) | 1.43 (1.11-1.85) | 0.005 |
| Radiological N stage [c] | | | | |
| 0, n (%) | 193 (77.2) | 57 (22.8) | 1.0 | |
| 1 | 388 (78.1) | 109 (21.9) | 0.99 (0.71-1.36) | 0.93 |
| 2 | 214 (71.8) | 84 (28.2) | 1.27 (0.91-1.78) | 0.17 |
| Radiological N status [c] | | | | |
| 0, n (%) | 193 (77.2) | 57 (22.8) | 1.0 | |
| 1-2 | 602 (75.7) | 193 (24.3) | 1.09 (0.81-1.47) | 0.56 |
| Lymph node ≥10 mm short axis | | | | |
| Absent, n (%) | 558 (78.0) | 157 (22.0) | 1.0 | |
| Present | 230 (71.0) | 94 (29.0) | 1.36 (1.05-1.75) | 0.02 |
| EMVI | | | | |
| Absent, n (%) | 323 (79.7) | 82 (20.3) | 1.0 | |
| Present | 465 (73.3) | 169 (26.7) | 1.37 (1.05-1.78) | 0.02 |

CI = confidence intervals; EMVI = extramural venous invasion; HR = hazard ratio; n = number of participants; N = lymph node stage; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

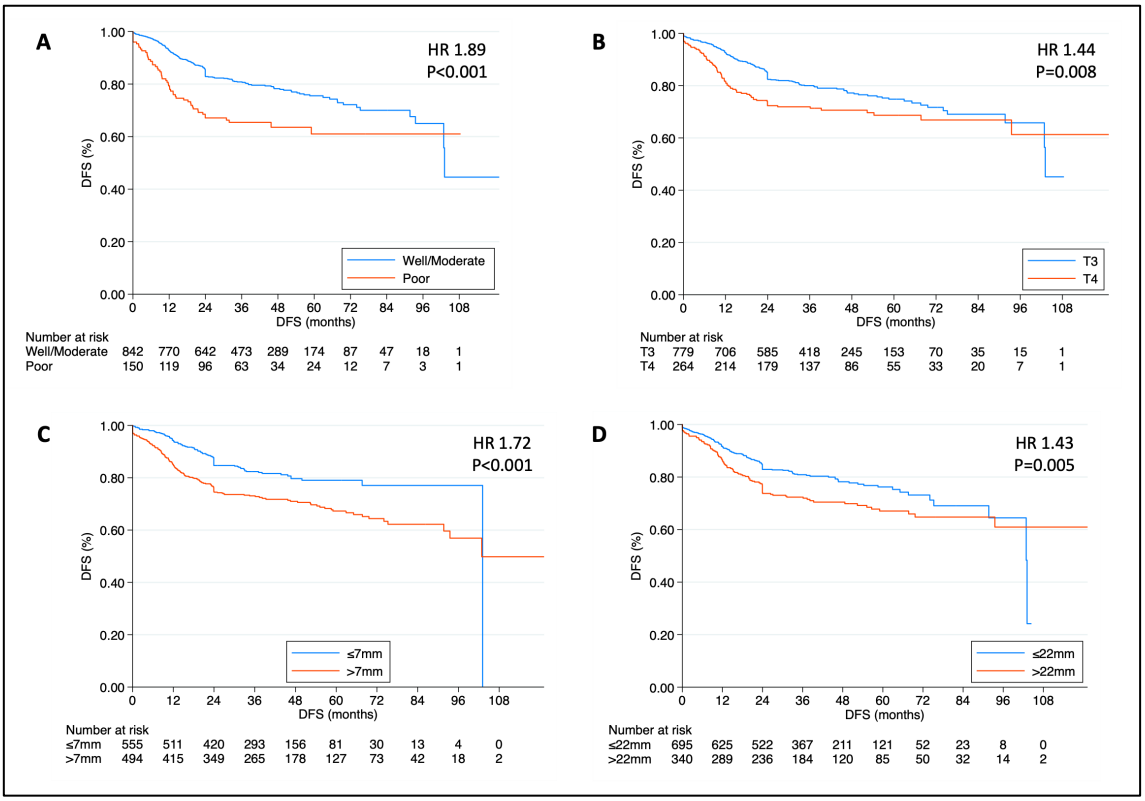



**Figure 3.1 Disease-Free Survival According to Individual Characteristics**
Kaplan-Meier survival curves for DFS according to select features. A) Tumour differentiation. B) Radiological T stage. C) Radiological extramural tumour extension. D) Radiological maximum tumour thickness. T = T stage; DFS = disease-free survival.

### 3.4.3 Subgroup Analyses

Tumour biology, treatment efficacy and prognosis are all influenced by MMR status.[29] On imaging, dMMR tumours also appear different to pMMR tumours with implications for radiology-pathology concordance.[28, 30, 31] Therefore, it is conceivable that prognostic factors may also differ between these groups with separate strategies required for neoadjuvant patient selection.

The univariable analyses were repeated according to MMR status (Table 3.3). In those with pMMR tumours, female sex (HR 0.67, 95% CI 0.48-0.95; P = 0.02), poor tumour differentiation (HR 2.28, 95% CI 1.51-3.45; P < 0.001), extramural extension >7 mm (HR 1.72, 95% CI 1.26-2.33; P = 0.001) and a lymph node ≥10 mm in short axis (HR 1.37, 95% CI 1.01-1.87; P = 0.04) were significantly associated with DFS. Conversely, in those with dMMR tumours, T4 (HR 1.87, 95% CI 1.00-3.47; P = 0.05) and N2 (HR 3.31, 95% CI 1.12-9.78; P = 0.03) stages were associated with a significantly worse DFS.

For pMMR tumours, poor tumour differentiation and extramural extension >7 mm were associated with significantly worse TTR (HR 2.61, 95% CI 1.72-3.95; P < 0.001 and HR 1.74, 95% CI 1.26-2.38; P = 0.001, respectively) and OS (HR 2.17, 95% CI 1.30-3.62; P = 0.003 and HR 1.97, 95% CI 1.32-2.94; P = 0.001, respectively); maximum tumour thickness >22 mm (HR 1.46, 95% CI 1.05-2.03; P = 0.02) was also associated with a significant reduction in TTR (Tables 7.13 & 7.15).

For dMMR tumours, N2 stage was associated with significantly worse TTR (HR 3.09, 95% CI 1.04-9.20; P = 0.04) and OS (HR 3.77, 95% CI 1.10-12.93; P = 0.04); T4 stage and maximum tumour thickness >22 mm were also associated with significantly worse TTR (HR 2.11, 95% CI 1.10-4.06; P = 0.03 and HR 2.30, 95% CI 1.17-4.51; P = 0.02, respectively); poor tumour differentiation was associated with a significant reduction in OS (HR 2.47, 95% CI 1.20-5.08; P = 0.01) (Tables 7.13 & 7.15). Significant interactions were detected for OS with N stage and N status (P = 0.05 for both).

When considering DFS according to treatment arm in the pMMR/MMR unknown cohort, the HRs for NAC versus upfront surgery were below 1 across most subgroups, with no significant main effects or interactions (Table 7.16 & Figure 7.2). Interestingly, while the presence of a lymph node ≥10 mm in short axis was significantly prognostic, there was no clear predictive value for the benefit of NAC over upfront surgery.

Table 3.3 Univariable Analysis of Disease-Free Survival According to MMR Status

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No DFS Event (n = 625) | DFS Event (n = 172) | HR (95% CI) | P value [a] | No DFS Event (n = 144) | DFS Event (n = 41) | HR (95% CI) | P value [a] | |
| Age (years) | | | | | | | | | |
| <50, n (%) | 42 (80.8) | 10 (19.2) | 1.0 | | 21 (80.8) | 5 (19.2) | 1.0 | | 0.68 |
| 50-59 | 133 (78.7) | 36 (21.3) | 1.05 (0.52-2.12) | 0.89 | 24 (82.8) | 5 (17.2) | 0.96 (0.28-3.30) | 0.94 | |
| 60-69 | 238 (77.0) | 71 (23.0) | 1.05 (0.54-2.04) | 0.88 | 57 (74.0) | 20 (26.0) | 1.51 (0.57-4.02) | 0.41 | |
| ≥70 | 144 (72.4) | 55 (27.6) | 1.36 (0.69-2.68) | 0.37 | 42 (79.2) | 11 (20.8) | 1.27 (0.44-3.67) | 0.66 | |
| Sex | | | | | | | | | |
| Male, n (%) | 357 (73.5) | 129 (26.5) | 1.0 | | 73 (74.5) | 25 (25.5) | 1.0 | | 0.86 |
| Female | 200 (82.3) | 43 (17.7) | 0.67 (0.48-0.95) | 0.02 | 71 (81.6) | 16 (18.4) | 0.73 (0.39-1.37) | 0.33 | |
| Performance status | | | | | | | | | |
| 0, n (%) | 440 (77.2) | 130 (22.8) | 1.0 | | 105 (80.8) | 25 (19.2) | 1.0 | | 0.22 |
| 1-2 | 117 (73.6) | 42 (26.4) | 1.14 (0.80-1.61) | 0.47 | 39 (70.9) | 16 (29.1) | 1.73 (0.92-3.24) | 0.09 | |
| Tumour differentiation | | | | | | | | | |
| Well/moderate, n (%) | 499 (77.6) | 144 (22.4) | 1.0 | | 90 (81.8) | 20 (18.2) | 1.0 | | 0.57 |
| Poor | 37 (57.8) | 27 (42.2) | 2.28 (1.51-3.45) | < 0.001 | 47 (71.2) | 19 (28.8) | 1.81 (0.97-3.39) | 0.06 | |
| *RAS* status | | | | | | | | | |
| Wild type, n (%) | 218 (73.9) | 77 (26.1) | 1.0 | | 82 (75.2) | 27 (24.8) | 1.0 | | 0.39 |
| Mutant | 165 (75.0) | 55 (25.0) | 0.90 (0.63-1.27) | 0.53 | 16 (69.6) | 7 (30.4) | 1.36 (0.59-3.13) | 0.46 | |
| Radiological tumour side [b] | | | | | | | | | |
| Right, n (%) | 205 (73.2) | 75 (26.8) | 1.0 | | 124 (78.5) | 34 (21.5) | 1.0 | | 0.58 |
| Left | 350 (78.6) | 95 (21.4) | 0.82 (0.60-1.11) | 0.19 | 18 (75.0) | 6 (25.0) | 1.13 (0.48-2.70) | 0.78 | |

Table 3.3 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No DFS Event (n = 625) | DFS Event (n = 172) | HR (95% CI) | P value [a] | No DFS Event (n = 144) | DFS Event (n = 41) | HR (95% CI) | P value [a] | |
| Radiological T stage [c] | | | | | | | | | |
| 3, n (%) | 435 (77.1) | 129 (22.9) | 1.0 | | 96 (82.8) | 20 (17.2) | 1.0 | | 0.26 |
| 4 | 117 (73.6) | 42 (26.4) | 1.18 (0.83-1.67) | 0.35 | 47 (70.1) | 20 (29.9) | 1.87 (1.00-3.47) | 0.05 | |
| Extramural tumour extension, mm | | | | | | | | | |
| Continuous, median (range) | 6 (0-45) | 8 (0-50) | 1.03 (1.01-1.04) | 0.001 | 8 (0-50) | 10 (1-40) | 1.02 (0.98-1.05) | 0.32 | |
| ≤7, n (%) | 330 (82.3) | 71 (17.7) | 1.0 | | 71 (81.6) | 16 (18.4) | 1.0 | | 0.39 |
| >7 | 226 (69.1) | 101 (30.9) | 1.72 (1.26-2.33) | 0.001 | 72 (74.2) | 25 (25.8) | 1.33 (0.71-2.50) | 0.37 | |
| Maximum tumour thickness, mm | | | | | | | | | |
| Continuous, median (range) [d] | 16 (1-96) | 18 (4-63) | 1.12 (0.99-1.27) | 0.07 | 20 (2-85) | 25 (4-88) | 1.13 (0.96-1.34) | 0.14 | |
| ≤22, n (%) | 402 (78.8) | 108 (21.2) | 1.0 | | 81 (82.6) | 17 (17.4) | 1.0 | | 0.48 |
| >22 | 147 (71.0) | 60 (29.0) | 1.30 (0.94-1.78) | 0.11 | 61 (71.8) | 24 (28.2) | 1.71 (0.92-3.19) | 0.09 | |
| Radiological N stage [c] | | | | | | | | | |
| 0, n (%) | 134 (76.6) | 41 (23.4) | 1.0 | | 34 (89.5) | 4 (10.5) | 1.0 | | 0.16 |
| 1 | 275 (78.1) | 77 (21.9) | 0.98 (0.67-1.43) | 0.91 | 67 (77.9) | 19 (22.1) | 2.23 (0.76-6.57) | 0.14 | |
| 2 | 146 (74.1) | 51 (25.9) | 1.12 (0.74-1.69) | 0.59 | 42 (70.0) | 18 (30.0) | 3.31 (1.12-9.78) | 0.03 | |
| Radiological N status [c] | | | | | | | | | |
| 0, n (%) | 134 (76.6) | 41 (23.4) | 1.0 | | 34 (89.5) | 4 (10.5) | 1.0 | | 0.07 |
| 1-2 | 421 (76.7) | 128 (23.3) | 1.03 (0.72-1.47) | 0.87 | 109 (74.7) | 37 (25.3) | 2.65 (0.95-7.45) | 0.06 | |

Table 3.3 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No DFS Event (n = 625) | DFS Event (n = 172) | HR (95% CI) | P value [a] | No DFS Event (n = 144) | DFS Event (n = 41) | HR (95% CI) | P value [a] | |
| Lymph node ≥10 mm short axis | | | | | | | | | |
| Absent, n (%) | 394 (78.5) | 108 (21.5) | 1.0 | | 94 (80.3) | 23 (19.7) | 1.0 | | 0.92 |
| Present | 156 (70.9) | 64 (29.1) | 1.37 (1.01-1.87) | 0.04 | 47 (73.4) | 17 (26.6) | 1.43 (0.76-2.68) | 0.26 | |
| EMVI | | | | | | | | | |
| Absent, n (%) | 233 (79.2) | 61 (20.8) | 1.0 | | 55 (84.6) | 10 (15.4) | 1.0 | | 0.36 |
| Present | 317 (74.2) | 110 (25.8) | 1.28 (0.94-1.75) | 0.12 | 86 (73.5) | 31 (26.5) | 1.80 (0.88-3.68) | 0.11 | |

CI = confidence intervals; dMMR = mismatch repair deficient; EMVI = extramural venous invasion; HR = hazard ratio; MMR = mismatch repair; n = number of participants; N = lymph node stage; pMMR = mismatch repair proficient; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

### 3.4.4 Impact of Multiple Prognostic Factors

To explore whether the presence of multiple prognostic factors influences DFS event risk, significant variables from the univariable analysis were combined into a simple cumulative risk score. Male sex and poor tumour differentiation were weighted as 2 according to its multivariable HR, and others (performance status 1-2, T4 stage, extramural tumour extension >7 mm, maximum tumour thickness >22 mm, lymph node short axis ≥10 mm and EMVI), weighted as 1, with a maximum score of 10. Scores were categorised into low- (0-2), medium- (3), high (4-5) and very high-risk (>5) groups (based on increments in risk and participant numbers) with distinct prognostic value (Table 3.4; Figure 3.2). The 95 participants with at least one missing risk score variable were not included. Compared to the low-risk group, participants in the high- and very high-risk groups had a significantly worse DFS (HR 1.95; P = 0.001 and HR 3.73; P < 0.001, respectively).

Table 3.4 Time to DFS event According to Cumulative Risk Score

| Risk Group | No DFS event (n = 728) | DFS event (n = 229) | HR (95% CI) | P Value [a] |
|---|---|---|---|---|
| Low, n (%) | 208 (87.0) | 31 (13.0) | 1.0 | |
| Medium | 164 (80.8) | 39 (19.2) | 1.51 (0.94-2.41) | 0.09 |
| High | 255 (75.0) | 85 (25.0) | 1.95 (1.29-2.95) | 0.001 |
| Very high | 101 (57.7) | 74 (42.3) | 3.73 (2.45-5.68) | < 0.0001 |

CI = confidence intervals; DFS = disease-free survival; HR = hazard ratio; n = number of participants.
[a] Cox proportional hazards.
Percentages represent rows.
Participants with one or more variable unknown not included.

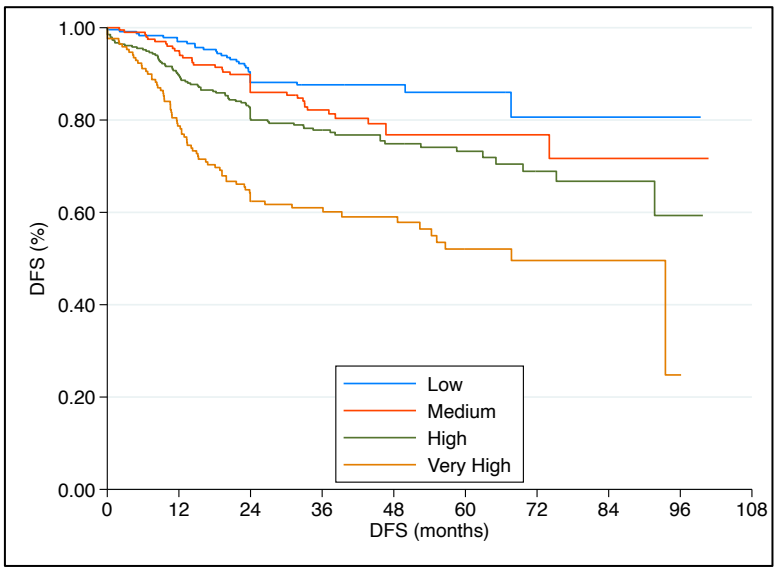



**Figure 3.2 Disease-Free Survival According to Cumulative Risk Group**
Kaplan-Meier survival curve for time to DFS event according to the risk score. DFS = disease-free survival.

## 3.5 Discussion

Neoadjuvant therapies could transform the treatment of CC and reliable prediction of the high-risk patient would greatly aid implementation.[18] While imaging is critical in patient selection, there is insufficient evidence for which radiological features confer greatest risk of recurrence or who is most likely to benefit from NAT. In this analysis of prospectively generated data from the phase III FOxTROT trial, several clinicopathological and imaging characteristics available at baseline were predictive of survival outcomes in locally advanced CC and add extra information beyond TNM staging. Importantly, like previous analyses in the adjuvant setting, these characteristics are prognostic within a population already considered to be high-risk, indicating that more refined and intentional patient stratification is possible.[32, 33] To the best of our knowledge, this is also the first published analysis to explore such prognostic factors according to MMR status, providing essential insights for neoadjuvant trials in molecularly defined populations. Emerging data in the adjuvant setting indicates the value of combining information across novel domains, including digital pathology and circulating tumour DNA (ctDNA).[34] Similar approaches to multimodal outcome prediction are likely to shape future neoadjuvant therapy delivery with the results of the current analysis representing a critical first step in this direction.

The current literature includes studies that have explored the prognostic effects of various radiological variables.[35-44] While these studies provide meaningful contributions to addressing this critical research question, the results are inconsistent with common limitations, including retrospective reporting, single-centre design, small sample size, lack of standardised reporting criteria, focusing on a specific colonic segment or individual feature, overlooking key biological distinctions (e.g. MMR status) and comprising evaluations from a single or small number of expert radiologists, which may not be generalisable to routine practice.

Radiological T stage is currently the main parameter for NAT patient selection.[1, 3] While previous studies have described relationships between radiological T stage and prognosis, they have either focused on the full range of T stages (T1-4) or created alternative dichotomies (e.g. T1-3b vs. T3c-4 [as seen in assessment of extramural extension in rectal cancer] and T1-3 vs. T4).[35, 37, 39-42, 45] The current analysis builds on this evidence, showing that radiological T4 staging significantly worsens DFS compared to T3 staging, exclusively within the locally advanced population. Despite the inability of CT to distinguish T3 from T4 tumours

pathologically, this radiological distinction appears prognostically relevant with potential value in baseline clinical decision-making.[28]

Depth of extramural extension is a well-recognised prognostic factor on pathology but is under-explored radiologically.[23] Cho et al. reported significantly lower DFS for T3 tumours of the ascending colon with radiological extramural extension >5 mm, yet this result is limited by small sample size, wide confidence intervals and not exploring alternative cut offs.[36] In the current analysis, depth of extramural extension was identified as the most prognostic imaging factor. The optimal cut off chosen was 7 mm, which differs from Cho et al. The prognostic value of maximum tumour thickness on imaging, which may represent a surrogate for tumour volume, has not been previously explored. Here, thickness >22 mm conferred a significantly worse DFS and offers the advantage of being an objective measure independent of the TNM system.

There may not be single best thresholds for evaluating tumour extension and thickness, as they could differ depending on the treatment and population being considered. Hence, data on a variety of cut-off points are provided in the Supplementary Data to guide future trial design and implementation. Dichotomising these variables provides a simple approach for application in clinical practice but introduces methodological limitations. Participants are considered equivalent despite potentially significant differences in risk within each group, resulting in the loss of valuable statistical power. Future predictive models may be improved by utilising these variables as continuous measures to optimise risk assessment. The interpretation and implementation of these measures in routine care may benefit from consultation and consensus from a group of experts and key opinion leaders.

EMVI is challenging to identify on CT, yet several studies have reported prognostic value.[38, 41, 44, 45] Small vessel invasion is unlikely to be visible on CT, while other features like tumour extension into pericolic fat, adjacent inflammation and lymphatic or perineural invasion can be misinterpreted as EMVI. Moreover, the absence of standardised criteria results in EMVI assessment being inherently subjective. Kato et al. found that patients with EMVI on standard CT had significantly higher recurrence risk than those without, yet only 10% were EMVI-positive on CT, compared to 69% pathologically.[41] This substantial discrepancy suggests the investigators used a high threshold for detection, potentially selecting only those with gross EMVI, which may be a particularly poor prognostic group.

The impact of a lymph node $\geq$10 mm in short axis on CT has not specifically been reported elsewhere. However, some studies have demonstrated adverse prognostic value for N1-2

status using pre-defined size thresholds. Huh et al. defined lymph node metastases as ≥10 mm in long axis or 7-10 mm in short axis alongside other pathological features; Tong et al. proposed a 5.8 mm short axis threshold.[39, 43] Other studies suggest prognostic importance for N staging without specific size criteria.[35, 40, 41, 45] In the current analysis, neither N stage nor status was associated with survival outcomes, in contrast to the other studies. This discrepancy may reflect the inclusion of exclusively advanced disease, variation in lymph node evaluation methods and the well-recognised limitations of CT for nodal evaluation.[25, 26] Using size criteria addresses some of the subjectivity involved with lymph node assessment and provides an evaluation that is not necessarily bound by its ability to predict pathological N stage. The contrast in prognostic value between lymph nodes ≥10 mm and N1-2 status may reflect high specificity for identifying lymph node metastases at this threshold. Alternatively, some enlarged lymph nodes ≥10 mm may not have been metastatic but instead indicate greater systemic inflammation which is known to confer a poor prognosis in CC.[46]

Consistent with previous research, participants with poorly differentiated tumours had a significantly worse survival outcomes than those with moderately or well differentiated tumours.[47] Definitive assessment of tumour differentiation is usually performed on the resection specimen, as was the case in FOxTROT, but in some instances may also be evaluated at baseline on the diagnostic biopsy. However, there are limitations and clinical considerations for the data presented in this analysis: biopsy assessment may not be fully representative due to tumour heterogeneity; assessing tumour differentiation is known to be limited by inter-observer variation and a lack of standardised criteria; NAC can affect tumour morphology for differentiation assessment, and in the small number of cases with a complete response, would prevent assessment altogether; and lastly, tumour differentiation is only relevant to adenocarcinoma and mucinous adenocarcinoma tumours, not those with rarer histological subtypes.[48, 49]

Around 15% of localised CCs are dMMR, with distinct biological, clinical and radiological characteristics, compared to pMMR tumours.[29, 30] CT staging performance is also affected, particularly for lymph node evaluation, where dMMR tumours are at greater risk of N over-staging.[31] Given these established differences, it is plausible that baseline prognostic features may vary by MMR status. In the current analysis, male sex, poor tumour differentiation, extramural tumour extension >7 mm, and the presence of a lymph node ≥10 mm in short axis were associated with a significantly worse DFS in pMMR tumours. Conversely, only T4 and N2 staging were significantly prognostic in those with dMMR tumours. Although these findings require validation, they highlight how risk factors may differ between

molecularly defined populations and impact the development of NAT in these groups. Dividing the dataset into subgroups also reduces statistical power, particularly for the smaller dMMR cohort, underscoring the need for dedicated datasets in these specific populations.

Exploring treatment effect across the same variable subgroups demonstrated inconsistencies in benefit. This analysis was limited to participants with proficient or unknown MMR status to best reflect current practice where patients with dMMR tumours would not be considered for NAC. Generally, participants with high-risk prognostic features derived greater benefit from NAC over surgery, particularly those related to the primary tumour (T4 stage, extramural extension >7 mm, maximum tumour thickness >22 mm). This pattern is in keeping with the main trial, which delivered a positive result in a population selected using primary tumour assessment.[1]

Developing a simple cumulative risk score illustrated that different risk factor combinations can confer additional adverse prognostic value and provide a more refined approach to individual risk assessment than TNM staging. Other risk scores have been developed but focus more on pathological variables evaluated post-operatively.[50] The purpose of the current analysis was to explore the cumulative effect of baseline prognostic features with a radiological focus, which will be validated in upcoming analyses within the FOxTROT platform. These data also indicate potential for developing multimodal predictive models, which could include more experimental approaches to imaging, such as radiomics and whole-body radiological profiling, ctDNA, digital pathology and applications of artificial intelligence.

Circulating tumour DNA is being widely tested across tumour types, including colorectal cancer, with a particular focus on the utility of minimal residual disease in adjuvant treatment decision making. Recent data has shown that combining ctDNA with other disciplines, such as digital pathology, in the adjuvant setting achieves greater prognostic value than either approach in isolation, highlighting the potential for multimodal approaches to treatment allocation.[34] However, there are numerous potential applications for ctDNA, including baseline patient evaluation. Integrating baseline ctDNA metrics with other modalities was recently shown to be feasible and provide clinically useful information for individual patient risk assessment.[51-53] These data must reframe how we consider patient selection, with multi-modal outcome prediction likely to represent the future of risk stratification and treatment selection in the neoadjuvant setting. Only through high-impact and methodologically robust research can this potential be realised and used to shape future clinical guidelines.

Training and education are fundamental to radiology practice and must be considered as new and clinically important features are identified.[54, 55] FOxTROT incorporated a bespoke training package to support radiologists in participant selection and to develop the dataset used in the current analysis. If the prognostic features identified are further integrated into neoadjuvant trial design and clinical practice, educational resources must re-calibrate accordingly. Other neoadjuvant trials should implement similar, tailored training strategies that align with their specific needs while utilising contemporary technologies and resources.

The current analysis is limited to a pre-defined selection of baseline variables chosen during trial design. Since FOxTROT only included participants with locally advanced disease, prognostic features in early CC (and thus the overall CC population) could not be assessed. Radiological central review was not feasible to provide quality assurance. However, with over 100 radiologists across 85 sites contributing to the radiological dataset, the findings from this analysis are likely to be more generalisable than those derived from central review.

With NAC now established as a standard treatment option for locally advanced CC and other NATs under investigation, optimising baseline patient evaluation is becoming increasingly important. As treatment shifts towards the neoadjuvant setting, effectively identifying high-risk patients will be essential to maximising benefit. This analysis highlights several baseline prognostic features, including new ways to utilise radiological assessment, which may support patient selection for NAT and inform future clinical trial design.

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# 4 Characterising Radiological Response to Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer in the International FOxTROT Trial

## 4.1 Abstract


### 4.1.1 Objectives

Neoadjuvant chemotherapy (NAC) represents a new treatment for locally advanced colon cancer (CC) where pathological regression closely informs recurrence risk. Response is also evaluable on imaging but is poorly understood and limited by the lack of a standardised framework. Here, radiological changes seen following NAC in the FOxTROT trial were explored.

### 4.1.2 Materials & Methods

FOxTROT was the first randomised controlled trial to establish NAC as safe and effective in patients with cT3-4 N0-2 M0 CC. Post-NAC CT scans were not required in the trial and were therefore only performed in a subset. Paired scans were retrospectively and independently evaluated by two reviewers for the following tumour variables: distance beyond the muscularis propria, maximum thickness (anywhere and invasive margin), length (along the colon and largest diameter), volume, mean attenuation and standard deviation (SD) of attenuation. Values were compared between pre- and post-NAC scans and according to pathological regression and mismatch repair (MMR) status using Wilcoxon signed-rank, paired-t, Mann-Whitney U and t-tests. Exploratory correlation analyses were performed between variables and reviewers.

### 4.1.3 Results

Fifty-three participants (mean age 62.7 $\pm$9.6; 32 men) were included. Significant reductions in all variables except SD of attenuation were seen for both reviewers ($P < 0.01$ for all). Other than volumetric reduction for one reviewer, no significant differences were seen by pathological regression or MMR status. Inter-observer analyses demonstrated moderate to strong agreement.

### 4.1.4 Conclusion

Six weeks of NAC induces significant measurable change on CT across several metrics, indicating potential utility for response assessment. The relationship between radiological and pathological response was limited and warrants further evaluation.

## 4.2 Introduction

The Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT; NCT00647530) trial was the first international phase III randomised controlled trial (RCT) to test neoadjuvant chemotherapy (NAC) in locally advanced colon cancer (CC).[1] Six weeks of NAC was associated with a significant reduction in the risk of residual disease or recurrence at two years, compared to upfront surgery. NAC was also associated with significant reductions in pathological stage, extramural venous invasion (EMVI) and peri-operative complications without any safety concerns. The safety and efficacy of NAC have since been reinforced by the OPTICAL trial and a recent meta-analysis, establishing a new treatment option for locally advanced but resectable CC.[2-5]

FOxTROT and OPTICAL also clearly demonstrated that the degree of pathological regression in the surgical specimen following NAC was closely related to long-term recurrence risk.[1, 2] Similar patterns are seen for neoadjuvant therapies in other tumour types, including rectal and gastro-oesophageal cancers, underscoring the importance of response to this initial phase of treatment.[6-9]

Imaging represents an alternative approach to response assessment with the advantage of *in vivo* evaluation prior to surgical resection. Like pathological assessment, radiological response to neoadjuvant therapies is associated with long-term outcomes in other tumour types.[10-12] Radiological response to NAC in CC has several potential applications, including prognostication, facilitating response-adapted treatment and indicating treatment sensitivity to guide adjuvant decision-making. However, there is no standardised framework for evaluating radiological response in primary colonic tumours, which have heterogenous and complex morphology.

Several clinically and molecularly distinct sub-populations of CC exist with differences in tumour behaviour, treatment-sensitivity and prognosis.[13] For example, tumours with deficient mismatch repair (dMMR) have a unique radiological phenotype and are more sensitive to immunotherapy than chemotherapy.[14-16] In FOxTROT, participants with dMMR

tumours did not benefit from NAC to the same degree, whereas remarkable pathological responses to neoadjuvant immunotherapy (NAI) have been seen in several early phase trials.[1, 17-24] Radiological response patterns specific to immunotherapy are now recognised, including pseudo-progression and pseudo-residual disease, indicating the importance of evaluating response within specific tumour- and treatment-related contexts.[25, 26]

There is a need to improve wider understanding of radiological response to neoadjuvant therapies in CC, with contemporary and high-quality trial datasets well placed to support this goal. For NAC, radiological changes seen following treatment should be comprehensively characterised and explored for associations with clinically relevant outcomes. In this analysis, a subset of participants from the FOxTROT trial with paired pre- and post-NAC CT scans were evaluated to provide initial insights into radiological response following six weeks of NAC.

## 4.3 Methods

### 4.3.1 Trial Design

The FOxTROT trial received National Health Service Research Ethics Committee approval, including prospective collection of CT scans and subsequent data analyses (MREC: 07/S0703/57). Written informed consent was obtained from all participants. The main trial results are published separately.[1]

FOxTROT was an international, phase III, RCT for patients with radiologically staged T3-4 (≥1 mm extramural tumour extension required) N0-2 M0 CC. Participants were identified from multidisciplinary team (MDT) meetings across 85 sites in the United Kingdom, Denmark and Sweden from 15$^{th}$ May 2008 to 23$^{rd}$ December 2016 and randomised 2:1 to NAC or upfront surgery. The NAC regimen comprised six weeks of a fluoropyrimidine (5-fluorouracil [5FU] or capecitabine) plus oxaliplatin. Adjuvant chemotherapy was given in both arms with an equivalent total of 24 weeks peri-operative chemotherapy.

### 4.3.2 Radiological Assessment

All participants underwent a baseline, portal venous phase, contrast-enhanced, spiral/multidetector CT of the chest, abdomen and pelvis with maximum 5 mm slices. Post-NAC scans were not mandated in the trial and were therefore only performed and submitted in a subset of participants, according to local site preference. The timing of post-NAC imaging

was also not protocolised or recorded. Participants with both scans available were included in this analysis.

Pre- and post-NAC CT scans for each participant in this sub-study were retrospectively and independently reviewed by a Research Fellow (JRP; Reviewer 1) and Specialist GI Radiologist (DJMT; Reviewer 2) with four- and twenty-years' experience in CC imaging, respectively. The reviewers were aware of the scan timing (pre- or post-NAC) but blinded to the paired scan images and clinicopathological data. At least a one-week interval was required between reviews of paired scans to ensure objective measurements of radiological features. A protocol for radiological assessment was developed in advance of the analysis, including variable definitions (Appendix 7.4.1).

The following primary tumour morphological features were evaluated on each scan: maximum distance beyond the muscularis propria (mm), maximum tumour thickness (mm), length of tumour along the colon (mm), and volume ($cm^3$). For a more comprehensive evaluation of thickness and length, maximum thickness at an invasive margin (mm) and single largest diameter in any direction (mm) were also recorded, even if equivalent. Volumetric segmentation was first completed slice-by-slice by Reviewer 1. In advance of the study, it was agreed that due to time constraints, Reviewer 2 would review and edit the existing segmentations from Reviewer 1, acknowledging that the segmentations would not be fully independent. Mean tumour attenuation (Hounsfield units [HU]) and standard deviation (SD) of the mean attenuation were also quantified from the segmentations. Scan reviews were performed using 3D Slicer (version 5.8.1, https://www.slicer.org/), including the Segment Comparison, Segment Editor, and Segment Statistics modules.[27]

### 4.3.3 Pathological Assessment

Tumour regression grade (TRG) was evaluated on resection specimens at both local and central laboratories, according to the Dworak system.[28] Central data were prioritised where specimens were available for review, with local data used for remaining cases. Consistent with the main trial publication, pathological regression was dichotomised in the current analysis into none or mild ('non-responder') versus moderate, marked or complete ('responder').[1] Mismatch repair (MMR) status was determined centrally using four protein immunohistochemistry.

### 4.3.4 Statistical Analysis

Descriptive statistics were generated for baseline characteristics and reported alongside the whole NAC trial arm for comparison. Tumour side was defined as right for those proximal to the splenic flexure and left for those at, or distal to, the splenic flexure. Radiological N stage was dichotomised as N status (N0 or N1-2) for clinical relevance. Local T stage, N stage and EMVI data were provided for the included subset to be most comparable to data for the whole NAC arm. Results are provided for both reviewers as there is no established ground-truth and to maximise insights.

Paired pre- and post-NAC measurements were compared using either Wilcoxon signed-rank tests or paired t-tests, depending on Shapiro-Wilk normality testing, and illustrated using box plots. Percentage change was calculated for each feature and reviewer for all participants and illustrated using waterfall plots. To explore the relationship between each response metric, Spearman's rho values were calculated and presented using heatmaps. Subgroup analyses were performed for pathological regression and MMR statuses using t- or Mann-Whitney U tests. P values $< 0.05$ were considered statistically significant for all analyses.

As an additional exploratory analysis, inter-observer variation between the two reviewers for each feature and scan timepoint was assessed using Spearman's rank correlation coefficients, intraclass correlation coefficients, Bland-Altman analyses, and Dice coefficients.

Statistical analyses were performed and visualisations prepared in Python (version 3.11.5, Python Software Foundation, Wilmington, DE, United States).

## 4.4 Results

### 4.4.1 Study Population

Baseline characteristics for the response analysis subset and the whole NAC arm are summarised in Table 4.1. Paired CT scans were available in 53 of 698 (7.6%) participants randomised to NAC, which comprised 32 men and 21 women with a mean age of 62.7 years at diagnosis (standard deviation 9.6) (Figure 4.1). Forty-three participants had proficient MMR (pMMR) tumours and 13 had dMMR tumours; for one participant, the MMR status was unknown. Disease recurrence occurred in 11 (20.8%) participants.

Table 4.1 Baseline Characteristics

| Characteristic | Response Evaluation Subset (n = 53) | Whole NAC Arm (n = 698) |
|---|---|---|
| Age, years | | |
| Mean (SD) | 62.7 (9.6) | 63 (9.8) |
| Sex | | |
| Male, n (%) | 32 (60.4) | 447 (64.0) |
| Female | 21 (39.6) | 251 (36.0) |
| MMR status | | |
| Proficient, n (%) | 43 (81.1) | 481 (68.9) |
| Deficient | 9 (17.0) | 117 (16.8) |
| Unknown | 1 (1.9) | 100 (14.3) |
| Tumour side [a, b] | | |
| Right, n (%) | 22 (41.5) | 333 (47.7) |
| Left | 31 (58.5) | 359 (51.4) |
| Radiological T stage [b, c] | | |
| 2 | 0 (0.0) | 1 (0.1) |
| 3 | 39 (73.6) | 523 (74.9) |
| 4 | 14 (26.4) | 169 (24.2) |
| Unknown | 0 (0.0) | 5 (0.7) |
| Radiological N status [b, c] | | |
| 0 | 17 (32.1) | 169 (24.2) |
| 1-2 | 36 (67.9) | 524 (75.1) |
| Unknown | 0 (0.0) | 5 (0.7) |
| Radiological EMVI status [b, c] | | |
| Negative, n (%) | 13 (24.5) | 280 (40.1) |
| Positive | 40 (75.5) | 408 (58.5) |
| Unknown | 0 (0.0) | 10 (1.4) |

EMVI = extramural venous invasion; MMR = mismatch repair; N = lymph node stage; NAC = neoadjuvant chemotherapy; SD = standard deviation; T = primary tumour stage; TNM = tumour, node metastasis.

[a] Right = proximal to splenic flexure; left = involving, or distal to, the splenic flexure.

[b] Local data provided for the subset to be most comparable to whole NAC cohort.

[c] According to TNM version 5.

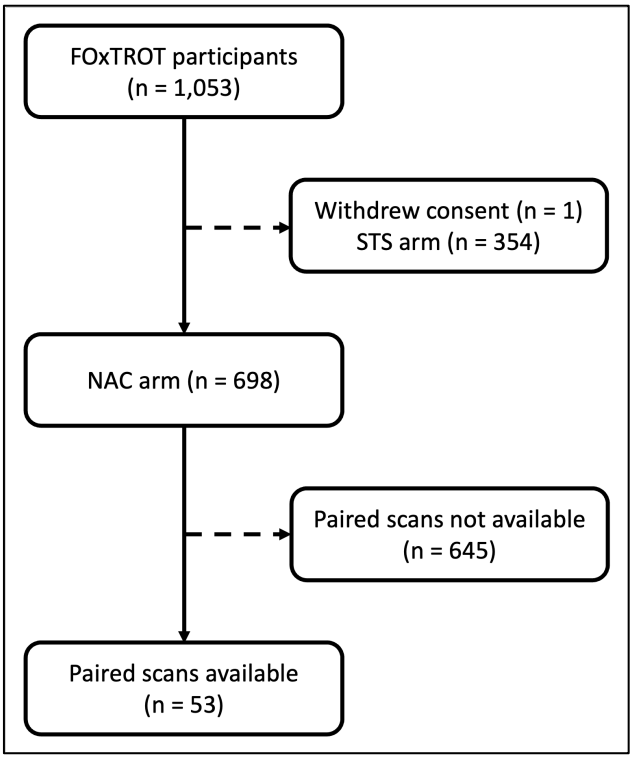



**Figure 4.1 Selection of Participants**
Evaluating radiological response was limited to those in the NAC arm of the trial. Post-NAC CT scans were not mandated as part of the trial, leaving a subset of participants with paired scans available for response assessment. NAC = neoadjuvant chemotherapy; STS = straight-to-surgery.

### 4.4.2 Radiological Changes Following Neoadjuvant Chemotherapy

Comparing pre- and post-NAC values, significant reductions in maximum distance beyond the muscularis propria, maximum tumour thickness, maximum tumour thickness at an invasive margin, length of the tumour along the colon, single largest diameter, tumour volume and mean tumour attenuation were seen for both reviewers ($P < 0.01$ for all; Table 4.2; Figures 4.2 & 7.3). Notably, as the feature most representative of the whole tumour, median tumour volume fell from 34.4 $cm^3$ to 15.9 $cm^3$ for Reviewer 1 and 33.1 $cm^3$ to 15.9 $cm^3$ for Reviewer 2 (Figure 4.3).

Table 4.2 Radiological Changes Following Neoadjuvant Chemotherapy

| | Reviewer 1 | | | Reviewer 2 | | |
|---|---|---|---|---|---|---|
| Characteristic | Pre-NAC | Post-NAC | P Value [a] | Pre-NAC | Post-NAC | P value [a] |
| Maximum distance beyond muscularis propria, mm | | | | | | |
| Median (IQR) | 7 (4-11) | 4 (2-9) | < 0.01 | 7 (5-13) | 5 (3-10) | < 0.01 |
| Maximum tumour thickness, mm | | | | | | |
| Median (IQR) | 20 (16-28) | 14 (10-20) | < 0.01 | 19 (15-25) | 15 (11-21) | < 0.01 |
| Maximum tumour thickness at invasive margin, mm | | | | | | |
| Median (IQR) | 17 (12-23) | 12 (9-17) | < 0.01 | 19 (14-25) | 14 (11-21) | < 0.01 |
| Length of tumour along colon, mm | | | | | | |
| Median (IQR) | 52 (40-65) | 45 (36-56) | < 0.01 | 53 (42-65) | 44 (35-57) | < 0.01 |
| Single largest diameter, mm | | | | | | |
| Median (IQR) | 52 (40-65) | 45 (36-56) | < 0.01 | 54 (43-65) | 45 (36-57) | < 0.01 |
| Tumour volume, $cm^3$ | | | | | | |
| Median (IQR) | 34.4 (21.3-57.7) | 15.9 (10.6-36.6) | < 0.01 | 33.1 (21.8-57.7) | 15.9 (10.5-34.4) | < 0.01 |
| Mean tumour attenuation, HU | | | | | | |
| Mean (SD) | 59.5 (11.5) | 55.3 (12.8) | < 0.01 | 59.3 (11.3) | 54.9 (12.8) | < 0.01 |
| Standard Deviation of tumour attenuation | | | | | | |
| Median (IQR) | 27.1 (24.2-32.3) | 26.7 (23.4-28.6) | 0.06 | 28.4 (24.2-32.8) | 27.0 (23.7-31.1) | 0.34 |

HU = Hounsfield units; IQR = interquartile range; NAC = neoadjuvant chemotherapy; SD = standard deviation.

[a] Wilcoxon signed-rank or paired t test according to variable distribution.

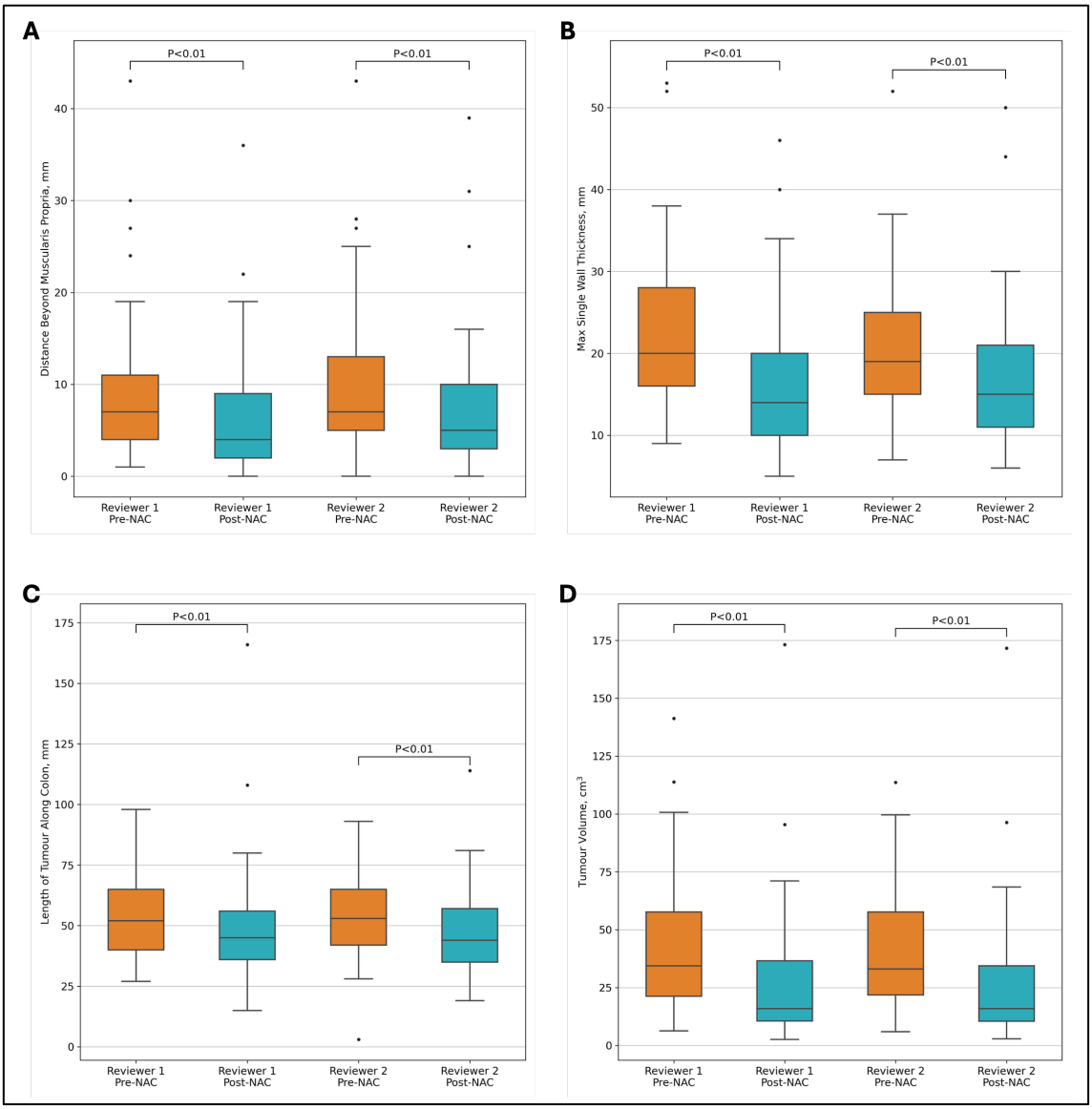



**Figure 4.2 Radiological Changes Following Neoadjuvant Chemotherapy**
Box plots showing pre- and post-NAC measurements for Reviewer 1 and Reviewer 2. Whiskers represent the largest and smallest datapoints within 1.5x the interquartile range. A) Maximum distance beyond the muscularis propria. B) Maximum tumour thickness. C) Length along the colon. D) Volume. NAC = neoadjuvant chemotherapy.

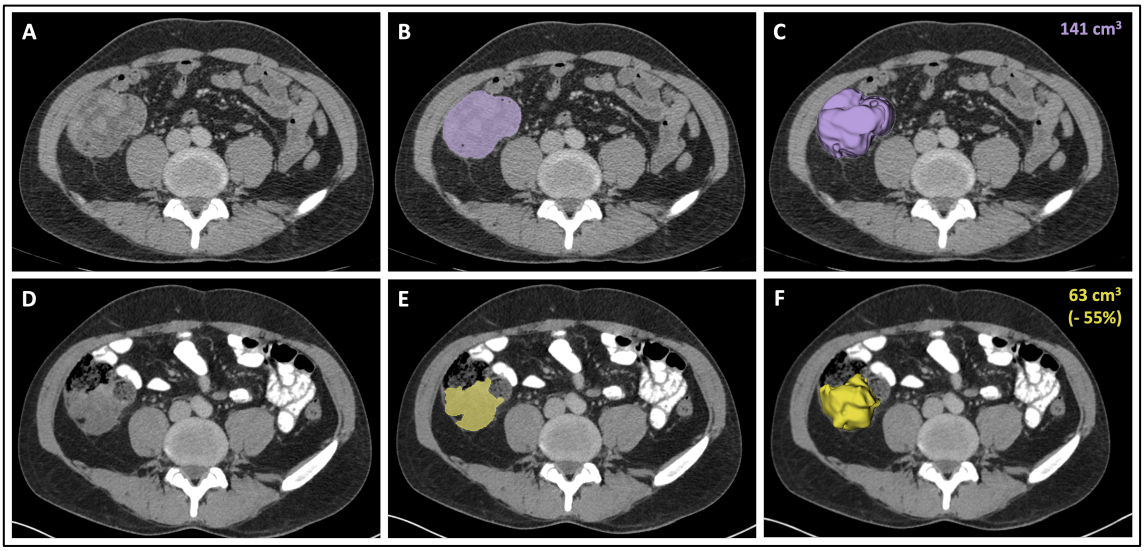



**Figure 4.3 Example of Volumetric Response**
Axial images of a locally advanced cancer in the caecum. A-C) Images along the top row represent the pre-NAC scan. D-F) Images along the bottom row represent the post-NAC scan. A and D) are plain images. B and E) are the same images plus a superimposed 2D segmentation. C and F) are the same images plus the completed 3D segmentation. A clear response to NAC can be seen which, using manual segmentation by Reviewer 1, was calculated to be a reduction in volume of 55%. 2D = 2-dimensional; NAC = neoadjuvant chemotherapy.

Other than tumour attenuation, each variable represents a different approach to measuring tumour size. To explore the relationships between changes in these measures, a correlation analysis was performed. Spearman's rank correlation ranged from weak to very strong across pairings (Figure 7.4). Change in tumour volume was most correlated with changes in length along the colon ($\rho = 0.71$) and largest single diameter ($\rho = 0.60$) for Reviewer 1 and Reviewer 2, respectively. Strong correlation was seen between the two measures of tumour thickness (anywhere vs. at an invasive margin) and length (along the colon vs. any diameter) for both reviewers, suggesting that these measurements were often taken from the same tumour region.

### 4.4.3 Subgroup Analyses

The degrees of change for each radiological measure were evaluated according to pathological regression and MMR status. For Reviewer 1, no significant differences in response metrics were seen between pathological responders and non-responders (Table 4.3). For Reviewer 2, reduction in tumour volume was significantly greater in pathological responders than non-responders (-56.2% vs. -44.9%, P = 0.04). However, when considered visually on an individual

patient level, no close relationship was seen between volumetric change and pathological regression status (Figure 4.4). It is also notable that clear radiological changes were seen in pathological non-responders, in whom NAC would normally be considered ineffective (Figures 7.5 to 7.11). For example, tumour volume reduced by 46.0% and 44.9% in pathological non-responders for Reviewer 1 and Reviewer 2, respectively. No significant differences in radiological changes were seen between participants with pMMR and dMMR tumours (Table 7.17).

Table 4.3 Radiological Responses According to Pathological Regression Status

| | Reviewer 1 | | | Reviewer 2 | | |
|---|---|---|---|---|---|---|
| Characteristic | Responder [a] (n = 10) | Non-Responder (n = 43) | P Value [b] | Responder (n = 10) | Non-Responder (n = 43) | P value [b] |
| Distance beyond MP, mm | | | | | | |
| Median percentage change, % (IQR) | -48.4 (-65.0 to -6.7) | -40.0 (-60.0 to -4.2) | 0.61 | -36.7 (-53.5 to 34.4) | -36.7 (-66.9-0.0) | 0.70 |
| Max tumour thickness, mm | | | | | | |
| Median percentage change, % (IQR) | -40.3 (-53.9 to -31.2) | -22.2 (-38.2 to -6.3) | 0.07 | -33.2 (-45.0 to 1.8) | -20.0 (-46.9 to 0.0) | 0.71 |
| Max tumour thickness at IM, mm | | | | | | |
| Median percentage change, % (IQR) | -31.9 (-54.4 to 8.3) | -25.0 (-48.4 to 1.5) | 0.95 | -33.2 (-45.0 to 1.8) | -21.4 (-46.9 to -1.9) | 0.71 |
| Length of tumour along colon, mm | | | | | | |
| Median percentage change, % (IQR) | -15.9 (-41.8 to 7.2) | -14.7 (-28.4 to 0.1) | 0.69 | -28.9 (-38.3 to -21.9) | -13.0 (-28.3 to 3.9) | 0.13 |
| Single largest diameter, mm | | | | | | |
| Median percentage change, % (IQR) | -15.9 (-41.8 to 7.2) | -14.7 (-26.8 to 0.1) | 0.64 | -28.9 (-38.3 to -21.9) | -13.0 (-27.3 to 3.9) | 0.07 |
| Tumour volume, $cm^3$ | | | | | | |
| Median percentage change, % (IQR) | -56.2 (-68.3 to -50.1) | -46.0 (-62.0 to -9.4) | 0.12 | -56.2 (-68.2 to -50.1) | -44.9 (-55.8 to -12.6) | 0.04 |
| Mean tumour attenuation, HU | | | | | | |
| Mean percentage change, % (SD) | -9.8 (11.2) | -4.9 (20.5) | 0.47 | -10.7 (9.7) | -5.3 (21.3) | 0.45 |
| SD of tumour attenuation | | | | | | |
| Median percentage change, % (IQR) | -13.2 (-23.7 to 13.9) | -3.3 (-12.0 to 8.9) | 0.63 | -4.1 (-24.6 to 21.2) | -3.7 (-11.6 to 8.3) | 0.85 |

HU = hounsfield units; IM = invasive margin; IQR = interquartile range; MP = muscularis propria; SD = standard deviation.
[a] Responder = Moderate, marked or complete pathological regression; Non-responder = No or mild pathological regression.
[b] Mann-Whitney U or t test according to variable distribution.

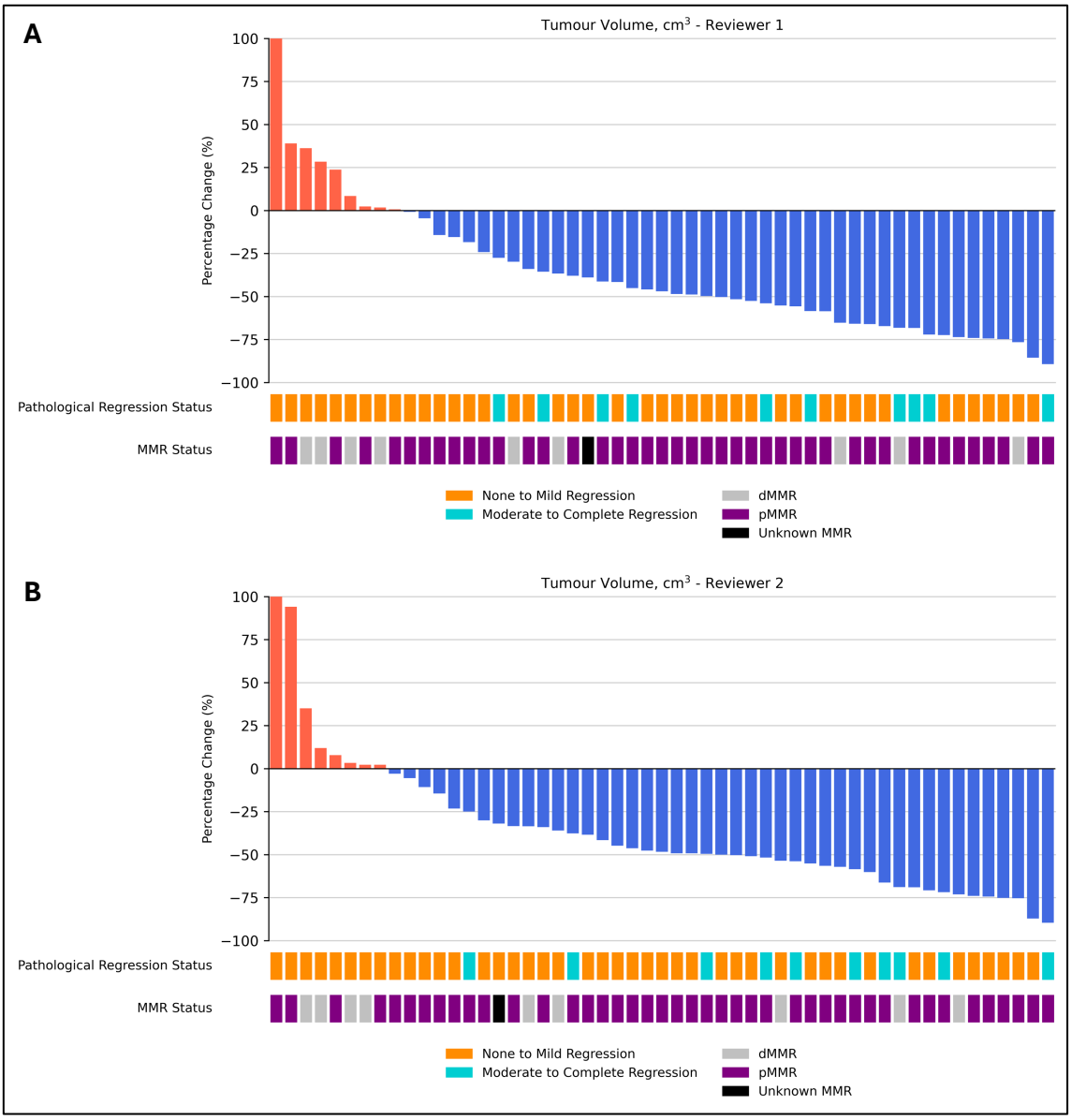



**Figure 4.4 Waterfall Plots for Volumetric Response by Subgroups**
Waterfall plots showing the degree of change in primary tumour volume after neoadjuvant chemotherapy for each participant according to pathological regression status (none or mild vs. moderate, marked or complete) and MMR status. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair' pMMR = proficient mismatch repair.

### 4.4.4 Inter-Observer Analyses

The inter-observer variation between Reviewer 1 and Reviewer 2 was evaluated for each feature on both pre- and post-NAC scans (Table 7.18). Moderate to very strong Spearman's rank correlation was achieved across measures, with greatest correlation for tumour volume ($\rho = 0.97$ and $\rho = 0.98$ for pre- and post-NAC values, respectively) (Figures 7.12 to 7.14). Considering segmentations were not fully independent between reviewers, the next strongest

correlation was for the single largest diameter (ρ = 0.72 and ρ = 0.78, respectively). ICCs were similarly strong, including values of 0.97 (95% confidence intervals [CI] 0.95-0.98) and 0.98 (95% CI 0.97-0.99) for pre- and post-NAC volume, respectively. Minimal systematic bias was detected across all features on Bland-Altman analysis; the largest being +2.38 for post-NAC tumour length, which is unlikely to be clinically significant (Figures 7.15 to 7.17). Finally, mean Dice coefficients of 0.96 and 0.93 demonstrate strong spatial consistency for pre- and post-treatment segmentations.

## 4.5 Discussion

In this preliminary analysis, six weeks of NAC was found to induce significant and measurable radiological change across several metrics, indicating potential utility for radiological response evaluation in locally advanced CC. There was no clear relationship between radiological responses and pathological regression status in this dataset, which suggests these are distinct assessments but also may reflect the limitations of each evaluation in isolation. Across different measures, correlation between reviewers was strong, suggesting generalisability of these objective radiological assessments. Together, these findings demonstrate a potential role for radiological response assessment following NAC which warrants further investigation as neoadjuvant therapies evolve.

There is little existing literature on radiological response to NAC, with the few published studies limited by small sample size, single site design, variation in patient population and treatment regimen, poorly defined or chosen radiological measures, and a lack of pathological correlation. In the current analysis, median tumour volume reduction mirrors two other studies despite differences in treatment duration and composition.[29, 30]

A multicentre Danish study of 67 patients also demonstrated significant changes in several morphological features following NAC.[31] However, limited information was provided for the definitions of, and methodological approaches to, specific measurements that are not standard-practice in CC evaluation, such as ‘axial tumour diameter’ and ‘largest tumour diameter’. All previous studies were published without detailed radiological protocols or guidance to determine reproducibility and validation, unlike the approach taken here. Along with two other studies, RECIST 1.1 criteria were also explored but without recognising potential limitations of this system in localised disease and luminal tumours.[30, 32] Longitudinal tumour size along the colon was included in the current analysis to mitigate the impact of luminal content and distension on these measurements. However, the results show

strong correlation between this measure and single largest diameter, indicating that these evaluations were often the same.

Complex three-dimensional shapes of colonic tumours, together with a hollow lumen and inconsistent bowel content, distension and movement, presents a major challenge to morphological response assessment. Other than volume, a single unidimensional measurement is unlikely to fully capture changes in tumour size. Identifying simple and reproducible surrogate measures for volume may, at least partially, overcome this issue. Je et al. took a different approach, devising a hierarchical CT-based TRG (ctTRG) system to combine morphological measures and generate a composite score from one to five.[33] Moderate correlation was seen between ctTRG and pathological TRG in this single centre study of 53 patients, suggesting potential utility for response assessment. However, the included parameters (unidimensional longest length, wall layer pattern, wall surface, and wall thickness) do not appear evidence-based, nor is the use of a hierarchical system where tumour length is the predominant component justified. In the current analysis, tumour length is one of the less responsive metrics with no clear advantage over others. Implementing a novel system like ctTRG would require a rigorous approach with multicentre validation, including alternative NAC regimens and inter-observer analyses.

Radiological changes and pathological regression status were not strongly related. Other than volumetric response for Reviewer 2, no significant differences were seen between pathological responders and non-responders; although, several numerical differences were seen and must be considered within the limited statistical power offered by this sample size. These data almost certainly reflect radiology and pathology being distinct assessments which evaluate different tumour properties. The limitations of pathological regression assessment may also partly contribute to this discrepancy and should be considered. First, several different pathological regression systems exist, resulting in a heterogeneous evidence-base and inconsistencies in practice; second, pathological regression is subjective with limited inter-observer agreement; third, existing TRG systems were developed in tumour sites other than CC and with different treatment regimens, although the prognostic effects of the Dworak system were validated in the main FOxTROT analysis; and fourth, no established dichotomy exists to categorise response versus no response.[1, 28, 34, 35] For the current analysis, it was particularly challenging to dichotomise the data, as most tumours were found to have ‘mild’ regression, meaning the ‘responder’ and ‘non-responder’ groups were imbalanced regardless of which way they were defined.

Any radiological response data must be considered within the specific contexts of tumour biology and treatment type; in this case, molecularly unselected locally advanced CC and six weeks of doublet NAC. A clear weakness of the existing literature is the variation in patient populations and treatment type and duration, all of which could influence radiological response. Different frameworks may be required for different treatment regimens and patient cohorts, rather than a one-size-fits-all approach. For example, with the increasing use of immunotherapy in dMMR CC, distinct patterns of response are already recognised, including pseudo-progression, pseudo-residual disease and stricturing.[25, 26, 36] On a molecular level, a novel serosa to mucosa response pattern has also been described.[37] There is also no standardised approach for assessing pathological response to NAI, with recent trials utilising residual tumour volume instead of a TRG system.[17, 38] Given these developments, and that dMMR tumours have different appearances on imaging, the findings of the current analysis may not be generalisable to this population.[14] The prospect of pursuing organ preservation following NAI for some patients with dMMR CC has placed even greater importance on understanding the specific nuances to radiological response in this population.[17]

The use of magnetic resonance imaging (MRI) and Fluorine-18 fluorodeoxyglucose (FDG) positron-emission tomography (PET)-CT has been investigated in localised CC, primarily for baseline radiological staging, with similar levels of diagnostic accuracy.[39] However, as neoadjuvant therapies provide new applications for imaging, the roles of these other modalities must be re-considered. MRI is used routinely in the post-treatment imaging of localised rectal cancer with advantages of being able to distinguish anatomical layers of the rectum, evaluate fibrotic treatment response and provide functional quantitative data, none of which is possible on CT.[40] Although using MRI for CC evaluation has logistical challenges, it may provide additional clinically important value for response assessment. The use of FDG PET-CT is currently limited to the assessment of indeterminate lesions during baseline staging but could also have a role in treatment response, particularly where microscopic pathological response precedes visible morphological changes on imaging. The use of FDG PET-CT for treatment response is already established in other tumour types, indicating potential utility that may translate to neoadjuvant therapies in CC.[41] In the study by Arredondo et al., 13 participants underwent additional FDG PET-CT scans pre- and post-NAC; in addition to morphological changes, a significant reduction in SUVmax was also detected.[29] Despite established practices, research into alternative imaging modalities must be prioritised to address new unanswered questions and optimise neoadjuvant therapy delivery.

Radiological response assessment is more developed in other tumour types but is closely related to treatment and imaging modalities used. In rectal cancer, MRI is used for neoadjuvant chemoradiotherapy response assessment to guide surgical intervention and the selection of patients for watch and wait pathways.[40] The magnetic resonance TRG (mrTRG) system has limited correlation with pathological regression and has not been universally adopted in routine practice.[42] Similar to CC, response assessment in gastro-oesophageal cancer is challenging due to complex tumour morphology. In this setting, radiological response assessment is routinely performed but primarily focuses on the exclusion of disease progression in the neoadjuvant phase.[43] In breast cancer, MRI is used to evaluate for progression during neoadjuvant therapy and identify patients suitable for breast-conserving surgery.[44]

The current analysis is limited by: a small (relative to the trial population) and potentially unrepresentative sample; smaller and imbalanced subgroup samples; evaluation by two reviewers; restricting tumour evaluations to standard views (axial, coronal and sagittal) rather than custom multiplanar reconstruction (MPR) to align with tumour orientation; lacking the statistical power to evaluate the prognostic value of radiological response metrics; and not considering the complementary or additive effects of measures combined together. It is unclear whether post-NAC scans were undertaken in this specific cohort for clinical reasons (e.g. concerns regarding progression or resectability) or simply due to local standard practices. As such, the included cases may not be representative of the wider trial population despite similar baseline characteristics (Table 4.1). Undertaking blinded evaluations is methodologically strong but is less generalisable to routine practice, where side-by-side comparison of pre- and post-NAC imaging would be expected. However, these novel and clinically relevant data provide a strong foundation from a multicentre trial cohort to guide larger-scale future research in this setting.

Six weeks of NAC induces significant radiological change across several measures. The relationship between these changes and pathological regression is inconsistent and warrants further investigation, in addition to evaluating the role of radiological response in prognostication and treatment delivery.

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# 5 Risk of Bowel Obstruction in Patients with Colon Cancer Responding to Immunotherapy: an International Case Series

## 5.1 Abstract


### 5.1.1 Background

Immunotherapy is used routinely for treating deficient mismatch repair (dMMR) colon cancer (CC). This case series highlights an emerging safety issue, where patients develop bowel obstruction associated with immunotherapy response.

### 5.1.2 Patients and Methods

Patients with dMMR CC who developed bowel obstruction while responding to immunotherapy were retrospectively identified. Data on patient, disease, treatment, and response-specific factors were explored for potential risk factors. Overall treatment numbers were used to estimate incidence.

### 5.1.3 Results

Nine patients from eight European centres were included. Common features were hepatic flexure location (5/9), T4 radiological staging (6/9), annular shape (8/9), radiological stricturing (5/9), and endoscopic obstruction (6/9). All received pembrolizumab and obstructed between 45 and 652 days after starting treatment. Seven patients underwent surgical resection; one was managed with a defunctioning stoma; and one was managed conservatively. One patient died from obstruction. Radiological response was seen in eight patients, including two complete responses. Pathological response was seen in all seven who underwent resection, including four complete responses. The overall incidence of immunotherapy response-related obstruction in these centres was 1.51%.

### 5.1.4 Conclusions

Bowel obstruction associated with immunotherapy response may represent a rare treatment-related complication in dMMR CC. Clinicians must recognise this safety signal and share experience to maintain patient safety.

## 5.2 Introduction

Colon cancer (CC) is the most common abdominal malignancy and a major cause of cancer-related death.[1] Localised disease typically requires surgical resection with or without additional chemotherapy, whereas metastatic CC is usually treated with systemic anticancer therapies alone.[2, 3] For deficient mismatch repair (dMMR) metastatic CC specifically, immunotherapy is now the established first-line treatment.[3]

Neoadjuvant chemotherapy (NAC) for locally advanced CC has been shown to be safe and effective in randomised trials, but lesser benefit has been observed for dMMR tumours.[4] Immunotherapy for dMMR localised colon and rectal cancers has been investigated in phase II trials with consistently remarkable rates of pathological complete response (pCR).[5, 6]

In CC, bowel obstruction is a major complication and the most common indication for emergency surgery, with significant morbidity and mortality.[7, 8] While obstruction risk is well recognised in the metastatic setting, the emergence of neoadjuvant therapies has placed a broader population at risk. In the international Fluorouracil, Oxaliplatin and Targeted Receptor pre-Operative Therapy (FOxTROT) trial, 4.3% of participants developed bowel obstruction during a short course of NAC; endoscopic obstruction and radiological or endoscopic stricturing were identified as independent baseline risk factors for bowel obstruction.[9] Interestingly, there was no association between obstruction risk and pathological regression, suggesting tumour phenotype may be more relevant in this setting than treatment response.

This case series was collated following observations from clinicians internationally that patients with dMMR CC were unexpectedly developing bowel obstruction after treatment with immunotherapy. However, rather than being associated with disease progression, they were associated with excellent immunotherapy response, an unusual phenomenon in CC.

## 5.3 Patients and Methods

Cases were identified retrospectively by clinicians across our international research network. While a specific period for inclusion was avoided to ensure maximal case identification, all instances of obstruction occurred within the past 3 years. Patients who developed bowel obstruction within a primary colonic tumour showing evidence of immunotherapy response (radiological and/or pathological) were included.

The following data were collected for each patient: age, sex, tumour site, radiological staging (according to the American Joint Committee on Cancer [AJCC] tumour-node-metastasis [TNM]

system, version 8), sites of metastases, tumour annularity, the presence of radiological stricturing, details of endoscopic evaluation, molecular biomarker testing results (*RAS*, *BRAF*, and MMR), tumour histology, type and duration of immunotherapy, clinical presentation, radiological response, relevant clinical outcomes (including surgical resection and/or defunctioning, colonic stenting, and adverse events), interval from starting immunotherapy to bowel obstruction, pathological staging (according to the AJCC TNM system, version 8), tumour regression score (TRS), and the total number of patients given immunotherapy for colorectal cancer within that centre.[10] One case was selected to provide greater detail, including imaging and histology.

Common features were explored for potential risk factors. The incidence of bowel obstruction associated with immunotherapy response was estimated by dividing the number of cases by the total number of patients given immunotherapy for colorectal cancer within these centres.

Written consent was obtained from all patients or next of kin. Local information governance policies were followed at each centre.

## 5.4 Results

### 5.4.1 Detailed Case

A 76-year-old woman presented with 8 months of abdominal pain, fatigue, and weight loss. After being found to have iron-deficiency anaemia, a computed tomography (CT) scan, colonoscopy, histological assessment of biopsies, and molecular testing diagnosed a dMMR, *BRAF V600E*-mutant, moderately differentiated hepatic flexure adenocarcinoma (Figure 5.1). The radiological staging was reported as T4b N1 M0 with predicted invasion of the peritoneum and duodenum. A specialist colorectal multidisciplinary team (MDT) confirmed that the tumour was unresectable and recommended attempted downstaging with immunotherapy. The patient tolerated 3 months of pembrolizumab well with toxicity limited to grade 1 immune-related dermatitis. A CT scan at this point showed a partial response within the primary tumour and no new disease (Figure 5.1). The treating team and patient agreed to complete a further 3 months of pembrolizumab before reassessing resectability with the MDT.

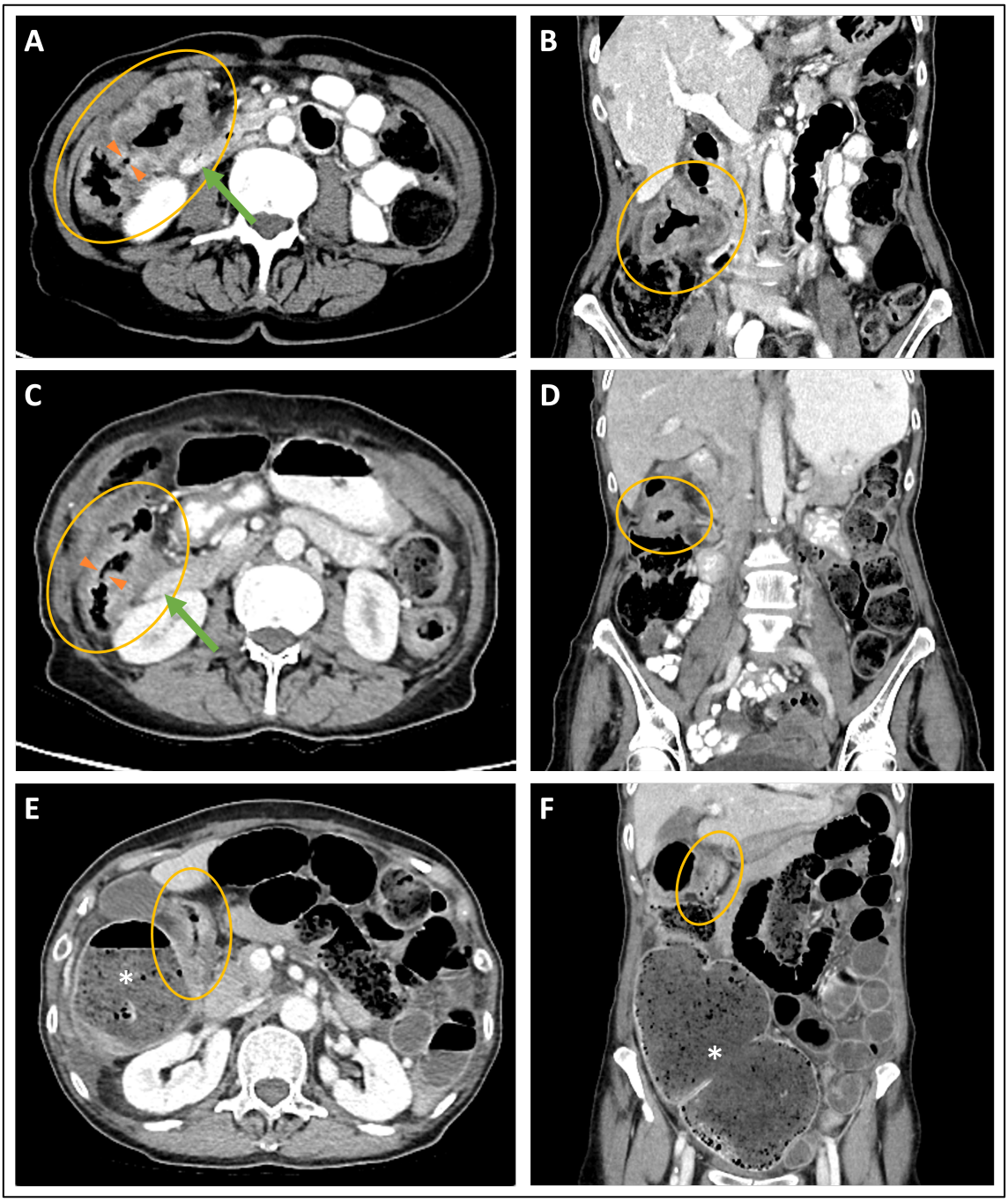



**Figure 5.1 Evolution of the Primary Tumour on Computed Tomography**
(A and B) Baseline images showing a cT4b tumour at the hepatic flexure with suspected involvement of the duodenum. Luminal narrowing is observed in the centre of the tumour. (C and D) Images following 3 months of pembrolizumab showing a partial response within the primary tumour and persistent luminal narrowing. (E and F) Images after a further 17 days showing an accelerated radiological response, with stricturing causing acute large bowel obstruction. Green arrows indicate the duodenum; orange triangles indicate area of narrowed colonic lumen; yellow rings indicate the primary tumour. The asterisk (∗) represents the dilated right colon.

Seventeen days after the most recent CT scan, the patient presented acutely with severe abdominal pain and vomiting. She also described a nonspecific change in bowel habit. On examination, she was haemodynamically stable but had obvious abdominal distension, with diffuse tenderness on palpation and absent bowel sounds. An urgent CT scan showed a significant reduction in the size of the hepatic flexure tumour, causing luminal stricturing and obstruction with gross distension of the ascending colon, caecum, and terminal ileum (Figure 5.1).

Following a sudden deterioration, the patient underwent an emergency laparotomy and right hemicolectomy. As a result of persistent intraoperative haemodynamic instability, damage control surgery with a laparostomy and delayed primary closure was carried out with the patient taken to intensive care for immediate organ support. She returned to theatre the following day for the formation of an end ileostomy and closure of the abdomen.

Macroscopic histopathological assessment of the surgical specimen confirmed the presence of a fibrotic stricture with a lumen diameter of 8 mm, surrounded by areas of necrosis and mucin. Microscopically, islands of residual adenocarcinoma were present and associated with an intense lymphoplasmacytic inflammatory reaction and large pools of acellular mucin (Figure 5.2). Residual cancer accounted for <20% of the tumour volume, indicating a good response to immunotherapy with a TRS of 2. The final pathological staging was ypT1 ypN1a ypR0.

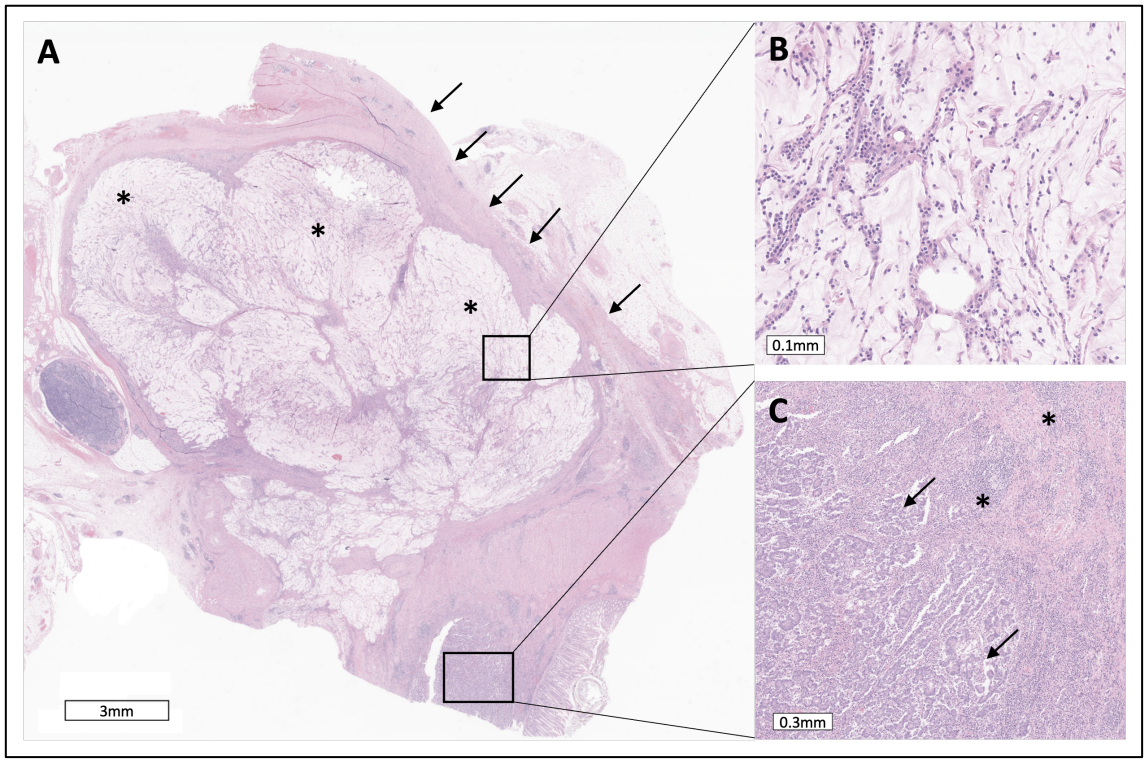



**Figure 5.2 Microscopic Features of Response to Immunotherapy**
Images were taken during the histological assessment of the right hemicolectomy specimen after immunotherapy. Residual cancer remains with evident tumour regression (tumour regression score of 2) and final staging of ypT1 ypN1a ypR0. A) Haematoxylin and eosin overview showing tumour regression with fibrosis extending beyond the bowel wall and into the subserosal fat (black arrows), large pools of mucin without viable tumour cells (black stars), an intense lymphocytic reaction, and a small region of residual moderately differentiated tumour. B) Acellular mucin pools with inflammatory cells but no viable tumour cells. C) A small area of residual moderately differentiated adenocarcinoma (black arrows) that is confined to the submucosa (ypT1) and surrounded by aggregates of lymphocytes (black asterisks).

### 5.4.2 Other Cases

A further eight cases were identified across seven European centres (Table 5.1). The primary tumour was located at the hepatic flexure in five patients, sigmoid in three patients, and descending colon in one patient. On radiological assessment, six tumours were staged as T4 and three as T3; eight were described as annular; and five were described as having radiological stricturing. Six tumours were not traversable at endoscopy.

Table 5.1 Summary of Cases

| Case (Age, Sex) | Tumour Site | Baseline TNM [a] | Annular | Rad Stricture [b] | Traversable [c] | Molecular Biomarkers | Lynch Syndrome Work-up | Histology | Rad Response | Time to Obst, days | Key outcomes | Path TNM & TRS [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1<br>66F | HF | T4b N1 M1 (liver) | Yes | No | No | MLH1, PMS2 loss<br>*BRAF* wild type<br>*KRAS A146T*<br>*NRAS* wild type | Awaiting somatic MLH1 promoter hypermethylation testing. | AC | PR | 183 | Defunctioning stoma, immunotherapy ongoing. | N/A |
| 2<br>77F | HF | T4a Nx M1 (peritoneum, lymph nodes) | Yes | Yes | No | MSI-h<br>*BRAF V600E*<br>*KRAS* wild type<br>*NRAS* wild type | N/A | AC | PR | 604 | Emergency surgery [d] | ypT0<br>ypN0<br>R0<br>TRS0 |
| 3<br>48F | DC | T4b N2 Mx | Yes | No | No | PMS2 loss<br>*BRAF* unknown<br>*KRAS* unknown<br>*NRAS* unknown | Awaiting germline testing. | AC | PR | 48 | First episode: managed conservatively. Second episode: defunctioning stoma, planned surgery. | ypT3<br>ypN0<br>R0<br>TRS1 |
| 4<br>45M | S | T3 Nx M1 (liver) | Yes | No | Yes | PMS2 loss<br>*BRAF* wild type<br>*KRAS G12A*<br>*NRAS* wild type | Awaiting germline testing. | M-AC | SD | 47 | Defunctioning stoma, planned surgery. | ypT0<br>ypN0<br>R0<br>TRS0 |
| 5<br>49M | S | T4b N2 M0 | Yes | Yes | No | MSH2, MSH6 loss<br>*BRAF* wild type<br>*KRAS* wild type<br>*NRAS* wild type | Unknown. | AC | CR | 53 | Defunctioning stoma, planned surgery. | ypT0<br>ypNx<br>R0<br>TRS0 |
| 6 [e]<br>76F | HF | T4b N1 M0 | Yes | Yes | Yes | MLH1, PMS2 loss<br>*BRAF V600E*<br>*KRAS* wild type<br>*NRAS* wild type | N/A | AC | PR | 81 | Emergency surgery [d], re-operation (within 30d), death (within 30d). | ypT1<br>ypN1<br>R0<br>TRS2 |
| 7<br>64F | HF | T3 N1 M1 (liver) | No | No | No | MSI-h<br>*BRAF* wild type<br>*KRAS* wild type<br>*NRAS* wild type | Unknown | AC | PR | 79 | Expedited surgery [d] | ypT0<br>ypN0<br>R0<br>TRS0 |

Table 5.1 (Continued)

| Case (Age, Sex) | Tumour Site | Baseline TNM [a] | Annular | Rad Stricture [b] | Traversable [c] | Molecular Biomarkers | Lynch Syndrome Work-up | Histology | Rad Response | Time to Obst, days | Key outcomes | Path TNM & TRS [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8<br>71F | HF | T4a N1 M0 | Annular | Yes | No | MLH1, PMS2 loss<br>*BRAF V600E*<br>*KRAS* wild type<br>*NRAS* wild type | N/A | AC | PR | 45 | Defunctioning stoma, expedited surgery [d] | ypT1<br>ypN0<br>R0<br>TRS2 |
| 9<br>24F | S | T3 N2 M1 (lymph nodes) | Annular | Yes | Yes | MLH1, PMS2 loss<br>*BRAF* wild type<br>*KRAS* wild type<br>*NRAS Q61R* | No abnormality on germline testing. | AC | CR | 652 | Managed conservatively without complication. | N/A |

AC = adenocarcinoma; CR = complete response; DC = descending colon; F = female; HF = hepatic flexure; M = male or metastasis stage; M-AC = mucinous adenocarcinoma; MSI-h = microsatellite instability high; N = lymph node stage; N/A = not applicable; Obst = obstruction; PR = partial response; Rad = radiological; S = sigmoid; SD = stable disease; T = primary tumour stage; TNM = tumour, node, metastasis; TRS = tumour regression score; yp, pathological stage following neoadjuvant treatment.

[a] According to AJCC TNM version 8.[10] Sites of metastatic disease are stated in brackets.

[b] Defined as clear luminal narrowing without upstream obstruction on baseline CT.

[c] On baseline endoscopy.

[d] Expedited surgery is defined as taking place sooner than initially planned but not within 48 hours of obstruction. Emergency surgery defined as taking place within 48 hours of obstruction.

[e] Detailed case.

All patients received pembrolizumab and developed bowel obstruction between 45 and 652 days (median 79 days; interquartile range 47-394 days) after starting treatment (Table 5.1). Seven patients underwent surgical resection of their tumours, including four who were initially defunctioned (Figure 5.3). Another patient was defunctioned and, at the time of writing, continues to receive immunotherapy. One patient was managed conservatively. Radiological response was seen in eight patients, including two radiological complete responses. Pathological response was seen in all seven who underwent resection of their tumour, including four pCRs. One patient died from bowel obstruction. At the time of writing, eight of these cases (one excluded due to unknown treatment numbers in this centre) occurred out of 529 (1.51%) similarly treated patients in these centres.

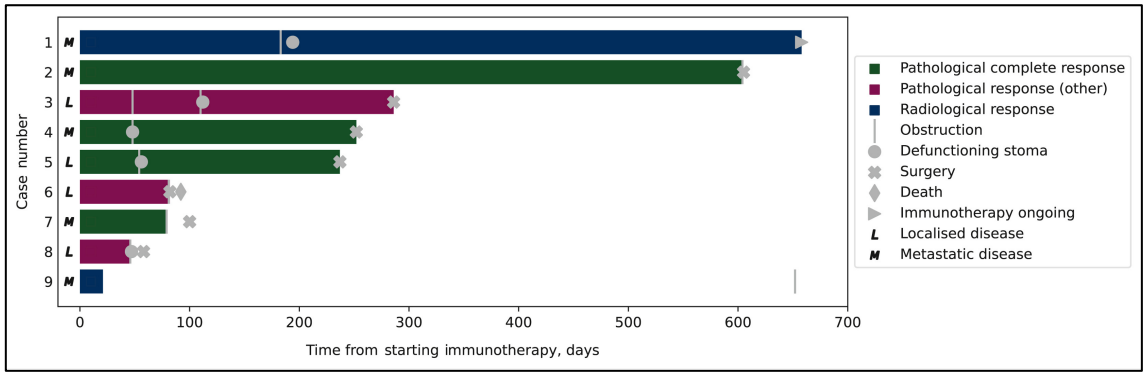



**Figure 5.3 Timing of Obstruction and Interventions**
Swimmer plot showing the timeline for developing obstruction and interventions, according to treatment response categories. Bar length represents time receiving immunotherapy. In case 3, bowel obstruction was initially managed with conservative measures before re-obstructing and requiring a defunctioning stoma. In case 9, immunotherapy was stopped after two cycles due to toxicity but bowel obstruction did not occur until much later.

## 5.5 Discussion

Immune checkpoint inhibitors are used routinely for dMMR metastatic CC and are emerging as a transformative treatment in the neoadjuvant setting for localised disease.[3, 5, 6] As new indications for immunotherapy in CC are confirmed, it is critical to recognise all treatment-related complications to support decision making and safe patient stratification. In this series, we have reported nine cases of bowel obstruction associated with immunotherapy response, an important safety signal not previously described. Identifying risk factors for obstruction must become a priority for the clinical community.

Bowel obstruction is a persistent risk for patients with metastatic CC. During periods of disease progression, patients may require acute intervention with surgical resection and/or defunctioning with a stoma or a colonic stent.[11] For rectal cancer, slowly evolving symptoms may be treated with radiotherapy.[12] With the development of neoadjuvant therapies, the risk of bowel obstruction also applies to localised CC.

In this case series, we have identified several themes: first, the primary tumour was located at the hepatic flexure in five out of nine cases, which is disproportionate to the wider dMMR CC population; second, radiological stricturing, endoscopic obstruction, and annular tumour shape were reported in most cases; and third, obstruction appeared to occur more quickly in those with localised, compared with metastatic, disease (Figure 5.3).[13] In FOxTROT, obstruction was most common in tumours of the hepatic and splenic flexures and transverse colon.[4, 9] As dMMR CCs predominantly arise in the right colon, hepatic flexure tumours may represent a specific group at high risk of obstruction associated with immunotherapy response.[13] Endoscopic obstruction and radiological or endoscopic stricturing were identified as independent risk factors for bowel obstruction in FOxTROT. As these features were also common in this series, they may represent risk factors applicable to either NAC or immunotherapy.

In contrast to the FOxTROT data, for each patient in this series, bowel obstruction was associated with a response to treatment, including four with a pCR.[9] dMMR and proficient MMR (pMMR) CCs are increasingly recognised as distinct entities, which may include patterns of response and associated complications.[14] While immunotherapy response in CC has not been well characterised, a recent report introduced the concept of an ‘inside-out’ pattern, where tumour regression transitions from serosa to mucosa with residual tumour most likely to remain on the luminal surface.[15] There is also evidence for differing radiological appearances between dMMR and pMMR tumours.[16] These distinctions may underpin the emergence of new treatment-related complications. Characterising tumour appearance and treatment response, according to MMR status, is therefore an important avenue of research.

Lynch syndrome accounts for ∼20% of dMMR CCs.[14] In this case series, six patients had initial molecular testing which raised the possibility of Lynch syndrome and required further germline testing. At the time of writing, only one patient had undergone germline testing, which showed no evidence of germline variants in the mismatch repair genes. For the other five, the underlying aetiology remains unclear. There is some evidence for better treatment response and prognosis in Lynch syndrome-associated, compared with sporadic, dMMR CC,

although this is not conclusive.[17] Given the potentially disproportionate representation of Lynch syndrome-associated tumours in this case series, it is possible that stricturing responses are more likely in this patient group.

It is challenging to illustrate the incidence of a rare complication in a relatively novel treatment area. Nevertheless, as an approximation, 8 of 529 (1.51%) patients given immunotherapy for colorectal cancer in these centres developed bowel obstruction associated with immunotherapy response. These numbers should be interpreted with significant caution; only centres where a case was identified were included, which may overestimate incidence; yet, many patients with metastatic disease will no longer have a primary tumour in situ, resulting in an underestimate. However, these data suggest that obstruction may be more common, and occur earlier, in patients with localised, compared with metastatic, disease and may explain the lack of cases seen in large metastatic trials (Figure 5.3).[18] In a phase II trial of neoadjuvant toripalimab for dMMR localised colorectal cancer, one patient developed bowel obstruction but this was considered unrelated to study treatment.[19]

While ongoing trials of neoadjuvant immunotherapy may provide additional insight into obstruction risk, this is unlikely to be definitive. Therefore, the pooling of clinical experience and existing cases is important to support the identification of patients at risk. For example, a retrospective review of the detailed case in this series identified luminal narrowing on CT, which may represent a risk factor for obstruction (Figure 5.1). Multidisciplinary input is likely to be essential for risk prediction on an individual level. Translational molecular research may also uncover patterns of immunological response specifically associated with obstruction risk. Furthermore, we must consider changes to established practice, which may include more frequent imaging, bowel motility assessments, or greater use of upfront surgical defunctioning and colonic stenting.

We propose that clinicians recognise the potential risk factors described in this case series, including hepatic flexure location, radiological stricturing, endoscopic obstruction, and annular tumour shape, and actively disseminate this information within local MDTs. While these data do not provide robust criteria for risk assessment on an individual level, routine radiological evaluation will include several of the aforementioned risk factors and may also demonstrate subtle signs of evolving obstruction, such as upstream colonic wall thickening. It is critical for patients and acute services to be aware of this phenomenon, necessitating clear safety-netting advice, informative documentation (e.g. alert cards) to indicate when a primary tumour is in situ and the provision of effective educational resources. Furthermore, until we better

understand this treatment-related complication, it may be reasonable to adopt a lower threshold for upfront defunctioning or more frequent imaging during treatment.

There are several limitations to this case series: the lack of a systematic approach to case identification may have resulted in an under-representation of the true affected population; bowel obstruction is poorly defined and may have impacted case identification; original local data was used without central review of imaging or pathology; and limited outcome data was available to illustrate the impact of bowel obstruction.[20] Nevertheless, the value of highlighting an important safety signal in this rapidly evolving treatment area should not be understated.

Bowel obstruction in CC secondary to a stricturing immunotherapy response, rather than progressive disease, is a novel phenomenon which has not been previously described. This case series provides a clear signal for a potential safety issue, which requires further evaluation to identify specific high-risk factors. Immunotherapy continues to transform the treatment of dMMR CC, but it is critical that progress is made while ensuring patient safety remains paramount.

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# 6 Discussion

## 6.1 Summary of Aims

Radiology currently plays a central but limited role in CC, primarily focusing on the baseline exclusion of distant metastatic disease, surgical planning and long-term surveillance. While neoadjuvant therapies have the potential to transform outcomes in localised CC, the roles of imaging must evolve to ensure the right patient receives the right treatment for the best chance of cure.

The aim of this thesis was to explore and refine the position of CT imaging within this evolving treatment landscape. Data from the landmark FOxTROT trial were used as the central resource to evaluate the performance of radiological staging when stratifying patients to NAC (Chapter 2), uncover potential imaging biomarkers to optimise the identification of high-risk patients (Chapter 3) and explore approaches to treatment response assessment (Chapter 4). In addition, a case series was developed in response to an emerging safety issue with major implications for patients treated with NAI, comprising a world-first report of bowel obstruction due to stricturing immunotherapy responses (Chapter 5).

As neoadjuvant therapies continue to evolve, this thesis will have strong value in a rapidly evolving area of research to personalise treatment and improve outcomes for patients with localised CC.

## 6.2 CT Staging Performance in an International Trial of Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer (Chapter 2)

### 6.2.1 Summary

Selecting patients with locally advanced CC for NAC using imaging represents a major shift in practice and, unlike using histopathological criteria in the adjuvant setting, relies on a suboptimal evidence-base. The concordance between radiological and pathological TNM staging has been extensively evaluated but with limitations.[1] No studies have explored this relationship within the context of identifying patients for neoadjuvant systemic therapies and few have considered clinically important subpopulations. This study was undertaken to address these issues and provide critical quality-control data for the FOxTROT trial.[2] As similar data have not been published for the other phase III trials in this setting, this analysis

stands alone in the literature to act as a guide for future neoadjuvant trial design and clinical implementation.[3-5]

In this analysis of prospectively collected staging data from 354 participants, CT performed strongly for the identification of pT3-4 tumours as a combined group. Limited performance was seen for alternative T staging evaluations (e.g. cT3 vs. cT4), N status and EMVI status, in keeping with wider literature.[1] Exploratory subgroup analyses highlighted that MMR status and primary tumour location may impact staging performance. In addition to the main findings from FOxTROT, which remains the only positive trial of NAC in CC to date, these data indicate that cT3-4 staging is the best available biomarker to select patients for NAC. The roles of lymph node and EMVI assessment in patient selection remain unclear and should be considered with caution given current evidence.

### 6.2.2 Limitations

Utilising a phase III RCT dataset has several advantages but, in this case, there are limitations which must be considered. As a consistent issue across clinical trials, the participant population is not fully representative of the real-world CC population, which may impact the generalisability of findings. In the control arm of FOxTROT, 63.6% of participants were male, compared to 56% of all CC diagnoses in the UK.[2, 6] Likewise, the mean age of participants included in this analysis was 63.2, whereas most new diagnoses in the UK are made between the ages of 70 and 74.[6] Overall health status is arguably more important than age in isolation, with around 50% and 10% of 70 year olds suffering from multimorbidity and frailty, respectively.[7, 8] In FOxTROT, 77.4% of participants had an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0 which, although a limited evaluation of health status, suggests a likely fitter and hyper-selected cohort than the real-world population.[2] As patient factors, such as body habitus and comorbidities, are known to impact image quality, and tumours are heterogeneous across different patient groups, it is conceivable that such shifts in population characteristics may affect the generalisability of these findings to the wider population.[9, 10] These data encompassing a population with exclusively advanced tumours must also be recognised when considering the findings within the context of wider literature and clinical practice. Furthermore, the requirement for >1 mm extramural tumour extension on CT may have resulted in the over-representation of tumours with grossly invasive disease, potentially enhancing performance metrics and shifting the population to which these results should be generalised.

Relative to other literature, which includes several meta-analyses and population-level studies, the sample of 354 participants used in this analysis is small with limited statistical power.[1, 11-17] In addition, although participants were recruited from 85 centres internationally, the infrastructure, specialist experience and clinical cultures of the included centres may not be representative on a global scale. A site's participation in the trial is likely to be a clear indicator of being well positioned to deliver neoadjuvant treatment pathways and potentially support this shift in clinical practice. These factors may not apply to centres and/or countries not included in the trial. A further limitation is the historic timeframe of the included staging data, where 2008 to 2016 may not be sufficiently contemporary to capture more recent improvements in scanning techniques and technologies.

The original trial used TNM version 5 for both radiological and pathological staging.[18] While this was appropriate at that time, all subsequent analyses are limited by this outdated system. Several differences exist between TNM version 5 and the current version 9.[19] For this analysis, the most important change is that tumour deposits ≤3 mm were previously assigned T3, compared to the current N1c, staging. Although this change may influence staging outcome data, tumour deposits are uncommon and therefore unlikely to have a major impact on the study findings. Other changes to the TNM system between these versions, which are less relevant to this analysis, include the subcategorisation of the N and M categories, combined staging subgroups and the addition of carcinoma in situ (Tis).

Beyond using an outdated version in the original trial, the wider limitations of the TNM system must be considered. While pathological TNM staging represents the gold-standard for post-operative evaluation, it is an imperfect system with limited prognostic and predictive ability.[20] It also excludes several established prognostic factors, some of which are now routinely evaluated, but not used, in making treatment decisions.[21] As such, each staging group represents a heterogeneous patient population not accounted for within the TNM system.[22] Finally, the use of TNM in practice primarily focuses on the selection of patients for doublet AC. However, in the modern era of precision oncology in CC, as treatment algorithms become more complex and nuanced, it remains unclear how effective TNM (radiological or pathological) will be for patient stratification.

The depth of extramural tumour extension is not formally included in radiological TNM staging but is recommended by ESGAR (≤5 mm or >5 mm) and sometimes reported.[23] As both oncology and radiology studies have previously applied this threshold, an exploratory analysis was conducted which showed a substantial reduction in PPV from 94.5% for T3-4 to 68.7% for

T3cd-4 (i.e. >5 mm extension).[3, 4, 24, 25] While these data highlight the challenge of identifying this higher-risk population, it potentially over-simplifies the relationship between radiological and pathological tumour extension and how this characteristic should be assessed and utilised. Instead, the results could be strengthened by a formal correlation analysis between the two variables and the testing of additional thresholds to identify areas of diagnostic strength and weakness. The analysis of T stage accuracy was further limited by a lack of other metrics due to the exclusion of participants with cT1-2 tumours from the original trial. As such, fewer conclusions can be drawn for T stage, compared to other parameters within the study.

Although at trial outset there were plans to collect baseline CT scans for further evaluation and central review, only a small proportion were acquired. The use of local staging data from many sites and radiologists has its merits and likely better reflects real-world practice and performance for routine implementation. However, the lack of central review prevented a key component of quality-control that is often expected of a large, practice-changing, phase III trial. Subsequent analyses were therefore limited to select data collected during the trial without the flexibility of undertaking exploratory research using original scans, which may have included additional subgroups or scanning parameters (e.g. impact of standard CT vs. CTC). Central imaging review would also have addressed the issue set out in the manuscript regarding discrepancies between initial MDT and final Radiology Lead evaluations.

### 6.2.3 Future Work

This analysis provides critical insights into the ability of CT to select patients for neoadjuvant therapies and transparent quality-control data for a landmark trial that has established a new treatment option for locally advanced CC. As patient selection is primarily based on radiological staging and clinicians have concerns regarding its clinical utility and risk of over-staging, it is surprising that other trials in this setting have not published similar analyses or raw data.[3, 4] To support the adoption of neoadjuvant therapies in routine practice, such analyses must be published to allow for rigorous appraisal in the context of patient stratification. These data would add clear value to the evidence-base and allow greater personalisation of treatment across relevant subgroups. As NAC can now be used as a standard treatment option, the collation of real-world data is crucial to illustrate how treatment pathways can be implemented and the accuracy of radiological staging in routine practice, as opposed to academic centres with specialist trial support.

While the value of this study is enhanced by the sparsity of other data available, several limitations of the original trial and subsequent analyses could be addressed in future research. First, efforts should be made to ensure trial populations are as reflective of the real-world population as possible. Previous trials in gastrointestinal cancers have demonstrated the feasibility of delivering dose-adapted chemotherapy in older adults or those with frailty, without compromising benefit.[26, 27] The ongoing FOxTROT 2 and FOxTROT 5 trials are testing this approach with neoadjuvant therapies in locally advanced CC to provide high-quality data in this setting.[28, 29] Future neoadjuvant trials should publish imaging analyses concurrently or soon after the main trial findings to ensure data reflect contemporary radiological practice and staging systems. Appointing trained Lead Radiologists alongside Principal Investigators at each site provides a layer of research quality and standardisation. Yet, this approach, where Lead reviews may follow an initial MDT evaluation, risks conflicting assessments with implications for trial participation and subsequent analyses. To avoid this issue, future trials could protocolise mandated local or central Lead Radiologist review for trial randomisation, an approach seen across many other trials. Unlike in FOxTROT, a robust approach to central scan collection should be prioritised to provide a validated and quality-controlled dataset to support main trial findings and subsequent exploratory research.

While the concordance between radiological and pathological staging has been well studied, the current analysis provides much-needed data on the impact of key patient subgroups.[1, 11-17] Future research should continue to explore the relationship between radiology and pathology across other clinically and molecularly defined populations, including meta-analyses for important cohorts, such as dMMR CC. Ongoing trials are testing neoadjuvant anti-BRAF therapies in *BRAF* mutant CC, in which primary tumours are known to have distinct radiological appearances.[30, 31] Yet, the impact of this radiological phenotype on staging concordance, a critical factor for neoadjuvant treatment delivery, is not known. Further subgroups of interest include: primary tumour location according to tumour side, intraperitoneal versus retroperitoneal segments, and individual segments (e.g. colonic flexures); sex; body habitus; tumours with a mucinous component; and HER2-positive tumours. Generating large datasets to evaluate staging in these groups would then support clear hypothesis-testing research to definitively understand the heterogeneity of radiological staging and areas of strength and weakness. Focused multireader studies should also be undertaken to identify areas of inconsistency for targeted educational interventions or avoidance in patient selection processes.

As most research into radiological and pathological concordance has focused on TNM, a system plagued by limitations, the development of new tools to support patient stratification is a research priority. For any novel features which have a pathological basis, the ability of imaging to accurately identify them must be determined. Tumour deposits illustrate this concept well; pathologically confirmed TDs are routinely identified and known to have adverse prognostic value.[32] Likewise, there is now some evidence that radiologically identified TDs also carry adverse prognostic value.[25] However, if TDs are to be used in multicentre clinical trials or routine clinical practice, there is a need to understand how accurately imaging can identify them and how this varies between radiologists.

## 6.3 Baseline Clinicopathological and Imaging Predictors of Disease Recurrence in Locally Advanced Colon Cancer: a Post Hoc Analysis of the FOxTROT Trial (Chapter 3)

### 6.3.1 Summary

Effective patient selection for neoadjuvant treatment requires an evaluation of individual risk using baseline information without insights from post-operative pathology. As surgical resection is the cornerstone of curative treatment in localised CC, and AC has proven efficacy for curing a proportion of high-risk patients, neoadjuvant therapies must target the remaining population who recur despite these established approaches. The diagnostic workup for localised CC provides a wealth of information that is under-utilised due to insufficient evidence to guide its use in patient stratification. The FOxTROT trial established that cT3-4 N0-2 M0 staging predicts NAC benefit but also highlighted that most patients are still cured from the standard upfront surgery approach.[2] As such, there are understandable concerns from clinicians that NAC may represent over-treatment, including some patients who could be spared chemotherapy entirely based on definitive pathological staging.[33] More effective tools to identify the highest-risk patients and those most likely to benefit from neoadjuvant therapies are therefore needed. To explore how patient selection for NAC could be refined, this study analysed the associations between baseline clinicopathological and radiological features and long-term survival outcomes.

Consistent with wider literature in localised CC, DFS was chosen as the primary endpoint for this analysis, with TTR and OS secondary endpoints.[34] Male sex, performance status 1-2 and poor tumour differentiation were the non-imaging variables associated with a significantly worse DFS. T4 staging, extramural tumour extension >7 mm, maximum tumour thickness >22

mm, the presence of a lymph node ≥10 mm and EMVI were the imaging variables associated with significantly worse DFS. Similar results were obtained for TTR and OS, reinforcing the value of DFS as a reliable surrogate endpoint.[34] A subgroup analysis by MMR status highlighted prognostic factors within each cohort which may inform future clinical trial design. To explore the impact of having more than one high-risk feature, a simple cumulative risk score was developed, which defined significantly different prognostic groups. Together, these findings show that baseline data in patients with CC are currently under-utilised and that it is possible to risk-stratify within a locally advanced population.

### 6.3.2 Limitations

The variables included in this analysis were limited to those chosen at trial outset due to incomplete central scan collection. Central validation of the radiological measures could therefore not be performed, nor additional imaging features with potential prognostic value identified. These findings are also limited to patients with cT3-4 N0-2 M0 staging (i.e. the FOxTROT population) and therefore may not be applicable to other cohorts. However, given the lack of evidence in this space, this multicentre and multireader analysis provides significant value to refine patient selection for neoadjuvant therapies in CC.

A pre-planned decision was made to focus on presenting univariable, instead of multivariable, results to provide clear data on prognostic signals, which may be otherwise obscured by collinearity between imaging variables. However, the lack of statistical adjustment limits the identification of independent high-risk features, which could be tested prospectively in clinical trials, and does not account for confounders. Due to the exploratory nature of the analysis, statistical multiplicity was also not accounted for, which should be considered when evaluating these results.

The subgroup analysis by MMR status was conducted to provide clinically relevant data that could be applied to clinical trial design in each population. However, this approach sacrificed statistical power, resulting in fewer significant associations for the smaller dMMR cohort, and further increases the risk of false-positive results due to multiplicity. These limitations highlight the need for larger datasets across distinct molecularly defined populations.

The simple risk score was included as a final step to explore the impact of having more than one risk factor, having hypothesised that this would be associated with poorer outcomes. As this part of the analysis was undertaken for illustrative purposes, rather than to develop a

robust model for clinical application, a simple scoring system based on HRs was used. However, this concept could be strengthened in future research by taking a focused and pre-planned approach to the development of a robust predictive model.[35]

### 6.3.3 Future Work

This analysis provides a strong foundation to improve patient selection for neoadjuvant therapies in localised CC, addressing some of the limitations of wider literature. To build on this work, future research must consider advances in tumour biology and how this underpins the shift towards personalised treatment. It is now clear that several distinct subpopulations of CC exist rather than it being a single disease.[36] Therefore, the identification and validation of novel prognostic biomarkers should be undertaken within these contexts. Ongoing neoadjuvant trials present excellent opportunities for this translational research, aligned to specific tumour biology and treatment type.

Baseline radiological evaluations provide critical but limited information to guide patient management. As the treatment landscape shifts to the neoadjuvant setting, imaging will be increasingly relied upon to provide information beyond what is currently offered. Novel features related to both the primary tumour and elsewhere should be explored to identify those with prognostic and predictive value. Whole-body radiological profiling is gaining traction with several potential applications but remains confined to the academic setting.[37, 38] Likewise, approaches to quantitative radiology, including radiomics, have not matured sufficiently to impact clinical practice.[39] As these alternative sources of information may provide clinically useful insights, translational research pipelines linked to neoadjuvant clinical trials should be leveraged to develop these techniques at pace. Furthermore, as radiology is unlikely to achieve optimal patient evaluation when used alone, multimodal models should be developed to utilise all available information, including emerging domains, such as circulating tumour DNA and digital pathology.[40, 41]

Due to global availability, acceptable performance for tumour localisation and traditional staging practices, and a lack of superior alternatives, CT remains the gold-standard imaging modality for CC.[42] However, the emergence and continued development of neoadjuvant therapies requires fresh insights from imaging and it is conceivable that alternative modalities may be better placed to meet these demands. Although MRI and FDG PET-CT have been explored for baseline staging, with similar performance to standard CT, it is possible that they

are more effective in evaluating novel prognostic features to improve patient stratification.[42] It is crucial that future research looks beyond CT, despite its position as the global standard.

## 6.4 Characterising Radiological Response to Neoadjuvant Chemotherapy for Locally Advanced Colon Cancer in the International FOxTROT Trial

### 6.4.1 Summary

Assessing response to neoadjuvant therapies on post-operative pathology has strong prognostic value across tumour types.[2, 43-45] In FOxTROT, pathological tumour regression grade, according to the Dworak system, correlated strongly with recurrence risk and has therefore been adopted as a surrogate endpoint in ongoing trials.[28] Imaging represents an alternative approach to response assessment with several potential applications, including further prognostication, demonstrating treatment sensitivity to guide adjuvant treatment decisions and in an adaptive neoadjuvant pathway with a stop-go decision point. However, the evidence-base for radiological response is under-developed with minimal translation to routine clinical practice.

In a subset of participants randomised to NAC in the FOxTROT trial, significant and measurable radiological changes were seen on CT across several different metrics. Significant reductions in extramural tumour extension, maximum tumour thickness (both anywhere and at an invasive margin), tumour length (both along the colon and any diameter), tumour volume and mean tumour attenuation following six weeks of NAC were demonstrated by two independent reviewers. Correlation between changes in individual variables was mixed but was strong for individual measures between reviewers, suggesting teachability and reliability in wider practice. In keeping with emerging data, these results also illustrated the lack of correlation between radiological and pathological response, including substantial changes on imaging in some patients without evidence of pathological regression.[46, 47]

### 6.4.2 Limitations

As the FOxTROT trial did not mandate post-NAC CT scans and undertook limited central scan collection, only a small sample of participants was available for the current analysis, limiting statistical power and the insights gained. Although significant differences were seen between pre- and post-NAC CT metrics, the precision of these data may be limited. Furthermore, when

evaluated by pathological regression status, several numerical differences were detected, which may have reached statistical significance with a more representative sample.

The relationship between radiological and pathological response was explored given the importance of this outstanding research question. While the results were consistent with other literature, the methodological and practical limitations of each system in isolation must be considered, as described in the manuscript. Survival data were not included as outcome prediction was neither the aim of this technical study, nor were there sufficient events to generate reliable conclusions. Considering the momentum behind neoadjuvant therapies and the drive for clinical application, not being able to provide strong data on pathological regression or survival outcomes represents a clear limitation.

Undertaking this research between two reviewers was planned to provide greatest transparency, identify metrics prone to variation and subjectivity, and deliver a degree of quality-control. However, compared to dedicated multireader studies, using just two reviewers, with vastly different experience levels, could be considered a limitation. This issue is compounded by the assessments of tumour volume not being fully independent.

The study aimed to evaluate changes in the primary tumour due to the known challenges presented by other features, such as lymph node assessment. However, assessing dynamic change is not the same as baseline staging and the omission of these other features may have excluded important information. The use of standard views, rather than implementing custom MPR to align with tumour orientation, may have provided suboptimal images with limitations for individual assessments. A pre-planned decision was also made for both reviewers to be blinded to the paired scan and clinical information to provide objective assessments of each variable. However, this is unlikely to reflect real-world practice and also risks corresponding measurements being taken from different tumour regions.

### 6.4.3 Future Work

To address the main limitation of this analysis, future research into treatment response must utilise a greater sample size with sufficient statistical power. The findings from the current analysis should be validated to provide definitive data on the true relationships with pathological response and long-term survival outcomes. It was notable from this study and the limited existing data that discordant radiological and pathological responses are increasingly recognised; understanding the clinical relevance of this pattern needs to be better understood.

As primary colonic tumours often have complex morphology, single unidirectional measures are unlikely to be fully representative of tumours at a single timepoint or as an evaluation of response. In the study by Je et al., a hierarchical approach was devised comprising several different measures.[47] While there were methodological limitations to this study, this type of approach should be further explored using rich neoadjuvant trial datasets. For assessments of tumour volume, manual segmentation represents a huge resource burden, highlighting the need to develop semi or fully automated tools for this purpose.

The response patterns described in the current study should be considered specific to six weeks of doublet NAC. It is likely that alternative treatment durations and NAC regimes impact response patterns, which are therefore unlikely to be universal across wider NAC use. This concept is particularly important for tumours with distinct tumour biology where alternative neoadjuvant therapies are being explored. The use of NAI in dMMR CC is a great example, where different patterns of response have already been observed.[48-50]

Novel approaches to imaging in this setting must also be explored. In the small study by Arredondo et al., where radiological responses to NAC on standard CT were described, 13 patients also underwent paired FDG PET-CT scans, with significant reductions in SUVmax following NAC.[51] Delta radiomics is also being investigated in other disease settings and may have utility in CC response assessment.[52] Future research into response assessment is likely to be multimodal, including the integration of endoscopic evaluation and ctDNA dynamics. For any new system, multireader studies should be undertaken to demonstrate reliability in wider practice.

## 6.5 Risk of Bowel Obstruction in Patients with Colon Cancer Responding to Immunotherapy: an International Case Series (Chapter 5)

### 6.5.1 Summary

Immunotherapy is the first-line standard of care for metastatic or unresectable dMMR CC and is emerging as a potentially transformative option in the neoadjuvant setting for localised disease.[36, 53] Recently, distinct patterns of response to immunotherapy have been recognised, including pseudo-progression, hyper-progression and pseudo-residual disease, each with implications for patient care.[48-50] In this case series, a new type of immunotherapy response is described for the first time, with nine patients experiencing luminal stricturing and bowel obstruction.

While it is not possible to identify statistical associations in a case series of this nature, several themes emerged which could influence patient care and provide a foundation for future research. Of the nine patients, most had a primary tumour that was located at the hepatic flexure, annular in shape, staged cT4 and non-traversable on baseline endoscopy. Surgical resection was performed in seven of the patients, all with evidence of pathological regression (including four pCRs), indicating high levels of treatment sensitivity. The case series describes, for the first time, that patients responding well to immunotherapy may be at risk of bowel obstruction if they have a primary tumour in situ. Hence, there are major implications for those treated in the neoadjuvant setting with localised disease. The publication of this case series serves as an effective platform to rapidly communicate an adverse safety signal to inform clinical practice and future research. Subsequent citations and further reports of such responses highlight the impact and clinical relevance of this case series in an evolving treatment context.[54, 55]

### 6.5.2 Limitations

As a study design at the lower end of the hierarchy of evidence, case series have several inherent limitations which apply to this published work. The retrospective approach to case identification, using existing professional networks, was not systematic and therefore is unlikely to have provided a representative sample. While several common themes emerged across the cases, these parameters were chosen in advance by investigators based on clinical judgement, which may introduce selection or confirmation bias. Estimates of incidence were provided based on the number of similarly treated patients in each centre; however, due to the case series design and resulting lack of a true denominator, these estimates are likely to be unreliable. Furthermore, the lack of a standardised definition for bowel obstruction and the likelihood of there being many cases of stricturing without obstruction will also have affected these estimates.

Due to the routine and international nature of the included cases, it was not possible to centrally review scans or pathological specimens. Therefore, local reporting through a standardised template was relied upon with no option to quality-control the submitted data and detect inconsistencies between centres. Outcome data were also limited, including long-term survival and the need for subsequent interventions.

### 6.5.3 Future Work

This case series further indicates the need to improve understanding of response to neoadjuvant therapies in CC. Ongoing trials of NAI should prioritise the collection of relevant data, including scans, to evaluate how primary tumours respond to specific NAI regimens. These studies may provide additional insights into the incidence and evolution of stricturing response. In the immediate term, collation of affected cases may demonstrate further trends to allow more focused and definitive research into this novel phenomenon. Subsequently, a case-control study would be the ideal design to explore these responses further and progress towards identifying true risk factors, which could then be applied in routine patient care. Understanding who is at greatest risk of these responses would allow clinical teams to re-consider or modify treatment plans, such as with more frequent monitoring or upfront surgical defunctioning.

## 6.6 Future Perspectives and Considerations

Neoadjuvant therapies have the potential to transform outcomes for patients with localised CC, but progress is dependent on engagement and support from across the MDT. Radiological research must be prioritised as a critical component of neoadjuvant treatment delivery within clinical trials networks and growing routine practice.

There are several outstanding questions surrounding neoadjuvant therapies in CC, including the optimal regimen (including treatment type and duration), target populations, timing of surgery and the need for adjuvant treatment. Furthermore, there are concerns regarding over-treatment, compromising patient fitness prior to surgery, inconsistencies in current clinical evidence and the challenges of neoadjuvant pathway implementation, particularly where upfront molecular testing is required. Fortunately, the successes of previous neoadjuvant trials have generated strong momentum in this space, with numerous academic groups undertaking clinical trials and translational research to address these key outstanding questions.[30]

One of the key lessons from FOxTROT was that radiology should not merely be seen as a service with which to select participants, but as an opportunity to collect rich translational data for exploratory research. Quality-assured digital infrastructure is critical for neoadjuvant trials to facilitate scan collection and the curation of high-quality datasets. Radiology research outputs, underpinned by a wider educational strategy, must also be developed with global reach in mind to provide reproducible, generalisable and valid data with impact beyond specialist academic centres.

The chapters in this thesis provide much-needed evidence for how radiology can shape neoadjuvant therapies in CC, using data from the landmark FOxTROT trial. However, due to the limitations of this research, some of the results should be regarded as foundational, rather than definitive, to provide a strong platform on which future imaging research can be developed. Future radiological research will be critical to improving outcomes for patients with CC but must not be confined to single discipline silos. For patient stratification and response assessment, it is clear from the data in this thesis that radiology continues to be under-utilised; further work to refine its role and combine with other data modalities is a priority. Emerging evidence suggests that multimodal approaches to outcome prediction using baseline data are feasible and achieve better performance than single modalities used alone.[56, 57] With advancements across disciplines including quantitative imaging, digital pathology and ctDNA, plus methodological innovations in fields such as AI, future research is likely to focus on multimodal approaches to addressing key questions around patient stratification, response assessment and surveillance.

The concepts of precision oncology and personalised medicine are increasingly relevant to the treatment of CC due to the availability and utility of molecular biomarkers. While these biomarkers have direct implications for treatment efficacy and patient outcomes, the impacts on imaging are poorly understood. As neoadjuvant treatments become increasingly tailored to individual tumour biology, there is a need to develop robust evidence for the role of radiology in these populations. Therefore, future radiological research in CC should be undertaken with specific patient cohorts in mind.[36]

Improving neoadjuvant treatments in CC requires a robust approach to high-quality, multidisciplinary research and a strong educational strategy to underpin new developments. Many of the challenges facing neoadjuvant treatments for CC relate to wider education, training and expertise. Prioritising education will ensure disciplines can advance together, adopting new techniques to support one another, and to achieve the ultimate goal: to cure more patients with CC worldwide.

## 6.7 Conclusions

This thesis has explored how CT imaging can support the delivery of neoadjuvant therapies in locally advanced CC using data from the landmark FOxTROT trial. These data show that CT is appropriately positioned as the gateway to patient selection for NAC but has untapped potential for patient stratification and response assessment. In addition, key opportunities are

identified to inform future research strategies across bespoke CC imaging research, multimodal translational studies and prospective clinical trials.

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# 7 Appendices

## 7.1 Supplementary Material for Chapter 2

Table 7.1 Differences Between MDT and Lead Radiologist Data for Whole Study Population

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Lead Radiologist Review** | | | | | | | | | |
| **MDT Radiology Review** | **Primary Tumour Site** | | | | | | | | | |
| | | Caecum | Ascending colon | Hepatic flexure | Transverse colon | Splenic flexure | Descending colon | Sigmoid | Rectosigmoid | Other/ missing | TOTAL |
| | Caecum | 168 | 9 | 0 | 0 | 0 | 1 | 2 | 0 | 2 | 182 |
| | Ascending colon | 10 | 170 | 1 | 0 | 0 | 1 | 0 | 0 | 3 | 185 |
| | Hepatic flexure | 0 | 7 | 55 | 1 | 1 | 0 | 0 | 0 | 2 | 66 |
| | Transverse colon | 0 | 2 | 7 | 71 | 0 | 1 | 0 | 0 | 0 | 81 |
| | Splenic flexure | 0 | 0 | 0 | 3 | 29 | 0 | 2 | 0 | 0 | 34 |
| | Descending colon | 0 | 0 | 0 | 0 | 1 | 52 | 1 | 0 | 1 | 55 |
| | Sigmoid | 4 | 0 | 0 | 0 | 5 | 3 | 350 | 8 | 1 | 371 |
| | Rectosigmoid | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 60 | 1 | 78 |
| | Other/ missing | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | TOTAL | 182 | 188 | 63 | 75 | 36 | 58 | 372 | 68 | 10 | 1052 |
| | **T Stage** | | | | | | | | | |
| | | T1 | T2 | T3 | T4 | Unknown | TOTAL | | | |
| | T1 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | T2 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | T3 | 0 | 2 | 743 | 35 | 4 | 784 | | | |
| | T4 | 0 | 0 | 36 | 229 | 3 | 268 | | | |
| | Unknown | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | TOTAL | 0 | 2 | 779 | 264 | 7 | 1052 | | | |
| | **Degree of Extramural Extension** | | | | | | | | | |
| | | <5 mm | ≥5 mm | Unknown | TOTAL | | | | | |
| | <5 mm | 257 | 37 | 2 | 296 | | | | | |
| | ≥5 mm | 33 | 722 | 1 | 756 | | | | | |
| | Unknown | 0 | 0 | 0 | 0 | | | | | |
| | TOTAL | 290 | 759 | 3 | 1052 | | | | | |
| | **N Stage** | | | | | | | | | |
| | | N0 | N1 | N2 | Unknown | TOTAL | | | | |
| | N0 | 210 | 26 | 5 | 2 | 243 | | | | |
| | N1 | 30 | 431 | 42 | 2 | 505 | | | | |
| | N2 | 5 | 31 | 249 | 2 | 287 | | | | |
| | Unknown | 5 | 9 | 2 | 1 | 17 | | | | |
| | TOTAL | 250 | 497 | 298 | 7 | 1052 | | | | |

MDT = multidisciplinary team; N = lymph node stage; T = primary tumour stage.

Table 7.2 Baseline Clinical and Staging Characteristics

| | NAC (n = 698) | STS (n = 354) | P-value [a] |
|---|---|---|---|
| Age, years (range; SD) | | | |
| Mean | 63.0 (27 – 82; 10) | 63.2 (31 – 83; 9) | 0.69 |
| Sex, n (%) | | | |
| Male | 447 (64.0) | 225 (63.6) | 0.88 |
| Female | 251 (36.0) | 129 (36.4) | |
| Tumour location, n (%) | | | |
| Caecum | 119 (17.0) | 63 (17.8) | 0.96 |
| Ascending colon | 124 (17.8) | 64 (18.1) | |
| Hepatic flexure | 41 (5.9) | 22 (6.2) | |
| Transverse colon | 49 (7.0) | 26 (6.2) | |
| Splenic flexure | 27 (3.9) | 9 (2.5) | |
| Descending colon | 36 (5.2) | 22 (6.2) | |
| Sigmoid | 250 (35.8) | 122 (34.5) | |
| Rectosigmoid junction | 46 (6.6) | 22 (6.2) | |
| Unknown | 6 (0.9) | 4 (1.1) | |
| Tumour side, n (%) | | | |
| Right | 333 (47.7) | 175 (49.4) | 0.57 |
| Left | 359 (51.4) | 175 (49.4) | |
| Unknown | 6 (0.9) | 4 (1.1) | |
| MMR status, n (%) [b] | | | |
| Proficient | 481 (68.9) | 248 (70.1) | 0.49 |
| Deficient | 117 (16.8) | 68 (19.2) | |
| Unknown | 100 (14.3) | 38 (10.7) | |
| Radiological T stage, n (%) [c] | | | |
| T2 | 1 (0.1) | 1 (0.3) | 0.42 |
| T3 | 523 (74.9) | 256 (72.3) | |
| T4 | 169 (24.2) | 95 (26.8) | |
| Unknown | 5 (0.7) | 2 (0.6) | |
| Radiological N stage, n (%) [c] | | | |
| N0 | 169 (24.2) | 81 (22.9) | 0.88 |
| N1 | 328 (47.0) | 169 (47.7) | |
| N2 | 196 (28.1) | 102 (28.8) | |
| Unknown | 5 (0.7) | 2 (0.6) | |
| Radiological EMVI, n (%) | | | |
| Present | 408 (58.5) | 226 (63.8) | 0.11 |
| Absent | 280 (40.1) | 125 (35.3) | |
| Unknown | 10 (1.4) | 3 (0.9) | |
| Pathological T stage, n (%) [c] | | | |
| T1 | 10 (1.4) | 1 (0.3) | 0.0004 |
| T2 | 70 (10.0) | 19 (5.4) | |
| T3 | 436 (62.5) | 224 (63.3) | |
| T4 | 142 (20.3) | 107 (30.2) | |
| Unknown | 40 (5.7) | 3 (0.9) | |
| Pathological N stage, n (%) [c] | | | |
| N0 | 406 (58.2) | 169 (47.7) | 0.0001 |
| N1 | 173 (24.8) | 87 (24.6) | |
| N2 | 104 (14.9) | 90 (25.4) | |
| Unknown | 15 (2.1) | 8 (2.3) | |
| Pathological EMVI, n (%) | | | |
| Present | 221 (31.7) | 156 (44.1) | 0.00004 |
| Absent | 456 (65.3) | 184 (52.0) | |
| Unknown | 21 (3.0) | 14 (4.0) | |

EMVI = extramural venous invasion; MMR = mismatch repair; N = lymph node stage; NAC = neoadjuvant chemotherapy; SD = standard deviation; STS = straight-to-surgery; T = primary tumour stage.

[a] Pearson Chi-squared or Fisher's exact test. Tests exclude unknowns.

[b] Tested using immunohistochemistry.

[c] According to TNM version 5.

Table 7.3 T Stage Agreement Including T3 <5 mm vs. T3 ≥5 mm

| | pT1 | pT2 | pT3 <5mm | pT3 ≥5mm | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| cT3 <5mm | 0 | 8 | 40 | 15 | 20 | 4 | 87 |
| cT3 ≥5mm | 1 | 7 | 48 | 61 | 42 | 10 | 169 |
| cT4 | 0 | 3 | 20 | 26 | 44 | 2 | 95 |
| Unknown | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| Total | 1 | 19 | 108 | 103 | 107 | 16 | 354 |

c = radiological; p = pathological; T = primary tumour stage.

Table 7.4 Agreement for pMMR Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 1 | 0 | 1 |
| cT3 | 1 | 12 | 126 | 44 | 0 | 183 |
| cT4 | 0 | 3 | 33 | 27 | 0 | 63 |
| Unknown | 0 | 1 | 0 | 0 | 0 | 1 |
| Total | 1 | 16 | 159 | 72 | 0 | 248 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 1 | 0 | 1 |
| cT3-4 | 16 | 230 | 0 | 246 |
| Unknown | 1 | 0 | 0 | 1 |
| Total | 17 | 231 | 0 | 248 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 30 | 15 | 12 | 0 | 57 |
| cN1 | 54 | 39 | 24 | 1 | 118 |
| cN2 | 28 | 12 | 31 | 0 | 71 |
| Unknown | 0 | 2 | 0 | 0 | 2 |
| Total | 112 | 68 | 67 | 1 | 248 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 83 | 68 | 3 | 154 |
| cEMVI absent | 34 | 57 | 0 | 91 |
| Unknown | 1 | 2 | 0 | 3 |
| Total | 118 | 127 | 3 | 248 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; pMMR = mismatch repair proficient; T = primary tumour stage.

Table 7.5 Agreement for dMMR Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT3 | 0 | 2 | 30 | 12 | 0 | 44 |
| cT4 | 0 | 0 | 11 | 12 | 0 | 23 |
| Unknown | 0 | 0 | 1 | 0 | 0 | 1 |
| Total | 0 | 2 | 42 | 24 | 0 | 68 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 0 | 0 | 0 |
| cT3-4 | 2 | 65 | 0 | 67 |
| Unknown | 0 | 1 | 0 | 1 |
| Total | 2 | 66 | 0 | 68 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 11 | 2 | 1 | 0 | 14 |
| cN1 | 17 | 8 | 8 | 0 | 33 |
| cN2 | 12 | 1 | 8 | 0 | 21 |
| Unknown | 0 | 0 | 0 | 0 | 0 |
| Total | 40 | 11 | 17 | 0 | 68 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 19 | 27 | 2 | 48 |
| cEMVI absent | 8 | 11 | 1 | 20 |
| Unknown | 0 | 0 | 0 | 0 |
| Total | 27 | 38 | 3 | 68 |

c = radiological; dMMR = mismatch repair deficient; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; T = primary tumour stage.

Table 7.6 Agreement for Right-Sided Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 1 | 0 | 1 |
| cT3 | 0 | 6 | 78 | 32 | 1 | 117 |
| cT4 | 0 | 0 | 31 | 25 | 1 | 57 |
| Unknown | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 6 | 109 | 58 | 2 | 175 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 1 | 0 | 1 |
| cT3-4 | 6 | 166 | 2 | 174 |
| Unknown | 0 | 0 | 0 | 0 |
| Total | 6 | 167 | 2 | 175 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 15 | 9 | 3 | 2 | 29 |
| cN1 | 41 | 25 | 19 | 2 | 87 |
| cN2 | 29 | 7 | 23 | 0 | 59 |
| Unknown | 0 | 0 | 0 | 0 | 0 |
| Total | 85 | 41 | 45 | 4 | 175 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 52 | 58 | 5 | 115 |
| cEMVI absent | 18 | 38 | 3 | 59 |
| Unknown | 0 | 1 | 0 | 1 |
| Total | 70 | 97 | 8 | 175 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; T = primary tumour stage.

Table 7.7 Agreement for Left-Sided Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT3 | 1 | 9 | 98 | 28 | 1 | 137 |
| cT4 | 0 | 3 | 16 | 19 | 0 | 38 |
| Unknown | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 1 | 12 | 114 | 47 | 1 | 175 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 0 | 0 | 0 |
| cT3-4 | 13 | 161 | 1 | 175 |
| Unknown | 0 | 0 | 0 | 0 |
| Total | 13 | 161 | 1 | 175 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 28 | 10 | 10 | 2 | 50 |
| cN1 | 40 | 26 | 15 | 0 | 81 |
| cN2 | 14 | 8 | 19 | 2 | 43 |
| Unknown | 0 | 1 | 0 | 0 | 1 |
| Total | 82 | 45 | 44 | 4 | 175 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 58 | 49 | 4 | 111 |
| cEMVI absent | 26 | 35 | 2 | 63 |
| Unknown | 1 | 0 | 0 | 1 |
| Total | 85 | 84 | 6 | 175 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; T = primary tumour stage.

Table 7.8 Agreement for pMMR Right-Sided Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 1 | 0 | 1 |
| cT3 | 0 | 4 | 50 | 21 | 1 | 76 |
| cT4 | 0 | 0 | 22 | 16 | 1 | 39 |
| Unknown | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 4 | 72 | 38 | 2 | 116 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 1 | 0 | 1 |
| cT3-4 | 4 | 109 | 2 | 115 |
| Unknown | 0 | 0 | 0 | 0 |
| Total | 4 | 110 | 2 | 116 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 7 | 8 | 2 | 2 | 19 |
| cN1 | 26 | 18 | 12 | 2 | 58 |
| cN2 | 17 | 6 | 16 | 0 | 39 |
| Unknown | 0 | 0 | 0 | 0 | 0 |
| Total | 50 | 32 | 30 | 4 | 116 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 37 | 33 | 3 | 73 |
| cEMVI absent | 11 | 29 | 2 | 42 |
| Unknown | 0 | 1 | 0 | 1 |
| Total | 48 | 63 | 5 | 116 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; pMMR = mismatch repair proficient; T = primary tumour stage.

Table 7.9 Agreement for pMMR Left-Sided Tumours

| | pT1 | pT2 | pT3 | pT4 | Unknown | Total |
|---|---|---|---|---|---|---|
| cT1 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT2 | 0 | 0 | 0 | 0 | 0 | 0 |
| cT3 | 1 | 9 | 96 | 27 | 1 | 134 |
| cT4 | 0 | 3 | 14 | 16 | 0 | 33 |
| Unknown | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 1 | 12 | 110 | 43 | 1 | 167 |

| | pT1-2 | pT3-4 | Unknown | Total |
|---|---|---|---|---|
| cT1-2 | 0 | 0 | 0 | 0 |
| cT3-4 | 13 | 153 | 1 | 167 |
| Unknown | 0 | 0 | 0 | 0 |
| Total | 13 | 153 | 1 | 167 |

| | pN0 | pN1 | pN2 | Unknown | Total |
|---|---|---|---|---|---|
| cN0 | 25 | 9 | 10 | 2 | 46 |
| cN1 | 39 | 25 | 14 | 0 | 78 |
| cN2 | 14 | 8 | 18 | 2 | 42 |
| Unknown | 0 | 1 | 0 | 0 | 1 |
| Total | 78 | 43 | 42 | 4 | 167 |

| | pEMVI present | pEMVI absent | Unknown | Total |
|---|---|---|---|---|
| cEMVI present | 54 | 47 | 4 | 105 |
| cEMVI absent | 25 | 34 | 2 | 61 |
| Unknown | 1 | 0 | 0 | 1 |
| Total | 80 | 81 | 6 | 167 |

c = radiological; EMVI = extramural venous invasion; N = lymph node stage; p = pathological; pMMR = mismatch repair proficient; T = primary tumour stage.

## 7.2 Published Response to Letter

A letter to the Editor of the British Journal of Radiology was published in response to this analysis, giving a general critical appraisal of NAC and the limitations of radiology to optimally stratify patients.[1] The published reply to this letter is copied below.[2]

We thank the authors for their letter regarding our analysis of radiological staging in the FOxTROT trial.[3] We would like to take this opportunity to provide clarity on aspects of the data and debate the critical issues raised.

Following the FOxTROT pilot (first 150 participants [14% of the total]), the eligibility criteria for radiological T3 tumours changed from requiring 5 mm extramural extension to 1 mm.[4, 5] While this detail may have added value to our discussion, it does not affect the analysis or

conclusions. Our analysis was not designed to specifically evaluate performance against trial eligibility (including the time-dependent shift in T stage criteria), but to explore radiology-pathology correlation in a representative, multicentre population of advanced tumours. The additional analysis using 5 mm for “positivity” was used to explore how a different threshold impacted performance and possible implications for future trial design and treatment delivery.

The letter cites PRODIGE-22 and NeoCol as a “strong case against the widespread implementation of NAC”, but these are not like-for-like studies.[6, 7] The OPTICAL trial, which is the only other published phase III randomised controlled trial (RCT) in this setting, was not discussed.[8] OPTICAL reported a similar absolute risk reduction in disease recurrence and improved overall survival. Both FOxTROT and OPTICAL also show that NAC is ineffective for the vast majority of mismatch repair deficient (dMMR) tumours, with enhanced outcomes seen specifically for tumours that are mismatch repair proficient (pMMR).[5, 8] While molecular stratification is therefore critical for evaluating the benefit of NAC, it must be emphasised that both trials still showed substantial patient benefit without this distinction. These findings are further reinforced by a recent meta-analysis.[9]

The issue of over-treatment is not limited to NAC in colon cancer; for example, adjuvant chemotherapy only benefits around 20% of those with stage III colon cancer.[10] This example demonstrates that pathological TNM is an imperfect system, with criteria that do not necessarily translate to the preoperative setting, and which radiological staging should not exclusively be benchmarked against. Our analysis shows that radiological T3-4 staging is reliable with minimal over-staging, providing a foundation on which to safely deliver NAC while also seeking additional biomarkers to improve patient selection.

FOxTROT was the first phase III RCT to be delivered in this setting. The trial met its primary endpoint, with NAC significantly reducing the risk of residual disease or recurrence at two years with no safety concerns.[5] The absolute risk reduction of 4.6% described as a “modest” benefit is comparable to that provided by adjuvant oxaliplatin, which is now considered a standard of care.[11] Furthermore, these results represent the first major therapeutic advancement in locally advanced colon cancer since the introduction of doublet adjuvant chemotherapy around 20 years ago.

## 7.3 Supplementary Material for Chapter 3

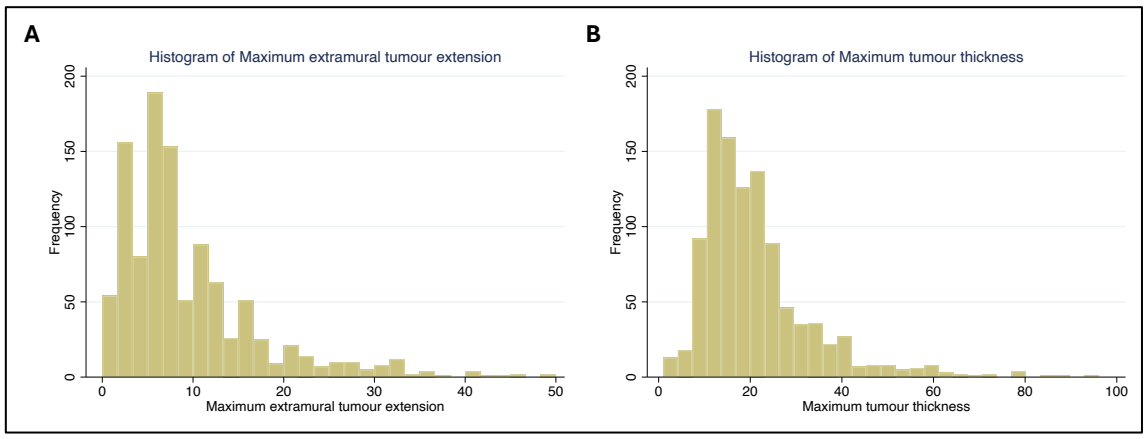



**Figure 7.1 Histograms for Maximum Extramural Tumour Extension & Tumour Thickness**
Distribution of baseline values in the study population. A) Maximum extramural tumour extension. B) Maximum tumour thickness.

Table 7.10 Evaluation of DFS Thresholds for Maximum Extramural Tumour Extension

| Threshold (mm) | Number of cases above threshold | Sensitivity | Specificity | Youden's J Statistic | DFS HR (95% CI) | P value |
|---|---|---|---|---|---|---|
| 2 | 925 (88%) | 0.89 | 0.12 | 0.01 | 1.23 (0.81-1.87) | 0.34 |
| 3 | 839 (80%) | 0.84 | 0.21 | 0.05 | 1.50 (1.05-2.14) | 0.03 |
| 4 | 763 (73%) | 0.79 | 0.29 | 0.08 | 1.58 (1.15-2.17) | 0.005 |
| 5 | 646 (62%) | 0.70 | 0.40 | 0.11 | 1.57 (1.19-2.07) | 0.001 |
| 6 | 570 (54%) | 0.64 | 0.49 | 0.13 | 1.66 (1.28-2.16) | 0.0002 |
| 7 | 494 (47%) | 0.58 | 0.56 | 0.14 | 1.72 (1.34-2.22) | 0.00003 |
| 8 | 417 (40%) | 0.49 | 0.63 | 0.12 | 1.61 (1.25-2.06) | 0.0002 |
| 9 | 368 (35%) | 0.45 | 0.68 | 0.12 | 1.63 (1.27-2.09) | 0.0001 |
| 10 | 310 (30%) | 0.38 | 0.73 | 0.11 | 1.66 (1.29-2.13) | 0.00008 |
| 12 | 240 (23%) | 0.32 | 0.80 | 0.11 | 1.73 (1.33-2.25) | 0.00004 |
| 14 | 190 (18%) | 0.25 | 0.84 | 0.09 | 1.74 (1.32-2.30) | 0.00009 |
| 16 | 138 (13%) | 0.19 | 0.88 | 0.08 | 1.83 (1.35-2.48) | 0.0001 |
| 18 | 113 (11%) | 0.15 | 0.90 | 0.05 | 1.71 (1.22-2.38) | 0.002 |
| 20 | 86 (8%) | 0.13 | 0.93 | 0.06 | 1.89 (1.32-2.71) | 0.0005 |
| 25 | 54 (5%) | 0.07 | 0.95 | 0.03 | 1.58 (1.00-2.50) | 0.05 |

DFS = disease-free survival; HR = hazard ratio.
Green shading represents chosen threshold based on Youden's J statistic.

Table 7.11 Evaluation of DFS Thresholds for Maximum Tumour Thickness

| Threshold (mm) | Number of cases above threshold | Sensitivity | Specificity | Youden's J Statistic | DFS HR (95% CI) | P value |
|---|---|---|---|---|---|---|
| 5 | 1019 (98%) | 0.99 | 0.02 | 0.01 | 2.12 (0.53-8.55) | 0.29 |
| 10 | 914 (88%) | 0.92 | 0.13 | 0.04 | 1.52 (0.96-2.40) | 0.07 |
| 15 | 632 (61%) | 0.67 | 0.41 | 0.08 | 1.38 (1.06-1.81) | 0.02 |
| 20 | 396 (38%) | 0.47 | 0.64 | 0.11 | 1.38 (1.08-1.77) | 0.01 |
| 21 | 370 (36%) | 0.45 | 0.67 | 0.11 | 1.39 (1.08-1.79) | 0.01 |
| 22 | 340 (33%) | 0.42 | 0.70 | 0.12 | 1.43 (1.11-1.85) | 0.005 |
| 23 | 312 (30%) | 0.38 | 0.72 | 0.11 | 1.42 (1.10-1.83) | 0.008 |
| 24 | 283 (27%) | 0.35 | 0.75 | 0.10 | 1.44 (1.11-1.87) | 0.006 |
| 25 | 243 (23%) | 0.32 | 0.79 | 0.11 | 1.47 (1.12-1.93) | 0.005 |
| 30 | 158 (15%) | 0.22 | 0.87 | 0.08 | 1.55 (1.14-2.09) | 0.005 |
| 35 | 106 (10%) | 0.13 | 0.91 | 0.04 | 1.36 (0.95-1.96) | 0.10 |
| 40 | 67 (6%) | 0.07 | 0.94 | 0.01 | 1.30 (0.83-2.04) | 0.25 |
| 45 | 50 (5%) | 0.06 | 0.96 | 0.02 | 1.55 (0.95-2.53) | 0.08 |
| 50 | 34 (3%) | 0.05 | 0.97 | 0.02 | 1.97 (1.15-3.38) | 0.01 |
| 55 | 26 (3%) | 0.04 | 0.98 | 0.02 | 2.31 (1.30-4.14) | 0.005 |
| 60 | 16 (2%) | 0.03 | 0.99 | 0.01 | 1.99 (0.94-4.22) | 0.07 |
| 65 | 11 (1%) | 0.02 | 0.99 | 0.008 | 1.70 (0.63-4.56) | 0.30 |

DFS = disease-free survival; HR = hazard ratio.

Green shading represents chosen threshold based on Youden's J statistic.

Table 7.12 Univariable Analysis of Time to Recurrence in All Participants

| | No Recurrence (n = 823) | Recurrence (n = 253) | HR (95% CI) | P value [a] |
|---|---|---|---|---|
| Age (years) | | | | |
| <50, n (%) | 80 (81.6) | 18 (18.4) | 1.0 | |
| 50-59 | 181 (79.7) | 46 (20.3) | 1.10 (0.64-1.89) | 0.74 |
| 60-69 | 338 (77.7) | 97 (22.3) | 1.21 (0.73-2.00) | 0.46 |
| ≥70 | 224 (76.7) | 68 (23.3) | 1.28 (0.76-2.16) | 0.35 |
| Sex | | | | |
| Male, n (%) | 513 (76.3) | 159 (23.7) | 1.0 | |
| Female | 310 (81.6) | 70 (18.4) | 0.78 (0.59-1.04) | 0.09 |
| Performance Status | | | | |
| 0, n (%) | 642 (79.8) | 162 (20.2) | 1.0 | |
| 1-2 | 181 (73.0) | 67 (27.0) | 1.46 (1.10-1.94) | 0.01 |
| Tumour differentiation | | | | |
| Well/moderate, n (%) | 676 (80.3) | 166 (19.7) | 1.0 | |
| Poor | 100 (66.7) | 50 (33.3) | 1.99 (1.45-2.73) | < 0.001 |
| *RAS* status | | | | |
| Wild type, n (%) | 349 (77.4) | 102 (22.6) | 1.0 | |
| Mutant | 203 (75.8) | 65 (24.2) | 1.08 (0.79-1.48) | 0.62 |
| MMR status | | | | |
| Proficient, n (%) | 572 (78.5) | 157 (21.5) | 1.0 | |
| Deficient | 148 (80.0) | 37 (20.0) | 0.94 (0.66-1.35) | 0.75 |
| Radiological Tumour side [b] | | | | |
| Right, n (%) | 391 (77.0) | 117 (23.0) | 1.0 | |
| Left | 424 (79.4) | 110 (20.6) | 0.88 (0.68-1.14) | 0.32 |
| Radiological T stage [c] | | | | |
| 3, n (%) | 627 (80.5) | 152 (19.5) | 1.0 | |
| 4 | 189 (71.6) | 75 (28.4) | 1.57 (1.19-2.07) | 0.001 |
| Extramural tumour extension, mm | | | | |
| Continuous, median (range) | 7 (0-50) | 9 (0-50) | 1.03 (1.02-1.04) | < 0.001 |
| ≤7, n (%) | 461 (83.1) | 94 (16.9) | 1.0 | |
| >7 | 359 (72.7) | 135 (27.3) | 1.67 (1.29-2.18) | < 0.001 |
| Maximum tumour thickness, mm | | | | |
| Continuous, median (range) | 17 (1-96) | 20 (4-88) | 1.16 (1.06-1.27) [d] | 0.001 |
| ≤22, n (%) | 567 (81.6) | 128 (18.4) | 1.0 | |
| >22 | 243 (71.5) | 97 (28.5) | 1.59 (1.22-2.07) | 0.001 |
| Radiological N stage [c] | | | | |
| 0, n (%) | 200 (80.0) | 50 (20.0) | 1.0 | |
| 1 | 398 (80.1) | 99 (19.9) | 1.02 (0.73-1.44) | 0.90 |
| 2 | 221 (74.2) | 77 (25.8) | 1.35 (0.94-1.92) | 0.10 |
| Radiological N status [c] | | | | |
| 0, n (%) | 200 (80.0) | 50 (20.0) | 1.0 | |
| 1-2 | 619 (77.9) | 176 (22.1) | 1.14 (0.83-1.56) | 0.41 |
| Lymph node ≥10 mm short axis | | | | |
| Absent, n (%) | 574 (80.3) | 141 (19.7) | 1.0 | |
| Present | 238 (73.5) | 86 (26.5) | 1.40 (1.07-1.83) | 0.01 |
| EMVI | | | | |
| Absent, n (%) | 332 (82.0) | 73 (18.0) | 1.0 | |
| Present | 480 (75.7) | 154 (24.3) | 1.41 (1.07-1.86) | 0.02 |

CI = confidence intervals; EMVI = extramural venous invasion; HR = hazard ratio; n = number of participants; N = lymph node stage; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

Table 7.13 Univariable Analysis of Time to Recurrence According to MMR Status

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No Recurrence (n = 572) | Recurrence (n = 157) | HR (95% CI) | P value [a] | No Recurrence (n = 148) | Recurrence (n = 37) | HR (95% CI) | P value [a] | |
| Age (years) | | | | | | | | | |
| <50, n (%) | 43 (82.7) | 9 (17.3) | 1.0 | | 21 (80.8) | 5 (19.2) | 1.0 | | 0.86 |
| 50-59 | 133 (78.7) | 36 (21.3) | 1.17 (0.56-2.43) | 0.67 | 24 (82.8) | 5 (17.2) | 0.96 (0.28-3.30) | 0.94 | |
| 60-69 | 245 (79.3) | 64 (20.7) | 1.11 (0.55-2.23) | 0.77 | 60 (77.9) | 17 (22.1) | 1.26 (0.46-3.40) | 0.65 | |
| ≥70 | 151 (75.9) | 48 (24.1) | 1.36 (0.67-2.76) | 0.40 | 43 (81.1) | 10 (18.9) | 1.11 (0.38-3.24) | 0.85 | |
| Sex | | | | | | | | | |
| Male, n (%) | 371 (76.3) | 115 (23.7) | 1.0 | | 77 (78.6) | 21 (21.4) | 1.0 | | 0.65 |
| Female | 201 (82.7) | 42 (17.3) | 0.73 (0.51-1.04) | 0.09 | 71 (81.6) | 16 (18.4) | 0.88 (0.46-1.68) | 0.69 | |
| Performance status | | | | | | | | | |
| 0, n (%) | 451 (79.1) | 119 (20.9) | 1.0 | | 108 (83.1) | 22 (16.9) | 1.0 | | |
| 1-2 | 121 (76.1) | 38 (23.9) | 1.16 (0.80-1.67) | 0.44 | 40 (72.7) | 15 (27.3) | 1.79 (0.93-3.46) | 0.08 | 0.23 |
| Tumour differentiation | | | | | | | | | |
| Well/moderate, n (%) | 514 (79.9) | 129 (20.1) | 1.0 | | 92 (83.6) | 18 (16.4) | 1.0 | | 0.38 |
| Poor | 37 (57.8) | 27 (42.2) | 2.61 (1.72-3.95) | < 0.001 | 49 (74.2) | 17 (25.8) | 1.80 (0.93-3.49) | 0.08 | |
| RAS status | | | | | | | | | |
| Wild type, n (%) | 228 (77.3) | 67 (22.7) | 1.0 | | 86 (78.9) | 23 (21.1) | 1.0 | | 0.31 |
| Mutant | 170 (77.3) | 50 (22.7) | 0.97 (0.67-1.39) | 0.86 | 16 (69.6) | 7 (30.4) | 1.59 (0.68-3.70) | 0.29 | |
| Radiological tumour side [b] | | | | | | | | | |
| Right, n (%) | 210 (75.0) | 70 (25.0) | 1.0 | | 128 (81.0) | 30 (19.0) | 1.0 | | 0.30 |
| Left | 359 (80.7) | 86 (19.3) | 0.76 (0.56-1.05) | 0.10 | 18 (75.0) | 6 (25.0) | 1.33 (0.55-3.19) | 0.53 | |

Table 7.13 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No Recurrence (n = 572) | Recurrence (n = 157) | HR (95% CI) | P value [a] | No Recurrence (n = 148) | Recurrence (n = 37) | HR (95% CI) | P value [a] | |
| Radiological T stage [c] | | | | | | | | | |
| 3, n (%) | 450 (79.8) | 114 (20.2) | 1.0 | | 99 (85.3) | 17 (14.7) | 1.0 | | 0.25 |
| 4 | 117 (73.6) | 42 (26.4) | 1.35 (0.95-1.92) | 0.10 | 48 (71.6) | 19 (28.4) | 2.11 (1.10-4.06) | 0.03 | |
| Extramural tumour extension, mm | | | | | | | | | |
| Continuous, median (range) | 6 (0-45) | 8 (0-50) | 1.03 (1.01-1.05) | < 0.001 | 8 (0-50) | 8 (0-50) | 1.02 (0.98-1.05) | 0.39 | |
| ≤7, n (%) | 335 (83.5) | 66 (16.5) | 1.0 | | 71 (81.6) | 16 (18.4) | 1.0 | | 0.25 |
| >7 | 236 (72.2) | 91 (27.8) | 1.74 (1.26-2.38) | 0.001 | 76 (78.4) | 21 (21.6) | 1.18 (0.61-2.26) | 0.62 | |
| Maximum tumour thickness, mm | | | | | | | | | |
| Continuous, median (range) [d] | 16 (1-96) | 18 (4-63) | 1.14 (1.00-1.29) | 0.05 | 20 (2-85) | 26 (4-88) | 1.19 (1.00-1.40) [d] | 0.045 | |
| ≤22, n (%) | 414 (81.2) | 96 (18.8) | 1.0 | | 85 (86.7) | 13 (13.3) | 1.0 | | 0.25 |
| >22 | 150 (72.5) | 57 (27.5) | 1.46 (1.05-2.03) | 0.02 | 61 (71.8) | 24 (28.2) | 2.30 (1.17-4.51) | 0.02 | |
| Radiological N stage [c] | | | | | | | | | |
| 0, n (%) | 139 (79.4) | 36 (20.6) | 1.0 | | 34 (89.5) | 4 (10.5) | 1.0 | | 0.22 |
| 1 | 281 (79.8) | 71 (20.2) | 1.03 (0.69-1.54) | 0.88 | 70 (81.4) | 16 (18.6) | 1.83 (0.61-5.49) | 0.28 | |
| 2 | 150 (76.1) | 47 (23.9) | 1.19 (0.77-1.84) | 0.42 | 43 (71.7) | 17 (28.3) | 3.09 (1.04-9.20) | 0.04 | |
| Radiological N status [c] | | | | | | | | | |
| 0, n (%) | 139 (79.4) | 36 (20.6) | 1.0 | | 34 (89.5) | 4 (10.5) | 1.0 | | 0.15 |
| 1-2 | 431 (78.5) | 118 (21.5) | 1.09 (0.75-1.58) | 0.65 | 113 (77.4) | 33 (22.6) | 2.32 (0.82-6.55) | 0.11 | |

Table 7.13 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No Recurrence (n = 572) | Recurrence (n = 157) | HR (95% CI) | P value [a] | No Recurrence (n = 148) | Recurrence (n = 37) | HR (95% CI) | P value [a] | |
| Lymph node ≥10 mm short axis | | | | | | | | | |
| Absent, n (%) | 402 (80.1) | 100 (19.9) | 1.0 | | 98 (83.8) | 19 (16.2) | 1.0 | | 0.49 |
| Present | 163 (74.1) | 57 (25.9) | 1.35 (0.98-1.87) | 0.07 | 47 (73.4) | 17 (26.6) | 1.72 (0.90-3.32) | 0.10 | |
| EMVI | | | | | | | | | |
| Absent, n (%) | 239 (81.3) | 55 (18.7) | 1.0 | | 56 (86.1) | 9 (13.9) | 1.0 | | 0.41 |
| Present | 326 (76.4) | 101 (23.6) | 1.32 (0.95-1.83) | 0.10 | 89 (76.1) | 28 (23.9) | 1.82 (0.86-3.85) | 0.12 | |

CI = confidence intervals; dMMR = mismatch repair deficient; EMVI = extramural venous invasion; HR = hazard ratio; MMR = mismatch repair; n = number of participants; N = lymph node stage; pMMR = mismatch repair proficient; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

Table 7.14 Univariable Analysis of Overall Survival in All Participants

| | No Recurrence (n = 823) | Recurrence (n = 253) | HR (95% CI) | P value [a] |
|---|---|---|---|---|
| Age (years) | | | | |
| <50, n (%) | 85 (86.7) | 13 (13.3) | 1.0 | |
| 50-59 | 203 (89.4) | 24 (10.6) | 0.79 (0.40-1.55) | 0.49 |
| 60-69 | 359 (82.5) | 76 (17.5) | 1.21 (0.67-2.18) | 0.53 |
| ≥70 | 237 (81.2) | 55 (18.8) | 1.46 (0.80-2.67) | 0.22 |
| Sex | | | | |
| Male, n (%) | 556 (82.7) | 116 (17.3) | 1.0 | |
| Female | 328 (86.3) | 52 (13.7) | 0.80 (0.58-1.11) | 0.18 |
| Performance Status | | | | |
| 0, n (%) | 697 (86.7) | 107 (13.3) | 1.0 | |
| 1-2 | 187 (75.4) | 61 (24.6) | 1.85 (1.35-2.54) | < 0.001 |
| Tumour differentiation | | | | |
| Well/moderate, n (%) | 728 (86.5) | 114 (13.5) | 1.0 | |
| Poor | 110 (73.3) | 40 (26.7) | 2.32 (1.61-3.33) | < 0.001 |
| *RAS* status | | | | |
| Wild type, n (%) | 371 (82.3) | 80 (17.7) | 1.0 | |
| Mutant | 216 (80.6) | 52 (19.4) | 1.07 (0.75-1.52) | 0.71 |
| MMR status | | | | |
| Proficient, n (%) | 619 (84.9) | 110 (15.1) | 1.0 | |
| Deficient | 153 (82.7) | 32 (17.3) | 1.20 (0.81-1.78) | 0.36 |
| Radiological Tumour side [b] | | | | |
| Right, n (%) | 410 (80.7) | 98 (19.3) | 1.0 | |
| Left | 465 (87.1) | 69 (12.9) | 0.66 (0.49-0.90) | 0.009 |
| Radiological T stage [c] | | | | |
| 3, n (%) | 672 (86.3) | 107 (13.7) | 1.0 | |
| 4 | 204 (77.3) | 60 (22.7) | 1.66 (1.21-2.27) | 0.002 |
| Extramural tumour extension, mm | | | | |
| Continuous, median (range) | 7 (0-50) | 10 (0-50) | 1.03 (1.02-1.05) | < 0.001 |
| ≤7, n (%) | 499 (89.9) | 56 (10.1) | 1.0 | |
| >7 | 382 (77.3) | 112 (22.7) | 1.95 (1.41-2.69) | < 0.001 |
| Maximum tumour thickness, mm | | | | |
| Continuous, median (range) | 17 (1-96) | 20 (4-88) | 1.17 (1.06-1.30) [d] | 0.003 |
| ≤22, n (%) | 600 (86.3) | 95 (13.7) | 1.0 | |
| >22 | 269 (79.1) | 71 (20.9) | 1.41 (1.03-1.92) | 0.03 |
| Radiological N stage [c] | | | | |
| 0, n (%) | 207 (82.8) | 43 (17.2) | 1.0 | |
| 1 | 430 (86.8) | 67 (13.5) | 0.81 (0.55-1.19) | 0.28 |
| 2 | 241 (80.9) | 57 (19.1) | 1.06 (0.71-1.58) | 0.77 |
| Radiological N status [c] | | | | |
| 0, n (%) | 207 (82.8) | 43 (17.2) | 1.0 | |
| 1-2 | 671 (84.4) | 124 (15.6) | 0.91 (0.64-1.29) | 0.59 |
| Lymph node ≥10 mm short axis | | | | |
| Absent, n (%) | 617 (86.3) | 98 (13.7) | 1.0 | |
| Present | 256 (79.0) | 68 (21.0) | 1.50 (1.10-2.05) | 0.01 |
| EMVI | | | | |
| Absent, n (%) | 349 (86.2) | 56 (13.8) | 1.0 | |
| Present | 524 (82.7) | 110 (17.3) | 1.29 (0.93-1.77) | 0.13 |

CI = confidence intervals; EMVI = extramural venous invasion; HR = hazard ratio; n = number of participants; N = lymph node stage; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

Table 7.15 Univariable Analysis of Overall Survival According to MMR Status

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No OS event (n = 619) | OS event (n = 110) | HR (95% CI) | P value [a] | No OS event (n = 153) | OS event (n = 32) | HR (95% CI) | P value [a] | |
| Age (years) | | | | | | | | | |
| <50, n (%) | 45 (86.5) | 7 (13.5) | 1.0 | | 23 (88.5) | 3 (11.5) | 1.0 | | 0.22 |
| 50-59 | 151 (89.3) | 18 (10.7) | 0.71 (0.30-1.70) | 0.44 | 26 (89.7) | 3 (10.3) | 0.93 (0.19-4.59) | 0.93 | |
| 60-69 | 262 (84.8) | 47 (15.2) | 0.83 (0.37-1.84) | 0.64 | 59 (76.6) | 18 (23.4) | 2.40 (0.71-8.15) | 0.16 | |
| ≥70 | 161 (80.9) | 38 (19.1) | 1.26 (0.56-2.82) | 0.58 | 45 (84.9) | 8 (15.1) | 1.65 (0.44-6.25) | 0.46 | |
| Sex | | | | | | | | | |
| Male, n (%) | 403 (82.9) | 83 (17.1) | 1.0 | | 80 (81.6) | 18 (18.4) | 1.0 | | 0.57 |
| Female | 216 (88.9) | 27 (11.1) | 0.67 (0.44-1.04) | 0.07 | 73 (83.9) | 14 (16.1) | 0.89 (0.44-1.78) | 0.73 | |
| Performance status | | | | | | | | | |
| 0, n (%) | 495 (86.8) | 75 (13.2) | 1.0 | | 110 (84.6) | 20 (15.4) | 1.0 | | 0.73 |
| 1-2 | 124 (78.0) | 35 (22.0) | 1.47 (0.98-2.21) | 0.06 | 43 (78.2) | 12 (21.8) | 1.57 (0.77-3.22) | 0.22 | |
| Tumour differentiation | | | | | | | | | |
| Well/moderate, n (%) | 551 (85.7) | 92 (14.3) | 1.0 | | 97 (88.2) | 13 (11.8) | 1.0 | | 0.68 |
| Poor | 46 (71.9) | 18 (28.1) | 2.17 (1.30-3.62) | 0.003 | 49 (74.2) | 17 (25.8) | 2.47 (1.20-5.08) | 0.01 | |
| RAS status | | | | | | | | | |
| Wild type, n (%) | 247 (83.7) | 48 (16.3) | 1.0 | | 87 (79.8) | 22 (20.2) | 1.0 | | 0.96 |
| Mutant | 180 (81.8) | 40 (18.2) | 1.05 (0.69-1.60) | 0.81 | 18 (78.3) | 5 (21.7) | 1.13 (0.43-2.98) | 0.81 | |
| Radiological tumour side [b] | | | | | | | | | |
| Right, n (%) | 227 (81.1) | 53 (18.9) | 1.0 | | 130 (82.3) | 28 (17.7) | 1.0 | | 0.99 |
| Left | 389 (87.4) | 56 (12.6) | 0.72 (0.50-1.06) | 0.10 | 20 (83.3) | 4 (16.7) | 0.83 (0.29-2.37) | 0.73 | |

Table 7.15 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No OS event (n=619) | OS event (n=110) | HR (95% CI) | P value [a] | No OS event (n=153) | OS event (n=32) | HR (95% CI) | P value [a] | |
| Radiological T stage [c] | | | | | | | | | |
| 3, n (%) | 484 (85.8) | 80 (14.2) | 1.0 | | 101 (87.1) | 15 (12.9) | 1.0 | | 0.40 |
| 4 | 130 (81.8) | 29 (18.2) | 1.24 (0.81-1.90) | 0.32 | 50 (74.6) | 17 (25.4) | 1.92 (0.96-3.84) | 0.07 | |
| Extramural tumour extension, mm | | | | | | | | | |
| Continuous, median (range) | 6 (0-45) | 9 (0-50) | 1.03 (1.01-1.05) | 0.005 | 8 (0-50) | 11 (1-40) | 1.03 (0.99-1.06) | 0.17 | |
| ≤7, n (%) | 364 (90.8) | 37 (9.2) | 1.0 | | 76 (87.4) | 11 (12.6) | 1.0 | | 0.29 |
| >7 | 254 (77.7) | 73 (22.3) | 1.97 (1.32-2.94) | 0.001 | 76 (78.4) | 21 (21.6) | 1.50 (1.72-3.12) | 0.28 | |
| Maximum tumour thickness, mm | | | | | | | | | |
| Continuous, median (range) [d] | 16 (1-96) | 19 (4-63) | 1.11 (0.95-1.30) | 0.21 | 20 (2-85) | 26 (4-88) | 1.17 (0.98-1.41) | 0.09 | |
| ≤22, n (%) | 441 (86.5) | 69 (13.5) | 1.0 | | 84 (85.7) | 14 (14.3) | 1.0 | | 0.83 |
| >22 | 168 (81.2) | 39 (18.8) | 1.18 (0.79-1.75) | 0.42 | 67 (78.8) | 18 (21.2) | 1.46 (0.72-2.94) | 0.29 | |
| Radiological N stage [c] | | | | | | | | | |
| 0, n (%) | 144 (82.3) | 31 (17.7) | 1.0 | | 35 (92.1) | 3 (7.9) | 1.0 | | 0.05 |
| 1 | 304 (86.4) | 48 (13.6) | 0.82 (0.52-1.29) | 0.38 | 73 (84.9) | 13 (15.1) | 1.95 (0.56-6.85) | 0.30 | |
| 2 | 167 (84.8) | 30 (15.2) | 0.79 (0.48-1.31) | 0.36 | 44 (73.3) | 16 (26.7) | 3.77 (1.10-12.93) | 0.04 | |
| Radiological N status [c] | | | | | | | | | |
| 0, n (%) | 144 (82.3) | 31 (17.7) | 1.0 | | 35 (92.1) | 3 (7.9) | 1.0 | | 0.05 |
| 1-2 | 471 (85.8) | 78 (14.2) | 0.81 (0.53-1.22) | 0.31 | 117 (80.1) | 29 (19.9) | 2.66 (0.81-8.74) | 0.11 | |

Table 7.15 (Continued)

| | pMMR | | | | dMMR | | | | P Interaction |
|---|---|---|---|---|---|---|---|---|---|
| | No OS event (n = 619) | OS event (n = 110) | HR (95% CI) | P value [a] | No OS event (n = 153) | OS event (n = 32) | HR (95% CI) | P value [a] | |
| Lymph node ≥10 mm short axis | | | | | | | | | |
| Absent, n (%) | 435 (86.7) | 67 (13.3) | 1.0 | | 100 (85.5) | 17 (14.5) | 1.0 | | 0.77 |
| Present | 177 (80.5) | 43 (19.5) | 1.37 (0.94-2.02) | 0.11 | 50 (78.1) | 14 (21.9) | 1.59 (0.78-3.22) | 0.20 | |
| EMVI | | | | | | | | | |
| Absent, n (%) | 255 (86.7) | 39 (13.3) | 1.0 | | 56 (86.1) | 9 (13.9) | 1.0 | | 0.85 |
| Present | 357 (83.6) | 70 (16.4) | 1.25 (0.84-1.85) | 0.27 | 94 (80.3) | 23 (19.7) | 1.42 (0.66-3.07) | 0.38 | |

CI = confidence intervals; dMMR = mismatch repair deficient; EMVI = extramural venous invasion; HR = hazard ratio; MMR = mismatch repair; n = number of participants; N = lymph node stage; pMMR = mismatch repair proficient; T = primary tumour stage.

[a] Cox proportional hazards.

[b] Right = located proximal to the splenic flexure; left = located at, or distal to, the splenic flexure.

[c] According to TNM version 5.

[d] Scaled down by a factor of 10.

Percentages represent rows.

Table 7.16 Effect of NAC vs. Upfront Surgery on DFS in pMMR/Unknown MMR by Subgroups

| | Participants (DFS events) | HR for NAC vs. Upfront Surgery (95% CI) | P value | P Interaction |
|---|---|---|---|---|
| Age (years) | | | | |
| Below median (65 years) | 481 (112) | 0.85 (0.58-1.26) | 0.42 | 0.25 |
| Above median | 386 (100) | 0.61 (0.41-0.91) | 0.02 | |
| Sex | | | | |
| Male | 574 (154) | 0.67 (0.48-0.93) | 0.02 | 0.47 |
| Female | 293 (58) | 0.89 (0.52-1.55) | 0.69 | |
| Performance Status | | | | |
| 0 | 674 (152) | 0.76 (0.54-1.05) | 0.10 | 0.47 |
| 1-2 | 193 (60) | 0.62 (0.36-1.05) | 0.07 | |
| Tumour differentiation | | | | |
| Well/moderate | 732 (164) | 0.78 (0.57-1.08) | 0.13 | 0.67 |
| Poor | 84 (34) | 0.77 (0.39-1.53) | 0.46 | |
| Radiological Tumour side | | | | |
| Right | 350 (95) | 0.87 (0.57-1.33) | 0.52 | 0.47 |
| Left | 510 (115) | 0.65 (0.45-0.95) | 0.03 | |
| Radiological T stage | | | | |
| 3 | 663 (152) | 0.77 (0.55-1.08) | 0.14 | 0.62 |
| 4 | 197 (59) | 0.69 (0.41-1.16) | 0.17 | |
| Extramural tumour extension, mm | | | | |
| ≤7 | 468 (84) | 0.90 (0.57-1.42) | 0.66 | 0.16 |
| >7 | 397 (128) | 0.62 (0.43-0.88) | 0.008 | |
| Maximum tumour thickness, mm | | | | |
| ≤22 | 597 (129) | 0.78 (0.54-1.12) | 0.17 | 0.55 |
| >22 | 255 (79) | 0.70 (0.44-1.09) | 0.12 | |
| Radiological N status | | | | |
| 0 | 212 (53) | 0.45 (0.26-0.78) | 0.004 | 0.08 |
| 1-2 | 649 (156) | 0.85 (0.61-1.18) | 0.32 | |
| Lymph node ≥10mm short axis | | | | |
| Absent | 598 (134) | 0.59 (0.42-0.84) | 0.003 | 0.07 |
| Present | 260 (77) | 1.05 (0.65-1.71) | 0.84 | |
| EMVI | | | | |
| Absent | 340 (72) | 0.76 (0.46-1.23) | 0.26 | 0.76 |
| Present | 517 (138) | 0.71 (0.50-0.99) | 0.05 | |

CI = confidence intervals; DFS = disease-free survival; EMVI = extramural venous invasion; HR = hazard ratio; MMR = mismatch repair; N = lymph node stage; NAC = neoadjuvant chemotherapy; pMMR = MMR proficient; T = primary tumour stage

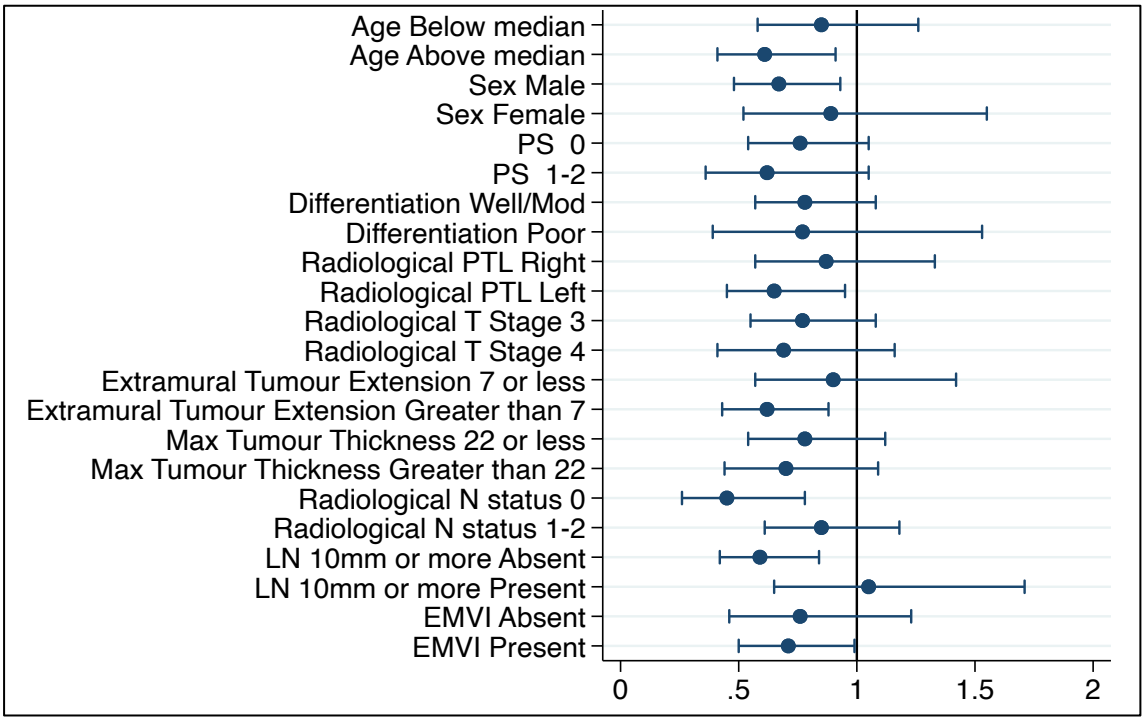



**Figure 7.2 Effect of NAC vs. Upfront Surgery by Subgroups**

Forest plot to illustrate the effect of NAC vs. upfront surgery on DFS in participants with pMMR/unknown MMR tumours by subgroups. Median = 65. Horizontal axis represents HR for NAC vs. upfront surgery. Circle represents HR estimate. Bars represent 95% confidence intervals. EMVI = extramural venous invasion; HR = hazard ratio; LN = lymph node; Mod = moderate; N = lymph node stage; PS = performance status; PTL = primary tumour location; T = primary tumour stage.

## 7.4 Supplementary Material for Chapter 4

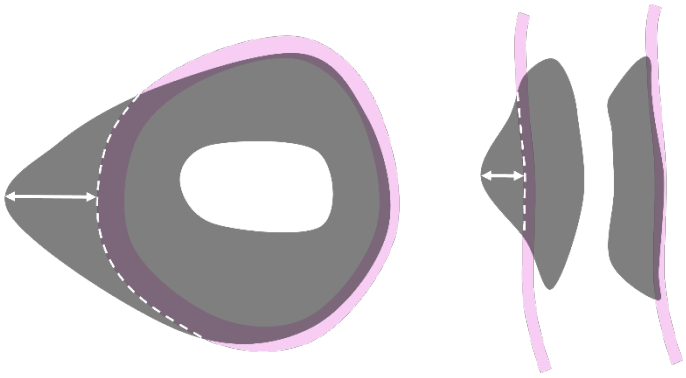
### 7.4.1 Protocol

**Primary Aims**

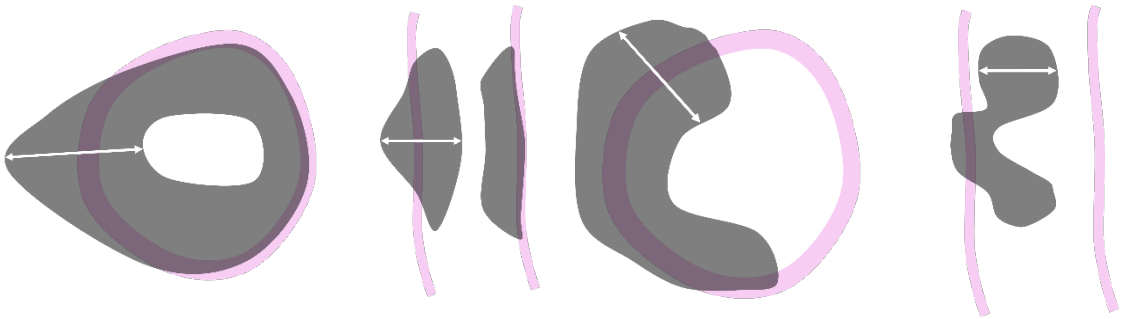
1. Comprehensively characterise the changes seen on imaging between paired baseline and interval scans, which may indicate treatment response.
2. Evaluate radiological responses according to pathological regression status.
3. Explore inter-observer variation for response metrics.

**Definitions**

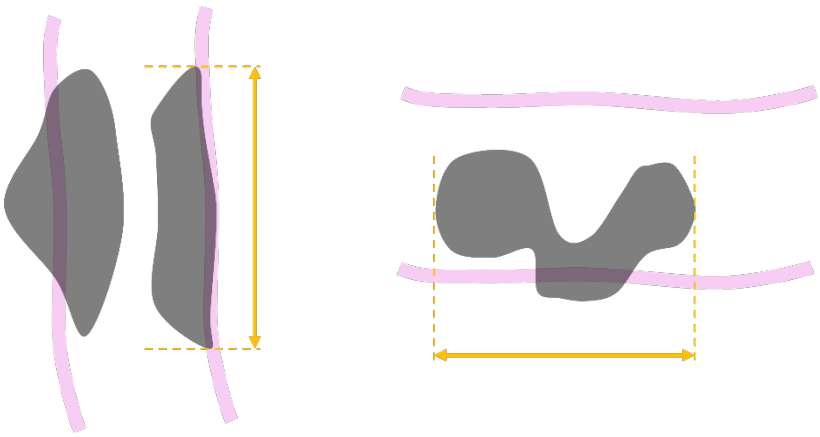
| | |
|---|---|
| Caecum | Proximal to, or involving, the ileocaecal valve (ICV).[12] |
| Ascending colon | Segment between the ICV and the initial angulation of the colon towards the hepatic flexure point.[12] |
| Hepatic flexure | From the initial angulation towards the hepatic flexure point to the proximal border of the falciform ligament.[12] |
| Transverse colon | From the proximal border of the falciform ligament to a threshold two thirds of the way to the splenic flexure point. |
| Splenic flexure | Segment comprising the remaining colon proximal to the splenic flexure point and 10cm beyond the splenic flexure point.[13] |
| Descending colon | 10cm from the splenic flexure point to the level of the left iliac crest. |
| Sigmoid | From the level of the left iliac crest to 15cm proximal to the anal verge.[12] |
| Maximum distance beyond the muscularis propria (mm) | The largest area of extramural tumour extension, measured perpendicular to the colonic lumen/bowel wall. |



| | |
|---|---|
| Maximum tumour thickness (mm) | The largest measurement of the primary tumour, from the colonic lumen to the outer edge of the tumour (i.e. measured perpendicular to the colonic lumen). A further measurement should be made at a suspected invasive margin. |



| | |
|---|---|
| Length along the colon (mm) | The maximum longitudinal measurement of the primary tumour parallel to the colonic lumen. A further measurement should be made of the largest diameter in any direction. |



| | |
|---|---|
| Tumour volume ($cm^3$) | Total volume of the primary tumour based on manual segmentation. |
| Mean tumour attenuation (HU) | Mean attenuation within the segmented primary tumour. |
| Standard deviation of tumour attenuation | Standard deviation of attenuation within the segmented primary tumour. |

### 7.4.2 Results

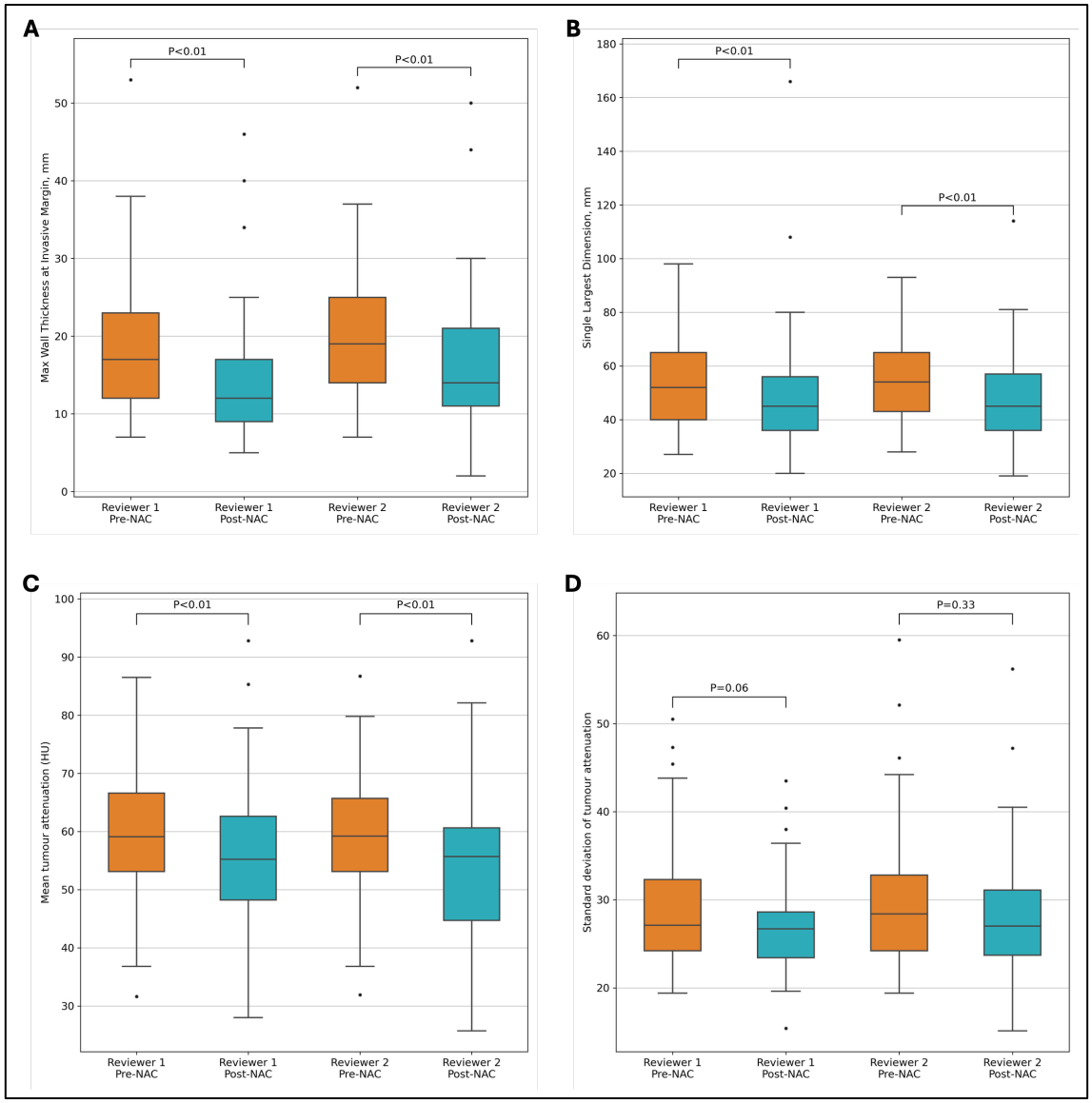



**Figure 7.3 Boxplots Comparing Pre- and Post-Neoadjuvant Chemotherapy Values**
A) Maximum wall thickness at an invasive margin. B) Single largest diameter. C) Mean tumour attenuation. D) Standard deviation of tumour attenuation. NAC = Neoadjuvant chemotherapy.

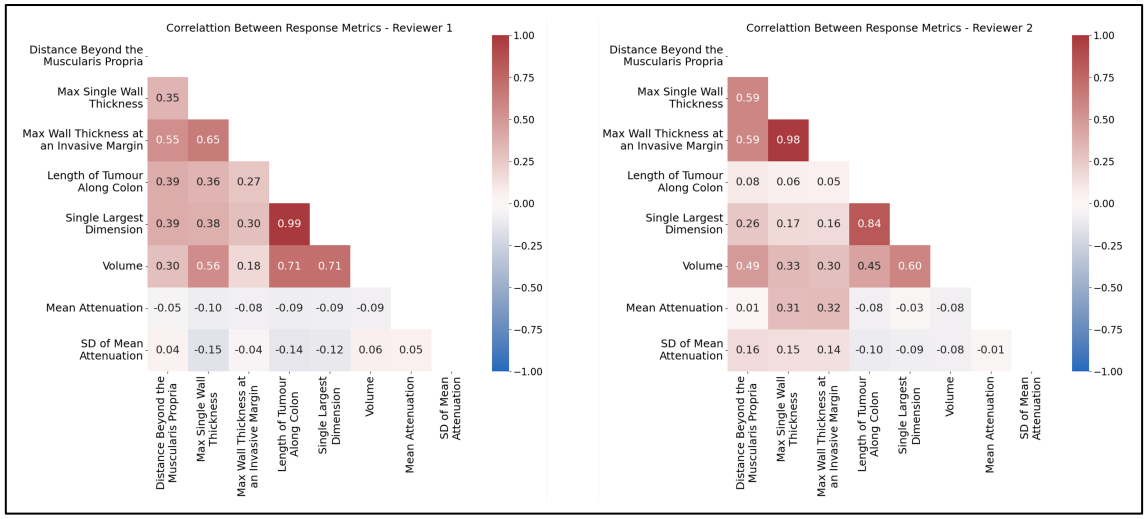



**Figure 7.4 Spearman's Rank Correlation Between Individual Response Metrics**
Each number represents the Spearman's rho value between percentage change values for the corresponding features. SD = standard deviation.

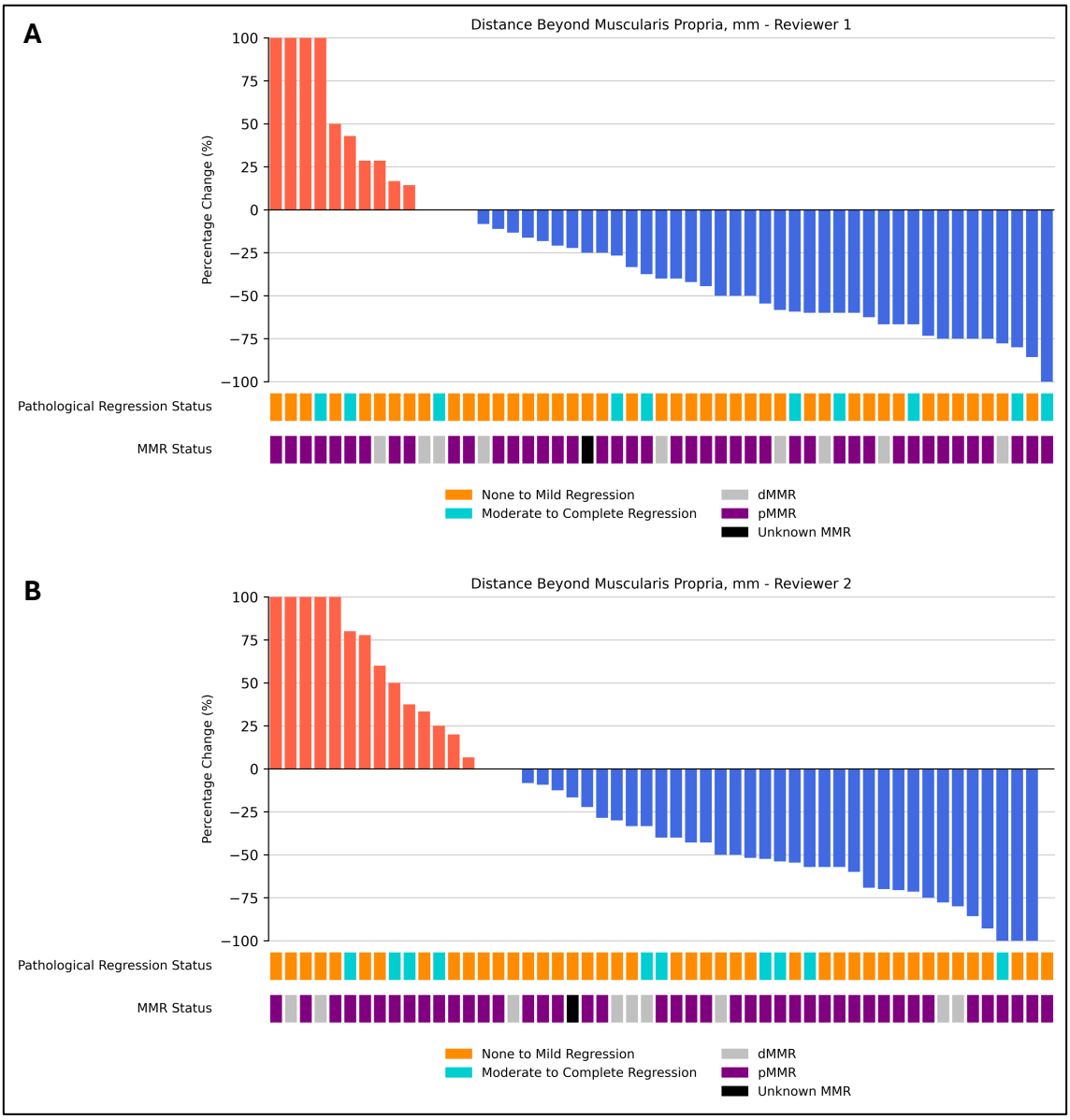



**Figure 7.5 Waterfall Plots for Change in Distance Beyond the Muscularis Propria**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

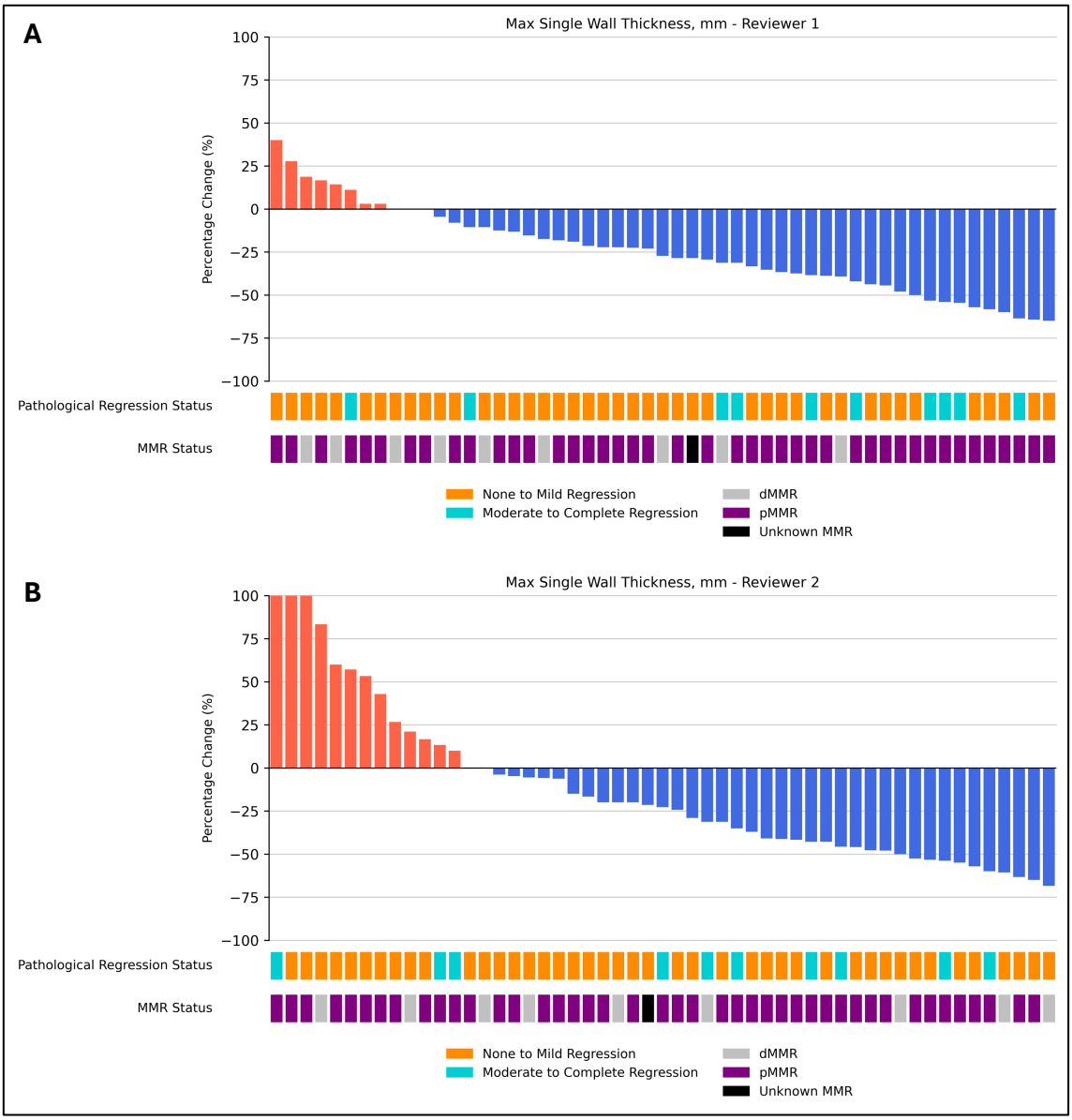



**Figure 7.6 Waterfall Plots for Change in Maximum Tumour Thickness**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

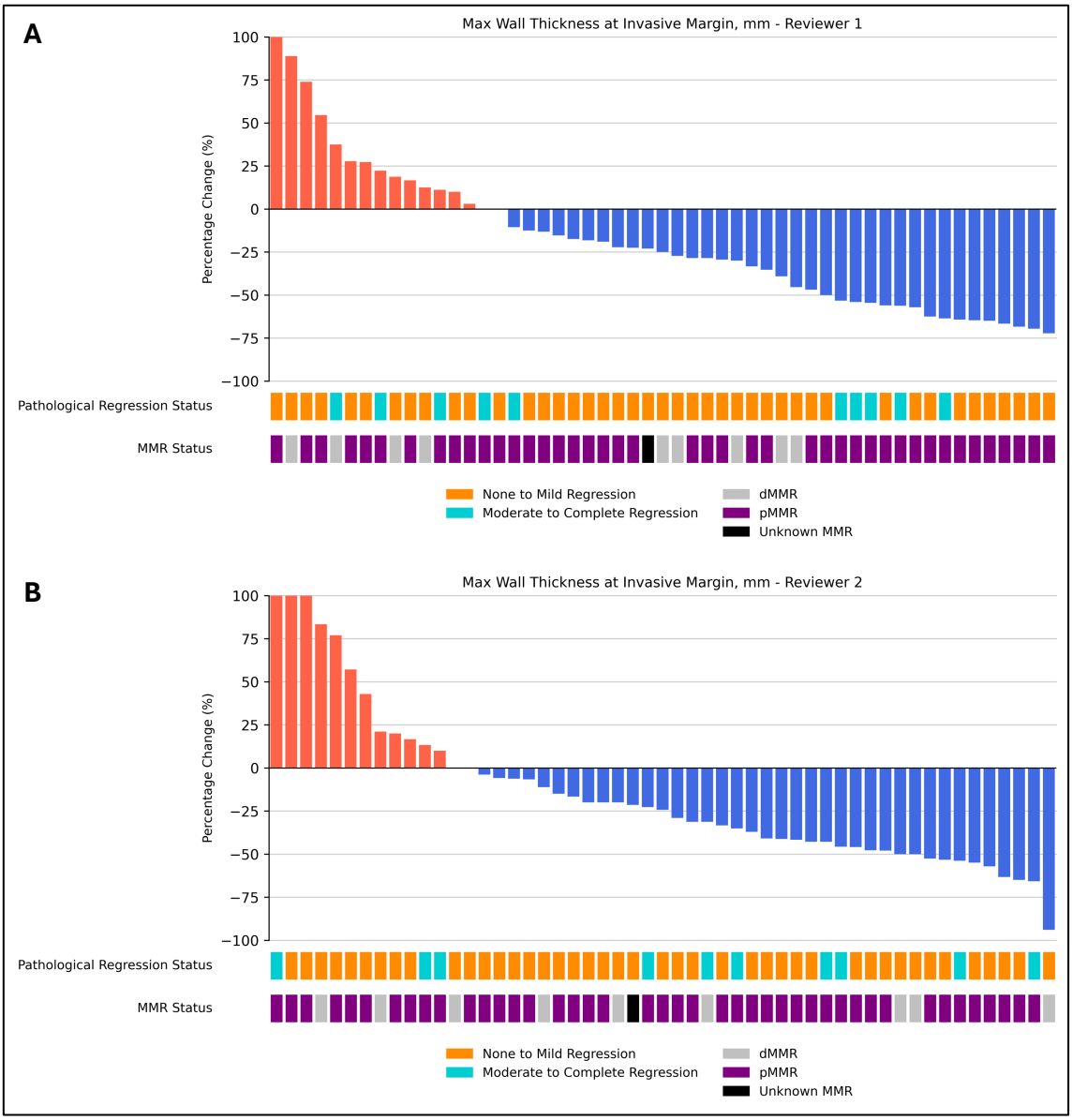



**Figure 7.7 Waterfall Plots for Change in Tumour Thickness at an Invasive Margin**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

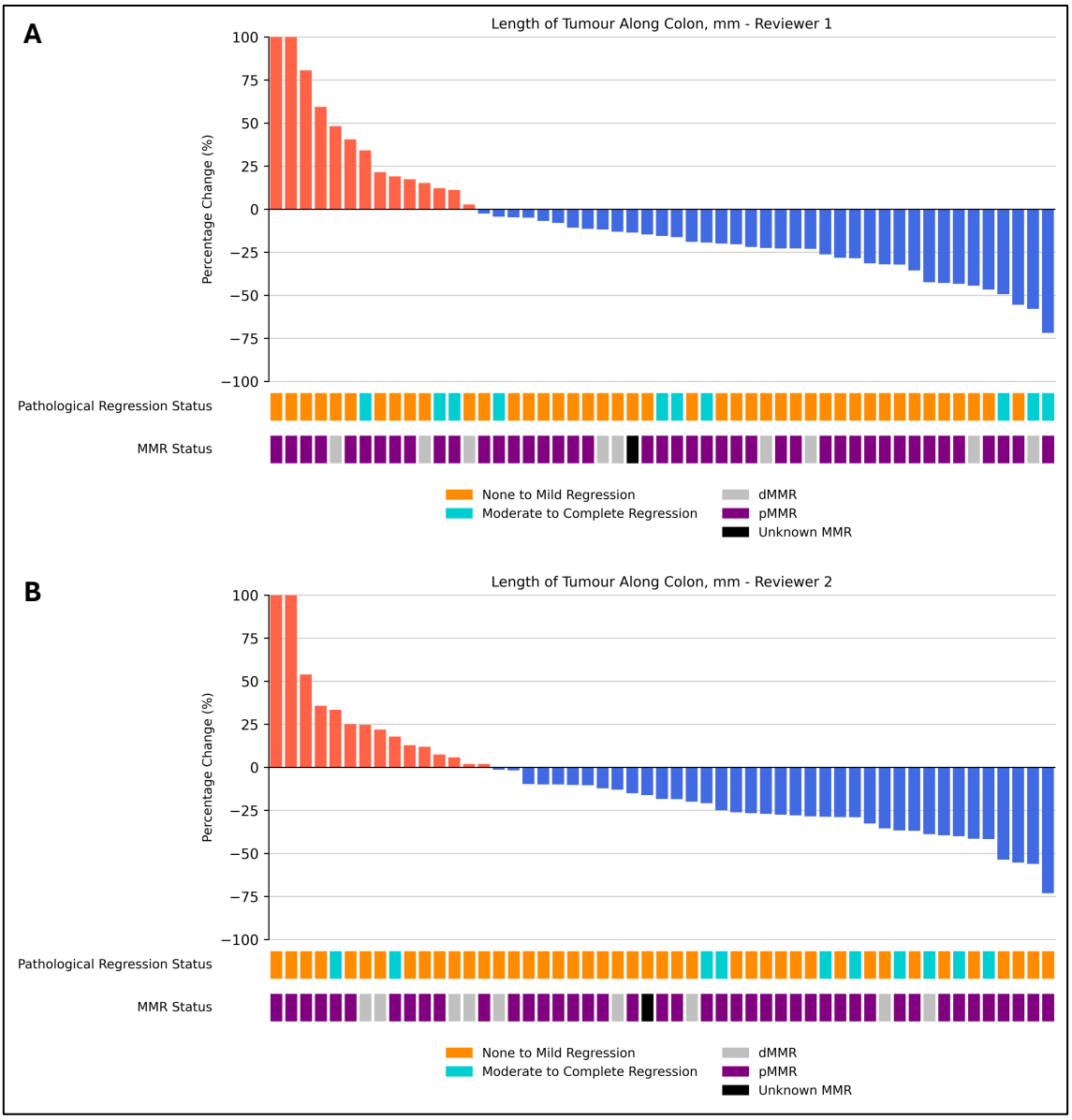



**Figure 7.8 Waterfall Plots for Change in Length of Tumour Along the Colon**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

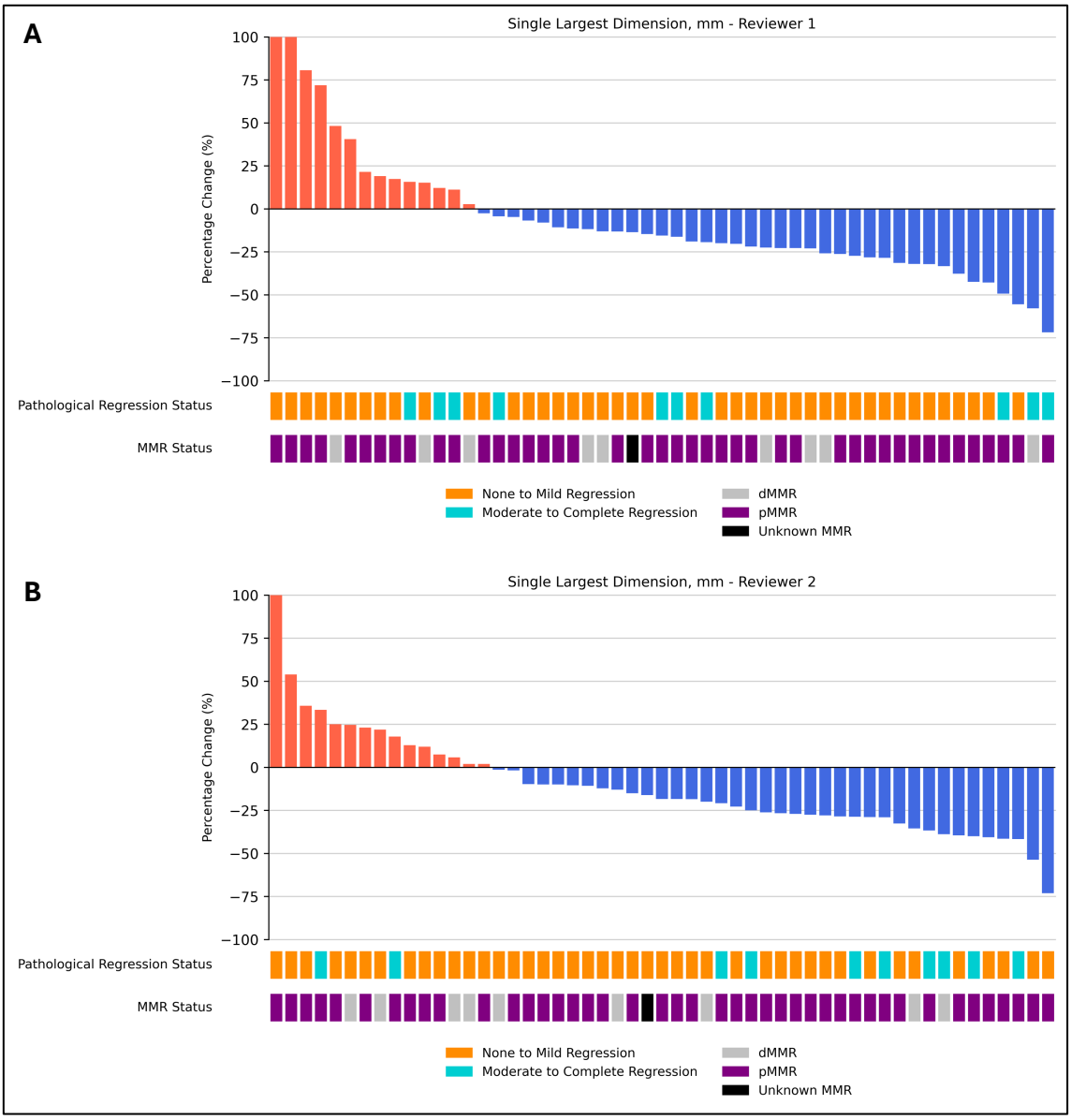



**Figure 7.9 Waterfall Plots for Change in Single Largest Diameter**

The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

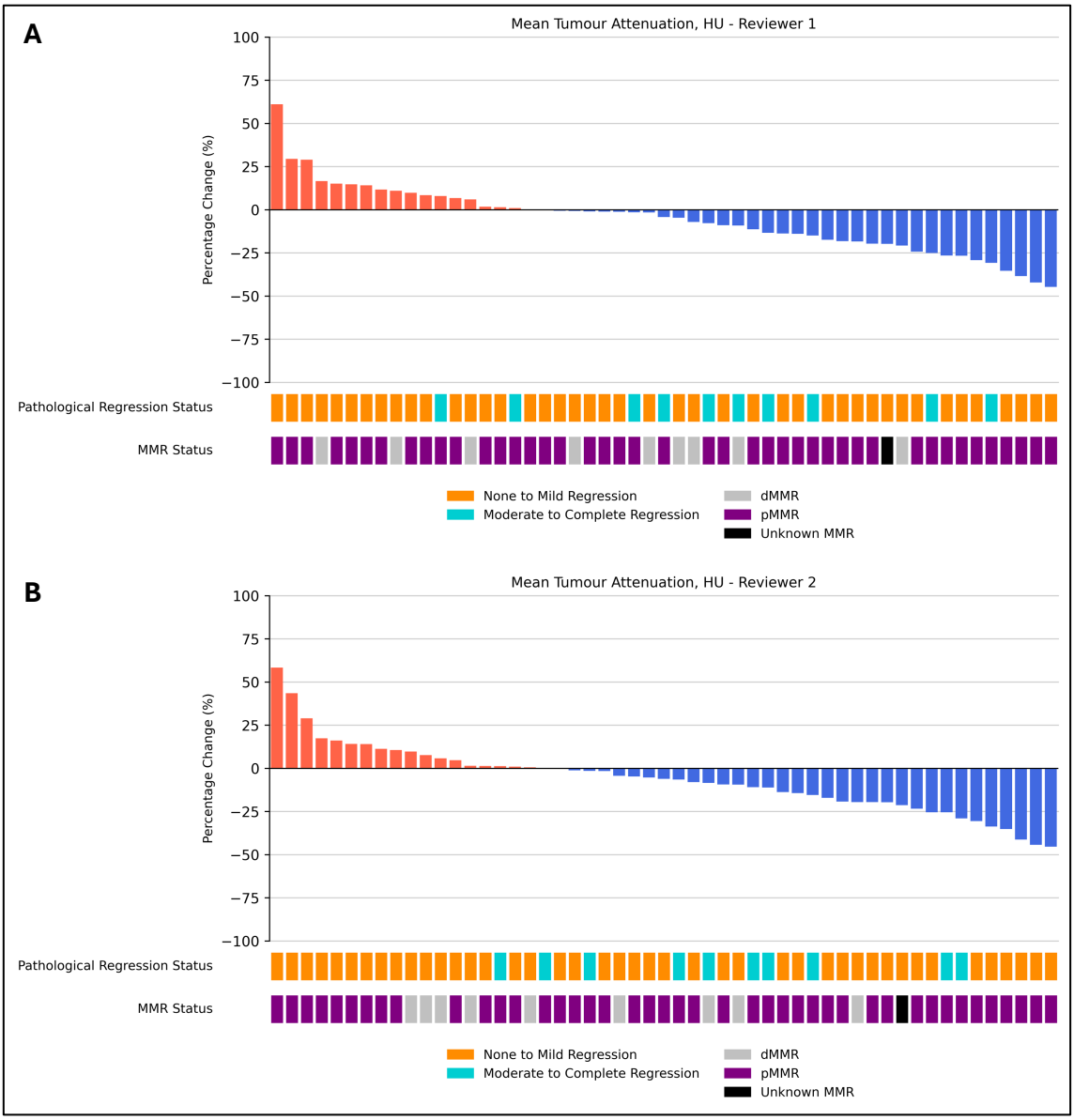



**Figure 7.10 Waterfall Plots for Change in Mean Tumour Attenuation**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

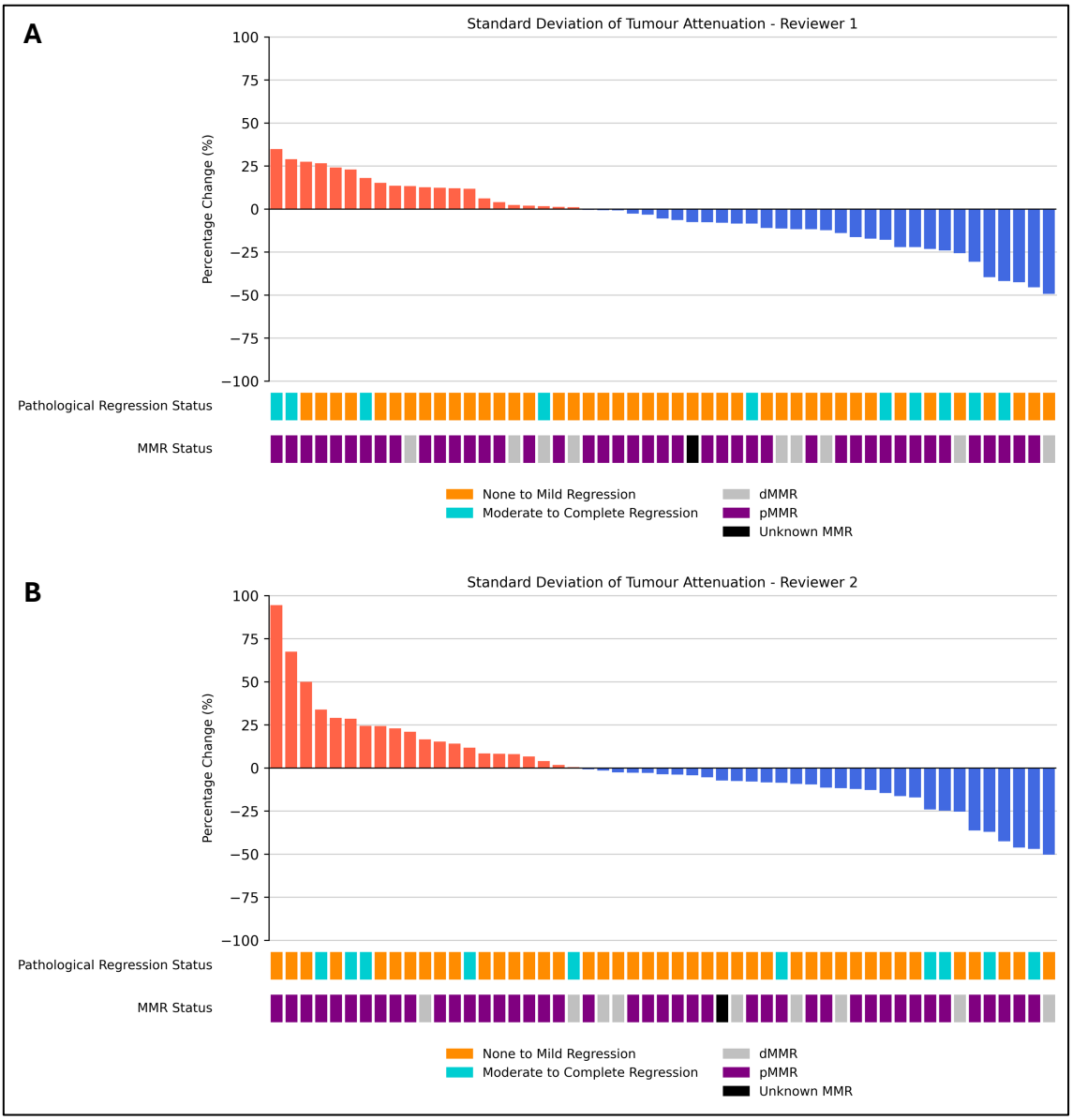



**Figure 7.11 Waterfall Plots for Change in Standard Deviation of Tumour Attenuation**
The depth of each bar represents the percentage change for an individual patient. Pathological regression and MMR statuses are indicated below each plot. A) Reviewer 1. B) Reviewer 2. dMMR = deficient mismatch repair; MMR = mismatch repair; pMMR = proficient mismatch repair.

Table 7.17 Radiological Responses According to MMR Status

| | Reviewer 1 | | | Reviewer 2 | | |
|---|---|---|---|---|---|---|
| Characteristic | pMMR | dMMR | P value [a] | pMMR | dMMR | P value [a] |
| Maximum distance beyond muscularis propria, mm | | | | | | |
| Median (IQR), % | -40.0% (-61.2 to -5.6) | -40.0% (-60.0 to 0.0) | 0.97 | -41.4% (-59.3 to 16.7) | -33.3% (-50.0 to 0.0) | 0.67 |
| Maximum tumour thickness, mm | | | | | | |
| Median (IQR), % | -29.4% (-46.2 to -12.9) | -10.5% (-27.3 to 0.0) | 0.06 | -24.3% (-45.8 to 5.0) | -20.0% (-50.0 to 0.0) | 0.92 |
| Maximum tumour thickness at invasive margin, mm | | | | | | |
| Median (IQR), % | -28.6% (-56.1 to 0.0) | -25.0% (-30.0 to 18.8) | 0.16 | -29.0% (-45.8 to -1.9) | -20.0% (-50.0 to 0.0) | 0.90 |
| Length of tumour along colon, mm | | | | | | |
| Median (IQR), % | -16.2% (-30.0 to 4.3) | -13.2% (-23.1 to 2.7) | 0.90 | -20.8% (-30.8 to 0.1) | -1.4% (-20.0 to 5.8) | 0.29 |
| Single largest diameter, mm | | | | | | |
| Median (IQR), % | -16.2% (-28.4 to 4.3) | -13.2% (-23.1 to 2.7) | 0.88 | -18.5% (-28.8 to 0.1) | -1.4% (-20 to 5.8) | 0.33 |
| Tumour volume, $cm^3$ | | | | | | |
| Median (IQR), % | -48.9% (-66.0 to -25.9) | -29.8% (-65.3 to 8.4) | 0.23 | -49.2% (-59.3 to -31.8) | -33.5% (-53.6 to 3.3) | 0.15 |
| Mean tumour attenuation, HU | | | | | | |
| Mean (SD), % | -6.5% (20.5) | -1.2% (10.6) | 0.46 | -6.9% (21.4) | -1.88% (8.9) | 0.50 |
| Standard Deviation of tumour attenuation | | | | | | |
| Median (IQR), % | -3.3% (-16.8 to 12.2) | -11.4% (-12.3 to 1.7) | 0.44 | -2.9% (-12.5 to 14.7) | -7.6% (-11.7 to -1.5) | 0.31 |

dMMR = deficient mismatch repair; IQR = interquartile range; MMR = mismatch repair; pMMR = proficient mismatch repair; SD = standard deviation.

[a] Mann-Whitney U or t test according to variable distribution.

Table 7.18 Inter-Observer Variation Analyses

| | Spearman's Rho (P value) | ICC (2,1) (95% CI) | Bland-Altman Bias (LoA) | Mean Dice Coefficient (SD) |
|---|---|---|---|---|
| Maximum distance beyond muscularis propria – Pre | 0.68 (< 0.01) | 0.82 (0.71-0.89) | -0.79 (-9.80-8.21) | - |
| Maximum distance beyond muscularis propria – Post | 0.61 (< 0.01) | 0.66 (0.48-0.79) | -1.34 (-12.49-9.81) | - |
| Maximum tumour thickness – Pre | 0.75 (< 0.01) | 0.81 (0.69-0.88) | 1.15 (-9.89-12.20) | - |
| Maximum tumour thickness – Post | 0.58 (< 0.01) | 0.64 (0.45-0.77) | -0.57 (-15.00-13.87) | - |
| Maximum tumour thickness at invasive margin – Pre | 0.70 (< 0.01) | 0.76 (0.62-0.85) | -1.58 (-13.62-10.45) | - |
| Maximum tumour thickness at invasive margin – Post | 0.51 (< 0.01) | 0.61 (0.41-0.75) | -2.06 (-16.86-12.75) | - |
| Length of tumour along colon – Pre | 0.65 (< 0.01) | 0.58 (0.37-0.74) | 0.11 (-29.09-29.32) | - |
| Length of tumour along colon – Post | 0.77 (< 0.01) | 0.71 (0.55-0.82) | 2.38 (-28.44-33.19) | - |
| Single largest diameter – Pre | 0.72 (< 0.01) | 0.69 (0.51-0.81) | -0.66 (-24.64-23.32) | - |
| Single largest diameter – Post | 0.78 (< 0.01) | 0.71 (0.54-0.82) | 1.64 (-28.80-32.08) | - |
| Tumour volume – Pre | 0.97 (< 0.01) | 0.97 (0.95-0.98) | 1.90 (-10.15-13.95) | 0.96 (0.05) |
| Tumour volume – Post | 0.98 (< 0.01) | 0.98 (0.97-0.99) | 0.78 (-9.44-11.01) | 0.93 (0.15) |

ICC = intraclass correlation coefficient; LoA = limits of agreement; SD = standard deviation.

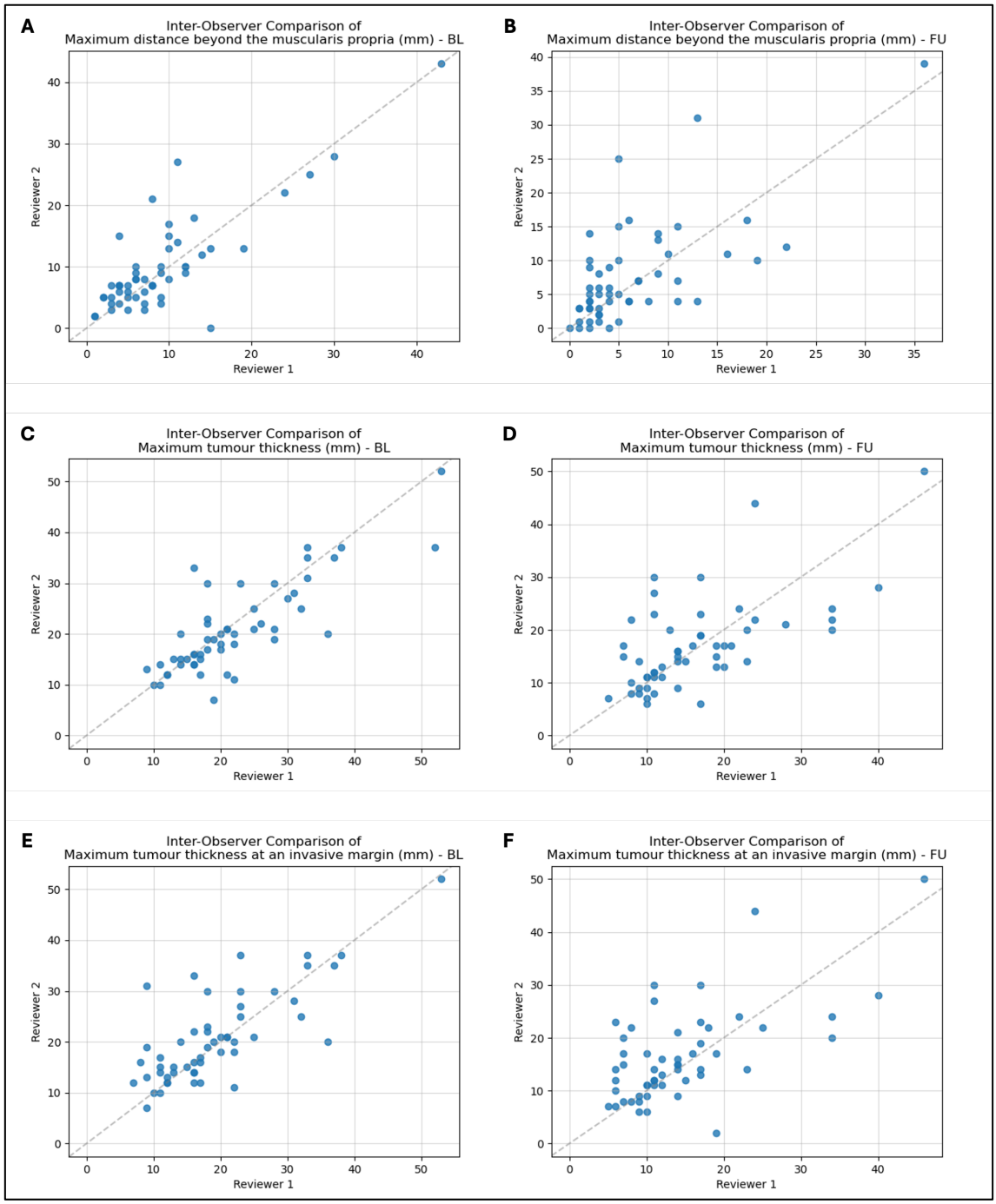



**Figure 7.12 Scatter Plots for Inter-Observer Variation 1**

A & B) Maximum distance beyond the muscularis propria. C & D) Maximum tumour thickness. E & F) Maximum tumour thickness at an invasive margin. Each blue dot represents a specific measurement across both reviewers. The dashed line indicates perfect agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); NAC = neoadjuvant chemotherapy.

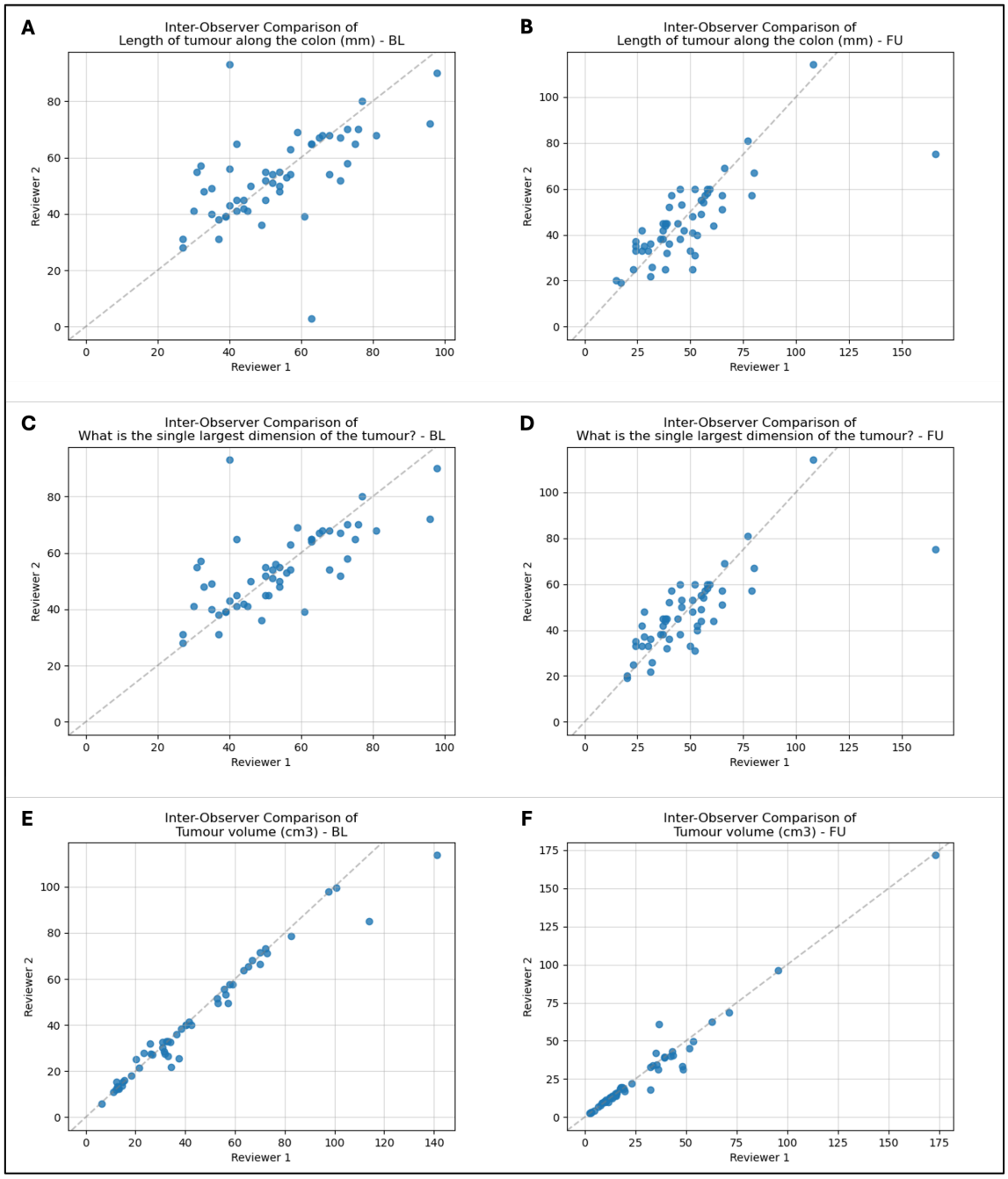



**Figure 7.13 Scatter Plots for Inter-Observer Variation 2**

A & B) Length of tumour along the colon. C & D) Single largest diameter. E & F) Volume. Each blue dot represents a specific measurement across both reviewers. The dashed line indicates perfect agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); NAC = neoadjuvant chemotherapy.

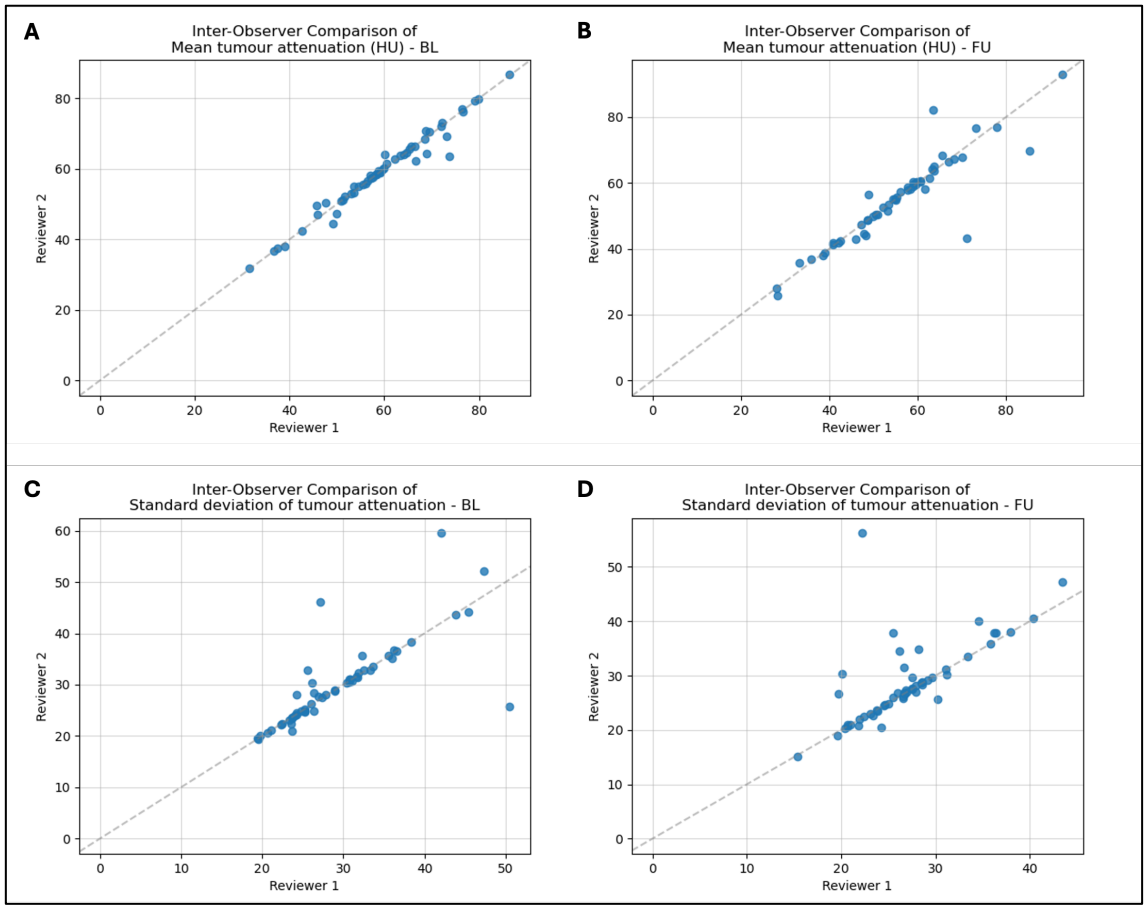



**Figure 7.14 Scatter Plots for Inter-Observer Variation 3**

A & B) Mean tumour attenuation. C & D) Standard deviation of tumour attenuation. Each blue dot represents a specific measurement across both reviewers. The dashed line indicates perfect agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); HU = Hounsfield units; NAC = neoadjuvant chemotherapy.

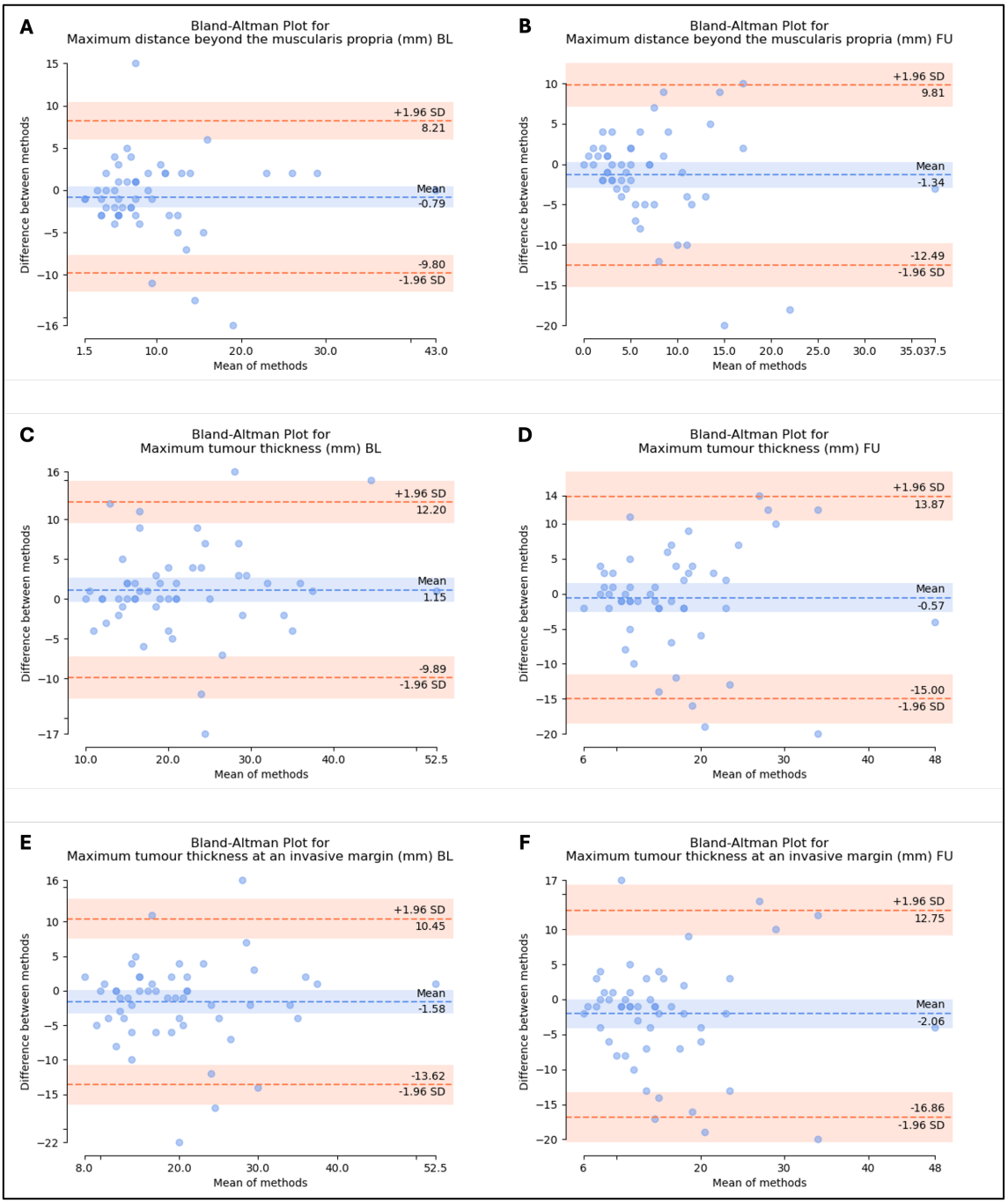



**Figure 7.15 Bland-Altman Plots for Individual Features 1**

A & B) Maximum distance beyond the muscularis propria. C & D) Maximum tumour thickness. E & F) Maximum tumour thickness at an invasive margin. Each blue dot represents the evaluation of a specific feature in an individual case. The blue line indicates the level of systematic bias. The red line indicates the limits of agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); NAC = neoadjuvant chemotherapy; SD = standard deviation.

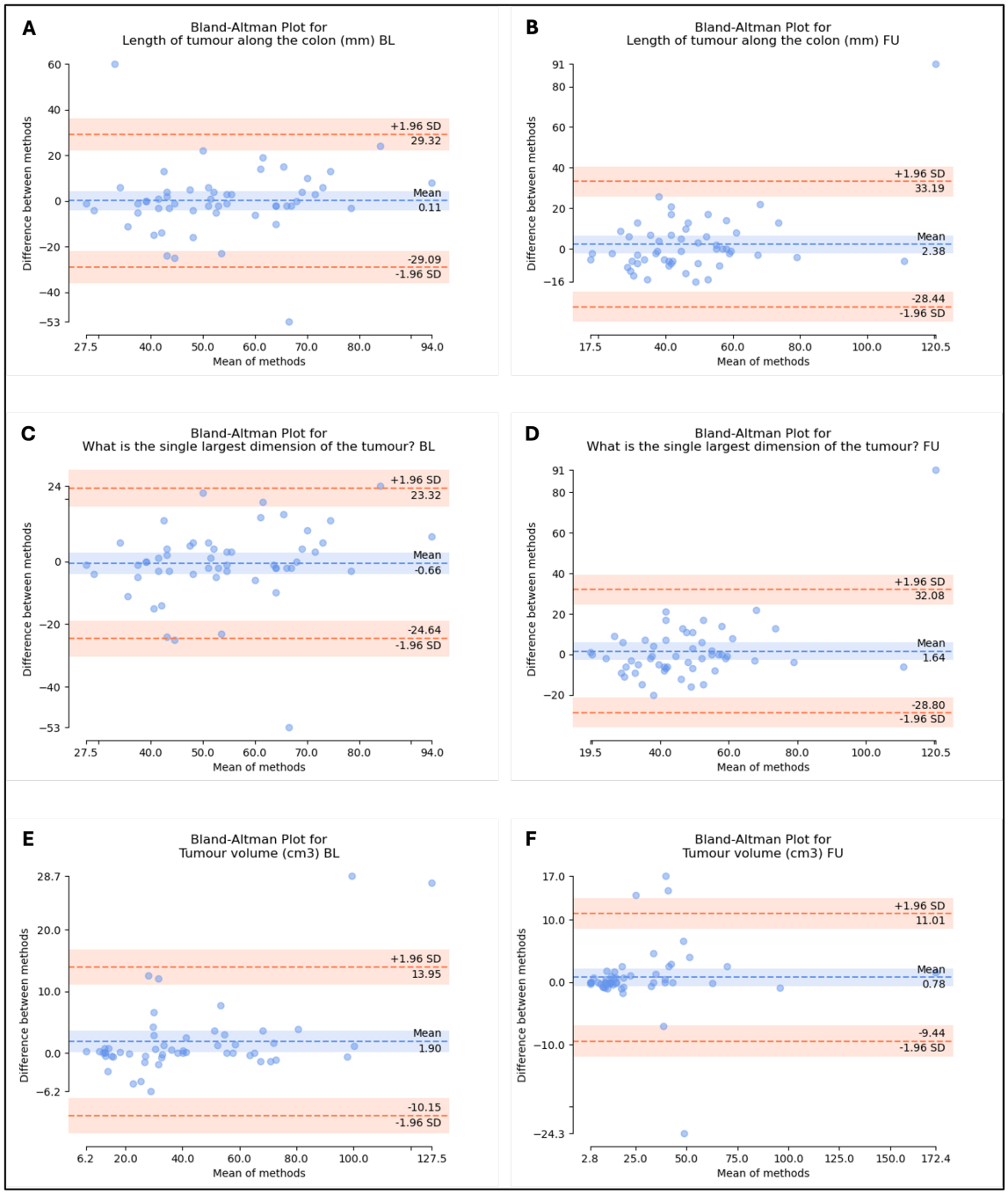



**Figure 7.16 Bland-Altman Plots for Individual Features 2**

A & B) Length of tumour along the colon. C & D) Single largest diameter. E & F) Volume. Each blue dot represents the evaluation of a specific feature in an individual case. The blue line indicates the level of systematic bias. The red line indicates the limits of agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); NAC = neoadjuvant chemotherapy; SD = standard deviation.

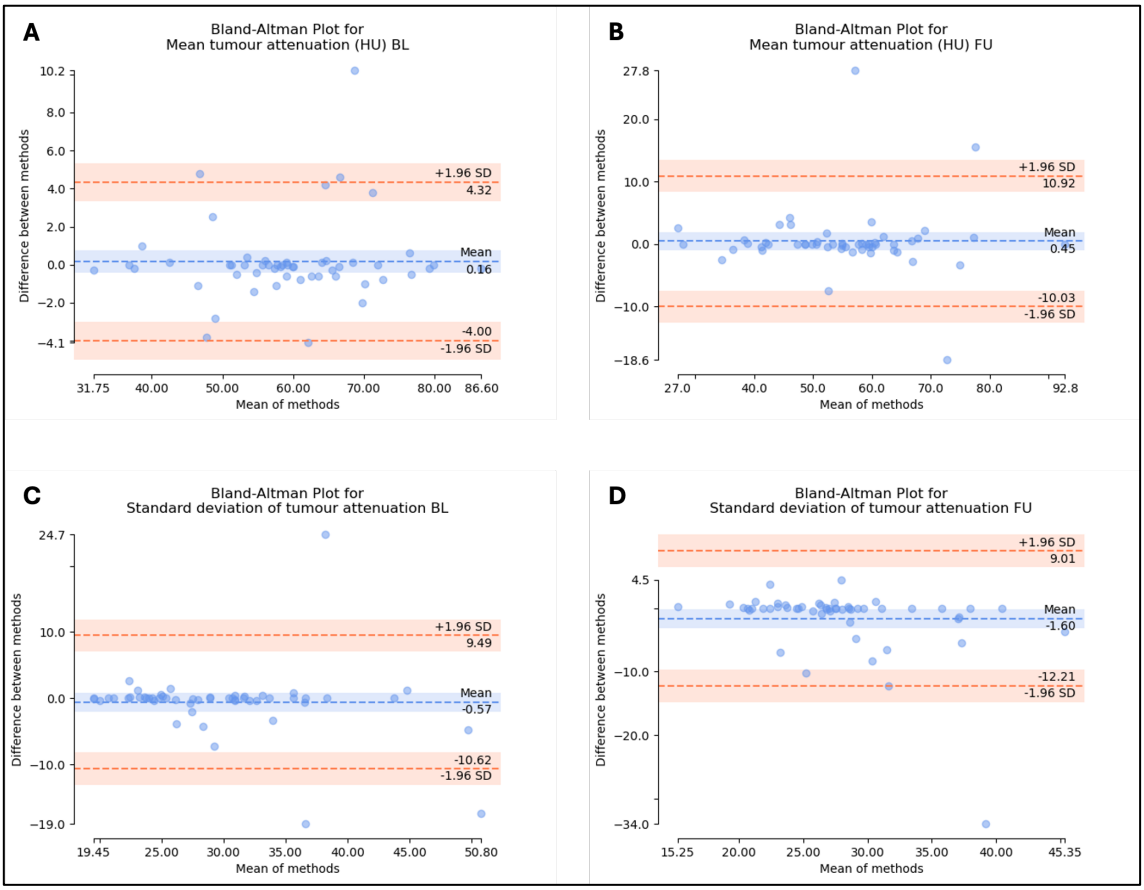



**Figure 7.17 Bland-Altman Plots for Individual Features 3**

A & B) Mean tumour attenuation. C & D) Standard deviation of tumour attenuation. Each blue dot represents the evaluation of a specific feature in an individual case. The blue line indicates the level of systematic bias. The red line indicates the limits of agreement. BL = baseline (pre-NAC); FU = follow-up (post-NAC); HU = Hounsfield units; NAC = neoadjuvant chemotherapy; SD = standard deviation.

### 7.4.3 References


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