Leeds Teaching Hospitals

Research Protocol

Short Title EMI-137 in laparoscopic colonic resections

Study Full Title: Intraoperative imaging of colon cancer using a fluorescent peptide (EMI-137) against the c-Met receptor

Sponsor Name: University of Leeds

Sponsor Number: GS16/87090

EudraCT Number: 2016-003128-22

IRAS Project ID Number: 212190

Version: 2.1 **Date:** 14th February 2018

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1.9 Registration

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1.10 Pharmacovigilance and Protocol Violation or Breach Reporting

To report any Serious Adverse Events (SAE) or SUSAR Suspected Unexpected Serious Adverse Reaction or possible pregnancy in a trial participant or serious breaches in trial conduct please contact:

UoL / LTHT Joint Sponsor QA Office (CTIMPs)

Research & Innovation Centre

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You will receive notification of receipt of SAE/SUSAR reporting.

Industrial Partner (EMI Ltd) pharmacovigilance reporting address:

globalSAEinbox@chiltern.com

Contents

2 Abbreviations

Abbreviation/ Acronym	Definition
AE	Adverse Event
AR	Adverse Reaction
ASA	American Society of Anaesthesiologists
BMI	Body Mass Index
CI	Chief Investigator
CME	Complete Mesocolic Excision
CRC	Colorectal Cancer
CT	Computerised Tomography
CTRU	Clinical Trials Research Unit
EMI	Edinburgh Molecular Imaging Limited
FL	Fluorescent light
FOBT	Faecal Occult Blood Test
GP	General Practitioner
GCP	Good Clinical Practice
HGF	Hepatocyte Growth Factor
HGFR	Hepatocyte Growth Factor Receptor
HTA	Human Tissue Act
IMP	Investigational Medicinal Product
IB	Investigator's Brochure
IV	Intravenous
LFT	Liver Function Test
LTHT	Leeds Teaching Hospitals Trust
MDT	Multi-Disciplinary Team
MET	Mesenchymal Epithelial Transition factor
MHRA	Medicines and Healthcare Regulatory Authority
MRI	Magnetic Resonance Imaging
R&I	Research and Innovation department
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
SJUH	St James' University Hospital
SOP	Standard Operating Procedure
U&E	Urea and Electrolytes
WL	White Light

3 Protocol Synopsis

General Information		
Name of Sponsor University of Leeds		
Sponsor Protocol Number GS16/87090	**EUDRACT Number** 2016-00312-22	**IRAS Project ID Number:** 212190
Investigatory Medical Product		
IMP Name	EMI-137	
IMP Supplier	Edinburgh Molecular Imaging Limited	
Trial Information		
Short Title	EMI-137 in Laparoscopic colonic resections	
Full title	Intraoperative imaging of colon cancer using a fluorescent peptide against the c-Met receptor	
Objectives	**Primary objective:** 1) To investigate the ability of EMI-137 to produce visible fluorescence of colon cancer during laparoscopic surgery. **Secondary objectives:** 1) To investigate the ability of EMI-137 to produce visible fluorescence in regional lymph nodes draining the colon cancer. 2) To investigate the concordance of visible fluorescence in colon cancer with histological stage and c-MET expression in resected specimens. 3) To investigate the concordance of visible fluorescence in cancer draining lymph nodes with histopathological evidence of metastasis. 4) To explore the tumour (signal) to background (noise) florescence 5) Investigation of the safety profile of EMI-137	

	6) Exploration of systemic, operative, and patient factors, which adversely affect EMI-137 fluorescence detection of colon cancer. 7) Study of in vivo imaging compared against ex vivo fluorescent detection.
Trial Design	Single-centre stage IIa developmental study to explore and evaluate the ability of EMI-137 to produce visible intra-operative fluorescence of primary colon cancer and lymph node metastases. In total 10 patients will be recruited. Patients will have a diagnosis of primary colon adenocarcinoma, confirmed on biopsy or on radiological imaging, and will have been discussed at the colorectal cancer MDT meeting. Imaging will have been performed less than 8 weeks prior to surgery. Patients will be fit for surgical resection of the primary cancer. They may have suspected or confirmed distant metastatic disease. The planned procedure may be of palliative or curative intent. Patients will be identified via the colorectal cancer MDT and approached to participate in the study. The research team will ensure that recruited patients me*et* all of the eligibility criteria. Patients will receive written and verbal information, and allowed at least 24 hours to consider their participation. Participating patients will provide written, informed consent. Consent will be obtained by an appropriately delegated member of team, any time up to the morning of surgery, prior to administration of the IMP. It is permissible to take trial specific consent at the same time as consent for the planned colonic resection. The patient's general practitioner will be informed of their involvement. Pre-operative patient demographics will be collected, including gender, age, BMI, baseline FBC, U&Es, LFTs and clotting profile, co-morbidities, ASA grade, and medication. All patients will undergo routine preoperative assessment, to include colonic imaging, and staging CT scan of chest, abdomen and pelvis. The planned procedure and radiological staging will be documented.

	Patients will be administered an intravenous bolus of EMI-137 at a dose of between 0.02mg/kg to 0.13mg/kg EMI-137 2.5 hours (range 1 to 3 hours) prior to the surgical procedure start time (this refers to expected "knife to skin time"). The maximum dose will be 0.13mg/mg per patient for the total operative period. The time of administration will be recorded. Patients will be closely monitored for any adverse reactions to the medicinal product (as per schedule below). The surgical procedure will be as per surgeon preference but must include laparoscopic surgery. The procedure must use a Near Infra-Red laparoscopic system such as the Karl Storz® D-Light endoscopic fluorescence imaging systems. It will be used to evaluate the colon cancer and regional lymph nodes under both white and red light. The presence of fluorescence within the primary cancer and any draining lymph nodes will be recorded relative to background fluorescence. The intensity of fluorescence will be recorded semi-quantitatively as: highly fluorescent, mildly fluorescent or isofluorescent to background. Mobilisation of structures to permit visualisation of the cancer and lymph nodes is permissible as long as the oncological principles of the operation are not compromised. Any fluorescent lymph nodes will be marked with a surgical clip (Ligaclip) to allow subsequent histopathological identification. Postoperative care will be as routinely provided. Serum blood tests for FBC and U&Es will be performed daily and LFTs, and clotting profile will be measured twice weekly as per standard care. Patients will be monitored for any adverse effects related to the medicinal product. Any adverse reactions as defined in the protocol below will be reported to the CI, clinical trial team, the industrial sponsor and where necessary, the appropriate regulatory bodies.

	The resected colon cancer specimen histopathology request form will be clearly labelled to record the patient's involvement in the clinical trial and the intra-operative use of EMI-137. The histopathologists involved in the trial will examine the specimen using routine histopathological techniques. Separate fresh tissue samples (unfixed cancer and normal mucosal tissue) will be taken, if accessible, and stored in a HTA-approved tissue bank on the St. James's Hospital site. Patients will also be consented for potential future translational research to include genomic/molecular of their specimen. Patients will be followed up as per routine practice. All patients will be reviewed in a colorectal clinic between 2 and 3 weeks following discharge, at which point their involvement in the study will cease. Patients will be allocated a unique trial number when recruited to the study and thereafter all data will be anonymised. All data will be anonymised and stored securely in accordance with University of Leeds policy for clinical trials data handling.
Trial Subject Information	
Study Population	Adult (≥18 years) patients undergoing elective surgery for primary colon cancer.
Number trial subjects	10 patients.
Age of trial subjects	Aged over 18 years at time of registration.
Inclusion criteria	• Age ≥ 18 years.

	• Patients with a diagnosis of colonic cancer (the disease can be of any radiological TNM stage and be located anywhere from the caecum up to but not including the rectosigmoid junction) • Patients with or without distant visceral or lymphatic metastatic disease. • Patients with synchronous colon cancers or polyps can participate. • American Society of Anaesthesiologists (ASA) classification ≤3. • Normal hepatic and renal function (eGFR ≥60 mls/min/1.73m^2) and bilirubin within institutional limits and/or ALT ≤2.5x upper limit of institutional normal value) on serum laboratory blood tests performed ≤30 days prior to EMI-137 administration. • Female participants who are surgically sterile (documented bilateral oophorectomy and/or hysterectomy), post-menopausal (cessation of menses for more than 1 year), or pre-menopausal with two negative urine pregnancy tests performed within 24 hours of administration of EMI-137 Injection. • Pre-menopausal female participants of child-bearing potential who agree to employ two method of contraception (as defined in eligibility criteria section 8.2) during the study period and for 90 days after EMI-137 administration. • Male participants with a non-pregnant female partner. Male participants with a pre-menopausal partner of child-bearing potential who agree to use two forms of contraception (as defined in section 8.2) during the study period and for at least 90 days after receiving EMI-137. (The only permissible exception would be if the participant had undergone documented bilateral orchidectomy or their female partner is post-menopausal (cessation menses >1 year) or has undergone documented bilateral oophorectomy and/or hysterectomy).

Exclusion criteria	• Patients who are participating in another intra-operative fluorescence study, or have participated in another fluorescence study within 3 months of the planned surgical procedure. • Received an investigational medicinal product at any dose within 28 days of planned EMI-137 administration • Patients with pre-existing inflammatory bowel disease. • Patients who have undergone neoadjuvant chemotherapy to treat the colon cancer. • Patients with impaired renal function (eGFR <60 mls/min/1.73m^2). • Patients with impaired liver function (Bilirubin above institutional limits and/or ALT >2.5x upper limit of normal). • Pregnant and breastfeeding woman. • Pre-menopausal woman planning to become pregnant within 90 days of receiving EMI-137; or pre-menopausal woman of child-bearing potential who refuse to use two forms of contraception for at least 90 days after receiving EMI-137 • Male patients with a currently pregnant partner or male patients who are planning to conceive a pregnancy with a female partner within 90 days of receiving EMI-137; or male participants who refuse to use two forms of contraception as defined in section 8.2 for at least 90 days after receiving EMI-137 with their female partner of child-bearing potential. • Poorly controlled or serious medical or psychiatric illness that, in the investigator's opinion, is likely to interfere with participation and/or compliance in this clinical trial. • Previous adverse reaction to fluorescent agents
Procedures	**Pre-operative routine care** Visualisation of the colorectum e.g. colonoscopy or CT colonography • CT scan (chest, abdomen, pelvis)

	• Local MDT discussion • Routine pre-operative assessment of fitness for surgery **Operative Procedure** Administration of 0.02mg/kg to 0.13mg/kg EMI-137 IV 2.5 hours before surgery (range 1 to3 hours). • Laparoscopic surgery for colon cancer as per MDT decision **Postoperative Procedures** • Routine postoperative care
Trial Treatment	0.02mg/kg to 0.13mg/kg EMI-137
Trial Timeline	
Expected Start Date	20th December 2017
Subject Enrolment phase	10 patients undergoing laparoscopic surgery for colon cancer – Anticipated 6 months recruitment
Follow-up duration	2 -3 weeks following hospital discharge
End of trial definition	The 'end of trial' will be defined as the last recruited patient's final data collection visit - The last visit of the last subject (LVLS). At this point, the 'end of trial notification' will be submitted to the relevant ethics committee and the Medicines and Healthcare Regulatory Authority (MHRA). The industrial partner; Edinburgh Molecular Imaging will also receive an end of trial notification.
Expected completion date	20th July 2018

4 Rationale

4.1 Colon Cancer

Colorectal cancer (CRC) is the fourth most common malignancy in the UK. In 2013, 41,112 cases were diagnosed. CRC is strongly related to age, with nearly 60% of cases diagnosed in patients over the age of 70 years. CRC continues to be the second highest cause of cancer related mortality in the UK, accounting for 10% of all cancer related deaths [1]. In the most deprived social groups this figure is greater. CRC mortality is 30% higher in males from the poorest areas than in their age-standardised counterparts from the least deprived areas. CRC poses significant disease burden, with the NHS continuing to see a year on year rise in cases, often in an older population with significant co-morbidities [1].

The anatomical distribution of CRC varies slightly between the genders and with age. However, there is a left-sided predominance, with over 30% of CRC seen in the rectum/rectosigmoid junction and a further quarter in the sigmoid or descending colon [1].

Survival rates in CRC are strongly correlated to the stage at diagnosis. One year survival data from 2012 shows 98% of patients presenting with stage I disease were alive after one year compared to only 46% with stage IV disease [2]. Long-term survival can usually only be achieved with curative resection. This involves segmental colectomy and en-bloc resection of the draining lymph node field. Currently there is significant variance of opinion regarding the radicality of resection and, in particular the extent of lymphadenectomy [3]. A method of accurately visualising the tumour and any associated metastatic lymph nodes in real-time would be invaluable for intra-operative decision making, allowing the surgeon to tailor the radicality of resection to the stage of disease.

4.2 National Screening

In 2006, the National Bowel Cancer Screening Program was introduced into the NHS [4]. In England, all patients aged 60-74 years registered with a GP are invited to undertake a Faecal Occult Blood test (FOBT) every two years. Those with a positive FOBT are offered further investigation by colonoscopy.

Ten-year longitudinal data from Scotland and Wales, who introduced FOBT screening in 2003 and for 50-74 year olds, shows a statically significant change in both detection rates and the stage at presentation. FOBT screening detects 18% of CRCs and reduces emergency presentations of CRC from 20 to 13% [4]. Importantly, the introduction of FOBT screening has led to an increase in the early detection of CRC. The percentage total of stage I CRC detected rose from 17% in the pre-screening to 28% in the post-screening era [4, 5, 6].

4.3 Surgical Resection Strategies

To maximise the chances of oncological clearance, the colon cancer needs to be removed with clear surgical margins and along with the draining veins, lymphatics and lymph nodes. The lymph nodes are divided into three tiers: tier I nodes are next to the bowel lumen (D1), tier II are contained within the mesentery along the supplying segmental artery (D2), and tier III are in close proximity the origin of the segmental vessels (D3). Resection of twelve or more lymph nodes is recommended for accurate histological assessment and staging [6, 7].

4.4 Laparoscopic Surgery

Laparoscopic colectomy for colonic adenocarcinoma was first trialled in the early 1990s [7]. After thorough evaluation and dismissal of concerns regarding increased port-site disease recurrence and inferior lymph node yields, laparoscopic colectomy became accepted and is now the preferred elective surgical approach for colorectal cancer in most centres. There are several advantages of laparoscopic as compared to open surgery, including earlier patient recovery, fewer complications, and shorter hospital stay [8, 9, 10, 11, 12]. Initially operation length was around a third longer with laparoscopic surgery. However, as colorectal surgeons have become more experienced in minimally invasive surgery and laparoscopic resection this difference has been reduced.

4.4.1 Complete Mesocolic Excision (CME)

Whilst survival rates in rectal cancer have markedly increased in recent years, rates for colon cancer have increased at a much lower rate. The improved survival seen in rectal cancer is attributed to the introduction of a standardised approach for low and middle rectal cancers, referred to as Total Mesorectal Excision (TME), along with the introduction of preoperative imaging, preoperative (chemo)radiotherapy and improved histopathological assessment. TME requires meticulous dissection of mesorectum along the mesorectal fascial plane and ligation of the supplying blood vessels at their origin [13, 14].

The same logic is being applied to colonic cancer and in an attempt to improve survival statistics [15], with the concept of Complete Mesocolic Excision (CME) and D3 lymphadenectomy. CME can be performed open or laparoscopically and involves dissection of the mesocolic envelope as an intact “package”. D3 lymphadenectomy refers to central ligation of the feeding vessels at their origin and adequate segmental colectomy to maximise lymph node clearance [3, 16, 17]. Several case series have shown CME with D3 lymphadenectomy to produce higher lymph node yields with a reduction in locoregional recurrence rates. In a recent Danish non-randomised population study, the cumulative 4-year disease-free survival for stages I-III was 85·8% after CME compared to 73·4% after non-CME surgery. Specifically, for the stage III cohort, the difference in survival was 73·5% compared

to 67·5% [17]. This compliments the low local recurrence rate of 3.6% described by Hohenberger *et al*. in their 2009 prospective study of CME [3].

CME with D3 lymphadenectomy may be beneficial in patients with lymph node involvement, but only 30% of patients in the UK present with lymph node metastases. With the National Bowel Cancer Screening Programme, this figure is likely to fall as a higher proportion of early stage I cancers are detected [4, 5]. CME with D3 lymphadenectomy also carries the risk of greater morbidity, meaning that 70% of patients without lymph node disease will be subjected to increased surgical risk. A selective approach is therefore required whereby patients with lymph node disease are offered radical CME with D3 lymphadenectomy, whilst patients without lymph node disease undergo a less radical CME with D2 resection. This requires a means of accurately identifying lymph node status either preoperatively or intraoperatively.

4.5 Colorimeteric Dye

To aid intra-operative identification of a colonic tumour, it is standard practice to preoperatively mark the site with a colorimetric dye (India ink) at colonoscopy. Although luminal tattooing is generally safe, inexpensive and quick to perform, it has some limitations: it is operator dependent and often imprecise.

Several studies have assessed the efficacy and safety of luminal tattooing. The most common problem is transmural inoculation and intraperitoneal spillage. Free India ink staining the peritoneal cavity and adjacent viscera negates the diagnostic yield and causes localised inflammation. Conversely, if the inoculation is too superficial it might not be visible at laparoscopy necessitating further intra-operative colonoscopy [18, 19].

The need for accurate intra-operative identification of colonic cancers, and the limitations of colorimetric tattooing, has driven the search for a real-time intraoperative method for cancer localisation.

5 Hepatocyte Growth Factor

5.1 Background

Hepatocyte growth factor (HGF), also known as Scatter Factor (SF) protein and c-met protein is secreted by mesenchymal cells as an inactive polypeptide and cleaved to its active form by serum protein kinases. The HGF ligand binds to the transmembrane C-Met receptor, it is also referred to MET and Hepatocyte Growth Factor Receptor (HGFR). C-Met is encoded by chromosome 7q.31 and expressed by the majority of epithelial and mesenchymal cells. Formation of the HGF-C-Met receptor-ligand complex activates phosphorylation of a tyrosine

kinase and is the first step in multiple cellular pathways. Normal function of HGF pathways is essential for cell motility in both embryogenesis and throughout adult life. HGF disrupts cadherin cell-to-cell adhesion allowing cell scattering. The overexpression and/or upregulation of both c-met protein and its receptor has been demonstrated in the stroma of several malignancies. Dysregulation of the c-met pathway is seen as a crucial step in angiogenesis and proliferation in colorectal and other cancers [20, 21, 22, 23, 24].

5.2 Tumour and Node Visualisation with EMI-137

A potential solution to the difficulties of intra-operative visualisation of colon cancers and lymph nodes is to target the c-Met receptor with a fluorescent molecular probe - EMI-137. EMI-137, developed by Edinburgh Molecular Imaging (EMI), has been shown to be safe and effective in human and animal studies. EMI-137 consists of a fluorescent cyanine dye coupled to a 26 amino acid cyclic peptide targeted to the C-Met receptor. It possesses photo-stability in white light, peak florescence at 653nm, and emission at 675nm. EMI-137 is highly selective and has a high affinity for the extra-cellular domain of human C-Met receptor owing to its low-nanomolar dissociation constant (~2 nM). It binds without activation of the c-Met receptor complex. It is renally excreted and shows low affinity for human plasma proteins [25, 26].

Burggraaf *et al*. have demonstrated the safety and efficacy of EMI-137 for the visualisation of colonic polyps at colonoscopy. This single centre study from Leiden, the Netherlands, used healthy volunteers and patients deemed at high risk of colorectal cancer to establish the optimal dose and timing of administration. This was found to be 0.13mg/kg of EMI-137 given intravenously 3 hours prior to colonoscopy. Higher doses (0.18mg/kg), although safe, created too much background (noise) fluorescence. Importantly, no serious adverse reactions to the EMI-137 were reported [26].

Using EMI-137 in patients at high risk of colonic neoplasms led to the visualisation of an additional 17 colonic polyps as compared to white light colonoscopy. These were mostly non-polypoidal and/or small lesions. Histopathological examination of the resected polyps showed a clear relationship between the degree of fluorescence and the histological dysplasia grade of the polyp. This was mirrored in the immunohistochemical examination of the resected polyps with dysplastic polyps and adenomas showing greater c-Met expression as compared to normal tissue. All of the adenomas identified with white light colonoscopy also showed increased fluorescence with EMI-137.

5.2 Risks and benefits

Participants will be selected on the basis that they have a diagnosis of colon cancer that is suitable for laparoscopic segmental colectomy. There will be the normal risk of surgical complications associated with general anaesthesia and surgical resection.

There are few risks specifically related to EMI-137. The safety profile for EMI-137 has been established by the Industrial partner EMI's own developmental research and from the clinical trial conducted by Burggraaf *et al*.

The c-Met amino acid sequence in Macaca mulatta (Rhesus monkey) is 98.2% homologous for human c-Met and is 89.5% homologous in mice. Macaca fascicularis (cynomolgus monkey) who show genetic similarity with rhesus monkeys were therefore selected by EMI for large animal model safety and pharmacology studies.

Using xenographed BALBc/A nude mice models of CRC with HT29 tumours; a subcutaneous injection of EMI-137 in to the tumour washed out of normal tissue within 120-240 minutes leaving only fluorescences in the tumour and in the kidneys. EMI-137 is rapidly cleared from the blood, with over 88% cleared within the first hour after administration. Both mouse and monkey models (using higher doses) showed peak accumulation in the renal cortex at around 4 to 6 hours after intravenous injection. Nearly all of the EMI-137 was cleared in the urine by 168 hours (7 days) after administration. Finally, using variable doses of EMI-137 between 0.02 and 0.18mg/kg in healthy volunteers demonstrated a clear dose-linear pharmacokinetic profile, similar to the animal models.

There is limited data on the reproductive toxicity of EMI-137. Edinburgh Molecular Imaging Ltd has performed repeat dose toxicity studies investigating the effects of EMI-137 on the reproductive organs of sexually mature cynomolgus monkeys. Males were administered repeated daily doses 16.8mg/kg (fifteen times the maximal clinical dose) before assessment of spermatogenesis in the testes and epididymides. In all cases no abnormalities were detected. Currently, there is no data on any potential tetratogenic effects of EMI-137 in pregnant females. Burggraaf's application of EMI-137 in 20 healthy volunteers and 15 patients at high risk of colorectal cancer undergoing colonoscopy found EMI-137 to be well tolerated with few adverse reactions. With a dose ranging from 0.02 to 0.18mg/kg, headache and somnolence were reported in 5 and 3 participants respectively. Extravasation of EMI-137 following intravenous injection caused a transient blue discolouration of the skin in 2 patients, which resolved spontaneously. Transient blue discolouration of the urine was seen in phase I animal studies, but not in a human phase II study, which is probably related to the lower dose

used. The maximum safe human dose of EMI-137 is thought to be 0.36mg/kg; in this study we will be using a maximum dose of 0.13mg/kg [23].

With a short half-life and complete urinary clearance of EMI-137 within one week, follow-up a minimum of two weeks after administration will ensure appropriate safety profile surveillance is conducted. The lack of reprotoxicity data on EMI-137 excludes pregnant woman and either partner in a couple unwilling to use two forms of barrier contraception or actively planning pregnancy within 90 days of receiving EMI-137. In human males the average duration of spermatogenesis is 74 days [27]; therefore a 90 day exclusion period is required.

There will be potential advantages to patients from taking part in the study. These include benefits arising from laparoscopic surgery that is of high quality and scrutinized by meticulous histopathological assessment. There might be a benefit from better intraoperative cancer imaging if the cancer proves to be fluorescent. Participants will also gain from a high level of monitoring vigilance in the postoperative period to detect any adverse consequences of the investigational medicine.

If the study aids the evaluation of the efficacy and safety of EMI-137 fluorescence cancer imaging, it might benefit future patients with colon cancer, enabling them to undergo tailored surgery appropriate to the biology of their cancer.

6 Aims & Objectives

The purpose of this study is to investigate the ability of EMI-137 to produce visible fluorescence of colon cancer during laparoscopic surgery. We hypothesize that colon cancer and/or metastatic lymph nodes will preferentially uptake the EMI-137 marker due to overexpression of c-Met receptor allowing accurate intraoperative localisation of the primary cancer and diseased lymph nodes.

Primary Objective

1) The primary aim of this trial is to investigate the ability of EMI-137 to produce visible fluorescence of colon cancer during laparoscopic surgery.

Secondary Objectives

1) To investigate the ability of EMI-137 to produce visible fluorescence in regional lymph nodes draining the colon cancer.
2) To investigate the concordance of visible fluorescence in colon cancer with histopathological stage and c-MET expression in resected specimens.
3) To investigate the concordance of visible fluorescence in cancer draining lymph nodes with histopathological evidence of metastasis.
4) To explore the tumour (signal) to background (noise) fluorescence
5) Investigation of the safety profile of EMI-137
6) Exploration of systemic, operative, and patient factors, which adversely affect EMI-137 fluorescence detection of colon cancer.
7) Study of in vivo imaging compared against ex vivo fluorescent detection

7 Overall Design

This is a single centre, stage IIa developmental study. 10 patients undergoing laparoscopic segmental resection for primary colon adenocarcinoma will be recruited to the study.

Pre-operative: 10 patients with primary colon adenocarcinoma will be identified in the Colorectal cancer MDT. They will be screened for eligibility and approached to participate. Potential participants will be provided with at least 24 hours to consider their involvement in the trial. Informed written consent will be obtained by a member of the research team; the It is permissible for written consent to be obtained on the morning of surgery when operative consent is re-confirmed as long as participants have been provided with the adequate information and allowed enough time to consider their involvement in the trial.

Day of Surgery: Trial participants will be admitted to SJUH and receive an IV bolus of 0.02 - 0.13mg/kg of the IMP EMI-137 2.5 (range 1 to 3) hours prior to planned surgery. Laparoscopic segmental colectomy will be performed using the a Near Infra-Red laparoscopic system such as the Karl Storz® D-Light or the NOVADAQ PINPOINT® endoscopic fluorescence imaging systems as per pre-operative plan. This might include one of: right hemicolectomy, extended right hemicolectomy, left hemicolectomy, sigmoid colectomy, high anterior resection, or subtotal colectomy. The ability of EMI-137 to produce visible fluorescence of colon cancer will be recorded semi-quantitatively as one of: highly fluorescent, mildly fluorescent or isofluorescent to background

Post-operative: Trial participants will undergo routine postoperative care. The resected specimen will undergo standard histopathological and additional trial specific

immunohistochemical and/or molecular assessment. Participants will be reviewed between 2 and 3 weeks following discharge after which their participation in the study will cease.

8.1 Centre

Leeds Teaching Hospitals NHS Trust will be the single centre for this study.

8.2 Eligibility

8.2.1 Inclusion Criteria

- Patients aged 18 years or over
- Patients with a diagnosis of colonic cancer (the disease can be of any radiological TNM stage and be located anywhere from the caecum to the up to but not including the rectosigmoid junction)
- Patients with or without distant visceral or lymphatic metastatic disease.
- Patients with synchronous colon cancers or polyps can participate.
- American Society of Anaesthesiologists (ASA) classification ≤3
- Normal hepatic and renal function on most recent blood tests on serum laboratory blood tests performed ≤30 days prior to EMI-137 administration). For the purposes of the trial normal hepatic renal function will be defined as:

  Total bilirubin within normal institutional limits and/or ALT < 2.5 X institutional upper limit of normal

  eGFR ≥60mls/min/1.73m^2

- Female subjects need to be either surgically sterile (have undergone bilateral oophorectomy and/or hysterectomy), post-menopausal (cessation of menses for more than 1 year), or pre-menopausal with two negative urine pregnancy tests performed within 24 hours of administration of EMI-137 Injection.
  Pre-menopausal female participants of child-bearing potential must also agree to employ two effective method of contraception for 90 days after EMI-137 administration, at least one of which must be a “highly effective contraceptive method”. “Highly effective contraception methods” are defined as methods that can achieve a failure rate of less than 1% per year when used consistently and correctly [28]. Such methods include:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
    - oral
    - injectable
    - implantable
    - intrauterine device (IUD)
    - intrauterine hormone-releasing system (IUS)
- Vasectomised partner
- Bilateral tubal occlusion
- Sexual abstinence when in line with the preferred and usual lifestyle of the patient. (Sexual abstinence defined as abstaining from all penetrative sexual intercourse for a continuous period of 90 days after receiving EMI-137. Any form of periodic abstinence for example around the date of presumed ovulation will not be considered as sexual abstinence and as such will not be deemed an appropriate method of contraception). The additional method of contraception used with an “effective method of contraception” as listed above can include barrier methods, including but not limited to male and female condoms. Female diaphragms and cervical caps when used with spermicide will also be considered an acceptable additional method of contraception. Importantly, all “natural family planning” and withdrawal methods of contraception will not be considered a suitable second method of contraception for the purposes of this trial. This includes but is not limited to, all methods of calendar abstinence; sympthermal monitoring; coitus interruptus; the use of spermicides alone and lactational amenorrhoea. Male participants with a non-pregnant female partner; and males with a female partner of child-bearing potential who agree to using two forms of contraception (as defined above, section 8.2) with this partner for at least 90 days after receiving EMI-137.

8.2.1 Exclusion criteria

- Participation in another intra-operative fluorescence study, or participation in another fluorescence study within 3 months of the planned surgical procedure.
- Received an investigational medicinal product at any dose within 28 days of planned EMI-137 administration.
- Previous adverse reaction to fluorescent agents.
- Pre-existing inflammatory bowel disease.
- Patients who have undergone neoadjuvant chemotherapy to treat the colon cancer.
- Patients with impaired renal function (eGFR <60 mls/min/1.73m^2).
- Patients with impaired liver function (elevated total serum bilirubin above normal institutional limits and/or serum ALT >2.5 X institutional upper limit of normal).
- Pregnant woman or breast feeding mothers.
- Pre-menopausal women planning to become pregnant within 90 days of receiving EMI-137 or female participants of child-bearing potential who refuse to employ two forms of contraception (as defined above, section 8.2) for at least 90 after receiving EMI-137.
- Male patients with a currently pregnant partner or male patients who are planning to conceive a pregnancy with a female partner within 90 days of receiving EMI-137: or male participants with a female partner of child-bearing potential who refuse to use two forms of contraception (as defined in section 8.2) for at least 90 days after receiving EMI-137.
- Poorly controlled or serious medical or psychiatric illness that, in the investigator's opinion, is likely to interfere with participation and/or compliance in this clinical trial.

8.3 Concurrent Clinical Trials

Patients will be screened for inclusion in other clinical trials. Providing there is no conflict, patients may be included in both this study and other trials not involving florescent medicinal products. Patients cannot participate in any other fluorescent trials intra-operatively, or in the 3 months prior to participation in this study. Participants may be screen for, and approached to participate in concurrent genetic studies.

8.4 Surgeon Eligibility

The surgeons participating in this study must meet the below eligibility criteria:

- Proficient with laparoscopic colon cancer surgery (defined as performing a minimum of 15 laparoscopic colonic resections/year for the purpose of this trial).
- Trained in Good Clinical Practice (GCP).

8 Recruitment Process

It will be the responsibility of the recruiting site, Leeds Teaching Hospitals NHS Trust (LTHT), to maintain GCP and meet all ethical standards during the recruitment process.

9.1 Informed Consent and Eligibility

Patients suitable for participation in this study will be identified via the local MDT meeting. They will be approached during their routine clinic appointment. They will be provided with verbal and written information about the trial, which will include the rationale behind the trial, possible risks and benefits of participation, and implications of their involvement.

Patients will usually be given a minimum of 24 hours to consider their involvement prior to surgery. However, an arbitrary 24 hour period will not be enforced if a participant or their consultee wished to indicate to participate sooner than this.

Patients who fulfil the eligibility criteria will be asked to give informed written consent. This will be obtained by a designated medically qualified member of the research team. It is permissible to obtain written consent on the morning of surgery when confirming operative consent, if the patient has been offered adequate information and time to consider their involvement in the trial. The patient's right to refuse consent without providing reason will be respected. At any time, a patient can withdraw from the trial without reason. Patients will be made aware that withdrawal from the study will not prejudice their care.

A record of the consent process will be filed in the Investigator Site File. A copy of the consent form will be given to the patient and a copy filed in the medical records.

9.2 Loss of capacity following informed consent

Where valid, informed consent is obtained from the patient and the patient subsequently becomes unable to give informed consent by virtue of physical or mental incapacity, the consent previously given when capable remains legally valid. Participants who lose capacity after informed consent has been obtained will continue with protocol treatment and follow-up at the discretion of the treating investigator.

9.3 Registration

Eligibility will be confirmed by a medically qualified doctor and written informed consent obtained prior to registration. To register a patient for this clinical trial, please contact the trial research fellow, Gemma Armstrong in the first instance. Alternatively contact, Catherine Moriarty, Senior Surgical Research Sister (as per Key contacts on page 3).

The following information will be required at registration:

- Name of person enrolling the participant
- Name of treating surgeon
- Participant details, including initials, date of birth, NHS number and gender
- Confirmation of eligibility
- Confirmation of written informed consent
- Planned operation (for example right hemicolectomy, extended right hemicolectomy, sigmoid colectomy)
- Planned operation date

9 Intervention

10.1 Pre-operative Investigations

All patients will undergo routine pre-operative investigation as per local protocol for the investigation and management of suspected CRC. Any additional investigations deemed necessary for the assessment of the patient prior to surgery will be permissible.

10.1.1 Radiological imaging

Pre-operative investigations will follow the routine local best practice pathway. All patients will undergo a staging CT scan of the chest, abdomen and pelvis less than 8 weeks prior to surgery. This may include CT colonography either where the patient has had an incomplete endoscopic assessment of the colon or where the initial colonic assessment has been performed radiologically. The CT results will be captured on a dedicated CRF, including:

- the radiological T-stage
- presence or absence of involved lymph nodes and their characteristics, size, and anatomical location
- any co-existent pathology
- the presence of metastatic disease

10.1.2 Colonoscopy and Colorimetric dye

Pre-operatively patients may undergo colonoscopy and endoluminal tumour tattooing with India ink. However, the application of a colorimetric tattoo is not essential for participation in the clinical trial.

10.2 Administration of EMI-137 marker

Two and a half hours (150 minutes) (range 1 to 3 hours (60 to 180 minutes)) prior to surgery, participants will be given an intravenous bolus of EMI-137. All participants will receive a dose between 0.02mg/kg and 0.13mg/kg body weight. The planned trial dose is also the maximum dose permissible, 0.13mg/kg as established by Burggraaf's application of EMI-137 in colonoscopy. If during the course of the clinical trial, the CI and clinical team identify this dose is inadequate, for example produces excessive background fluorescence and impairs intra-operative visualisation subsequent patients will be administered a lower dose of the IMP. The total dose administered will not exceed the safe dose of 0.13mg/kg in any participant. The time of administration will be recorded accurately and patients will be observed closely for any signs of adverse reaction.

10 Surgery: laparoscopic or laparoscopic assisted colectomy

The initial surgical procedure will be a diagnostic laparoscopy performed using a Near Infra-Red laparoscopic system such as the Karl Storz® D-Light or the NOVADAQ PINPOINT® endoscopic fluorescence imaging systems in white light (WL) mode. This will visualise the primary tumour site and any visible suspicious lymph nodes or peritoneal metastases. The laparoscopic system will then be switched to red light mode. The operating surgeon will assess the degree of tumour fluorescence (signal) to background (noise) generated by the EMI-137 marker. This will be recorded semi-quantitatively as: highly fluorescent, mildly fluorescent, and isofluorescent to background colonic resection will be performed as per the pre-operative plan and as per surgeon preference. The oncological principles of colonic resection will be respected.

11 Histopathological and molecular assessment

The resected colonic specimen and any additional resected lymph nodes will be subject to routine histopathological assessment as per institutional policy. This will include fresh and fixed specimen photography. In addition to routine procedures, the lymph nodes will be dissected out and mapped on to the photographs to allow correlation with the fluorescence status.

In addition, specimens will be transported to the university and may undergo:

- Assessment of C-Met and its receptor expression by immunohistochemistry in the primary cancer and any resected lymph nodes (this refers to nodes that exhibiting intra-operative (in-vivo) fluorescence and those that are isofluorescent to background (not exhibiting increased intra-operative fluorescence)).
- Correlation of the degree of fluorescence in the fixed specimen & nodes using fluorescent microscopy.
- Correlation of c-Met expression and degree of fluorescence.
- Step sectioning and immunohistochemistry for cytokeratins on all fluorescent nodes that were negative on standard histopathological examination.
- Scanning of the glass slides and digitisation of specimen photographs to form a permanent record of the pathology (the original glass slides will be returned to LTHT after scanning).

All standard histopathology assessment will be conducted on-site within the LTHT pathology department as per appendices 1 & 2 in accordance with Royal College of Pathologists best practice guidance. LTHT pathology department is accredited to ISO 15189. Additional trial specific pathological assessment will be conduct in University of Leeds laboratories. The university pathology laboratory adheres to national GCP standards and its own pathology quality policy.

12 Post-operative care

Post-operative care will be as per the standard pathway. Participants will be monitored for the unlikely event of any adverse effects attributable to EMI-137. This will include the standard plan of daily U&E and FBC measurement and twice weekly LFTs and clotting profile blood tests. The frequency may be increased after individual patient assessment.

Patients will be followed up between 2 and 3 weeks following discharge from hospital, after which their involvement in the study will cease.

13 Withdrawal criteria

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of the clinical team or the participants themselves. All patients will be able to withdraw from the study at any time, and will be reassured that this will not influence their care.

Any participant who is withdrawn by the trial team, selects to self-withdraw, or is offered an alternative treatment will continue to be offered follow-up assessment. The appropriate CRFs will continue to be completed.

If a trial participant withdraws for any reason, the trial team will endeavour to fulfil the wishes of the participant regarding continued contact and follow-up. The research fellow attached to this trial and/or the lead investigator should be informed of any withdrawal as they will be responsible for ensuring the appropriate level of contact is provided to these participants and for ensuring the relevant documentation is completed.

It will be made clear to any participant specifically withdrawing consent that safety data will continue to be collected for regulatory reporting and will be included in any safety analysis. The participant will also be made aware that if any significant new information becomes available with regard to the treatment they have received trial it may be necessary to contact them in the future.

14 Trial Medical Product Management

EMI-137 is classified as an Investigational Medicinal Product (IMP).

14.1 Investigational Medicinal Products

EMI-137 will be administrated by intravenous bolus at a dose of 0.02mg/kg to 0.13mg/kg body weight. The expected trial dose is 0.13mg/kg. This may be adjusted during the course of the clinical trial with evolving results from background to signal ratio.

No placebo or comparative drug will be used.

14.2 IMP supply, labelling & handling

Edinburgh Molecular Imaging Limited (EMI) will supply the IMP, EMI-137, to LTHT pharmacy. It will be supplied with a trial specific label, which will conform to Directive 2001/20/EC and Medicines for Human Use (Clinical Trials) Regulation. The IMP will be delivered to LTHT pharmacy department who will inspect the stock for signs of damage, verify the quantity, and identify of the trial drug delivered. The Pharmacy department will ensure the IMP is stored

securely and separately from all normal hospital stock medication and as per manufacture instructions. They will be responsible for dispensing the prescribed IMP to the research team on the day of surgery.

All EMI-137 material containers will be returned to the supplier EMI or destroyed at site.

The IMP must be stored at 5°C (between 2°C and 8°C) and protected from direct light. Once reconstituted EMI-137 is photo-stable for 24 hours and must be used within this time period. If the EMI-137 is not used within 24 hours of reconstitution or before the expiry date of the dry product, the EMI-137 will be disposed of as per manufacturer and local pharmacy guidelines.

14.3 EMI Preparation

EMI-137 will be prepared in accordance with manufacturer instructions.

14.4 EMI-137 administration

The investigator at LTHT will be responsible for ensuring the IMP is reconstituted and administered as per manufacture instructions. The IMP will be reconstituted with 5mL of sterile water for injection immediately prior to intravenous injection. The sterile water used for reconstitution will be an authorised stock product, supplied by, and in accordance with standard pharmacy policy at the clinical site, LTHT. The supply, and adequacy of the sterile water used for the reconstitution of the IMP will be entirely the responsibility of the clinical site team. When reconstituted each vial contains a clear dark blue solution of 4.8mg/mL of EMI-137 in a 50mM phosphate buffer. All trial participants will receive a maximum dose of 0.13mg/kg two and a half hours prior to the start of surgery (permissible range 1 to 3 hours).

15 Assessment and data collection

The trial site, LTHT, will be responsible for producing and maintaining the essential documentation relating to the on-going trial. The site will store copies of the completed CRFs securely and comply will all data protection laws and University of Leeds clinical trial data regulations.

15.1 General CRF completion guidance

The CI, or clinical staff authorised by the CI, will collect data using paper CRFs. A record of authorised personnel will be recorded in the trial specific Authorised Personnel Log. Data will

be collected from the patient's medical records and directly from source data obtained intra-operatively and entered in to the dedicated CRF.

It will be the responsibility of the Authorised Personnel completing the CRFs to ensure the forms are anonymised and the confidentiality of participants is maintained. Completed CRFs should contain the unique trial number allocated to each participant, their initials and date of birth only. The exception will be the consent forms; the participant name and signature must not be removed from these forms. The consent forms should be returned to the LTHT Research Fellow separately from the anonymised CRFs. Digital copies of the forms may also be made. Digital and paper copies of trial documents will be stored securely in accordance with University of Leeds trial data handling regulations and in full compliance with the Data Protection Act 2008.

15.2 Perioperative assessment and data collection

The CI and Authorised Trial Personnel will have overall responsibility for assessing patients for eligibility. A separate CRFs will be completed for each trial participant, pre-operatively, intra-operatively and a post-operatively.

15.3 Pre-operative assessment and data collection

General assessment for fitness for surgery and for the surgical procedure required will be as per institutional policy. The clinical trial team will be responsible for ensuring the patients me*et a*l of the eligibility criteria prior to registration and enrolment.

Informed trial specific written consent will be obtained by an appropriately delegated member of the research team prior to the commencement of any trial specific interventions.

15.4 Operative Assessment and data collection

An intra-operative CRF will be completed for each trial participant. This CRF will collect data relating to:

- Consultant Surgeon operating
- ASA grade
- Assessment of tumour +/- node fluorescence
- Assessment of tumour signal to background noise ratio

- Presence or absence of colorimetric endo-luminal tattoo.
- Outcome of surgical procedure (curative, palliative or unresectable)
- The surgical procedure performed
- Whether there was a change to the pre-planned procedure intra-operatively
- Any intra-operative complications

15.5 Histopathology assessment

The resected colonic specimens will be assessed according to local pathology department protocols. This may include:

Fresh and fixed specimen photographs including cross-sectional slices

Number and size of detected lymph nodes (involved and non-involved)

Estimated position of lymph node station according to the Japanese system

Final histopathological report

The specimens will also undergo trial specific immunohistochemical assessment. This may include:

Assessment of tumour and lymph node expression of c-met receptor

Assessment of normal tissue expression of c-met (HGF)

Step sections and cytokeratin assessment of fluorescent lymph nodes that are negative on standard pathological analysis

Fluorescence obtained from fixed specimen

During the consent process, participants will be asked whether they agree to storage of their pathology specimen (formalin fixed and fresh frozen tumour and normal tissue) for the purposes of additional future translational research, to include genomic/molecular assessments. Refusal of this additional step will not alter a patients' eligibility to participate in the trial. These specimens will be stored securely in a HTA licensed facility at University of Leeds. Formalin fixed samples will be labelled with unique trial number and local pathology

number. Fresh frozen samples will be labelled with unique pathology identifier, trial number and date of birth.

15.6 Post-operative assessment and data collection

The frequency of post-operative observations will follow institutional guidelines for the standard post-operative care of surgical patients.

The early warning score will be calculated from the respiratory rate, oxygen saturations, heart rate, blood pressure and temperature for each patient. The frequency of these observations will be tailored to the clinical needs of each patient throughout their post-operative recovery. If these values are satisfactory the frequency of observation will be reduced to a minimum of four times a day.

Daily blood tests for FBC and U&E will be performed for the first five days and clotting and LFT will be measured weekly unless there is an individual clinical need to increase the frequency of serum blood analyses.

15.7 Post-operative follow-up arrangements

All trial participants will have a single follow-up visit at between 14 and 21 days following discharge, after which their involvement in the study will cease. The purpose of this visit will include assessment for any post-operative complications, including adverse events relating to EMI-137. This follow-up appointment will coincide with routine postoperative follow-up to minimise patient inconvenience.

15.8 End of Trial

The end of the trial will be when the last recruited patient has completed their post-operative clinic review appointment and the CRF is filed with the clinical trial team. This conforms to the European Clinical Trial Directive definition. The end of trial notification will be filed with the regulatory competent authority, ethics committee, trial sponsor and industrial partner.

If the study is terminated prematurely, an end of trial notification will be filed with a report outlining the reason(s) for the deviation from the trial schedule and factors negating termination.

16 Safety Reporting

The safety of the IMP will be actively assessed from registration through to the follow-up clinic appointment. After active trial monitoring has ceased, the site clinical trial team will still have an ongoing responsibility and duty of care for safety reporting should they be notified of any SAE/SUSAR at a later date. Safety relates to any issue arising from the administration of the IMP or to surgical mortality and morbidity. The safety data will be collected via CRFs.

If any serious or potentially serious adverse events arise during the trial, this data will be expedited to the Sponsor QA office, the Clinical trials team and the IMP supplier EMI, as a matter of urgency. All SAE/SUSAR will be reported to the Sponsor Quality Assurance (QA) Office as per contact details in section 1.10 within 24 hours of becoming aware of the event and to EMI Ltd as per their approved pharmacovigilance reporting system. Notification of receipt of reported adverse event will be issued by the QA team. The QA team will perform onward reporting to the appropriate regulatory authorities where necessary.

16.1 General Definitions

The following section makes reference to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 2009 publication [28] and to accepted and documented known risks and unavoidable complications of laparoscopic and colonic surgery. This includes the operative experience of the colorectal team at SJUH.

16.1.1 Adverse Events (AE)

An adverse event is defined as any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product. The adverse event may not necessarily have a causal relationship with the study drug or intervention. An AE can therefore be considered as any unfavourable or unintended sign, symptom or disease process occurring in a trial participant, either related to or entirely unrelated to their participation in the trial.

16.1.2 Adverse reactions (AR)

An adverse reaction is defined as an untoward and unintended response to an investigational medicinal product related to any dose administered. This definition implies a reasonable possibility of a causal relationship which is supported by facts, evidence or arguments to suggest a causal relationship. This definition includes medication errors and uses outside that foreseen in the protocol (i.e. if an AR occurs as a result of a medication error).

16.1.3 Serious Adverse Events (SAE)

Any untoward medical occurrence or effect that:

- results in death,

- is life-threatening,

- requires hospitalisation or prolongation of existing hospitalisation,

- results in persistent or significant disability or incapacity,

- is a congenital anomaly or birth defect,

- jeopardised the subject or required intervention to prevent one of the above,

- is otherwise considered medically significant by the Investigator.

Medical and scientific judgement will be exercised in deciding whether an event is serious). These characteristics / consequences will be considered at the time of the event and will not refer to an event which hypothetically may have caused one of the above.

Where an SAE is deemed to have been related to the IMP used in the trial, the event is termed as a Serious Adverse Reaction (SAR). In this trial any SAE at least possibly related to the IMP will be considered and reported as an SUSAR.

16.1.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)

An adverse reaction, the nature and severity of which is not consistent with the applicable product information as issued by the IMP manufacturer and supplier, Edinburgh Molecular Imaging (EMI). Severity describes the intensity of the event. EMI Ltd will supply an IB including detailed "Reference Safety Information (RSI)" to the clinical site LTHT. The CI will assess all suspected SAEs and make reference to the RSI provided. All SAEs at least possibly related to the IMP regardless of whether they are considered expected and listed in the RSI will be reported via the pharmacovigilance pathway (section 1 contact details) as an SUSAR.

In all previous clinical applications of EMI-137 no SAE were reported. Therefore, all SAEs at least possibly related to the IMP – EMI-137 will be considered unexpected and reported as SUSARs

16.1.5 Operational Definition and Reporting Adverse Events/Reactions

Any adverse event/reaction detected by participant self-reporting, clinical examination, laboratory blood testing or other investigation will be recorded on the relevant CRF. Data on adverse reactions/events will be recorded from the point of registration to the end clinic visit. The reported AE/AR will be evaluated for intensity and severity according to the National

Cancer Institute Common Terminology Criteria for Adverse Events V4.0 (NCI-CTCAE) [29] definition.

At the end of study, the industrial partner EMI Ltd, will receive an end of study report detailing all recorded AEs/ARs regardless of causality.

16.1.6 Operational Definition and Reporting SAEs/SARs and SUSARs

ALL SAE, SAR and SUSARs occurring from the time of registration to the end review appointment will be recorded on the appropriate form and the CI and research fellow will be made aware within 24 hours of identification. The Sponsor's Office QA office will be notified using the correct form within 24 hours of awareness of the event. Once the concern has been resolved, the original form should be returned to the research fellow and a copy filed in the patient's clinical notes.

If the clinical team are made aware of an SAE/SAR or SUSAR after the period of active monitoring has ceased; they remain responsible for reporting this event to the appropriate regularity body and if required, acting upon the information received to ensure the safety of the individual patient and all future trial participants.

It will remain the responsibility of the LTHT research team and primarily the clinical trial research fellow to notify the Industrial Partner of any SAEs/SAR or SUSARS reported to the team, the Sponsor and if appropriate, the regulatory agencies.

For each **SAE, SAR, and SUSAR** the following information will be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator)*
- whether the event would be considered expected or unexpected.

*Assessment of causality and preliminary expectedness will be made by the CI. If the CI is unavailable, initial reports without causality and expectedness assessment will be submitted urgently and will be followed up by medical assessment as soon as possible thereafter. The CI may classify the causality as related; probable; possible; unlikely or unrelated. The assessment of causality and expectedness will be using the clinical experience of the CI and

made against the Reference Safety Information (RSI) supplied by the industrial partner EMI Ltd in the current version of the Investigator's Brochure for the IMP, EMI-137.

All SAEs assigned with a suspected and unexpected relationship to IMP-treatment will be classified as SUSARs and will be subject to expedited reporting to the Medicines and Healthcare products Regulatory Authority (MHRA).

Each event must be reported separately and not combined on one SAE form.

16.1.7 Expected AEs

The following events will be classed as expected surgical AEs. Any such events meeting the seriousness criteria should be reported as SAEs but will not be reportable as SUSARs unless the Investigator considers the severity to be unexpected:

Perioperative

Operative

- Several serious and/or frequently occurring risks/complications directly related to general anaesthesia and/or colonic resection surgery and/or immobility during the post-operative period, entirely irrespective of the administration of the IMP - EMI-137 will be considered as "expected perioperative adverse events". This are all well documented complications of major oncological colonic surgery and in the opinion of the CI and research team can be "expected"; Damage to organ/structure e.g.
    - Bowel
    - Bladder/ureter
    - Major vessel
    - Nerves
- Faecal contamination
- Haemorrhage
- Surgical emphysema
- Failure of surgical equipment (laparoscopic equipment)

Post-operative

- Anastomotic leak
- Gastrointestinal fistula
- Gastrointestinal ischaemia/necrosis
- Gastrointestinal obstruction

- Gastrointestinal perforation
- Gastrointestinal stricture/stenosis
- Gastrointestinal ulceration
- Haemorrhage
- Hernia
- Ileus
- Intra-abdominal/pelvic abscess
- Post-operative peritonitis
- Stoma prolapse/necrosis
- Urinary dysfunction
- Urinary retention
- Wound infection
- Wound dehiscence

Cardiorespiratory

(May be operative or post-operative)

- Respiratory, including
- Acute respiratory distress syndrome/respiratory failure
- Aspiration
- Atelectasis
- Bronchospasm
- Pleural effusion
- Pneumonia/chest infection
- Pulmonary embolus (or DVT)
- Cardiac, including
- Arrhythmia
- Cardiac failure

Ischaemic heart disease/ myocardial infarction / cardio-respiratory arrest

Other

- Acute renal failure

- Back pain
- Cerebrovascular attack/stroke
- Disseminated intravascular coagulation
- Distal limb ischaemia/compartment syndrome
- Metabolic acidosis
- Necrotising fasciitis
- Pressure sore
- Pseudomembranous colitis
- Sepsis
- Subcutaneous emphysema
- Urinary tract infection
- Delirium

16.2 Pregnancies and Suspected Pregnancies

Pregnancy and breastfeeding are contra-indications to the administration of EMI-137. If a participant or partner of a participant becomes pregnant or is suspected to be pregnant while taking part in this clinical trial, or up to three months following the last administration of EMI-137 the key contacts for pharmacovigilance (section 1.9) must be notified within 24 hours of becoming aware of the pregnancy. All patients will be provided with contact details of who to contact in the event of pregnancy in three months following EMI-137 administration. This will be clarified in the patient information sheet. The GP information letter will also provide contact details for pregnancy reporting should a participant's GP become aware of pregnancy in their patient. The sponsor, industrial partner and appropriate regulatory authorities will be subsequently notified. If the participant is pregnant or is suspected to be pregnant, EMI-137 must be stopped immediately. Participants or partner of a participant will be followed through the outcome of the pregnancy. The Investigator will be required to report the outcome of the pregnancy. If the outcome of the pregnancy meets a criterion for immediate classification as an SAE - spontaneous abortion (any congenital anomaly detected in an aborted foetus is to be documented), stillbirth, neonatal death, or congenital anomaly—the Investigator should repeat the procedures for expedited reporting of SAEs as outlined above.

17 Responsibilities

- Chief Investigator – the CI will have overall responsible for the design, establishment and running of the trial. The CI will be responsible for monitoring SAEs and using their medical judgement to assign seriousness, causality and expectedness to the SAE. The CI will perform the end of trial sign off requirements.

- The Research Fellow – will be responsible for the daily running and administration of the clinical trial. This responsibility extends to contributing to the design of the study, the development of the trial protocol, the CRF, and to all of the associated documents necessary for the trial set-up and running. The research fellow will also produce and maintain the trial database, produce regular progress reports under supervision of the CI, and perform all of the statistical analysis with oversight from the trial statisticians. The research fellow will also be responsible for notifying the industrial partner of any SAE/SUSAR once the event has been appropriately managed and the overseeing regulatory agencies notified.

- Industrial Partner- Edinburgh Molecular Imaging Ltd (EMI) **-** is responsible for supplying and labelling of the Study Drug according to the Drug Transfer Agreement**.** The pharmaceutical company must inform the CI of all important new safety information or quality defects for the Study Drug without delay as soon as they are made of aware of them. They must also ensure the Investigator and clinical site promptly receive the most current version of the Investigator's Brochure (IB) and Investigational Medicinal Product Dossier (IMPD) if and when these documents are amended and/or updated.

- CTRU – will provide statistical advice and support on the planned trial analyses. If EMI-137 proves effective, CTRU will assist in the subsequent development and administration of a larger randomised controlled trial.

- Radiologist - will be responsible for reviewing and formally reporting the pre-operative radiological imaging of each trial participant.

- Pathologist – will perform or supervise the performance of the histopathological dissection and reporting of the specimens, specimen photography, fresh tissue sampling, development of the histopathology protocol and completion of the histopathology CRFs. They will also assist in the performance and interpretation of downstream translational studies.

- Pharmacy **-** will be responsible for ordering, receipt and storage and temperature monitoring, dispensing and accountability and destruction or return to manufacturer at the

end of the study. The IMP (EMI-137) will be stored and handled according to the Manufacturer's recommendations as detailed in the current product data sheet/Summary of Product Characteristics (SmPC). Pharmacy will ensure processes are in place to conduct the trial in accordance with current Good Clinic Practice guidelines, Good Manufacturing Guidelines, The Medicines for Human Use (Clinical Trials) Regulations 2006 standards and the Clinical Trial Authorisation thorough adherence to procedures outlined in the current version of the protocol.

- The Sponsor QA office – will be responsible for recording and acknowledging all SAE/SUSAR or protocol violations/breaches reported to them and for onward reporting to the appropriate regulatory authority where necessary.

18 Endpoints

Primary endpoint

1. Investigation of the ability of EMI-137 to produce visible fluorescence of colon cancer during laparoscopic surgery.

At the end of trial the ability of EMI-137 to produce visible fluorescence in colonic tumours during laparoscopic resection will be appraised. Using the semi-quantitative descriptive terms of; highly fluorescent, mildly fluorescent and isofluorescent to background we will assess the intra-operative appearance of the tumour 2.5 (range 1 to 3) hours after receiving the IMP. The opinion of the senior operative surgeon performing the EMI-137 assisted cases regarding the benefit will also be taken in to consideration.

Secondary endpoints

1. To investigate of the ability of EMI-137 to produce visible fluorescence in regional lymph nodes draining the colon cancer.

At the end of this trial the ability of EMI-137 to produce visible fluorescence in lymph nodes draining colonic tumours during laparoscopic resection surgery will be appraised. Using the semi-quantitative descriptive terms of; highly fluorescent, mildly fluorescent and isofluorescent to background we will assess the intra-operative appearance of draining lymph nodes 2.5 (range 1 to 3) hours after receiving the IMP. The appearance of the tumour and the lymph nodes will be appraised singly and as a combined endpoint. The

opinion of the senior operative surgeon performing the EMI-137 assisted cases regarding the benefit of using a c-Met targeted peptide will also be taken in to consideration.

2. To investigate the concordance of visible fluorescence in colon cancer with histological stage and c-MET expression in resected specimens.

   The resected specimen(s) will undergo thorough standard and trial-specific histopathological assessment as outlined in the trial design and appendices. Cryosections will be taken from fresh frozen samples and analysed by fluorescent microscopy. The fluorescences obtained will be appraised using the same semi-quantitative scale; highly fluorescent, mildly fluorescent and isofluorescent. The tumour stage will be ascertained and compared against the pre-operative radiological stage. The degree of fluorescence obtained may also be compared against the level of c-Met expression and the morphological and functional characteristics of the tumour. All comparisons will be made against the gold standard- standard histopathological assessment of the tumour, lymph nodes and the ascribed pathological stage of the tumour.

3. To investigate the concordance of visible fluorescence in cancer draining lymph nodes with histological evidence of metastasis.

As part of the pathological assessment, the pre-operative predicted lymph node status will be compared against the pathological lymph node status and the intra-operative lymph node appearance with the administration of EMI-137. Step sectioning and cytokeratin staining of fluorescent nodes will be performed if metastatic disease is not identified during standard histopathological analysis.

4. To explore the tumour (signal) to background (noise) florescence

During each operative case using EMI-137 the background (noise) appearance of nearby structures will be assessed, Factors likely to be negatively influencing the tumour to signal ratio will be appraised and may be adjusted on an individual case basis within the limits of the trial design.

5. Investigation of the safety profile of EMI-137

All trial participants will be monitored for any adverse events/reactions relating to, or suspected to be related to the administration of EMI137. Any adverse reactions will be reported immediately and investigated thoroughly to increase the body of knowledge related to the safety profile of EMI-137.

6. Exploration of systemic, operative, and patient factors, which adversely affect EMI-137 fluorescence detection of colon cancer.

Baseline demographic data will be collected for all patients participating in the trials. This will include, age, weight and height to calculate body mass index (BMI), previous surgical history, current medication and co-morbidities. In addition intra-operative data including timing of administration and dose received will be collected. This data will be analysed and reviewed to identify potential patient and operative factors that negatively influence intra-operative visualisation with EMI-137. With several previous intra-operative imaging methods, intra-abdominal/mesenteric obesity has been disadvantageous to visualisation. Therefore, BMI and assessment of intra-abdominal fat will be specifically reviewed.

7. Study of in vivo imaging compared against ex vivo fluorescent detection

Using methods of fluorescent microscopy the fresh frozen biopsies from the resected specimen will be analysed for fluorescence. This will be compared and contrasted against the intra-operative appearance of the tumour and draining lymph nodes with EMI-137. Again, all specimens will be compared against the gold standard – histopathological assessment of tumour characteristics and TNM (tumour, node and metastases) stage.

If this developmental study yields promising results and EMI-137 is deemed able to accurately detect colonic tumours and any metastatic lymph nodes intra-operatively it may lead to a larger randomized control trial.

19.1 Study definitions

- "Patient factors" refers to individual patient specific variables that adversely affect the fluorescence obtained from EMI-137. For example, this may include raised BMI or previous abdominal surgery.
    - Safety profile refers to;

i) Review of any AE/AR relating to the IMP EMI-137.

Review of intra-operative and post-operative mortality and morbidity during the trial follow-up period relating to the surgical resection in any way.

20 Statistical considerations

20.1 Sample size

As per study design. 10 patients will be recruited from the single site, LTHT.

20.2 Planned recruitment rate

The single site, LTHT will recruit 10 patients over a 6 month period. LTHT is a large tertiary referral colorectal centre treating 200 patients with colon cancer per year; therefore the target of recruiting 10 patients during this time period is feasible and realistic.

21 Statistical analysis

Statistical analysis will be the responsibility of the Research Fellow with support from the CTRU. Statistical analysis will be conducted after the trial is completed; this refers to the study endpoint as defined above.

21.1 Frequency of analysis

Safety will be continually assessed. If any excessive rates of untoward outcomes are identified during the trial, there will be a prompt investigation. Specific attention will be paid to rates of open conversion, anastomotic leak, positive resection margins or mortality in the study cohort. If necessary, the trial may be suspended or terminated early to ensure the safety of trial participants.

The prompt reporting of SAE, SAR or SUSAR will allow real-time evaluation of the safety profile of EMI-137. Again, if rates are suspected to be unacceptably high, the trial may be suspended or terminated early.

22 Data Monitoring

The trial itself and all staff involved in the trial will adhere to GCP and to all relevant guidelines as outlined by the UK Research Governance Framework. Because this is a small feasibility study, governance will be undertaken by a Trial management team, consisting of the CI, Research Fellow, Clinical Research Nurse and CTU representative.

22.1 Data monitoring

Data will be monitored for quality and completeness by the research team at LTHT. The research fellow will chase up all missing data confirm when data is deemed unavailable/unobtainable for any reason. Intermittent quality assurance checks may be carried out to ensure the quality and accuracy of data collected

23 Clinical governance issues

The principles of clinical governance will be respected and adhered to throughout the clinical trial. Any clinical governance issue identified during routine clinical care will be brought to the attention of the trial management team and sponsor.

23.1 Quality Assurance

The trial itself and all staff involved in the trial will adhere to GCP and to all relevant guidelines as outlined by the UK NHS Research Governance Framework and to local standard operating procedures (SOPs) guidelines.

23.2 Protocol Deviation and Major Breaches

Any protocol deviations or major breach of protocol practice will be reported to the Quality Assurance Sponsor Office (contact details in section 1.10) in accordance with Standard Operating Procedure document “Researchers guide to protocol deviations, violations and potential GCP breaches [30]

24 Ethical considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human participants adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 52nd World Medical Association General Assembly, Edinburgh, Scotland, October 2000 [31]. All trial participants will give informed written consent prior to registration. The autonomy of trial participants will be respected at all times. Patients can refuse consent to participate or withdraw their consent at any time without reason. Their refusal will not prejudice their on-going treatment in any way. The trial protocol will be submitted to, and approved by the clinical site Research and Innovation department (R&I) and from the Health Research Authority (HRA).

24.2 Confidentiality

All trial data will be viewed and treated as strictly confidential. Both paper and electronic copies of trial data will be stored securely and confidentially in accordance with the 1998 Data

Protection Act and with University of Leeds trial data handling guidelines. This clinical site will ensure;

- consent from participants to record personal details including name, date of birth, NHS ID, hospital ID
- appropriate storage, restricted access and disposal arrangements for participant personal and clinical details
- consent from participants for access to their medical records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to trial participation
- Consent from participants for the data collected for the trial to be used to evaluate safety and develop new research.
- When data forms need to be transferred, it will be the responsibility of the clinical site team to ensure the forms are anonymised and transferred in a secure manner. This will ensure confirmation of receipt by the recipient site/individual.
- Only the consent form will contain the participant's name and signature. This will be stored securely and separately from other trial data forms at LTHT.
    - All other data collection forms will be coded with the participant's unique trial number and will include two participant identifiers, usually the participant's initials and date of birth.

25 Archiving

At the trial end-point all data will be archived for 15 years. This will be stored securing in accordance with local policy. After 15 years data will be destroyed confidentially and in adherence to data protection regulations.

26 Statement of indemnity

Legal indemnity and liability relating specifically to the trial design will be provided by the University of Leeds. LTHT remain liable for clinical negligence occurring during NHS clinical care, this extends to medical care and interventions received as part of a clinical trial.

This is a clinician-led trial and therefore there are no arrangements for no-fault compensation.

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27 Appendices

Appendix 1: Histopathological Specimen Assessment Proforma

1. Introduction

The guidance below is provided to assist the key role of the histopathologist in the assessment of the excised colon cancer specimens.

In the study, the local histopathologist will be involved in the following:

i) Taking digital photographs of the whole intact fresh resection specimen including both anterior and posterior views (any clips identifying fluorescent lymph nodes should be clearly visible)
ii) Sampling of fresh tumour and normal mucosal tissue (if accessible and patient has consented)
iii) Taking digital photographs of the whole formalin-fixed resection specimen including both anterior and posterior views (any clips identifying fluorescent lymph nodes should be clearly visible)
iv) Taking digital photographs of the serial cross-sectional slices from the resection specimen
v) Submitting a specimen sketch detailing the estimated position of all lymph nodes (positive and negative) according to station number (see diagram below)
vi) Submitting the final histopathology report with full histopathological staging data for review
vii) Submitting all of the H&E stained glass slides (or copies) for scanning and central review
viii) Submitting the formalin-fixed paraffin-embedded tissue blocks of all of the lymph nodes (and the additional two blocks of tumour and one of normal mucosa if the patient has consented)

Thank you for your efforts and for participating. The key issues are photography, consistent dissection and making the slides/blocks available for central review.

2. Preparation of the specimen and photography

Dissection should be undertaken using the method described here which is consistent with the Royal College of Pathologists guidelines for reporting colorectal cancer and other colon cancer clinical trials including MRC CLASICC and NCRI FOxTROT.

The fresh specimen should be carefully examined by the local pathologist as soon as possible after surgery to determine the plane of surgery and measure the length of the different vascular

ties. If there is to be a delay before delivery of the specimen to the pathology department, it is acceptable to refrigerate the fresh specimen for up to 24 hours rather than undertake immediate fixation. The whole intact fresh specimen should have the front and back surfaces digitally photographed prior to inking of any non-peritonealised surfaces to allow central review of the plane of surgery (see separate photography protocol for guidance). Any clips identifying fluorescent lymph nodes should be clearly visible.

The photographs should not contain any direct identifiers (e.g. name or date of birth) but should be identifiable by trial number, and local histopathology number. .

The specimen should then be opened along the anterior aspect down to just above the tumour (but not through the tumour). The anterior surface in the area of the tumour should be preserved to allow assessment of this surface for direct and peritoneal spread. All non-peritonealised surfaces should be painted with ink e.g. India ink *(figure 1).* It should be remembered that the non-peritonealised resection margin only applies to the surgically incised mesocolic planes (e.g. the retroperitoneal margin in right sided specimens and the upper mesorectal margin in left sided specimens) and not to the peritonealised surfaces.

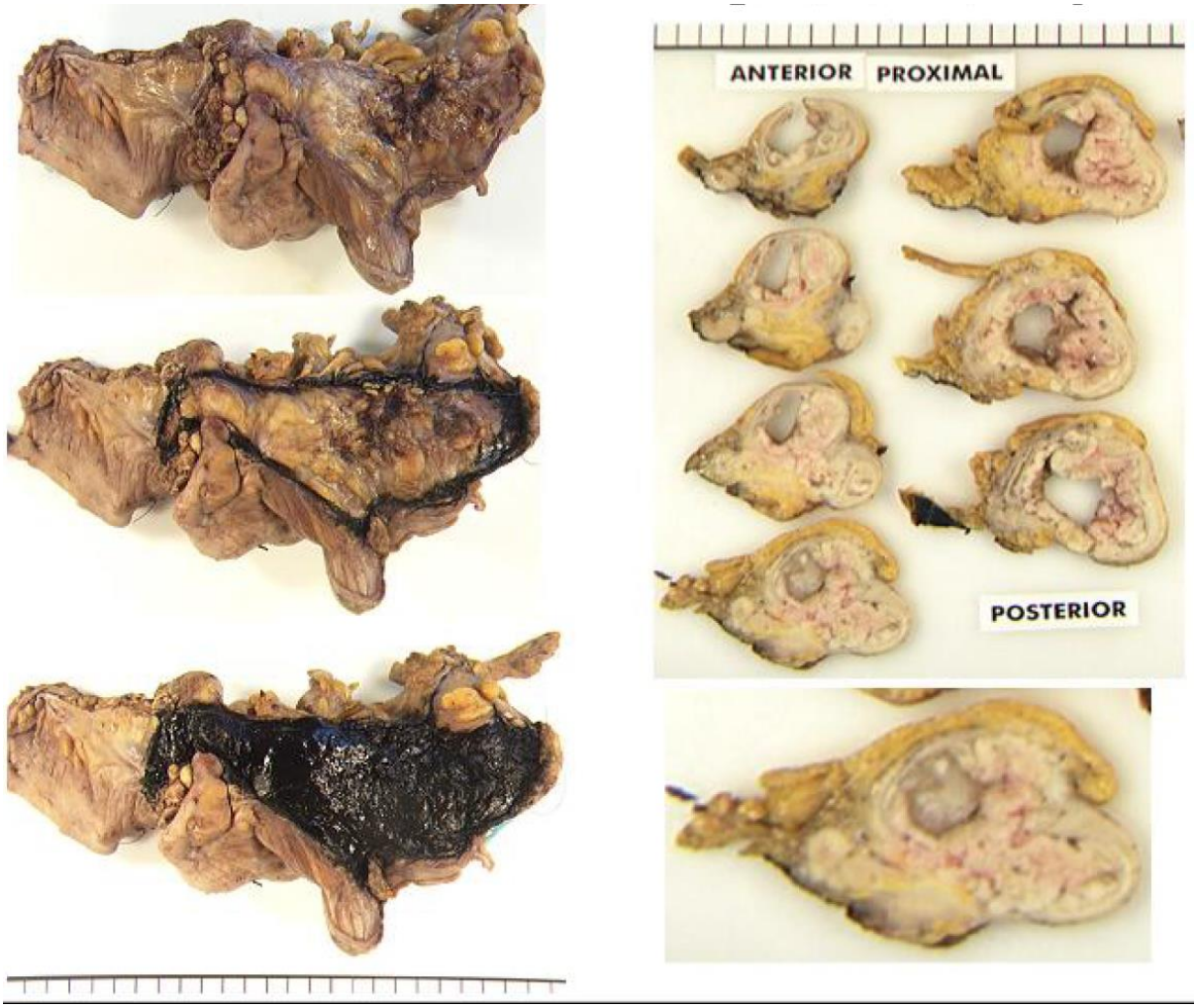


Figure 1: Dissection method showing inking of the retroperitoneal margin and cross sectioning to assess tumour spread. Note the metric scale in the images.

3. Specimen dissection, cross-sectional slicing and photography

After the non-peritonealised surfaces have been inked, and the specimen fixed in formalin for a minimum of two days, it should then be described in detail and a confirmation of the quality of surgery should be undertaken (see below for further details). The formalin fixed specimen should also be photographed if possible prior to further dissection. Again, any clips identifying fluorescent lymph nodes should be clearly visible. The specimen should then be carefully assessed for the presence of a perforation, which may be either through the tumour (tumour perforation) or through the bowel wall proximal/distal to the tumour segment (bowel perforation). The site of the tumour and its maximum dimension should be described along with the minimum distance to the nearest longitudinal margin. A measurement should be taken on the fixed specimen between the tumour and the closest high tie vessel. The specimen should then be thinly (3-4 mm) sliced transversely from 20 mm below to 20 mm above the

tumour. The slices should also be photographed as a valuable demonstration of the quality of the surgery (see separate photography protocol).

Not opening the specimen through the tumour segment facilitates comparison of the cross sectional slices with MRI/CT imaging. The slices should be carefully inspected and the position of the tumour recorded according to the quadrants of involvement. The distance of direct spread outside the muscularis propria and the distance from the tumour to the nearest non-peritonealised margin should be recorded along with the tumour thickness. These measurements should be made initially from the slices and confirmed histologically e.g. by using the Vernier scale. Large blocks should be taken from the area closest to the circumferential margin wherever possible and also from any area where the tumour extends to within 3 mm of the non-peritonealised margin. Other blocks should be taken to allow at least five blocks of tumour (including large blocks) to confirm presence or absence of extramural venous invasion. Likewise the peritoneal surface should be sampled by a minimum of two blocks if the tumour impinges on it (figure 2).

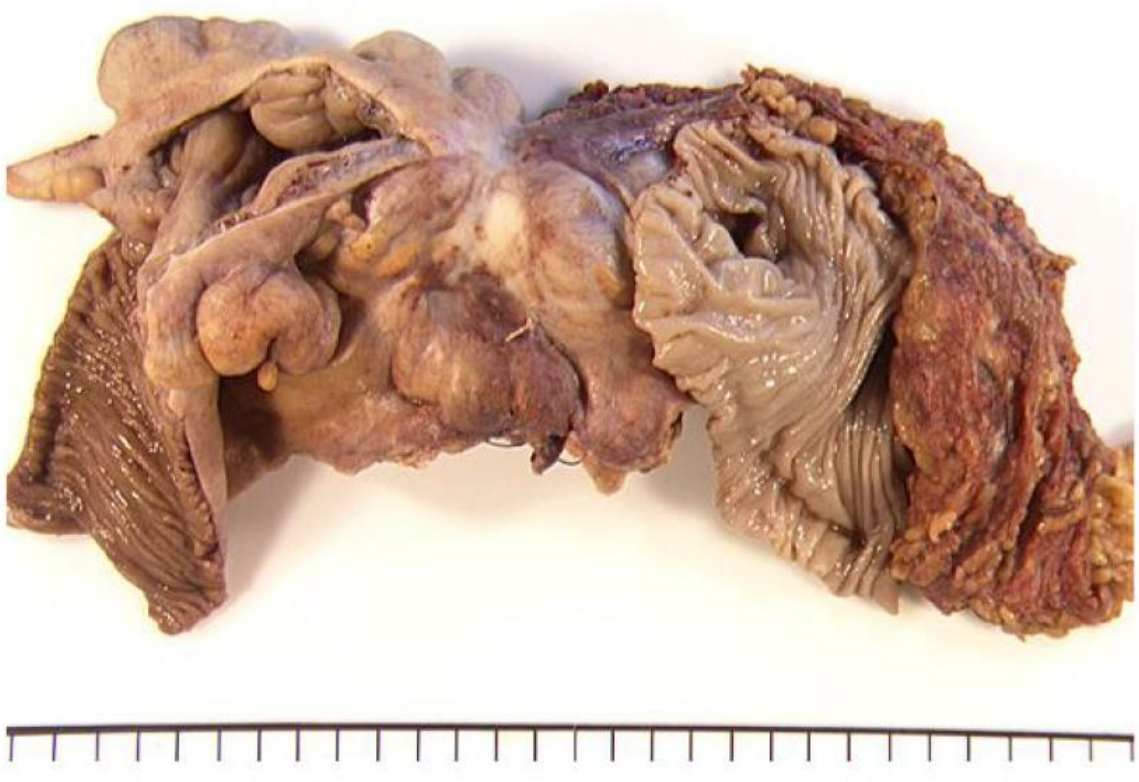


Figure 2: Suspected peritoneal/serosal involvement by tumour.

All of the local lymph nodes in the specimen should be identified, mapped, embedded and examined. The high tie (apical node) should be blocked separately as should any lymph nodes marked (e.g. with a clip) by the surgeons to allow comparison with the intra-operative staging. The number of lymph nodes identified in each station (see below) should be noted on a diagram for subsequent mapping. If the nodes have not been marked by the surgeons, multiple nodes from the same station may be embedded in one block to cut down on the workload.

4. Histological reporting

We are using TNM 8 for histopathological staging in this study as is routine from 1st January 2018. Thus we will be reporting tumour deposits, although these will be captured separately to the lymph nodes on the CRF.

The non-peritonealised resection margin is considered involved (i.e. an incomplete R1 excision) if the tumour extends to within 1 mm of the inked margin. Measurement can easily be made by using a sheet of graph paper that is photocopied onto a sheet of acetate and cut to size. This may be more easily used than the Vernier scale. This is demonstrated in figure 3. No distinction is currently made between the various modes of non-peritonealised resection margin involvement, e.g. direct spread, lymph node spread, venous, perineural, etc. Although all are associated with an increased local recurrence rate, this is lower in the case of involvement by tumour within a lymph node.

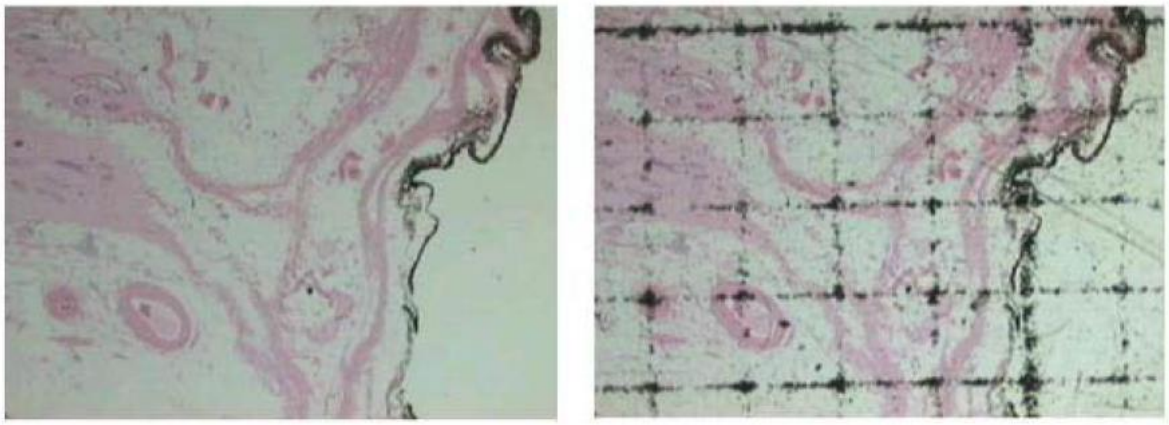


Figure 3: Rapid measurement of the distance from the tumour to the non-peritonealised resection margin by overlaying a simple grid.

Peritoneal involvement (pT4a) should be diagnosed if tumour cells penetrate the peritoneal surface (figure 4).

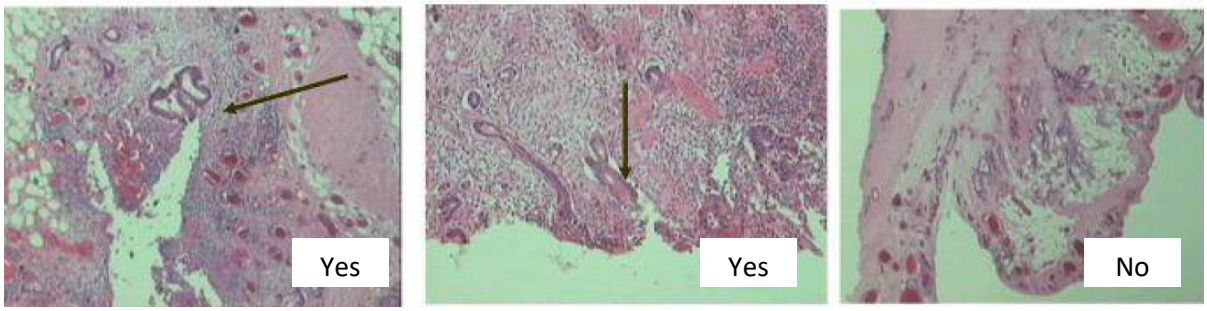


Figure 4: Peritoneal involvement should be assessed by the method of Shepherd *et al* 1995 whereby tumour cells should be seen to penetrate through the serosa to lie on the surface of the specimen.

The grade of differentiation of the tumour should be defined by the predominant area of tumour and not on the area of the worst grade. Other types of differentiation, i.e. mucinous adenocarcinoma, signet ring cell and undifferentiated carcinoma should be documented.

5. Grading the plane of surgery (mesocolic resection)

The plane of the mesocolic resection can be easily assessed using a combination of assessing the fresh specimen, fixed specimen and cross sectional slices. We recommend a three grade classification (table 1, figures 5-6), first used in the MRC CLASICC trial and subsequently used in NCRI FOxTROT. These systems have been demonstrated to be usable in the context of phase III clinical trials and have been shown to predict a higher risk of local recurrence in MRC CLASICC and overall survival in a large Leeds study. A second assessment of surgical planes will be made during the central review to identify inter-observer agreement. This will be done on the digital photographs.

2 Plane of dissection	**3 Short description**	**4 Detailed description** **5**
6 Mesocolic **7** plane	**8** Good quality specimen	**9** There should be an intact and smooth mesocolic surface with only minor irregularities. Any peritoneal or fascial defects must be no deeper than five millimetres. There should be smooth retroperitoneal and mesocolic resection margins on the cross-sectional slices

10 Intramesocolic **11** plane	**12** Moderate quality specimen	**13** There may be moderate bulk to the mesocolon but significant irregularity of the peritoneal or fascial surface in at least one area that is deeper than five millimetres. The muscularis propria should not be visible. There may be moderate irregularity of the retroperitoneal and mesocolic resection margins on the cross-sectional slices
14 Muscularis propria **15** plane	**16** Poor quality specimen	**17** There may be little bulk to the mesocolon and there will be extensive defects that extend down to the muscularis propria. The retroperitoneal and mesocolic resection margins may be partially formed by the muscularis propria on the cross-sectional slices

Table 1: A detailed description of the pathological grading system used to describe the plane of dissection in colon cancer specimens.

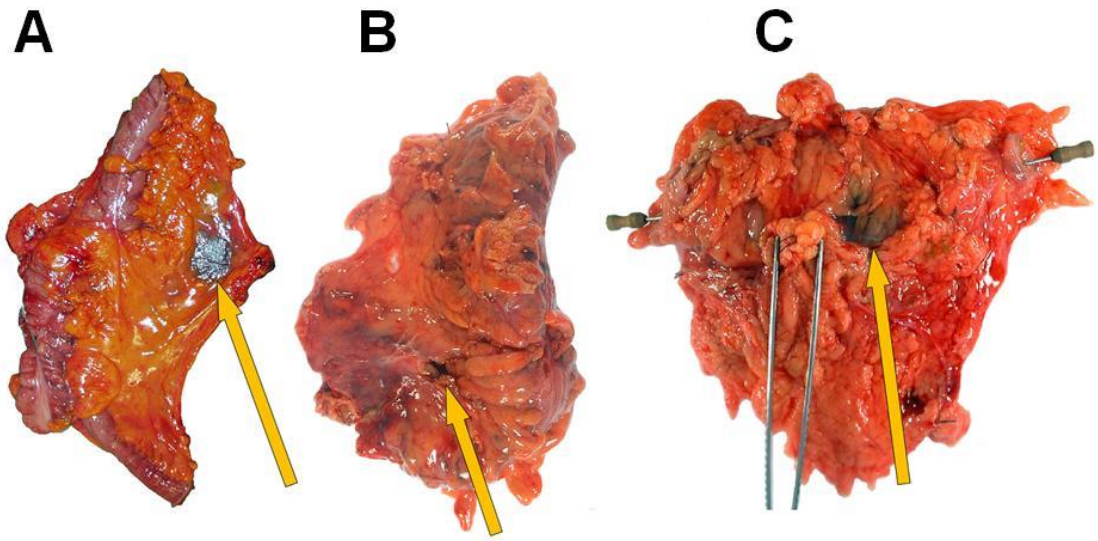


Figure 5: Three fresh colon cancer specimens resected in the (A) mesocolic plane with an intact peritoneal window (arrow), (B) intramesocolic plane with a significant mesocolic defect (arrow), and (C) muscularis propria plane with a posterior perforation of the specimen (arrow).

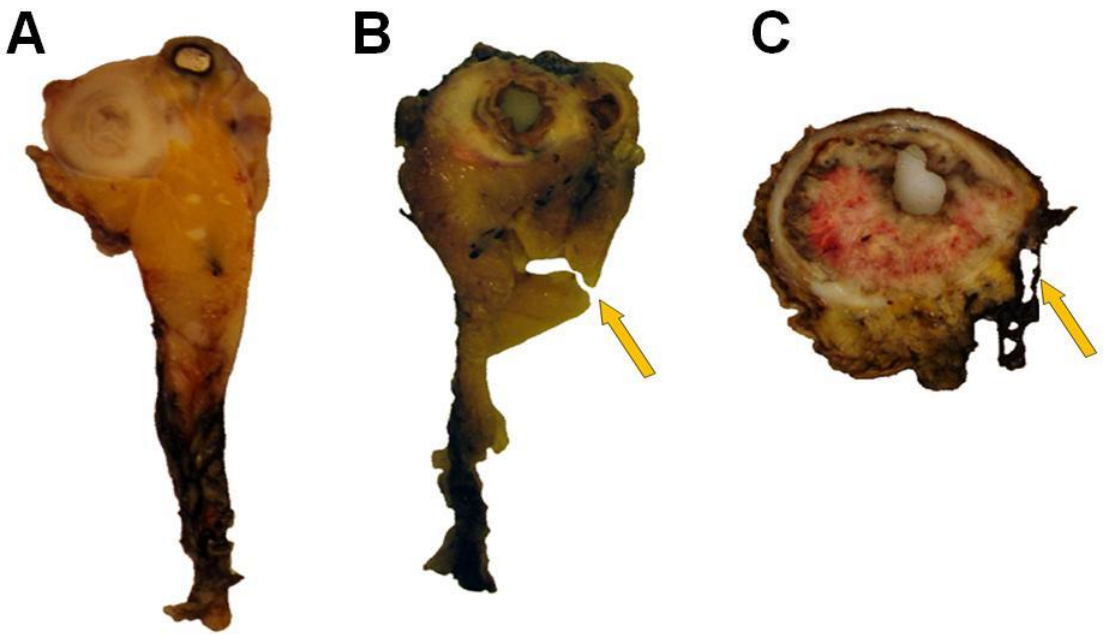


Figure 6: Cross-sectional slices from three specimens resected in the (A) mesocolic plane, (B) intramesocolic plane with a significant mesocolic defect (arrow), and (C) muscularis propria plane with a defect extending down to the muscularis propria (arrow).

6. Mapping the lymph node stations

The specimen will be carefully dissected to map the location of all of the lymph nodes identified using the Japanese station sub-groupings (figure 7, table 2). The pathologist will mark on a specimen diagram and lymph node mapping table the number of nodes in each station and how many of these were fluorescent intra-operatively. An accurate block key will be kept so that it is clear which nodes belong to which station and whether or not they were fluorescent. The D1 nodes will be subdivided into those within 5cm of the tumour, those that lie between 5 and 10cm of the tumour and those that are more than 10cm beyond the tumour. Any non-fluorescent nodes in the same station may be embedded with more than one node in each block. Each fluorescent node will be blocked out individually. The apical node(s) will also be blocked separately.

As stated in TNM 8, extramural tumour deposits are recorded separately to involved lymph nodes. The number of tumour deposits should be indicated in the report (either 0, 1, 2, 3, 4, 5 or >5). Any tumour deposits that are macroscopically visible and believed to be lymph nodes at the time of dissection should also be mapped and blocked carefully.

The local pathologist will cut a single section from each lymph node/macroscopic tumour deposit block and report the number of positive and negative nodes/macroscopic tumour deposit in each station using conventional histopathology. A copy of the diagnostic glass H&E slides, pathology report, photographs, specimen sketches and fluorescent lymph node blocks will then be sent to the University of Leeds for central review and to create a permanent record of the pathology. Formalin fixed blocks of the tumour and normal mucosa will also be sent if the patient has consented to future translational research. Deeper levels and immunohistochemical testing will be performed on the fluorescent lymph node blocks (where standard pathology was negative) to confirm their status and detect missed metastases, micrometastases and isolated tumour cells. A final lymph node report will be issued identifying how many lymph nodes/macroscopic tumour deposits in each station were detected, how many were involved by tumour, and how this related to the intra-operative fluorescent nodes.

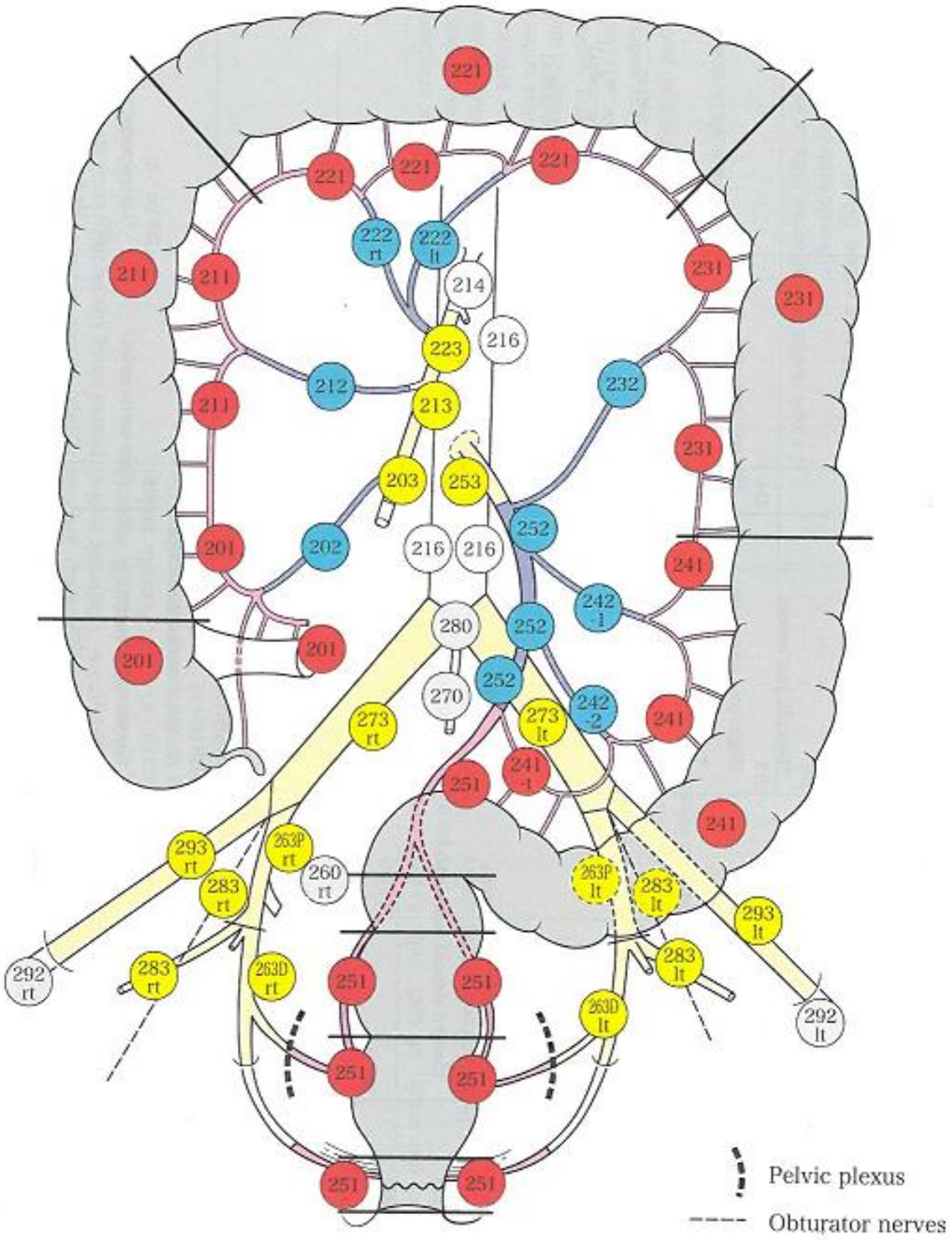


Figure 7: Japanese staging subgroups - Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Station number	All nodes	Notes	Fluorescent	Cassette	Involved
201	1	5-10cm	No	A1	No
	2	5-10cm	No	A1	No
	3	5-10cm	No	A1	No
	4	<5cm	Yes	A2	Yes
	5	<5cm	No	A3	No
	6	<5cm	No	A3	No
202	1		No	A4	No
	2		No	A4	No
	3		Yes	A5	No
203	1	Apical (ileocolic)	No	A6	No
	2		No	A7	No
	3		No	A7	No
	4		No	A7	No
211	1	>10cm	No	A8	No
	2	>10cm	No	A8	No
	3	5-10cm	No	A8	No
	4	5-10cm	No	A8	No
	5	<5cm	No	A9	No
	6	<5cm, tumour deposit	No	A9	No
	7	<5cm	No	A9	No
212	1		No	A10	No
	2		No	A10	No
	3		No	A10	No
	4		No	A11	No
	5		No	A11	No
213	1	Apical (right colic)	No	A12	No
	2		No	A13	No
TOTAL	**27 nodes**		**2 nodes**		**1 node**

Table 2: Example of how to map the lymph nodes for central review for a right hemicolectomy specimen including the station number, number of nodes in each station, whether the node was fluorescent, cassette the node has been embedded in and whether or not it is involved. Note that comments have been provided to identify the apical nodes (there may be more than one apical node if the tumour lies between two vascular ties) and any macroscopic tumour

deposits. Also the pericolic (D1) nodes are sub-classified into those >10cm from the tumour, 5-10cm from the tumour and <5cm from the tumour. The apical node(s) and any fluorescent nodes are embedded separately. All other nodes from the same station can be embedded in the same block, however, please do not embed more than four nodes in the same cassette.

Appendix 2: Histopathology specimen photography proforma

Title: Photography of colonic cancer specimens taken for EMI-137 in laparoscopic colonic resections trial

General principles

1. Photographs should be taken with a high resolution digital camera which is ideally mounted on a fixed stand to minimise movement artefacts.

2. Photographs should be taken directly above the specimen and not at an angle to reduce distortion artefact.

3. A white background is ideal although any other plain colour is acceptable.

4. We require photographs of the **WHOLE FRESH AND FIXED SPECIMEN** (front and back) and the serial **FIXED CROSS SECTIONAL SLICES** (can be all together or individually).

5. Images should not contain any direct patient identifiers (names, date of birth etc).

6. The image files should be saved in a generic format (JPEG, TIF etc) and labelled by unique trial number and local pathology number, which can be linked by the host institution to the patient's clinical details if necessary. Sequential images from the same case should contain a relevant suffix in brackets e.g. (1), (2), (3) etc.
   .

7. **ALL IMAGES MUST INCLUDE A METRIC SCALE TO ALLOW CALIBRATION WHEN USING THE IMAGE ANALYSIS SOFTWARE**. This can be a simple ruler placed below the specimen or slices.

<u>Whole specimen images</u>

1. The whole specimen photographs should be performed before the specimen is opened.

2. The specimen should be quickly washed and dried to remove any blood/faecal debris.

3. We require fresh whole specimen images for the measurements but would also like whole specimen images of the fixed specimen prior to slicing in addition if possible.

4. **THE MESENTERY MUST BE LAID OUT FLAT** (please make sure that it is not folded or over-stretched). If the specimen is distorted, please try to lay it out as flat as it will go but do not divide any adhesions prior to fixation.

5. **THE SITE OF THE TUMOUR AND THE SITE OF THE HIGH VASCULAR TIES MUST BE CLEARLY VISIBLE ON THE PHOTOGRAPHS** (e.g. by using forceps or labels to point them out).

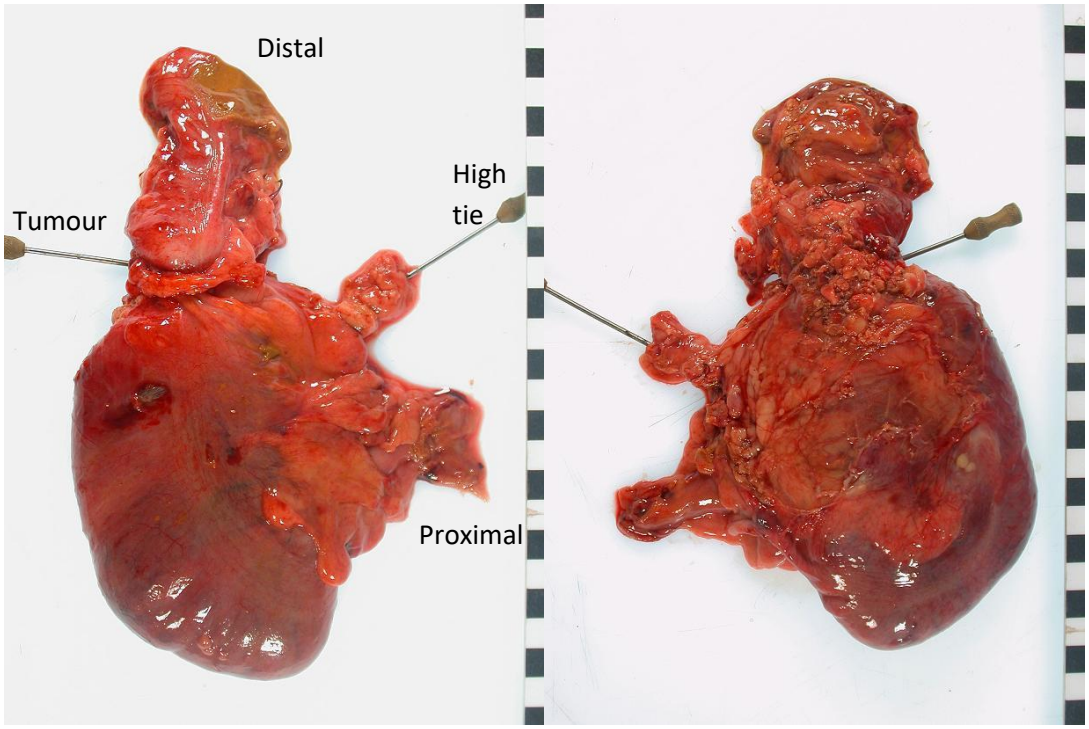
6. The whole specimen should be visible in the image.

7. Ideally the distal and proximal aspects should be labelled - you can use card labels with written annotations placed alongside the bowel.

8. Additional close up pictures of any mesocolic defects or perforations would be helpful

9. Whole specimen images of the fixed specimen should be taken in the same way as the fresh specimen photographs, although it is recognised that the specimen will likely have been opened above and below the tumour for cleaning prior to fixation. It is important that the tumour segment is not opened to facilitate intact cross sectional slicing.

Example of whole specimen images from a right-sided resection:



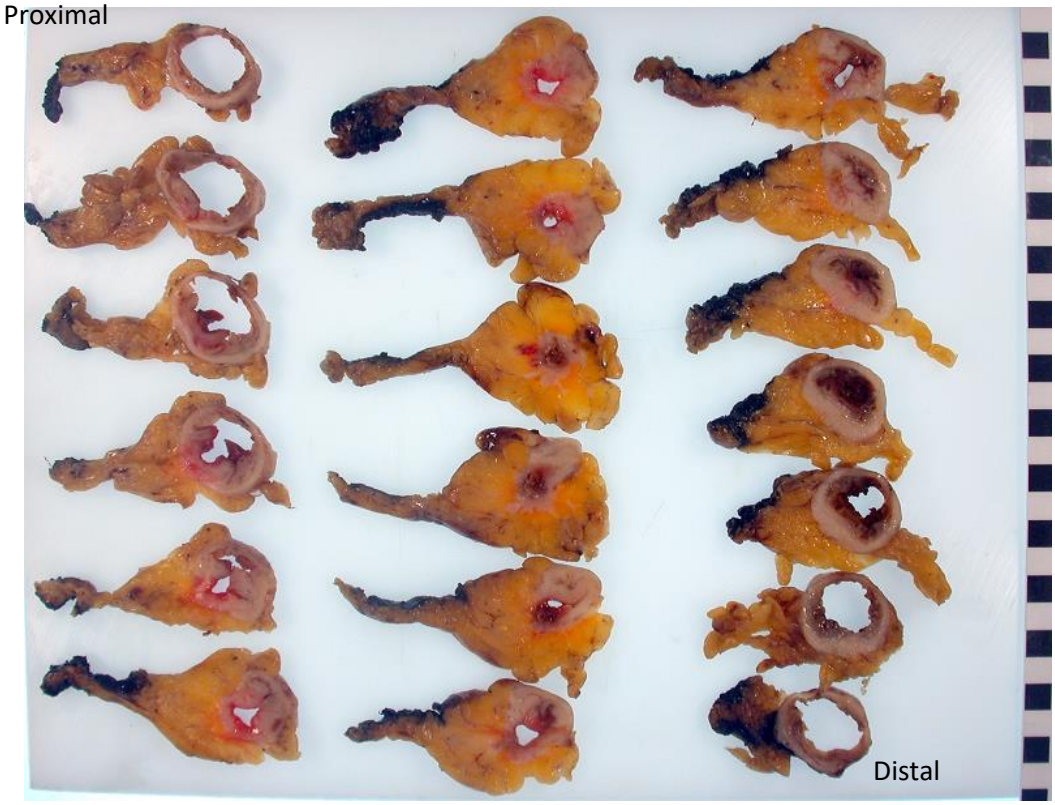
Fixed cross-sectional slice images

1. The slices should be laid out sequentially and the picture should include all slices (slices should be made every 3 to 4 mm and include the entire tumour segment). Alternatively, or in addition, slices can be photographed individually.

2. It is helpful to label the proximal and the distal slice on the photographs.

3. It is helpful if the retroperitoneal margin in the right colon is either inked black or indicated by a label.

Example of a fixed cross sectional slices image:



If you have any queries or require clarification of any of the points in this proforma then please contact:

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Appendix 3: Radiological Assessment Proforma

All patients will have baseline staging CT of the thorax, abdomen and pelvis as part of standard care at a maximum of 8 weeks prior to surgery. Abdominal imaging will be performed in the portal venous phase at 65 seconds. Reconstructed slice thickness 5mm axial & 3mm coronal planes for the abdomen.

All primary tumours will be given a proposed radiological TNM staging (according to TNM 8) and include assessment for extramural vascular invasion (V).

Tumour location will be indicated according to the following locations:

- ***Caecum*** (segment proximal to or involving ileocaecal valve)
- ***Ascending colon*** (segment distal to ileocaecal valve and including the hepatic flexure proximal to the border of the falciform ligament)
- ***Transverse colon (***segment distal to the hepatic flexure and falciform ligament to the splenic (left) flexure
- ***Descending colon*** (segment distal to the splenic flexure to the left iliac crest)
- ***Sigmoid colon*** (segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)

Tumour length (mm): _____

T stage

□ **T1** tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria)

□ **T2** tumour invades muscularis propria

□**T3** tumour invades into subserosa or into non-peritonealised pericolic or perirectal tissues but not breaching serosa

□**T4a** tumour perforates visceral peritoneum

□**T4b** tumour directly invades other organs or structures

N stage

Number of Malignant Nodes_____

□ **NX:** regional lymph nodes cannot be assessed

□ **N0:** no regional lymph node metastasis

□ **N1:** metastasis in 1 - 3 regional lymph nodes

□ **N1a:** metastasis in 1 regional lymph node

□ **N1b:** metastasis in 2 - 3 regional lymph nodes

□ **N1c:** no regional lymph nodes are positive but there are tumour deposits in the subserosa, mesentery or nonperitonealised pericolic or perirectal / mesorectal tissues

□ **N2:** metastasis in 4 or more regional lymph nodes

□ **N2a:** metastasis in 4 - 6 regional lymph nodes

□ **N2b:** metastasis in 7 or more regional lymph nodes

Size of largest malignant (mm) node_____

Node location: According to lymph node mapping – see Figure 1.

V stage

□**V1** - Vascular invasion present or probably present

□**V0** - Vascular invasion probably absent or absent

M stage

□ **M0:** no distant metastasis by imaging; no evidence of tumour in other sites or organs

□ **M1:** distant metastasis

- **M1a:** metastasis confined to 1 organ or site without peritoneal metastasis
- **M1b:** metastasis to 2 or more sites or organs is identified without peritoneal metastasis

- **M1c:** metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Metastases location:

□**Liver**

□**Lung**

□**Peritoneum**

Other _____

Mapping the lymph node stations

The cross sectional imaging will be carefully examined to map the location of all the lymph nodes identified using a modified Japanese station sub-grouping. The radiologist will mark on a specimen diagram (Figure 1) and lymph node mapping table (Table 1) whether the dominant node at each lymph node station appears:

0. No node present
1. Benign
2. Probably Benign
3. Probably malignant
4. Malignant

Size of nodes will be recorded as the maximum short axis diameter. Based on node size and morphology the radiologist will decide how likely they feel the node is to be malignant.

Coding for lymph node stations (1)

- In the superior and inferior mesenteric arterial system, the first figure of the code indicates the position of the lymph nodes, expressing the epicolic and paracolic (D1) nodes as 1Δ (marked in red on figure 1), the intermediate (D2) nodes as 2Δ (marked in blue on figure 1), the main (D3) nodes as 3Δ(marked in yellow on figure 1) and the

para-aortic nodes as 4Δ (marked in white on figure 1)

- The second figure indicates the position of the lymph nodes along the main trunk artery; Δ1 is used for the nodes along the ileo-colic artery, Δ2 is used for nodes long the right colic artery, Δ3 for those along the middle colic artery, Δ4 for those along the left colic artery and Δ5 for the sigmoid artery and Δ6 for the superior rectal artery

- The lymph nodes at the root of the superior mesenteric artery are expressed as 214

- The inferior mesenteric nodes are expressed as 253

- Para-aortic nodes are marked as 216, 280, 270 and 292, and iliac nodes as 263, 273, 283 and 293, (left and right) – presence of nodes at these stations can be commented on in Table 1.

Presence of additional pathology

The presence of additional pathology can be commented on in Table 1.

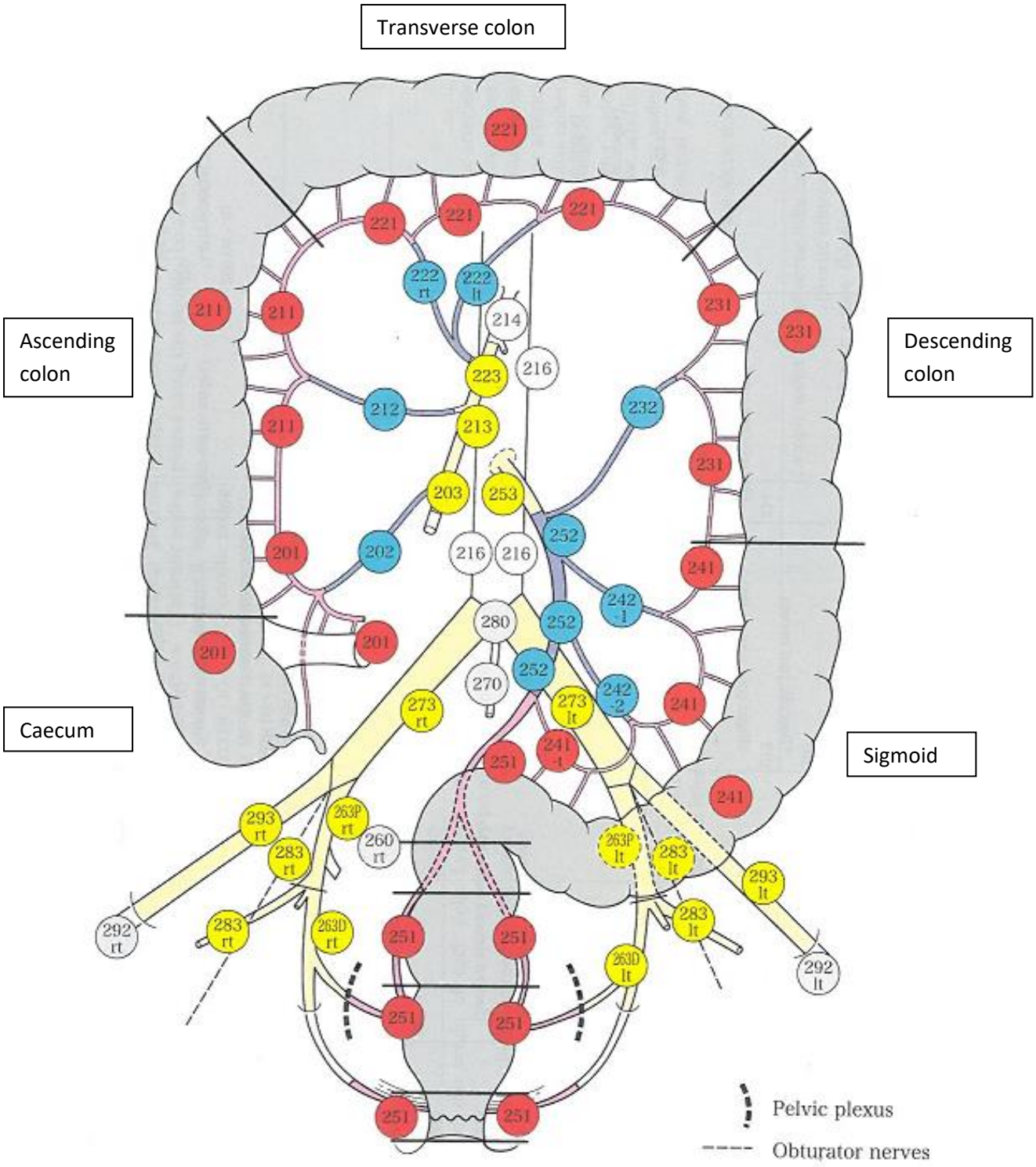

Figure 1. Modified Japanese staging subgroups - Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Station number	**Node**	**Estimate size of node:** maximum short axis diameter (mm)	**Appearance of dominant node**
201	1 2 3		e.g. 0.No node present 1.Benign 2.Probably Benign 3.Probably malignant 4.Malignant
201			
203			
211			
212			
213			
221			
222			
223			
214			
231			
232			
241			
242			
251			

Station number	**Node**	**Estimate size of node:** maximum short axis diameter (mm)	**Appearance of dominant node**
252			
253			
216			
292			
280			
270			
273			
283			
263			
TOTAL			

Table 1: Example of how to map the lymph nodes including the station number, vessel, number of nodes in each station plus size and appearance of nodes.

Comments to identify the apical nodes will be added. There may be more than one apical node if the tumour lies between two vascular ties. *N.B*. Right colic vessel may not be present.