END OF TRIAL SUMMARY REPORT

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

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Abbreviations and Acronyms

Acronym	
AE	Adverse Event
ASA	American Society of Anesthesiologists Grade
BMI	Body Mass Index
HRA	Health Research Authority
LTHT	Leeds Teaching Hospitals Trust
MHRA	Medicines and Healthcare Products Regulatory Authority
PI	Principal Investigator
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction

1 CLINICAL TRIAL DESIGN

The clinical feasibility study of EMI-137 in laparoscopic colonic resections is an early phase clinical trial to explore the feasibility of EMI-137 as an intraoperative imaging agent in laparoscopic colonic tumours was conducted at LTHT following the in-vitro results described above.

1.1 Design

This trial was designed as an unblinded, non-randomised, phase IIa feasibility study. The trial was conducted at the single site, St James' University Hospital in Leeds, with support from the University of Leeds. The eligibility criteria are defined in table below.

The primary aim of the study was to investigate the ability of the novel c-Met targeted fluorescent imaging agent, EMI-137, to produce visible fluorescence in colon cancer during laparoscopic surgery. The planned secondary endpoints included exploration of fluorescent in metastatic lymph nodes, dose optimisation, safety and feasibility outcome measures.

The intraoperative image obtained with the IMP and ability to detect metastatic lymph nodes will be compared to the final pathological stage (TNMv8.0) as the gold standard.

1.2 Aims and Objectives

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

1.2.1 Primary Objectives

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

1.2.2 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

1.3 Eligibility criteria

Inclusion criteria	Exclusion 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 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 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.

- 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

• 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).	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

Table 1: Eligibility Criteria for Intraoperative Imaging of Colon Cancer using a Fluorescent peptide (EMI-137) against the c-Met receptor feasibility study

1.4 Regulatory authorisation

1.4.1 Initial approval

MHRA granted clinical trial authorisation on 29th August 2017. HRA granted favourable approval on 4th December 2017.

1.4.2 AMENDMENTS

1.4.2.1 Substantial Amendments

Introduction of the Union for International Cancer Control TNM v8 classification system Tumour Node Metastasis (TNM) v8.0 classification system on 1st January 2018 at LTHT necessitated a substantial amendment to the trial protocol. Regulatory approval was granted

for this prior to trial commencement by REC and MHRA on 5th January and 10th January 2018 respectively.

The Investigator Brochure (IB) and Investigational Medicinal Product Dossier (IMPD) were updated by the Industrial Partner and drug supplier Edinburgh Molecular Imaging Ltd (EM Imaging Ltd) to incorporate batch re-test results and new safety information discovered. This facilitated shelf-life extension of the EMI-137 batch supplied to LTHT. MHRA approved the first substantial amendment to these supporting documents on 15th March 2018 and 28th March 2019.

1.4.2.2 Non substantial amendments

1. Incorporated a change to external pharmacovigilance reporting processes. This was approved on 14th February 2018.
2. The expected recruitment period was extended by a further six months. This was approved on 4th September 2018.
3. The expected recruitment period was extended by a further six months. This was approved on 15th February 2019.

1.5 Immunohistochemistry method

Five micron slices of formalin fixed paraffin embedded (FFPE) tumour, normal and metastatic lymph node blocks were prepared using a microtome and mounted on to Superfrost Plus glass slides and allowed to dry overnight in a drying cabinet at 40℃. To ensure resistance to mechanical manipulation slides were fixed by heating at 78℃ for 10 minutes on the Leica HI1220 flattening table (Leica Geosystems Holdings AG; St. Gallen, Switzerland) and allowed to cool at RT. The prepared slides were then de-paraffinised in xylene and rehydrated in 99.8% v/v ethanol according to laboratory standard operating procedure (SOP) in preparation for specific staining and antibody probing.

1.5.1 Antigen retrieval

Heat induced epitope retrieval was performed by microwaving the slides for 10 minutes at 900W power with a citric acid buffer solution corrected to pH6.2.

1.5.2 C-Met expression immunohistochemistry

Recombinant monoclonal Anti-Met (c-Met) antibody [EP1454Y] (ab51067) (Abcam Plc, Cambridge CB2 0AX) was used as the primary anti c-Met antibody throughout. Slides were incubated with 100µL of the antibody at RT for 1 hour then repeatedly washed in TBS and TBS-T solution.

A rabbit specific labeled HRP immunoenzymatic antigen detection system was used for protein detection and chromogenic visualisation. Slides were incubated with 100µL of Horseradish Peroxidase (HRP) containing Equilibrate SignalStain® Boost IHC Detection Reagent (Cell Signalling Technology Inc, Leiden, The Netherlands. Cat: 8114S) as the secondary antibody for 30 minutes in a humidified chamber. Slides were repeatedly washed in TBS solution. Enzymatic chromogenic detection was performed with the SignalStain® 3,3'-diaminobenzidine (DAB) Substrate Kit (Cell Signalling Technology Inc, Leiden, The Netherlands. Cat: # 8059S). Slides were incubated with 100µL of the prepared chromogen reagent for 10 minutes in a humidified chamber at RT. Development of brown tissue colouration was observed.

1.5.3 Counterstaining

Counterstaining was performed according to local laboratory protocol. Prepared tissue slides were incubated with Meyer's hematoxylin solution (Sigma-Aldrich Scientific Inc., Cat: H9627-25G) for 60 seconds, washed under running tap water before incubation with Scott's tap water (laboratory stock reagent). Slides were dehydrated in ethanol and cleaned in xylene, then mounted on to glass cover slides with DePeX Mounting medium for histology and allowed to cure overnight at RT.

1.5.4 Control normal tissue preparation

The matched normal tissue was prepared in an identical manner with the exception of incubation with the primary anti-c-Met antibody.

1.5.5 Imaging

Bright-field microscopy imaging of IHC stained and prepared slides was performed using the Nikon Eclipse MicroscopyU E1000 automated microscope (Nikon Instruments Inc., New York, USA) at x20 magnification.

2 CLINICAL TRIAL RESULTS

2.1 Recruitment

2.1.1 Screening

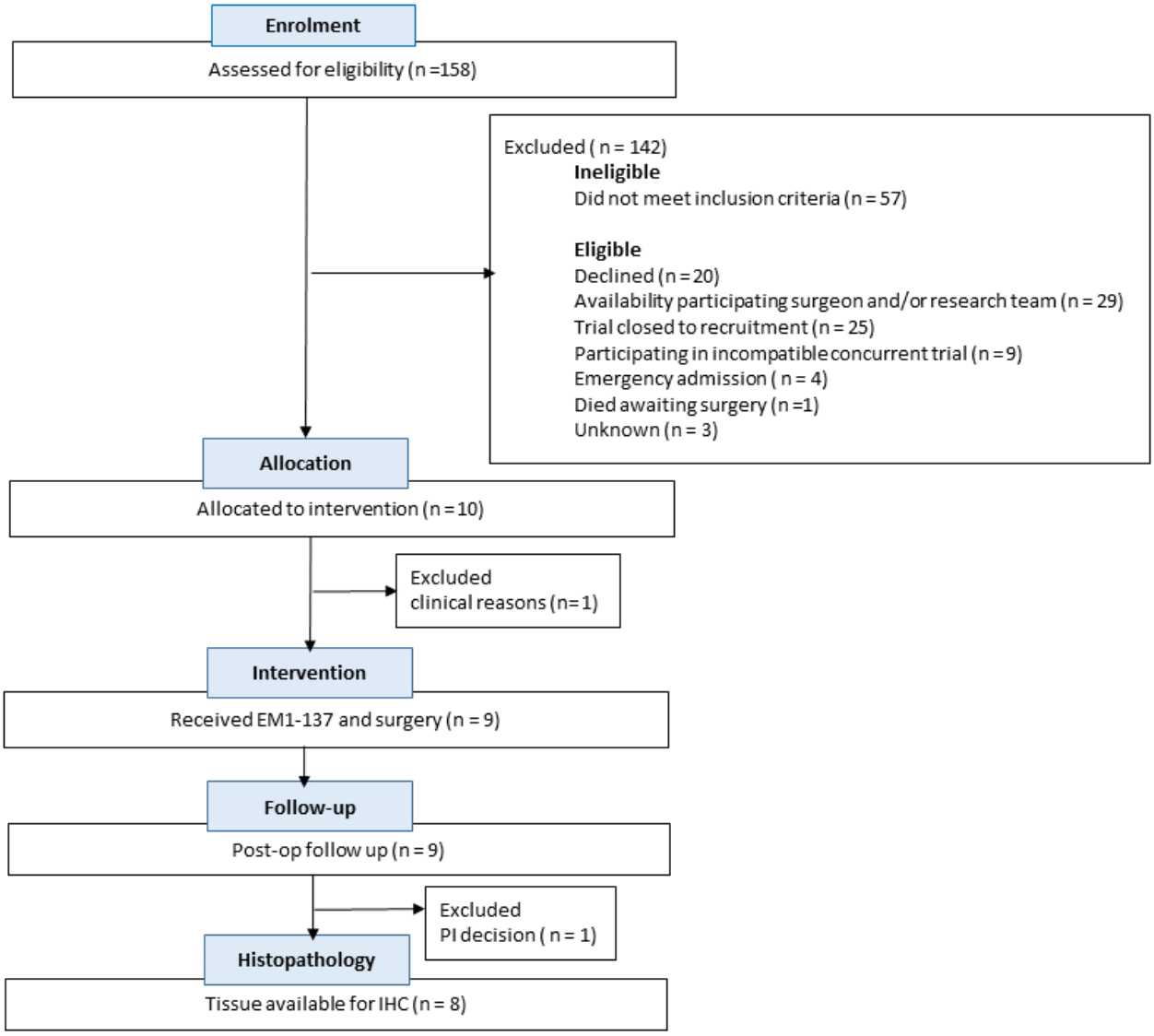


Figure 1: trial recruitment consort diagram

2.1.2 Recruitment timeline

The first patient was recruited to the trial on 14th February 2018. A total of 10 patients were recruited to the trial. The last patient underwent fluorescent guided surgery on 29th July 2019. The last visit of the last subject was conducted on 28th August 2019.

2.2 Baseline participant demographics

Table 2 shows the baseline demographics of the nine participants enrolled in to the trial and underwent trial specific laparoscopic colonic resection surgery with the addition of EMI-137.

(N = 9)		**Mean average**	**S.D**
Age (years)		72	± 3.9
Gender M:F	4 : 5		
Body Mass Index (m^2)		26.5	± 4.1
ASA grade			
1	1		
2	4		
3	4		
Co-morbidities			
Cardiovascular disease Yes : No	6 : 3		
Peripheral vascular disease Yes : No	0 : 9		
Respiratory disease Yes : No	3 : 6		
Diabetes Yes : No	0 : 9		
Colonoscopy			
Tumour location			
Ascending colon	2		
Transverse colon	1		
Hepatic flexure	1		
Descending	1		
Sigmoid colon	4		
Tumour morphology			
Polypoidal	2		
Semi annular	1		
Circumferential	6		
Colorimetric tattoo applied	9 : 0		

Table 2: Summary of participant baseline demographics

2.3 Participant summary

Subject ID	Gender M/F	Age (y)	BMI (m^2)	ASA grade	Tumour location	Operation performed	Interval to assessment (hh:mm)	pT stage	Tumour fluorescence	pN stage	Lymph node fluorescence
EM001	F	75	24.8	2	Ascending colon	Right hemicolectomy	02:31	pT3	Isofluorescent to background	pN0	None
EM002	M	76	22.7	3	Sigmoid	High anterior resection	01:36	pT3	Isofluorescent to background	pN0	None
EM003	F	67	23.0	1	Descending colon	Left hemicolectomy	01:40	pT3	Mildly fluorescent	pN1b	None
EM004	M	77	31.5	3	Sigmoid	High anterior resection	01:15	pT2	Mildly fluorescent	pN1a	None
EM005	M	73	19.8	3	Hepatic flexure	Left hemicolectomy	01:50	pT4b	Isofluorescent to background	pN2a	None
EM007	F	70	30.5	2	Sigmoid	Left hemicolectomy	01:09	pT3	Isofluorescent to background	pN1c	None
EM008	M	67	28	2	Ascending colon	Right hemicolectomy	01:55	pT3	Isofluorescent to background	pN0	None
EM009	F	68	27.3	2	Sigmoid	Robotic anterior resection	01:49	pT1	Mildly fluorescent	pN0	None
EM010	M	71	30.8	3	Transverse colon	Extended right hemicolectomy	02:14	pT3	Mildly fluorescent	pN1b	None

Table 3: Key characteristics of trial subjects. Abbreviations: pT pathological Tumour stage; pN pathological node stage; M male; F Female; m^2 metre^2: y years; ASA American Society of Anesthesiologists Grade

2.4 Intra-operative data

Table 4 outlines the key intra-operative findings for the nine participants who underwent laparoscopic colonic resection surgery with EMI-137 at LTHT in the clinical trial.

(n = 9)	**Frequency *n***	**Mean average**	**± S.D**
EMI-137 dose received			
0.13mg/kg	8		
0.09mg/kg	1		
Dose volume administered (mg)		9.5	± 1.4
Dosing interval (hh:mm)		01:46	± 00:17
Tumour fluorescence			
Presence of fluorescence in tumour Y:N	9 : 0		
Highly fluorescence to background	0		
Mild fluorescent to background	4		
Isofluorescent to background	5		
Lymph node fluorescence			
Presence of fluorescence in LN Yes:No	0 : 9		
Surgical procedure performed			
Right hemicolectomy	2		
Extended right hemicolectomy	1		
Left hemicolectomy	3		
High anterior resection	3		
Laparoscopic length of procedure (hh:mm)		02:30	00:47
Curative resection performed? Yes: No	9 : 0		
Serious intra-operative complications? Yes: No	0 : 9		

Table 4: Summary of Intraoperative results. (Abbreviations: hh:mm Hour hour:minute minute)

2.5 Pathological results summary

(N = 9)		**Mean ± S.D**	**Range**
Tumour differentiation			
Well/moderately	9		
Poorly	0		
Tumour histological sub-type			
Adenocarcinoma	8		
Adenocarcinoma variant - mucinous	1		
Max tumour diameter (mm)		34.2 ± 20.3	15 - 80
Max distance tumour extends beyond muscularis propria (mm) (≥pT3) *(n = 7)*		2.5 ± 2.3	1 - 7
Plane of excision			
Mesocolic	5		
Intramesocolic	4		
Complete resection at all margin achieved?			
R0 (yes)	9		
R1 (no)	0		
*Tumour T stage**			
pT1	1		
pT2	1		
pT3	6		
pT4b	1		
Distance to circumferential margin (mm)		25.4 ± 27.4	2.5 – 75
Lymph node yield		24.3 ± 10.7	5 – 38
Total number lymph nodes involved			
0	5		
1	1		

2	1		
3	1		
*Tumour N stage**			
pN0	4		
pN1a	1		
pN1b	2		
pN1c	1		
pN2a	1		
Apical node involvement			
Yes	0		
No	9		
Size largest malignant node (mm) *(n = 4)*		7.0 ± 1.2	6 – 9
Deepest level of venous invasion			
None	6		
Intramural	0		
Extramural	3		
Deepest level of lymphatic invasion			
None	4		
Intramural	0		
Extramural	5		
Deepest level of perineural invasion			
None	7		
Intramural	0		
Extramural	2		
Histologically confirmed distant metastatic disease Yes: No	0 : 9		
*Tumour M stage**			

M0	9		
M1 a/b/c	0		

Table 5: Summary of subject histopathology results

Abbreviations: S.D standard deviation.

* TNM staging (tumour, node metastasis) of colorectal carcinoma (AJCC 8th edition) (Loughrey et al. 2017)

Loughrey, M, P Quirke, Neil A NA Shepherd, and Gloucestershire Royal Hospital. 2017. Royal College of Pathologists., *G049 Dataset for Histopathological Reporting of Colorectal Cancer*.

3 Safety data

Expected Adverse Events (AEs)	
Respiratory	
Hospital acquired pneumonia	2
Gastrointestinal	
Constipation	1
Prolonged ileus	2
Biochemical: deranged liver function tests	1
Cutaneous	
Phlebitis at cannula site	1
Genito-urinary	
Urinary tract infection	1
Unexpected Adverse Event	
Mechanical fall at home	1
Total events	**9**

Serious Adverse Events (SAE)	
Neurological	
Vasvovagal syncope episode	1

Suspected Unexpected Serious Adverse Reactions (SUSARs)	
Total	0

Table 6: Summary of adverse events

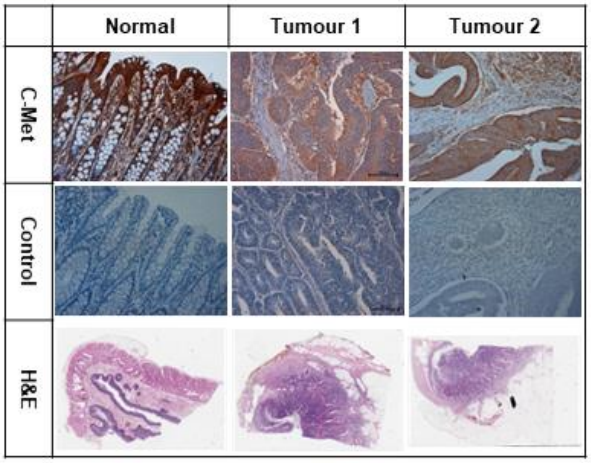
4 Protocol adherence

There were two protocol deviations reported.

Participant ID	Deviation	Reason	Outcome
EM003	Fresh frozen tumour samples not obtained for long-term storage and potential future translation research.	Patient safety. Tumour was too small to safety take tissue for storage and ensure adequate tissue for standard NHS histopathology assessment.	No fresh tumour stored. Normal colon tissue stored.
EM006	Withdrawn from trial after consent obtained.	Patient safety. A lack of high dependency post-operative beds at the clinical site, LTHT meant surgery was cancelled on the day. Surgery was urgently rescheduled but the research team could not support the new date.	Participant was withdrawn from the trial. Underwent standard care surgery without EMI-137.

Table 7: Protocol deviations reported during trial active phase.

EM002



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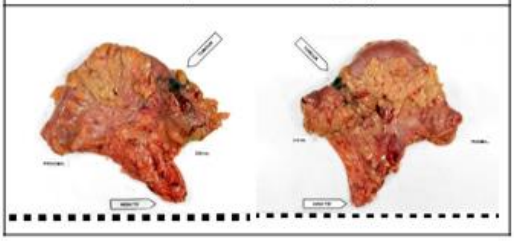

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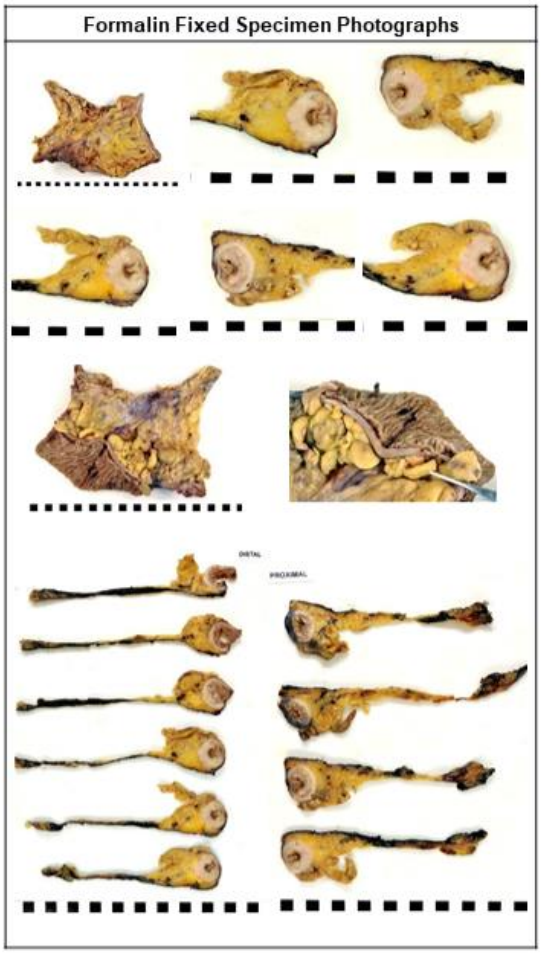
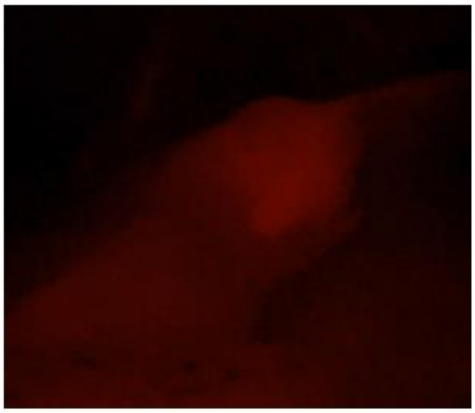
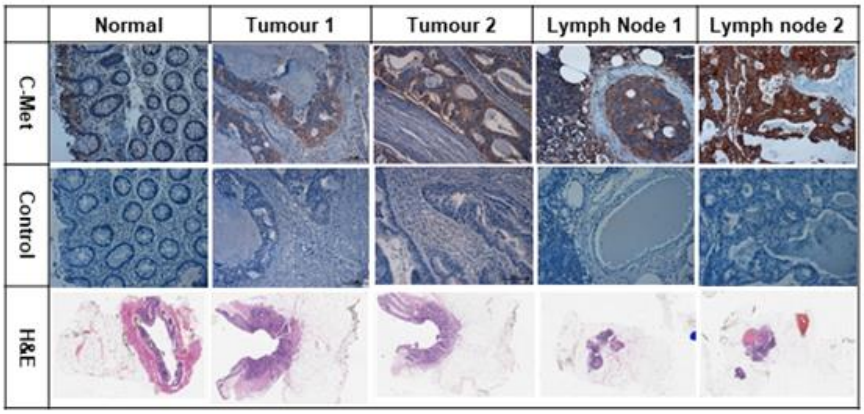
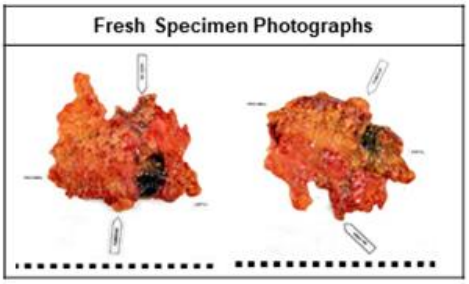


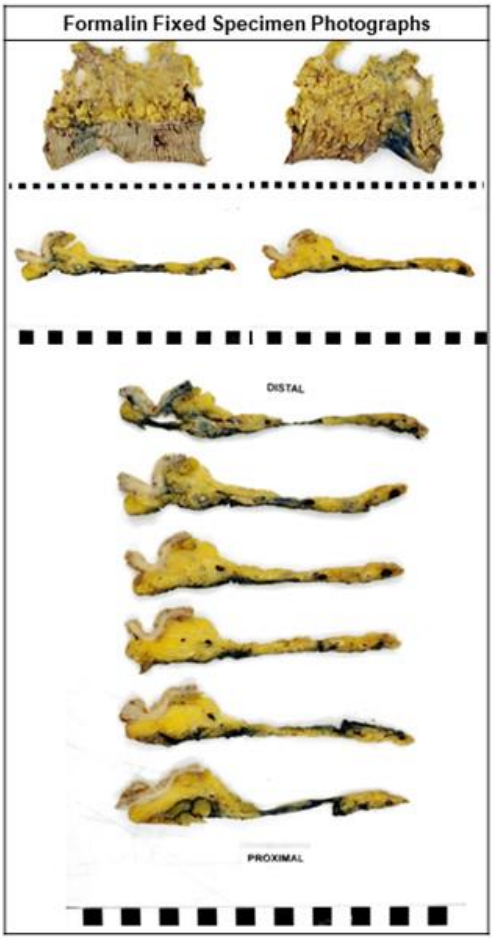
EM002	
Age (years)	76
Gender	Male
Tumour location	Sigmoid colon
Maximum tumour diameter (mm)	25
Pathological stage	pT3 N0 (0/19) M0 R0 V0
Plane of excision	Mesocolic
Histological sub-type	Adenocarcinoma
Differentiation	We///moderately differentiated
Tumour fluorescence	Isofluorescent to background
Lymph Node fluorescence	None
Interval to assessment (hh:mm)	01:36



EM003

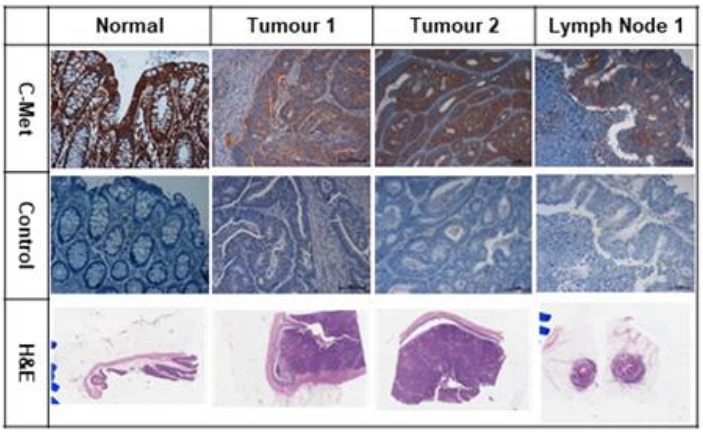






EM003	
Age (years)	67
Gender	Female
Tumour location	Descending colon
Maximum tumour diameter (mm)	15
Pathological stage	pT3 N1b (2/14) M0 R0 V0
Plane of excision	Mesocolic
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Mildly fluorescent
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:40

EM004



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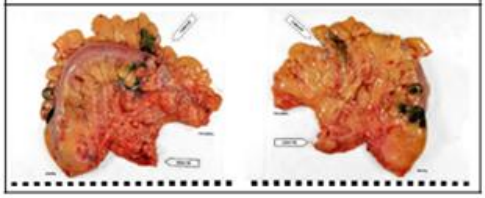

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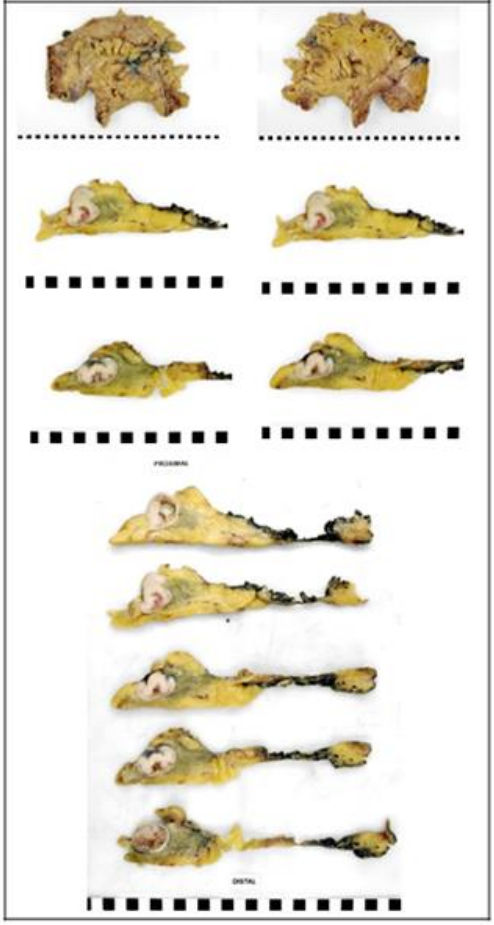
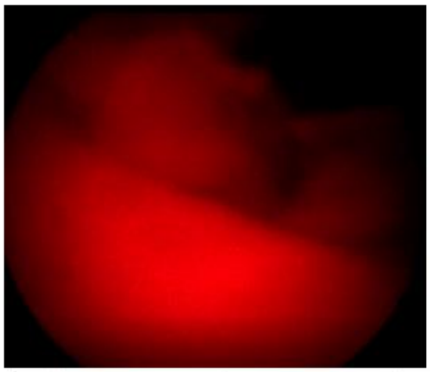
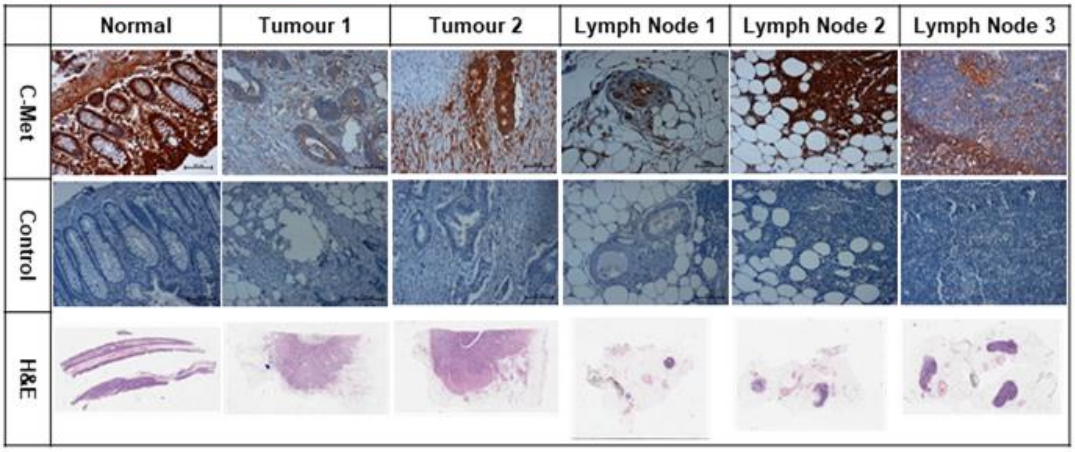


EM004	
Age (years)	77
Gender	Male
Tumour location	Sigmoid colon
Maximum tumour diameter (mm)	35
Pathological stage	pT2 N1a (1/21) M0 R0 V0
Plane of excision	Intramesenteric
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Mildy to background
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:15



EM005



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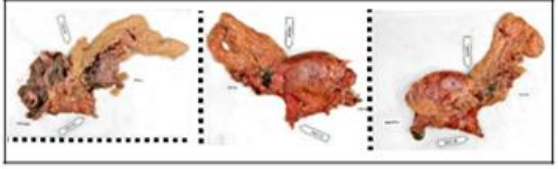

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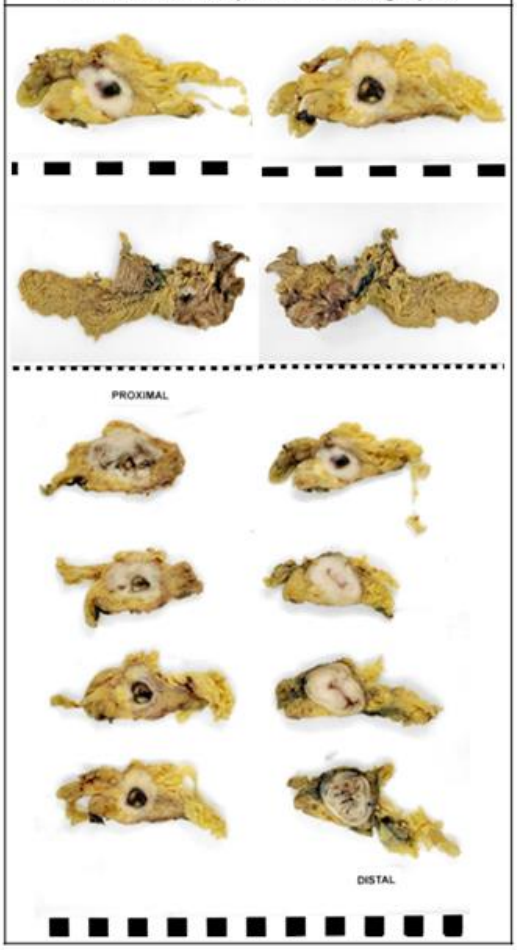
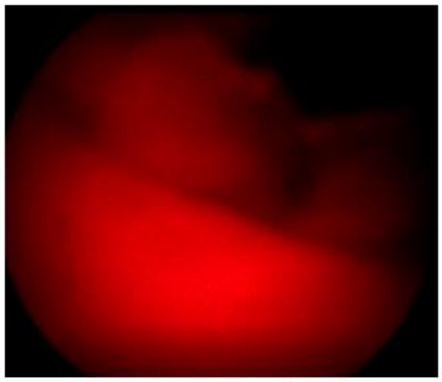
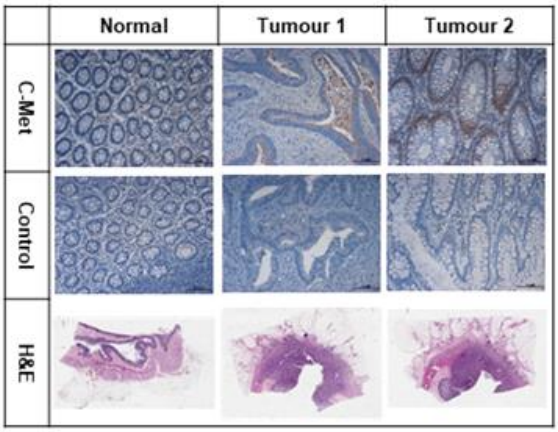


EM005	
Age (years)	73
Gender	Male
Tumour location	Hepatic flexure
Maximum tumour diameter (mm)	38
Pathological stage	pT4b N2a (4/28) M0 R0 V1
Plane of excision	Mesocolic
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Isofluorescent to background
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:50



EM007



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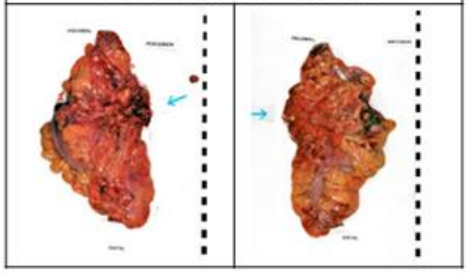

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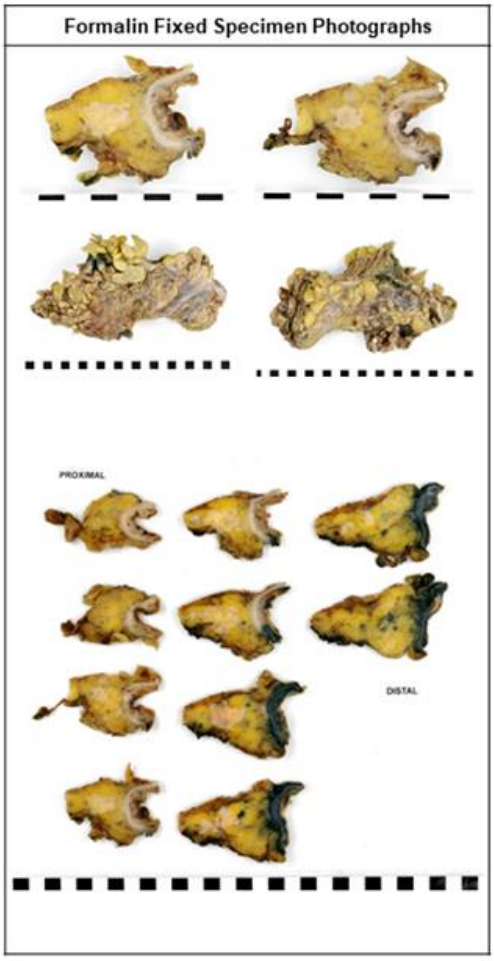
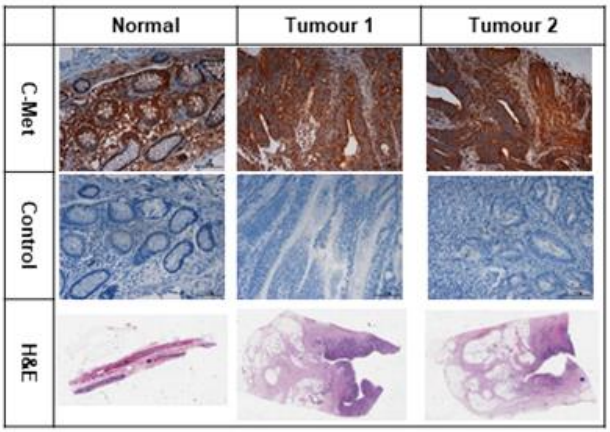
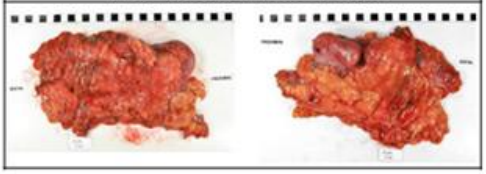


EM007	
Age (years)	70
Gender	Female
Tumour location	Sigmoid colon
Maximum tumour diameter (mm)	17
Pathological stage	pT3 N1c (0/35) M0 R0 V1
Plane of excision	Mesocolic
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Isofluorescent to background
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:09

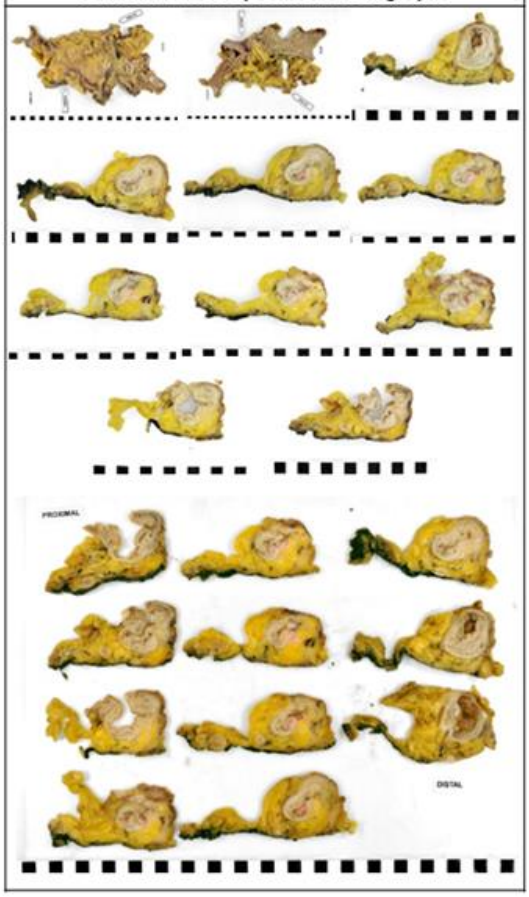
EM008



Fresh Specimen Photographs

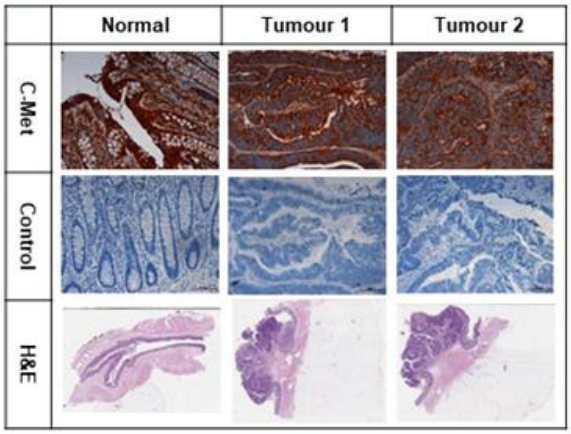


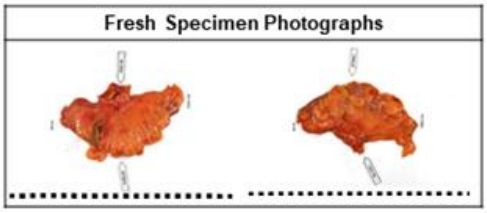
Formalin Fixed Specimen Photographs

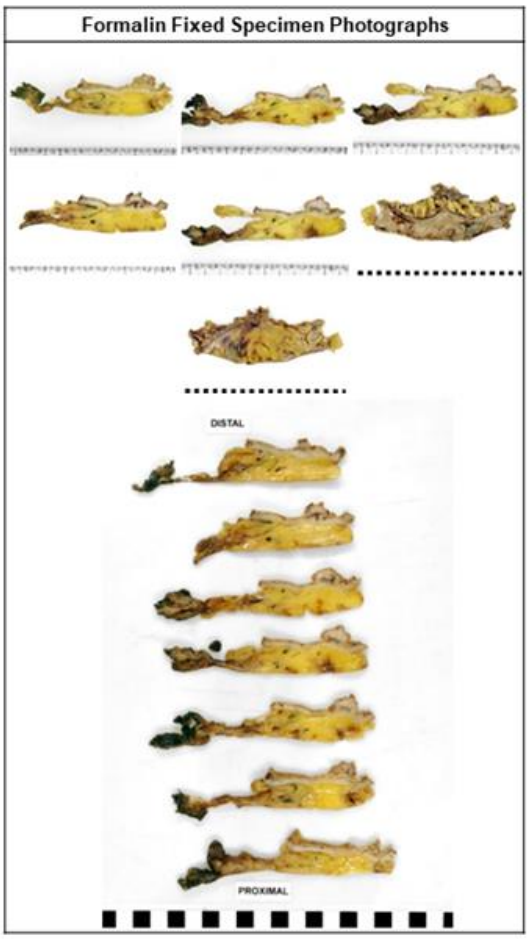


EM008	
Age (years)	67
Gender	Male
Tumour location	Ascending colon
Maximum tumour diameter (mm)	55
Pathological stage	pT3 N0 (0/38) M0 R0 V0
Plane of excision	Intramesenteric
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Isofluorescent to background
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:55

EM009

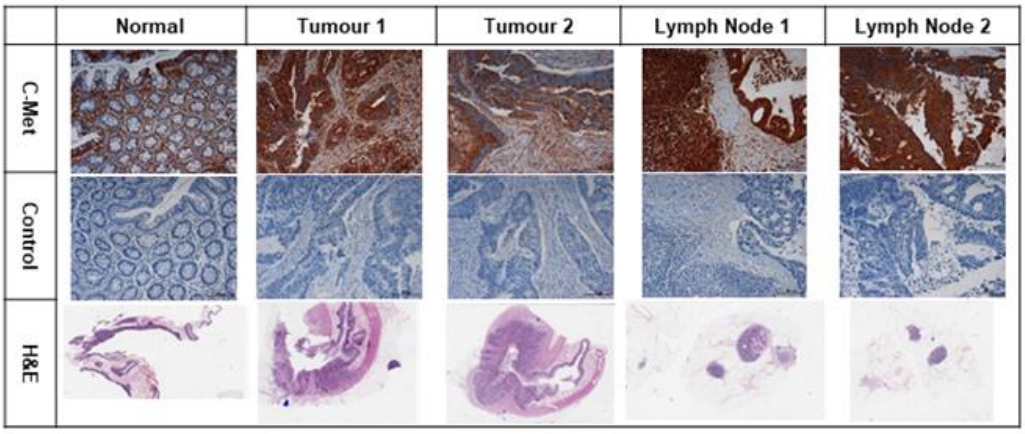


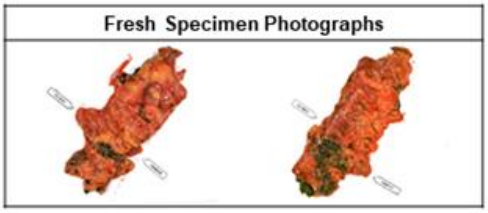


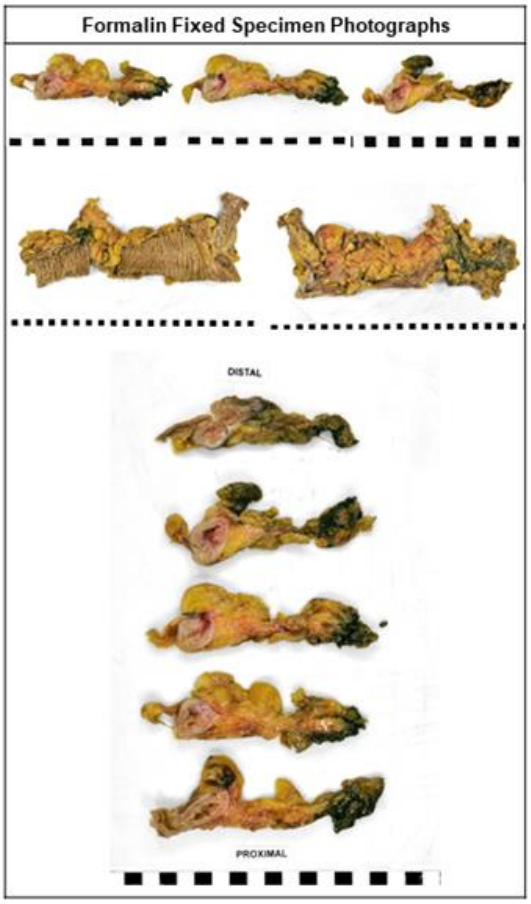


EM009	
Age (years)	68
Gender	Female
Tumour location	Sigmoid colon
Maximum tumour diameter (mm)	28
Pathological stage	pT1 N0 (0/5) M0 R0 V0
Plane of excision	Intramesenteric
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Mildly fluorescent
Lymph node fluorescence	None
Interval to assessment (hh:mm)	01:49

EM010







EM010	
Age (years)	71
Gender	Male
Tumour location	Transverse colon
Maximum tumour diameter (mm)	15
Pathological stage	pT3 N1b (3/21) M0 R0 V1
Plane of excision	Intramesenteric
Histological sub-type	Adenocarcinoma
Differentiation	Well/moderately differentiated
Tumour fluorescence	Mildly fluorescent
Lymph node fluorescence	None
Interval to assessment (hh:mm)	02:14

Figure 2: Summary of individual participants' intra-operative imaging and pathological assessment.