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GLiSten

5-ALA in Bowel Cancer Surgery

Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery - Developmental phase

Sponsor: University of Leeds (GS11/9681)

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Trial Registration

Only authorised members of staff from hospital sites with appropriate trial approvals have permission to register patients into the trial.

Tel: CTRU +44 (0)113 343 4930 (Monday – Friday 9am – 5pm except Public and University holidays)

Reporting SAEs, SARs and SUSARs

Complete the relevant CRF for all SAEs occurring in the trial and fax to the CTRU within 24 hours of becoming aware of the event:

Fax: CTRU +44 (0)113 343 7985

A receipt will be sent for all received SAEs. ***If a receipt is not received within two working days contact the CTRU.***

Reporting Pregnancies

Report all pregnancies to the coordinating Research Fellow at SJUH using the Notification of Pregnancy CRF as soon as you become aware of the event:

Fax: +44 (0)113 206528

A receipt will be sent for all received pregnancy reports. ***If a receipt is not received within two working days contact SJUH Research Fellow***.

Pregnancies resulting in a congenital anomaly must also be reported to CTRU as an SAE

2 CONTENTS

3 PROTOCOL SYNOPSIS

Name of Sponsor University of Leeds		
Sponsor Protocol Number **GS11/9681**	**EUDRACT Number** 2012-002623-15	**ISRCTN number** TBC
Short Title GLiSten		
Full Title	GLiSten: Next generation intraoperative lymph node staging for stratified colon cancer surgery – development phase.	
Objectives	**Primary objective:** To optimise the dose of oral 5-ALA administration for intra-operative fluorescence diagnosis of metastatic lymph nodes in colon cancer. **Secondary objectives:** 1) Establish a reliable and repeatable methodology for fluorescence diagnosis of lymph node metastasis by standardisation of: i) pre-operative CT lymph node reporting ii) intra-operative fluorescence detection system iii) surgical technique for laparoscopic segmental colonic resection with D3 lymphadenectomy iv) histopathogical examination of resected specimens. 2) identify systemic, operative and patient factors which adversely affect the intraoperative detection of lymph node fluorescence.	
Trial Design	First phase of a two part design incorporating: i) developmental phase to optimise the use of 5-ALA for intraoperative lymph node staging in colon cancer, and ii) evaluation phase in which patients with colon cancer will be recruited	

	to determine sensitivity, specificity and diagnostic accuracy of 5-ALA intraoperative lymph node staging as compared to in-depth histopathology. In this developmental phase, initially two cohorts of 10 patients with positive lymph nodes (as verified on postoperative histology) will be treated with different doses of 5-ALA. As lymph nodes can only be verified on post-operative histology it is anticipated that up to 16 patients may need to be treated to identify 10 with positive lymph nodes. We will attempt to enrich the study population to contain patients with lymph node disease, but it is emphasised that this cannot be done accurately on preoperative CT staging. As a result, more than 10 patients may have to be recruited to each cohort to achieve the required 10 with positive lymph node disease. Sixteen patients per cohort has been anticipated as there is only 25% - 30% lymph node positivity in colorectal cancer. With enrichment of recruitment, to include more lymph node positive patients using the FOxTROT radiology criteria, we estimate that 10 out of 16 patients will be informative with positive lymph nodes. In patients without lymph node disease, fluorescence of the primary cancer will act as a positive control of efficacy of 5-ALA FD of colorectal cancer. Table 1 in section 8.1 provides a guide to the doses that may be used. The two participating centres will work together in this regard, so as to co-ordinate between themselves, but the exact dose administered to each patient will be at the discretion of the treating surgeon. We will start with the predicted optimal dose of administration i.e. 20mg/kg (the most commonly used dose and timing in the literature – Appendix 1). A second cohort will be assessed at a lower dose of 10mg/kg if 20mg gives an indication of activity, or a higher dose of 30mg/kg if 20mg/kg does not give adequate indication of activity. In this way, by the time we have recruited 20 patients with positive lymph nodes we will have optimised the dose of administration.

	Within the recruitment of the first 20 patients with positive lymph nodes, the ability of 5-ALA to detect positive nodes as compared to histology in at least 2 of 10 patients (in either cohort) will be required to progress to the next part of the study (see below). This corresponds to the upper boundary of the 99% exact confidence interval of the sensitivity being at least 60% i.e. a good indication of activity given that the technical protocols and techniques may not yet be fully optimised. A 99% confidence interval has been chosen to reflect the additional uncertainty in 10 patients. As the dose of 5-ALA is assessed, work will be carried out to standardise the pre-operative radiological assessment, the technique of laparoscopic D3 lymphadenectomy, and the pathological lymph node mapping. If 5-ALA detects positive lymph nodes in at least 2 of 10 patients (in either cohort) then, from these initial results, the most promising dose of 5-ALA will be identified. At least a further 10 patients with lymph nodes with metastatic disease as judged by histological evaluation will then be recruited and treated with this schedule, with flexibility to include further patients to confirm the validity of the technique before proceeding to the evaluation phase. The exact number of patients will be determined in discussion with the TSC and DMEC prior to the start of this stage of the trial but, at their discretion, may be increased during this stage of the trial. We will aim to recruit up to 52 patients in total for the developmental phase. However, if necessary, more than this number of patients will be recruited to obtain this number with metastatic disease as judged by histological evaluation. The ability of 5-ALA FD to reliably detect lymph nodes with metastatic disease as judged by histopathological evaluation will be assessed by requiring

	that, to progress to the evaluation phase, the upper bound of the 99% (Clopper Pearson) confidence interval of the sensitivity (in the patients with positive nodes recruited in this stage of the trial) is at least the target value of 80%. This analysis will be versus histopathology but on a per patient basis, considering whether a patient has at least 1 positive tumour node identified by 5-ALA. A 99% confidence interval has been used to reflect the additional uncertainty in a relatively low number of patients. To allow for the optimization of the other variables (e.g. fluorescence detection system), however, this analysis will not include patients treated with this schedule before it was identified as the most promising schedule.
Trial Population	Adult patients undergoing elective surgery for colon adenocarcinoma
Inclusion Criteria	• Patients undergoing elective surgery for right or sigmoid colon cancer amenable to laparoscopic surgery incorporating D3 lymphadenectomy, including those with metastatic disease. • American Society of Anaesthesiologists (ASA) classification ≤ 3 • Normal hepatic and renal function
Exclusion Criteria	• Patients with a past history of hypersensitivity reactions, hepatic or renal dysfunction, and acute or chronic porphyria due to the risk of side effects related to 5-ALA administration. • Patients with cancers of the transverse and descending colon due to difficulties in defining D3 lymphadenectomy in these anatomical locations • Co-existent colonic pathology, such as inflammatory bowel disease, which might influence the lymphatic uptake of 5-ALA or patients with synchronous colonic or rectal cancer (but not benign polyps)

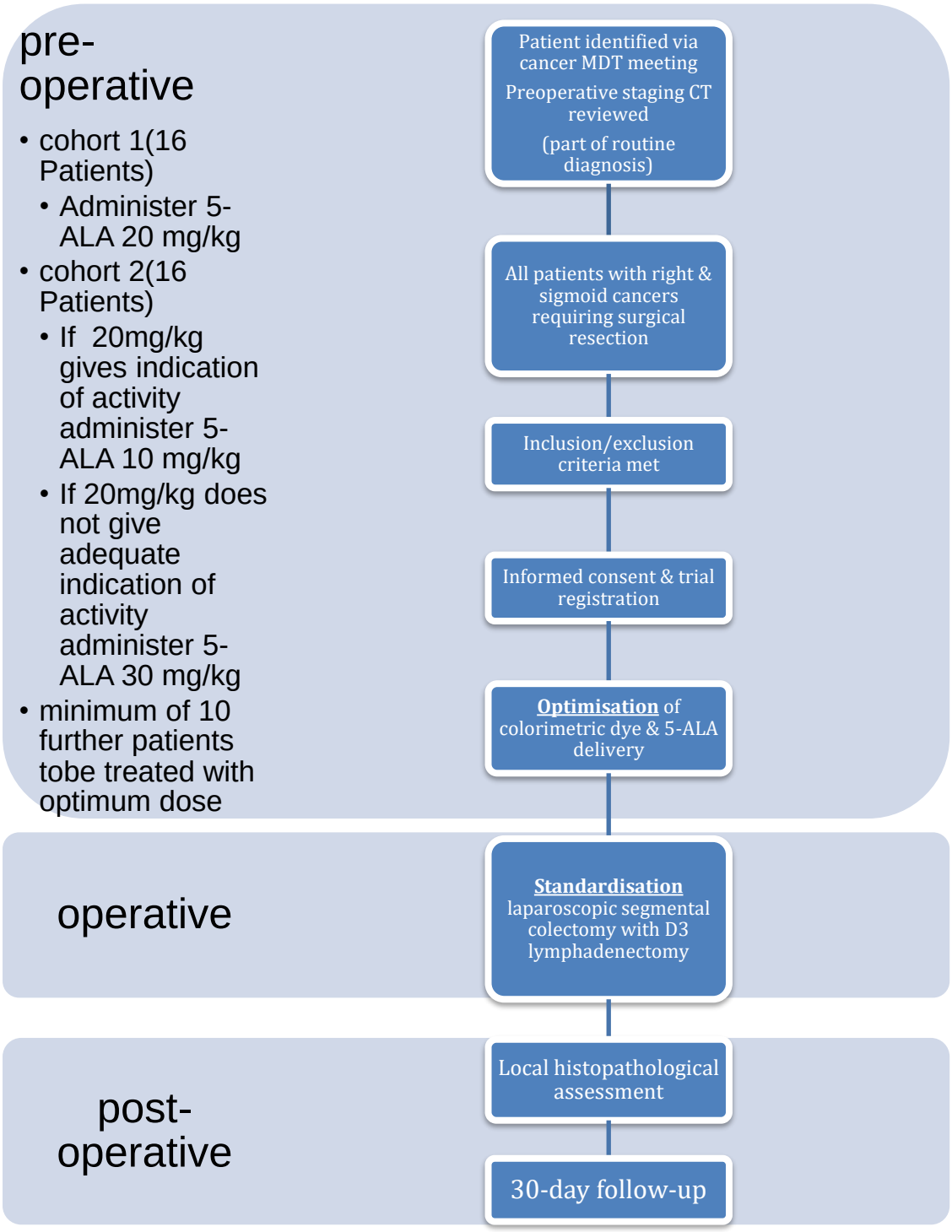
Procedures	• Preoperative CT scan as per routine care • Endoscopic administration of Indian ink at routine colonoscopy • Perioperative 5-ALA administration • Laparoscopic right or sigmoid colectomy with D3 lymphadenectomy
Trial Treatment	• Oral 5-ALA administration
Statistical Methods	See, “trial design,” above.

4 LIST OF ABBREVIATIONS

ACRONYM	DEFINITION
AE	Adverse Event
ALT	Alanine Transaminase
AR	Adverse Reaction
AST	Aspartate Transaminase
CI	Chief Investigator
CI	Confidence Interval
CRF	Case Report Form
CT	Computerised Tomography
CTA	Clinical Trial Authorisation
CTCAE v4.0	Common Terminology Criteria for Adverse Events version 4.0
CTRU	Clinical Trials Research Unit
DMEC	Data Monitoring and Ethics Committee
FBC	Full blood count
FD	Fluorescence diagnosis
GCP	Good Clinical Practice
ICMJE	International Committee of Medical Journal Editors
IMP	Investigational Medicinal Product
LFT	Liver Function Tests
MHRA	Medicines and Healthcare Products Regulatory Agency
NCRI	National Cancer Research Institute
NIHR	National Institute for Health Research
NHS	National Health Service

REC	Research Ethics Committee
RUSAE	Related Unexpected Serious Adverse Event
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SSOP	Study Site Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
UE	Urea and Electrolytes

5 TRIAL FLOW DIAGRAM



6 INTRODUCTION

6.1 Colorectal cancer

Colorectal cancer is the third most common cancer in the UK with an incidence of over 41,142 new cases per annum with over two thirds occurring in the colon (26,330 cases)(1); as such it represents a substantial burden on healthcare resources. In comparison to rectal cancer, which has seen improvement in survival over the past decade, the survival from colon cancer has remained largely unchanged with 5-year overall survival around 50%. In terms of segmental distribution, the right colon is the most common site harbouring ~25% of colorectal cancers, followed by the sigmoid colon with ~20%. Curative resection involves segmental colectomy to resect the primary cancer and the draining lymphatic field. Emerging evidence suggests that the standard of segmental colectomy as performed in the UK is of variable quality and that improvement in technique may help to improve survival outcomes with a survival advantage of up to 27% in patients with lymph node involvement (2). If this is correct, then a change in surgical technique to complete mesocolic resection and extended D3 lymphadenectomy would make a substantial contribution to improving the prognosis of patients with colon cancer (3-5). The corollary is that only ~25% of patients have lymph node involvement, meaning that in the other 75% of patients extended D3 lymphadenopathy potentially represents overtreatment. Such surgical changes can be quickly and cheaply introduced into practice and even very modest improvements in outcome, way below the potential that could be achieved, would be highly cost effective.

6.2 Strategies to improve colon cancer surgery

Segmental colectomy is the current standard of surgery for colon cancer based on resection of the primary cancer along with the draining lymphatic field. In this way, the primary cancer is removed along with any regional lymph node metastases so as to minimize the chance of local tumour recurrence. Emerging evidence suggests that survival outcomes following colon cancer surgery can be improved by increasing the

radicality of lymphadenectomy and respecting oncological planes of resection. The technique of complete mesocolic resection with extended lymphadenectomy, as described by Hohenberger (3), has reported local recurrence rates of 3.6% and 5-year disease free survival of 89.1%, attributed to removal of more tissue and lymph nodes in the correct surgical planes. These figures compare favourably to accepted local recurrence rates of 8-10% reported elsewhere (6). Further, West et al showed a survival advantage from mesocolic plane surgery of 15% across all disease stages and up to 27% in patients with lymph node involvement (4), an observation that has been validated in the MRC CLASICC trial (7). In the series reported by West et al, only 32% of the specimens appeared optimal, suggesting substantial room for improvement in surgical technique (4).

Current standard surgery for colon cancer differs from that described by Hohenberger in the extent of the lymphadenectomy performed. Standard surgery would generally involve a “D2 lymphadenectomy” whereby the second tier of draining lymph nodes are removed but the central high ligation required for “D3 lymphadenectomy” is not routinely practiced. Attempts to reproduce Hohenberger’s results have been encouraging with a series from Bergen, Norway, reporting an improvement in overall survival from 58% to 78% following the introduction of standardized D3 lymphadenectomy in 2007 (personal communication). There is therefore a strong case that standardized radical surgery with D3 lymphadenectomy in all cases of colon cancer will improve long-term outcomes.

However, this “one size fits all” approach fails to take into account the biological variation of colon cancer or the fitness and expectations of the patient. Only 25% of cancers have metastatic disease to the lymph nodes, suggesting that D3 lymphadenectomy is overtreatment in the majority. There is the added concern that the majority of colorectal cancer patients are elderly with comorbidity, and that a universal policy of radical resection will lead to unnecessary morbidity. This concern is reinforced by data from the Karolinska Institute, Sweden, with a significant increase in major complications from 19.3% to 28.3% and re-operation rates from

2.9% to 8.6% following the introduction of standardized D3 lymphadenectomy in 2004/5 (personal communication). Other series, however, such as that from Hillerød Hospital, Denmark, have reported improved oncological resection without any increase in morbidity (5). Another factor that needs to be taken into account when determining future surgical strategy is the changing pattern of disease presentation with the introduction of screening programmes. In the UK, the introduction of a national bowel screening programme has seen a shift in incidence of early cancers (Dukes' stage A) from 10.1% prior to screening to 45.3% following implementation (8). As the incidence of lymph node metastases in Dukes' A cancer is less than 10%, a policy of radical D3 lymphadenectomy for all cannot be justified and is unlikely to produce any survival benefit. It therefore seems sensible to adopt a more selective approach whereby patients with lymph node involvement are offered D3 lymphadenectomy, whilst those without nodal involvement undergo a more conventional D2 lymphadenectomy. Currently, there is no reliable method for determining lymph node status either pre-operatively or intra-operatively. The decision to carry out D2 or D3 lymphadenectomy therefore remains at the discretion of the operating surgeon. It is only with an accurate and objective system for intra-operative lymph node staging that the level of resection can be tailored to individual patients. The proposed research will evaluate the merits of 5-ALA fluorescence for this purpose.

6.3 Preoperative lymph node staging

The difficulty in implementing a selective strategy for surgical resection, however, is in accurately defining preoperative lymph node status. No clear radiological definition of a malignant lymph node is agreed. A common definition is any node greater than 1cm or a cluster of three or more nodes less than 1cm. Some studies have used a cut-off size of 1.5cm, or have used contrast enhancement to distinguish positive nodes. The presence of micrometastases within normal sized lymph nodes and benign enlargement of nodes due to inflammation are known to contribute to inaccuracies of size-based criteria. In a recent prospective audit of 84 patients with colon cancer undergoing preoperative multi-detector CT scanning, the accuracy, sensitivity, and specificity for detection of lymph node disease was 58 (95%CI: 48,

68), 64 (95%CI: 48, 77), and 53 (95%CI: 39, 67) per cent respectively, with poor inter-observer agreement for node status (9). Further, when nodes were assessed according to TNM classification by separating into N0, N1, and N2 disease, the accuracy, sensitivity, and specificity fell to 50% for all values. A meta-analysis of 19 studies has reported an overall sensitivity and specificity for CT detected malignant lymph nodes to be 70% (95%CI: 59, 80) and 78% (95%CI: 66, 86), with a diagnostic odds-ratio of 8.1 (95%CI: 4.7, 14.1) (10). Attempts to improve lymph node staging by combining functional imaging, such as positron-emission tomography, with CT or using functional diffusion-weighted MRI, have shown some promise, but are not universally available and therefore as yet have limited application.

The Japanese Society for Cancer of the Colon and Rectum has circumvented this dilemma to some extent by recommending surgical resection based on primary tumour T-stage, with D3 lymphadenectomy recommended for T3/4 disease (11). A similar strategy was adopted in the NCRI FOxTROT study; a randomised trial investigating the use of pre-operative chemotherapy +/- anti-EGFR monoclonal antibody in high risk operable colon cancer (12). Although still not perfect, CT imaging is more accurate in determining T-stage than it is for nodal status and within the context of FOxTROT has been shown to accurately select patients for pre-operative chemotherapy (13). The accuracy, sensitivity, and specificity of CT scan in selecting patients with “bad” T3/4-stage cancers was reported to be 74% (95%CI:64–82), 78% (95%CI:65–87), 67% (95%CI:49–81) respectively (9). Although this gets around, to some extent, the problem of inaccurate pre-operative staging of local disease, there is no absolute correlation between “bad” T-stage and lymph nodal status; some 50% of T4 cancers will not have metastatic disease to the lymph nodes.

6.4 Strategies for intraoperative lymph node staging

- **Sentinel lymph node mapping**

The ideal solution to the problem of accurate preoperative lymph node staging is to develop a reliable means of intraoperative staging. This would enable a real-time

assessment of lymph node status. The concept of intra-operative lymph node staging is not new, but has received much interest with the introduction of sentinel lymph node (SLN) mapping techniques. The sentinel lymph node was defined by Morton et al as the first regional lymph node encountered by metastasising tumour cells in a study using SLN mapping in melanoma (14). It was proposed that if the sentinel lymph node was free of tumour, then it could be assumed that the remaining lymph nodes would also be tumour-free and hence unnecessarily extensive resections could be avoided in patients with early stage disease. Early studies of SLN mapping were performed by injecting a dye, such as isosulphan blue or patent blue-V (14, 15) into the primary lesion, which was taken up into the lymphatics so that the lymph nodes could be visualised at operation. Subsequently, lymphoscintigraphy was added to blue dye, whereby a locally injected radionucleotide was detected intra-operatively using a gamma probe, enabling higher detection rates (16, 17). This concept has proven validity in some cancers, most notably breast cancer, where the absence of an involved sentinel node spares the patient more radical axillary clearance and associated morbidity. The Association of Breast Surgery Guidelines now recommends sentinel node biopsy for the majority of patients with early invasive breast cancer (18). SLN mapping was first reported in colorectal cancer in 1999 with blue dye injected around the tumour prior to resection and histological examination of the lymph nodes for cytokeratin expression. The results from early series were not encouraging with SLN detection rates of 58%-98%, sensitivity rates of 40%-100%, and false negative rates between 0%-60% (19). More recent studies have failed to improve a great deal on these results with large variations in the reported detection rates, sensitivity, and false negative rates, which have been variously attributed to heterogeneity in detection techniques, definition of the SLN, time interval between injection and SLN detection, histopathological techniques, and patient characteristics, including tumour stage and body mass index (20).

Importantly, concerns have been expressed regarding the high false negative rates and whether metastases from colon cancer follow an orderly spread through tiers of lymph nodes, or rather metastasize as skip lesions. The variability in anatomical

site of the first metastatic lymph node was highlighted by Tan et al, who found that in 48% of cases metastatic tumour cells skipped the pericolic lymph nodes or were 5cm beyond the longitudinal tumour margin in 18% of cases (21). Further, Park et al reported that in 6% of caecal cancers lymph node metastases occurred along the right branch of the middle colic artery, which would frequently not be included in a standard D2 segmental resection (22, 23)

Currently, therefore, sentinel lymph node mapping is not in routine use in colon cancer, although it may have a role in focused histological ultra-staging, whereby positive nodes are subjected to detailed step sectioning in order to increase the detection of micro-metastatic disease (23). The main disadvantage of SLN mapping techniques, however, is that although they may identify potentially involved lymph nodes, they are not specific for metastatic tumour.

- **Photodynamic diagnosis with 5 aminolevulinic acid (5-ALA)**

A potentially neat solution to intra-operative lymph node staging involves agents used in photodynamic diagnosis (PDD) and therapy (PDT). Probably the most studied agent in this respect is 5-aminolevulinic acid (5-ALA), which is present in virtually all human cells. 5-ALA is a pro-drug, taken up into the mitochondria of cells, where it serves as a precursor of protoporphyrin IX (PpIX), which in turn is the direct precursor of heme. PpIX is a fluorescent molecule, which when exposed to blue-violet light of excitation wavelength 405nm, emits a characteristic red fluorescence at a wavelength of 630 to 700 nm.

5-ALA is preferentially taken up by cancer cells and metabolized to Protoporphyrin IX (PpIX). The tendency for cancer cells to accumulate PpIX, is enhanced by exogenous administration of 5-ALA. When administered in high doses, and irradiated with blue-violet light, 5-ALA is cytotoxic to cancer cells, and hence its PDT effects. In lower doses, the emitted fluorescence can be used for PDD.

The reasons for the selective uptake of 5-ALA in tumour cells have not been fully elucidated. In normal cells, 5-ALA synthesis is regulated by a feedback control system that is driven by high intracellular concentrations of free heme. This system is overridden when exogenous 5-ALA is administered. Accumulation of PpIX in tumour relative to normal cells has been attributed to alterations in the activity of the rate limiting enzymes porphobilinogen deaminase (PBGD) and ferrochelatase (FC) (24, 25). The former is increased and the latter decreased in tumour cells. Alterations in enzymatic activity may also be due to reduced availability of intracellular Fe2+ as a result of rapid cell division. Additionally, several biochemical and structural changes in tumour cells have been linked to their avidity for PpIX, including reduced pH, up-regulation of low-density lipoprotein receptors, and stromal abnormalities including large interstitial space, leaky vasculature, and large amounts of newly synthesised collagens and lipids (26).

Following systemic administration, 5-ALA is metabolised in the liver and excreted in the bile and urine. Much of the 5-ALA is used by the liver to synthesize PpIX, whilst the remaining 5-ALA circulates to other body sites where all other cell types (except those without mitochondria) convert it to PpIX. Intracellular PpIX returns to normal levels up to 48hrs post application.

A substantial body of work has accumulated on the use of 5-ALA and its derivatives in the fluorescence detection of solid cancers. In humans, 5-ALA fluorescence has been extensively used as a diagnostic aid in transitional cell carcinoma of the bladder (26), in neurosurgery to guide malignant glioma resection, and in gynaecology to detect endometriosis (27, 28), peritoneal metastases from ovarian cancer (29) and cervical squamous intraepithelial lesions (30).

In Urology, several studies have shown the superior detection of transitional carcinoma of the bladder using photodynamic diagnosis as compared to white light cystoscopy. Both 5-ALA and its hexyl-derivative, Hexaminolaevulinate (HEX), have been evaluated in bladder cancer. Burger et al reported reduced residual tumour

and increased recurrence free survival in patients undergoing photodynamic diagnosis with 5-ALA or its HEX, as compared to white light alone(31). Residual tumour was present in 33% with white light, 15% with 5-ALA and 9% with HEX. 3 year recurrence-free survival was 67% with white light, 80% with 5-ALA and 82% with HEX. Differences between 5-ALA and HEX were non-significant. Similar promising results were reported by Daniltchenko et al in 115 patients with superficial bladder cancer(32), but a larger randomised study of 300 patients failed to demonstrate a difference in tumour recurrence(33).

In Neurosurgery, a randomised controlled phase III multicentre trial was performed to investigate the safety and efficacy of fluorescence-guided resection in malignant gliomas (34). The study involved oral administration of 5-ALA (20 mg/kg) 3 hours (range 2-4) before induction of anaesthesia. A modified neurosurgical microscope was used that allowed switching between conventional white xenon illumination and violet-blue excitation light. Follow-up (median 35.4 months) was available for 139 fluorescence-guided resections and 131 resections carried out with conventional white light microsurgery. Fluorescence-guided resection was associated with a reduced risk of death or progression compared with white light microsurgery (hazard ratio 0·73 [0·57–0·94], p=0·01).

In colorectal cancer and its metastases, PpIX has been identified as the predominant endogenous fluorophore, and even in the absence of exogenous 5-ALA can distinguish involved from uninvolved lymph nodes with a sensitivity and specificity of 62% and 78% respectively (35). In a small pilot study in humans, two patients with inoperable rectal adenocarcinoma were given 30 mg/kg 5-ALA orally, and a patient with sigmoid colon carcinoma was given 60 mg/kg. Serial biopsies taken over 24 hours after photosensitisation were examined by fluorescence microscopy and showed maximum PpIX accumulation after 4-6 hours of administration of 30 mg/kg 5-ALA, with tumour PpIX being greater in the patient who had received 60 mg/kg 5-ALA. In another pilot study involving a mixed group of 18 patients with inoperable adenocarcinomas or adenomas of the oesophagus,

duodenum, and colon, patients were administered 30-60 mg/kg 5-ALA by the oral route (36). In colorectal cancer patients, flexible sigmoidoscopy was performed with serial biopsies. Peak fluorescence in the large bowel tumours was achieved within 6 hours, with good selectivity between tumour and normal mucosa at a ratio 5:1. Similarly effective results were seen in oesophageal and duodenal lesions, but with a lower dose of 30mg/kg 5-ALA.

An important issue with the clinical use of any photosensitizing agent is the potential for side effects. This problem appears to be confined to systemic administration when high doses are used for photodynamic therapy as opposed to lower doses used in fluorescence diagnosis. When used systemically, it is important to establish a safe dose of photosensitiser according to the interval drug to light time to avoid photosensitivity side effects. Normal precautions include keeping the patient out of direct sunlight for 24-48 hours following drug administration. Occasionally reported side effects following systemic administration include nausea, vomiting, tachycardia, hypotension, photosensitivity for up to 48 hours, and elevated liver enzymes (25). A recent trial involving 351 patients given oral 5-ALA (20mg/kg) prior to fluorescent glioma resection showed no difference in the number of adverse events between patients given 5-ALA and those who didn't receive it (37). Following systemic administration 5-ALA is associated with a transient rise in aspartate transaminase and bilirubin levels, which return to normal after 72 hours.

In a study examining the clinical pharmacokinetics of 5-ALA in normal healthy volunteers and patients at high risk of recurrent bladder cancer the peak plasma concentration of 5-ALA following oral administration were achieved at 0.83 ± 0.20h. Plasma 5-ALA concentrations declined with a terminal half-life of approximately 45 minutes (38).

- **Proposed strategy for 5-ALA intraoperative lymph node detection**

We aim to combine existing techniques in SLN mapping using colorimetric dyes, to

provide an overall lymphatic map, with the tumour-specific properties of 5-ALA fluorescence diagnosis. To our knowledge, this approach has never before been tried. This combination is particularly attractive in the context of laparoscopic surgery with both agents visible with the Storz D-Light Laparoscopic System, which combines white light (colorimetric dyes) and blue light (5-ALA) modes together with enhanced stereoscopic magnification. We will focus on cancers of the right and sigmoid colon and perform segmental colectomy with D3 lymphadenectomy. Resection specimens will be scrutinized using routine and enhanced histopathological methods to determine the sensitivity, specificity, and positive and negative predictive values for 5-ALA fluorescence diagnosis as compared to histological analysis.

In this study, we have chosen to use 5-ALA systemically via oral administration as ranges for the optimal dose and time of preoperative administration in humans have been previously determined (Appendix 1). The supplier of 5-ALA for oral administration will be Photonamic GmbH & Co. Germany.

6.5 Risks and benefits

Participants for this study will be selected on the basis that they have a diagnosis of colon cancer that requires surgical resection, and are suitable for segmental colectomy with D3 lymphadenectomy. There will be the normal risk of surgical complications associated with general anaesthesia and surgical resection. There is a small risk of photosensitivity reactions related to 5-ALA administration. This includes transient derangement of liver function tests (returning to baseline at 24 – 72 hours post-administration) and skin hypersensitivity reactions due to exposure to UV-light. Other mild and transient side effects associated with 5-ALA administration include nausea, vomiting, tachycardia, and hypotension. When 5-ALA is administered in conjunction with surgical resection of malignant brain tumours, a potential side effect is brain oedema. In response to these potential adverse effects, all patients will be kept out of direct sunlight for at least the first 48 hours following

surgery and monitored for changes in vital signs and liver function (this is normal postoperative care). During the operation patient's eyes and skin will be protected from the operating lights, using standard methods such as sterile drapes and tape to keep their eyes closed.

The above risks to patients will be offset by the advantages arising from taking part in the study. These include potential benefits arising from laparoscopic surgery that is of high quality and scrutinized by pathological assessment, high calibre histopathological analysis of the resection specimen and in particular the lymph node status, and close monitoring during the postoperative period.

The main beneficiaries, should 5-ALA fluorescence diagnosis prove to be effective, will be future generations of colon cancer sufferers. The ability to accurately tailor the radicality of surgery to the stage of the primary cancer should enable optimal oncological resection whilst minimizing the risk of unnecessary postoperative morbidity.

7 AIMS AND OBJECTIVES

The purpose of this study is to optimise the use of 5-ALA for intra-operative fluorescence diagnosis (FD) of metastatic lymph nodes in colon cancer. We hypothesize that 5-ALA FD provides an accurate means of intraoperative lymph node staging and that it is more accurate than preoperative CT staging.

7.1 Primary Objective

- To optimize the dose and timing of oral 5-ALA administration for intra-operative fluorescence diagnosis of metastatic lymph nodes in colon cancer.

7.2 Secondary Objectives

1. Establish a reliable and repeatable methodology for fluorescence diagnosis of

lymph node metastasis by standardisation of:

i) pre-operative CT lymph node reporting

ii) intra-operative fluorescence detection system

iii) surgical technique for laparoscopic segmental colonic resection and with D3 lymphadenectomy

iv) histopathogical examination of resected specimens.

2. Identify systemic, operative and patient factors, which adversely affect the intraoperative detection of lymph node fluorescence.

8 DESIGN

8.1 Study Design

This developmental study will focus on optimizing the technique of intraoperative lymph node staging using a combination of colorimetric dyes and 5-ALA. Colorimetric dye (India ink) is in routine use for SLN mapping.

Parameters to be optimised during the development phase include:

i) The dose of 5-ALA.
The most effective dose, with best-tolerated adverse effect profile from previous clinical trials, is 20 - 30mg/kg. There is some evidence that doses of 10mg/kg are equally effective. Higher doses up to 40mg/kg have been used with no increase in FD, but a higher incidence of adverse effects. (See Appendix 1 for evidence of current trials using 5-ALA)

ii) The dose and technique of administration of India ink for optimal lymphatic mapping.

Experience will be drawn from previously established sentinel lymph node mapping techniques.

iii) Standardisation of radiological reporting to include evaluation of preoperative lymph node staging.

iv) Practicalities involved in the use of the Storz D-Light laparoscopic system in detecting lymph nodes marked with India ink (white light mode) and 5-ALA fluorescence (blue light mode).

Support will be provided by the company and advice sought from urological colleagues who use the same system for transurethral resection of bladder cancers.

v) Standardisation of the techniques for segmental colectomy with D3 lymphadenectomy between the 2 centres involved in the development phase.

vi) Optimisation and standardisation of the techniques for pathological lymph node mapping and step sectioning for histological lymph node staging.

viii) Assessment of patient factors that might influence colorimetric and 5-ALA intra-operative lymph node detection, such as advanced versus early tumours, presence of other colonic pathology, patient body mass index etc.

The findings from this preliminary study will feed directly into the trial protocol of the subsequent multicentre evaluation phase. The developmental work will also result in detailed guidance and training videos to limit any operator effect when the technique is used in other trial centres.

Initially two cohorts of 10 patients with positive lymph nodes (as verified on postoperative histology) will be treated with different doses of 5-ALA. As lymph nodes can only be verified on post-operative histology it is anticipated that up to 16 patients may need to be treated to identify 10 with positive lymph nodes. As previously stated, we will attempt to enrich the study population to contain patients

with lymph node disease, but it is emphasised that this cannot be done accurately on preoperative CT staging. As a result, more than 10 patients may have to be recruited to each cohort to achieve the required 10 with positive lymph node disease. Sixteen patients per cohort have been anticipated as there is only 25% - 30% lymph node positivity in colorectal cancer. With enrichment of recruitment, to include more lymph node positive patients using the FOxTROT radiology criteria, we estimate that 10 out of 16 patients will be informative with positive lymph nodes. In patients without lymph node disease, fluorescence of the primary cancer will act as a positive control of efficacy of 5-ALA FD of colorectal cancer. Table 1 below provides a guide to the doses that may be used. The two participating centres will work together in this regard, so as to co-ordinate between themselves, but the exact dose administered to each patient will be at the discretion of the treating surgeon. We will start with the predicted optimal dose of administration i.e. 20mg/kg (the most commonly used dose and timing in the literature – Appendix 1). A second cohort will be assessed at a lower dose of 10mg/kg if 20mg gives an indication of activity, or a higher dose of 30mg/kg if 20mg/kg does not give adequate indication of activity. In this way, by the time we have recruited 20 patients with positive lymph nodes we will have optimised the dose of administration.

Table 1

Schedule	**5-ALA dose**
Cohort 1	20 mg/kg
Cohort 2	10 mg/kg or 30 mg/kg depending on activity of 5-ALA at 20 mg/kg

Information will be collected for each dose level including

- toxicity
- 5-ALA identified LN
- Histopathologically confirmed LN status

Within the recruitment of the first 20 patients with positive lymph nodes, the ability of 5-ALA to detect positive nodes as compared to histology in at least 2 of 10 patients (in either cohort) will be required to progress to the next part of the study (see below). This corresponds to the upper bound of the 99% exact confidence interval of the sensitivity being at least 60% i.e. a good indication of activity given that the technical protocols and techniques may not yet be fully optimised. A 99% confidence interval has been chosen to reflect the additional uncertainty in 10 patients.

As the dose of 5-ALA is assessed, work will be carried out to standardise the pre-operative radiological assessment, the technique of laparoscopic D3 lymphadenectomy and the pathological lymph node mapping.

If 5-ALA detects positive lymph nodes in at least 2 of 10 patients (in either cohort) then, from these initial results, the most promising dose of 5-ALA will be identified. Both patient and node-level information and practical considerations will be used to further inform this decision. At least a further 10 patients with lymph nodes with metastatic disease as judged by histological evaluation will then be recruited and treated with this schedule, with flexibility to include further patients to confirm the validity of the technique before proceeding to the evaluation phase. The exact number of patients will be determined in discussion with the TSC and DMEC prior to the start of this stage of the trial but, at their discretion, may be increased during this stage of the trial. We will aim to recruit up to 52 patients in total for the developmental phase. However, if necessary, more than this number of patients will be recruited to obtain this number with metastatic disease as judged by histological evaluation. The ability of 5-ALA FD to reliably detect lymph nodes with metastatic disease as judged by histopathological evaluation will be assessed by requiring that, to progress to the evaluation phase, the upper bound of the 99% (Clopper Pearson) confidence interval of the sensitivity (in the patients with positive nodes recruited in this stage of the trial) is at least the target value of 80%. This analysis will be versus histopathology but on a per patient basis, considering whether a patient has at least

1 node identified by 5-ALA. A 99% confidence interval has been used to reflect the additional uncertainty in a relatively low number of patients.

To allow for the optimization of the other variables (e.g. fluorescence detection system), however, this analysis will not include patients treated with this schedule before it was identified as the most promising schedule.

Centres

Two centres will collaborate in the developmental work: Prof David Jayne (Leeds) and Mr Ronan Cahill (Dublin). Each centre will recruit patients, aiming to ensure an enriched sample with lymph node metastases. It is anticipated that each centre will recruit 8 patients to each of the initial two cohorts (anticipated total of 16 patients per cohort).

9 ELIGIBILITY

9.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria to be eligible to enrol in this trial.

1. Able to give informed consent and willing to follow trial protocol
2. Aged over 18
3. Patients with cancers of the right and sigmoid colon amenable to laparoscopic resection incorporating D3 lymphadenectomy, as agreed by MDT discussion following histopathological diagnosis and radiological staging.
4. Patients with distant metastatic disease will be eligible, provided laparoscopic resection of the cancer is part of routine clinical care.

5. Fit for standard laparoscopic resection
6. American Society of Anesthesiologists (ASA) classification ≤ 3
7. Normal hepatic and renal function on most recent blood tests (to be within 30 days prior to surgery). For the purposes of the trial a normal hepatic renal function can be defined as:
    i. Total bilirubin within normal institutional limits
    ii. AST/ALT < 2.5 X institutional upper limit of normal
    iii. GFR ≥ 60 mls/min/1.73m2 or Creatinine within 10% of upper value for normal institutional limits

9.2 Exclusion Criteria

Participants meeting any of the following exclusion criteria are not eligible to enrol in this trial.

1. Patients with cancers of the transverse and left colon (due to difficulty in defining D3 lymphadenectomy in these anatomical locations)
2. Past history of hypersensitivity reactions to 5-ALA or colorimetric dye.
3. Acute or chronic porphyria or a family history
4. Patients with synchronous colonic or rectal cancer (but not benign polyps)
5. Patients with co-existent inflammatory bowel disease, such as Crohn's disease, ulcerative colitis or active diverticulitis, which may influence the lymphatic uptake of 5-ALA
6. Pregnant (positive pregnancy test) or breast feeding. 5-ALA has unknown teratogenic and abortifacient effects.

    In women of childbearing potential (defined as any female who has experienced menarche and who is not postmenopausal or permanently

sterilised e.g. tubal occlusion, hysterectomy, bilateral salpingectomy). a pregnancy test will be performed and evidence of a negative result will be required prior to entry into the study. In females of child bearing potential, or those patients with partners of child bearing potential trial investigators will advise on appropriate contraception (e.g. barrier method) from the point of registration to 30 days postop.

7. Received an investigational medicinal product at any dose within 28 days before registration
8. 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.

Where possible, the study population will be enriched with locally advanced colon cancers (CT findings of a T4 tumour, T3 tumours with an extramural depth of >5mm, as per FOxTROT definition) to obtain as much information as possible on 5-ALA for lymph node metastases.

Concerns regarding a patient's eligibility can be discussed with the SJUH Research Fellow or Chief Investigator.

9.3 Concurrent clinical trials

Patients will be screened for inclusion in other clinical trials. Providing there is no conflict, then patients may be included in both GLiSten and other trials. A notable exception is patients recruited to FOxTROT in which preoperative chemotherapy is given. This can down-stage the disease and reduce the probability of detecting lymph node metastases.

9.4 Surgeon eligibility

The two surgeons participating in this developmental study:

- are proficient with laparoscopic colon cancer surgery.
- are able to recruit a minimum of 24 patients each over a period of 18 months.

- have appropriate and willing radiological and histopathological support.
- are familiar with the principles of Good Clinical Practice.
- have the necessary research support infrastructure

10 RECRUITMENT PROCESS

Both research sites will be required to have obtained ethical, competent authority and management approval prior to opening to recruitment.

10.1 Informed Consent and Eligibility

Patients will be identified via cancer multidisciplinary team meetings and approached in outpatient clinics for participation in the study. Patients will be approached during standard clinic visits for management of their disease and will be provided with verbal and written details about the trial. This will include detailed information about the rationale, design and personal implications of the trial.

Following information provision, patients will have as long as they need to consider participation (normally a minimum of 24 hours) and will be given the opportunity to discuss the study with their family and other healthcare professionals before they are asked whether they would be willing to take part in the study.

Provision of information regarding the trial is permitted by any member of the site research team approved to do so by the Principal Investigator, although the Principal Investigator should be informed of any patients approached to participate by any other member of the site research team.

Assenting patients will then be assessed for eligibility and invited to provide informed, written consent. The Principal Investigator or any other clinically qualified member of the trial team who has received GCP training, and who is approved by

the Principal Investigator, is permitted to take informed consent. The right of the patient to refuse consent without giving reasons will be respected. Further, the patient will be free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment.

A record of the consent process detailing the date of consent and all those present will be kept in the patient notes. The original consent form will be filed in the Investigator Site File, a copy of the consent form will be given to the patient and a copy will be returned to Research Fellow at SJUH

10.1.1 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.

10.2 Registration

Eligibility must be confirmed and written informed consent for entry into the trial must be obtained prior to registration.

Registration will be administered by the CTRU, using a telephone system within office hours (Monday – Friday 9am – 5pm except Public and University holidays). The following information will be required at registration:

- Hospital name and NIHR site code
- 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 (right hemicolectomy, extended right hemicolectomy, sigmoid colectomy, high anterior resection)
- Planned operation date

The participant will be allocated a trial number by CTRU

Direct line for registration during office hours

(Monday – Friday 9am – 5pm except Public and University holidays)

+ 44 (0) 113 343 4930

Please ensure you have completed the Eligibility Checklist and Enrolment CRF before phoning

After registration, the trial research site will provide each participant with a Trial ID Card which they should be instructed to carry with them at all times and present to medical staff should they be admitted to hospital during their time on the trial.

11 Intervention Details

11.1 Pre-operative investigations and preparation

11.1.1 Preoperative CT staging:

All participants will undergo preoperative thoracic, abdominal and pelvic staging CT according to usual practice. The CT scan should be performed within 8 weeks of surgery. Reporting of the CT scans for trial purposes will be on a dedicated proforma and will report:

- 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 (patients with evidence of metastatic disease are only eligible in this developmental phase)

Any other radiological imaging and preoperative work-up will be as per routine cancer practice.

11.1.2 Colorimetric dye and 5-ALA administration

As part of their normal investigation for bowel cancer, all patients undergo endoscopic examination. It is usual practice on finding a colon cancer to tattoo the lesion with India ink as a means of identifying it during laparoscopic surgery. We will standardise the method by which India ink tattooing is performed so as to be consistent between all patients recruited into GLiSten.

Participants will be admitted to hospital and undergo bowel preparation according to institutional preference. Colorimetric dye (India ink) will be administered at colonoscopy at the time of cancer investigation and diagnosis. This is routine practice for tumour localisation tattooing prior to laparoscopic colorectal surgery.

Oral 5-ALA solution will be administered at 4-6 hrs prior to operation.

11.2 Surgery: Segmental colectomy with D3 lymphadenectomy

Participants will undergo initial laparoscopic assessment to identify the overall lymphatic anatomy (colorimetric dye) and the presence of involved lymph nodes (5-ALA). Fluorescent lymph nodes will be marked with small surgical clips (e.g. Ligaclips) to facilitate subsequent pathological identification. Patients will undergo segmental colectomy with D3 lymphadenectomy. For cancers of the right colon, segmental colectomy with D3 lymphadenectomy will include central dissection at the origin of the ileocolic, right colic (when present) and middle colic vessels, with high vascular ligation and division. Specimen extraction and ileo-colic anastomosis will be as per surgeon's preference. For cancers of the sigmoid colon, segmental colectomy with D3 lymphadenectomy will include high ligation and division of the inferior mesenteric vessels proximal to the origin of the left colic vessels. Specimen extraction and colo-rectal anastomosis will be as per surgeon's preference.

11.3 Histological assessment of lymph node status

The sites of any fluorescent nodes will be marked at operation using small surgical clips. Photographs of the fresh specimen with the clips in place with a cm scale will be taken in theatre to assist the pathologists. Resection specimens will be subjected to in depth histopathology, as per the Royal College of Pathologists guidance. See appendix 3 for further details.

Additional assessment will include:

i) specimen photography and assessment of the completeness of mesocolic resection

ii) distance of tumour and bowel wall to vascular ligation and length of vessels

iii) area of lymphadenectomy

iv) level of lymphadenectomy based on vascular anatomy

v) histological analysis and mapping of lymph nodes identified by colorimetric

dye/5-ALA for metastatic deposits.

vi) Assessment of whether it is possible to visualise the fluorescent nodes at the time of cut up to further assist localisation of at the time of microscopy by fluorescent illumination.

11.4 Postoperative care

Participants will undergo standard postoperative care, with additional monitoring for side-effects to colorimetric dye/5-ALA, including measurement of liver function tests.

As follow-up is restricted to 30-days postoperative, for the purpose of identifying adverse events, problems with loss to follow-up will be minimal.

11.5 Withdrawal criteria

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of attending clinicians or the participants themselves. All participants withdrawn from treatment or prescribed alternative treatment will still attend for follow-up assessments unless unwilling to do so and CRFs will continue to be completed. The PI, or delegate should make every effort to ensure that the specific wishes of any participant who wishes to withdraw consent for further involvement in the trial are defined and documented using the Withdrawal CRF in order that the correct processes are followed by the SJUH Research Fellow and site following the withdrawal of consent. It should be made clear to any participant specifically withdrawing consent for further data collection in a CTIMP that data pertaining to safety will continue to be collected for regulatory reporting purposes and will be included in any safety analysis. In addition it is suggested that the participant is made aware of the fact that if any significant new information becomes available in regard to the treatment they have received in the trial it may be necessary to contact them in the future.

All patients will be able to withdraw from the study at any time, and will be reassured that this will not influence their subsequent standard of care. Specific reasons for patient withdrawal might include:

- Concern relating to involvement in a clinical trial of an investigational product.
- Concern regarding adverse effects of 5-ALA administration.

12 Trial Medicinal Product Management

12.1 Investigational Medicinal Products

The following are classified as Investigational Medicinal Products (IMPs).

- **5-ALA**

5-ALA (in the form of its hydrochloride, 5-ALA HCl) has been used in humans for fluorescence diagnosis and photodynamic therapy in a variety of cancers. It preferentially accumulates in cancer cells where it is metabolised to Protoporphyrin IX (PpIX), a component of the Heme pathway and an endogenous photosensitiser (22).

5-ALA is safe for human administration; it is used topically in dermatological malignancies, intravesically to detect bladder cancers, and orally in premalignant oesophageal lesions and malignant gliomas (23). It has also been administered intradermally, intraperitoneally and intravenously in experimental studies without adverse effects. 5-ALA is available in various forms depending on the intended mode of application. When administered orally in high doses for photodynamic therapy, side-effects may include nausea, tachycardia, hypotension, photosensitivity, and a transient rise in aspartate transaminase and bilirubin When 5-ALA is administered orally in conjunction with surgical resection of malignant brain tumours side effects can include brain oedema. 5-ALA is cleared by the liver and kidneys with a short half-life and return to baseline levels by 24 hours. Much smaller doses are required for fluorescence diagnosis; typically 20 mg/kg.

Please refer to the Pharmacy and IMP Trial Site Operating Procedure for full details of the trial IMP management requirements, including details of IMP labelling,

destruction, accountability and disposal records.

12.2 IMP supply, labelling and IMP handling

5-ALA will be supplied by photonamic GmbH & Co.KG, Germany with a trial specific label, in line with Directive 2001/20/EC and the Medicines for Human Use (Clinical Trials) Regulations 2004 (amended 2006). It must be ring fenced for this trial in a separate area to non-trial products and records retained in the Pharmacy Site File noting the location of the storage.

Please refer to the Pharmacy and IMP Management Trial Site Operating Procedure for full details of the trial IMP management requirements.

12.3 IMP Preparation

5-ALA will be prepared and handled in line with manufacturers' recommendations.

12.4 5-ALA Administration

5-ALA will be supplied in a sterile powder format to be reconstituted by dissolving in tap water immediately prior to oral administration. The dose of 5-ALA will be administered at either10 mg/kg, 20 mg/kg or 30 mg/kg.

12.5 Concomitant Medications

The only absolute contra-indications to the use of 5-ALA are

- A previous history of hypersensitivity to photosensitising drugs
- A previous history of acute or chronic porphyria
- Hepatic or renal dysfunction
- Pregnancy

5-ALA should be used with caution:

- With other medications known to have a photosensitising effect e.g. tetracylines, sulphonamides, quinolones. Patients should not be exposed to any photosensitizing agent up to 2 weeks after administration of 5 ALA
- With other medications associated with acute porphyria e.g. diclofenac, barbiturates, carbamazepine, phenytoin
- With other medication associated with hepatic or renal dysfunction e.g. NSAIDs, ACE-inhibitors, loop diuretics, phenytoin

13 ASSESSMENT AND DATA COLLECTION

Participating sites will be expected to maintain a file of essential trial documentation (Investigator Site File, ISF). Sites will keep copies of completed CRFs for the trial, or a file note to their location, within the ISF.

13.1 General CRF completion guidance

Investigations in this trial will use the results of local assessments. Data will be collected using paper case report forms (CRFs). CRFs must only be completed by personnel authorised to do so by the Principal Investigator, as recorded on the trial-specific Authorised Personnel Log. The original 'wet ink' version of the completed CRFs should be returned to the Research Fellow at SJUH at the address given in the Investigator Site File.

It is the responsibility of staff at participating sites to obliterate all personal identifiable data on any hospital reports, letters, etc, prior to sending to SJUH. Such records should only include Trial Number, initials and date of birth to identify the participant. The exception to this is the participant consent form, where the participant name and signature must not be obliterated. If signed consent forms are posted to SJUH, they must be sent in a separate envelope and not accompanied by any CRFs containing clinical data.

13.2 Pre-operative assessments and data collection

Preoperatively, patients will be assessed for inclusion into the study according to the criteria given in Section 9 and at the time of outpatient review. General assessment regarding cancer treatment and fitness for surgery will be as per institutional protocol.

Written informed consent must be obtained prior to the commencement of trial-specific assessments.

13.3 Radiological assessment

A specific baseline radiological CRF will be completed with details from the baseline screening CT.

13.4 Operative assessments and data collection

An operative CRF will be completed. This will collate data relating to the operation including:

Surgeon

ASA status

Details of D-Light 5-ALA lymph node assessment

Settings of Storz D-light laparoscopic system for 5-ALA fluorescence detection
Type of operation performed

Whether outcome of operation curative, palliative or unresectable in the opinion of the surgeon at the time of operation

Type of anastomosis

Any intra-operative complications, including open conversion

As patients will be undergoing cancer operations, it will not be possible to blind the surgeon to the result of the preoperative CT scan. The preoperative CT scan is fundamental to selection of the patient for curative surgery.

13.5 Pathology Assessment

Histopathological analysis of the colonic resection specimens will be carried out according to internationally agreed criteria (39). Further details are provided in Appendix 3.

This will include:

Fresh specimen photographs
Formalin-fixed specimen photographs
Cross-sectional slice photographs

Specimen sketch with lymph node mapping

- Number and size of detectable metastatic lymph nodes
- Estimated position of lymph nodes (D1/D2/D3) marked on diagram, using Japanese sub-group classification of lymph node stations

Final histopathology report with full histopathological staging data

Central review of specimens will require a complete set of glass H&E slides (either originals or an extra set) and all of the lymph node blocks and 2 blocks from the tumour. Further lymph node levels will be subjected to immunohistochemical analysis to confirm lymph node status and detect missed metastasis.

As a quality assurance measure, copies of all histopathology reports will be submitted to SJUH. **All personal identifiable information must be obliterated from reports prior to sending to SJUH.** However, the following patient information should be clearly marked on all histopathology reports to enable tracking and processing:

- Unique trial number (with site number obscured)
- Initials
- Date of birth
- Histopathology report number

As an optional item on the consent form, patients will be asked for consent to obtain their redundant tumour samples from the pathology laboratories in order to subject them to tissue microarray analysis. Research staff from SJUH will request the fixed tumour block from the Dublin centre pathologist in order to maintain confidentiality. On receipt, the sample will be anonymised and securely stored in a Human Tissue Authority (HTA, UK) licensed facility at St James's University Hospital, Leeds. These slides/tissue blocks will be identified by the patient's unique trial number, date of birth, and initials.

13.6 Post-operative Assessment and Data Collection

Post-operative care will be in line with nationally agreed guidance. (e.g. every 30 minutes for the next two hours, and hourly for a 12 hour period thereafter). Following this in the first 2 post-operative days observations will be Routine observations (temperature, pulse, oxygen saturation, blood pressure, and early warning score) will be performed most frequent initially (e.g. every 15 minutes for the first hour), becoming less frequent over time performed 4 hourly. After this period if a patient in considered stable with normal physiological parameters observations will be performed 4 times daily. The frequency and exact content of the assessment will be tailored to the individual patient.

Routine blood tests including Urea and Electrolytes and Liver Function Tests will be performed on a daily basis for 5 days post-operatively.

A single follow-up visit to the outpatient clinic at 30 days postoperative (or review in hospital if still an in-patient) will complete patient involvement in the study. Data collected at this visit will include:

Post-operative complications to 30 days and severity

Adverse events related to colorimetric dye / 5-ALA administration

There will be no trial follow up after 30 days post-operative and no data will be collected beyond this point.

13.7 Follow up after 30-day discharge from the study

After 30 days patients will undergo standard follow up for their colon cancer as per institutional guideline – involvement in the study will not change this standard practice. Data from these investigations and review will not be collected.

13.8 End of Trial

The ‘end of trial’ for the purposes of the European Clinical Trials Directive will be defined as the last recruited patient’s last data collection visit. At this point, the ‘end of trial notification’ will be submitted to the relevant regulatory authorities (e.g. UK MHRA) and ethics committees.

14 SAFETY REPORTING

Safety will be assessed by review of adverse events from the time of registration into trial to 30 days post-operatively, whether due to IMP administration or intra- and post-operative complications and mortality.

Collection of this data will be via the Case Report Forms and may require expedited reporting depending on seriousness of event.

14.1 General Definitions

Adverse Events (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Adverse Reactions (AR)

All untoward and unintended responses 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 what is foreseen in the protocol (i.e. if an AR occurs as a result of a medication error).

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 must be exercised in deciding whether an event is serious (see protocol section 14.5 for Responsibilities). These characteristics / consequences must be considered at the time of the event and do not refer to an event which hypothetically may have caused one of the above.

Where an SAE is deemed to have been related to an IMP used within the trial the event is termed as a **Serious Adverse Reaction (SAR)**.

Suspected Unexpected Serious Adverse Reactions (SUSAR)

An adverse reaction, the nature and severity of which is not consistent with the applicable product information in the summary of product characteristics. Severity describes the intensity of the event.

14.2 Operational Definition and Reporting Adverse Events/Reactions

Information about adverse events/reactions whether volunteered by the participant, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded on the relevant CRF.

Adverse events /reactions will be collected for all participants from the time of registration into the trial until 30 days post-operatively and will be evaluated for duration and intensity according to the National Cancer Institute Common Terminology Criteria for Adverse Events V4.0 (NCI-CTCAE). A copy is provided in the Investigator Site File and may be obtained at:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

Published date: 28 May 2009.

14.3 Operational Definition and Reporting SAEs/SARs and SUSARs

All SAEs, SARs and SUSARs occurring from the time of registration until 30 days post-operatively must be recorded on the relevant form and faxed to the CTRU within 24 hours of the research staff becoming aware of the event. Once all resulting queries have been resolved, the original form should also be posted to the Research Fellow at SJUH and a copy to be retained on site.

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 must be made by a doctor. If a doctor is unavailable, initial reports without causality and expectedness assessment should be submitted to CTRU within 24 hours, but must be followed up by medical assessment as soon as possible thereafter.

Any change of condition or other follow-up information should be faxed to the CTRU as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

All SAEs assigned as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Medicines and Healthcare products Regulatory Authority (MHRA) and Irish Medicines Board (IMB). The CTRU will inform the MHRA, IMB the main REC and the Sponsor of SUSARs within the required expedited reporting timescales.

Please ensure that each event is reported separately and not combined on one SAE form.

Events not classed as SAEs

The following events **will not** be recorded as SAEs within this trial:

Hospitalisation for:

- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- Treatment that was elective or pre-planned, for a pre-existing condition not associated with any deterioration in condition.
- Admission to hospital or other institution for general care not associated with any deterioration in condition.

- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions for serious as given above and not resulting in hospital admission.
- Disease progression

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:

Operative

- 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

- Altered bowel habit
- 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
- Sexual dysfunction
- 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
- Scrotal swelling
- Sepsis
- Subcutaneous emphysema
- Urinary tract infection
- Delerium

Fax Number for reporting SAE/SARs and SUSARs:

+ 44 (0) 113 343 7985

14.4 Pregnancies and Suspected Pregnancies

If a participant becomes pregnant while enrolled in this clinical trial or up to three months following administration of 5-ALA the investigator or designated site personnel must notify the CTRU within 24 hours of learning of the pregnancy. The Sponsor will be responsible for notifying SJUH Research Fellow. Pregnancy is a contraindication to administration of 5-ALA. Participants, spouses, or partners will be followed through the outcome of the pregnancy. The Investigator will be required to report the results to the Sponsor.

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.

14.5 Responsibilities

Principal Investigator (PI):

1 Checking for AEs and ARs when participants attend for treatment / follow-up.

2 Using medical judgement in assigning seriousness, causality and preliminary expectedness using the Reference Safety Information approved for the trial.

3 Ensuring that all SAEs and SARs (including SUSARs) are recorded and reported to the CTRU within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.

4 Ensuring that SAEs and SARs (including SUSARs) are chased with CTRU if a record of receipt is not received within 2 working days of initial reporting.

5 Ensuring that AEs and ARs are recorded and reported to the CTRU in line with the requirements of the protocol.

CTRU

1 Provide office hours cover for receipt of all initial and follow up SAEs, SARs, and SUSARs. The CTRU will conduct an initial review of the core dataset prior to forwarding to CI/Research Fellow for medical review.

2 Expedited reporting of SUSARs to the relevant Competent Authorities (MHRA and Irish Medicines Board [IMB]), Main RECs in UK and Ireland and Sponsor within required timelines.

3 Pass all data to the Research Fellow for databasing.

SJUH Research Fellow:

1 Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol onto a database.

2 Preparing annual progress reports to Main RECs in UK and Ireland and periodic safety reports to TSC and DMEC as appropriate.

3 Reporting safety information to the independent oversight committees identified for the trial (Data Monitoring & Ethics Committee (DMEC) and / or Trial Steering Committee (TSC)) according to the Trial Monitoring Plan.

4 Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the MHRA, IMB and Main REC in UK and Ireland.

5 Notifying Investigators of SUSARs that occur within the trial.

6 Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.

7 Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring

Plan.

8 Reporting events to collaborating pharmaceutical company in accordance with the trial contract.

9 Any duties as delegated by the CI

Chief Investigator (CI) / delegate or independent clinical reviewer:

1 Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.

2 Using medical judgement in assigning seriousness, causality and expectedness of SAEs where it has not been possible to obtain local medical assessment.

3 Immediate review and determination of expectedness for all events assessed as related to the IMP in the opinion of the local investigator.

4 Review of specific SAEs and SARs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.

5 Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.

6 Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data and liaising with the DMEC regarding safety issues.

Data Monitoring & Ethics Committee (DMEC):

In accordance with the Trial Terms of Reference for the DMEC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

Pharmaceutical company:

1 Supply of the Study Drug according to the Drug Transfer Agreement

2 Labelling of the Study Drug which with the label provided by the sponsor

3 Information of the Principal Investigator on all important new safety information for the Study Drug without delay.

4 Information of the Principal Investigator on relevant Quality defects as soon as they are known.

15. ENDPOINTS

15.1 Primary Endpoints

- Identification of the optimal dose for oral administration of 5-ALA for the accurate intra-operative detection of lymph node metastases

15.2 Secondary Endpoints

- Standardisation of preoperative CT reporting, with emphasis on lymph node evaluation
- Standardisation of operative procedure including D3 lymphadenectomy
- Optimisation and standardisation of fluorescence detection system.
- Standardisation of pathological lymph node mapping and step sectioning for in depth lymph node evaluation.
- Patient factors affecting the accuracy of 5-ALA fluorescence diagnosis.
- Safety

15.3 Study Definitions

- Standardisation of operative procedure including D3 lymphadenectomy will be achieved through the production of a training video to standardise segmental right and

sigmoid colectomy with D3 lymphadenectomy.

- Patient factors affecting the accuracy of 5-ALA fluorescence diagnosis will be identified and recorded e.g. BMI and visceral obesity.
- Safety will be assessed by review of:
    - i) adverse events from the time of colonoscopic application of colorimetric dye and oral administration of 5-ALA to 30 days post-operatively,
    - ii) intra-and post-operative complications and mortality up to 30 days and re-operation rate

16. STATISTICAL CONSIDERATIONS

16.1 Sample size

See Section 8.1 (study design).

16.2 Planned recruitment rate

Both centres will recruit at least 24 patients over a period of 18 months (total of at least 48 patients), aiming to ensure an enriched sample of patients with lymph node metastases (Dukes' stage C). Both investigators undertake around 30 resections for colon cancer per annum, so a minimum target of 24 patients per centre in 18 months should be readily achievable

17 STATISTICAL ANALYSIS

Statistical analysis is the responsibility of the Research Fellow, with oversight from the CTRU Statistician. A full statistical analysis plan will be written before any analyses are undertaken. The analysis plan will be finalised and agreed by the following people: the Research Fellow, the CTRU Statistician, CTRU Supervising Statistician, the Chief Investigator, the CTRU Principal Investigator and the Senior Trial Manager. Any changes to the finalised analysis plan, and reasons for changes, will be documented.

Statistical analysis will be descriptive for the endpoints defined in Section 15.

The primary analysis population will include all participants who have received 5-ALA.

17.1 Frequency of analyses

See Section 8.1 for details of the study design.

Within the recruitment of the first 20 patients with positive lymph nodes, the ability of 5-ALA to detect positive nodes as compared to histology in at least 2 of 10 patients (in either cohort) will be required to progress to the next part of the study.

If 5-ALA detects positive lymph nodes in at least 2 of 10 patients (in either cohort) then, from these initial results, the most promising dose of 5-ALA will be identified. At least a further 10 patients with lymph nodes with metastatic disease as judged by histological evaluation will then be recruited and treated with this schedule, with flexibility to include further patients to confirm the validity of the technique before proceeding to the evaluation phase. The ability of 5-ALA FD to reliably detect lymph nodes with metastatic disease as judged by histopathological evaluation will be assessed by requiring that, to progress to the evaluation phase, the upper bound of the 99% (Clopper-Pearson) confidence interval of the sensitivity (in the patients with positive nodes recruited in this stage of the trial) is at least the target value of 80%. To allow for the optimization of the other variables (e.g. fluorescence detection), however, this analysis will not include patients treated with this schedule before it was identified as the most promising schedule.

Safety will be assessed on an ongoing basis. Particular attention will be paid to the rates of conversion, resection margin positivity and post-operative outcomes of laparoscopic segmental colectomy with D3 lymphadenectomy as markers of safety. Any rates deemed to be excessive (e.g. conversion rates >50%; resection margin positivity >30%; morbidity >50%; anastomotic leak rates ≥10%; major complication

rate ≥30%; re-operation rate ≥10%, 30-day mortality rate ≥5%) will prompt further investigation and, if necessary, the suspension or withdrawal of individual sites or termination of the entire trial. Adverse events suspected to be related to 5-ALA will be reviewed in real time by the DMEC. Serious adverse events (SAEs) not suspected to be related to 5-ALA will also be reviewed in real time by the DMEC

18. DATA MONITORING

Trial supervision will be established according to the principles of GCP and in line with the relevant Research Governance Framework within the UK (and any relevant research governance requirements in non-UK countries). This will include establishment of a core Project Team, Trial Management Group (TMG), a Trial Steering Committee (TSC) and Data Monitoring and Ethics Committee (DMEC).

18.1 Data Monitoring and Ethics Committee

An independent DMEC will review the safety and ethics of the study.

Safety will be assessed on an ongoing basis by SJUH. Particular attention will be paid to the rates of conversion, resection margin positivity and post-operative outcomes of laparoscopic segmental colectomy with D3 lymphadenectomy as markers of safety. Any rates deemed to be excessive (e.g. conversion rates >50%; resection margin positivity >30%; morbidity >50%; anastomotic leak rates ≥10%; major complication rate ≥30%; re-operation rate ≥10%, 30-day mortality rate ≥5%) will prompt further investigation and, if necessary, the suspension or withdrawal of individual sites or termination of the entire trial. Adverse events suspected to be related to 5-ALA will be reviewed in real time by the DMEC. Serious adverse events (SAEs) not suspected to be related to 5-ALA will also be reviewed in real time by the DMEC.

The DMEC will be provided with detailed reports containing the information agreed in the data monitoring analysis plan. Particular attention will be paid to the rates of conversion, resection margin positivity and post-operative outcomes of laparoscopic segmental colectomy with D3 lymphadenectomy as markers of safety, as detailed above.

18.2 Data Monitoring

Data will be monitored for quality and completeness by SJUH. Missing data will be chased until it is received, confirmed as not available or the trial is at analysis. SJUH/Sponsor will reserve the right to intermittently conduct source data verification exercises on a sample of participants, which will be carried out by staff from SJUH/ Sponsor. Source data verification will involve direct access to participant notes at the participating hospital sites and the collection of copies of consent forms and other relevant investigation reports.

18.3 Clinical Governance Issues

To ensure responsibility and accountability for the overall quality of care received by participants during the trial period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the TSC and, where applicable, to individual NHS Trusts.

19 QUALITY ASSURANCE AND ETHICAL CONSIDERATIONS

19.1 Quality Assurance

The trial will be conducted in accordance with the principles of Good Clinical Practice in clinical trials, as applicable under UK regulations, the NHS Research Governance Framework (*and Scottish Executive Health Department Research Governance Framework for Health and Social Care 2006 for studies conducted in Scotland),* and through adherence to CTRU Standard Operating Procedures (SOPs) for those duties undertaken by CTRU.

SJUH, CTRU and Sponsor have systems in place to ensure that serious breaches of GCP or the trial protocol are picked up and reported. Investigators are required to

promptly notify the CTRU of a serious breach (as defined by Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of. A “serious breach” is a breach which is likely to effect to a significant degree –

the safety or physical or mental integrity of the participants of the trial; or

the scientific value of the trial.

For further information, the Investigator should contact the Research Fellow at SJUH.

19.2 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. Informed written consent will be obtained from the participants prior to registration into the trial. The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment. The trial will be submitted to and approved by a main Research Ethics Committee (main REC) and the appropriate Site Specific Assessor for each participating centre prior to entering participants into the trial. The CTRU will provide the main REC with a copy of the final protocol, patient information sheets, consent forms and all other relevant trial documentation.

20 CONFIDENTIALITY

All information collected during the course of the trial will be kept strictly confidential. Information will be held securely on paper and electronically at SJUH. SJUH will comply with all aspects of the 1998 Data Protection Act and operationally this will include:

- 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.
- Participant name will be collected on the consent form when a participant is registered into the trial, but all other data collection forms that are transferred to or from SJUH will be coded with a trial number and will include two participant identifiers, usually the participant's initials and date of birth.
- where central monitoring of source documents by SJUH (or copies of source documents) is required (such as scans or local blood results), the participant's name must be obliterated by site before sending
- where anonymisation of documentation is required, sites are responsible for ensuring only the instructed identifiers are present before sending to SJUH

If a participant withdraws consent from further trial treatment and / or further collection of data, their data and samples will remain on file and will be included in the final study analysis.

The trial staff at the participating site will be responsible for ensuring that any data / documentation sent to the SJUH is appropriately anonymised as per instructions given by SJUH in accordance with the trial procedures to conform with the 1998 Data Protection Act.

21 ARCHIVING

At the end of the trial, data will be securely archived in line with the Sponsor's procedures for a minimum of 15 years. Site data and documents will be archived at the participating centres. Following authorisation from the Sponsor, arrangements for confidential destruction will then be made.

22 STATEMENT OF INDEMNITY

This trial is sponsored by The University of Leeds and the University of Leeds will be liable for negligent harm caused by the design of the trial.

In the UK the NHS has a duty of care to participants treated, whether or not the participant is taking part in a clinical trial, and the NHS remains liable for clinical negligence and other negligent harm to participants under this duty of care.

In Ireland the study will be covered under the national Clinical Indemnity Scheme and all Irish doctors participating will have their own annual medical malpractice insurance.

As this is a clinician-led trial, there are no arrangements for no-fault compensation; however, usual product liability will be covered by the manufacturer under the Consumer Protection Act 1987.

23 TRIAL ORGANISATIONAL STRUCTURE

Responsibilities

Chief Investigator - The Chief Investigator will have overall responsibility for the design and set-up of the trial, the investigational drug supply, pharmacovigilance, management and conduct of the trial in accordance with relevant GCP standards.

The Research Fellow will be responsible for the day-to-day running of the trial including trial design, clinical set-up, protocol development, CRF design, trial administration, organisation of oversight groups (TSC and DMEC) set up and maintenance of the Trial Master File, database administrative functions, data management, training, monitoring and progress reports (funder, MREC, Sponsor, DSUR), promotion of the trial. The Research Fellow will be responsible for all statistical analyses, with oversight from the CTRU.

Clinical Trials Research Unit – The CTRU will lead the IRAS and NIHR CSP submissions and provide input into protocol development in accordance with CTRU SOPs and the GCP Conditions and Principles as detailed in the UK Medicines for Human Use (Clinical Trials) Regulations. The CTRU will house the office hours registration service, provide pharmacovigilance reporting to MHRA, IMB and MREC in accordance with regulatory timelines, provide a database and oversight of statistical analysis.

Operational Structure

Trial Management Group - The TMG, comprising the Chief Investigator, Research Fellow, CTRU team and co-investigators will be assigned responsibility for the clinical set-up, on-going management, promotion of the trial, and for the interpretation of results. Specifically the TMG will be responsible for (i) protocol completion, (ii) CRF development, (iii) obtaining approval from the main REC and supporting applications for Site Specific Assessments, (iv) submitting a CTA

application and obtaining approval from the MHRA, (v) completing cost estimates and project initiation, (vi) appointing and facilitating the TSC and DMEC, (vii) reporting of serious adverse events, (viii) monitoring of screening, recruitment, treatment and follow-up procedures, (ix) auditing consent procedures, data collection, trial end-point validation and database development

Trial Steering Committee (TSC) – The TSC, with an independent Chair, will provide overall supervision of the trial, in particular trial progress, adherence to protocol, participant safety and consideration of new information. It will include an Independent Chair, not less than two other independent members and a consumer representative. The CI and other members of the TMG may attend the TSC meetings and present and report progress. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee: The independent DMEC will review the safety and ethics of the trial during the dose expansion phase by reviewing interim data during recruitment. The Committee will meet or communicate via teleconference approximately annually, with discussion of safety reports conducted via email.

24 PUBLICATION POLICY

The trial will be registered with an authorised registry, according to ICMJE Guidelines, prior to the start of recruitment. The success of the trial depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contributorship. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data
- drafting the article or revising it critically for important intellectual content
- and final approval of the version to be published
- and that all these conditions must be met (www.icmje.org).

In light of this the Chief Investigator, Beaumont Hospital Principal Investigator, Co-Investigators and relevant senior CTRU staff will be named as authors in any publication. Dependent on the number of participating sites and journal restrictions, wherever possible at least two CTRU staff will be named as authors. Notwithstanding this, all publications will be written on behalf of the GLiSten research collaborative, and all participants will be listed by name in the publication, giving details of roles in planning, conducting and reporting the trial.

The release of any data must be approved by the Trial Management Group, and the release of any data out with that described above must be approved by the Data Monitoring and Ethics Committee. In addition, individual collaborators must not publish data concerning their participants which is directly relevant to the questions posed in the trial until the long-term results of the trial have been published and following written consent from the Sponsor.

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26 APPENDICES

Appendix 1: Current trials using 5-ALA

Study Title	Aim	Phase	Dose of 5-ALA	Timing prior to surgery	Recruiting/completed	Sponsor and Country of origin of Study
Fluorescence guided surgery for low and high grade gliomas (BALANCE)	To determine the impact of intra-operative fluorescence and confocal microscopy on the volume of residual disease following resection of a newly diagnosed glioma.	Phase 3 Single – blinded Randomised Placebo controlled	20 mg/kg	3 hours	Recruiting	St. Joseph's Hospital and Medical Center Phoenix America
More complete removal of malignant brain tumours by fluorescence guided surgery	To determine the safety and utility of 5-ALA for identifying brain tumours during surgery.	Phase 2	20 mg/kg	3-5 hours	Recruiting	Emory University America
A study of the specificity and sensitivity of 5-ALA	To identify borders of malignant gliomas intra-operatively using 5-ALA and fluorescence to assess the	Phase 1 Phase 2	10mg/kg 20 mg/kg 30 mg/kg 40 mg/kg	Not stated	Recruiting	Southern Illinois University America

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fluorescence in malignant brain tumors	toxicity, sensitivity and specificity of 5-ALA.		50 mg/kg			
Study Title	**Aim**	**Phase**	**Dose of 5-ALA**	**Timing prior to surgery**	**Recruiting/completed**	**Sponsor and Country of origin of Study**
Fluorescence guided resection of malignant gliomas with 5-ALA	To determine how accurately contrast agent-accumulating tumour can be removed by primary surgery and to assess the clinical usefulness of this method.	Phase 3 Single – blinded Randomised	1.5 g	2-4 hours	Completed	Medac GmbH University of Munich Germany
5-ALA in visualizing a Tumor During Surgery in Patients With glioblastoma multiforme	To investigate the safety and performance of 5-ALA for visualizing glioblastoma multiforme intra-operatively in comparison to intra-operative MRI.	Phase 2	Not stated	2-4 hours	Recruiting	Case Comprehensive Cancer Center Ohio America

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Study of 5-ALA for Fluorescence-guided Resection of malignant gliomas	To assess the positive predictive value of 5-ALA induced tissue fluorescence, safety and pharmacokinetics following a single dose 5-ALA for surgery in patients with newly or recurrent malignant glioma.	Phase 3	20mg/kg	2-4 hours	Completed	Nobelpharma Japan
Study Title	**Aim**	**Phase**	**Dose of 5-ALA**	**Timing prior to surgery**	**Recruiting/completed**	**Sponsor and Country of origin of Study**
Safety Study of 5-ALA to improve visibility of brain tumors during surgery	To assess whether 5-ALA induced fluorescence allows for a more complete resection of malignant brain tumours.	Phase 2	20 mg/kg	3 hours	Recruiting	West Penn Allegheny Health System Pennsylvania, United States,
Gliadel wafer and fluorescence-guided surgery with 5-ALA	To study the side effects of fluorescence-guided surgery with 5-ALA given together with gliadel wafer, followed by radiation therapy and	Phase 2	Not stated	Not Stated	Recruiting	Cancer Research UK Cambridge United Kingdom

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followed by radiation therapy and temozolomide in treating patients with primary glioblastoma	temozolomide, in treating patients with primary glioblastoma.					
Study Title	**Aim**	**Phase**	**Dose of 5-ALA**	**Timing prior to surgery**	**Recruiting/completed**	**Sponsor and Country of origin of Study**
A Study of 5-ALA to enhance visualization and resection of malignant glial tumors of the brain	To determine the safety and utility of using 5-ALA in removing brain tumors during surgery.	Phase 2	30 mg/kg	4 hours	Recruiting	Legacy Health System Oregon America

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Appendix 2: Radiology 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 5 due to its reproducibility) 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 proximal to the initial angulation of the hepatic flexure)

□ ***Hepatic Flexure*** (segment distal to the initial angulation of the hepatic flexure to the proximal border of the falciform ligament)

□ ***Sigmoid colon*** (segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)

Tumour morphology

Polypoidal□ Flat□ Semi annular□ Annular□

Tumour length (mm): _____

T stage

□**T2** or less (limited by muscularis propria)

□**T3** not breaching serosa

□**T4a** penetration of serosa with extension into adjacent organs

□**T4b** penetration of serosa and peritoneal surface with perforation of bowel

N stage

Number of visible nodes_____

Number of malignant nodes

□**N0** - none

□**N1** - 1-3 regional lymph nodes appear malignant

□**N2** – 4 or more regional lymph nodes appear malignant

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 metastases

□**M1a** - distant metastases one organ

□**M1b** - peritoneal or distant metastases to more than one organ or distant nodes

Metastases location:

□**Liver**

□**Lung**

□**Peritoneum**

Other _____

Right colon vascular anatomy assessment

□Ileocolic artery present

□Right colic artery present (arising directly from the SMA ***not*** ileocolic)

□Middle colic artery present

□Artery crosses anterior to SMV

□Artery crosses posterior to SMV

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 41

- The inferior mesenteric nodes are expressed as 36

- Para-aortic nodes are marked as 4, and iliac nodes as 3 – 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.

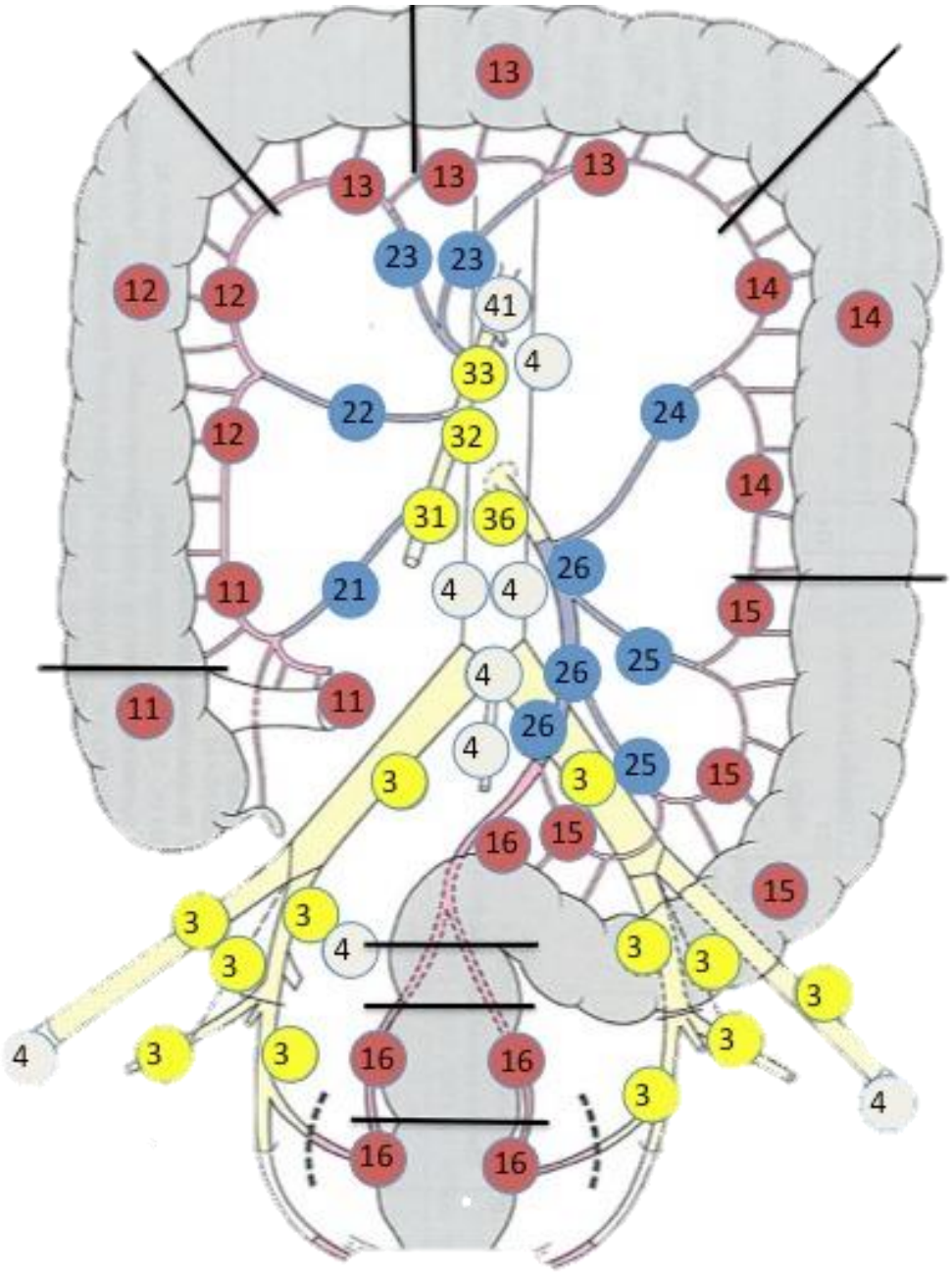

Figure 1. Modified Japanese staging subgroups - Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

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Station number	**Node**	**Estimate size of node:** maximum short axis diameter (mm)	**Appearance of dominant node**
11	1 2 3		e.g: 0.No node present 1.Benign 2.Probably Benign 3.Probably malignant 4.Malignant
21			
31			
12			
22			
32			
13			
23			
33			
41			
14			
24			
15			
25			
16			

Station number	**Node**	**Estimate size of node:** maximum short axis diameter (mm)	**Appearance of dominant node**
26			
36			
4			
3			
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. 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). N.B. Right colic vessel may not be present - anatomy to be drawn in on Figure 2.

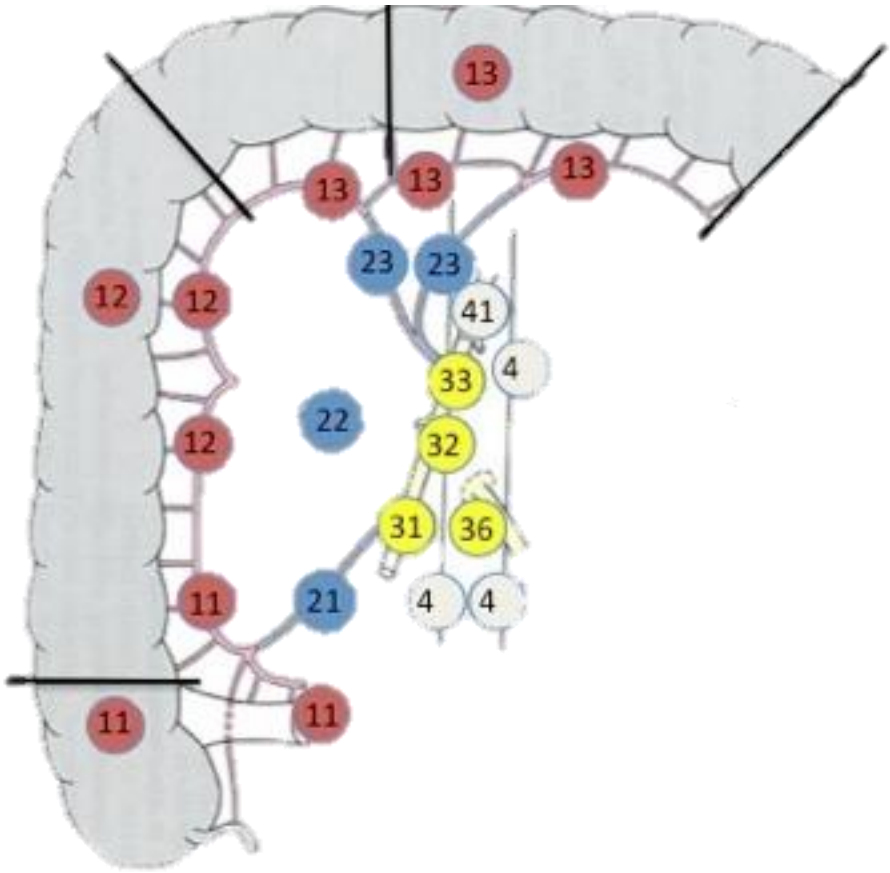

Figure 2. Right-sided resection. Modified Japanese staging subgroups

To draw in vascular anatomy of right colic artery

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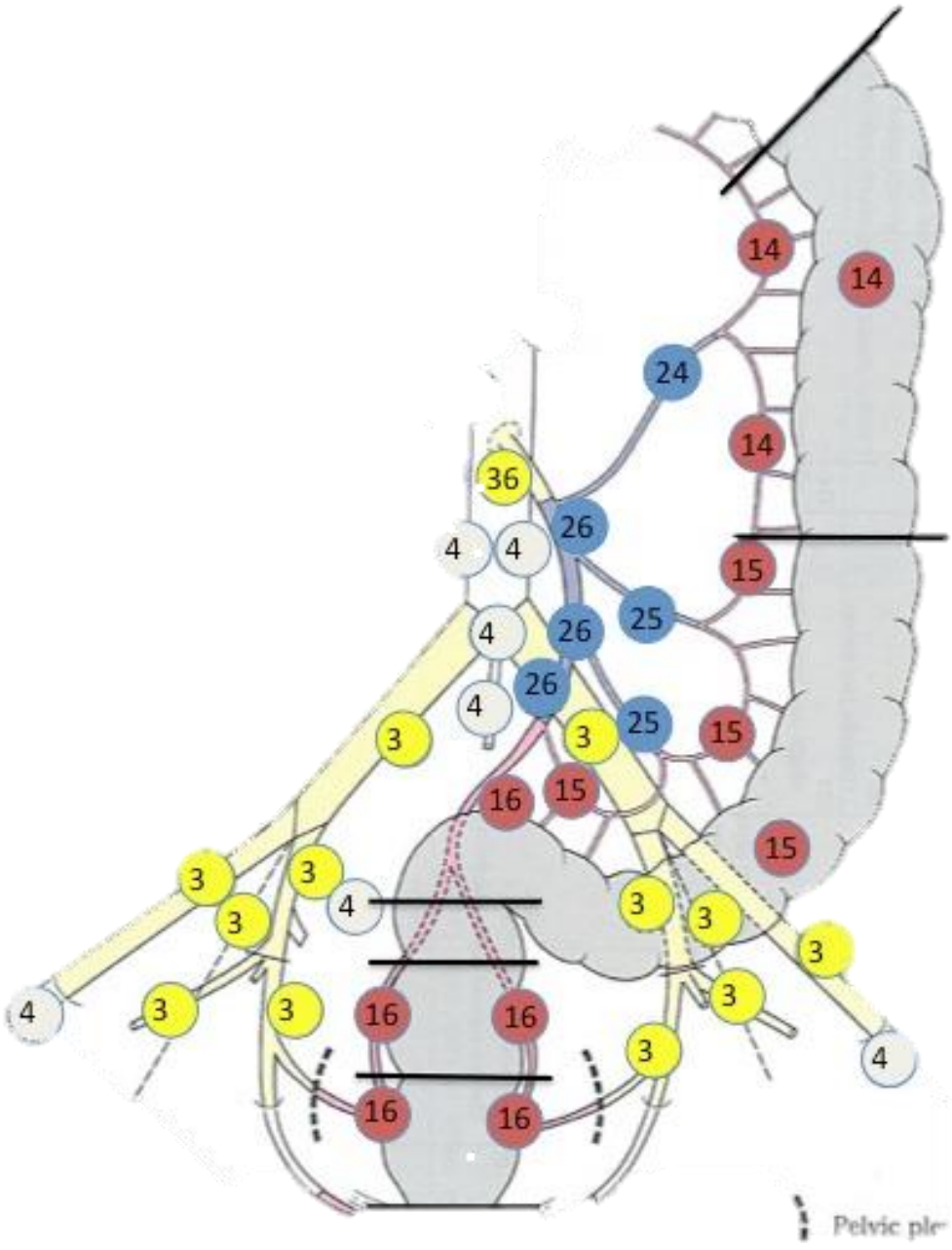

Figure 3. Left-sided resection. Modified Japanese staging subgroups.

To note any anomalies involving a left sided resection.

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Appendix 3: Histopathology proforma

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) 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)

iii) Taking digital photographs of the serial cross-sectional slices from the resection specimen

iv) Submitting a specimen sketch detailing the estimated position of all lymph nodes (positive and negative) according to station number (see diagram below)

v) Submitting the final histopathology report with full histopathological staging data for review

vi) Submitting all of the H&E stained glass slides (or copies) for central review

vii) 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.

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 histopathologist 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 quality of surgery. Any clips identifying fluorescent lymph nodes should be clearly visible. Additional pictures of any mesocolic defects or perforations would be helpful. All photographs **must** include a metric ruler scale (for calibration) and the site of the tumour and the high vascular ties should be marked (e.g. with forceps or pre-printed labels). The whole specimen should be visible in the image and mesentery should be laid out flat (not folded or over stretched). The proximal and distal aspects can also be labelled if not obvious. Photographs should be taken directly above the specimen to reduce distortion and while a white background is ideal, any other plain colour is acceptable (see figure 1).

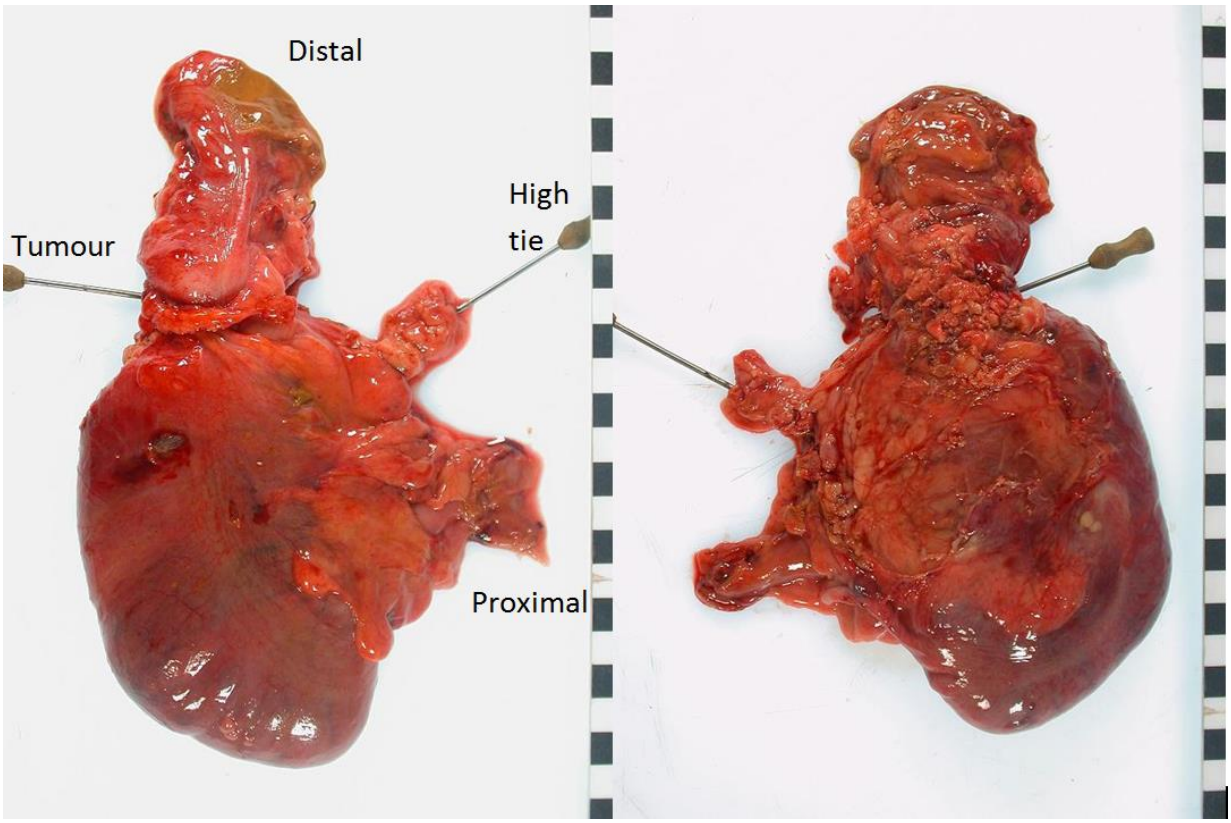

Figure 1: Photography of the whole fresh resection specimen including anterior (left) and posterior (right) views alongside a metric scale with the tumour and high tie identified.

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

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 2). It should be remembered that the circumferential 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.

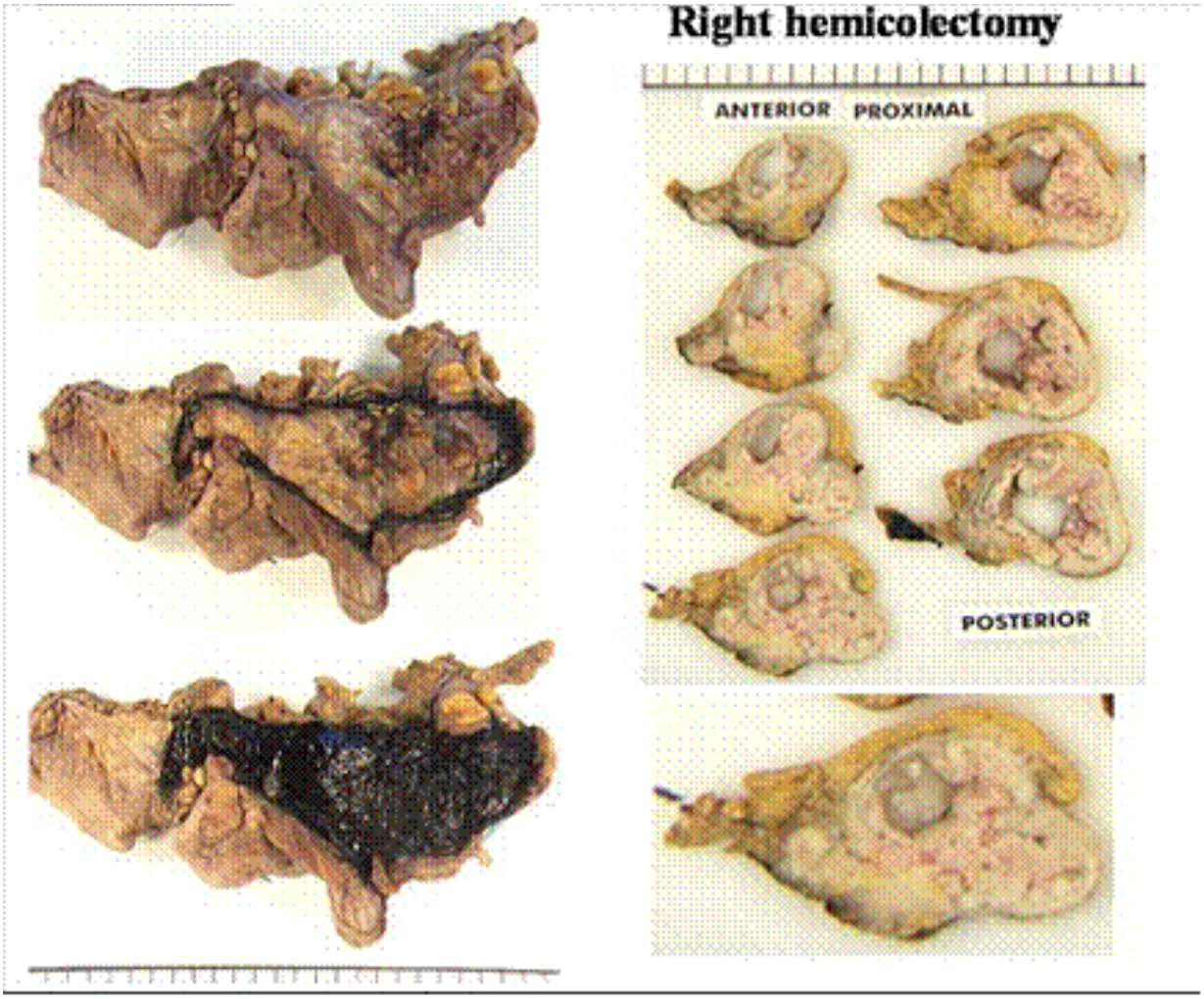

Figure 2: Dissection method showing inking of the retroperitoneal margin and cross sectioning to assess tumour spread. Note the metric scale in the images.

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 2 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 (3-5mm) sliced transversely from 2 cm below to 2 cm above the tumour. The slices should also be photographed as a valuable demonstration of the quality of the surgery. These can be taken all together as shown in figure 2. Additional close ups of all tumour bearing slices would be helpful or alternatively all of the slices may be photographed individually. It should be clear which are the most proximal and distal slices (e.g. by using labels) and all photographs must contain a metric scale e.g. ruler (figure 2).

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 5 blocks of tumour to confirm presence or absence of extramural venous invasion. The number of any large blocks containing tumour can be subtracted from these 5. Likewise the peritoneal surface should be sampled by a minimum of 2 blocks if the tumour impinges on it (figure 3).

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 to allow Dukes' staging to be conducted 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.

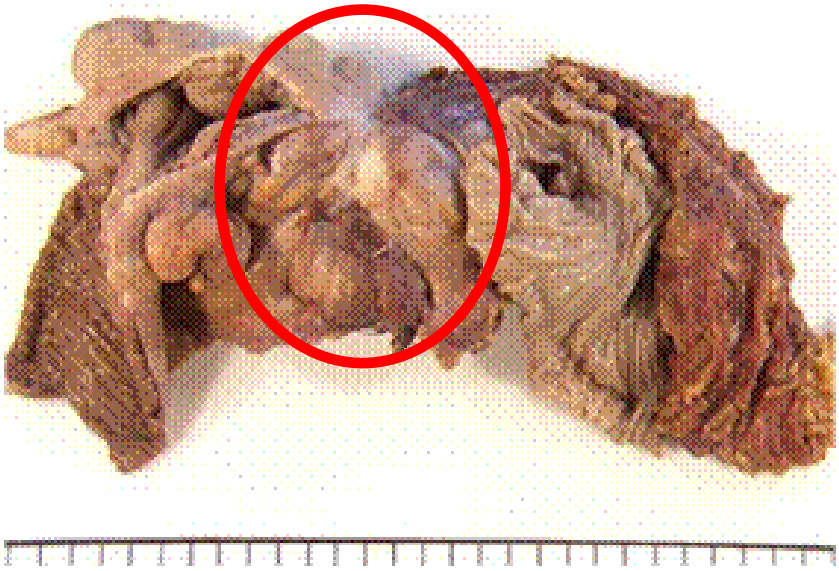

Figure 3: Suspected peritoneal/serosal involvement by tumour.

Histological reporting

We are using both Dukes' and TNM 5 for histopathological staging in this study, not TNM 6 or 7. This is because of the poor reproducibility of the TNM 6 definitions of extramural vascular invasion and lymph nodes. Thus we will be using the 3mm rule for nodal involvement, although these will be captured separately on the CRF. This allows the study to be consistent with other trials such as the Dutch TME trial and MRC CR07 trial. Dukes' staging allows easy communication between surgeons and the clinical team whereas TNM 5 gives more prognostic information, especially with respect to early tumours and local spread, e.g. peritoneal and direct spread (figure 3).

The non-peritonealised resection margin is considered involved (i.e. an incomplete excision) if the tumour extends to within 1 mm of the inked margin. Measurement is best made by using a sheet of graph paper that is photocopied onto a sheet of acetate and cut to size. This can be provided if required and may be more easily used than the Vernier scale. This is shown in figure 4 and can also be used for measuring the EMVI to see if it is greater than 3 mm. No distinction is currently made between the various modes of non-peritonealised resection

margin involvement, e.g. direct spread, lymph node spread, vascular, etc. Although all are associated with an increased local recurrence rate, this may be lower in the case of involvement by tumour within a lymph node.

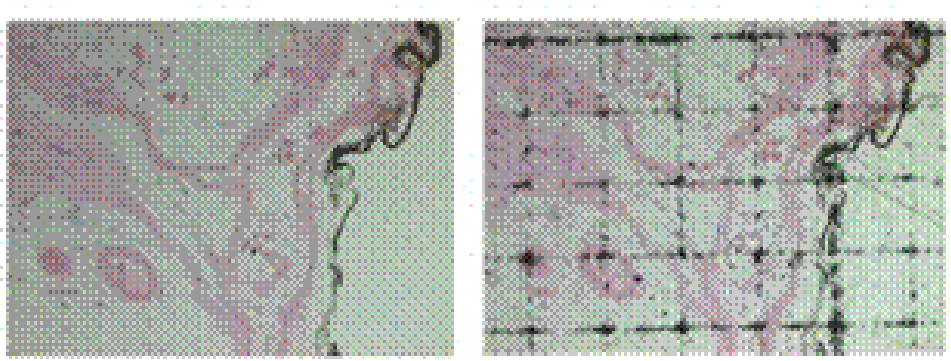

Figure 4: Rapid measurement of the distance from the tumour to the non-peritonealised resection margin by overlaying a simple grid.

Peritoneal involvement (pT4) should be diagnosed if tumour cells penetrate the peritoneal surface (figure 5).

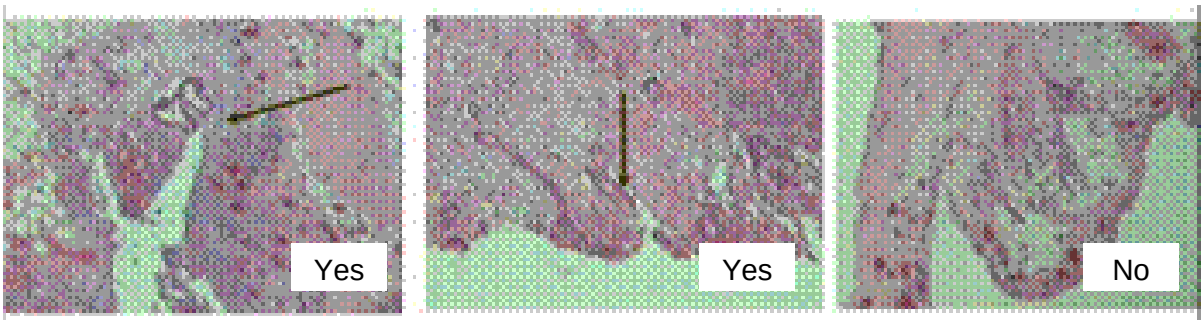

Figure 5: Peritoneal involvement should be assessed by the method of Shepherd et al 1995 whereby tumour cells should be seen to penetrate through the serosa to lie on the surface of the specimen.

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 adenocarcinomas, signet ring and undifferentiated should be documented.

Grading the quality of surgery (mesocolic resection)

The quality 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 6/7), 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.

Plane of dissection	**Short description**	**Detailed description**
Mesocolic plane	Good quality specimen	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
Intramesocolic plane	Moderate quality specimen	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
Muscularis propria	Poor quality specimen	There may be little bulk to the mesocolon and there will be extensive defects that extend down

plane		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.

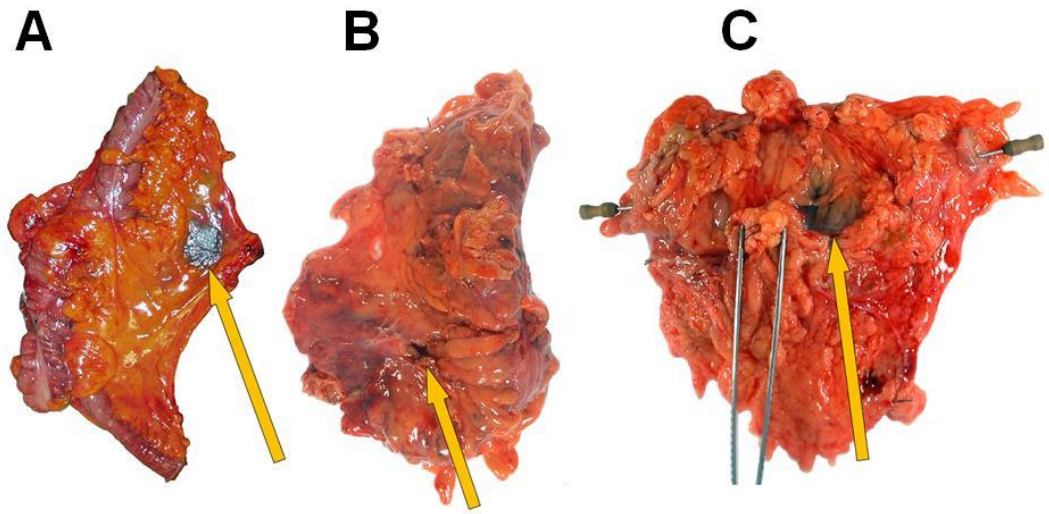

Figure 6: Three fresh colon cancer specimens resected in the (A) mesocolic plane with an intact peritoneal window (arrow), (B) intramesocolic plane with a significant mesocolic defect (arrow), and (C) muscularis propria plane with a posterior perforation of the specimen (arrow).

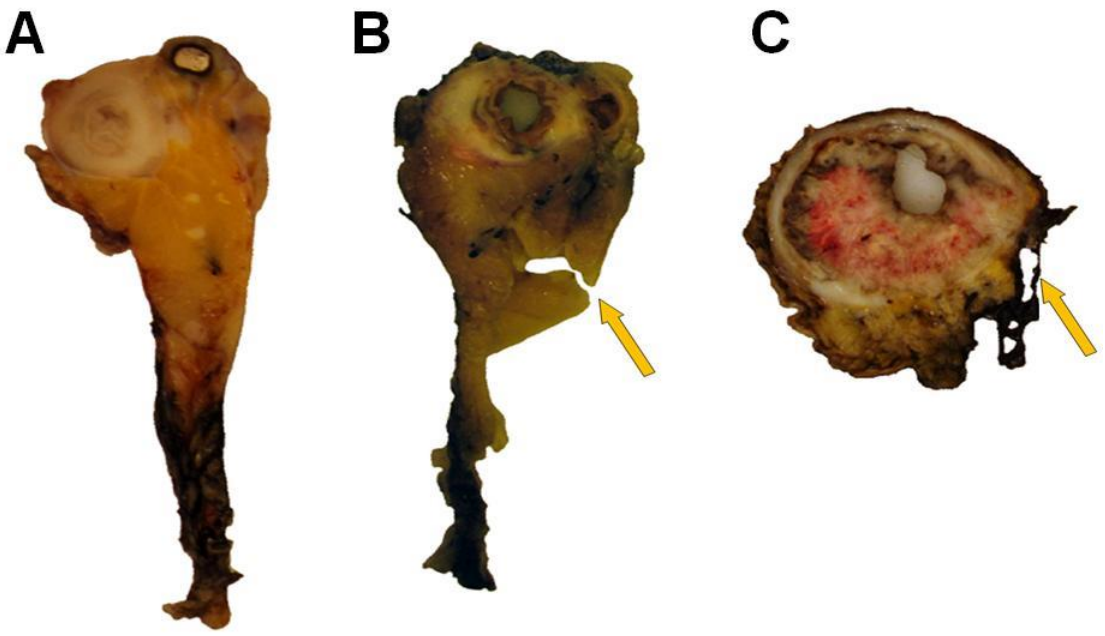

Figure 7: Cross-sectional slices from three specimens resected in the (A) mesocolic plane, (B) intramesocolic plane with a significant mesocolic defect (arrow), and (C) muscularis propria plane with a defect extending down to the muscularis propria (arrow).

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 8, 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 to enable Dukes' staging.

As stated in TNM 5, extramural tumour deposits measuring ≥3 mm in maximum size are

counted as involved lymph nodes even if no residual lymph node structure can be identified. Smaller deposits are regarded as apparent discontinuous extensions of the main tumour. The number of ≥3mm tumour deposits classified as lymph nodes should be indicated in the report. Any tumour deposits measuring >3mm (and therefore counted as lymph nodes under TNM 5) should also be mapped and blocked carefully.

The local pathologist will cut a single section from each lymph node block and report the number of positive and negative nodes in each station using conventional histopathology. A copy of the diagnostic glass H&E slides, pathology report, photographs, specimen sketches and lymph node blocks will then be sent to Leeds for central review. Deeper levels and immunohistochemical testing will be used on the lymph node blocks 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 in each station were detected, how many were involved by tumour, and how this related to the intra-operative fluorescent nodes.

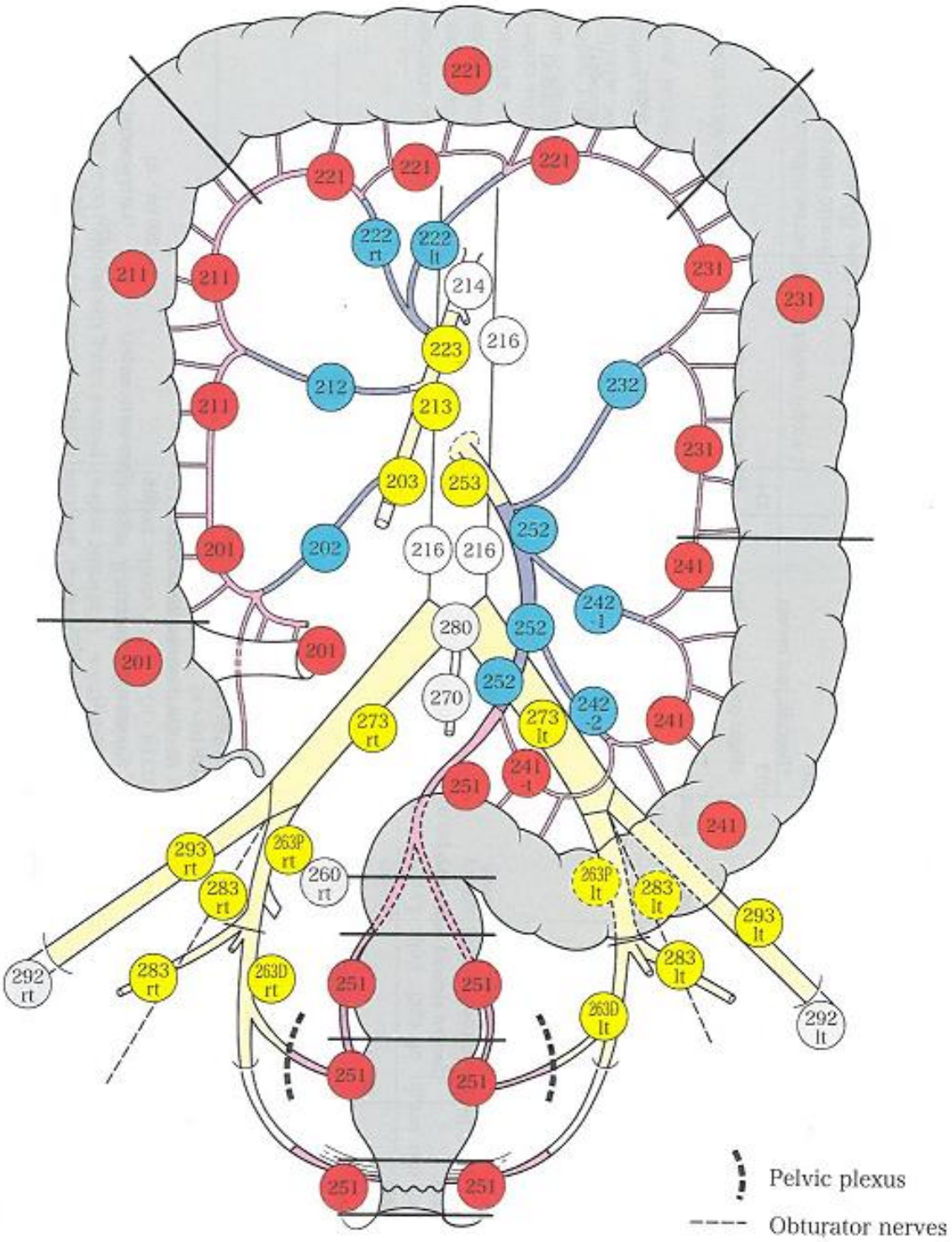

Figure 8: Japanese staging subgroups - Pericolic, D1 lymph nodes (red); Intermediate D2

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, deposit >3mm	No	A9	No
	7	<5cm	No	A9	No
212	1		No	A10	No

	2 3 4 5		No No No No	A10 A10 A11 A11	No No No No
213	1 2	Apical (right colic)	No No	A12 A13	No 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, the 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 nodes that are tumour deposits >3mm. Also the pericolic (D1) node 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 4: Surgical proforma

Introduction

The guidance below is provided with the aim of establishing a standard surgical procedure with regards to documentation of areas of fluorescence and extent of dissection and resection.

Operative assessment

The surgeon's details will be documented along with the patient's ASA.

The dosage and timing of administration of 5-ALA will be noted prior to initial laparoscopy, thus compiling a set of data to optimise the protocol during the evaluation phase. The settings of the Storz D-light laparoscopic system for 5-ALA fluorescence detection will also be documented.

The method of establishing pneumoperitonum and port site location and size will be performed according to the surgeon's preference.

Participants will undergo initial laparoscopic assessment to identify the overall lymphatic anatomy (colorimetric dye) and the presence of fluorescence (5-ALA) of lymph nodes, tumour and any additional areas within the parietal or visceral peritoneum.
Fluorescent lymph nodes will be marked with ligaclips to facilitate subsequent pathological identification.
The estimated location of the tumour and position of any fluorescent nodes (D1/D2/D3), including their size and number will be marked on the diagram (modified Japanese sub-group classification of nodal stations) in figure 1 and noted on table 1.The anatomy of the right colic artery will be noted on figure 2. Any anomalies involving a left sided resection will be noted on figure 3. Any additional areas of fluorescence; size, approximate location and whether excised as separate specimen will be noted on table 1.
Photos and videos will be taken via the laparoscopic stack to document areas of fluorescence.
Following initial laparoscopy patients will undergo segmental colectomy with D3 lymphadenectomy. A note will be made of the type of operation performed.

Right Hemicolectomy

For cancers of the right colon (caecum to the medial border of the falciform ligament) segmental colectomy with D3 lymphadenectomy will include:

- Incision of the mesocolon at the root of the ileocolic vessels, extending towards the planned site of terminal ileal division.
- Medial to lateral dissection in the retroperitoneal plane with ventral displacement of the right ureter and duodenum. Proximal dissection of the ileocolic vessels to their superior mesenteric origin.
- High, central ligation of ileocolic artery and vein
- High, central ligation of the right colic vessels, when present as separate branches
- Dissection of the middle colic vessels. For hepatic flexure cancers, the middle colic vessels will be taken at their origin. For caecal and ascending colon cancers, the right branch of the middle colic vessels will be taken at their origin
- Division of the terminal ileum
- Division of the lateral peritoneal attachments and mobilisation of the splenic flexure.
- Division of the transverse colon.
- Exteriorisation of the specimen through a wound of the surgeon's choosing
- Extracorporeal ileocolic anastomosis, by the surgeon's preferred method.
- Return to the bowel to the peritoneal cavity and closure of the wound using the surgeon's preferred technique.

Sigmoid Colectomy

For cancers of the sigmoid colon (distal to the level of the left iliac crest to 15 cm proximal to the anal verge) segmental colectomy with D3 lymphadenectomy will include:

- High ligation and division of the inferior mesenteric artery proximal to the origin of the left colic vessels.
- High ligation of the inferior mesenteric vein immediately below the inferior border of the pancreas
- Medial to lateral dissection with extension laterally under the left paracolic peritoneal

reflection, caudally over the sacral promontory to the mesorectal plane and superiorly to the transverse mesocolon

- Mobilisation of the splenic flexure where necessary
- Method of bowel division, specimen extraction and colorectal anastomosis will be as per surgeon's preference.

Operative procedure

The outcome of surgery, according to the opinion of the surgeon at time of operation, whether curative, palliative or unresectable will be noted. Any intra-operative complications, including excessive blood loss or open conversion will also be documented. Conversion to an open procedure will not affect the initial laparoscopic assessment and marking of fluorescent nodes; such patients will still be included in the trial. A note will be made of the duration of the operation, type of anastomosis, and extraction site of specimen.

Training video

Standardisation of operative procedure including D3 lymphadenectomy will be achieved through the production of a training video to standardise segmental right and sigmoid colectomy with D3 lymphadenectomy for the evaluation phase(4).

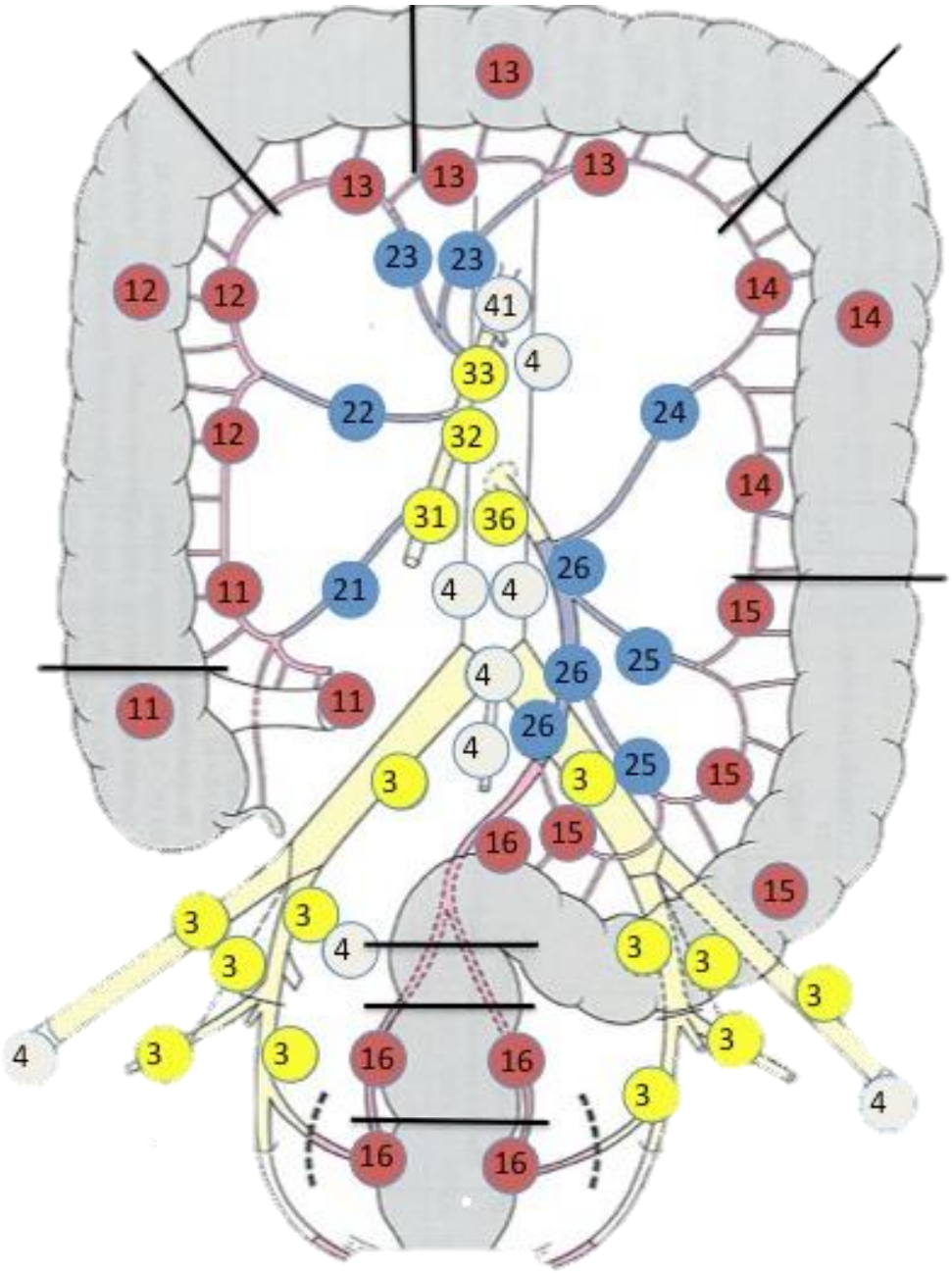

Figure 1. Modified Japanese staging subgroups - Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

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Station number	**Vessel**	**Node**	**Estimate size of node (mm)**	**Comments e.g. Apical node**	**Colorimetric dyevisible Yes/No**	**Fluorescent Yes/no**
11	Ileocolic D1	1 2				
21	Ileocolic D2	1 2				
31	Ileocolic D3	1 2		e.g.Apical (ileocolic)		
12	Right colic D1	1 2				
22	Right Colic D2	1 2				
32	Right Colic D3	1 2 3		e.g.Apical (right colic)		
13	Middle Colic D1	1 2				
23	Middle Colic D2	1 2				
33	Middle Colic D3	1 2		e.g.Apical node (middle colic)		

Station number	**Vessel**	**Node**	**Estimate size of node (mm)**	**Comments e.g. Apical node**	**Colorimetric dyevisible Yes/No**	**Fluorescent Yes/no**
41	Origin of SMA	1 2		Comment whether fluorescent (not excised)		
14	Left Colic D1	1				
24	Left Colic D2	1				
15	Sigmoid branches D1	1 2				
25	Sigmoid Branches D2	1 2				
16	Superior rectal D1	1				
26	IMA	1				
36	Origin of IMA	1		e.g.Apical node (IMA)		

Station number	**Vessel**	**Node**	**Estimate size of node (mm)**	**Comments e.g. Apical node**	**Colorimetric dyevisible Yes/No**	**Fluorescent Yes/no**
4	Any para-aortic nodes	1 2 3		Comment whether fluorescent (not excised)		
3	Any iliac nodes	1 2 3		Comment whether fluorescent (not excised)		
TOTAL						
Additional Comments: Free text to add any additional areas of fluorescence – size, approximate location and whether excised as separate specimen.						

Table 1: Example of how to map the lymph nodes including the station number, vessel, number of nodes in each station, whether colorimetric dye was present, whether the node was fluorescent. 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). N.B. Right colic vessel may not be present - anatomy to be drawn in on Figure 2.

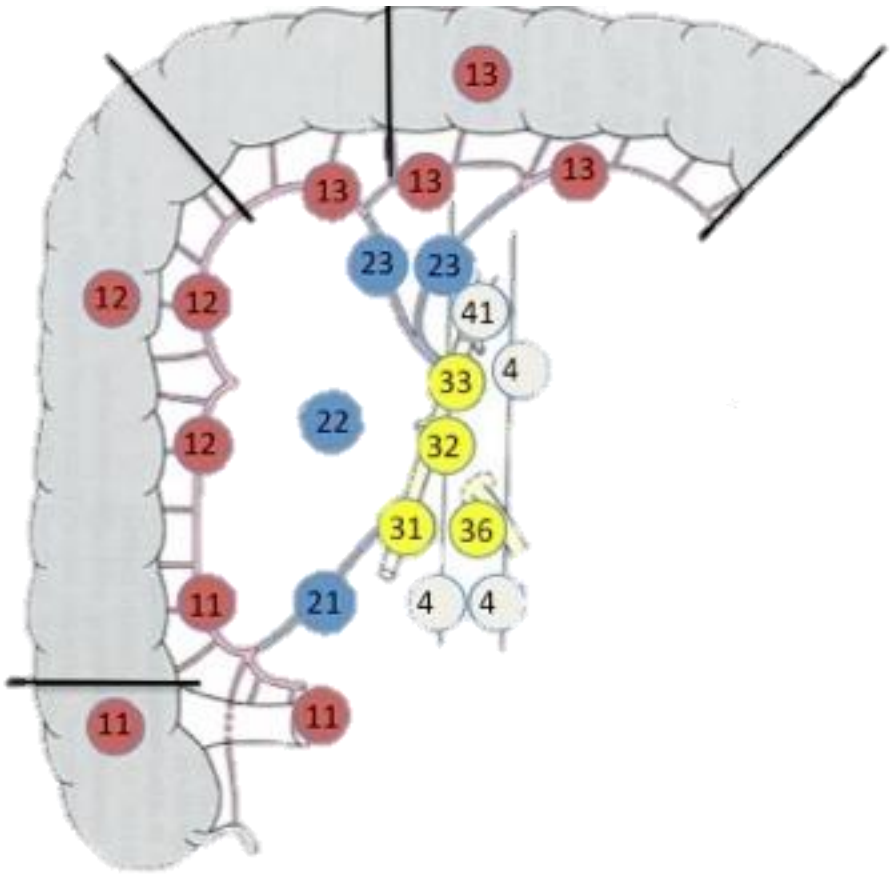

Figure 2. Right-sided resection. Modified Japanese staging subgroups.

To draw in vascular anatomy of right colic artery.

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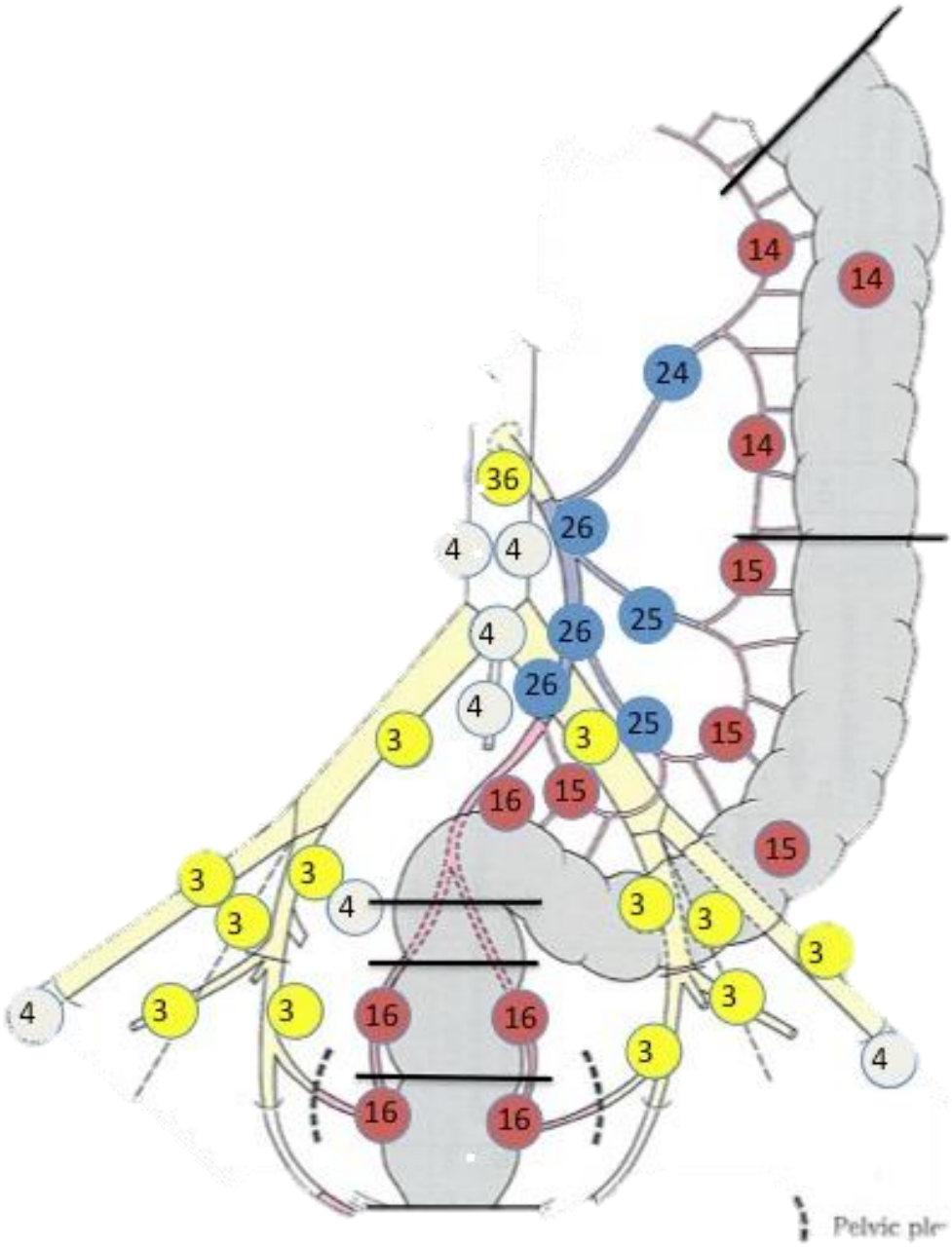

Figure 3. Left-sided resection.Modified Japanese staging subgroups.

To note any anomalies involving a left sided resection.

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